



MORPHOLOGICAL CHANGES IN TWO TOBACCO AND PETUNIA CULTIVARS UNDER EXPOSURE TO TROPOSPHERIC OZONE

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The study assessed the effect of cumulative tropospheric ozone on the morphology of an ozone-sensitive (Bel W3) and an ozone-resistant (Bel B) tobacco cultivar, and two petunia cultivars (Mirage, White Cascade). The plants were exposed at two sites differing in tropospheric ozone level for two months during the 2008 growing season. Similar sets of plants were cultivated in control conditions. Morphological parameters of the plants were measured every week during the experiment. The correlation between the recorded results and the cumulative concentrations of tropospheric ozone measured at the two exposure sites was estimated. The ozone-sensitive tobacco cultivar showed increased visible damage after four weeks of the experiment, although ozone was relatively low during the preceding weeks, possibly confirming the cumulative effect of ozone on the plant response. The ozone-resistant tobacco cultivar showed higher mean plant growth and leaf growth than the ozone-sensitive one throughout the experimental period, but at the exposure sites the ozone-sensitive cultivar showed plant growth similar to or higher than the controls, especially at the forest site where ozone concentrations were higher. This suggests a plant defense against reduction of leaf assimilation area (i.e., against leaf necrosis). *Petunia* cv. Mirage showed lower growth at the control site and had fewer flowers than White Cascade at all sites. White Cascade had more flowers than Mirage in the last week of the experiment at the forest site where tropospheric ozone was higher. Its mean growth was higher at the forest site than at the other exposure site.

Key words: Tobacco, petunia, morphology, tropospheric ozone.

INTRODUCTION

The increase of motor vehicle traffic has brought an increase of air pollution from auto exhaust. Transport air pollution sources are responsible for emission of such pollutants as nitrogen oxides, carbon oxides and volatile organic compounds, all of which can become precursors of ground-level ozone. The highest tropospheric ozone concentrations are observed during summer, when the conditions for its creation occur (high solar radiation, high temperature). Tropospheric ozone concentrations are predicted to increase during the coming decades in almost every area of the world due to steady or increased levels of its precursors (IPCC, 2007). Ozone is a greenhouse gas which plays an important role in climate change. It is one of the most reactive air pollutants, and can cause visible plant damage as well as internal changes in plants leading to altered morphology and yield losses. The economic loss of crop yield due to ozone for four major commodities has been estimated at 14-26 billion USD per year

(van Dingenen et al., 2009). Yield reduction has been noted in many crop plant species, such as wheat (Rai et al., 2007; Feng et al., 2008), potato (Vandermeiren et al., 2005), rice (Shi et al., 2009) and cotton (Hassan and Tewfik, 2006).

Ozone affects the plant at the leaf surface and also enters the plant via stomata, disturbing metabolic processes. The main effects of ozone on a plant are leaf damage and alteration of normal growth. The extent of ozone's effects on a plant is correlated with the duration of exposure, ozone concentration, plant height and meteorological conditions (Long and Naidu, 2004). Previous studies have reported changes in plant morphology due to ozone fumigation. The several measured parameters generally have shown similar results: ozone affected plant growth, leading to yield reduction. The observed changes in plant morphology include reduced leaf size in tree species (Dizengremel, 2001; Oksanen, 2003; Riikonen et al., 2004; Riikonen et al., 2010), field mustard (Kleier et al., 1998) and Pima cotton (Grantz and Young, 1995); reduced leaf area and

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plant height in cotton (Zouzoulas et al., 2009) and tobacco (Saitanis and Karandinos, 2002); decreased leaf area ratio and specific leaf area in soybean (Morgan et al., 2003); and reduction of plant height in cucumber (Agrawal et al., 1993) and chickpea (Welfare et al., 2002). These results come mainly from experiments in controlled or semi-controlled conditions. There are fewer studies done in completely natural conditions. Earlier work with two tobacco cultivars (ozone-sensitive and ozone-resistant) grown outdoors revealed higher plant and leaf growth of the ozone-sensitive cultivar (Borowiak et al., 2010). That experiment was conducted in a twoweek exposure series; the morphological parameters were measured before and after each exposure period. Hence we could not measure the cumulative effect of ozone exposure over an entire growing season. In the present study the same plants were exposed for a two-month period, during which the plants' morphological characteristics were noted every week for an assessment of the cumulative effect of ozone on the plants.

Negative effects of ozone have also been noted in horticultural plant species showing visible symptoms of ozone influence. Necrotic damage to leaves together with reduction of biomass production and number of flowers were observed in butterfly bush and petunia (Booker et al., 2009). Such symptoms decrease their economic value, so research on the effect of ambient ozone on those plants is also needed.

