Proc. Estonian Acad. Sci. Biol., 1992, 41, 1, 35—43
https://doi.org/10.3176/biol.1992.1.05

UDC 581.111/.112 + 581.2.02

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EFFECT OF OZONE ON THE REHYDRATION OF BEAN LEAVES

Bean (*Phaseolus vulgaris* L.) plants were fumigated with 0.37 to 0.60 ppm ozone for 3 to 4 hours or with 0.16 to 0.30 ppm for 24 to 48 hours. After ozonation, the stem was cut near the base, and the shoot was allowed to transpire until wilting of leaves was evident. Then the cut end of the stem was immersed in water in a pressure bomb, and a leaflet was fitted into the leaf chamber. The pressure in the bomb was increased stepwise, and the leaf water content, transpiration rate and leaf temperature were monitored.

The leaf water content and stomatal conductance (guard cell water content) of ozonated leaves increased much less in response to a pressure increment than in control leaves. The difference between water content values at full turgor and at turgor loss in ozonated leaves was also significantly lower than that in control leaves. Ozonation effect was more pronounced if plants were treated with higher ozone concentration for a shorter time.

It can be concluded that rehydration of mesophyll and guard cells of ozonated bean plants was diminished because ozone induces changes in the properties of cell walls (increases volumetric elastic modulus) and/or in the properties of plasmalemma (increases leakage of solutes).

It has been shown by several authors that stomata close under ozone pollution (Dugger, Ting, 1970; Heath, 1980). This indicates that the turgor pressure of guard cells decreases during ozonation. Data about the influence of ozone on water relations of mesophyll cells are scanty. There is some evidence that the leaf water content declines under ozone (Evans, Ting, 1974). It is believed that ozone causes changes in cell walls or plasmalemma (Heath, 1980). As changes in the water content of mesophyll cells depend directly on the characteristics of plasmalemma and cell wall (Zimmermann, 1978), I hypothesize that changes in these cell characteristics could be detected in experiments, where changes in the leaf water content are monitored during pressurization of ozonated leaves via cut petioles. In the present paper results of such experiments by using the leaf chamber, beta-gauge and pressure bomb techniques, are described.

Materials and methods

Plant material. Dwarf bean (*Phaseolus vulgaris* L. *cv. Oregon*) plants were grown in a growth cabinet singly in 1000 cm^3 stainless steel pots containing a mixture of quarz sand and vermiculite (2:1 by volume) and normal Knop solution. Moisture was held at 60% of mixture full water capacity. Light/dark period was 14/10 h, PAR density 160 W/m^2 (mercury fluorescent lamp). There was normal ambient CO_2 concentration, 25/25 °C day/night temperature and 50—60% relative humidity in the growth cabinet. Experiments were performed when plants had 3 to 4 trifoliate leaves (14 to 18 d from emergence).

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Experimental procedures. For ozone treatment, a plant was transferred to an open-system air- and temperature-controlled twocompartment (for shoots and roots) plant chamber (Moldau et al., 1990) supplemented with an ozone generator (spark discharge in pure oxygen). Ozone was added to the air flow at the shoot-compartment entrance through teflon tubing. The root-compartment was not ozonated. During the treatment, ozone concentration around shoots (equal to that in outgoing air) was recorded by Dasibi UV absorption ozone photometer, model 1003-AH. Two plants were ozonated for a relatively shorter time (3 and 4 hours) under higher (0.37 and 0.60 ppm, respectively), two others for a longer time (24 and 48 hours) under lower (0.30 and 0.16 ppm) ozone concentration.

