

## KINETICS OF THE CO<sub>2</sub> EMISSION BY VEGETATION UNDER STRESS CONDITIONS

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Received January 13, 1997*

*The response of vegetation to stress is characterized by the activation of respiration process and by an increase of the carbon dioxide emission.*

*The results of a series of measurements of kinetics of the carbon dioxide emission by herbaceous plants and leaves of various trees are discussed for cases of the impact from ethylene and carbon dioxide of high concentration as well as for low air pressure influences (so-called hypobaria). The measurements of the CO<sub>2</sub> emission kinetics have been performed using a tunable cw CO<sub>2</sub> laser-based photoacoustic spectrometer. These results, supplemented with biochemistry data, allow one to detect the structure and functional changes in the plant studied under stress environmental conditions.*

The respiration process activation accompanied by an increase in the CO<sub>2</sub> emission by plants under the extreme conditions, characterizes a response of plants to the environmental stress.<sup>1</sup> Analysis of kinetics of the plant gas cycle allows the investigation of the ontogenesis process as well as the estimation of a concrete plant resistance to the environmental influences: e.g., drought, frosts, soil salinity,<sup>2</sup> pollution of the atmosphere by industrial gases and aerosols.<sup>3,4</sup>

Usually the gas-resistance of plants inversely depends on a gas exchange intensity and the rate of the gas impurities absorption, as well as directly depends on the toxicant concentrations on leaf surfaces.<sup>5</sup>

The plant gas exchange kinetics characterizes the stress of both natural and anthropogenic origin that depend on a plant species. Among various types of natural and anthropogenic stress, let us consider effects of high concentration of toxic gases and low atmospheric pressure (hypobaria) on the CO<sub>2</sub> kinetics during the period of dark respiration of plants. The increase of the CO<sub>2</sub> emission may influence the greenhouse effect.

As a rule, IR gas analyzers with broad-band thermal sources of radiation designed for detecting a particular gas<sup>2</sup> are used in the investigations of the plant gas exchange. Generally, such gas analyzers are designed for industrial purposes and do not allow simultaneous measurements of other atmospheric constituents emitted during the gas exchange processes, e.g. ethylene (C<sub>2</sub>H<sub>4</sub>) taking part in the hormone balance, or ammonia (NH<sub>3</sub>), characterizing the protein metabolism. That is why, the monitoring of the above mentioned gases as well as of other

volatile matters (metabolites) is performed by biochemistry testing methods.<sup>7</sup>

In recent years, the papers devoted to applications of the laser photoacoustic methods to investigations of the photosynthesis (Ref. 8) and detection of ethylene emitted by plants (Refs. 4 and 9) have been published in foreign literature.

The methods and technologies of laser photoacoustic spectroscopy that are being developed at the Institute of Atmospheric Optics SB RAS during already 25 years, enabled highly sensitive investigations of the plants gas exchange kinetics including simultaneous measurements of concentrations of CO<sub>2</sub>, C<sub>2</sub>H<sub>4</sub>, NH<sub>3</sub>, H<sub>2</sub>O, O<sub>3</sub> in different atmospheric conditions and dosed impacts of contaminating gases and aerosols on plants.<sup>10–12</sup>

This paper describes some results of a series of experiments on kinetics of carbon dioxide emission by herbs and leaves of various trees, in case of enhanced C<sub>2</sub>H<sub>4</sub> and CO concentration hypobaria corresponding to the atmospheric pressure at different altitudes. All measurements have been performed using a frequency tunable CO<sub>2</sub> laser. These results, supplemented with biochemistry data, allow the detection of structure and functional changes in the cells, organs, and tissues of plants under stress conditions.

### *Types of natural and anthropogenic stress*

Climatic (natural) stress:

- 1) overheating and supercooling,
- 2) hypobaria (a low atmospheric pressure in mountains),
- 3) increase of the solar UV radiation flux,
- 4) drought.

Anthropogenic stress:

- 1) contamination gases,

- 2) increase of the atmospheric ozone concentration,
- 3) industrial aerosols and photochemical smog,
- 4) increase of the total concentration of CO<sub>2</sub> and global climate change.

