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VISIBLE TOBACCO LEAF INJURY INDICES AS INDICATORS OF CUMULATIVE TROPOSPHERIC OZONE EFFECT

ANNA BUDKA[1]*, DARIUSZ KAYZER[1], KLAUDIA BOROWIAK[2], JANINA ZBIERSKA[2], AGNIESZKA WOLNA-MARUWKA[3], ANITA SCHROETER-ZAKRZEWSKA[4], ANNA CHLEBOWSKA[2]

[1]Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, ul. Wojska Polskiego 28, 60-637 Poznań, Poland
[2]Department of Ecology and Environmental Protection, Poznan University of Life Sciences, ul. Piątkowska 94C, 60-649 Poznań, Poland
[3]Department of General and Environmental Microbiology, Poznan University of Life Sciences, ul. Szydłowska 50, 60-656 Poznań, Poland
[4]Department of Ornamental Plants, Poznan University of Life Sciences, ul. Dąbrowskiego 159, 60-594 Poznan, Poland
*Corresponding author's email: budka@up.poznan.pl

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Abstract: Tropospheric ozone is one of the most reactive air pollutants, which causes visible injuries, as well as biomass and yield losses. The negative effect of ozone is cumulative during the growing season; hence crops are the most sensitive plants. Visible symptoms and biomass losses can cause economic losses. Tobacco plants have been recognized as one of the best bioindicators, but data on the cumulative effect of ozone on this species are limited. Results of an experiment with ozone-sensitive tobacco plants grown on sites varying in ozone concentration are presented in this paper. Two indices were used for data presentation of visible leaf injury degree. Higher solar radiation was the main cause of higher ozone concentration at the rural site. Higher tropospheric ozone concentrations were noted in 2010 in comparison to 2011, which was reflected in visible leaf injury. Canonical variate analysis did not reveal highly significant differences between sites, however, differences were observed in certain investigation periods. Moreover, higher leaf injury was noted at the rural site at the end of the experiment in both experimental years. This indicates the cumulative effect of ozone during the growing season. However, higher injury variability was noted at the urban site, even though lower ozone concentrations were noted there. Lower variability of injury at the rural site might suggest lack of influence of particulate matter and occurrence of higher injury even though lower ozone concentrations occurred. Better detection of ozone injury was shown by the first index based on three mean values.

INTRODUCTION

During the twentieth century there was a great increase in the number of motor vehicles, which emit huge amounts of air pollutants, such as carbon oxides, nitrogen oxides, and hydrocarbons. This also led to increased creation of ozone, because the above-mentioned pollutants are its precursors [5, 15, 25]. Although the emission of ozone precursors is decreasing due to the use of catalysers, there is not a corresponding decrease of ozone concentration [14]. This is because ozone precursors can be transported over great distances, mainly from North America and Asia, causing systematic elevation of tropospheric ozone concentrations [12].

Several-year investigations have confirmed the negative effects of ozone on plants, biodiversity, animals and materials [17]. The effects on ecosystems and crop plant species are revealed as visible leaf injury, and reduction of crop yield and seed productivity [33, 34]. Ozone is also one of the greenhouse gases which reacts with other photochemical pollutants in the troposphere [31]; hence its concentrations can change very fast.

Ozone is created during photochemical reactions, and several factors can influence this processes, such as sufficient air relative humidity, high temperature (>18°C) and high solar radiation [3]. Moreover, emission of ozone precursors, such as nitrogen oxides, volatile organic compounds, and carbon oxide, is also required [1, 3].

The effect of tropospheric ozone on plants is cumulative during the growing season, and plant responses vary according to plant age and duration of exposure [7]. Visible leaf injuries can occur on both leaf sides, and necrosis usually appears between veins. It has been found that the degree of ozone injury is related to the level of ozone in the air. However, the plant response is also connected with other factors which influence stomatal opening [25]. Visible leaf injury, as well as reduction of yield and quality, can cause economic losses [1, 2, 4, 30].