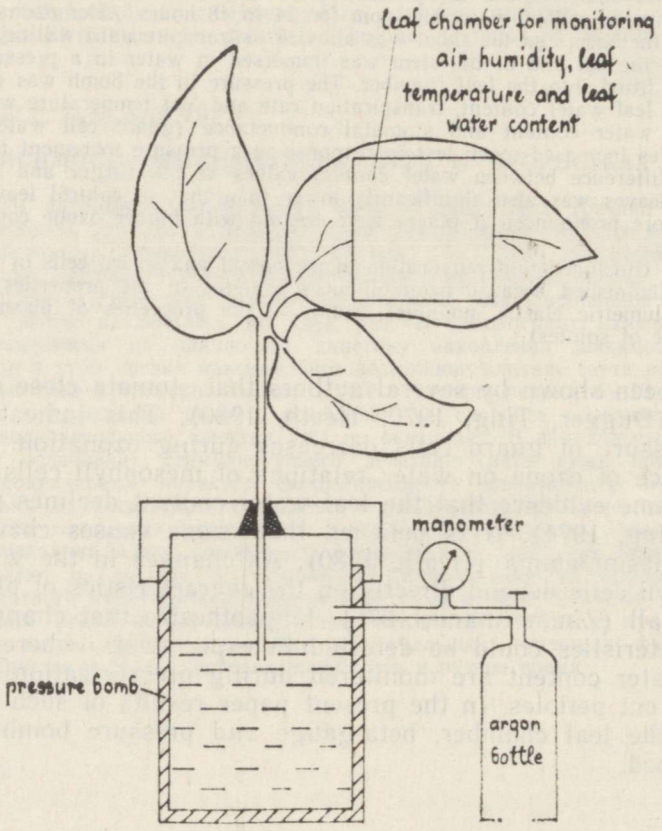

Fig. 1. A cheme of experiment.

After ozonation, the shoot of the plant was cut near the stem base. The cut end was driven through the rubber seal of a pressure bomb (Рахи, 1973). The shoot was allowed to transpire until wilting of leaves became apparent. Thereafter, the cut end of the stem was immersed in water in the bomb and recut under water. The bomb was closed, and a leaflet of the first trifoliate was fitted into a leaf chamber (Fig. 1). The chamber used has been designed for simultaneous measurements of the relative changes in the water content and transpiration rate of the leaf under the given air humidity and CO_2 concentration (Сыбер, Молдау,

1977). After recording the intensity of beta radiation corresponding to the initial value of the leaf water content, the pressure in the bomb was increased stepwise by 50 kPa until the water content stopped to increase, and infiltration had not yet begun. (Infiltration began at pressures between 150 and 500 kPa, depending on transpiration rate). Changes in the leaf water content were monitored with the help of β-gauge technique, air humidity by means of micropsychrometer, and the leaf temperature by means of infrared thermometer (Сыбер, Молдау, 1977).

Stomatal conductance g_s was calculated by using transpiration, humidity, and leaf temperature data:

$$g_s = \frac{(r_c - r_l)}{r_c \cdot r_l}; \tag{1}$$

with

$$r_l = \alpha \cdot \frac{(e_l - e)}{E} - r_a,$$

with

$$E = \frac{v}{S}(e - e_0),$$

where r_l — leaf resistance to water vapour (stomatal and cuticular resistances in parallel), r_a and r_c — boundary layer and cuticular resistances, e_l — saturating humidity at leaf temperature, e_0 and e — humidities without and with the leaf in leaf chamber, E — transpiration rate, α — mass-flow correction, v — air flow rate and S — area of the leaf in leaf chamber.

Absolute values of the leaf water content w (g/cm^2) cannot be registrated with sufficient sensitivity because of the different absorption and reflection of beta-radiation by leaves with different dry-matter content m_d (g/cm^2) and different structure. At the same time relative difference H of current water content w from a certain water content w_0 of given experiment can be calculated by using measured intensities (counting interval 100 seconds) of beta radiation:

$$H = (w - w_0)/w_0 = (\ln I_0 - \ln I)/(\ln I' - \ln I_0), \tag{2}$$

where I and I' — intensities of β-radiation with and without leaf in the leaf chamber and I_0 — beta radiation intensity at the moment, when $w = w_0$ and m_d is assumed to be constant.

