THE LONG AND SHORT TERM EFFECT OF OZONE ON THE PHOTOSYNTETIC PIGMENT CONTENT FROM CORN (*ZEA MAYS*)

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The purpose of the present study was to determine the modifications of the content of the photosynthetic green (chlorophyll a and chlorophyll b) and carotenoid pigments as a consequence of long and short exposure to ozone. Firstly (the long term experience) there took place an exposure of the seeds to ozone (0,03 ppm) for 15 minutes (in alternation with 45 minute breaks) and then an exposure of the Zea mays seedlings, for 14 days. Secondly (the short term experience) the control seedlings, aged 14 days, were continuously exposed to ozone (0,03 ppm) for 15, 150 and 195 minutes. After this we determined the photosynthetic pigment content from the apical zone of the leaves. In the case of long term exposure, the content of pigments from the ozone exposed seedlings were 1,736mg/g, in comparison with the control, where the content was 3,41mg/g. In the case of samples exposed to ozone for 15, 150 and 195 minutes, the content of the photosynthetic pigments was 3,25 mg/g, 3,12 mg/g and 2,70 mg/g. As a result of the study, we established a direct correlation between the duration of exposure to ozone and the content in green and carotenoid pigments.

***Key words**: ozone, chlorophyll, Zea mays*

Ozone is one of the major air pollutants causing physiological damage to both animals and plants. Ozone can significantly reduce crop yield [5] and is responsible for more crop losses than any other air pollutant. It is formed through photochemical reactions between nitrogen oxides, carbon monoxide and hydrocarbons, released primarly through the burning of fossil fuels in urban areas [10].

Ozone is a powerful oxidizing agent, wich is able to react directly with lipids and proteins. The primary site of ozone interaction with plant cells is the extracellular matrix where ozone challenges the antioxidant protection of the cells [1]. Ozone increases peroxidase activity in sensitive seedlings.

Long term exposure of rice to 10–20 nl/L ozone resulted in grain yield losses up to 40% [7, 8, 16]. Understanding the physiological responses to ozone and the mechanisms that confer tolerance to ozone should help prevent crop yield reduction. In plants, many physiological processes can be affected by exposure to ozone. Ozone can reduce quantum yield, electron transport of photosystem II,

membrane permeability, stomatal conductance, the function of enzymes for CO_2 fixation and, ultimately, photosynthesis [4, 6]. Initially, the damage may be caused by active oxygen species and hydrogen peroxide released by ozone attack on apoplastic structures [6]. However, plants may ameliorate ozone injury through stomate closure and induction of the Halliwell-Ashada pathway for scavenging active oxygen species and hydrogen peroxide [13, 14].

MATERIAL AND METHOD

Plants and ozone treatment

The corn seeds (*Zea Mays)* were provided by the Seed Center in Oradea, harvested in the fall of 2008 and are of the Danubiu variety. The seeds were disinfected by using Twenn 80, washed over three times with sterile water and set to germinate on Wathmann No.1 filter paper in Petri dishes.

Firstly (the long term experience) there took place an exposure of the seeds to ozone (0,03 ppm, measured at a distance of 25,4 mm from the ozone generator) for 15 minutes (in alternation with 45 minute of break) and then an exposure of the Zea Mays seedlings, for 14 days. The ozone was obtained by using a comercially available ozone generator. The determination of the vegetable pigments was made by spectrophotometer by taking 2 cm of the apical zone of leaves. At the same time, there were germinated seeds without ozone exposure, which represent the control samples. Secondly (the short term experience) the control seedlings, aged 14 days, were continuously exposed to ozone (0,03 ppm) for 15, 150 and 195 minutes, after which the levels of vegetable pigments were determined from the apical zone of the leaves. *Fig.1* shown the flow sheet of ozone treatment of *Zea mays.*

