Biometrical Letters
Vol. 47 (2010), No. 2, 147-154

Study of ozone-caused leaf injury degree in different physiological phases of tobacco plants exposed to ambient air conditions

Anna Budka[1], Klaudia Borowiak[2], Dariusz Kayzer[1], Janina Zbierska[2]

[1]Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland, e-mail: budka@up.poznan.pl; dkayzer@up.poznan.pl;
[2]Department of Ecology and Environmental Protection, Poznan University of Life Sciences, Piątkowska 94C, 60-649 Poznań, Poland, e-mail: klaudine@up.poznan.pl; jzbier@up.poznan.pl;

SUMMARY

An ozone-sensitive cultivar of tobacco plants was exposed at two sites – urban and rural – for eight weeks in the 2008 growing season. Visible injuries caused by tropospheric ozone were measured every two weeks of the experiment. The differences in damage at every two-weekly observation are presented here. Three different ozone injury indexes were examined to establish the future usefulness of one of these indexes in similar tropospheric ozone bioindicative investigations. For this purpose the T^2-test for equality of mean vectors method was applied. The calculations revealed the most suitable index for this kind of investigation, taking account of five maximum values for leaf injury on each plant exposed at an individual site.

Key words: Hotelling's T^2 test, leaf ozone injures, tobacco plants

1. Introduction

The concentration of tropospheric ozone has been increasing in recent times. An adverse ozone effect is observed especially on plants which are affected by ozone continuously during their lifetime – for several years in the case of trees (Ashmore, 2004; Sanz, 2002), or during the growing season in the case of crop plants (Elagöz and Manning, 2005; Ribas and Peñuelas, 1999; Kostka-Rick et al., 2002). Tropospheric ozone is created in photochemical reactions of its precursors, such as nitrogen oxides, carbon oxides and volatile organic compounds. Hence the ground level ozone concentration is connected with emissions of these air pollutants and with favorable meteorological conditions (high solar radiation, high temperature) (Barret et al., 1998). For this reason the highest ozone concentrations occur during the summer season, especially when there is a high level of solar radiation.

The Bel W3 tobacco cultivar is a well-known tropospheric ozone bioindicator. Visible leaf injury can be treated as an ozone level detector (Ashmore, 2004). The ozone effect on plants cumulates during the growing season, and the plant's response differs depending on physiological age and time of exposure. Visible leaf injury is observed on both sides of the leaf as necrosis between veins. The degree of damage is correlated with the ozone level in the ambient air, as well as being connected with meteorological conditions, which also influence the stomatal closure of plants (Ribas and Penuelas, 2004).

The aim of the study presented here was to compare the degree of ozone leaf injury on the same tobacco plants at two selected exposure sites measured at two-week intervals over an eight-week experiment. To achieve this aim, a comparison of three proposed indexes describing degree of tobacco leaf injury for one plant is presented in this paper.

2. Materials and methods

2.1 Study area

The experiment was conducted in the 2008 growing season. The tobacco plants were cultivated in a greenhouse for eight weeks in 1.5 liter pots, and afterwards exposed to ambient air conditions for another eight weeks, from June 16 until August 11. Two exposure sites were selected, having different ozone levels, and where observations of air pollution and meteorological parameters are carried out.

One site was located within the city of Poznan, in the Botanical Garden, and the second in a forest area 80 km west of Poznan. Five tobacco plants (replications) were located at each exposure site. The ozone-related leaf injury was measured as the ratio of damaged leaf area to the whole leaf area. For the purposes of the present work we used values in the interval from 0 to 1. Every two weeks the degree of visible injury caused by tropospheric ozone was measured on the tobacco leaves, starting from the fourth leaf counted from the bottom of a plant (separately for each time of observation). Values of differences in degree of leaf injury between selected times of observations (periods) are analyzed in this paper. The value of a suggested index for one plant is treated here as an experimental unit. The injury level was measured four times for each plant, hence each plant is characterized by four injury values (after the 2nd, 4th, 6th and 8th week of exposure).

2.2 Leaf ozone injury indexes

The characteristic value of leaf injury degree for each plant had to be determined for further comparison of tobacco plants' response at the two exposure sites.