In principle H can be calculated in relation to any moment during the experiment. For example calculating H in relation to water content at full turgor w_s, we get water saturating deficit H_s. To find current values of the absolute water content w (mg/cm^2), we calculate H_e from formula (2) where index e denotes H, w, and I at the end of experiment:

$$w = w_e(1 + H_e), \tag{3}$$

where w_e is obtained directly by weighing fresh and dried leaf segments after the experiment.

Water content w also depends on leaf dry-matter content m_d. Variability in m_d was large, and the reduced parameter — **leaf specific water content**

$$h = \frac{w}{m_d}, \tag{4}$$

was introduced,

Calculating H from formula (2) in relation to the water content at turgor loss w_t, i. e. relative to the water content at the beginning of experiment, we get a parameter defined as **relative turgidity**

$$H_t = \frac{w - w_t}{w_t} = \frac{h - h_t}{h_t}, \tag{5}$$

where index t denotes the state of turgor loss.

The slope of H_t against pressure p was calculated to compare the values of H_t after different pressure changes, and defined as **swelling capacity**

$$S_{wt} = \frac{\Delta H_t}{\Delta p} = \frac{\Delta H_t}{\Delta p_t} \cdot \frac{\Delta p_t}{\Delta p} = \frac{1}{\varepsilon} \cdot \frac{\Delta p_t}{\Delta p}, \tag{6}$$

where p_t is turgor pressure in mesophyll cells. The term $\Delta H_t / \Delta p_t$ is equal to the complience, or reciprocal of volumetric elastic modulus ε (Zimmermann, 1978). The swelling capacity is equal to the complience $\frac{1}{\varepsilon}$, if pressure changes in the bomb are transferred to equal changes in turgor pressure. But S_{wt} may differ from $\frac{1}{\varepsilon}$, if osmotic pressure is changing after pressure changes in the apoplast.

Results

The time courses of the stomatal conductance and the relative turgidity after the first increase in pressure are presented in Fig. 2. Different curves correspond to the leaves from differently ozonated plants, curves 1 and 1′ describe the behaviour of the leaves of the two control plants. Pressure increase induced remarkable stomatal opening and an almost 15 per cent increase in relative turgidity of the control leaves. The leaves of the plants which had previously been ozonated for 3 or 4 hours under higher ozone concentration showed smaller stomatal opening and absorbed water with a significantly lower rate and to a smaller extent (curves 2 and 2′) than the leaves of the control plants. In addition, in the case of curve 2, the increase in relative turgidity was only temporary, as, after two hours under excess pressure, relative turgidity dropped to its initial value again and continued to decrease. However, turgor was not lost. The leaves of plants exposed to the lower ozone concentration for a longer period (24 or 48 hours) showed intermediate changes in stomatal conductance and relative turgidity. In general, the greater were the changes in the water content of the leaves, the greater were those in the stomatal conductance. This indicates that ozone had a similar effect on the rehydration of both the mesophyll cells and guard cells.

In our previous paper (Сыбер, Рахи, 1989) with nonozonated leaves it has been shown that the relative change in the leaf water content in response to an equal pressure increase (i. e. swelling capacity, calculated from formula (6)) was lower when the initial leaf water content was nearer to that of the water-saturated leaf. Swelling capacity S_{wt} as a function of the leaf specific water content h (Eq. 4) for differently ozonated leaves is shown in Fig. 3. It can be seen that ozone does not influence the shape of the dependence between S_{wt} and h. The zero value of S_{wt} corresponds to the specific water content h_s of the water-saturated leaf. The highest value of S_{wt} of every individual leaf corresponds to the first pressure rise at turgor loss, when the leaf specific water content is h_t.