Tobacco plant (*Nicotiana tabacum* L.), and its two cultivars Bel W3 and Bel B, have been recognized as the best ozone bioindicators. High tropospheric ozone pollution can cause tissue death, and also visible necrosis in sensitive cultivars [22]. Visible leaf injuries caused by ozone appeared on both sides of a sensitive cultivar (Bel W3), and were 2–3 times higher than for resistant cultivars [16, 22]. In the case of resistance, defence mechanisms occur such as metabolism of plants and production of antioxidants, which protect plants against negative effects of ozone [27]. In some cases, when the ozone level is very high, visible injuries occur even on ozone-resistant tobacco leaves, although only on the upper leaf side [21, 22]. Bioindicator plants are an effective and simple way to assess the level of air pollution when automatic measurements are not available [19]. Normally plants are exposed to certain time intervals and injuries are assessed after these periods [6]. There is a paucity of investigations on cumulative ozone effects on plants. The present study is an attempt to fill this gap. The aim of the study was to evaluate visible leaf injuries of an ozone-sensitive tobacco cultivar during continuous exposure at two sites differing in ozone concentration, as well as to examine the cumulative ozone effect during the growing season in relation to meteorological conditions. Moreover, the mean leaf injury was compared between two exposure sites in the growing season of 2010 and 2011.

MATERIALS AND METHODS

Experimental design

An ozone-sensitive tobacco cultivar (Bel W3) was used in our investigations. Plants were cultivated in greenhouse conditions for 8 weeks and afterwards they were transported to exposure sites for the growing season beginning at the end of June. Investigations were carried out in 2010 and 2011. Two exposure sites differing in tropospheric ozone concentrations were selected for our experiment. The monitoring of air pollution and meteorological parameters was performed at both sites by Wielkopolska Environmental Agency. This was one reason for choosing these places for our experiment, due to free access to the above-mentioned data and comparison with our results. One site was located in an urban area (Poznan city, Poland) and the second one in a rural area (about 80 km north-east from Poznan city). For the purposes of this paper, the sites were named respectively urban and rural sites. Eight plants were exposed at each site. Plants were placed in pots filled with standard soils mixture (peat and sand 8:1) with slowly released fertilizer sufficient for whole cultivation time and exposure period. Pots were located in styrofoam and then on plastic tray filled with water. Glass fibre wicks placed in pots and trays provided continuous water supply. Plastic trays with styrofoams and plants were placed on specially constructed aluminium racks and covered by shadow fabric protecting plants against too high solar radiation and winds. Visible leaf injury was evaluated each week from the 4th leaf counting from the bottom of the plant. Overall, four investigation periods are presented in this paper. Leaf injury degree is determined as the ratio of damaged leaf area to the whole leaf area and presented on a 0–1 scale. Due to the great and varying number of leaves on plants, two indices were analyzed to find the best indicator of cumulative ozone effect on visible plant response.

Leaf ozone injury indices

Characteristic values of leaf injury degree for each plant had to be determined for further comparison of tobacco plant response in the two exposure sites.

Two indices of leaf injury of tobacco plants were created for the purposes of this paper:

- the first index of leaf injury – the arithmetic mean value from three maximum values of leaf injury degree in an individual observation period from all leaf injury values for one plant,
- the second index of leaf injury – the arithmetic mean value from five maximum values of leaf injury degree in an individual observation period from all leaf injury values for one plant.

Let us assume the structure of a model for index y_{ijkl} coming from the i-th observation period ($i = 1,.., I$; here $I = 4$), the j-th exposure site ($j = 1,\dots, J$; here $J = 2$), the k-th year ($k = 1,\dots, K$; here K=2), and the l-th replication ($l= 1,\dots, L_{jk}$; here $L_{11} = L_{12} = L_{21} = 5, L_{22} = 6$):

$$y_{ijkl} = \mu_i + \xi^1_{ij} + \xi^2_{ik} + \xi^{12}_{ijk} + e_{ijkl} \quad (1)$$

where for the i-th selected (one of four) observation period,

μ_i is the grand mean,
ξ^1_{ij} is the j-th exposure site effect,
ξ^2_{ik} is the k-th year effect,
ξ^{12}_{ijk} is the jk-th effect of exposure site × year interaction,
e_{ijkl} is the random error.

In addition, let:

$$N = \sum_{j=1}^{J} \sum_{k=1}^{K} L_{jk} \quad (2)$$

Ozone impact on plants is cumulative during the growing season; hence the plant response in certain exposure series is an effect of ozone concentrations in previous and present series. According to the above assumptions and relations, it is convenient to treat the results of leaf injury degree in an individual observation period for one plant as multidimensional variates. The multivariate linear model can be written in the form:

$$\mathbf{Y} = \mathbf{1}_N \boldsymbol{\mu}^{\mathrm{T}} + \mathbf{X}_1 \boldsymbol{\Xi}_1 + \mathbf{X}_2 \boldsymbol{\Xi}_2 + \mathbf{X}_{12} \boldsymbol{\Xi}_{12} + \mathbf{e} \tag{3}$$

where:

