LIFE07 ENV/DE/000218 "FutMon"

Results of the 10th UNECE/ICP-Forests & FutMon Combined Intercalibration Course on the Assessment of Ozone Visible Injury

Report: Action C1-O3-24 (ES)

Report Prepared by Fundación CEAM
and the Swiss Federal Institute WSL

Course hosted by the Hungarian
Forest Research Institute (ERTI)

1. INTRODUCTION

As part of FutMon action C1-O3-24 (ES), an Intercalibration Course on the Assessment of Ozone Visible Injury was carried out in Budapest, on 21-24 September 2009, hosted by the Hungarian Forest Research Institute (ERTI). 27 delegates of the following countries have participated: Austria, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Japan, Romania, Slovakia, Slovenia, Spain and Switzerland.
Progress reports of the activities related with air quality and visible injury were presented by the different countries/FutMon beneficiaries. Detailed information on the program and on the reports can be found at: http://www.ozoneeffects.org/Training2009_EN. In the present report, we will focus on results of the exercises in the context of Quality Assurance/Quality Control actions of the FutMon project.

The intercalibration course included three exercises:
1) Photo-exercise with 54 pictures of symptomatic leaves and plants. The participants scored each pictorial sample in two categories: symptoms are a.) ozone-induced or b.) not ozone-induced.
2) Exercise with fresh plant material, during which leaves of 20 plants were scored as injured by ozone or not.
3) Field exercise, during which the incidence of visible injury in native vegetation at a forest edge in Matra Mountains was assessed, applying the methods of the FutMon field protocols (see http://www.ozoneeffects.org/Downloads_EN)

2. RESULTS OF THE PHOTO EXERCISE

A total of 54 pictures of plants with typical ozone-induced symptoms and with other types of injury (abiotic or biotic) were assessed as ozone-symptomatic or not-ozone symptomatic by the different individual teams. Four of the plants were removed from the analysis due to unclear description of the symptoms, so that the analysis presented here covers 50 slides.
For all the 50 pictures, the mean agreement per slide compared to the control (one of the chairmen, who provided some of the pictures, some of them from controlled conditions) was 80%. For some species, a 100% agreement was recorded (Figure 1). All these pictures except of *Viburnum lantana* (4) showed symptoms caused by other

agents. *Viburnum lantana* (4) was a leaf with very clear and typical ozone symptoms. The lowest agreement was with *Cornus mas*, and with some pictures of *Fagus sylvatica* and *Viburnum lantana*. The former species is well-known to be problematic due to its natural reddening, difficult to discriminate from ozone-induced visible injury. In severely injured *Fagus sylvatica* leaves with necrotic areas (e.g. *Fagus sylvatica*, 1), agreement was above 90%, but for leaves showing diffuse bronzing there was some discrepancy in the assessment. In this course, F. Bussotti presented results of a study, demonstrating that bronzing was observed even under low ozone levels but under high light intensities; concomitant exposure to ozone increased bronzing. Therefore, assessment of this type of symptoms in *Fagus* is problematic. It is expected that expert advice using the on-line tool for ozone-like symptom validation (see http://www.ozoneeffects.org/Links_EN) will help establishing clear criteria for the assessment of such symptoms. Pictures of symptoms from different parts of Europe will be assessed by several experts, providing a homogeneous advice on how to score the symptoms for difficult species.

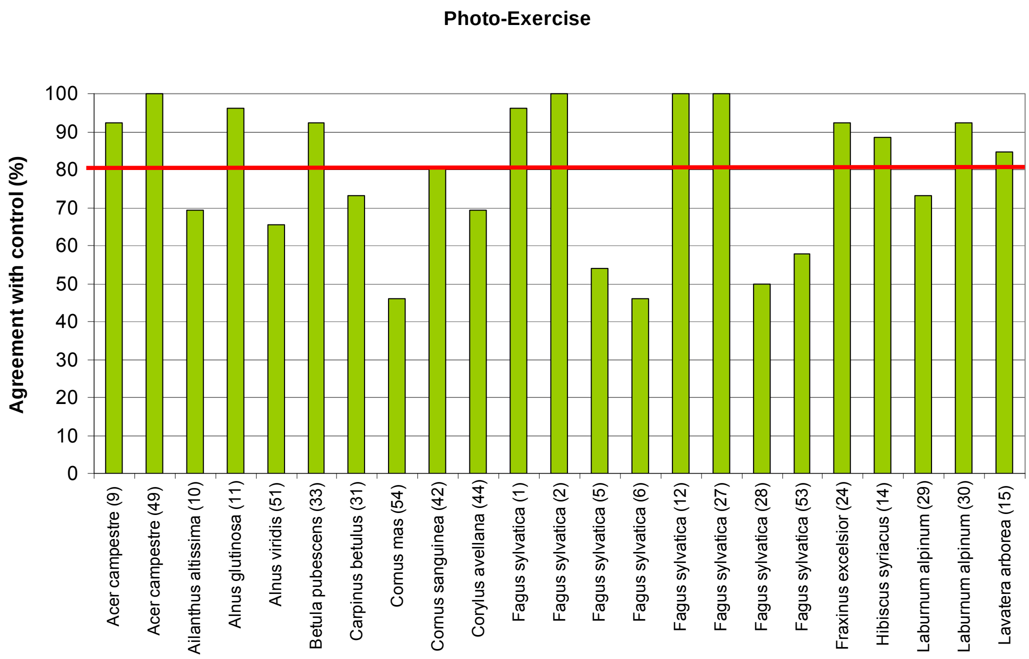

Figure 1 (part 1). Percentage of agreement with the control for the different pictures of plants showing ozone injury and other types of symptoms. Teams assessed each picture as ozone-symptomatic or not. Line in red = mean agreement for all species (80%).

