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Oxidative stress enzymes in tobacco during a long-term exposure to ambient ozone at two different sites

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Abstract: Tropospheric ozone is a harmful air pollutant which may cause oxidative stress in plant cells, leading to biochemical and physiological changes and yield reduction. The aim of the study was to examine the cumulative effect of long-term ozone stress on the activity of antioxidative enzymes in leaves of ozone sensitive and resistant tobacco cultivar growing at the sites of various ozone concentrations. The one-month experiment was conducted in the 2009, 2010 and 2011 growing seasons with different meteorological conditions and ozone concentrations. The activity of SOD, APX and GuPX was measured four times at weekly intervals. The highest tropospheric ozone concentration was recorded in 2010 along with high level of solar radiation and temperature. The enhanced ozone level caused the increase in the activity of all examined enzymes. However, at more elevated ozone level the activity of all enzymes was higher in sensitive (Bel W3) than resistant (Bel B) tobacco cultivar. On the other hand, at lower ozone level the activity of examined enzymes was rather similar in both cultivars or even higher in ozone-resistant one, which was especially valid for SOD. A positive correlation between the activity of all enzymes and ozone concentration was shown in both cultivars. The presented results show that meteorological conditions modify tropospheric ozone concentration and real plant response to this environmental pollutant. It is highly important to interpret week-by-week plant response and environmental conditions bearing in mind the cumulative ozone effect resulting from previous weeks conditions.

Introduction

Ozone is a naturally occurring chemical compound playing a double role in the atmosphere: a positive one in the stratosphere and a negative one in the troposphere. The bulk of ozone molecules (about 90%) is found in the stratospheric ozone layer where they act as a screen to protect all living organisms from deleterious effects of solar UV radiation. The rest of the atmospheric ozone (ca. 10%) is found in the troposphere (Revell et al. 2015). There are several sources of tropospheric ozone: (1) influx from stratosphere, (2) long-range transport of ozone within troposphere from distinct pollutant sources, (3) sunlight-driven photochemical reactions of nitrogen oxides (NO_x) with organic volatile compounds (VOCs) and carbon monoxides (CO). The latter is responsible for approximately 90% of tropospheric ozone production (Crutzen et al. 2002, Vingarzan 2004, Zeng et al. 2008, Revell et al. 2015). The above mentioned ozone precursors may be produced naturally in soil, during vegetation and forest fire or may be released as a result of human activities (Vingarzan 2004). The global tropospheric ozone level is expected to rise in the 21st century in many countries, mainly due to high level of its precursors emitted from automobiles and many industrial sources, like fossil fuel combustion or refineries (Crutzen et al. 2002). The only way to reduce ozone creation is to decrease the emission of ozone precursors, as it is already happening in some developed countries (Monks et al. 2015). Moreover, high level of ozone in troposphere may be caused by the rise of CO_2 concentration leading to the enhancement of ozone exchange between the layers of stratosphere and troposphere. In addition, elevated CO_2 concentration affects the temperature increase which favours ozone production from its precursors (Zeng et al. 2008). The level of tropospheric ozone existing in different areas depends on the weather conditions, season or geographical region (Vingarzan 2004). In the Northern Hemisphere the daily mean of annual background ozone concentration is at the level of 25–40 ppb (Reid et al. 2008). The increased concentration of tropospheric ozone above the natural ground value is considered as air pollution. In urban areas increased ozone level usually occurs during warm and sunny spring, and summer days and reaches its peak in mid- to late afternoon (Vingarzan 2004, Reid et al. 2008).

Ozone, as a very reactive air pollutant, may cause biochemical and physiological plant disturbances which can affect plant morphology, as well as biomass and yield decrease, and in consequence economic losses (Biswas et al. 2008). Ozone enters the plant tissue through the open stomata and apoplast of leaf mesophyll cells (Castanga and Ranieri 2008). After getting

into apoplast, ozone is dissolved in apoplastic fluid and rapidly converted into reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\cdot-}$) and hydroxyl radicals (OH^-) which may react with cell wall components and cell membranes (Castanga and Ranieri 2008, Baier et al. 2005). Apart from direct ROS formation from ozone, this air pollutant may cause oxidative stress in plant cells as a result of the imbalance between production and scavenging of ROS (Iriti and Faoro 2008). These highly reactive oxidizing agents are able to absorb electrons from essential cell organic molecules, leading to their oxidation (Kley et al. 1999). As a result of oxidative stress, damages of DNA and proteins, as well as lipid peroxidation, can be observed. The damages of cell membrane lipids and proteins lead to changes in membrane selectivity, disruption of cellular functions and uncontrolled cell death, resulting in faster leaf senescence (Heath 2009).