Three indexes of leaf injury to tobacco plants were created for the purposes of this work:

- the first index of leaf injury – the maximum value of leaf injury degree in a single observation period from all leaf injury values on each plant;
- the second index of leaf injury – the arithmetical mean of three maximum values of leaf injury degree in a single observation period from all leaf injury values on each plant;
- the third index of leaf injury – the arithmetical mean of five maximum values of leaf injury degree in a single observation period from all leaf injury values on each plant.

2.3 Test for equality of means (Hotelling's T^2 Test)

We wished to compare the mean leaf injury degree of tobacco plants between two exposure sites for one experimental time divided into *p* two-week observation periods. Because the calculated values of an index for one plant may be correlated, it is convenient to treat them as multidimensional variates.

Let $\mathbf{y}_{Ri}$ denote the vector of leaf injury values for plants located at the rural site in the *i*-th replication, and let $\mathbf{y}_{Ui}$ denote the vector of leaf injury values for plants located at the urban site in the *i*-th replication. Let *n* denote the number of replications in either of the experiments.

We assume that random vectors $\mathbf{y}_{R1}, \mathbf{y}_{R2}, ..., \mathbf{y}_{Rn}$ and $\mathbf{y}_{U1}, \mathbf{y}_{U2}, ..., \mathbf{y}_{Un}$ have multivariate normal distribution $N_p(\boldsymbol{\mu}_R, \boldsymbol{\Sigma}_R)$ and $N_p(\boldsymbol{\mu}_U, \boldsymbol{\Sigma}_U)$ respectively. Let $\overline{\mathbf{y}}_R = \sum_{i=1}^{n} y_{Ri} / n$ and $\overline{\mathbf{y}}_U = \sum_{i=1}^{n} y_{Ui} / n$ denote the vectors of mean leaf injury degree for the rural and urban exposure site respectively. Let $\mathbf{A}_R = \sum_{i=1}^{n} (\mathbf{y}_{Ri} - \overline{\mathbf{y}}_R)(\mathbf{y}_{Ri} - \overline{\mathbf{y}}_R)'$, $\mathbf{A}_U = \sum_{i=1}^{n} (\mathbf{y}_{Ui} - \overline{\mathbf{y}}_U)(\mathbf{y}_{Ui} - \overline{\mathbf{y}}_U)'$ and $\mathbf{S} = (2n-2)^{-1}(\mathbf{A}_R + \mathbf{A}_U)$ denote the pooled estimator of the covariance matrix (Krzyśko, 2000; Rencher, 1998; Seber, 1984).

We are interested in testing the hypothesis $H_0 : \boldsymbol{\mu}_R = \boldsymbol{\mu}_U$. The testing of mean values will be preceded by testing of the equality of the dispersion matrices $\boldsymbol{\Sigma}_R$ and $\boldsymbol{\Sigma}_U$. This equality is tested using the test statistics $u = -2(1-c)\log M$, where $c = (2p^2 + 3p - 1) / 3n(p+1)$ and $M = 2^{np} |\mathbf{A}_R|^{n/2} |\mathbf{A}_U|^{n/2} / |\mathbf{A}_R + \mathbf{A}_U|^n$. The statistic *u* has an approximate χ^2-distribution with $0.5p(p+1)$ degrees of freedom (Rencher, 1998).

To test $H_0 : \boldsymbol{\mu}_R = \boldsymbol{\mu}_U$ we use the test statistic $T^2 = \frac{n}{2}(\overline{\mathbf{y}}_R - \overline{\mathbf{y}}_U)' \mathbf{S}^{-1} (\overline{\mathbf{y}}_R - \overline{\mathbf{y}}_U)$. We reject H_0 if

$$\frac{2n-p-1}{(2n-2)p} T^2 > F_{p,2n-p-1}(\alpha).$$

In the case where we consider the contrast between elements of vector $\boldsymbol{\mu}_R$ and corresponding elements of vector $\boldsymbol{\mu}_U$, we test the hypothesis $H_{0\mathbf{C}} : \mathbf{C}(\boldsymbol{\mu}_R - \boldsymbol{\mu}_U) = 0$, where $\mathbf{C}$ denotes the $1 \times p$ vector of contrast (Krzyśko, 2002). The test statistic for $H_{0\mathbf{C}}$ is $T^2 = \frac{n}{2}(\mathbf{C}\overline{\mathbf{y}}_R - \mathbf{C}\overline{\mathbf{y}}_U)' (\mathbf{CSC}')^{-1} (\mathbf{C}\overline{\mathbf{y}}_R - \mathbf{C}\overline{\mathbf{y}}_U)$ (Rencher, 1998).