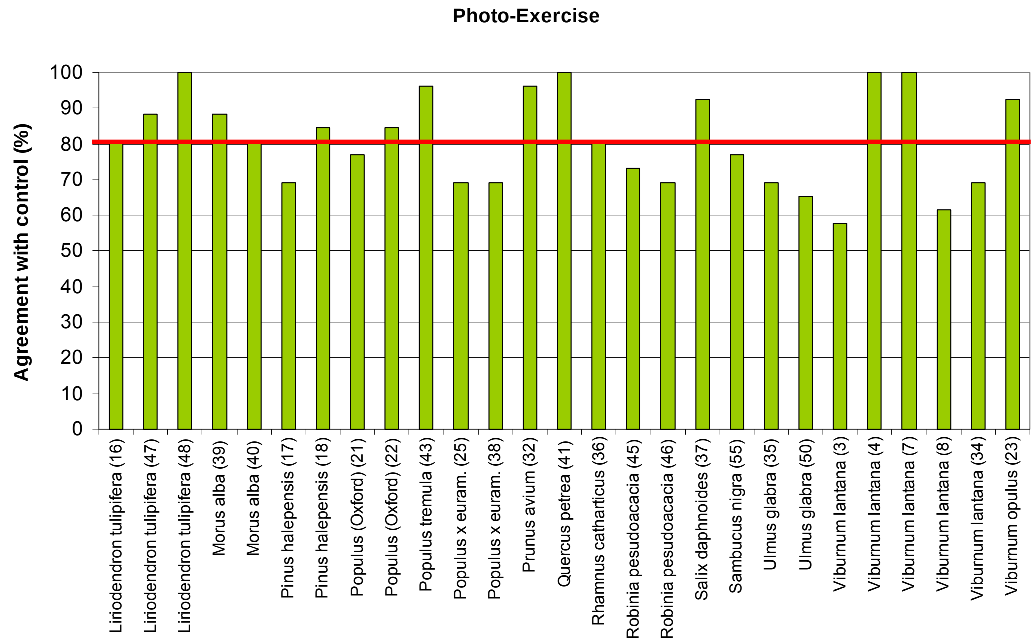

Figure 1 (part 2). [Cont. of Figure 1 (part 1)]. Percentage of agreement with the control for the different pictures of plants showing ozone injury and other types of symptoms. Teams assessed each picture as ozone-symptomatic or not. Line in red = mean agreement for all species (80%).

With respect to the teams, 65.4% of the teams showed an agreement of ≥ 80% with the control (Figure 2). When the threshold was set at ≥ 70%, 80.8% of the teams achieved this objective. The five teams below this limit attended the course for the first time, suggesting that previous intercalibration courses have been successful in increasing the comparability and calibration among the teams.

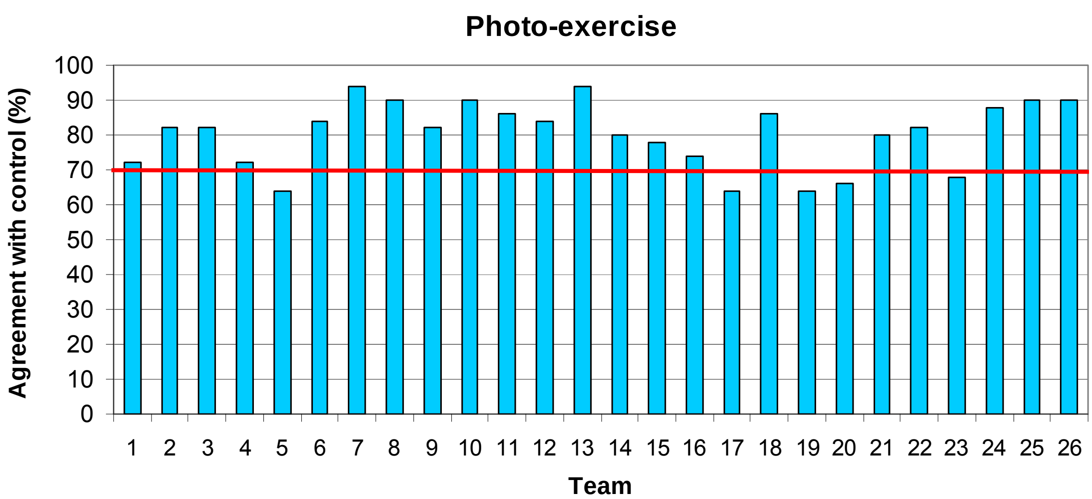

Figure 2. Percentage of agreement of the teams with the control for the 50 slides scored.