Plants have evolved efficient scavenging mechanisms which remove ROS from cellular components (Gill and Tuteja 2010). There are two types of antioxidant compounds: low molecular non-enzymatic and enzymatic antioxidants. The first group includes glutathione, tocopherol, carotenoids, ascorbic acid and flavonoids. The enzymatic antioxidants include, among others, superoxide dismutase (SOD, EC 1.15.1.1) and peroxidases. SOD represents a class of antioxidant enzymes which prevent the generation and accumulation of $O^{-}_{2}\cdot$ by providing its conversion to O_2 and less reactive H_2O_2 lowering the risk of their conversion to highly destructive hydroxyl radicals (Gill and Tuteja 2010, Kumari et al. 2015). SOD is localized in many parts of plant cells and is recognized as a key enzyme regulating plant responses to ozone and other stresses causing the excess in ROS accumulation, and its activation often leads to stress tolerance (Kumari et al. 2015). Guaiacol peroxidase (GuPX) belongs to a large group of peroxidases (EC 1.11.1.7) which oxidize several substrates in the presence of hydrogen peroxide (Hiraga et al. 2001). It occurs in the cell wall and cytosol and is characterized by broad specificity with respect to substrate, and both guaiacol and pyrogallol have been used as electron donors in the determination of their activity (Mika and Luthje 2003). Ascorbate peroxidase (APX, EC 1.11.1.11) is characterized by a high degree of specificity for ascorbate as electron donor and its activation is recognized as one of the most efficient ROS scavenging responses, playing a fundamental role in the antioxidant system because of its presence in at least four cell compartments (stroma, thylakoid membrane, microbody, cytosol) and its high affinity to H_2O_2 (Castanga and Ranieri 2008, Gill and Tuteja 2010). Activation of APX in ozone-stressed plants is considered important by many authors in reducing O_3-derived toxic H_2O_2 concentrations (Ranieri et al. 2003). The results of many experiments have shown that enhanced activity of antioxidant enzymes may protect plants against oxidative stress and leaf damage under the conditions of increased ozone levels in ambient air (Iriti and Faoro 2008, Scebba et al. 2003).

There are numerous studies focused on plants response to ozone, but most of them concern the impact of short-term or severe ozone stress usually imposed under controlled conditions (Borowiak et al. 2010, Ueda et al. 2013, Wohlgemut et al. 2002). Little is known about the response of plants to ambient ozone concentration under natural conditions during the growing season (April-September in the Northern Hemisphere), especially concerning the changes in the activity of the antioxidant system. Plant response to ozone depends on its concentration in ambient air and time of exposition (ozone dose). The effect of ozone cumulates in plants exposed to this air pollutant continuously during the growing season (Budka et al. 2014).

The critical value of ozone concentration for plants and ecosystems has been evaluated at the level of 40 ppb (Official Journal UE 2008). European Union law has been adjusted to the tropospheric ozone parameter AOT40 (the accumulated exposure over the threshold of 40 ppb) which informs whether ozone pollution can be hazardous for plants. Tobacco (*Nicotiana tabacum* L.), especially Bel W3 cultivar, is considered as sensitive to ozone and has been recognized as a useful bioindicator of this pollutant (Verge et al. 2002).

The aim of the study was to examine the cumulative effect of long-term ozone stress on the activity of antioxidative enzymes in leaves of ozone sensitive (Bel W3) and resistant (Bel B) tobacco cultivar growing at the sites of various ozone concentrations, i.e. the forest and the city. The relations between solar radiation, temperature and ozone concentration during the three four-week summer experiment periods and their effect on the activity of above mentioned enzymes have been evaluated.

Materials and methods

Experimental design

The experiments were carried out in the July of 2009, 2010 and 2011. At the beginning of May seeds of tobacco cultivars (Bel B, Bel W3) were sown into pots of 1.5 dm^3 filled with a standard mixture of peat and sand with slowly released fertilizer and were cultivated in greenhouse conditions. After eight weeks (at the end of June) twenty plants of each cultivar were transported to two exposure sites and left there for 4 weeks. One site was located in a forestry area, about 80 km north-east of Poznan (called here the "forest site"), and the second one was located in the Botanical Garden of Poznan (called here the "city site"). In each location plants were placed in special aluminium racks 90 cm above the ground level. The top part of the rack was covered with shadow fabric for protection against excessive insolation. A continuous water supply was guaranteed by glass fibre wicks placed in pots and in trays with water located under the styrofoam pallets with pots. The sites differed in tropospheric ozone concentrations and meteorological conditions. The air monitoring system operated by the Provincial Environmental Agency was located at both sites to measure NO_x, and ozone concentration, as well as meteorological parameters. The solar radiation ($W \cdot m^{-2}$) and air temperature were taken into consideration. For the above mentioned purposes the hour mean values of air pollutants and meteorological parameters were undertaken and on this basis average values, as well as the sum of selected parameters for one--week periods were calculated. A similar set of plants remained during the entire experimental period in a greenhouse (ozone-free conditions) and was considered as a control combination.

Sample collection

Three randomly chosen plants of each cultivar from both exposure sites and control combination were transported to the laboratory at the end of 1, 2, 3 and 4-week period of the experiment. Samples of fully matured leaves (sixth or seventh leaf, counted from the bottom of the plant) were used for the estimation of antioxidant enzymes activity. The activity of APX and GuPX was determined

immediately after the collection of samples. Plant material for the estimation of SOD activity was frozen in liquid nitrogen and stored at -20°C until analysis. The analyses were carried out using three independent biological replicates. Each replicate was a sample of plant material derived from a different plant.

Enzyme extraction and assay

The samples (500 mg) were homogenized in a chilled mortar with 4 cm^3 0.1 M potassium phosphate buffer (pH 7.0) with 20 mg of Polyclar AT added and centrifuged at 16 000 × *g* for 30 min at 4°C. The supernatant was used for the determination of APX and GuPX activities and protein content.