3. Analysis of results

Visible leaf injury of tobacco plants caused by tropospheric ozone was observed at both exposure sites, from the first period of observation onwards.

The calculated values of the statistics used for testing equality of the dispersion matrices $\mathbf{\Sigma}_R$ and $\mathbf{\Sigma}_U$ are given in Table 1. Based on these values we cannot reject the zero hypothesis of the equality of the covariance matrix for each of the indexes. For this reason we use the test for comparison of mean vectors, in case of equality of the covariance matrix. The results of testing for equality of mean ozone-related tobacco leaf injury between urban and rural sites are given in Table 1. We can observe from them that the mean ozone-related injury degrees for plants of tobacco cultivar Bel W3 exposed at the two selected sites are significantly different (this is true for all of the indexes calculated).

Table 1. Values of testing statistics (empirical significance levels in brackets)

	First index	Second index	Third index
Hypothesis: $H_0 : \mathbf{\Sigma}_R = \mathbf{\Sigma}_U$			
Value of *u* statistic	13.62 (0.191)	3.38 (0.971)	5.44 (0.860)
Hypothesis: $H_0 : \boldsymbol{\mu}_R = \boldsymbol{\mu}_U$			
Value of T^2 statistic	71.94	130.78	47.47
Value of *F* statistic	11.24 (0.010)	20.43 (0.003)	7.42 (0.025)

The results shown in Table 2 reveal that in the first observation period, for the second and the third index, leaf injury degrees at the two exposure sites are significantly different. Plants exposed at the rural site sustained higher ozone damage than these exposed at the urban site.

Table 2. Results of comparison between two exposure sites. (Differences: site located in Poznań city minus site located in forest.)

	First index	Second index	Third index
Day 0-14 – 1st observation period	-0.099	-0.373**	-0.387**
Day 15-28 – 2nd observation period	0.124	0.113	0.040
Day 29-42 – 3rd observation period	0.304**	0.131	0.058
Day 43-56 – 4th observation period	0.164*	0.161	0.107
Contrast: $\frac{1}{4}\mathbf{1}'_4(\boldsymbol{\mu}_U - \boldsymbol{\mu}_R)$	0.123**	0.008	-0.045*

* significant at level α=0.05;
** significant at level α=0.01

There are no differences between exposure sites in the 2nd, 3rd and 4th observation periods as far as the second and third index are concerned.

The first index revealed significant differences between the degrees of ozone injury to the tobacco plants at both sites in the 3rd and 4th observation periods. Slightly higher injuries on plants exposed at urban sites are observed in the 2nd, 3rd and 4th periods.

The results obtained for this index are uncommon in bioindicative research, since higher ozone injury is normally observed at rural sites, because ozone and its precursors are transported over long distances (Klumpp et al., 2004, Klumpp et al., 2006). In some cases, in spite of high ozone concentration in the ambient air, low degrees of visible ozone injury are recorded. This might be connected with high solar radiation and stomatal closure to decrease water loss in the plant tissue, which simultaneously reduces the possibility of ozone affecting the plants (Manning, 2003; Long and Naidu, 2004). Moreover, lower ozone injuries may occur in the case of meteorological conditions which are unfavorable to ozone creation, such as low solar radiation and high rainfall (Bandurska et al., 2009). In the case of our study, high solar radiation was observed, with a high ozone concentration which surely affected the exposed plants, and the normal effect of higher ozone injury at the rural site would be expected to be observed. Hence very interesting results are revealed by the contrasts analysis $\frac{1}{4}\mathbf{1}_4'\left(\boldsymbol{\mu}_U - \boldsymbol{\mu}_R\right)$, where a comparison is made of the sums of leaf injury degrees for plants at the rural and urban sites. The results of this analysis revealed a higher degree of ozone injury to tobacco plants at the urban site, if we consider the first index; however in the case of the second index there are no differences between sites, and the third index indicated that it was the rural site where higher visible leaf damage occurred (Table 2). This is valid when ozone and its precursors are transported far from the city and greater ozone impact on plants is observed in rural and forest areas – these conditions existed during our experiment. Hence we can deduce that this could be a good indicator for further consideration in similar tropospheric ozone bioindicative investigations.