$\mathbf{Y} = \begin{bmatrix} y_{1111} & \cdots & y_{I111} \\ \cdots & & \cdots \\ y_{1JKL_{JK}} & \cdots & y_{IJKL_{JK}} \end{bmatrix}$ is the $N \times I$ matrix of one of the indices,

$\mathbf{1}_N$ is the vector of every element equal to 1,

$\boldsymbol{\mu} = [\mu_1 \ldots \mu_I]^{\mathrm{T}}$ is the $I \times 1$ vector of general means,

$\boldsymbol{\Xi}_1 = \begin{bmatrix} \xi_{11}^1 & \cdots & \xi_{I1}^1 \\ \xi_{12}^1 & \cdots & \xi_{I2}^1 \end{bmatrix}$ is the $2 \times I$ matrix of exposure site parameters,

$\boldsymbol{\Xi}_2 = \begin{bmatrix} \xi_{11}^2 & \cdots & \xi_{I1}^2 \\ \xi_{12}^2 & \cdots & \xi_{I2}^2 \end{bmatrix}$ is the $2 \times I$ matrix of year parameters,

$\boldsymbol{\Xi}_{12} = \begin{bmatrix} \xi_{111}^{12} & \cdots & \xi_{I11}^{12} \\ \cdots & & \cdots \\ \xi_{1JK}^{12} & \cdots & \xi_{IJK}^{12} \end{bmatrix}$ is the $JK \times I$ matrix of exposure site × year interaction parameters,

$\mathbf{X}_1 = \begin{bmatrix} \mathbf{1}_{L_{11}} & \mathbf{0}_{L_{11}} \\ \mathbf{1}_{L_{12}} & \mathbf{0}_{L_{12}} \\ \mathbf{0}_{L_{21}} & \mathbf{1}_{L_{21}} \\ \mathbf{0}_{L_{22}} & \mathbf{1}_{L_{22}} \end{bmatrix}$, $\mathbf{X}_2 = \begin{bmatrix} \mathbf{1}_{L_{11}} & \mathbf{0}_{L_{11}} \\ \mathbf{0}_{L_{12}} & \mathbf{1}_{L_{12}} \\ \mathbf{1}_{L_{21}} & \mathbf{0}_{L_{21}} \\ \mathbf{0}_{L_{22}} & \mathbf{1}_{L_{22}} \end{bmatrix}$, $\mathbf{X}_{12} = \begin{bmatrix} \mathbf{1}_{L_{11}} & \mathbf{0}_{L_{11}} & \mathbf{0}_{L_{11}} & \mathbf{0}_{L_{11}} \\ \mathbf{0}_{L_{12}} & \mathbf{1}_{L_{12}} & \mathbf{0}_{L_{12}} & \mathbf{0}_{L_{12}} \\ \mathbf{0}_{L_{21}} & \mathbf{0}_{L_{21}} & \mathbf{1}_{L_{21}} & \mathbf{0}_{L_{21}} \\ \mathbf{0}_{L_{22}} & \mathbf{0}_{L_{22}} & \mathbf{0}_{L_{22}} & \mathbf{1}_{L_{22}} \end{bmatrix}$ are design matrices (in our case),

$\mathbf{e} = \begin{bmatrix} e_{1111} & \cdots & e_{I111} \\ \cdots & & \cdots \\ e_{1JKL_{JK}} & \cdots & e_{IJKL_{JK}} \end{bmatrix}$ is the $N \times I$ matrix of errors.

Let us consider the hypotheses: $\mathrm{H}_{0,s} : \mathbf{C}_s \boldsymbol{\Xi}_s \mathbf{M}_s = \mathbf{0}$ (s=1,2,12), where $\mathbf{C}_1 = \mathbf{I}_J - \frac{1}{J}\mathbf{1}_J\mathbf{1}_J^{\mathrm{T}}$, $\mathbf{C}_2 = \mathbf{I}_K - \frac{1}{K}\mathbf{1}_K\mathbf{1}_K^{\mathrm{T}}$, $\mathbf{C}_{12} = \mathbf{I}_{JK} - \frac{1}{JK}\mathbf{1}_{JK}\mathbf{1}_{JK}^{\mathrm{T}}$. Then the best linear unbiased estimator for particularc $\boldsymbol{\Xi}_s$ is $\hat{\boldsymbol{\Xi}}_s = \left(\mathbf{X}_s^{\mathrm{T}}\mathbf{X}_s\right)^{-}\mathbf{X}_s^{\mathrm{T}}\mathbf{Y}$, $\mathbf{M}_1 = \mathbf{I}_J$, $\mathbf{M}_2 = \mathbf{I}_K$, $\mathbf{M}_{12} = \mathbf{I}_{JK}$, and $\mathbf{I}_J$ is the identity matrix of order J [9, 10, 32].