In Ref. 6 one can find a more complete classification of stress.

**EXPERIMENTAL INSTRUMENTATION AND MEASUREMENT TECHNIQUES**

Method of the photoacoustic gas analysis is based on the photoacoustic (PA) effect, when acoustic waves are generated in a substance absorbing laser radiation. When a modulated laser radiation passes through a PA cell with a gas mixture under study, the molecules absorbing the light are excited and then they either emit or relax without emission. The radiationless relaxation results in a gas heating which, in its turn, generates a pressure wave in the closed volume of a PA cell. The pressure pulse is recorded with a sensitive microphone and the microphone electrical signal can be processed using standard instrumentation for measuring pulse and periodic electric signals.<sup>10</sup>

In the general case, the amplitude of a PA signal is described by the expression:

$$U(\lambda_i) = \left( \sum_{j=1}^m k_{ij}x_j + \beta(\lambda_i) \right) \alpha P(\lambda_i), \quad i = 1, \dots, n, \quad (1)$$

where  $\lambda_i$  is the wavelength of laser radiation,  $P(\lambda_i)$  is the radiation intensity at the same wavelength;  $\alpha$  is the sensitivity of a PA gas analyzer,  $k_{ij}$  is the absorption coefficient of the  $j$ th gas at the wavelength  $\lambda_i$ ;  $x_j$  is the  $j$ th gas concentration, and  $\beta(\lambda_i)$  is the value of the background absorption which, as a rule, is determined by the absorption of laser radiation by the windows of a PA cell (Ref. 10).

The value of  $\alpha$  is determined from the gas analyzer calibration. The calibration means measurements of the PA signal amplitude from a reference gas mixture involving absorbing gas molecules with known concentrations and absorption coefficients (see Ref. 15).

The concentrations of the gas mixture components sought are derived, as a solution of the inverse problem, i.e. by calculating the  $x_j$  values in terms of measured values of  $U(\lambda_i)$ . The least squares method is used to resolve this problem, and the vector of the concentrations  $X$  sought is expressed as<sup>13</sup>:

$$X = (K^T W K)^{-1} K^T W U, \quad (2)$$

where  $K$  is the matrix of the absorption coefficients of gases under study,  $W$  is the matrix of weighting factors calculated on data from several measurements. The accuracy of the concentrations restoration

depends on the precision of the components of the matrix  $K$  as well as on the value of the random error of measurements.<sup>13,15</sup> Equation (1) shows that if the absorption gas spectra overlap, each gas of the mixture contributes to the PA signal amplitude. This fact results in certain restrictions on a multicomponent mixture analysis. To increase the analysis accuracy of such mixtures, different techniques like chemical extraction of one or some constituents from the mixture, special mathematical techniques for the PA measurements processing,<sup>14</sup> etc.

The use of a CO<sub>2</sub>-laser based PA gas analyzer is preferable to analyze such components of the plant gas exchange cycle as CO<sub>2</sub>, C<sub>2</sub>H<sub>4</sub>, and NH<sub>3</sub>, since all these molecules have strong vibrational-rotational absorption lines within the CO<sub>2</sub> laser generation band. In addition, the CO<sub>2</sub> lasers generate intense continuous radiation that provides high sensitivity of gas analysis, since according to Eq. (1), the amplitude of a PA signal is directly proportional to the laser radiation intensity.

Table I gives minimum concentrations of the CO<sub>2</sub>, C<sub>2</sub>H<sub>4</sub>, and NH<sub>3</sub> gases which can be detected with a PA gas analyzer (according to Ref. 15). The last column of Table I gives maximum permissible concentrations of these gases for industrial areas.<sup>16</sup>

TABLE I. Values of concentrations of some gases detected with a CO<sub>2</sub> laser-based PA spectrometer.