The activity of APX was determined according to Nakano and Asada (1987). The reaction mixture contained 2.3 cm^3 of 0.1 M potassium phosphate buffer (pH 7.0), 0.2 cm^3 of 5 mM L-ascorbate (ASC), 0.3 cm^3 of 1 mM H_2O_2 and 0.2 cm^3 of enzyme extract. The hydrogen peroxide-dependent oxidation of ASC was followed by a decrease in absorbance at 290 nm (absorption coefficient 2.8 $mM^{-1} \cdot cm^{-1}$). APX activity was expressed in nkat · mg^{-1} protein.

The activity of GuPX was estimated according to Hammerschmidt et al. (1982). The reaction mixture contained 0.5 cm^3 of enzyme extract, 0.5 cm^3 of 3.4 mM guaiacol and 0.5 cm^3 of 0.9 mM H_2O_2. The oxidation of guaiacol to tetraguaiacol in the presence of H_2O_2 was measured as an increase in absorbance recorded at 470 nm. The enzyme activity was calculated using the absorption coefficient for tetraguaiacol (absorption coefficient 2.66 $mM^{-1} \cdot cm^{-1}$) and it was expressed in nkat · mg^{-1} protein.

SOD activity was assayed by the method of Beauchamp and Fridovich (1971). Plant samples (500 mg) were homogenized in a chilled mortar with 4 cm^3 of buffer (50 mM sodium phosphate buffer pH 7.0 containing 1% polyvinylpolypyrrolidone, 1 mM EDTA-Na and 0.5 M NaCl) and centrifuged at 16 000 × *g* for 25 min at 4°C. The supernatant was used for the estimation of enzyme activity and protein concentration. The incubation mixture contained 2.35 cm^3 of 50 mM sodium phosphate buffer (pH 7.8) with addition of 0.1 mM EDTA-Na, 0.4 cm^3 of 97 mM methionine, 0.1 cm^3 of 2 mM NBT (nitro blue tetrazolium), and 0.05 cm^3 of enzyme extract. Finally, 0.1 cm^3 of 120 µM riboflavin was added and the samples were placed under fluorescent lamps for 10 min. At the same time, a blank without the enzyme extract was prepared. Absorbance was measured at 560 nm, and the unit of activity was taken as the quantity of enzyme reducing absorbance to 50% of the blank.

Protein concentration in supernatants was determined according to the method applied by Bradford (1976), with bovine serum albumin as a standard.

Statistical analysis

Repeated measures analysis of variance was performed separately for each year in order to compare certain variables: APX, GuPX and SOD activities in leaves of examined tobacco cultivars. Tukey's test was employed to analyse differences between measured parameters. A graphical presentation of Tukey's test results is provided in the present study. To determine the structure and relations between variables (APX, GuPX, SOD, mean ozone concentration, AOT 40, solar radiation, temperature, NO_x) principal component analysis (PCA) was used. In this analysis the orthogonal transformation of observed variables to a new set of non-correlated variables (components) is performed. The data were analysed with the statistical software *STATISTICA 13.1* and the *R* computational platform (R Core 2014).

Results

A quite similar level of tropospheric ozone concentration in 2009 and 2011 was noted (Fig. 2). It was however lower than in 2010 when much more favourable meteorological conditions (higher average solar radiation and air temperature) for tropospheric ozone creation during photochemical reactions of its precursor were detected (Fig. 1). Instead of comparable concentration

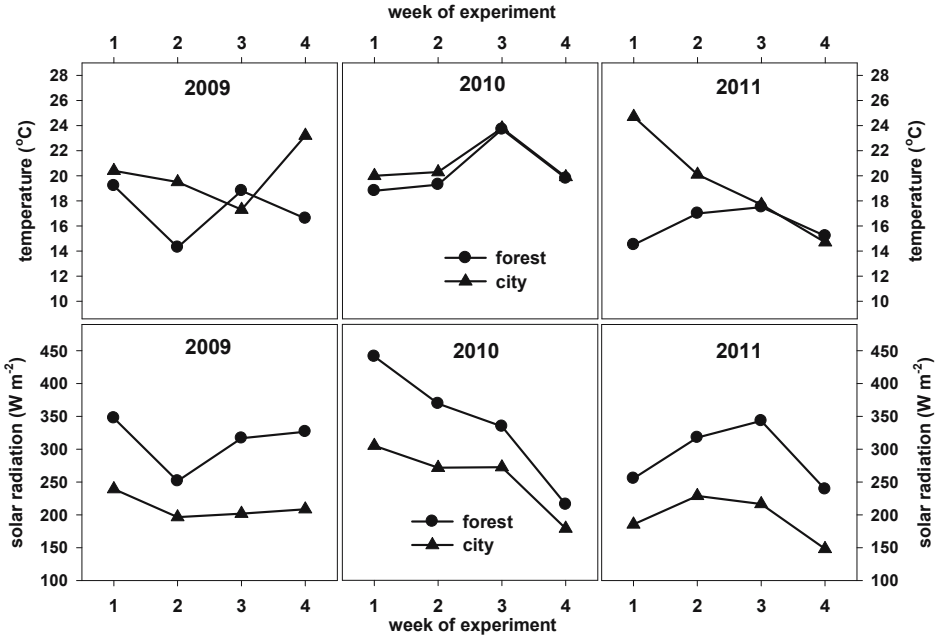

Fig. 1. Mean week values of temperature and solar radiation measured by the Provincial Environmental Agency at two exposure sites (forest and city)