With hypothesis $H_{0,1}$ the differences between visible leaf injury degree at the urban area and the rural area are tested. The hypothesis $H_{0,2}$ tests whether leaf injury degree differs between the years when the experiment was conducted. The hypothesis $H_{0,12}$ tests whether the leaf injury degree of an experimental object (results from the particular exposure site and the particular year), reduced by the exposure site × year interaction effect (means of leaf injury degrees in both exposure sites over both years), is equal to zero. The elements of the matrix $\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12}$ are differences between a selected (one of four) leaf injury degree in an individual observation period and mean values across both exposure sites of both years. The hypothesis was tested using Lawley-Hotelling's statistic [23] in the form:

$$T_{12}^2 = (N - JK)\,trace\left(\mathbf{E}^{-1}\mathbf{H}_{12}\right) \tag{4}$$

where $\mathbf{H}_{12} = \left(\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12}\right)^{\mathrm{T}}\left[\mathbf{C}_{12}\left(\mathbf{X}_{12}^{\mathrm{T}}\mathbf{X}_{12}\right)^{-1}\mathbf{C}_{12}^{\mathrm{T}}\right]^{-}\left(\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12}\right)$ and $\mathbf{E} = \mathbf{Y}^{\mathrm{T}}\left(\mathbf{I}_N - \mathbf{X}_{12}\left(\mathbf{X}_{12}^{\mathrm{T}}\mathbf{X}_{12}\right)^{-1}\mathbf{X}_{12}^{\mathrm{T}}\right)\mathbf{Y}$.

After rejection of $H_{0,12}$ the parameters which are responsible for this were determined. Tests of the hypothesis $H_{0,12,l\bullet} : \mathbf{c}_l^{\mathrm{T}}\boldsymbol{\Xi}_{12}\mathbf{M}_{12} = \mathbf{0}^{\mathrm{T}}$ ($\mathbf{c}_l^{\mathrm{T}}$ being the l-th row of matrix $\mathbf{C}_{12}$) make it possible to identify which rows of the matrix $\mathbf{C}_{12}\boldsymbol{\Xi}_{12}\mathbf{M}_{12}$ caused the rejection of this hypothesis. This hypothesis was tested applying the statistic:

$$T_{0,12,l\bullet}^2 = \left[\mathbf{c}_l^{\mathrm{T}}\left(\mathbf{X}_{12}^{\mathrm{T}}\mathbf{X}_{12}\right)^{-1}\mathbf{c}_l\right]^{-}\Delta_l \tag{5}$$

where

$$\Delta_l = (N - JK)\mathbf{c}_l^{\mathrm{T}}\hat{\boldsymbol{\Xi}}_{12}\,\mathbf{E}^{-1}\hat{\boldsymbol{\Xi}}_{12}^{\mathrm{T}}\mathbf{c}_l \tag{6}$$

is the Mahalanobis distance.

Canonical variate analysis is a method which transforms the matrix $\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12}$ into a set of new variables, which carry similar information, but are distributed in a multivariate Euclidean space. Following the transformation, the matrix $\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12}$ is presented in the form:

$$\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12} = \sum_{h=1}^{v}\lambda_h^{-1/2}\boldsymbol{\psi}_h\boldsymbol{\varphi}_h^{\mathrm{T}} \tag{7}$$

where $v = \min(I, JK - 1)$.

The vectors $\boldsymbol{\Psi}_h$ are called the h-th canonical coordinates, and the vectors $\lambda_h^{-1/2}\varphi_h$ are called the h-th dual canonical coordinates. The results of the experiment are presented in the space of canonical variates, where the Mahalanobis distances (Δ) are the distances between the origin and points representing the individual experimental object.