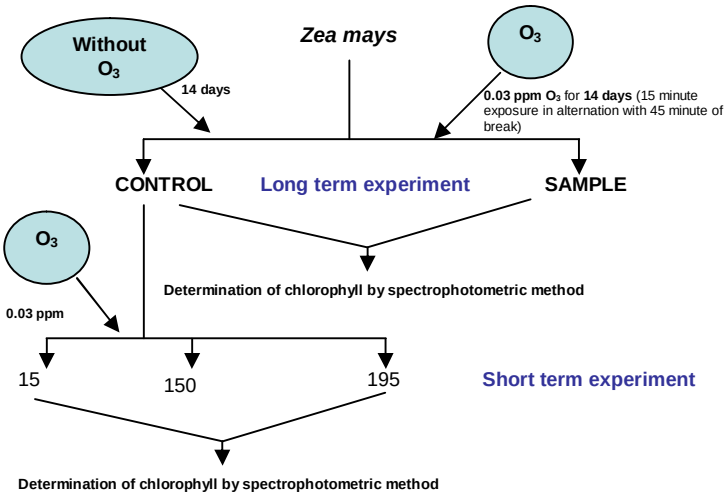


Figure 1 **Flow sheet of ozone treatment of Zea mays**

Spectrofotometric determination of chlorophyll pigments

After 14 days we determined the chlorophyll pigments content of maize seedling leaves, using N,N-dimethylformamide 99.9% for the extraction [11]. For extraction, 50 mg fresh weight of primary leaves were collected separately from each sample, and were blended with 5ml DMF and then cooled at 4˚C for 72 hours. The supernatant was

separated and the content of the pigment was determined using a Shimadzu – UV-mini – 1240 spectrophotometer at 664nm wave length for chlorophyll a, 647 nm for chlorophyll b, and 480 nm for carotenoid pigments. The data obtained after the spectrophotometrycal determination, was mathematically processed using formulae proposed by [12]:

Chlorophyll a (mg/g sp) = (11.65 A_{664} – 2.69 A_{647}) • V/sp

Chlorophyll b (mg/g sp) = (20.81 a_{647} – 4.53 a_{664}) • V/sp

Carotenoid (mg/g sp) = (1000 A_{480} – 0,89 clorofila a – 52,02 clorofila b)/ 245 • v/G•

When the spectrophotometer resolution was 1-4 nm, and where:

A_{647} – the value read with a 647 nm filter

A_{664} – the value read with a 664 nm filter

A_{480} – the value read with a 480 nm filter

V – ml of solvent used

sp – mg of material used for one extraction/sample

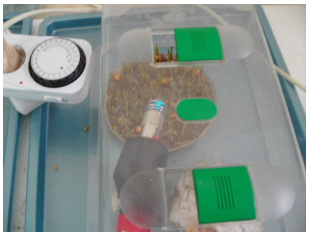
Statistical analysis - the results are averages of 3 determinations and were statistically processed using the "t- test" using *prisma 5 for windows*. The values of the probabilities were determined from tables using the values of the "t" distribution and the freedom degrees based on which the variance of the empiric series was calculated.

RESULTS AND DISCUSSIONS

The corn seedlings, of Danubiu variety, were kept at a temperature of 24°C and at a relative humidity of 80%, under plastic micro-hothouses. The seedlings were watered dayly with 4 ml of distilled water, applied on each Petri dishe's filter paper. The effects of ozone on germination were studied by exposing half of the seedlings to a concentration of 0.03ppm, measured at a distance of 25.4 mm from the ozone generator (*fig. 2 a)*. Ozone exposure was performed intermittently for a period of 15 minutes every hour, after which the cycle was repeated. The number of germinated seedlings was determined and expressed in a percentage, after 47 and respectively 68 hours, both in treated and untreated lots. The obtained results are presented in *fig. 2 b*.

The length of roots and shoots was measured in both lots with digital callipers, after 91, 115, 139 and 163 hours.

a



b

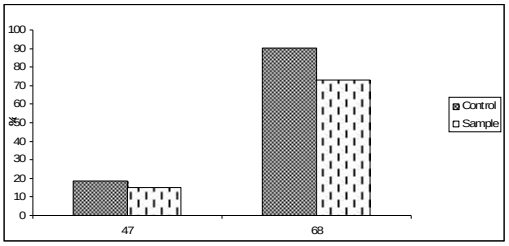


Figure 2 a. **The use of the ozone generator on the corn samples** b. **The procent of germination (%) in the case of control (the maize without exposure with ozone) and samples (the maize with exposure with ozone)**

For the biometrical determination we measured the length of the roots (*fig.3 a*), and shoots (*fig.3 b*) after 91; 115; 139 and 163 hours of germination, and we made 3 repetitions for each determination.

