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Stomatal closure during tobacco leaf desiccation as affected by ozone

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Abstract

The effect of acute ozone exposure on the stomatal conductance and leaf water content during rapid desiccation was examined in leaves of two tobacco cultivars, ozone sensitive cv. BelW3 and ozone tolerant cv. Samsun. The relative rate of stomatal closure was constant during leaf desiccation in cv. Samsun but decreased in cv. BelW3 in both ozonated and control plants. Ozone exposure increased the relative rate of stomatal closure and transpiration rate (measured on the following day) in cv. Samsun, but reduced the respective parameters in cv. BelW3. As a result, the plants of ozone-sensitive cultivar, treated with ozone, lost more water during desiccation than control plants.

Additional key words: leaf water content, *Nicotiana tabacum*, sensitive and tolerant cultivars, stomatal conductance.

Introduction

Ozone pollution disturbs several physiological processes, including stomatal behaviour. Not only steady-state values of stomatal conductance but also stomatal kinetics plays a certain role in regulation of leaf gas exchange. For example, the rates of stomatal movements are important during the formation and disappearance of sunflecks (Tinoco-Ojanguren and Pearcy 1993), during water stress (Maier-Maercker and Koch 1991, 1992) as well as during development of xylem embolism (Sperry 1986, Zotz *et al.* 1994). Ozone differently affects the stomata of different species and cultivars (Evans and Ting 1974, Heath 1988, Moldau *et al.* 1993). The present study was undertaken to examine impact of ozone on stomatal responses to leaf desiccation in different tobacco cultivars.

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Materials and methods

Two cultivars of tobacco (*Nicotiana tabacum* L.) differing in ozone-sensitivity were used: cv. BelW3 as a sensitive cultivar (this cultivar has been employed for the biomonitoring of ozone since the year 1960 and is probably one of the best-known ozone bioindicators; Skarby 1978, Heggestad 1991) and cv. Samsun as a tolerant cultivar. Tobacco plants were grown in a growth chamber (mercury fluorescent lamp providing irradiance of 410 μmol m^{-2} s^{-1}, 14-h photoperiod, CO_2 concentration of 350 μmol mol^{-1}, temperature of 25 °C, and relative humidity of 50 - 60 %).

Ozone exposure was performed in a CO_2 and temperature controlled plant chamber with two compartments (for shoots and roots) (Moldau *et al.* 1990). Ozone was added by an ozone generator (spark discharge in pure oxygen) to the incoming air of the shoot compartment through a teflon tubing. The root compartment was not ozonated. During the treatment, ozone concentration around shoots (equal to that in outgoing air) was recorded with a *Dasibi 1003-AH* (*Dasibi Environmental Corporation*, Glendale, USA) UV absorption ozone photometer, and was about 400 nmol mol^{-1}. Exposure lasted 6 - 8 h. Control plants were fumigated with charcoal-filtered air for the same time. After the fumigation the plants were returned back into the growth chamber overnight. In the following morning several visible injuries were observed in the leaves of the ozone-sensitive but not the ozone-tolerant plants. Mature leaves of similar ages were cut, cut edges were placed into the water and leaves were placed into the leaf chamber for the registration of water vapour exchange, leaf temperature, and leaf water content. As soon as the values of stomatal conductance became stable, the leaf petiole was taken off from water and stomatal conductance and leaf water content were monitored during leaf desiccation. Irradiance was 980 μmol m^{-2} s^{-1}, relative humidity 50 -70 %, CO_2 concentration 330 - 350 μmol mol^{-1}, leaf temperature 24 - 26 °C. Leaf water content was monitored by the β-gauge technique (for details see Sõber 1992, Moldau *et al.* 1993, Sõber 1997). Stomatal conductance, g_s [mol m^{-2} s^{-1}], was calculated from air humidity and leaf temperature measurements (for details see Sõber and Moldau 1977, Sõber 1992).

The relative rate of stomatal closure, v_g [s^{-1}], was calculated from the slope of the time course of g_s after leaf detachment:

$$v_g = dg_s/(dt \times g_s)$$

and was used as an indicator of stomatal sensitivity to desiccation. The relative (not absolute) rate of stomatal closure was introduced assuming that g_s decreased (after the transient increase) exponentially in time.