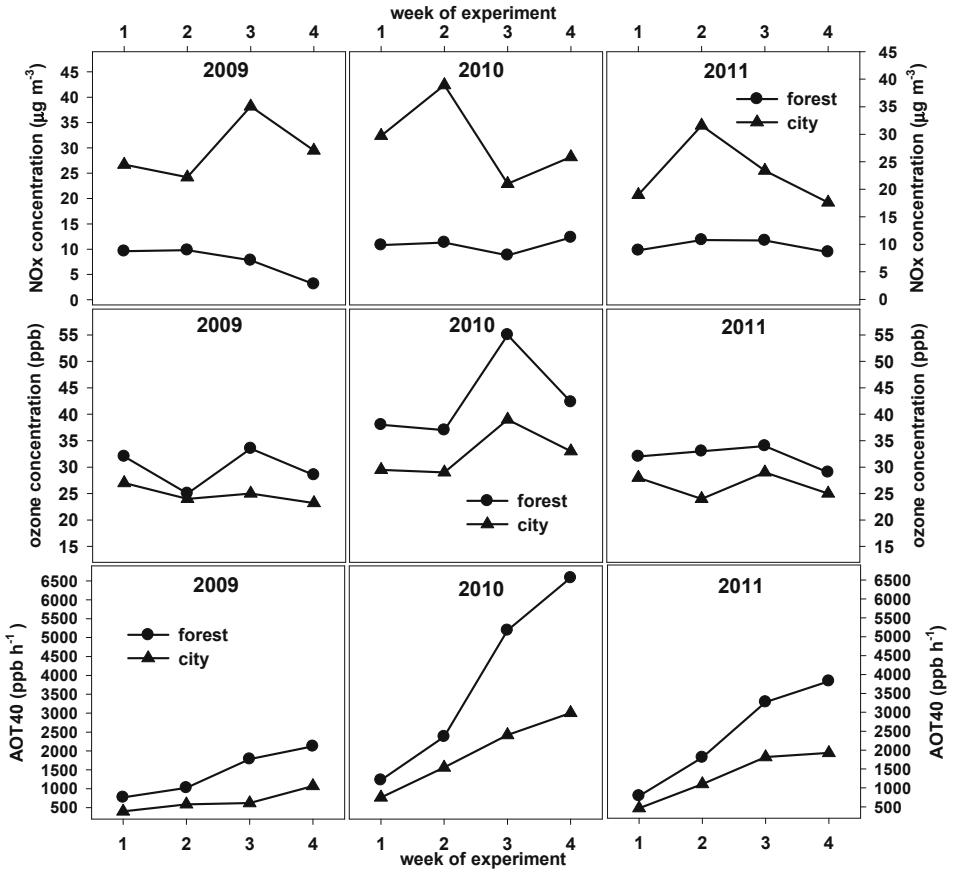

Fig. 2. Mean week value of NO_x and ozone concentration measured by the Provincial Environmental Agency, as well as calculated accumulation of ozone over the threshold of 40 ppb (AOT 40) at two exposure sites (forest and city)

of NO_x during the first weeks of the experiment (Fig. 2), the higher solar radiation in 2010 than in 2009 and 2011 resulted in the formation of greater ozone amounts in 2010. Moreover, in all years higher ozone concentration was observed in the forest site with higher intensity of solar radiation but lower concentration of ozone precursor (NO_x) than in the city site. As a result, summarized ozone concentration (AOT40) in all vegetation seasons was twice as high at most time points at the forest as at the city site. What is more, at both exposition sites it increased 3 to 5-fold at the end of experiments (Fig. 2).

Repeated measures analysis of variance revealed the significant effect ($\alpha \leq 0.05$) of exposure site and duration of plant exposition to the activity of antioxidative enzymes. Indeed, higher activity of examined enzymes in most dates was shown in the plants growing at exposition sites (forest, city) than in a greenhouse. Furthermore, the different pattern of the changes in the activity of examined enzymes was observed throughout the four weeks of the experiment and at both exposition sites. The differences in the course of these changes were noted between 2009 and 2011 where the ozone level was rather similar but lower than in 2010 (Fig. 2). A substantially highest activity of investigated enzymes at both the city and the forest site in all dates of the experiment was observed in 2010 but the lowest was in 2011 (Figs 3–5).

In 2009 a significant increase in the activity of APX was revealed only at the city site (Fig. 3). In ozone-resistant 'Bel B' the activity of the enzyme increased throughout the whole exposure period and only after 21 days of exposition in ozone-sensitive 'Bel W3'. However, in 2011 the activity of APX in 'Bel W3' increased at the forest site progressively from the fourteenth day of the experimental period. At the city site it increased after 14 days of exposure, did not change after 21 days but decreased to the level only slightly higher than in plants grown in a greenhouse. On the other hand, the activity of APX in leaves of 'Bel B' increased at the city site after 14 days and at the forest site after 21 days of exposition. While on the last date it decreased at the city site, reaching the level of control plants, at the forest site it remained at a level significantly higher than in the control combination. In 2010 when the highest tropospheric ozone concentrations were noted, the APX activity in both tobacco cultivars increased significantly in leaves of plants grown at both exposure sites just after one week of exposition. Moreover, higher activity of APX was observed in the ozone-sensitive tobacco 'Bel W3' cultivar (Fig. 3).