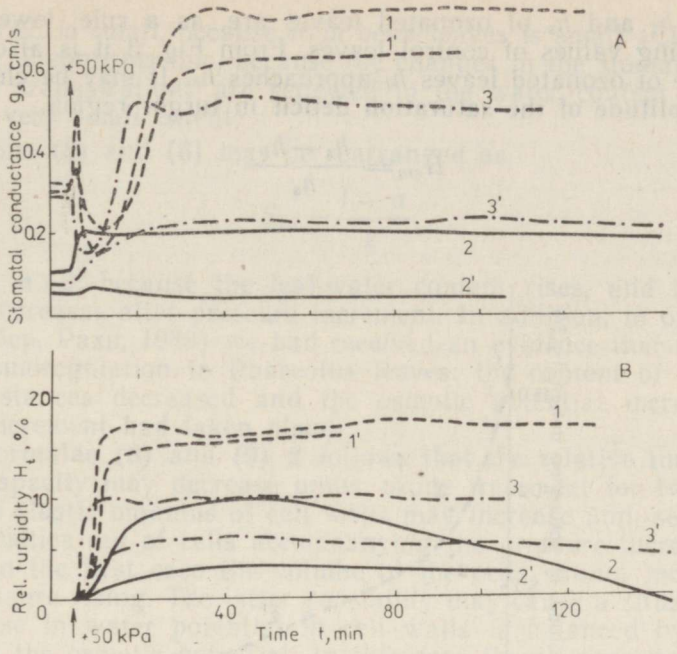

Fig. 2. Time courses: *A* — stomatal conductance g_s (Eq. 1) and *B* — relative turgidity H_t (Eq. 5) of Phaseolus leaves after an abrupt increase in xylem water potential by 50 kPa at the moment indicated by an arrow. Curves *1* and *1′*, control plants; *2* and *2′*, plants treated with ozone for 3 or 4 hours under the high (0.37 or 0.60 ppm respectively) ozone concentration; *3* and *3′*, plants treated with ozone for 24 or 48 hours under the lower (0.30 or 0.16) ozone concentration.

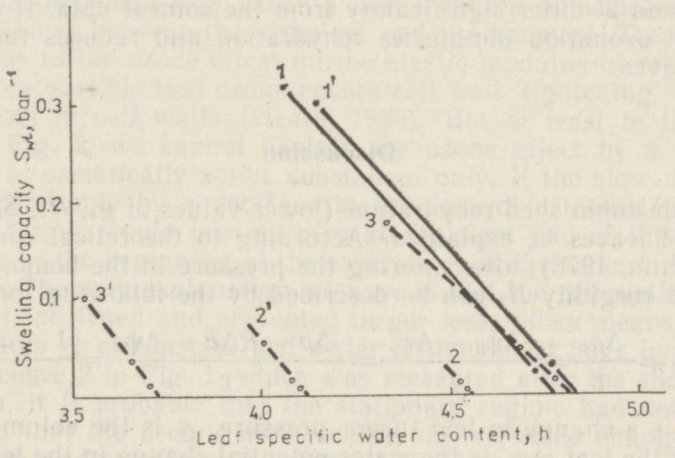

Fig. 3. The dependence of the swelling capacity (Eq. 6) on the leaf specific water content *h* (Eq. 4). Curves are numerated like in Fig. 2, closed symbols denoting control plants, intersection with the abscissa gives h_s, the maximum leaf specific water content at which swelling capacity approaches zero.

Values of h_t and h_s of ozonated leaves are, as a rule, lower than the corresponding values of control leaves. From Fig. 3 it is also seen that in the case of ozonated leaves h_t approaches h_s. It may be characterized by the **amplitude of the saturation deficit** in turgor region.

$$H_{sm}=\frac{h_s-h_t}{h_s}. \tag{7}$$

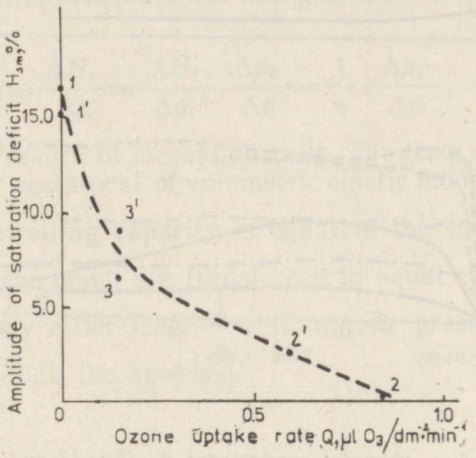

Fig. 4. The dependence of the amplitude of the saturation deficit H_{sm} (Eq. 7) on the mean rate of ozone uptake Q. Numeration as in Fig. 2.