RESULTS AND DISCUSSION

Ozone-caused visible leaf injuries of tobacco plants were much higher in 2010 in comparison to 2011 (Fig. 1) due to favourable meteorological conditions for ozone creation, such as higher solar radiation and temperature (Table 1). Due to cumulative ozone effect on plants ozone concentration is presented here as AOT 40 critical dose, which means the sum of the difference between hourly concentrations greater than 80 μg m^{-3} (= 40 parts per billion) and μg m^{-3} over a given period using only the one-hour values measured between 8.00 and 20.00 Central European Time (CET) each day [13]. The AOT 40 value did not reach target value (18 000 μg m^{-3} h^{-1}), however this limit value is calculated for May–July season, while our investigations were conducted only for one month. Hence we can assume that during 2010 season at rural site a critical dose could be exceeded. On the other hand at urban site even long term objective critical dose (6 000 μg m^{-3} h^{-1}) was not exceeded in both experimental years (Table 1).

Table 1. Mean week values of selected air pollutants and meteorological parameters influencing ozone creation and sum of ozone concentrations (AOT 40)

Investigation period	Mean O_3 [μg m^{-3}]	PM10 [μg m^{-3}]*	NO_X [μg m^{-3}]	UVB radiation [W m^{-2}]	Temperature [°C]	AOT40 [μg m^{-3} h^{-1}]
	Mean					Cumulative
Rural site						
2010						
28.06–04.07	76.3	15.7	10.8	440.9	18.8	2464.7
05.07–11.07	74.2	19.8	11.3	369.1	19.3	4763.2
12.07–18.07	110.1	14.2	8.8	334.4	23.7	10390.9
19.07–25.07	84.6	20.9	12.3	215.5	19.8	13166.6
2011						
27.06–03.07	63.5	14.3	8.9	255.5	14.5	785.6
04.07–10.07	65.1	22.3	10.8	317.7	17.0	1803.8
11.07–17.07	68.5	18.1	10.7	343.2	17.5	3270.0
18.07–24.07	58.0	13.0	8.6	239.1	15.2	3825.2
Urban site						
2010						
28.06–04.07	59.6	19.5	32.4	305.0	20.0	1543.0
05.07–11.07	58.0	21.2	42.4	271.5	20.3	2930.1
12.07–18.07	78.1	21.8	22.9	272.3	23.8	4852.8
19.07–25.07	65.4	21.0	28.2	178.9	19.9	6018.8
2011						
27.06–03.07	55.7	14.6	19.0	185.5	24.7	463.1
04.07–10.07	47.2	26.6	31.6	228.9	20.1	1101.2
11.07–17.07	57.9	17.4	23.4	216.5	17.7	1816.1
18.07–24.07	49.5	16.2	17.6	148.2	14.7	1925.3

* PM10 – particulate matter ≤10 μm, evaluated at Poznan city, Botanical Garden (urban site) and in another rural site with comparable environmental properties as Witkowo

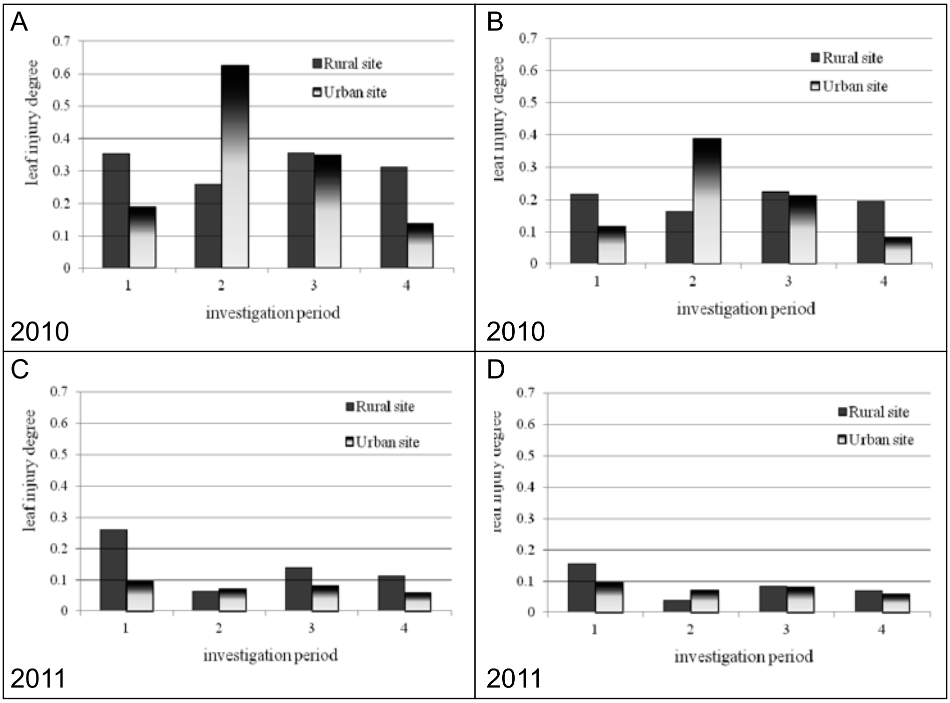

Fig. 1. Mean values of first (A and C) and second (B and D) index of maximum leaf injury degree of individual leaf in observation period for individual tobacco plant