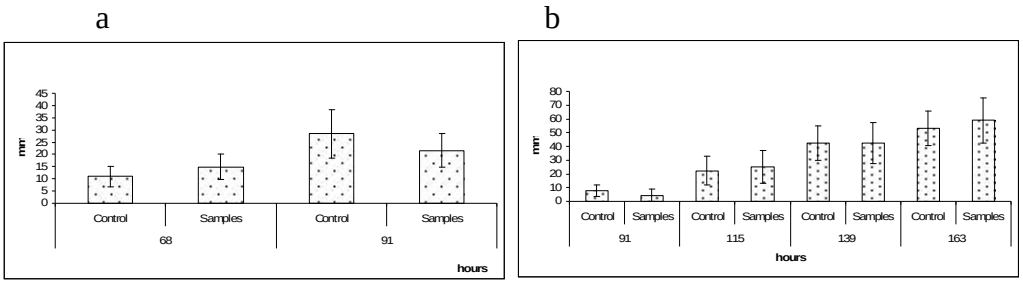


Figure 3 **Biometrical measurement. a. Measuremt of roots (mm); b. Measurement of shoots (mm)**

Data obtained after 98 hours of germination (*tab. 2*) shows that the mean growth of roots in the ozone treated sample is 21.62 mm, 23.79% less than the untreated sample (28.37 mm).

An even larger difference was recorded after this interval regarding shoots growth, in which case the ozone treated sample had a 42.6% decrease in growth (a mean of 4.45 mm as opposed to 7.73 mm in the untreated control lot). An interesting evolution was recorded regarding the shoots growth in the four succesive measurment days. If after 96 hours the mean growth of shoots in untreated control samples was significantly superior to the treated samples, after 115 hours this difference was significantly reduced, as we recorded a slight advance in the treated samples' growth mean (22.33 mm the control sample mean, as opposed to 24.94 mm the treated sample mean). After 139 hours, the 2 means are almost equal, the recorded difference being within the margin of error (42.26 mm as opposed to 42.47 mm). After 163 hours, the mean growth of the control sample surpasses the treated sample by 9.89%. Based on these observations we can state that, up to a point, ozone exposure can have a stimulating effect on corn plant growth. In our experiment, between hours 91 and 139, the differences between samples gradually reduced, the growth means of the two samples becoming almost equal after 139 hours.

In the case of short term treatment there is an observeable decrease of total green pigment quantity (*tab.1*), directly proportional with exposure time, as well as a decrease in the proportion of a/b clorophyll, which indicates senescence or poor plant growth [2, 15]. The lower ratio chlorophyll a and b under O_3 treatment was due to the decrease in chlorophyll a content and increase in chlorophyll b content.

In the case of long term treatment, the total quantity of green pigments in ozone treated samples was 1.736 mg/g, comparatively much less than in samples expozed for a shorter period of time (minutes).

Similar values were recorded regarding carotenoid pigments. The recorded values were 0.423 mg/g in the control sample and 0.392; 0.281 and 0.258 mg/g in corn plant samples exposed to ozon for 15, 150 and 195 minutes respectively.

Tabel 1

The level of green pigments from leaves of *Zea mays* with (control) and without exposure to ozone

Sample	Green pigments (mg/g)				Carotenoid pigments (mg/g)
	Clorophyll a	Clorophyll b	Clorophyll a + b	Clorophyll a / b	
Control	2.18	1.33	3.51	1.64	0.423
Short term experiment					
15'	1.57	1.68	3.25	0.93	0.392
150'	1.48	1.63	3.11	0.91	0.281
195'	1.19	1.51	2.70	0.79	0.258
Long term experiment					
14 days	0.336	1.40	1.736	0.24	0.236

Exposure to ozone determines the inhibition of biomass growth by modifying the stomatal conductance, the inhibition of some enzimes involved in photosynthesis, reducing the assimilation rate of carbon per leaf unit and te reduction of glucid delively to the radicular system.

Clorophyll levels in leaves of *Beta Vulgaris* and *Brassica Napus*, exposed to doses of ozone ranging from 40 μg O_3 m^{-3} and 220 μg O_3 m^{-3} showed a decrease of up to 13% under short-term, high-concentration ozone fumigation [9].

CONCLUSIONS

The conclusions that can be drawn from this study are:

1. After 47 hours, the germination percentage in the treated sample is 20% smaller than the control sample, whereas after 68 hours, the germination percentage is 19.08 % smaller. In both situations (47 and 68 hours) the proportion between control and treated samples is aproximately equal (1.2:1). The data obtained after 98 hours of germination shows that the mean growth of roots in ozone treated samples is 21.62 mm, 23.79% less than the control sample (28.37mm).

2. According to obtained results, there is a clear correlation between the lenghts of exposure to ozone and the quantity of clorophyll in the leaves. In case of ozone exposure of 195 minutes, clorophyll a is most affected, registering a 45.4% decrease compared to the control sample, while clorophyll b registered a 13.5% increase compared to the control sample.

3. The content of carotenoid pigments also decreases along with the increase of exposure time, therefore at an exposure of 195 minutes the decrease was of 39%.

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