Ozone-induced Visible Foliar Injuries in Lithuania

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In 2002 the survey was carried out on 25 Light Exposed Sample Sites (LESS) situated throughout Lithuania (network 52x52 km). Methods of ICP-Forests were used. For the first time in Eastern Europe ozone-induced leaf injuries on plants (*Rubus idaeus* L., *Alnus incana* L., *Salix caprea* L. and *Frangula alnus* Miller) were recorded on 10 LESS. The rate of injured plants per LESS varied from 1 to 15%. More visible ozone injuries were found in the eastern part of Lithuania, where higher tropospheric ozone concentrations were recorded. The results suggest that the present relatively low level of tropospheric ozone in Eastern Europe is enough to have a negative effect on vegetation. However, it is difficult to determine exposure/response relationship, different ozone sensitivity of species and individuals, and the influence of site conditions on plant response to ozone complicate these investigations. The coherent research on distribution of tropospheric ozone concentrations and ozone-induced injuries of plants in Lithuanian forests will be continued.

Key words: tropospheric ozone, visible foliar injury, forest vegetation

Introduction

Tropospheric (ground level) ozone (O_3) is considered to be one of the today's most important air pollutants affecting forests. On the global scale the concentrations of tropospheric ozone are rising year by year. It is estimated that ozone concentration has increased by about 35% since the pre-industrial era (some regions experienced larger and some smaller increases) (UNECE and EC 2003). Today the natural level of ozone varies from 20 to 80 μg/m^3. However, in 1980 peak ozone concentrations of 196-294 μg/m^3 were registered in many parts of Europe (Zierl 2002). According to the EU ozone directive, the threshold values of 65 μg/m^3 for daily mean and 200 μg/m^3 for hourly mean are set up (Hjellbrekke 1997). It is stated that ozone concentrations exceeding these limits can have a negative impact on the ozone sensitive species, and visible foliar injuries are one of these. The comparison of areas with occurrence of visible symptoms (based on field and experimental evidences) and modelled ozone concentrations in the growing season on a worldwide scale seems to support the above statement. In many areas of Europe (in such as Austria, Switzerland and especially in the Mediterranean) in summer the value that is recognised as an accepted standard for the protection of forest trees from adverse ozone effects is frequently and repeatedly exceeded (Sanz *et al.* 2001, de Vries *et al.* 2003). Although in Eastern Europe the critical levels of ozone are not exceeded frequently, it is expected that transboundary pollution and local sources will determine fast growth of ozone concentration in this part of Europe as well (Szaro *et al.* 2002b). Furthermore, ozone has been recognised as one of the predicting factors of ash dying in Eastern Europe (Ozolinčius 2002).

Tropospheric ozone is a secondary air pollutant that forms as a result of photochemical reactions between other pollutants, mainly NO_x and volatile organic compounds. The highest ozone levels generally occur in the daytime during the summer season, when sunlight is stronger and stagnant meteorological conditions can cause reactive pollutants to remain in an area for several days (Skelly *et al.* 1987, Manahan 1994, Innes *et al.* 2001). In Europe, the greatest rise in ozone levels occurred in the period 1940-1960, when the concentrations of ozone precursors from exhaust gases and industry increased 4 times. Since 1980 the level of industrial pollutants in Europe has decreased and resulted in a growing importance of photochemical pollutants (such as ozone). It is predicted that in 2100 nearly 49% of world forests (17 mil. km^2) will be affected by ozone concentration capable to provoke negative effects (Szaro *et al.* 2002, Percey 2002).

High ozone levels were first related with spots on the leaves of garden plants and crops around Los Angeles in 1944, USA (Hill *et al.* 1970). Later on the Ponderosa pine in the forests of the San Bernardino Mountains displayed spots on their needles. These forests finally died off in 1970 after a bark beetle attack (Alonso *et al.* 2002). Since 1950–1960 tropospheric ozone as the main photochemical compound has been recognised in North America, since 1980 in Western Europe, and since 1990 in the Mediterranean region (Bytnerowicz *et al.* 2002). In 1997, based on ten years of monitoring forest condition in Europe for estimating direct effects by air pollution on forest trees, the UNECE concluded that tropospheric ozone is the main parameter to be considered. In 2001, the Intergovernmental Panel on Climate Change (IPCC) classified tropospheric ozone as the third most important greenhouse gas after carbon dioxide and methane (de Vries *et al.* 2003).