This study examined the cumulative effect of tropospheric ozone on visible leaf damage in tobacco and petunia plants and on their morphological characteristics, and estimated the correlation between the recorded results and the ozone concentrations measured at two sites differing in ozone exposure.

MATERIAL AND METHODS

Two Nicotiana tabacum L. cultivars (ozone-sensitive Bel W3, ozone-resistant Bel B) and two Petunia × hybrida L. cultivars (Mirage, White Cascade; both ozone-sensitive cultivars) were used in this experiment. Tobacco seeds were purchased from Landwirtschaftliches Technologiezentrum Augustenberg, Germany, and petunia seeds from W. Legutko Breeding & Seed Company, Poland. Plants were cultivated in greenhouse conditions for eight (tobacco) and six (petunia) weeks in 1.5 L pots with a standard soil mixture of peat and sand. Slowrelease fertilizer was added to the pots in an amount sufficient for cultivation and the exposure period. After cultivation the plants were transported to two exposure sites differing in tropospheric ozone concentration. One site was located in the Witkowo Forest Division ~80 km west of the city of Poznań (called "forest site" here), and the other in Poznań in the Botanical Garden of Adam Mickiewicz University ("city site"). Air pollution and basic meteorological monitoring is carried out at both sites by the Provincial Environmental Agency. Air pollution monitoring followed government guidelines (Dz.U. 5, poz. 31, 2008). Tropospheric ozone was measured by standard methods using UV photometry (PN-EN 14625:2005).

Nine plants of each cultivar were exposed at each site. Similar sets of plants were cultivated in control conditions without ozone, under cultivation conditions (water supply, temperature, irradiance) similar to outside conditions. Temperature in the greenhouse (19–24°C) usually was very close to outside temperature, and ozone levels (0–20 $\mu g \ m^{\text{-}3}$) were below background levels but varied as visitors opened the greenhouse doors for entry. The ozone-exposed plants were mounted on specially constructed aluminum racks 90 cm high. Continuous water supply was provided through fiberglass wicks on trays under the pots. The plants were protected against high solar radiation and strong winds with 50%-shading fabric.

The experiment was carried out from June 16 to August 11, 2008. Morphological parameters were measured weekly: plant growth, tobacco leaf width and length, and number of petunia flowers. Leaf growth of tobacco plants was measured from the 4th leaf up from the base of the plant. The differences in plant and leaf growth between successive weeks were calculated and are presented here. The "day 0" values for plant height and leaf parameters are from measurements made before exposure. As the plants grew the number of tobacco leaves increased, complicating the task of calculating a proper value for leaf growth. Based on the method and results in Budka et al.'s (2010) study of tobacco leaf injury, leaf growth was averaged from the four highest leaves values of each plant each week. Ozone-sensitive tobacco cultivars show visual symptoms of ozone effects; the degree of leaf damage was assessed along with the other morphological parameters every week. The differences in leaf damage from week to week are presented here on a 0-1 scale. Ozone damage was calculated as for tobacco leaf growth (averaged from the four highest leaves' values of each plant each week). The number of petunia flowers was counted only for new flowers developed in a given week.

The correlations between morphological data and tropospheric ozone concentrations at both exposure sites were analyzed. Ozone concentrations are given in AOT 40 units, a measurement standard adopted by the European Union. AOT 40 is the accumulated ozone concentration over a threshold of 40 ppb, the critical threshold for plants and ecosystems under Polish regulations, measured here

TABLE 1. Maximum tropospheric ozone concentrations (O_3 max.) and mean values for nitrogen oxides (NO_x), UVB solar radiation and temperature (Temp) in each experimental week

	City site				Forest site			
Day of experiment	O ₃ max [µg m ⁻³]	NO _X [μg m ⁻³]	UVB [W m ⁻²]	Temp [°C]	O ₃ max [μg m ⁻³]	NO _X [μg m ⁻³]	UVB [W m ⁻²]	Temp [°C]
7	115	49.0	228.9	16.5	111	10.9	313.1	18.7
14	90	26.6	264.8	17.6	135	9.0	336.8	16.3
21	108	24.1	279.0	18.4	158	8.5	379.5	19.1
28	113	20.3	200.6	16.5	121	10.8	290.3	16.9
35	92	30.0	194.7	17.0	95	13.9	307.8	16.2
42	113	16.6	259.1	19.9	105	8.4	385.3	15.4
49	149	23.3	237.5	23.0	156	10.6	357.7	21.6
56	94	40.8	202.6	18.9	113	12.0	345.8	17.5

between 8 a.m. and 8 p.m. It is useful for presenting cumulative ozone effects on plants during the growing season. The plant data were also analyzed versus levels of nitrogen oxides (ozone precursor) and some meteorological parameters (temperature, solar radiation) that may affect the plant response and may also affect ozone creation.