3. RESULTS OF THE FRESH MATERIAL EXERCISE

The fresh material exercise represented an important complement of the photo-exercise. The fresh material exercise allows assessing several leaves per plant, examining both side of the leaf, and looking for possible remnants of insects. This information is partly missing in the photo-exercise. Out of 20 samples scored, two herbs were removed due to the presence of atypical (*Parietaria judaica*) or non-specific (*Taraxacum officinale*) ozone symptoms. Analysis was therefore based on 18 samples (Figure 3). Plants were collected in the field or from open-top chambers, where they were fumigated with elevated ozone levels. On average, 86% of the scores were coincident with the control. As in the photo-exercise, the *Cornus* species turned out to be most difficult to assess: ozone may enhance natural reddening, but this symptom is not specific as it can occur even under low ozone levels. Ozone symptoms in *Pinus halepensis* consisted of a diffuse and faint mottling that can be easily confused with traces from mites; considerable training is needed to separate both types of symptoms and microscopy may be applied as an additional tool/method for validation.

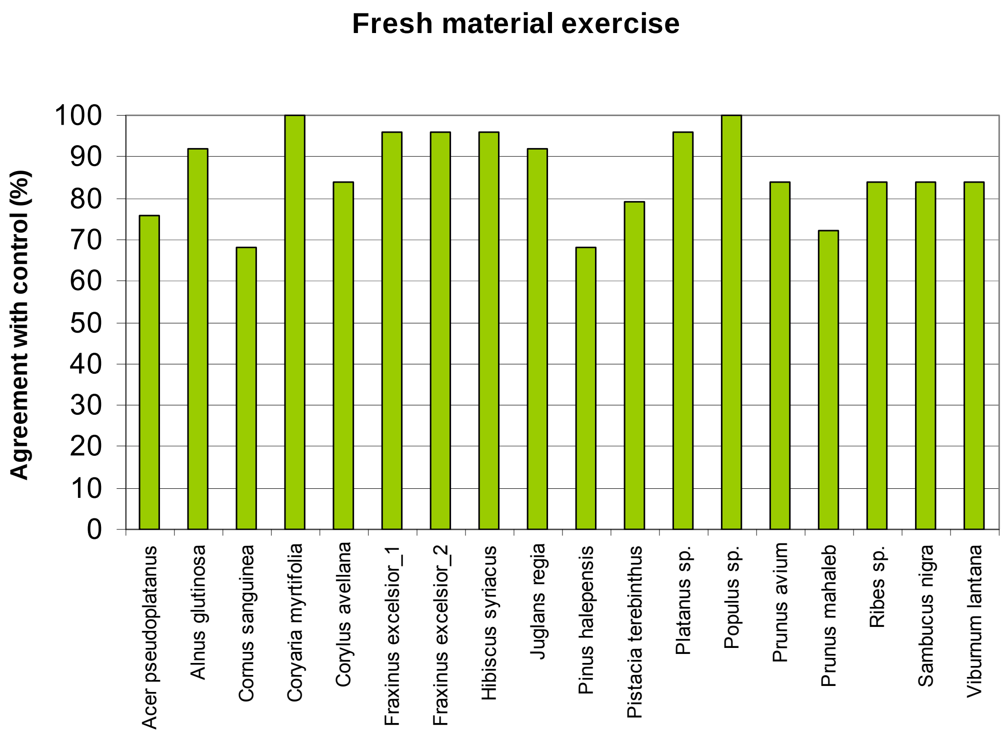

Figure 3. Percentage of agreement with the control for the different samples of plants showing ozone injury and other types of symptoms. Teams assessed each sample as ozone-symptomatic or not.

A total of 25 individual teams scored each of the 18 fresh samples. 84.0% of the teams agreed with the control assessment of ≥ 80%, while 92.0% of the teams agreed with the control assessment of ≥ 70% (Figure 4). Three of the four teams which did not reach the 80% threshold of agreement attended the visible injury intercalibration meeting for the first time or only occasionally. The results of this exercise showed a better agreement with the control than the photo-exercise, most likely due to the fact that more information can be gathered (as outline above) when fresh material is assessed compared to two-dimensional slides. However, the nature of the samples (species with difficult vs. easily identifiable ozone symptoms), may also have an effect on the results.

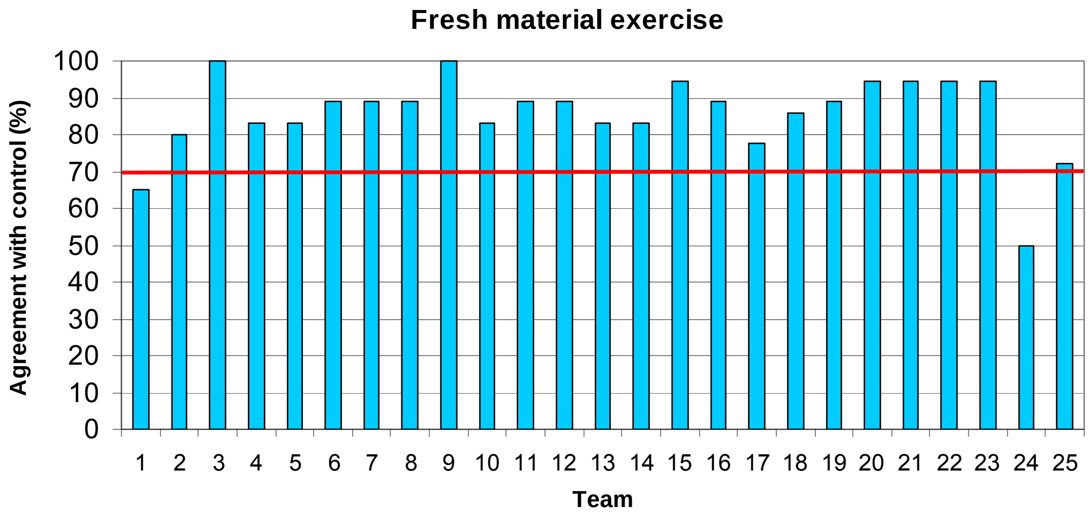

Figure 4. Percentage of agreement of the teams with the control for the 18 samples scored.

