# KINETICS OF $CO_2$ EVOLUTION FROM HERBACEOUS AND CONIFEROUS PLANTS UNDER THE EFFECT OF OZONE

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*Results of photoacoustic measurements of dark respiration kinetics of plants under the effect of enhanced ozone concentration are presented. A method for determination and monitoring of the amount of $O_3$ in the surrounding air is described. The experiments have been carried out for herbaceous (pea) and coniferous (cedar) plants. The increase of $CO_2$ evolution from plants under the effect of ozone has been established.*

Beginning in the 50s, interest is not decreased to a problem of plant damage as the atmospheric ozone concentration increases. The increase of $O_3$ concentration in the ground layer in various regions is caused by different reasons. For example, in industrial zones with enhanced concentration of hydrocarbons and nitrogen oxides the $O_3$ concentration is 2–3 times higher than its background value (40–80 μg/m$^3$ for the middle latitudes) and is increasing by ~1–2% per year.[1] In regions surrounded by large forest areas the increase of $O_3$ concentration may be caused by biogenic hydrocarbons (isoprene, terpenes, and so on) evolved by woody plants.[2]

Investigations into the influence of enhanced $O_3$ concentrations demonstrate that plants are damaged. This results in chlorosis and necrosis of leaves.[3–5] Under chronic effects the photosynthesis intensity and respiration decrease and the growth of roots slows down.[1,6,7] A character of observed changes depends on the specific, age, and methabolic features of objects,[4,5,8,9] conditions of their growth, accompanying factors (light, temperature, and so on), and ozone dozes.[6,10,11] Comparative investigations have shown that the maximum sensitivity to the ozone have crops; they are followed by deciduous species and then by coniferous ones.[1,4] However, sensitivity of species of each group varies in wide limits as a function of $O_3$ doze.

The results of investigations into $CO_2$ evolution from pea germinants, coniferous needles, and cedar gemmae in the dark respiration process after the effect of enhanced ozone concentrations are presented in the paper. $CO_2$ evolution from examined plants under stress conditions was measured by the method described in Refs. 12–13.

## EXPERIMENTAL SETUP

Experimental setup (Fig. 1) comprised a photoacoustic spectrometer on the basis of a $CO_2$ laser, an ozone generator, a device for measuring ozone concentration, and a fumigation chamber. The ozone generator produced ozone-enriched air flow. The PR–7 compressor pumped this air with a rate of 2 l/min through the chamber in which the VSB–2 high-frequency mercury lamp was installed. The $O_3$ concentration in the flow could be varied by control of the voltage applied at the lamp.

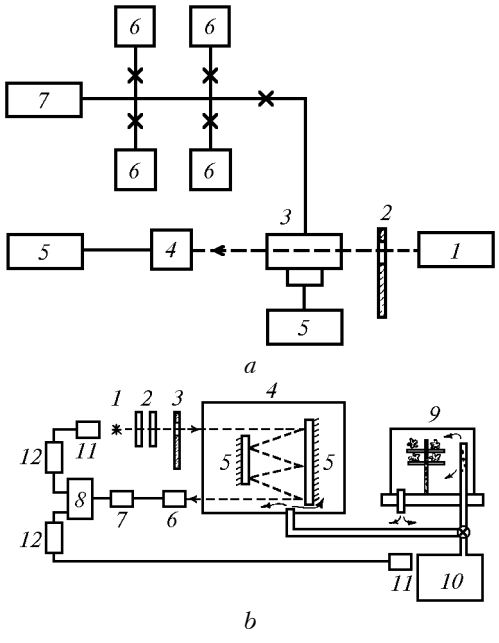


*FIG. 1. Experimental setup (a): $CO_2$ laser (1), modulator (2), photoacoustic detector (3), power meter (4), recording system (preamplifier, selective amplifier, voltage converter, and plotter) (5), exposure chambers (6), vacuum valve (7). Block diagrams of the mercury gas analyzer (MGA) and the fumigation chamber (b): UV lamp (1), light filters (2), modulator (3), cell (4), mirrors (5), photomultiplier (6), microvoltmeter (7), PC (8), fumigation chamber (9), ozone generator (10), photodiode (11), amplifier (12).*

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To measure the $O_3$ concentration in air flow, we used a modified analyzer of mercury vapor developed at the Design and Technology Institute "Optika" (Tomsk).[14] The modified MGA was capable of measuring the attenuation of the mercury lamp radiation with $\lambda = 254$ nm by ozone-air mixture that filled the hermetic MGA cell. Overall dimensions of MGA (70×35×10 cm$^3$) were not large, but a four-mirror six-pass Chernin[15] optical system built in the gas cell increased the beam propagation path length up to $L = 270$ cm.