The results were analyzed by multifactorial ANOVA, with cultivar, site and observation day as fixed factors. Mean values and standard errors were calculated for presentation of the differences between certain factors. The threshold of statistical significance in this work is p=0.05. Statistical analyses employed Statistica 9.1.

RESULTS

AIR POLLUTANT CONCENTRATIONS AND METEOROLOGICAL CONDITIONS

The mean surface ozone concentrations were highest in the first weeks of the experiment: ozone levels of 90 μg·m⁻³ at the forest site and 80 μg·m⁻³ at the city site were recorded at week 2. Mean ozone concentrations were higher at the forest site except at week 5 (Fig. 1a). The effect of ozone on plants is cumulative during the growing season, so it is important to capture AOT 40 values over consecutive weeks. In this case the difference between the two exposure sites increased, and at the end of the experiment the cumulative ozone concentration was twice higher at the forest site than at the city site (Fig. 1b). The maximum ozone concentration was noted on day 21 of the experiment at the forest site, and the ozone level generally was higher there. NO_v was higher at the city site. Solar radiation was higher at the forest site but temperature was not always higher there (Tab. 1).

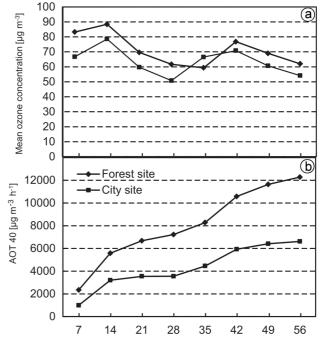


Fig. 1. Mean ozone concentrations (**a**) and AOT 40 values (**b**) in consecutive weeks of the experiment.

TOBACCO PLANT MORPHOLOGY

Visible leaf damage caused by ozone appeared only in the sensitive cultivar. Multifactorial ANOVA revealed a significant effect of day of experiment on leaf damage degree (Tab. 2). Although ozone concentrations were higher at the forest site, leaf damage was not always higher there. There was no significant difference in leaf damage between days 14 and 42. A difference in leaf damage was noted after days 49 and 56. Differences in ozone effect might

TABLE 2. Multifactorial ANOVA for morphological parameters, with site and day of experiment as fixed factors

Parameter	Site	Day of experiment	Interaction
Leaf injury	ns	***	***
Bel B plant growth	***	***	*
Bel W3 plant growth	ns	***	*
Bel B leaf length growth	***	***	***
Bel B leaf width growth	***	***	***
Bel W3 leaf length growth	ns	***	*
Bel W3 leaf width growth	*	***	***
Petunia cv. White Cascade plant growth	***	ns	*
<i>Petunia</i> cv. Mirage plant growth	*	ns	***

^{**} p< 0.001; ** p< 0.01; * p < 0.05; ns - not significant

have been expected especially at days 14, 21 and 28 of the experiment, when mean ozone concentrations decreased, but leaf damage increased at both exposure sites. Another interesting observation was that at days 35 and 42 of the experiment, when ozone concentrations increased, leaf damage was much less than in the three preceding weeks (Fig. 2).

The effects of site and observation day on Bel B tobacco cultivar plant height and leaf growth were

highly significant (p≤0.001) (Tab. 2). Plant growth was highest at the control site on almost all measurement days, though not always significantly higher. Plant growth was high at day 28 at both exposure sites, when the mean ozone concentration was lowest, and systematically decreased thereafter. The low plant growth in the last week might be related to gradual senescence together with the onset of flowering (Fig. 3a). Bel B plant height increase was negatively correlated with cumulative ozone concentration (Tab. 3). Bel B leaf length and width growth was highest at day 28 when one of the lowest ozone concentrations was noted at both sites. In previous weeks, leaf growth had been lower and ozone levels were moderate. The second peak of leaf growth at both sites was at day 42, when mean ozone concentrations were relatively low. The differences in leaf growth between sites are not so conspicuous (Fig. 4). Tobacco Bel B leaf growth values were negatively but not significantly correlated with cumulative ozone concentrations at both exposure sites (Tab. 3). Since the plant response is also related to other factors such as temperature and solar radiation, the correlations between the measured morphological parameters and those two factors were analyzed. Except for temperature and tobacco Bel B plant growth at the city site, there was no significant correlation between those weather components and the measured plant parameters. There was a slightly negative correlation between temperature and plant growth, but a detailed analysis of plant and leaf growth showed no such trend. Growth was higher on some days at sites where temperature was lower (days 7 and 14), and vice versa (day 35) (Tab. 2, Fig. 3).