3. RESULTS OF FIELD EXERCISE

The field exercise was emphasizing the procedures and guide lines for the assessment of ozone visible injury at the light exposed sampling sites (LESS) as outlined in the FutMon field protocol. It consisted of the assessment of the vegetation in 21 quadrates (2 x 1 m), randomly distributed along a 300 m forest edge, with an estimated sampling error of 20%. In each quadrate, all woody plants were assessed and scored for ozone injury (species symptomatic inside the quadrate or not). Eleven teams composed by 1 to 3 people (per country) participated in the assessment. In addition to practicing the procedures for the assessment of visible injury in the field, the exercise was also oriented towards an assessment of the variability due to adverse field conditions, i.e. variability due to the way each team determined the randomly selected quadrates. This information is complementary to previous exercises carried out in previous years, during which the variability in the same quadrates was scored (e.g. Bussotti et al., 2006).

Applying this methodology, on average 8 (varying from 5 to 10) different species were assessed by 11 teams (Table 1). As team 11 was considered the reference, 70% of the teams were inside the interval of 9 ± 20% (7.2-10.8). Species assessed for visible injury by each team that were common to those scored by the control was on average 62.2%, and varied from 88.9% to 44.4% in the different teams.

Table 1. Total number of species assessed by each team and percentage of species common to control.

Team	Number of species	Percentage of species common to control
1	6	44.4
2	9	66.7
3	8	66.7
4	10	88.9
5	5	44.4
6	9	55.6
7	8	66.7
8	7	66.7
9	9	55.6
10	8	66.7
11	9	Control
Mean	**8.0**	**62.2**
SD	**1.5**	**13.0**

The relationship between number of species and number of quadrates is presented in Figure 5. With 21 quadrates, the average number of species does not seem to increase anymore.

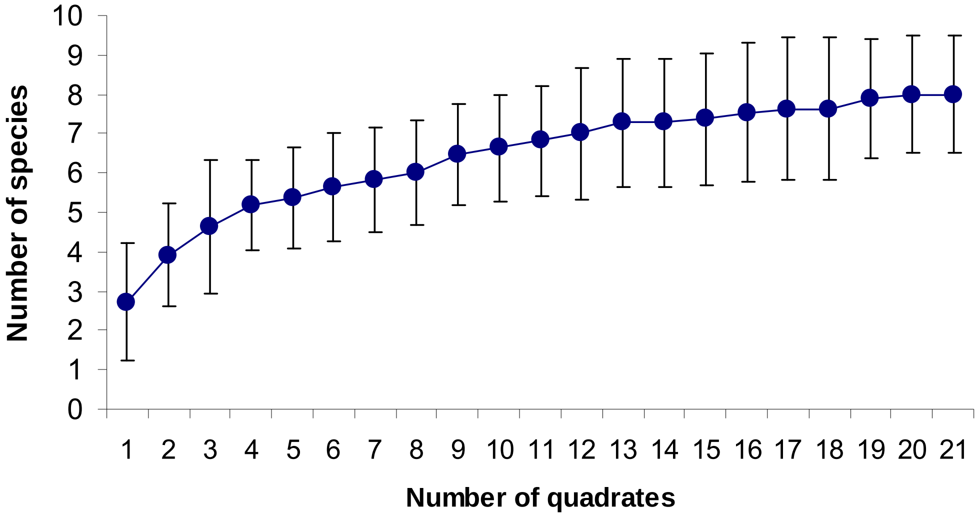

Figure 5. Increasing number of species found by the 11 teams (mean ± standard deviation) with increasing number of quadrates.

Although the approach with an error of 20% (21 quadrates for a 300 m of forest edge) is considered as being acceptable (see FutMon field protocols, Table 1, p. 18), large variation for the frequency of occurring species among the teams can be observed (Table 2). For homogenously distributed species such as *Carpinus betulus* (found in 20 out of 21 quadrates, by all teams) and *Rubus* sp. was found in 16 quadrates by one team and never by other teams. This potential source of variation may become crucial when considering the case of *Salix caprea*, which was the only species having been considered as symptomatic. To reduce the above outlined variability, it has been agreed on to revise Table 1 of the FutMon field protocol by omitting the 20% sampling error and considering the 10% sampling error only, leading to an intensified sampling of more quadrates per LESS (See Minutes in the Annex).

Table 2. Frequency of occurrence for each species within the 21 quadrates, both in number of quadrates (mean and standard deviation of the 11 teams) and in percentage. Maximum and minimum number of quadrates in which each species was recorded by the 11 teams.