The activity of GuPX increased in the examined tobacco cultivars during all four-week experiment periods but not at all exposure sites (Fig. 4). Only in 2010 GuPX activity increased significantly in both cultivars at both exposure sites. This increase was found after three weeks of exposure and was higher in ozone-sensitive 'Bel W3'. In 2009 a significant increase of GuPX activity was observed in both cultivars but only at the city site. In contrast, in 2011 the activity of GuPX increased in both cultivars at the forest site but to a lesser extent in 'Bel B' than in 'Bel W3'. However, at the city site it increased during the first three weeks of the experiment but only in 'Bel W3'.

SOD activity increased in both tobacco cultivars at both exposure sites in 2010 and 2011, while in 2009 the increase was revealed only in resistant 'Bel B' (Fig. 5). Moreover, it is noteworthy that in this resistant 'Bel B' a greater and earlier increase in SOD activity was shown in all three years in the city, in comparison to the forest site. At the forest site the activity of this enzyme increased more or less evenly, reaching the highest level after four weeks of exposure. Both in 2010 and 2011 the activity of SOD in the ozone-sensitive 'Bel W3' cultivar increased at the city and forest site, reaching the highest level at the end of the experiment. However, in 2009 the activity of SOD in this cultivar throughout the four-week experiment period was lower in plants growing at the city and forest site compared to the plants grown in the greenhouse.

Graphical data presentation obtained as a result of the principal component analysis (PCA) explained a significant part of data variability – over 70% of variability in both tobacco cultivars. Data from every week in all four-week experiment periods were taken into consideration. A separate analysis for each tobacco cultivar was done. The analysis was performed to show relations between the activity of examined enzymes, mean ozone concentrations and AOT40 value, as well as temperature and solar radiation. PCA revealed a very close positive correlation between the activity of all examined enzymes and AOT 40, and temperature but the lack of correlation between the enzymes activity and solar radiation. Similar relations were recorded for both tobacco cultivars. A close positive correlation was also noted between AOT 40 and temperature and slightly weaker correlation between solar radiation and mean ozone concentration (Fig. 6).

Discussion and conclusions

The results of numerous studies have shown that antioxidative enzymes play an important role in plant response to increased concentrations of ozone but the changes in their activity depend on the development stage and/or rhythms of intrinsic metabolic processes (Baier et al. 2005, Iriti and Faoro 2008). Moreover, plant response to ozone may be also modified by multiple environmental factors, such as solar radiation, temperature and air humidity, which affect the influx of O_3 into the leaves through stomata (Filella et al. 2005, Karlsson et al. 2004). There is little literature data regarding the changes in antioxidative enzymes activities in response to cumulative ozone which are based on experiments conducted for a long time in ambient air. Most of the experiments are carried out under control air conditions with only one stress factor influencing the plants (Scebba et al. 2003, Ueda et al. 2015). Such approach makes the conclusions confusing because they do not reflect the real plant response to the examined stress factor under natural conditions. Even when the investigations are performed under field conditions they are usually carried out for a short time period and plants responses are examined only at the beginning and/or the end of the exposure (Bandurska et al. 2009, Vyšniauskienė and Rančelienė 2008). The effect of long-term plant exposure to low ozone concentrations is different than the effect of short-term exposure to acute ozone concentrations (Chen et al. 2009). Chronic long term ozone exposure causes the acceleration of leaf senescence, reduction of photosynthesis rate and growth restrictions but on the other hand short term acute ozone effect is rather similar to

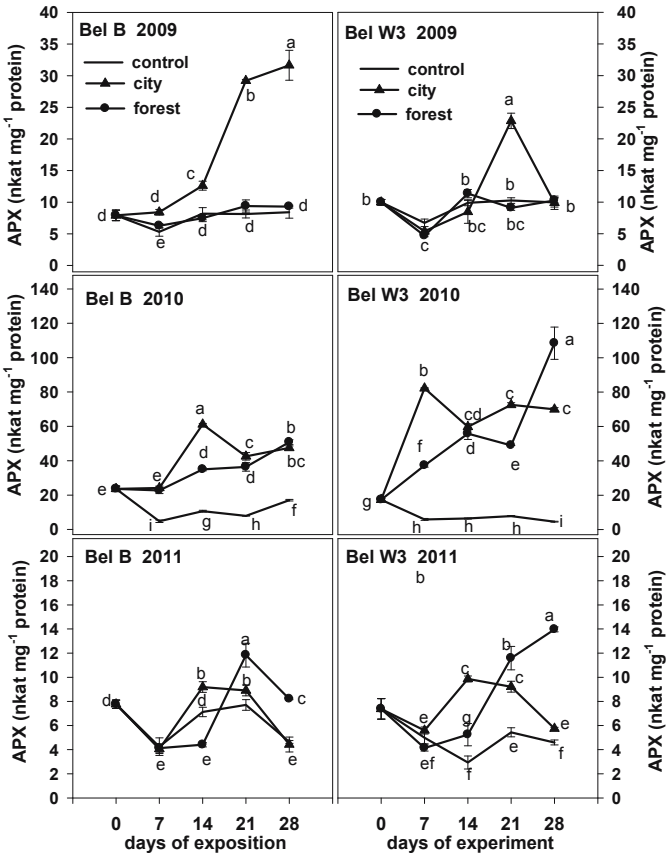

Fig. 3. APX activity (means ±SE) in tobacco 'Bel W3' and 'Bel B' in four observation dates during three growing seasons at two exposure sites (forest, city) and control (greenhouse). Different letters indicate significant differences between means at p = 0.05