Gas	C <sub>min</sub> , ppb		C <sub>m.p.c.</sub> , ppb
	N <sub>2</sub> + gas	Air + gas	
CO <sub>2</sub>	7400	—	5·10 <sup>6</sup>
C <sub>2</sub> H <sub>4</sub>	0.3–3	5	4.3·10 <sup>4</sup>
NH <sub>3</sub>	0.4–4	1–3	2.9·10 <sup>4</sup>

The PA gas analyzer based on a discrete frequency tunable continuous CO<sub>2</sub> laser<sup>11</sup> has been used to observe the kinetics of CO<sub>2</sub> emitted by plants. Block-diagram of the gas analyzer is shown in Fig. 1.

This gas analyzer uses a commercial CO<sub>2</sub> laser ILGN-705. For making the wavelength tuning, the laser output mirror was replaced by a combination of a diffraction grating (100 grooves/mm) and a 100% reflecting mirror. The mirror was adjusted so that the first-order reflected radiation should fall back on the diffraction grating and then back to spherical mirror (with 100% reflectivity), tightly welded to the laser gas-discharge tube. Radiation was emitted through the zeroth order of the grating diffraction. The laser wavelength was tuned by tuning the plane mirror.

The resonator design allowed the generation at the 1P(10)-1P(32) and 1R(12)-1R(22) laser lines. A panoramic spectral analyzer with the scale graduated in absolute wavelength values was used for the laser generation lines identification. Amplitude-modulated laser radiation was directed to PA cell through the diaphragm of 3 mm in diameter. The PA cell was 100 mm long and 10 mm in diameter, with BaF<sub>2</sub> windows. The plane

condenser microphone mounted on the vertical wall of the PA cell was used to record the pressure pulsations. The electrical signal from the microphone amplified with a pre-amplifier was transmitted to the input of the recording system consisting of a lock-in amplifier, synchronous detector, and a recorder. The reference signal from the modulator was entered into the

synchronous detector. The non-selective PA detector, designed for measurements of the laser radiation intensity, was mounted behind the PA cell with a gas under study. Both reference and tested groups of plants were placed in the same exposure chambers whose inner volume have been connected to the vacuum system, the settling chamber, and the PA cell.

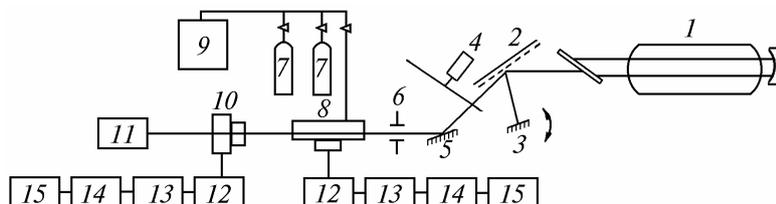


FIG. 1. Block diagram of the setup: gas-discharge tube (1), diffraction grating (2), the resonator mirror (3), modulator (4), beam folding mirror (5), diaphragm (6), exposure chambers (7), a photoacoustic cell (8), vacuum post (9), power meter (10), spectrum analyzer (11), pre-amplifier (12), lock-in amplifier (13), synchronous detector (14), and recorder (15).

To perform the measurements, the air samples from the exposure chambers were placed into the pre-evacuated PA cell. The ratio of values  $U$  and  $P$  was obtained during the experiment as follows:

$$A = U/P = \alpha k, \quad (3)$$

where  $\alpha$  is the sensitivity of the PA cell;  $\alpha$  depends on the total gas pressure in the cell. We have shown that the maximum value of  $\alpha$  was observed at 60 Torr of the total gas pressure, therefore all measurements were carried out at this pressure. To observe the kinetics of the  $\text{CO}_2$  and  $\text{C}_2\text{H}_4$  emission, caused by the gas exchange process, the values of  $A$  were derived from measurements at two laser wavelengths  $\lambda_1 = 10.591$  ( $P(20)$ ) and  $\lambda_2 = 10.532$   $\mu\text{m}$  ( $P(14)$ ). These wavelengths were chosen because the absorption by carbon dioxide (at  $\lambda_1$ ) and ethylene (at  $\lambda_2$ ) make the main contribution to the PA signal. The carbon dioxide was extracted from samples with ascarite, a chemical absorber of the  $\text{CO}_2$ , in order to confirm that it is the  $\text{CO}_2$  that absorbs the radiation at the wavelength  $\lambda_1$ .