		Quadrates present (from a total of 21)			
	Mean (in % of 21 quadrates)	**Mean**	**SD**	**Max**	**Min**
Acer campestre	15.2	3.2	1.9	6	0
Alnus glutinosa	2.6	0.5	1.8	6	0
Betula pendula	3.0	0.6	1.2	3	0
Carpinus betulus	97.0	20.4	0.5	21	20
Clematis sp.	0.9	0.2	0.6	2	0
Clematis vitalva	0.4	0.1	0.3	1	0
Crataegus monogyna	0.9	0.2	0.6	2	0
Euphorbia sp.	1.7	0.4	0.9	3	0
Fagus sylvatica	29.0	6.1	1.2	8	5
Fraxinus excelsior	0.4	0.1	0.3	1	0
Fraxinus ornus	0.4	0.1	0.3	1	0
Populus tremula	6.1	1.3	1.4	4	0
Prunus avium	0.4	0.1	0.3	1	0
Prunus sp.	0.4	0.1	0.3	1	0
Quercus cerris	1.3	0.3	0.6	2	0
Quercus petraea	38.5	8.1	3.4	13	2
Quercus robur	1.3	0.3	0.9	3	0
Ribes sp.	0.9	0.2	0.6	2	0
Rosa sp.	3.5	0.7	1.1	3	0
Rubus idaeus	0.4	0.1	0.3	1	0
Rubus sp.	25.1	5.3	7.3	16	0
Salix caprea	41.1	8.6	3.6	16	4
Salix sp.	1.7	0.4	0.8	2	0
Sambucus racemosa	0.4	0.1	0.3	1	0

Despite the presence of confusing ozone-like symptoms in different species (e.g. *Carpinus betulus*, *Fagus sylvatica*), the teams considered only one species, i.e. *Salix caprea* as being symptomatic. The percentages of symptomatic species, symptomatic quadrates, and symptomatic species per quadrate are provided in Table 3. Symptomatic plants of this *Salix caprea* were only found in one of the randomly selected quadrates by 2 out of the 11 teams. The other teams did not observe symptoms in the other quadrates in which *S. caprea* was present. Given the low incidence of ozone-induced symptoms, all teams were between class 0 (no symptoms found) and 1 (> 0 & ≤ 10) when transferring percentage of symptomatic quadrates and number of symptomatic species per quadrate into classes (in 10% increments). The percentage of symptomatic species (with regard to the total number) recorded by each team was more sensitive to changes in classes: teams which did not find any symptoms were assigned to class 0 (0% symptoms) and those that found one symptomatic species to class 2 (> 10 & ≤ 20). In terms of quality control, the latter two parameters should be preferred for being more representative for ozone impacts as they are related to the surface affected.

Table 3. Percentage of species scored as symptomatic with regard to the total number of species found by each team, percentage of symptomatic quadrats and mean percentage of symptomatic species per quadrat. Each percentage is assigned to a classe (* Class 0=0%; Class 1>0 & ≤10; Class 2>10 & ≤20, etc).

	Symptomatic species			Symptomatic quadrats		Symptomatic species per quadrat	
Team	**Number**	**% of total**	**Class***	**%**	**Class***	**%**	**Class***
1	0	0.0	0	0	0	0.0	0
2	1	11.1	2	5	1	1.6	1
3	0	0.0	0	0	0	0.0	0
4	0	0.0	0	0	0	0.0	0
5	1	20.0	2	5	1	2.4	1
6	0	0.0	0	0	0	0.0	0
7	0	0.0	0	0	0	0.0	0
8	0	0.0	0	0	0	0.0	0
9	0	0.0	0	0	0	0.0	0
10	0	0.0	0	0	0	0.0	0
11-Control	0	0.0	0	0	0	0.0	0

* Class 0=0%; Class 1>0 & <=10; Class 2>10 & <=20, etc

4. DATA QUALITY CONTROL

For the photo exercise, 80.8% of the teams showed an agreement of ≥ 70% compared to the control, while 65.4% of the teams the agreement was ≥ 80% (Table 4). For the fresh material exercise, the agreement of 92.0% (for ≥ 70%) with the control was higher. This finding suggests that the more thorough observation of both, the lower and upper side of the leaves as well as the observation of several leaves seem to allow a better discrimination between ozone-induced and other symptoms. However, the outcome may also depend on the species included in the exercises: as indicated before, there are clearly defined symptom expressions such as the hypersensitive response of *Populus*, compared to more symptoms which are more difficult to assess correctly such as reddening or bronzing of e.g. *Fagus*, which are hard to discriminate from natural senescence.

Under the given low level of symptom occurrence at the respective LESS of the field exercise, each team except for two considered no symptoms being present, and two teams found symptoms on *Salix caprea*. Consistently, symptoms that could be confused with ozone in *Carpinus* and *Fagus* were considered as non ozone-induced symptoms by all teams. As the Measurement Quality Objectives (MQO) accepted a deviation of 1 class above and below control (in Class 0, no symptoms), all groups fitted the data quality requirements. Under conditions with a higher number of symptomatic species, however, more discrepant values are to be expected.

Table 4. Summary of all exercises.

Investigation	MQO	Type	Number of Teams	Achivements of DQ (%)
Photo-exercise	≥ 70 % agreement with control	Photo	26	80.8
Fresh material exercise	≥ 70 % agreement with control	Leaves	25	92.0
Fiel exercise*	Control ± 1 class	Percentage of symptomatic quadrates	10	100
Fiel exercise*	Control ± 1 class	Percentage of symptomatic species per quadrate	10	100

MQO= Measurement Quality Objective [e.g 70% of a team fit with control team]
*Considering a sampling error of 20% and different, ramdomly selected quadrates for each team

CONCLUSIONS

- For a threshold of agreement with the control of ≥ 70% (MQO), 80.8% of the teams fulfilled these MQO in the photo-exercise and 92.0% in the fresh material exercise. With respect to the scoring based on a single picture, the fresh material exercise provides additional information on the symptom distribution over several leaves that seems to improve the data quality by the teams. This conclusion also underlines the importance of **assessing the entire branch or plant** for a possible “age effect” for proper symptom identification (see FutMon Field protocol, Annex III).