TABLE 3. Linear correlation coefficients and significance levels for relation between morphological parameters of plants and cumulative ozone concentration, temperature and solar radiation at forest and city sites

Parameter	Ozone	Temp	Solar radiation	Ozone	Temp	Solar radiation
		Forest site			City site	
Bel B plant growth	r = -0.870	r = -0.300	r = -0.463	r = -0.878	r = -0.742	r = -0.001
	p = 0.015	p = 0.470	p = 0.248	p = 0.004	p = 0.035	p = 0.999
Bel W3 plant growth	r = -0.931	r = 0.092	r = -0.271	r = -0.843	r = -0.608	r = 0.323
	p = 0.001	p = 0.829	p = 0.516	p = 0.009	p = 0.109	p = 0.435
Bel B leaf length growth	r = -0.364	r = -0.605	r = -0.223	r = -0.437	r = -0.458	r = -0.348
	p = 0.375	p = 0.112	p = 0.596	p = 0.278	p = 0.254	p = 0.398
Bel B leaf width growth	r = -0.646	r = -0.541	r = -0.449	r = -0.540	r = -0.554	r = -0.332
	p = 0.083	p = 0.167	p = 0.265	p = 0.167	p = 0.154	p = 0.421
Bel W3 leaf length	r = -0.323	r = -0.331	r = -0.366	r = -0.305	r = -0.385	r = -0.199
growth	p = 0.435	p = 0.424	p = 0.373	p = 0.462	p = 0.346	p = 0.636
Bel W3 leaf width growth	r = -0.499	r = -0.363	r = -0.446	r = -0.181	r = -0.334	r = -0.172
	p = 0.208	p = 0.377	p = 0.268	p = 0.667	p = 0.418	p = 0.683
<i>Petunia</i> cv. White	r = -0.480	r = -0.559	r = -0.320	r = 0.507	r = 0.338	r = -0.506
Cascade plant growth	p = 0.229	p = 0.149	p = 0.439	p = 0.200	p = 0.413	p = 0.201
Petunia cv. Mirage plant growth	r = -0.227	r = 0.028	r = -0.274	r = 0.037	r = 0.018	r = 0.188
	p = 0.590	p = 0.948	p = 0.512	p = 0.931	p = 0.967	p = 0.656

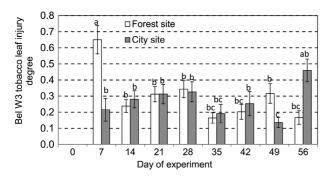


Fig. 2. Visible leaf damage (means \pm SE) in ozone-sensitive (Bel W3) tobacco plants exposed to ozone at forest and city sites. Letters denote significant differences between means at p=0.05.

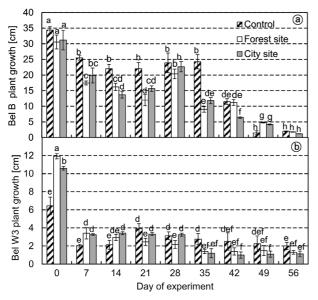


Fig. 3. Plant height growth (means \pm SE) of ozone-resistant (a) and ozone-sensitive (b) tobacco cultivar on consecutive days of the experiment. Letters denote significant differences between means at p=0.05.

The effect of site on Bel W3 tobacco plant height and leaf length growth was not significant or of low significance, but the effect of measurement day was highly significant (p≤0.001) (Tab. 2). There were no such clear trends of plant height increase as for the Bel B tobacco cultivar. Growth was slightly higher at exposure sites in the first four weeks than in the last four. Plant growth at the control site was very clearly higher in the last four weeks (Fig. 3b). As with the ozone-resistant cultivar, Bel W3 leaf growth peaked after day 28, but there was no such clear second peak after day 42 (Fig. 5). As in the case of Bel B, a both leaf growth parameters were negatively correlated with tropospheric ozone concentration (Tab. 3).

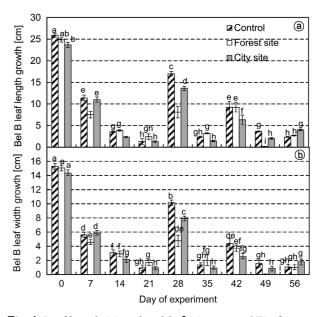


Fig. 4. Leaf length (**a**) and width (**b**) (means \pm SE) of ozone-resistant tobacco cultivars on consecutive days of the experiment. Letters denote significant differences between means at p=0.05.