### PREPARATION AND TECHNIQUE FOR A BIOCHEMICAL TESTING OF THE OBJECTS

The 8-day-old pea, wheat, and barley seedlings as well as 14-day-old maize and pine seedlings were used as objects for our investigation. The plants were grown under luminescent lamps LDC of  $40 \text{ W/m}^2$  intensity and 12-hour photoperiod. Some experiments dealt with measurements of the  $\text{CO}_2$  emission from needles of 4-year-old plants of siberian pine, pine, larch as well as by leaves of birch and aspen (adult plants).

The presence and tendencies of the respiration metabolism were recorded based on the analysis of principal ferments and the exchange products

concentration in leaves of the second or third levels. The measurements were performed whether immediately after the experiment termination in the exposure chamber, or later, using methods for the plants fixation. The activity was measured of: alcohol-dehydrogenase (ADH-n.f.1.1.1.1.), glucose-6-phosphate dehydrogenase (GPDH-n.f.1.1.1.49.), isocitrate dehydrogenase (IDH-n.f.1.1.1.41.), phosphoenolpyruvate carboxylase (PEPC-n.f.4.1.1.31.) ferments using a photospectral method, in a homogenate, described in Refs. 17, 18 and modified to meet the specimen features. The content of the lactate, malate, and pyruvate was measured using the enzymatic method, after the leaf fixation by the perchloric acid<sup>19</sup> whereas the starch content was measured using the sulphasocil method.<sup>20</sup> The chemical reagents from the "Reanal" company (Hungary) along with Russian mineral chemical pure salts were used in the experiments. A series of 3 experiments was performed under two biologically identical conditions. The results of experiments on the gas exchange are shown in figures (in relative units). The results of biochemical tests are given in tables as mathematical expectation values and their standard errors calculated using the data from all the experiments performed.

### RESULTS AND DISCUSSION

#### Kinetics of the $\text{CO}_2$ Emission under Hypobaria

The plants response to the stresses depends on species, age, and individual features. Our experiments showed that the  $\text{CO}_2$  emission from various groups of plants depended on plant species under conditions of hypobaria.<sup>12</sup>

The results obtained show that the absorption of  $\text{CO}_2$  laser radiation by an air sample, taken from the exposure chamber, is more strong in the case of

lower pressure in the chamber. This is valid for all types of the plants tested. Thus, the experiments just described show the increase in CO<sub>2</sub> emission due to the gas exchange between a plant and the environment (the emission from the plant surfaces and from the interior cells of plants).

The temporal dependences of the PA signal amplitudes are shown in Fig. 2 for pea seedlings at pressures of 8 and 54 kPa. The dotted lines correspond to the PA signal level at the standard atmospheric pressure. The values of received signals were normalized to 1 gram of fresh mass. At the pressure of 8 kPa (high hypobaria) an explicit two-peak curve of the CO<sub>2</sub> evolution is seen. At the same time, this curve is seen to be more smooth under moderate hypobaria ( $P = 54$  kPa). In 24 hours since the exposure start the maximum CO<sub>2</sub> emission takes place and then it declines. We think that the first peak of the CO<sub>2</sub> emission rate can be attributed to the emission rate increase from intercellular spaces.<sup>17,18</sup> The second peak can be explained by intensification of its intracellular formation due to activation of reactions of the endogenous substrate decarboxylation under hypobaria.<sup>19</sup>

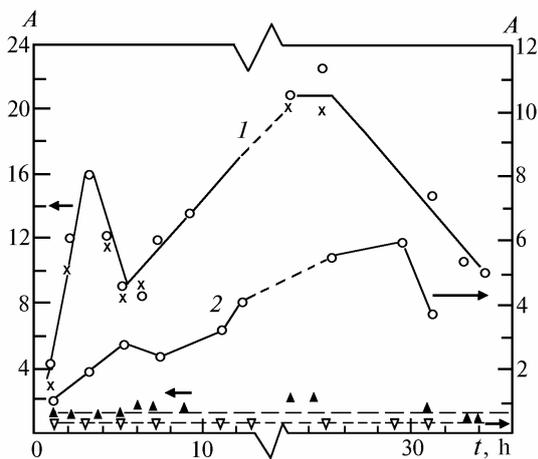


FIG. 2. Temporal dependence of photoacoustic signal for pea seedlings at pressures 8 (1) and 54 kPa (2) (▲, ▽ correspond to the control plants at  $P = 101$  kPa). --- the night-time interval free of measurements; ○ -  $\lambda_1(P(20))$ , × -  $\lambda_2(P(14))$ .