Elimination of illumination and selection of radiation with $\lambda = 254$ nm for which the $O_3$ absorption coefficient is well known[16] were performed by interference and UFS–5 filters. The radiation was modulated with a frequency of 120 Hz and at the exit from the cell was recorded with a photomultiplier. Data were recorded and processed on a personal computer. To determine the ozone concentration, the amplitudes of signals from the photomultipliers recorded when air was pumped through the cell ($I_0$) and when ozone-air mixture was pumped through the cell ($I$) were compared. The $O_3$ concentration in a flow was determined after stabilization of the transmission $T = I/I_0$ in the cell and was equal to $C = 8$ mg/m$^3$.

Figure 2 shows the variations of the MGA cell transmission as functions of time when the ozone-air mixture (curve *1*) and air (curve *2*) that removed $O_3$ were pumped through it. From Fig. 2 it can be seen that the transmission can be measured reliably with the help of the MGA 5–10 min after the start of air circulation. Moreover, it was established that after termination of ozone-air mixture circulation, the $O_3$ decays sufficiently fast and in ~ 8–10 min the transmission in the cell was doubled.

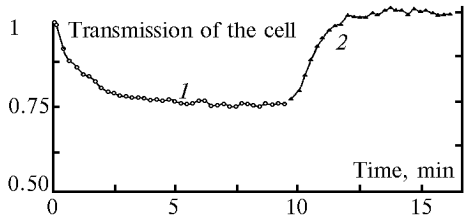


*FIG. 2. MGA cell transmission when the ozone-air mixture produced by the generator (1) and the air (2) were pumped through it. Unity is taken to be the transmission of the cell filled with air. Air circulation began after establishing the maximum absorption by the ozone mixture.*

To study the effect of $O_3$ on plants, a closed fumigation chamber (FC) having a volume of 6.5 l was fabricated through which the air enriched by $O_3$ was pumped. To investigate coniferous needles and cedar gemmae, a rod with small trays attached to it was placed at the center of FC. This attachment can be easily removed and pots with pea germinants occupied its place. The fumigation chamber had teflon pipes for the input and output of the ozone-air mixture. During the experiment, the $O_3$ concentration was measured at the FC input and output.

## EXPERIMENTAL PROCEDURE AND RESULTS

We investigated 9-day pea germinants grown under laboratory conditions[13] as well as coniferous needles of fast (F) and slowly (S) growing morphotypes of Tomsk (T) and Altay (A) cedar reproduction. After exposure to $O_3$, the plants were placed in clear closed exposition chambers with a volume of 0.5 l (for coniferous needles and gemmae) and 1l (for pea) at atmospheric pressure and were held there up to the beginning of measurements (1 or 2 h). The $CO_2$ evolution was measured with a photoacoustic spectrometer (PAS) whose cell was filled with the examined gas from the exposure chambers to a pressure of 8 kPa, at which the PAS has the maximum sensitivity. After probe sampling the pressure deficiency in the chamber was compensated by air to the atmospheric pressure ($P = 101$ kPa).

In the experiment the reference plants were under standard aeration conditions and were placed in the exposure chambers simultaneously with the examined material. In Figs. 3 and 4*a* and *b* the measurements are shown of $CO_2$ concentration evolved from plants as functions of time $t$ during which the plants were held in the closed exposure chamber.

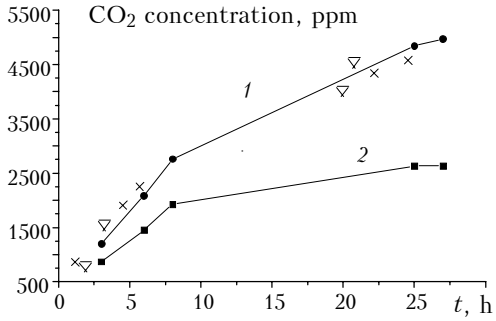


*FIG. 3. Dynamics of $CO_2$ evolution from pea germinants: ozonized (1) and reference plants (2).*

Each experimental cycle included the $CO_2$ concentration measurements in the atmospheric air, for which it is well known[17]: $C_{CO_2} = 345$ ppm. Based on this information and considering that the PAD sensitivity is linear in the range of measurable $CO_2$ concentrations, we presented the dynamics of $CO_2$ evolution in units of ppm (and for comparison of different cycles, in ppm/g). Investigations of the pea germinants demonstrated the activation of dark respiration of ozonized plants (Fig. 3), which is likely caused by the increase of energy required for compensation and reparation processes.[1]