In Fig. 4 H_{sm} is presented as a function of the mean rate of ozone uptake by a plant, Q. There is a smooth relationship between H_{sm} and Q. From the data of our previous work (Молдау, Сыбер, 1988) we calculated that H_{sm} of nonozonated bean leaves was commonly $14\pm4\%$. For this reason, although only two experiments were made at every fumigation regime in the present series, we can say that data from ozonation experiments 2 and 2′ differ significantly from the control data. It can be concluded that ozonation diminishes rehydration and reduces turgor region of bean leaves.

Discussion

How can diminished rehydration (lower values of g_s, H_t, S_w and H_{sm}) in ozonated leaves be explained? According to theoretical considerations (Zimmermann, 1978), after altering the pressure in the bomb, changes in the relative turgidity H_t can be described by the following formula

$$\Delta H_t=\frac{\Delta p_t}{\varepsilon}=\frac{\Delta\psi_a-\Delta\psi_0}{\varepsilon}=\frac{\Delta p-R\Delta E-\Delta\psi_0}{\varepsilon}=\frac{1-a}{\varepsilon}\Delta p, \tag{8}$$

where Δp_t is a change in leaf turgor pressure, ε is the volumetric elastic modulus of the leaf, $\Delta\psi_a$ is the water potential change in the leaf apoplast, Δp is a pressure change in the pressure bomb, R is the hydraulic resistance in xylem, ΔE is the transpiration change, $\Delta\psi_0$ is the change in the osmotic potential of the leaf and $a=\Delta\psi_0/\Delta p$ is a coefficient which determines changes in the osmotic potential of the leaf after a pressure change in the xylem has taken effect.

Term $R\Delta E$ is small, because R in bean leaves is small (Сыбер, Рахи, 1989). Fig. 2 confirms this, because the changes in the stomatal conductance (and transpiration) are reflected in the water content of the leaf only to a very small extent.

Equations (8) and (6) may be rearranged as

$$S_{wt}=\frac{1-a}{\varepsilon}. \qquad (9)$$

Coefficient $a>0$, because the leaf-water content rises, and the osmotic potential increases after pressure increment. In addition, in our previous work (Сыбер, Рахи, 1989) we had received an evidence that there exists a rapid osmoregulation in Phaseolus leaves: the content of osmotically active substances decreased and the osmotic potential increased after pressure increment had taken place.

From formulae (8) and (9) it follows that the relative turgidity and swelling capacity may decrease under ozone treatment for two reasons. Firstly, the elastic modulus of cell walls may increase and, secondly, the leak of osmotica out of cells accompanying the pressure increment, may increase. In the first case the volume of the cells cannot increase with turgor pressure rising. The latter possibility may cause a situation where any increase in water potential in cell walls is balanced by an equal increase in the osmotic potential. In this case there arises no difference between the apoplast and symplast water potentials, and the leaf water content does not increase after pressure increment.

Turgor region (or H_{sm} in Fig. 4) may be reduced if the content of osmotically active substances in the symplast decreases under ozone. Turgor region can be also reduced in the case if the effect of ozone on ε at turgor loss and at full turgor is different. The latter possibility does not seem very likely, because ozone did not affect the shape of dependence between S_{wt} and h (Fig. 3).