The variations in the PA signal (i.e. value of  $A$ ) at two of the above mentioned wavelengths were measured to perform the analysis of the respiration kinetics and identification of CO<sub>2</sub> and C<sub>2</sub>H<sub>4</sub>, taking part in the gas exchange process. The ratio  $A(\lambda_1)/A(\lambda_2)$  was found to remain approximately constant ( $\approx 1.2$ ) during the experiment. Thus, the plants, being in the state of dark respiration under low pressure, mainly emit carbon dioxide, and suppress the emission of other gases appearing under conditions of anaerobiosis.<sup>7,21</sup> This phenomenon observed for pea seedlings is also typical for plants from other systematic groups.

The results of measurements of the kinetics of CO<sub>2</sub> emission (case of hypobaria) by wheat, maize, and barley seedlings are shown in Fig. 3. The consideration of these results shows that CO<sub>2</sub> emission increased with increasing duration of the low pressure (54 kPa) action. Moreover, for these plants the temporal dependence of the CO<sub>2</sub> concentration differs from that for pea. Only one explicit maximum is observed (see Fig. 3). Probably, the same type of the response may be attributed to close structurally-functional peculiarities of these plants. However, it is worth noting that CO<sub>2</sub> emission from maize quickly reaches a saturation level and then remains constant (see the curve 3 in Fig. 3). This fact may be explained by the structural-functional peculiarities of the C<sub>4</sub>-type plants.

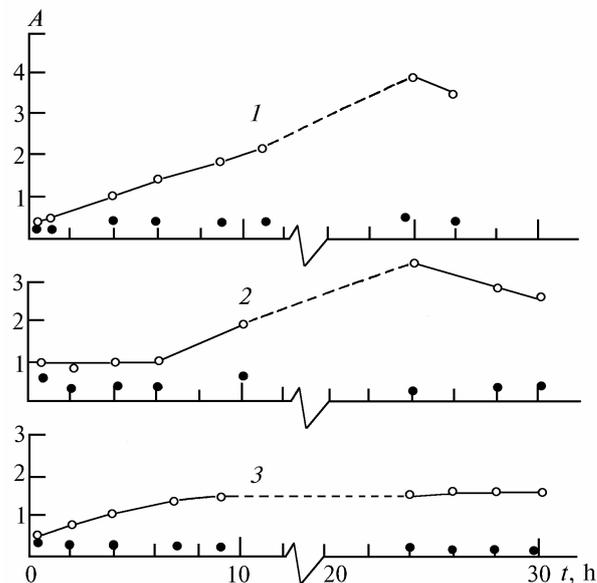


FIG. 3. Temporal dependence of photoacoustic signal for wheat (1), barley (2), and maize (3) seedlings at pressure 54 kPa (○), ● correspond to the control plants at  $P = 101$  kPa. ---- the night-time interval free of measurements.

Hypobaria is a stress for the above considered herbs. At the same time, the hypobaria is a natural condition of existence for a lot of trees, e.g. for mountain forests. That is why, one can assume that there are differences in physiological processes accompanied by CO<sub>2</sub> emission in the mountain and low land forests. The quantitative results of investigations on the kinetics of CO<sub>2</sub> emission from the mountain forests are of interest for the atmospheric CO<sub>2</sub> balance research. Finally, the kinetics of the CO<sub>2</sub> emission is an integral index of the plants gas-exchange. It can be used to study the plants physiology in mountains as well as to investigate their resistance to stress.

Let us mention some results of measurements of the kinetics of CO<sub>2</sub> emission from the tree leaves (according to Ref. 25). The temporal dependences of the CO<sub>2</sub> evolution are shown in Fig. 4. The value  $B$

is the ratio of the CO<sub>2</sub> concentration at low pressure in the exposure chamber to that at standard atmospheric pressure of 101 kPa. *B* has been normalized to a unit fresh mass.