Plants respond to ozone when it enters the leaf through the stomata. Within the leaf, ozone is transformed, producing a variety of free radicals, which damage the cell. The toxic effects of ozone depend on the amount of uptake into plants (Pell *et al.* 1997), which is determined by stomatal conductance and depends on the microclimatic factors (Skärby *et al.* 1998, Innes *et al.* 2001). Stomatal conductance is maximal during periods of high light intensity, high temperatures, low vapour pressure deficits and sufficient soil water supply (Zierl 2002). Furthermore, plant species and individual plants within species vary greatly in their ozone tolerance (Innes *et al.* 2001, UNECE and EC 2003). Therefore it is difficult to determine a clear link between the occurrence of high ozone concentration and the extent of damage on vegetation (Skärby *et al.* 1998, Zierl 2002).

Nevertheless, the results of many different experiments show that the high ambient ozone concentrations (more than 65 $\mu g/m^3$) can cause a range of effects to vegetation. These are: decreased foliar chlorophyll content and photosynthesis, reduced carbohydrate production, altered carbon allocation (Skärby *et al.* 1998, Günthardt-Goerg *et al.* 2000, Vollenweider *et al.* 2003), decreased nutrient availability, accelerated leaf senescence, lower vigour, slow growth (Oksanen 2001, Matsumura 2001, Muzika *et al.* 2002, Zierl 2003), yield reduction, visible foliar injury (Skelly *et al.* 1987, Innes *et al.* 2001, Sanz *et al.* 2001), and altered sensitivity to biotic and additional abiotic stressors (Skärby *et al.* 1994, Fuhrer *et al.* 1997, Skärby *et al.* 1998, Grodzki *et al.* 2002, Laurence and Andersen 2003, UNECE and EC 2003). It can be concluded that long-term impact of ozone on trees may impair the function of forest ecosystems (Dalstein *et al.* 2002, Zierl 2002), the aesthetic appearance of the landscape (Laurence and Andersen 2003).

The data we have today strongly suggest that tropospheric ozone causes visible foliar injury to sensitive plants. Visible injury is regarded as a result of oxidative stress, leading to a cascade of adverse effects. Ozone (unlike e.g. fluoride or sulphur dioxide pollution) leaves no elemental residue that can be detected by analytical techniques. Therefore, visible ozone-induced injury on needles and leaves is easily detectable ozone pollution evidence in the field. Visible injury does not include all the possible forms of injury to plants (i.e. pre-visible physiological changes, reduction in growth, etc.). Nevertheless, observation of typical symptoms on above ground plant parts in the field has turned out to be a valuable tool for the assessment of ozone injury in sensitive species in Europe (Innes *et al.* 2001, Sanz *et al.* 2001, de Vries *et al.* 2003). Since 1993 more than 100 species in Switzerland, Spain and Italy have been recognised as ozone sensitive species (Innes *et al.* 2001); surveys have recorded ozone-like symptoms on native tree, shrub, and herbaceous species in Greece, and France (de Vries *et al.* 2003).

However, little information is available on the effects of ozone on the native forest plant species throughout the rest of Europe (Fuhrer *et al.* 1997). Therefore, in 2001 the EU/ICP Forests programme launched a test phase to explore the monitoring of ozone concentrations and ozone-induced injuries on intensive monitoring plots (UNECE and EC 2003). Thus far in Lithuania tropospheric ozone was not approached as one of the forests condition influencing factors (Ozolincius and Stakenas 1996). In 2002 the first investigations were started. The object of our survey was to assess the impact of tropospheric ozone on forest vegetation in Lithuania, that is, to examine whether any visual ozone-like injury can be found on plants in Lithuania, which species are affected and what is the rate of the visible injury.

Materials and methods

On August 27 and September 11, 2002 the first pilot study on the visible ozone-like injuries was carried out. The assessment of visible ozone injury was based on the EU/ICP Forests methods - "Submanual on Assessment of Ozone Injury on Intensive Monitoring Plots" (UNECE 2001). The territory of Lithuania was divided into equal squares (network – 52x52 km). In the middle of each square observation plots - Light Exposed Sampling Sites (LESS) - of 25x20 m were set in a nearby southern forest edge (altogether - 25 LESS) (Fig. 1). Mostly clear cutting areas were used for LESS establishments.