Unfortunately, our experiments did not allow us to distinguish between the effects of ozone on the elastic properties of cell walls or on the amount of osmotically active substances in symplast. There is some evidence concerning the ozone effect on plasmalemma permeability (Evans, Ting, 1974; Heath, 1980; Heath, Frederick, 1980). The changes in the permeability of plasmalemma may cause a redistribution of osmotically active substances (ions) between the symplast and the apoplast. There seems to be no data as to the ozone effect on the elastic modulus of cell walls. In principle, it is possible that ozone causes cell wall 'tightening' via changing Ca^{2+} level in cell walls (Heath, 1988). But at least in the case of curve 2 in Fig. 2, we cannot explain the ozone effect by a change in the amount of osmotically active substances only. If the slow decrease of H_t were caused only by a decrease in the amount of osmotically active substances, turgor would become zero at the right end of this curve, as was the case at the beginning of the experiment at the same h. But turgor was not lost. The only explanation we have is that the cell walls of ozone-treated plants stiffened and prevented turgor loss, which means that their elastic modulus ε at constant leaf water content must have increased. In the case of curve 2 in Fig. 1, which was measured after the shortest time of ozonation, it is probable that the stationary regime had not yet been reached, and that the decline of the curve still reflected changes in cell-wall elasticity and/or membrane permeability induced by ozone.

The possibility remains that both the properties of cell walls and those of plasmalemma are changing under ozone treatment. It is even more likely because the ion transport properties of plasmalemma and elastic as well as plastic properties of cell walls are known to be interdependent (Taiz, 1984; Zimmermann, 1978).

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Presented by U. Margna

Received
June 20, 1991

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OSOONI MÕJU AEDOA LEHTEDE VEESISALDUSE TAASTUMISELE

Aedoa taimede maapealseid osi fumigeeriti lühemat aega (3—4 tundi) üsna kõrge (0,37—0,60 ppm) osoonisisaldusega õhus ja pikemat aega (1—2 ööpäeva) veidi madalama (0,16—0,30 ppm) osoonisisaldusega õhus. Pärast osoneerimise lõpetamist lõigati taime vars läbi ja lasti taim kuni lehtede turgori kadumiseni kuivada. Rehüdreerimiseks pandi taime vars läbi tihendi survekambrisse vette ja tõsteti survekambris rõhku. Samaaegselt registreeriti esimese kolmikgrupi keskmisel lehel β-kiirguse neeldumise meetodil veesisalduse muutused. Registreeriti ka transpiratsiooni, õhuniiskust lehe kohal ja lehe temperatuuri ning arvutati nende suuruste järgi õhulõhede juhtivus.

Osoneeritud taimede lehtede veesisaldus ja õhulõhede juhtivus suurenesid aeglasemalt ja vähemal määral kui kontrolltaimede vastavad parameetrid. Osooni mõju oli maksimaalne juhul, kui taimi osoneeriti lühemat aega kõrgemal kontsentratsioonil. Katsetulemuste analüüsi põhjal järeldati, et mesofülli- ja sulgrakkude rehüdratsioonivõime vähenemist põhjustab rakuseinte jäigemaks muutumine ja/või plasmalemma ioonläbilaskvuse suurenemine osooni mõjul.

Ану СЫБЕР

ВЛИЯНИЕ ОЗОНА НА ВОССТАНОВЛЕНИЕ СОДЕРЖАНИЯ ВОДЫ В ЛИСТЬЯХ ФАСОЛИ

Надземную часть растения фасоли фумигировали в течение 3—4 ч при относительно высокой (0,37—0,60 ppm) концентрации озона или в течение более длительного периода (1—2 сут) при несколько меньшей (0,16—0,30 ppm) концентрации озона.

После фумигации надземную часть растения отрезали и оставляли до потери тургора листьев в камере для выращивания растений. Для регидрации конец стебля вводили через уплотнитель в заполненную водой камеру давления. В ходе скачкообразного повышения давления в камере на листке первого тройчатого листа регистрировали изменение содержания воды (методом поглощения β-излучения). Одновременно регистрировали транспирацию, влажность воздуха и температуру листа и вычисляли на основе этих параметров проводимость устьиц.

Установлено, что содержание воды и проводимость устьиц у листьев озонированных растений повышаются при регидрации в меньшей степени, чем у контрольных растений. Влияние озона было наибольшим в случае кратковременной фумигации при высокой концентрации.

На основе анализа полученных данных сделан вывод, что причиной уменьшения способности восстановления содержания воды мезофильных и замыкающих клеток является увеличение жесткости клеточных оболочек и/или увеличение ионной проницаемости плазмалеммы под влиянием озона.