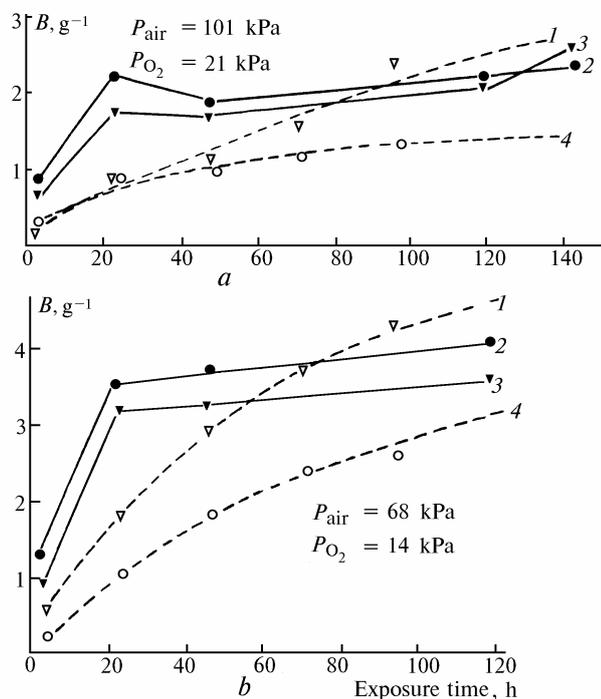


FIG. 4. Intensity of CO<sub>2</sub> emission from leaves of trees: larch (1), aspen (2), birch (3), and siberian pine (4).

The measurements of the CO<sub>2</sub> emission rate from the tree leaves at the standard pressure have confirmed its dependence on a plant species. Results of the experiments show that birch and aspen leaves intensively emit carbon dioxide during the first

24 hours of the experiment, then the CO<sub>2</sub> concentration slightly decreases, and remains approximately constant. As to the conifer trees (siberian pine, larch), the constant increase of the CO<sub>2</sub> concentration is observed during the experiment.

Under low pressure conditions, relative value of the CO<sub>2</sub> emission from larch is higher than that from siberian pine from the start of the experiment till its termination. As to the leaf-bearing trees, initially, the rate of CO<sub>2</sub> emission increases and then it remains constant. For conifer trees, the rate of the CO<sub>2</sub> emission increases constantly and then reaches the level of saturation.

The increase of CO<sub>2</sub> emission from trees, along with the transpiration and photosynthesis decrease as well as a lot of other changes in physicochemical parameters under stress conditions, may be considered as manifestation of a protective response providing a considerable resistance to a given stress. The results obtained show weaker sensitivity of siberian pine to the low pressure conditions that explains its wide spread in mountains.

Along with experiments on CO<sub>2</sub> emission from some plants at low pressure, we have studied some reactions of the respiration metabolism. The dark respiration of pea leaves under hypobaria are accompanied by changes of the ferments activity as well as the content of organic acids. In particular, a pronounced activation of alcohol dehydrogenase (a ferment turning acetaldehyde to ethanol at the terminal stage of anaerobic respiration) was observed already at a small air rarefaction. The enzymatic activity is directly proportional to an increase in hypobaria (see Table II). The alcohol dehydrogenase reactions are known to be connected with glycolic reactions in the plant tissues. Analysis of the respiration substrata concentrations in pea leaves shows that the hypobaria (*P* = 54 kPa) leads to a decrease in malate and pyruvate concentration as well as to an increase in the lactic acid concentration (see Table III).

TABLE II. Activity of alcohol dehydrogenase in pea leaves at rising hypobaria. Exposure time 24 h.

Treatments	Air pressure, kPa	Alcoholdehydrogenase	
		μmol NADH mg <sup>-1</sup> protein min <sup>-1</sup>	%
Control	101	1.53 ± 0.09	100
Hypobaria	54	2.76 ± 0.14	181
	29	4.98 ± 0.18	326
	8	14.29 ± 0.41	932

TABLE III. Activity of organic acids in pea leaves at the hypobaria.