The results of testing whether leaf injury degree varied for selected experimental factors are presented in Table 2. The results revealed that the level of visible symptoms caused by tropospheric ozone is dependent on the exposure site and the year of exposure. The results of comparison of leaf injury degree for urban and rural sites revealed high variability during the whole experiment period. However, we can observe some tendencies. Both used indices showed that higher ozone-caused injuries occurred after the second investigation period at the urban site, while during the rest of the series higher levels were observed at the rural site. Moreover, highly significant differences were noted only for the second investigation period in the 2010 growing season. Both indices revealed that mean differences between sites for all periods were lower in 2010 than in 2011 (Table 3). This might be connected with specific meteorological conditions and plant response. Ozone is created when high solar radiation occurs together with high emissions of ozone precursors [3]. However, some ozone precursors (such as peroxyacetyl nitrate – PAN) can be transported over great distances and when appropriate solar radiation and temperature conditions occur ozone can be created [11]. Hence, usually higher tropospheric ozone concentrations were noted in rural areas compared to an urban site [8, 28]. Most experiments on ozone effects on visible injury in typical biomonitoring projects with several exposure series using new plants have revealed higher damage of tobacco leaves at rural sites [19, 20]. Our results are in agreement with them, which means the ozone effect on plants is accumulative during the growing season, and relates to real ozone levels, which is proved by higher ozone injuries in the last two periods (Table 3). The second investigation period might be crucial for the plant response, when higher injuries were noted at the urban site in both experimental years. This might also suggest a cumulative ozone effect after two week exposure, when plants might be weaker, than in the first week. Moreover, in the second investigation period the highest nitrogen oxide concentrations were noted in both experimental years in the urban site (Table 1). This might be connected with emissions from car sources, due to higher traffic in the holiday season in the city. On the other hand, the ozone concentrations were not the highest at the urban site during this period, even though higher solar radiation was noted in 2011. This means that several factors influence ozone creation. Nevertheless, the total plant response throughout the entire experimental time was higher at the rural site.

Table 2. Results of hypothesis testing for first (a) and second (b) indices of tobacco leaf injury degree

Experimental factor	Index	The hypothesis	T_s^2	p
Exposure site	a	$\mathbf{C}_{1a}\boldsymbol{\Xi}_{1a}\mathbf{M}_{1a} = \mathbf{0}$	4.312	<0.05
	b	$\mathbf{C}_{1b}\boldsymbol{\Xi}_{1b}\mathbf{M}_{1b} = \mathbf{0}$	2.750	p=0.07
Year	a	$\mathbf{C}_{2a}\boldsymbol{\Xi}_{2a}\mathbf{M}_{2a} = \mathbf{0}$	61.665	<0.01
	b	$\mathbf{C}_{2b}\boldsymbol{\Xi}_{2b}\mathbf{M}_{2b} = \mathbf{0}$	50.848	<0.01
Exposure site × year interaction	a	$\mathbf{C}_{12a}\boldsymbol{\Xi}_{12a}\mathbf{M}_{12a} = \mathbf{0}$	21.964	<0.01
	b	$\mathbf{C}_{12b}\boldsymbol{\Xi}_{12b}\mathbf{M}_{12b} = \mathbf{0}$	17.380	<0.01

Table 3. Comparison of leaf injury degree ozone-sensitive tobacco for urban and rural sites $\left(\mathbf{c}_l^{\mathrm{T}}\hat{\boldsymbol{\Xi}}_{12}\mathbf{m}_i\right)$

"rural site" – "urban site"		Investigation period				All periods
		1st	2nd	3rd	4th	
$\mathbf{c}_l$	$\mathbf{m}_i^{\mathrm{T}}$	[1 0 0 0]	[1 0 0 0]	[1 0 0 0]	[1 0 0 0]	$[\frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}]$
First index						
2010	[1 –1 0 0]	0.164	-0.364**	0.007	0.175	-0.005
2011	[0 0 1 –1]	0.164*	-0.008	0.060	0.055	0.068*
Second index						
2010	[1 –1 0 0]	0.101	-0.224**	0.011	0.114*	0.001
2011	[0 0 1 –1]	0.060	-0.034	0.004	0.013	0.011