- The quality of symptom assessment may strongly depend on the type of symptoms of the species included in the LESS and annual exercises, as the proper identification of symptoms is known to be more or less difficult depending on the species specific symptom expression. **Regular participants of previous Intercalibration Courses however, showed a higher level of agreement with the control assessment**.

- A recently developed **on-line tool for ozone-like symptom validation** at http://www.ozoneeffects.org/Links_EN may further contribute to a harmonization of the symptom assessment for difficult species. Pictures of symptomatic species taken in the field by the teams/countries will be cross-checked by several experts, who will then provide a synthesis report to the respective team/country.

- In the field exercise, each team assessed ozone symptoms in woody species in different quadrates randomly distributed along the forest edge, considering an error of 20% (here, 21 quadrates for 300 m). The total number of species scored by each team varied from 5 to 10. Common species with the control was on average 62.2%. The frequency of some species in the quadrates showed considerable variation among teams due to random quadrate selection. As the species of the forest edge were woody plants, easily to determine, the contribution to increased variability due to misidentification can be considered as very low. These results suggest that a sampling error from 20% may be too high, and a reduction to 10% will provide more comparable results in terms of both the frequency of each species in the quadrates and the total number of species assessed. Consistently with these comments, **the Working Group decided in this meeting to consider only a 10% sampling error** (20% error will be omitted and not accepted any more).

- The restriction of the analysis to **woody species only** will also reduce the variability among the teams/countries (skilled teams able to recognize a wider range of species compared to teams with less knowledge of the flora).

- Under the low incidence level of ozone injury in the forest edge used for this field exercise, all groups fulfilled the data quality requirements of a deviation of 1 class either above or below the control. Eight teams (including the control) showed no symptoms, and two teams found symptoms in one species (*Salix caprea*). The percentage of symptomatic quadrates was between 0 and 5% (classes 0 and 1 respectively; in 10% increments), and the percentage of symptomatic species per quadrate between 0 and 2.4% (classes 0 and 1; in 10% increments). Future exercises should test the effect of a forest edge with a higher number of symptomatic species. It is expected that the final percentage of teams fulfilling the MQO may decrease with an increasing number of symptomatic species.

REFERENCES

Bussotti F., Schaub M., Cozzi A., Gerosa G., Novak K., Hug C. (2006) Sources of errors in assessing ozone visible symptoms on native vegetation. Environmental Pollution. *Environmental Pollution* 140:257-268.

ANNEX: MINUTES OF THE COURSE

Draft Minutes

10th UNECE/ICP-Forests & FutMon Intercalibration Course on the Assessment of Ozone Visible Injury

FutMon Action C1-O3-24(ES)

21-24 September 2009, Budapest & Mátrafüred (Hungary)

prepared by

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Host: Judit Sitkey

ERTI-Hungarian Forest Research Institute, Budapest

Participants:
Austria (Ferdinand Kristöfel); Cyprus (Marios Menelaou, Konstantinos Rovanias); Czech Republic (Vaclav Burianek, Radek Novotny); France (Laurence Dalstein, Nicolas Vas); Germany (Willy Werner); Greece (Constantinos Kaoukis, Constantina Tsagari); Hungary (Judit Sitkey); Italy (Filippo Bussotti, Fabiana Cristofolini, Rosanna Desotgiu, Silvia Ferlazzo, Marco Ferretti, Elena Gottardini, Martina Pollastrini); Japan (Aoki Masatoshi); Romania (Stefan Neagu, Diana-Maria Silaghi); Slovakia (Hana Pavlendová, František Máliš); Solvenia (Matej Rupel); Spain (Vicent Calatayud); Switzerland (Marcus Schaub, Pierre Vollenweider)

Monday, 21 September 2009

Morning: Opening of the meeting and introductory statements

- Welcome by the NFC representative of Hungary. A total of 28 participants from 14 countries attended the meeting.

- Introduction to the course program by Judit Sitkey.

- Marcus Schaub welcomed the participants and exposed the objectives, methods and tools related to air quality and visible injury assessment under FutMon project. A field manual to carry out such activities has been developed and is available under FutMon webpage (http://www.futmon.org). Guidelines for measuring air pollutants with passive samplers and continuous analyzers were discussed. Methods for setting up the plots for visible injury assessment were commented. Data quality objectives and limits have partially been established and will be implemented along the next months. Submission forms for data collected under FutMon have also been revised. In order to facilitate the flow of information between coordinators and the very experts of the beneficiaries/countries conducting the air pollutant measurements and visible injury activities, the following web page has been developed: http://www.ozoneeffects.org. It contains updated information on intercalibration activities, field forms, links to pages containing reference materials on ozone injury (http://www.ozone.wsl.ch and http://www.ozoneinjury.org) and scientific literature, as well as files to help with data submission (e.g. plant species codes). A new online tool (web album) allows the exchange of pictorial information and will be implemented as an open source discussion platform for the assessment of ozone visible injury: http://gallery.me.com/marcusschaub#100288. Beneficiaries/countries will be required to submit pictures of ozone injury of all those plants that show visible symptoms. Pictures will be revised by independent experts that will provide advice and ensure a harmonized assessment of the symptoms throughout Europe. If needed, surveys on difficult or interesting species using additional tools such as microscopy or controlled fumigation studies will be launched. Complementary, a Google discussion group has been established at http://groups.google.ch/group/ozone_effects.