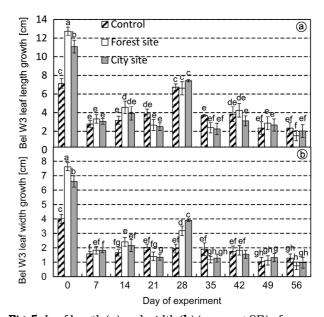


Fig. 5. Leaf length (**a**) and width (**b**) (means \pm SE) of ozone-sensitive tobacco cultivars on consecutive days of the experiment. Letters denote significant differences between means at p=0.05.

Throughout the experimental period and at all sites, both plant height and leaf growth were significantly higher in Bel B, the resistant cultivar, than in Bel W3. Ozone-exposed Bel B plants had lower values for plant and leaves growth than control Bel B plants, while the growth of the ozone-sensitive Bel

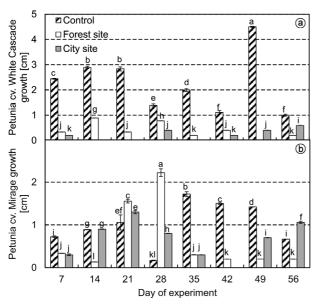


Fig. 6. Plant height growth (means $\pm SE$) of petunia cv. White Cascade and cv. Mirage on consecutive days of the experiment. Letters denote significant differences between means at p=0.05.

W3 cultivar under ozone exposure was similar to or higher than in the control plants. Leaf growth of Bel W3 was higher in plants exposed at the forest site, where tropospheric ozone concentrations were higher (Fig. 7a,b,c). As with the Bel B cultivar, there were no statistically significant relations between temperature, solar radiation and Bel W3 growth parameters. There were periods when plant growth was lower at a site with lower temperature, and vice versa (Tab. 3, Fig. 3). Hence I conclude that ozone was the main factor influencing plant growth.

PETUNIA MORPHOLOGY

Multifactorial ANOVA revealed a highly significant (p≤0.001) effect of site on cv. White Cascade plant growth and a less significant effect of site on cv. Mirage growth. Plant growth of both petunia cultivars did not vary according to the effect of day of observation (Tab. 2). Growth of White Cascade was highest at the control site throughout the experimental period, was higher at the forest site than at the city site until day 49, and was higher at both exposure sites after day 28 (Fig. 6a). For Mirage there were no such clear trends but an increase of growth was noted at day 28 (Fig. 6b). In both cultivars there was a negative but nonsignificant correlation between ozone concentration and plant growth at the forest site, and a positive correlation at the city site. There were no correlations between petunia growth and temperature or solar radiation (Tab. 3).

TABLE 4. Mean number of flowers for petunia cv. Mirage and cv. White Cascade

	Number of flowers						
Day of experiment	cv.	Mirage		cv. White Cascade			
	Control	Forest site	City site	Control	Forest site	City site	
7	0	1	1	0	0	0	
14	1	1	1	5	6	6	
21	1	1	1	6	5	5	
28	1	2	1	8	6	6	
35	1	1	1	5	4	3	
42	1	1	2	4	2	3	
49	1	3	2	4	6	3	
56	1	1	1	1	8	3	

TABLE 5. Multifactorial ANOVA for morphological parameters, with plant cultivar and site as fixed factors

Parameter	Cultivar	Site	Interaction
Tobacco height growth	*	***	**
Tobacco leaf width growth	ns	***	**
Tobacco leaf length growth	ns	***	*
Petunia growth	ns	Ns	*

^{***} p< 0.001; ** p< 0.01; * p< 0.05; ns - not significant

The number of petunia flowers increased during the experiment, especially in White Cascade, whose flower number peaked at day 28 at the city and control sites and on the last day at the forest site. It had many more flowers than Mirage, which showed no such clear trends of flower number (Tab. 4). Throughout the experiment, plant growth of Mirage was higher at the exposure sites than at the control sites, while White Cascade showed lower plant height at the exposure sites, especially the city site, than at the control site (Fig. 7d).

For all studied species there was a statistically significant effect of exposure site on plant morphological response (p=0.05).