Treatments	Air pressure, kPa	Pyruvate	Malate	Lactate
		μmol fresh weight g <sup>-1</sup>		
Control	101	2.08 ± 0.24	0.90 ± 0.01	1.83 ± 0.16
Hypobaria	54	1.08 ± 0.04	0.49 ± 0.01	2.34 ± 0.16
	29	3.47 ± 0.12	1.47 ± 0.12	2.58 ± 0.21
	8	4.55 ± 0.13	2.64 ± 0.11	1.04 ± 0.12

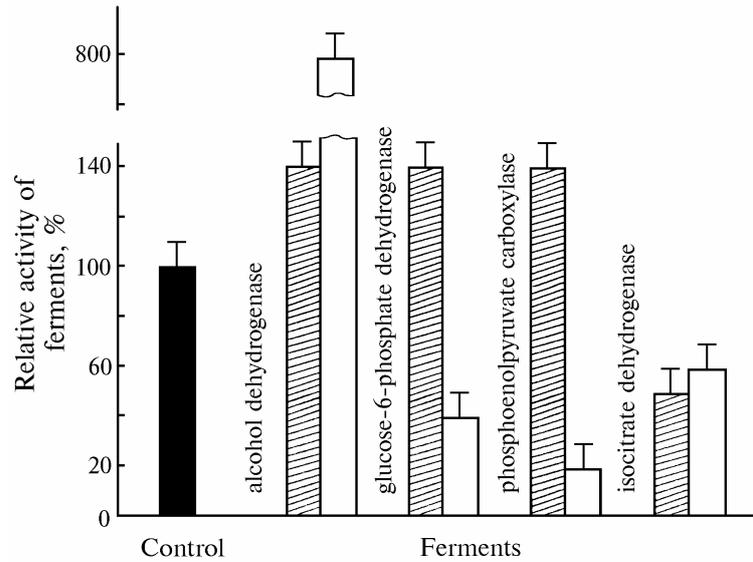


FIG. 5. Relative activity of ferments (%) in pea leaves at  $P = 8.3$  kPa. Exposure time: ▨ – 6 h, □ – 24 h.

The content of all the above mentioned metabolites increases at  $P = 29$  kPa. Strong increase of pyruvate and malate content as well as a decrease of lactate content takes place at  $P = 8$  kPa (high hypobaria).

Investigations showed that relative activity of alcohol dehydrogenase under hypobaria increased at 6-hour exposure time, and increased markedly at 24-hour exposure time (Fig. 5). For GPDH and PEP-carboxylase, 6-hour activation is observed. But after 24 hour exposure an inhibition of these enzymes is seen. The lowering of isocitrate dehydrogenase activity observed at 6-hour exposure remains after 24-hour experimenting. The same situation is observed with starch. Content as to other organic substances like an instant protein, pyruvate, and malate, their content was found to increase both at 6 hour and 24 hour exposure time.<sup>24</sup>

Hypobaria can lead to the structural changes in plants. For example, the tested plants increased the leaves area and stem length under conditions of 48 hour dark hypobaria. The measurements results are given in Table IV.

TABLE IV. Influence of the hypobarical hypoxia in combination with dark on pea leaves area and stem length.  $P = 54$  kPa,  $P_{O_2} = 11$  kPa. Exposure time is 48 h.

Experimental variants	Leaves area, cm <sup>2</sup>	%	Stem length, cm	%
Initial	18.0 ± 1.2	100	13.4 ± 0.9	100
Control	23.4 ± 1.2	130	14.5 ± 1.3	108
Test	27.6 ± 1.8	153	16.6 ± 1.2	123

These changes are accompanied by an increase in the leaf tissue cells volume (Table V). The cells

hypotrophia was shown to be accompanied by an increase in size of the leaf epidermis stoma slits. The above mentioned processes are favorable for the CO<sub>2</sub> evolution activation under conditions of hypobaria.

TABLE V. Influence of the hypobarical hypoxia in combination with dark on pea leaf cells volume.  $P = 8$  kPa,  $P_{O_2} = 2$  kPa. Exposure time is 20 h.