The experience of ozone injury surveys in the US as well as in Europe, showed the difficulty of discrimination ozone injury from confounding symptoms, associated with biotic (fungi, insects, etc.) or abiotic (i.e. edaphic) stress factors. To facilitate this, tools

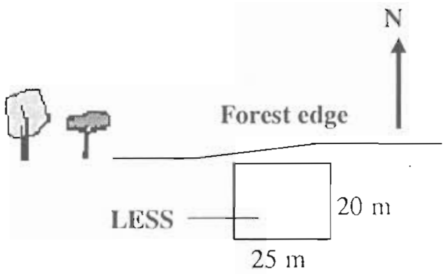

Figure 1. A schematic view of the Light Exposed Sampling Site (LESS)

such as a sensitive species list, photo gallery, and flow chart for injury discrimination (Innes *et al.* 2001) were created. Therefore, the assessment of ozone injury during our survey was based on these tools as well.

According to the "List of European Ozone Sensitive Species" (http://www.gva.es/ceam/icp-forests) a preliminary "List of Lithuanian Ozone Sensitive Species" was prepared, including 30 species of trees and shrubs and 16 perennial herbs (46 species in total). Foliage of these species was screened for ozone injury with respect to the following parameters: *abundance of the ozone sensitive species per LESS* (scale of 5%) and *number of injured plants per LESS* (scale of 5%). Visible leaf injury assessment was performed according to the flowchart for the diagnosis of ozone-induced injury on broadleaved species (Fig. 2) as well as comparing found symptoms with the pictures of ozone injuries (Innes *et al.* 2001, Sanz *et al.* 2001).

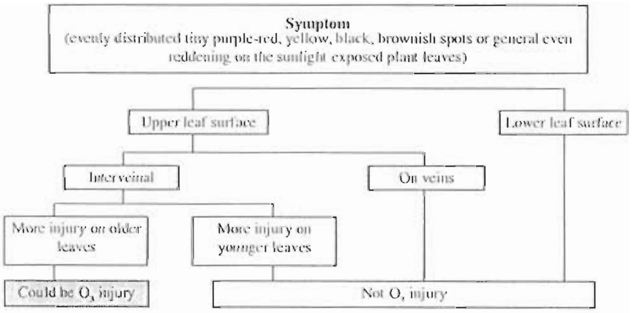

Figure 2. Flowchart for the diagnosis of ozone-induced injury on broadleaved species (according to Innes *et al.* 2001)

The symptoms found on the plants in the LESS were defined as ozone-induced injury when: 1) the symptoms (evenly distributed tiny purple-red, yellow, black, brownish spots or general even reddening) occurred only on the mature leaves exposed to sunlight, 2) were found only on the upper leaf surface, 3) were interveinal, and 4) more of the injury were found on older leaves comparing to the younger ones. In addition the "shade effect" (when two leaves overlap and the shaded leaf portions do not show injury) is considered to be the evidence that the injury is induced by ozone.

Results

On 25 Light Exposed Sampling Sites (LESS) 21 tree and shrub and 12 perennial herbs species included in the preliminary "List of Lithuanian Ozone Sensitive Species" were found (Tab. 1). *Rubus* spp., *Betula pendula* Roth. and *Quercus robur* L. were the most frequently found species.

Table 1. A preliminary list of Lithuanian species sensitive to ozone

N°	Trees and shrubs	Number of LESS, in which the species were found
1	*Acer platanoides* L.	4
2	*Acer pseudoplatanus* L.	
3	*Alnus glutinosa* (L.) Gaertn.	7
4	*Alnus incana* L.	5
5	*Betula pendula* Roth.	20
6	*Carpinus betulus* L.	
7	*Cornus* spp.	
8	*Corylus avellana* L.	13
9	*Crataegus* spp.	
10	*Euonymus europaeus* L.	2
11	*Frangula alnus* Miller	10
12	*Fraxinus excelsior* L.	7
13	*Larix decidua* Mill.	
14	*Lonicera xylosteum* L.	1
15	*Picea abies* (L.) H. Karst.	14
16	*Pinus sylvestris* L.	8
17	*Populus tremula* L.	10
18	*Prunus avium* L.	
19	*Prunus spinosa* L.	
20	*Quercus robur* L.	17
21	*Rhamnus catharticus* L.	1
22	*Ribes* spp.	2
23	*Rosa canina* L.	
24	*Rubus* spp.	21
25	*Salix* spp.	9
26	*Sambucus* spp.	
27	*Sorbus aucuparia* L.	15
28	*Tilia cordata* Miller	1
29	*Ulmus* spp.	1
30	*Viburnum opulus* L.	2
N°	**Ground vegetation**	**Number of LESS, in which the species were found**
31	*Agrimonia eupatoria* L.	1
32	*Artemisia vulgaris* L.	7
33	*Convolvulus arvensis* L.	1
34	*Conyza canadensis* (L.) Cronq.	2
35	*Epilobium angustifolium* L.	12
36	*Geranium sylvaticum* L.	3
37	*Hieracium* spp.	5
38	*Lamium* spp.	
39	*Lapsana communis* L.	
40	*Mycelis muralis* (L.) Dumort.	4
41	*Myosotis* spp.	1
42	*Oenothera biennis* L.	
43	*Plantago* spp.	5
44	*Rumex obtusifolius* L.	4
45	*Stachys officinalis* (L.) Trevisan	
46	*Trifolium pratense* L.	4