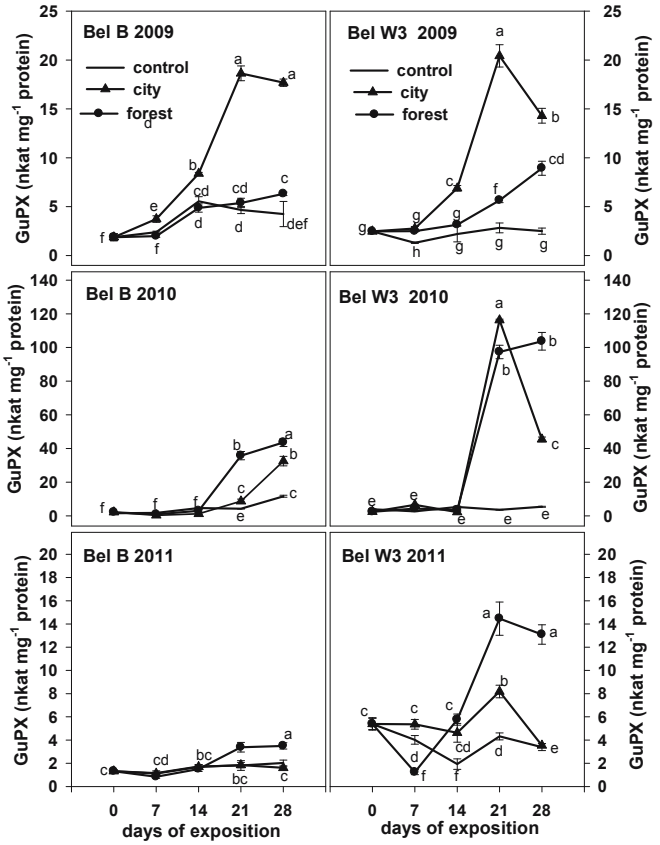

Fig. 4. GuPX activity (means ±SE) in tobacco 'Bel W3' and 'Bel B' in one-week observation period during three growing seasons at two exposure sites (forest, city) and control (greenhouse). Different letters indicate significant differences between means at p = 0.05

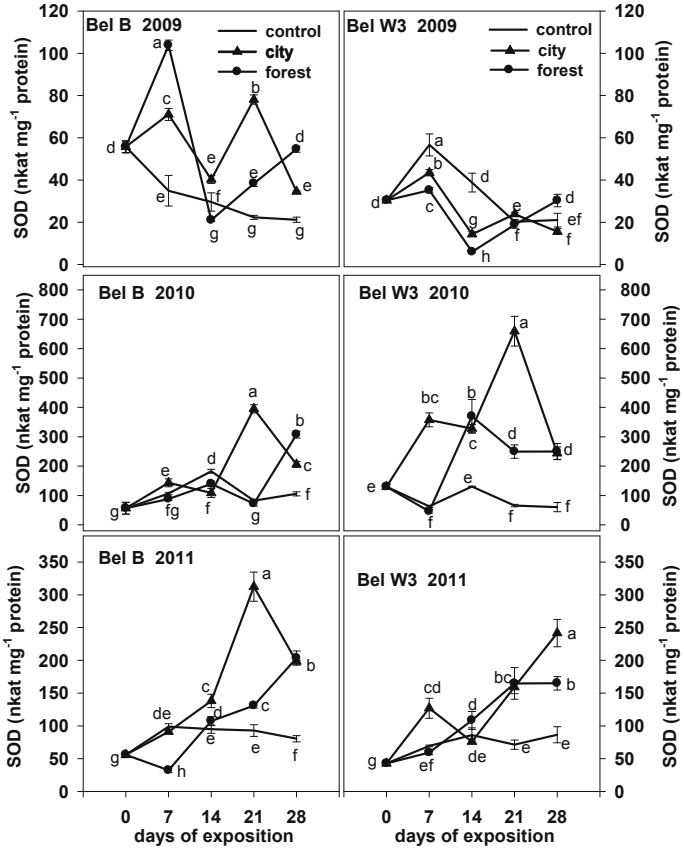

Fig. 5. SOD activity (means ±SE) in tobacco 'Bel W3' and 'Bel B' in one-week observation period during three growing seasons at two exposure sites (forest, city) and control (greenhouse). Different letters indicate significant differences between means at p = 0.05