It is interesting to note that experimental data on $CO_2$ evolution from germinants obtained under conditions of $O_3$ fumigation during 3 and 6 hours fell

well on curve *1*, that is, the plants under conditions of 3–6 hours stress evolve the same amount of $CO_2$ as plants after an hour ozonation (~ 1.5 times larger than the reference plants). The absence of the difference between the given results is likely connected with the effect of stoma closing manifested even in case of short-time fumigation by ozone, and the further $O_3$ incoming in the leaf tissue may be limited[1] in spite of high $O_3$ concentration in the surrounding medium.

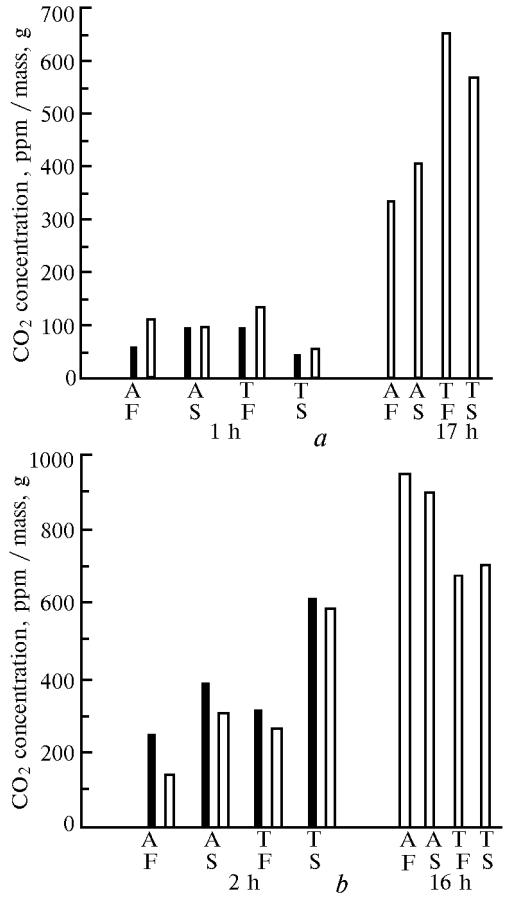


*FIG. 4. Measurements of* $CO_2$ *concentration, ppm (normalized to unit mass) for four specimens of cedar coniferous needles (a) and cedar gemmae (b): control (■); and experiment (□).*

Measurements of cedar coniferous needles also demonstrated that $CO_2$ evolution from all morphotypes was intensified as the time of ozone action increased (Fig. 4*a*), however, relative differences were most pronounced for the fast growing cedar of Tomsk reproduction (TF). The reaction of gemmae on the ozone influence (Fig. 4*b*) was different. The intensity of their respiration under standard aeration conditions was higher than that of coniferous needles and their resistance to the enhanced $O_3$ concentrations was lower. Differences between the intensities of $CO_2$ evolution from gemmae were also manifested for cedar morphotypes of the Altay and Tomsk reproductions. From Fig. 4 it can be seen that short-time influence of $O_3$ (1 – 2 h) inhibits the $CO_2$ evolution from cedar gemmae, which was not observed for the coniferous needles of the same specimens. A comparison between $CO_2$ concentrations for gemmae and coniferous needles for long exposures to the ozone atmosphere (16 – 17 h) demonstrated that AF and AS morphotypes had identical responses for both coniferous needles and gemmae.

Intraspecific differences for plant organs under conditions of ozone stress were observed by Moldau[1] and Tuomainen et al.[5] who noted that degradation processes in growing tissue under the effect of $O_3$ started earlier and manifested stronger.

Thus, our results demonstrate that the intensity of the respiratory exchange is the sensitive indicator of the effect of the enhanced ozone concentrations. Entering the plant tissue, $O_3$ reacts with the water phase producing active oxygen forms: free radicals, hydroxyl ions, and hydrogen peroxide, which affects directly or indirectly the functional activity of a cell. Enrichment of tissue by oxygen produced in the process of $O_3$ decomposition changes the carbon balance. That primarily affects the aerobic phase of respiration. To elucidate mechanisms of ozone influence on the plants, further investigations are necessary not only under laboratory, but also under field conditions. Accumulated database can be used for modeling of atmospheric-biospheric interactions.

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