DISCUSSION

Most studies of the effect of ozone on plants are short-term, lasting from a few days to a few weeks (Elkiev and Ormond, 1979; Cuny et al., 2004; Klumpp et al., 2006; Bandurska et al., 2009). Few papers report long-term observations of changes in plants during the growing season which might reflect the cumulative effect of ozone on plant growth. A modest ozone concentration over a pro-

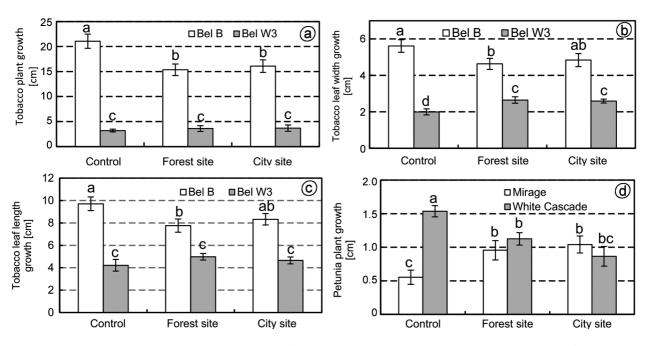


Fig. 7. Tobacco plant height growth (a), leaf length (b) and width (c), and petunia plant height growth (d) through the experimental period (means $\pm SE$). Letters denote significant differences between means at p=0.05.

longed period can inhibit photosynthetic activity (Fiscus et al., 2005) and cause a reduction of leaf area, lowering crop yield.

Some plant species recognized as ozone-sensitive show visible symptoms of the effect of ozone. These symptoms can result in economic losses. A positive correlation between cumulative ozone concentration and visible damage has been noted in the ozone-sensitive Bel W3 tobacco cultivar (Borowiak et al., 2012) and was confirmed in this study at both exposure sites. Leaf injury in this cultivar might be employed as an indicator of cumulative ozone effects potentially damaging to plants. The ozone concentration was higher at the forest site, in agreement with previous findings from several countries (Godzik, 2000; Forlani et al., 2005), and is connected with meteorological conditions (solar radiation) and ozone precursor concentrations in remote areas (Barett et al., 1998; Kley et al., 1994). The lower levels of NO_x at the forest site might be due to its consumption during ozone creation.

Interestingly, the ozone-sensitive tobacco cultivar showed an increase of ozone-caused damage at both exposure sites between days 14 and 28, when the ozone concentrations there were relatively low. In a similar investigation, ozone-caused damage was found to be higher at the city site when the ozone concentration was low (Borowiak et al., 2012). The ozone-resistant tobacco cultivar showed high growth after four weeks of the experiment. This peak was preceded by three weeks of relatively low ozone con-

centration; later the second peak of Bel B growth occurred when the mean ozone concentration was quite high. The ozone-sensitive tobacco cultivar showed the same growth trend at four weeks. For the forest site growth increase might also be connected with increased solar radiation; at the city site the temperature rose (Tab. 1). Negative but not always significant correlations between plant growth parameters and cumulative ozone concentrations were noted in both cultivars at both exposure sites. Some investigations have found that ozone affects plants by reducing leaf area and plant growth (Agrawal et al., 1993; Saitanis and Karandinos, 2002; Morgan et al., 2003; Zouzoulas et al., 2009). The reduction of leaf area might be connected with higher stomatal density which would lead to higher ozone uptake by leaves and intensified detoxification (Pääkonen et al., 1995; Hetherington and Woodward, 2003).

The ozone-resistant tobacco cultivar showed higher leaf and plant growth, as was also found in an experiment with a two-week exposure series (Borowiak et al., 2010a). On the other hand, the ozone-sensitive cultivar showed similar or higher plant and leaf growth in plants exposed at the forest and city sites. A similar tendency was observed in a short-term experiment (Borowiak et al., 2010b).

Negative effects of ozone have been reported in many horticultural species such as petunia and nasturtium. In this study the White Cascade petunia showed higher growth at the control site. Plants exposed in the forest where ozone concentrations were higher had higher growth. Tropospheric ozone can affect plant growth as well as flower yield (Elkiev and Ormond, 1979). This study confirmed that in part. At both exposure sites the number of flowers was highest on day 28, which was preceded by three weeks of relatively low ozone concentrations. A second peak of flower number occurred in White Cascade on the last day of the experiment at the forest site, when the ozone concentration increased. Such was not the case at the other sites, nor in cv. Mirage. Possibly this suggests a plant defense accelerating generative development under stress, as well as the cumulative effect of ozone. Usually a high ozone concentration is connected with high insolation or temperature, which can also influence plant flowering. Hence a synergistic effect may be involved. The aim of this study was to assess the ozone effect in natural conditions, which allows a number of uncontrolled factors to influence the plant response to ozone.