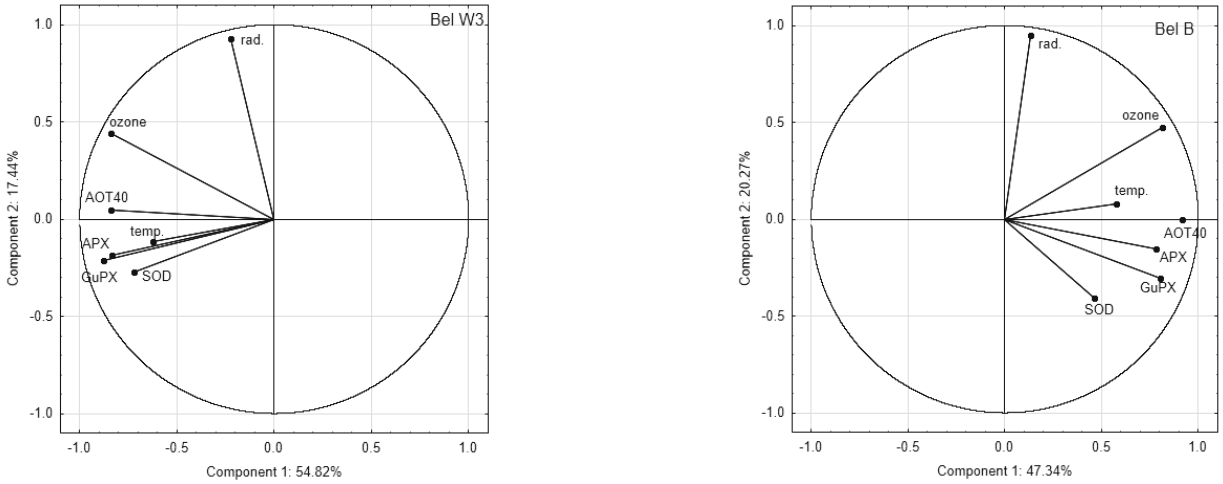

Fig. 6. Principal component analysis of air pollutants, meteorological parameters and enzymes activity in tobacco ozone-sensitive 'Bel W3' and ozone-resistant 'Bel B'

the hypersensitive response (HR) and can be noted as a result of programmed cell death (Castanga and Ranieri 2008).

Our long term and three-year study revealed that ambient ozone concentration was higher at the forest than at the city site and it increased during experimental periods. Whereas, the concentration of ozone precursors (NO_x) was higher at the city site and decreased during experimental periods. Ozone precursors emitted at urban areas may be transported to the regions far from the source of emissions where under favourable meteorological conditions (such as higher solar radiation and temperature) they are involved in ozone creation (Holoway 2003, Otero et al. 2016). It might be assumed that higher ozone concentration at the forest site may be caused by the transport of ozone precursors from the pollution sources occurring in the city site. Higher solar radiation and air temperature (Fig. 1) at the forest area were effective in ozone creation and its higher concentration at this exposition site was observed. Bearing in mind the literature data, we can assume that increased temperature caused higher volatile organic compounds (VOCs) emission from trees in the forest site (Simon et al. 2001) which could be an ozone-precursor leading to higher ozone concentration at this site. It was also revealed that the year 2010 with higher solar radiation and temperature was characterised by higher tropospheric ozone concentrations, while the remaining two years (2009, 2011) had lower levels of ozone. The results presented here confirm that ambient air ozone concentration depends on environmental factors, such as temperature or solar radiation which may modify plant response to this stress under natural conditions as it has been shown by other researchers (Silva et al. 2012). The increase of ozone concentration caused oscillation in the activity of all examined enzymes in leaves of both tobacco cultivars. The pattern of observed changes was depended on the cultivar, exposure sites and the year of study. Esposito et al. (2009) have shown that the activity of SOD and peroxidases (POD) in leaves of tobacco 'Bel W3' exposed to ozone in São Paolo city increased with elevated ozone concentrations and plant response was closely related to meteorological conditions. Our results revealed that the activity of all examined antioxidative enzymes was the highest in 2010 which could be due to the higher ambient air temperature, solar radiation and tropospheric ozone concentration noted during that year. The results presented here also revealed cultivar--related effect of increased ozone concentration on the changes in the activity of examined enzymes. It is worth emphasizing that the increase in the activity of examined enzymes, observed in both cultivars, well correspond with the rise of cumulative ozone concentration (AOT40).

The activity of APX and SOD increased to a greater extent in ozone-sensitive tobacco ('Bel W3') than in resistant 'Bel B', but only in the year 2010 with the highest AOT40. However, in the year 2009 with the lowest AOT40, the increase of APX and SOD activities in ozone-resistant tobacco ('Bel B'), and slight changes in sensitive one were revealed. It is worth to notice that higher increase of both SOD and APX activity in 2009 was mainly observed at city site where the lower ozone concentration and AOT40 were noted. These results may indicate that activation of antioxidative system in leaves of sensitive tobacco cultivar depends on ambient air temperature and ozone concentrations than in resistant one. Previous study has shown that APX activity increased in the ozone-resistant 'Bel B' along with increased ozone levels resulting in limitation of oxidative damage which confirms the protective role of this enzyme against visible symptoms of leaf injury (Bandurska et al. 2009). An important role in the protection of a plant against the development of ozone-caused leaf injuries is also played by SOD (Van Camp et al. 1998). The average level of both APX and SOD activities was higher in O_3 tolerant than sensitive soybean cultivar (Chernikova et al. 2000). Likewise, Scebba et al. (2003) showed lack of damages in ozone-resistant clover cultivar exposed to increased ozone level and characterized by greater increase of APX activity than in sensitive one. Contrary to APX, the activity of SOD increased in ozone-resistant and decreased in the ozone-sensitive clover cultivar. On the other hand, some investigators found a decline of SOD activity in ozone-resistant bean cultivar (Guidi et al. 2010). The present results showed the decline in the activity of this enzyme in ozone-sensitive tobacco cultivar in the year when ozone concentration and AOT40 were the lowest (2009) and increase for the other years (2010, 2011). On the other hand, in ozone-resistant cultivar the activity of SOD increased in each year of the study.