Ozone-like symptoms were found on 9 tree and shrub and on 1 perennial herbs species. After consultations with experts from Swiss Federal Research Institute WSL the visible ozone-induced symptoms were declared on 4 tree and shrub species - *Rubus idaeus* L., *Alnus incana* L., *Salix caprea* L. and *Frangula alnus* Miller (Fig. 3; see page 95).

The rate of affected plants varied from 1 to 15% per LESS (Table 2). Most of the ozone-like visible injury was found on *Rubus idaeus* L. (on 6 out of 25 LESS). The injury was manifested as an even upper surface interveinal reddening. The injury on *Alnus incana* L. was found in three plots and was seen as a purple-red intense stippling. The injuries on *Frangula alnus* Miller and *Salix caprea* L. were found on 2 LES sites and were displayed as a tiny brown stipples.

Table 2. The amount of the identified symptomatic species

Scientific name	Number of LESS in which the injuries were found	Abundance of the species per LESS, %	Number of injured plants per LESS, %
Rubus idaeus L.	6	5 – 35	1 - 15
Alnus incana L.	3	1 – 10	1 – 5
Salix caprea L.	2	5 – 10	1 – 5
Frangula alnus Miller	2	1 – 5	1 – 5

The locations of different plant species on which the visible ozone-like injury was found are presented in Figure 4. The ozone-induced injuries (especially on *Rubus idaeus* L.) were found almost in the whole country, however relatively more injuries were found in the southern, eastern, and northeastern parts of Lithuania. Most of the affected *Salix caprea* L. plants were found in the eastern part of Lithuania and injured *Frangula alnus* Miller plants - in southern one.

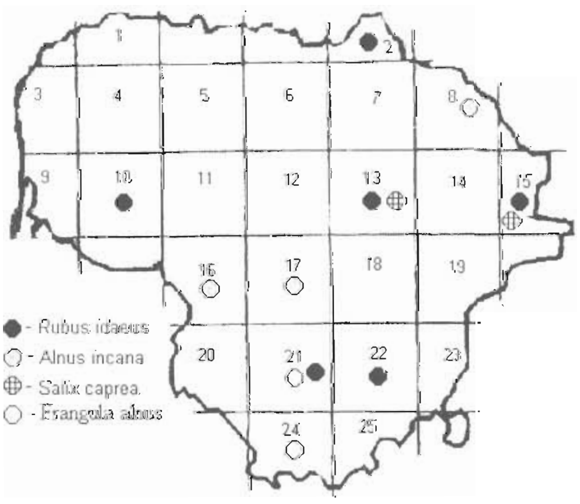

Figure 4. Distribution of identified visible ozone-induced symptoms on *Rubus idaeus* L., *Alnus incana* L., *Salix caprea* L., and *Frangula alnus* Miller in Lithuania, 2002

Discussion

The emerging of visible ozone-induced injuries generally depends on: 1) the ambient ozone concentration, 2) the uptake into the leaf, 3) environmental factors that greatly influence the uptake, and 4) on the species and individuals sensitivity to ozone (Innes *et al.* 2001).