Significance level ** α = 0.01; * α = 0.05

Similarly, leaf injury degree between years was compared. The analysis revealed that higher values were always noted in 2010 for all investigation periods. The highest statistically significant differences occurred after the second investigation period at the urban site. This was also valid for the average value of all periods, especially for the first index (Table 4). Differences between years revealed higher variability of leaf injury degree between experimental years at the urban site (Table 4), while basing on summarized ozone concentrations we should expect higher variability at the rural site (Table 1). This might suggest an effect of the influence of higher solar radiation on stomatal closure, due to prevention of higher water losses in plants exposed in the rural site [24, 25]. Moreover, lower variability means that at the rural site higher levels of ozone injury can occur when lower ozone concentrations are noted. This might be an effect of high concentration of particulate matter in the city (Tab. 1), which chokes stomata, while ozone does not affect internal plant tissue [29]. This clearly indicates that the level of ozone effect on visible leaf injury is strongly connected with growing conditions, and indirectly with occurrence of other pollutants, such as particulate matter, which can block stomata and disturb the ozone effect on plants.

Table 4. Comparison of leaf injury degree of ozone-sensitive tobacco for 2011 and 2010 $\left(\mathbf{c}_l^{\mathrm{T}}\hat{\mathbf{\Xi}}_{12}\mathbf{m}_i\right)$

"2011" – "2010"		Investigation period				All periods
		1st	2st	3st	4st	
$\mathbf{c}_l$	$\mathbf{m}_i^{\mathrm{T}}$	[1 0 0 0]	[1 0 0 0]	[1 0 0 0]	[1 0 0 0]	$[\frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}]$
First index						
Rural	[–1 0 1 0]	-0.094	-0.197*	-0.214**	-0.199**	-0.176**
Urban	[0 –1 0 1]	-0.094	-0.552**	-0.267**	-0.079	-0.248**
Second index						
Rural	[–1 0 1 0]	-0.062	-0.126*	-0.139**	-0.126**	-0.113**
Urban	[0 –1 0 1]	-0.021	-0.316**	-0.132**	-0.025	-0.124**

Significance level ** $\alpha = 0.01$; * $\alpha = 0.05$

The above-mentioned relationships were confirmed by the experimental analysis. The main direction of variability was connected with the effect of years. The highest variability was again noted after the second investigation period at the urban site, which varied from -0.183 to 0.369 and from -0.094 to 0.223 for the first and second index respectively (Table 5). This variability is graphically presented in the space of canonical variates, where original values from Table 5 were transformed to the Euclidean space. Also, over 90% of variability was connected with the first coordinate, which "represents" the effect of year (Fig. 2). Moreover, based on analysis of Mahalanobis distances the first index revealed higher differences between sites in 2011, when lower tropospheric ozone concentrations occurred (Table 5). This suggests that this index is also very useful to detect and show differences at low ozone concentration levels.

CONCLUSIONS

The two-year investigations of the cumulative tropospheric ozone effect on visible leaf injury revealed higher values of leaf lesions in plants exposed in the rural site in the last two investigation periods, which is in agreement with previous experiments of traditional biomonitoring systems with separate exposure series. However, higher injury variability

Table 5. Evaluation of experimental object effects (the mean degree of leaf injury for the exposure site and year) in comparison to mean values of all object effects $\mathbf{C}_{12}\hat{\boldsymbol{\Xi}}_{12}$

Exposure site × year	Investigation periods				Δ^l
	1st	2nd	3rd	4th	
First index					
Rural site × 2010	0.129*	0.005	0.124**	0.157**	10.2**
Urban site × 2010	-0.035	0.369**	0.117**	-0.018	22.1**
Rural site × 2011	0.035	-0.191**	-0.090*	-0.042	6.8**
Urban site × 2011	-0.129**	-0.183**	-0.150**	-0.097*	23.8**
Second index					
Rural site × 2010	0.071*	-0.001	0.073**	0.095**	8.9**
Urban site × 2010	-0.030	0.223**	0.062*	-0.019	18.6**
Rural site × 2011	0.010	-0.127**	-0.066*	-0.031	9.8**
Urban site × 2011	-0.051	-0.094**	-0.070**	-0.044	13.6**