- Marco Ferretti stressed the importance of Quality Assurance (QA) and quality control (QC) for the FutMon project. In "Action C1-QAC-15(IT): Objectives and expectations", M. Ferretti provided an overview of the actions taken by the different working groups. For air quality and visible injury assessment, the first intercomparison of passive samplers which has been carried out by CEAM under FutMon, revealed important results for QA/QC. This intercomparison should be conducted on a regular basis in the future. The attendance of the Intercalibration Course on the Assessment of Ozone Visible will be mandatory as it helps assessing the differences among countries as well as sources of errors. Data quality requirements will have to be identified before 30 April 2010.

- Vicent Calatayud presented the results of the intercomparison of passive samplers (O_3, SO_2, NO_2, NH_3 ongoing) carried out under FutMon Action C1-O3-24(ES). Results of two exposure periods are available. The objectives were to assess the variability between a.) brand names, b.) countries, and c.) replicates including possible effects due to shelters. With few exceptions, countries are using commercial passive samplers that are not analyzed by the country itself. Coefficient of variation between replicates was ≤ 10% for most of the cases. For ozone, exposure of passive samplers without adequate shelters was identified to be the most important cause of variation (i.e. overestimation). Shelters have to be used to ensure comparability among brands. It was decided that countries and brand names would be kept anonymous in external reports, but used for internal QA/QC within the group only.

- Vicent Calatayud reported the changes in the data submission forms for air quality and visible injury, which will be available at the end of 2009 and should be used starting for the 2007 data submission. Pre-formatted Excel files will be posted at http://www.ozoneeffects.org/index_EN. These files will provide examples and will help in processing the data and adjusting the format of the final submission forms. Another Excel file with species code names is already available at http://www.ozoneeffects.org/index_EN. Changes with regard to previous forms include: a.) the submission of replicates separately for passive samplers, b.) hourly data for continuous analyzers, c.) the lowest altitude within a 2.5 and 5 km radius from the site of ozone measurements, and d.) new forms for co-located passive samplers and blanks.

Afternoon: Countries' presentations and photo exercise

- **Cyprus:** Konstantinos Rovanias presented the activities related with ozone in Cyprus. Concentrations from passive samplers and active samplers are relatively high, especially during the summer period. Plants with ozone symptoms have been found in the past at humid areas (e.g. *Ailanthus altissima*, *Robinia pseudoacacia*, *Sambucus nigra* and *Alnus orientalis*), but not in 2009. The poplar clone Oxford was also asymptomatic this year. Ozone monitoring will be continued in the future.

- **Slovakia:** Hana Pavlendová and František Máliš reported the status of monitoring for ozone effects on intensive monitoring plots in Slovakia. In 2009, ozone and other pollutants were measured using passive samplers (O_3 and NH_3 at 8 plots, NO_2 and SO_2 at 2 plots). The use of a new type of shelter improved the quality of ozone data. Symptoms were observed on the LESS, while never on main tree species in the canopy (MTS). Future plans include continuing with the monitoring of ozone levels and visible injury assessment at all eight level II plots, an intensification at core plots, but a reduction of the measurements of other pollutants due to the low levels measured during 2009.

- **Japan:** Aoki Masatoshi presented results from several studies and field observations in Japan regarding ozone injury. Recently, the concentrations of ozone and peroxides have become high in middle May, especially in south-west Japan, due to air mass transferred from China.

- **Greece:** Constantina Tsagari and Constantinos Kaoukis presented results from Greek. Ozone concentrations were measured and ozone-induced visible injury was assessed at two sites. Pictures of the species showed different types of symptoms from biotic and abiotic causes. Ozone-like injury was identified in *Fagus sylvatica*. In *Cistus incanus,* observed symptoms were most likely not due to ozone.

- **Romania:** Stefan Neagu and Diana-Maria Silaghi presented results from Romania under FutMon. Ozone is measured with passive samplers at 4 sites and at 1 site with a continuous analyzer. They also participate in the FutMon passive sampler intercomparison exercise. ICAS analyzes the passive samplers by ion chromatography. Romania considered the use of 2 replicate filters to be sufficient. In case of dubious cases these data are rejected. The use of portable ozone analyzers with low power requirements as used by Romania are a valuable alternative to passive samplers.

- **Germany:** Willy Werner presented the status report of ozone measurements and assessment of ozone visible injury in Germany under the FutMon project. Ozone measurements with passive samplers were done on 42 plots with measurements of 3 parallel samplers per plot on 12 plots. Out of a total of 36 plots investigated for ozone visible injury, only at 5 plots ozone injury was detected on leaves (or needles). Validation of samplers is ongoing. Parallel active and passive ozone measurements are realized in year 2009 on 8 plots across 5 different federal states. Investigation of vegetation (LESS) is done on 15 plots, and injury was found in 4 plots. W. Werner suggested ring-tests for active monitors. However, due to nationally regulations and quality standards (certificates), this suggestion was declined.