The data on daily mean tropospheric ozone concentrations during June 1 – September 10, 2002 from the two integrated monitoring sites in Lithuania (Plateliai in the western part of the country and Rūgšteliškės in the eastern part) were provided by the Institute of Physics, Lithuania. The average ozone concentrations for the whole period in Rūgšteliškės and Plateliai were 58 and 54 μg/m³, respectively. The ozone concentration in Rūgšteliškės was higher than 65 μg/m³ during 26 days, that is 25,5% of the time, in Plateliai – 19 days, that is 18,6% of the time. The highest concentrations were recorded in August: on Rūgšteliškės site the daily mean ozone concentration was 94 μg/m³ (15.08.2003) and in Plateliai ozone concentration was 84 μg/m³ (12.08.2003). In the period 1990 – 1994, 35% of the time on summer days the average one-hour tropospheric ozone concentrations were higher than 80 μg/m³, and 10% of the time they were higher than 100 μg/m³ (Girgždys *et al.* 1999). It is apparent that ozone concentrations in Lithuania are high enough to be of potential risk to vegetation. Higher ozone concentrations in the eastern part of Lithuania could be one of the reasons why more visible ozone injuries were found in this part of the country comparing to the western part, where almost no symptoms were recorded.

However, it is widely recognised, that plant response is actually more closely related to the internal ozone dose, i.e. the ozone taken into the plant through the stomata, which in turn depends on a variety of ecological factors (Skärby *et al.* 1998, Zierl 2002, de Vries *et al.* 2003). The summer of 2002 in Lithuania distinguished by rather high air temperature and lack of precipitation especially in August. In the biggest part of the country the meteorological conditions were defined as dry (LHT 2002). These factors are favourable for ozone formation (Innes *et al.* 2001), however they are unfavourable for stomata opening and thus for ozone uptake (Skärby *et al.* 1998, Zierl 2002). There are some evidences that plants growing in drought conditions showed less visible ozone symptoms than those in normal conditions (Skärby *et al.* 1998, Vollenweider *et al.* 2003). In the future, it is important to link the data on ambient air quality and ozone effects (de Vries *et al.* 2003).

The tolerance of species to ozone is another important factor. It is not surprising that most of the injuries were recorded on *Rubus idaeus* L. According to the literature (Skelly *et al.* 1987, Innes *et al.* 2001, Sanz *et al.* 2001) *Rubus* spp. is indicated as one of the most sensitive species and as a possible bioindicator. In 2001 during the EU/ICP Forests programme test phase of monitoring ozone-induced injuries at Intensive monitoring plots (UNECE and EC 2003) *Rubus* spp. as well showed up to be one of the most affected species.

In 2002, out of the total number of established 25 LESS in Lithuania, ozone symptoms were observed on one or more species in 10 LESS (40%). In 2001 LESS were established in Austria (1), France (10), Germany (16), Greece (4), Italy (8), Spain (10), Switzerland (15), and the Slovak Republic (3) and vegetation screened according to the sensitive species list for ozone injury. Table 3 gives an overview of the species showing symptoms on the LESS observations for the year 2001 in Europe. Out of the total number of 67 plots in which LESS had been established, ozone symptoms were observed on one or more species in 37 LESS (55%), whereas at 30 LESS no ozone injury was found. Many of the species registered with ozone symptoms were not known to be ozone sensitive before (18 out of 61). Especially in Switzerland, surveys were carried out very intensively, showing the highest density of plots as well as species with ozone injury (de Vries *et al.* 2003). The assessment of ozone injury has to be considered as the first phase to implement a unique effects monitoring system on a European scale, based on validated field observations (UNECE and EC 2003).

Table 3. Species showing ozone injury on light exposed sampling sites in Europe, 2001 (de Vries *et al.* 2003)