Overall, tropospheric ozone negatively affected the growth of the resistant tobacco cultivar. Although the ozone-resistant tobacco cultivar showed higher mean plant and leaf growth throughout the experiment, the ozone-sensitive tobacco cultivar had similar or higher plant growth at the exposure sites, especially at the forest site where ozone concentrations were higher. This may indicate a plant defense preventing leaf necrosis and consequent reduction of the assimilation area. The ozonesensitive tobacco cultivar showed increased visible injury after four weeks, despite relatively low ozone concentrations in the preceding weeks. This seems to confirm the cumulative effect of ozone on the plant response. Petunia cv. White Cascade showed lower growth at the exposure sites but higher flower number in the last week of the experiment at the forest site where the tropospheric ozone concentration was higher; also its mean growth at the forest site was higher than in the other petunia cultivar. Those results point to a plant defense against stress factors such as tropospheric ozone.

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REFERENCES

AGRAWAL M, KRIZEK DT, AGRAWAL SB, KRAMER GF, LEE EH, and MIRECKI RM. 1993. Influence of inverse day/night temperature on ozone sensitivity and selected morphological and physiological responses of cucumber.

- Journal of American Society for Horticulture Science 118: 649–654.
- Bandurska H, Borowiak K, and 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.
- Barret LA, Bunce NJ, and Gillespie TJ. 1998. Estimation of tropospheric ozone production using concentrations of hydrocarbons and NOx, and a comprehensive hydrocarbons reactivity parameter. *Journal of Photochemistry and Photobiology* A 113: 1–8.
- Borowiak K, Drzewiecka K, Gąsecka M, Zbierska J, and Grajczyńska O. 2010a. Investigations of selected parameters of tobacco plants response to tropospheric ozone in ambient air conditions. *Fresenius Environmental Bulletin* 19(11): 2480–2489.
- BOROWIAK K, ZBIERSKA J, and DRAPIKOWSKA M. 2010b. Differences in morpho-anatomical structure of ozone-sensitive and ozone-resistant tobacco cultivars. *Acta Biologica Hungarica* 61(1): 90–100.
- Borowiak K, Kayzer D, Budka A, Zbierska J, Drzewiecka K, Bandurska H, and Goliński P. 2012. Cumulative tropospheric ozone effect on visible tobacco leaf injury. Fresenius Environmental Bulletin 21(2a): 509–517.
- BOOKER F, MUNTIFERING R, McGrath M, BURKEY K, DECOTEAU D, FISCUS E, MANNING W, KRUPA S, CHAPPELKA A, and GRANTZ D. 2009. The ozone component of global change: potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. *Journal of Integrative Plant Biology* 51(4): 337–351.
- Budka A, Borowiak K, Kayzer D, and Zbierska J. 2010. Study of ozone-caused leaf injury degree in different physiological phases of tobacco plants exposed in ambient air conditions. *Biometrical Letters* 47(2): 147–154.
- CUNY D, DAVRANCHE L, VAN HALUWYN PLAISANCE H, CARON B, and MALRIEU V. 2004. Monitoring ozone by using tobacco, automated network and passive sampler in an industrial area in France. In: Klumpp A, Klumpp G, Ansel W [eds.], Urban Air Pollution. Bioindication and Environmental Awareness, 89–96. Cuvillier Verlag, Göttingen.
- DIZENGREMEL P. 2001. Effects of ozone on the carbon metabolism of forest trees. *Plant Physiology and Biochemistry* 39: 729–742.
- Dz.U. 5, poz. 31. 2008. Rozporządzenie Ministra Środowiska w sprawie dokonywania oceny poziomów substancji w powietrzu.
- ELKIEY T, and ORMROD DP. (1979) Petunia cultivar sensitivity to ozone and sulphur dioxide. *Scientia Horticulturae-Amsterdam* 11: 269–280.
- Feng ZZ, Kobayashi K, and Ainsworth EA. 2008. Impact of elevated ozone concentration on growth, physiology and yield of wheat (*Triticum aestivum* L.): a meta-analysis. *Global Change Biology* 14: 2696–2708.
- FISCUS EL, BOOKER FL, and BURKEY KO. 2005. Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant, Cell and Environment* 28: 997–1011.