4. Conclusions

In this paper we examined the usefulness of proposed indexes for comparison of the degree of ozone leaf injury to tobacco plants exposed in urban and rural/forest areas. For this purpose the T^2-test for equality of mean vectors method was applied. It seems that the best solution is to use the third index for indication of differences in leaf injury degree between two exposure sites, because a larger number of leaves are considered. The contrast analysis revealed higher ozone injury at rural sites, which is reasonable in the case of our investigations. Based on the results presented in this paper we can conclude that the third index could be used in future analysis of the cumulative effect of ozone on plants, because of the higher ozone effect on plants in rural areas. This is a normal situation when high solar radiation occurs during the summer season and ozone is created far from its precursors' sources.

REFERENCES

Anderson T. W. (2003): An Introduction to Multivariate Statistical Analysis. Third Edition, John Wiley and Sons, New York.

Ashmore M.R. (2004): Effects of oxidants at the whole plant and community. In: Air pollution and plant life. (Eds.) Bell J.N.B., Treshow M., John Wiley and Sons, Chichester: 89-119.

Bandurska H., Borowiak K., Miara M. (2009): Effect of ozone exposure on the activity of antioxidative enzymes in leaves of two tobacco cultivars with contrasting ozone sensitivity. Acta Biologica Cracoviensia, series Botanica, 51(1): 37-44.

Elagöz V., Manning W.J. (2005): Responses of sensitive and tolerant bush beans (Phaseolus vulgaris L.) to ozone in open-top chambers are influenced by phenotypic differences, morphological characteristics, and the chamber environment. Environmental Pollution. 136: 371-383.

Klumpp A., Ansel W., Klumpp G., Vergne P., Sifakis N., Sanz M.J., Rasmussen S., Ro-Poulsen H., Ribas A., Penuelas J., Kambezidis H., Heg S., Garrec J.P., and Calatayud V. (2006): Ozone pollution and ozone biomonitoring in European cities. Part II. Ozone-induced plant injury and its relationship with descriptors of ozone pollution. Atmosphere Environment 40: 7437–7448.

Klumpp A., Klumpp G., and Ansel W. (2004): Urban air quality in Europe – results of three years standardized biomonitoring studies. In: Klumpp A [eds], Urban air pollution, bioindication and environmental awareness, 25-58. Cuvillier Verlag, Göttingen.

Kostka-Rick R., Bender J., Bergmann E., Weigel H.J. (2002): Symptoms of ozone-induced foliar injury on horticultural crops. Bioindication and air quality in European cities. Red. Klumpp A., Fomin A., Klumpp G., Ansel W. Verlag Günter Heimbach. Stuttgart: 191-196.

Krzyśko M. (2000): Multivariate statistical analysis. (In Polish). UAM, Poznań.

Long S.P., Naidu S.L. (2004): Effects of oxidants at the biochemical, cell and physiological level, with particular reference to ozone. Bell J.N.B., Treshow M. (Eds.), In: Air pollution and plant life. John Wiley and Sons, Chichester: 69-89.

Manning W.J. (2003): Detecting plant effects is necessary to give biological significance to ambient ozone monitoring data and predictive ozone standards. Environmental Pollution. 126: 375-379.

Morrison D. F. (1967): Multivariate Statistical Methods. John Wiley and Sons, New York.

Rencher A.C.(1998): Multivariate Statistical Inference and Applications. John Wiley and Sons, New York.

Ribas A., Peñuelas J. (1999): Effects of ethylene diurea as a protective antiozonant on beans (Phaseolus vulgaris cv Lit) exposed to different tropospheric ozone doses in Catalonia (Ne Spain). Water, Air and Soil Pollution: 263-271.

Ribas A., Peñuelas J. (2004): Ozone biomonitoring in rural stations of Catalonia (NE Spain). Urban air pollution, bioindication and environmental awareness. Red. Klumpp A., Ansel W., Klumpp G., Cuvillier Verlag, Göttingen: 137-146.

Sanz M.J. (2002): Efforts of standardisation of ozone bioindication in Europe. Bioindication and air quality in European cities. Red. Klumpp A., Fomin A., Klumpp G., Ansel W. Verlag Günter Heimbach, Stuttgart: 87-94.

Seber G.A.F. (1984): Multivariate Observations. Wiley, New York.