Significance level ** α=0.01; * α=0.05

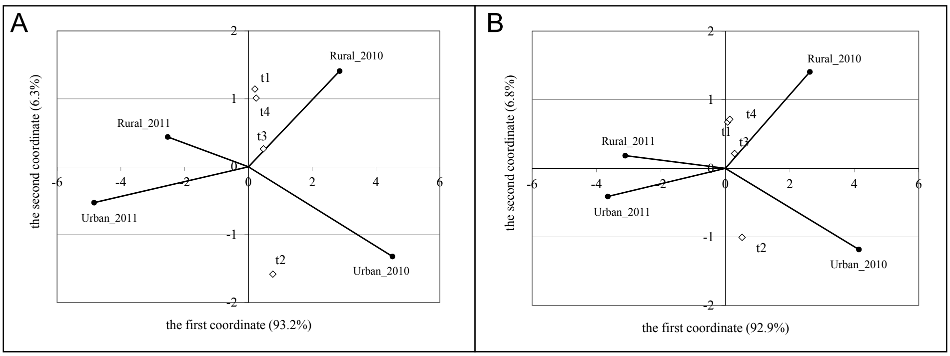

Fig. 2. Position of selected experimental objects differing in level of leaf injury degree (A – first index; B – second index) in the space of the first two canonical variates and spacing of the investigation periods in the dual space of canonical variates (values of canonical coordinates multiplied by 15)

was noted at the urban site, even though lower ozone concentrations were noted there. Lower variability of injury might suggest a lack of influence of particulate matter and the occurrence of higher injuries even though lower ozone concentrations occurred. Better detection of ozone injury was shown by the first index. Higher ozone injury was observed in 2010, when also higher tropospheric ozone concentrations were noted.

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INDEKSY WIDOCZNYCH USZKODZEŃ LIŚCI TYTONIU JAKO WSKAŹNIKI KUMULATYWNEGO STĘŻENIA OZONU TROPOSFERYCZNEGO

Ozon troposferyczny jest jednym z najbardziej reaktywnych zanieczyszczeń powietrza, przyczyniającym się do powstawania widocznych uszkodzeń, jak i zmniejszenia przyrostu biomasy i plonowania roślin. Ozon nie kumulują się w powietrz, ale jego negatywne oddziaływanie może się zwiększać wraz ze zwiększaniem czasu ekspozycji, stąd rośliny uprawne są najbardziej wrażliwe. Widoczne uszkodzenia roślin, jak i strata biomasy powodowane przez ozon mogą przyczynić się do strat ekonomicznych. Tytoń szlachetny uznany jest za jeden z lepszych bioindykatorów ozonu, jednakże mało poznana jest odpowiedź tego gatunku na wpływ ozonu przez dłuższy okres czasu. Dotychczas prowadzone badania opierały się na krótkotrwałej ekspozycji i jednorazowego pomiaru po zakończeniu doświadczenia. W pracy przedstawiono wyniki badań ekspozycji tytoniu (odmiana wrażliwa na ozon Bel W3) na dwóch stanowiskach (miejskim i leśnym) różniących się stężeniem tego zanieczyszczenia powietrza. Do prezentacji wyników posłużono się dwoma indeksami widocznych uszkodzeń liści. Na stanowisku leśnym zanotowano wyższe stężenia ozonu oraz sprzyjające warunki do jego tworzenia (większe promieniowanie słoneczne). W 2010 roku stwierdzono również wyższy poziom badanego zanieczyszczenia niż w 2011 roku, co ma odzwierciedlenie w uzyskanych wynikach badań biomonitoringowych. Analiza zmiennych kanonicznych nie wykazała różnic pomiędzy stanowiskami w reakcji na ozon, jednakże wyniki stopni uszkodzenia liści w poszczególnych terminach badawczych wykazały, że pod koniec eksperymentu występowały większe uszkodzenia na stanowisku leśnym. Wskazuje to na kumulatywny wpływ ozonu w cza-

sie sezonu wegetacyjnego. Większe zróżnicowanie zanotowano na stanowisku miejskim, na co nie wskazuje zróżnicowanie poziomu ozonu w danym roku badawczym. Niższe zróżnicowanie stopnia uszkodzenia liści na stanowisku leśnym może sugerować brak wpływu pyłu zawieszonego i wystąpienia wyższych uszkodzeń, nawet przy niższych stężeniach ozonu. Pierwszy indeks uszkodzenia liści, bazujący na średniej z trzech wartości, z rośliny wykazał większą czułość na niższe poziomy ozonu.