N°	Species	Country	Total N° of plots showing ozone injury	N°	Species	Country	Total N° of plots showing ozone injury
1	Acer campestre	Switzerland	1	32	Plantago lanceolata	France	2
2	Acer pseudoplatanus	Switzerland	2	33	Plantago major	France	1
3	Alnus glutinosa	France, Switzerland	3	34	Populus tremula	Switzerland	2
4	Alnus incana	Switzerland	2	35	Prunus avium	Switzerland	1
5	Alnus viridis	Switzerland	1	36	Prunus spinosa	Switzerland	4
6	Aquilegia vulgaris	France, Switzerland	2	37	Robinia pseudoacacia	Switzerland	1
7	Artemisia campestris	France	1	38	Rosa canina	Switzerland	3
8	Astrantia major	Italy	1	39	Rosa sp.	France	1
9	Betula pendula	Germany	1	40	Rubus fructicosus	France, Switzerland	6
10	Carpinus betulus	Switzerland	4	41	Rubus idaeus	France, Germany, Switzerland	5
11	Centaurea nigra	Italy	1	42	Rubus spp.	Italy	1
12	Cirsium helenioides	Italy	1	43	Salix alba	Switzerland	1
13	Cornus sanguinea	Switzerland	4	44	Salix caprea	Germany, Switzerland	2
14	Corylus avellana	France, Switzerland	4	45	Salix sp.	Switzerland	1
15	Crataegus laevigata	Switzerland	2	46	Sambucus nigra	Switzerland	1
16	Crataegus monogyna	France, Switzerland	4	47	Sambucus racemosa	Switzerland	2
17	Crataegus oxyacantha	France	1	48	Senecio nemorensis	Germany	1
18	Euonymus europaeus	Switzerland	1	49	Senecio ovatus	Switzerland	1
19	Fagus sylvatica	France, Germany, Switzerland	13	50	Sorbus aria	Switzerland	2
20	Frangula alnus	Switzerland	1	51	Sorbus aucuparia	Switzerland	2
21	Fraxinus excelsior	Austria, France, Switzerland	8	52	Sorbus chamaemespilus	Germany	1
22	Helleborus niger	Italy	1	53	Sorbus mougeotti	Switzerland	1
23	Heracleum sphondylium juranum	Switzerland	1	54	Spiraea ulmaria	France	1
24	Impatiens parviflora	Switzerland	1	55	Taxus baccata	Switzerland	1
25	Lamium spp.	Italy	1	56	Tilia cordata	Switzerland	1
26	Lonicera nigra	Switzerland	1	57	Ulmus glabra	Switzerland	1
27	Lonicera xylosteum	Switzerland	4	58	Vaccinium myrtillus	Switzerland	2
28	Oenothera biennis	Switzerland	1	59	Vaccinium uliginosum gaultherioides	Switzerland	1
29	Picea abies	Germany, Switzerland	4	60	Viburnum lantana	Switzerland	2
30	Pinus halepensis	Spain	1	61	Viburnum opulus	France, Switzerland	2
31	Pinus sylvestris	Switzerland	1				

In order to make detailed reliable conclusions more precise investigations and assessment should be carried out. In further investigations the influence of the lack of experience should be eliminated as well. The data presented here have therefore a rather limited value and the evaluation has to be considered as preliminary. However, it shows that in the near future the problem of ozone effects on forest vegetation will have to be investigated in detail.

Conclusions

1. For the first time visible ozone-like injuries were found in Lithuania. They are the first ozone-induced vegetation damage symptoms in Eastern Europe as well.

2. Ozone induced leaf injuries were recorded on 10 Light Exposed Sampling Sites (40% of sites investigated).

3. The rate of injured *Rubus idaeus* L., *Alnus incana* L., *Salix caprea* L. and *Frangula alnus* Miller plants varied from 1 to 15% per LESS.

4. More visible ozone injuries were found in the eastern part of Lithuania, where higher tropospheric ozone concentrations were recorded.

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ВИЗУАЛЬНО ОПРЕДЕЛЯЕМЫЕ ПОВРЕЖДЕНИЯ ЛИСТВЫ ОЗОНОМ В ЛИТВЕ

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Резюме

Исследования проводились в 2002 г. на 25 открытых к солнцу пробных площадках (ОСПП), расположенных по всей территории Литвы (сеть 52x52 км). ОСПП заложены по европейской методике мониторинга лесов (ICP-Forests). На каждой ОСПП при опозновании симптомов повреждений исползован прелиминарный список чувствительных к тропосферному озону видов растений. Список включает 30 видов деревьев и кустарников и 15 видов травянистых растений. Симптомы озонных повреждений были найдены на *Rubus idaeus* L. (6 ОСПП), *Alnus incana* L. (3 ОСПП), *Salix caprea* L. (2 ОСПП) и *Frangula alnus* Miller (2 ОСПП). Количество поврежденных растений на ОСПП составило 1-15%. Наибольшая часть ОСПП, где зарегистрированы симптомы повреждений, расположены в восточной части Литвы. Здесь зафиксированы и наибольшие концентрации тропосферного озона.

Симптомы озонных повреждений зарегистрированы в Литве впервые. Их также можно считать первыми и в Восточной Европе. Это свидетельствует о том, что даже в регионах со сравнительно невысокими средними концентрациями тропосферного озона на листьях растений проявляются симптомы его повреждений.

Ключевые слова: тропосферный озон, симптомы повреждений, лесная растительность