Experimental variants	Paling parenchyma	Spongy parenchyma
Volume of cells μm <sup>3</sup> · 10 <sup>3</sup>		
Control	27.4 ± 2.3	17.8 ± 1.5
Experiment	45.1 ± 3.9	31 ± 3.0

### Kinetics of the CO<sub>2</sub> Emission at Air Pollution

Ethylene is a gas of the environmental interest because it is the only gaseous hormone regulating physiological processes at all stages of the plants development. Moreover, ethylene is one of hydrocarbons polluting the atmosphere due to activity of oil-chemical enterprises.<sup>16</sup> We have carried out the investigations of effect of ethylene high concentration on kinetics of CO<sub>2</sub> evolution by pea seedlings. Initially, the seedlings were placed into the air medium with a fixed ethylene concentration (exposure time is 48 h), then they have been put in an exposure chamber with a pure air at standard temperature and pressure. After this, we measured the CO<sub>2</sub> evolution by tested plants in comparison with that due to reference (control) plants. The latter have not been affected by ethylene influence. The ethylene effect on tested plants is noted to result in double increase of CO<sub>2</sub> evolution (see Fig. 6). Available data<sup>26</sup> confirm the activation of the plants respiration when an exogenously applied ethylene influencing.

We also studied the influence of another important atmospheric toxicant, the CO, on the plants respiration. Barley seedlings were used as tested objects at 66 hour of exposure time. Figure 7 shows kinetics of CO<sub>2</sub> emission from reference (control) and tested plants.

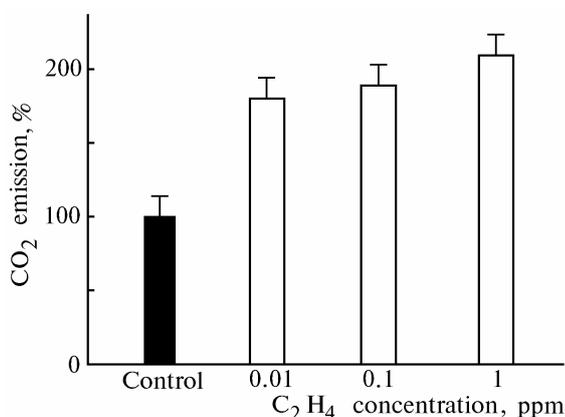


FIG. 6. Influence of exogenously applied ethylene on CO<sub>2</sub> emission by pea seedlings.

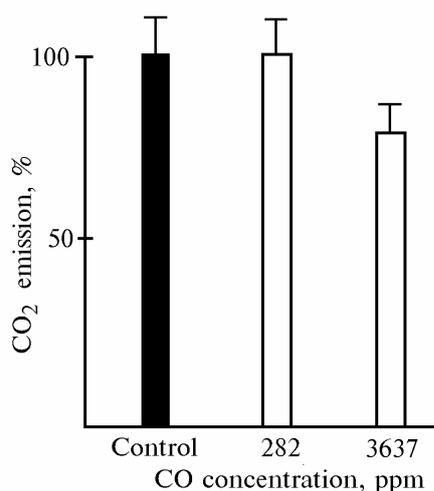


FIG. 7. Influence of the CO on CO<sub>2</sub> emission from barley seedlings.

The experiment did not reveal any explicit changes in CO<sub>2</sub> evolution at moderate CO concentration. Moreover, a tested group of plants exposed to air with a maximum CO concentration of 3640 ppm (in the course of the given experiment) exhibited a decrease in CO<sub>2</sub> emission. These results demonstrate the selective dependence of the plants response to a concrete toxicant stress. This property can be used for selection of most steady plants to be grown under unfavorable environmental conditions. Let us note that the plants response to CO is revealed only at its high concentration exceeding considerably the limiting allowed for industrial areas (17 ppm).

## ACKNOWLEDGMENTS

The authors would like to thank Academician V.E. Zuev for his attention and support. Our thanks to T.A. Zaitseva, A.P. Zotikova, and N.A. Vorobi'eva for their participation in some parts of this work.

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