- Region (Southern Italy). Fresenius Environmental Bulletin 14(6): 478–483.
- Godzik B. 2000. The measurement of tropospheric ozone concentrations in southern Poland using the passive samplers and plant bioindicators. *Archives of Environmental Protection* 26(2): 7–19.
- Grantz DA, and Young S. 1995. Effect of ${\rm O_3}$ on hydraulic architecture in Pima cotton: biomass allocation and water transport capacity of roots and shoots. *Plants Physiology* 112: 1649–1657.
- Hassan IA, and Tewfik I. 2006. ${\rm CO_2}$ photoassimilation, chlorophyll fluorescence, lipid peroxidation and yield in cotton (Gossypium hirsutum L. cv Giza 65) in response to ${\rm O_3}$. World Review of Science, Technology and Sustainable Development 3(1): 70–78.
- HETHERINGTON AM, and WOODWARD FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424: 901–908.
- IPCC 2007. Intergovernmental Panel on Climate Change Fourth Assessment Report Climate Change 2007: Synthesis Report, University Press, Cambridge, Great Britain.
- Kleier C, Farnsworth B, and Winner W. 1998. Biomass, reproductive output, and physiological responses of rapid-cycling Brassica (*Brassica rapa*) to ozone and modified root temperature. *New Phytologist* 139: 657–664.
- KLEY D, GEISS H, and MOHNEN VA. 1994. Tropospheric ozone at elevated sites and precursor emission in the United States and Europe. *Atmospheric Environment* 28(1): 149–158.
- Klumpp A, Ansel W, Klumpp G, Vergne P, Sifakis N, Sanz MJ, Rasmussen S, Ro-Poulsen H, Ribas A, Penuelas J, Kambezidis H, Heg S, Garrec JP, 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.
- Long SP, and Naidu SL. 2004. Effects of oxidants at the biochemical, cell and physiological level, with particular reference to ozone. In: Bell JNB, Treshow M [eds.], *Air Pollution and Plant Life*, 69–89. John Wiley and Sons, Chichester.
- Morgan PB, Ainsworth EA, and Long S. 2003. How does elevated ozone impact soybean? A meat-analysis of photosynthesis, growth and yield. *Plant, Cell and Environment* 26: 1317–1328.
- Oksanen E. 2003. Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed to six growing seasons and potted

- seedlings exposed for one season. Tree Physiology 23:603-614.
- PÄÄKKÖNEN E, METSÄRINNE S, HOLOPAINEN T, and KÄRENLAPMI L. 1995. The ozone sensitivity of birch (*Betula pendula* Torh.) in relation to the developmental stage of leaves. *New Phytologist* 132: 145–154.
- PN-EN 14625: 2005. Reference method of analysis the level of substances. Atmospheric air quality. Standard method measurement of ozone concentration with using UV photometry.
- RAI R, AGRAWAL M, and AGRAWAL SB. 2007. Assessment of yield losses in tropical wheat using open top chambers. Atmospheric Environment 41: 9543–9554.
- RIIKONEN J, LINDSBERG M-M, HOLOPAINEN T, OKSANEN E, LAPPI J, PELTONEN P, and VAAVOURI E. 2004. Silver birch and climate change: variable growth and carbon allocation responses to elevated concentration of carbon dioxide and ozone. *Tree Physiology* 28: 243–254.
- RIIKONEN J, PERCY KE, KIVIMÄENPÄÄ M, KUBISKE ME, NELSON ND, VAPAAVOURI E, and KARNOSKY DF. 2010. Leaf size and surface characteristics of Betula papyrifera exposed to elevated ${\rm CO}_2$ and ${\rm O}_3$. Environmental Pollution 158: 1029–1035.
- SAITANIS CJ, and KARANDINOS MG. 2002. Effects of ozone on tobacco (*Nicotiana tabacum* L.) varieties. *Journal of Agronomy and Crop Science* 188: 51–58.
- Shi G, Yang L, Wang Y, Kobayashi K, Zhu J, Tang H, Pan S, Chen T, Liu G, and Wang Y. 2009. Impact of elevated ozone concentration on yield of four Chinese rice cultivars under fully open-air field conditions. *Agriculture, Ecosystems and Environment* 131: 178–184.
- Vandermeiren K, Black C, Pleijel H, and De Temmerman L. 2005. Impact of rising tropospheric ozone on potato: effects on photosynthesis, growth, productivity and yield quality. *Plant, Cell and Environment* 28: 982–996.
- VAN DINGENEN R, DENTENER FJ, FRANK R, MAURTEN CK, EMBERSON L, and COFALA J. 2009. The global impact of ozone on agricultural crop yield under current and future air quality legislation. Atmospheric Environment 43: 604–618.
- Welfare K, Yeo AR, and Flowers TJ. 2002. Effects of salinity and ozone, individually and in combination, on the growth and ion contents of two chickpea (*Cicer arietinum* L.) varieties. *Environmental Pollution* 120: 397–403.
- ZOUZOULAS D, KOUTROUBAS SD, VASSILIOU G, and VARDAVAKIS E. 2009. Effects of ozone fumigation on cotton (*Gossypium hirsutum* L.) morphology, anatomy, physiology, yield and qualitative characteristics of fiber. *Environmental and Experimental Botany* 67: 293–303.