The induction of GuPX activity is a common plant response to environmental stresses and is often associated with sensitivity to certain stress. Highly significant and positive correlation between GuPX activity and ozone concentration as well as leaf damage in ozone-sensitive Bel W3 tobacco cultivar was shown (Bandurska et al. 2009). Greater increase of GuPX activity in ozone-sensitive than tolerant cultivars of soybean and bean exposed to increased ozone level was also revealed (Chernikova et al. 2000). The results presented in this paper show that the changes in the activity of GuPX in the examined tobacco cultivars proceeded differently than the activity of APX and SOD. An increase of GuPX activity in sensitive 'Bel W3' was usually higher in exposure site (forest), dates and years (2010, 2011) characterized by higher level of AOT40. In ozone-resistant 'Bel B' the activity of GuPX also increased as a result of increased ozone concentration but the increase was significantly lower. The above results confirm that the increase of GuPX activity is associated with sensitivity to elevated ozone concentration. However, similar changes in the activity of GuPX in both tobacco cultivars during the year (2009) and at the exposition site (city) with the lowest ozone concentration and AOT40 can indicate that at these circumstances the other factors may have an impact on the activity of GuPX.

Generally, it is possible to conclude that meteorological conditions affect the changes in tropospheric ozone concentration and plant response to this stress, as we noted the relations between solar radiation, air temperature and AOT40 which had an effect on the antioxidative enzymes activity. Long-time exposure to increased tropospheric ozone concentration under natural conditions caused changes in antioxidative enzyme activities. A positive correlation between the activity of all enzymes and ozone concentration was revealed. Under the conditions of high tropospheric ozone level the activity of antioxidative enzyme was higher in ozone-sensitive tobacco cultivar, while at lower ozone level similar changes in both cultivars or higher in ozone-resistant were recorded. Under the conditions of lower ozone concentrations the meteorological conditions could have a greater effect on the activity of examined enzymes. Probably for this reason the response of resistant cultivar was in some cases similar to the sensitive one. The results obtained provide evidence that plant response to elevated ozone concentration under natural conditions is modified by environmental factors and especially by temperature. It is always highly important to interpret the week-by-week plant response and environmental conditions bearing in mind the cumulative effect of ozone and meteorological conditions in previous weeks.

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Kumulatywny wpływ ozonu na aktywność enzymów antyoksydacyjnych u tytoniu rosnącego na dwóch stanowiskach ekspozycyjnych

Streszczenie: Zwiększone stężenie ozon troposferycznego w powietrzu jest szkodliwe dla roślin, gdyż przyczynia się do powstania stresu oksydacyjnego prowadzącego do zmian biochemiczno-fizjologicznych oraz redukcji plonów. Celem pracy było zbadanie wpływu długotrwałego stresu ozonowego na aktywność enzymów antyoksydacyjnych w liściach wrażliwej oraz odpornej na ozon odmiany tytoniu rosnących na stanowiskach ekspozycyjnych różniących się stężeniem tego zanieczyszczenia. Przeprowadzono trzy trwające jeden miesiąc doświadczenia w latach 2009, 2010 i 2011 charakteryzujących się różnymi warunkami meteorologicznymi oraz różnym stężeniem ozonu. Aktywność SOD, APX oraz GuPX oznaczano czterokrotnie (w odstępach tygodniowych) w czasie ekspozycji roślin. Najwyższe stężenie ozonu stwierdzono w roku 2010, kiedy odnotowano również wysoki poziom promieniowania słonecznego oraz temperatury powietrza. Zwiększone stężenie ozonu przyczyniło się do wzrostu aktywności badanych enzymów. Większą aktywność enzymów w warunkach podwyższonego stężenia ozonu stwierdzono u wrażliwej (Bel W3) niż odpornej (Bel B) odmiany tytoniu. Przy niższym stężeniu ozonu, aktywność badanych enzymów była zbliżona u obu odmian, lub nawet wyższa u odmiany odpornej, co było szczególnie widoczne w przypadku SOD. U obu odmian stwierdzono pozytywną korelacje pomiędzy aktywnością badanych enzymów oraz stężeniem ozonu. Uzyskane wyniki wskazują, że warunki atmosferyczne wpływają na stężenie ozonu troposferycznego, co z kolei wpływa na rzeczywistą odpowiedź rośliny na to gazowe zanieczyszczenie powietrza. Niezwykle ważne jest, aby uzyskane dane dotyczące odpowiedzi roślin na zanieczyszczenie powietrza ozonem interpretować z uwzględnieniem czasowych zależności, w szczególności efektu kumulowania się wpływu ozonu w poprzedzającym przedziale czasowym.