- **Italy:** Elena Gottardini, Fabiana Cristofolini, Antonella Cristofori and Marco Ferretti showed results from the ozone monitoring network in Trentino (Italy) with 15 level I plots, including 6 plots of observation of visible injury. The measured variability of ozone concentrations within different grids (1 x 1 km grid, 15 x 15 km grid) lead to very different AOT40 estimates even within the same EMEP grid cell. Ongoing studies also focus on morphological and physiological responses of *Viburnum lantana* to O_3.

  Filippo Bussotti, Rosanna Desotgiu, Martina Pollastrini and Chiara Cascio presented a study on the interaction of ozone and high light radiation with particular focus on beech. In beech leaves, symptoms become evident during late season when the capacity to cope with the excess of excitation declines. The onset of symptoms is more likely dependent from the physiological state of the leaf rather than from the flux. Symptoms are enhanced by the presence of ozone, but they are intrinsically caused by high light. It was suggested to consider the extensive reddening not as ozone symptoms (code 2), and to consider the more doubtful manifestations (red stippling, bronzing in beech leaves) as “unknown” (code 4). In their opinion, the north facing forest edges should not be excluded when establishing the LESS (see Figure 3 a and b, ICP-Forests Manual, Part B).

- **Photo exercise:** Participants were asked to assess 55 standard pictures of symptoms, caused by ozone or other causes. The evaluation will be analyzed and used as one of different indicators for QA, i.e. variation among the different countries' and experts' assessments.

Tuesday, 22 September 2009

Morning: Discussion of the photo exercise, fresh material exercise, and final conclusions.

- **Photo exercise:** Comments on the photo exercise. The most problematic pictures were discusses. Results demonstrate that the agreement with a reference (control), in general increased for countries that have already participated in the previous courses.

- **Fresh material exercise:** The participants assessed 20 samples of fresh leaves collected from the field and from Open Top chambers. They decided whether the symptoms were due to ozone or due to any other biotic or abiotic agent. Each of the samples was commented and discussed in plenum.

- **Discussion and final conclusions:** Following issues have been discussed and suggestions made to be implemented for the FutMon field protocol, becoming effective in 2010:

  - **Change of passive samplers:** Given the difficulties of the countries to follow a common calendar for changing the passive samplers, synchronic changes will not be mandatory. However, it is strongly recommended that for ozone passive sampler changes always take place during even weeks (2, 4, 6, …).

  - **Number of replicates:** The question of the use of three replicates was raised as some countries are using only 1 or 2 replicates for passive samplers to reduce the costs. The intercomparison of passive samplers and the use of 3 replicates of different brands in the FutMon project will contribute towards a better understanding of the precision of the different brands under different climatic conditions. Based on the findings of the intercomparison, respective changes for the number of replicates and confidence interval will be considered in the FutMon field protocol. However, the minimum number of 2 replicates for each brand and each country remains mandatory. Data for ozone measurements based on one passive sampler replicate will not be considered.

  - **Main Tree Species (MTS):** Based on the assessment of MTS during the previous years, results show very little ozone effects in the canopy of mature trees. This may be due to a.) differing micrometeorological conditions and b.) time of sampling (June-July) together with foliar analysis sampling. Also, due to the low number of samples (5 trees), any results may not be representative for the very objectives of the program. For the future, the assessment of ozone visible symptoms within the canopy of MTS will be optional. MTS assessments can be restricted to plots where interesting results have been obtained so far and, for these cases, it would be interesting to adjust the sampling date to the most appropriate data for ozone symptoms observation and even to increase the number of sampled trees.

  - **Light Exposed Sampling Sight (LESS):** Due to the high diversity of species composition for herbaceous species and due to little knowledge on ozone induced symptom development for herbaceous species, it has been suggested to assess woody species (minimum height yet to be defined) only to reduce the error on a.) correct identification of species, and b.) correct identification of symptoms. It is also proposed to reduce the sampling error to 10% only (20% will be omitted). For LESS of 100 m and longer, the number of quadrates will be 33. Table 4 of the FutMon Field Protocol/ICP-Forests Manual and reporting forms will be revised accordingly. This measure is expected to increase QA as it decreases the error as outlined above (a. and b.).

  - **Location of the LESS:** For establishing the LESS, the transect should start at the forest edge, closest to the meteorological field station (passive samplers) and may also include north facing forest edges.

  - **OTS form:** Observation of symptoms outside the quadrates (OTS form) is mandatory. A list of symptomatic species may be provided and divided for woody and herbaceous species. The data submission forms will be revised accordingly.

- **2010 Intercalibration Course in Valencia (Spain):** Vicent Calatayud proposed Valencia (Spain) for the Intercalibration Course. The proposal was accepted.

Wednesday, 23 September 2009

- **Field exercise:** The participants applied the method for assessing visible injury along a forest edge (LESS). Vegetation was sampled by using 2 x 1 quadrates randomly distributed along a 300 m transect. All woody plants inside the quadrates were examined and assessed for ozone injury. Complementary observations on other species of this area were also carried out. The exercise was concluded with discussing and explaining the various symptom expressions being found on different species.

Thursday, 24 September 2009

- Excursion to the National Park of Hortobágy.

- Closure of the course