Decrease in water content at the moment t (after leaf detachment) is the sum (integral) of transpired water (E) over time t:

$$x - x_0 = \Sigma\ (E\ dt)$$

As E decreases due to g_s, relative changes in water content can be (in the first approximation) described by the formula:

$$(x - x_0)/x_0 = A + (E_{max}/x_0 v_g) \times (g_s - g_{s0})/g_{s0}$$

where A is relative water loss during transient stomatal opening. If $(g_s - g_{s0})/g_{s0} = -1$ (stomata closed), maximal relative change in leaf water content should be

$$[(x - x_0)/x_0]_{max} = A + (E_{max}/x_0 v_g)$$

Thus, relative changes in leaf water content, which occur simultaneously with certain changes of stomatal conductance are more pronounced in the case of prolonged transient opening and low v_g, however, these changes depend also on the ratio of initial transpiration to leaf water content values.

Results and discussion

Initial values of the stomatal conductance of control plants did not differ significantly between the two cultivars. Ozone exposure increased stomatal conductance (measured on the following day) in the ozone-tolerant cultivar but reduced it in ozone-sensitive cultivar (Table 1, Fig.1). Water content of the leaves was significantly higher in cv. Samsun compared with the cv. BelW3. After ozone exposure a decrease in leaf water content was observed in cv. BelW3, and an increase in leaf water content was observed in cv. Samsun, but these changes were small (Table 1). Apparently osmotic adjustment occurred (increase of osmotic pressure or loosening of cell walls) in ozone-tolerant plants, but not in ozone-sensitive plants.

Table 1. Steady state values of stomatal conductance, g_{s0}, maximal values of relative rate of stomatal closure, v_{gmax}, and water content, x_0, in cotrol and ozone-treated leaves of two tobacco cultivars.

	BelW3 control	ozone	Samsun control	ozone
g_{s0} [mol m^{-2} s^{-1}]	0.432 ± 0.056	0.299 ± 0.036	0.254 ± 0.064	0.439 ± 0.082
v_{gmax} [s^{-1}]	0.0030 ± 0.0004	0.0028 ± 0.0003	0.0013 ± 0.0003	0.0032 ± 0.0006
x_0 [g m^{-2}]	217 ± 6	193 ± 8	248 ± 7	260 ± 3

A short-time opening of stomata occurred immediately after leaf detachment in most cases (Fig. 1). This transient opening was higher in ozone-tolerant control plants, and in ozone-sensitive ozonated plants (Figs. 1,3) than in other plants. It is reasonable to analyse the measured differences in the light of existing models. The dependence of stomatal conductance on water deficit is determined by changes in pressure potential in guard cells, P_g and epidermal cells, P_e (DeMichele and Sharpe 1973, Dewar 1995, Haefner *et al.* 1997):

$$g_s = c_1 P_g - c_2 P_e$$

where c_1 and c_2 are coefficients which depend on structure and elastic properties of guard and epidermal cell walls. The transient increase in g_s after leaf detachment (Fig. 1) is caused by mechanical advantage of epidermal cells ($c_2 > c_1$). As ozonated leaves of ozone-sensitive cv. BelW3 lost more water during transient stomatal opening (Fig. 3*A*), c_2 can be increased or c_1 can be decreased by ozone in ozone-sensitive plants.

The relative rate of stomatal closure, v_g achieved its maximal value during the first 7 - 11 and 11 - 14 min in cvs. BelW3 and Samsun, respectively. In both cultivars, maximal v_g was achieved more rapidly when initial transpiration rate was higher (Figs. 1,2).

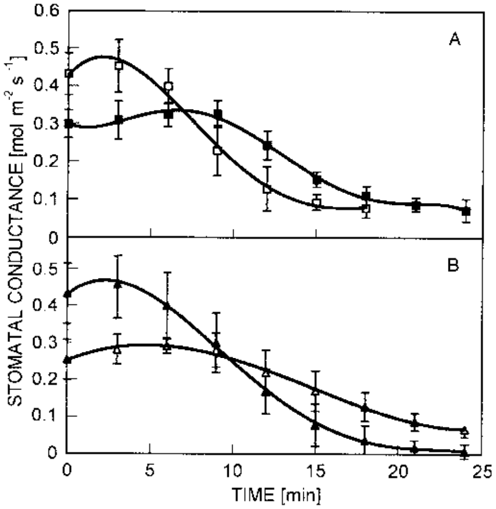

Fig. 1. Time courses of stomatal conductance of the leaves of the control (*open symbols*) and ozonated (*closed symbols*) plants. *A* - ozone-sensitive cultivar BelW3, *B* - ozone-tolerant cultivar Samsun. The leaf base was taken out of water at a moment t = 0. *Bars* denote SE.

It is probable, that stomatal conductance started to decrease after additional P_g decrease when hydroactive processes were initiated in guard cells. Stomata closed with increasing rate probably until pressure potential of subsidiary epidermal cells was zero. It should take actually more time when initial transpiration was lower.

After achieving the maximal value, v_g decreased in cv. BelW3, but remained constant in cv. Samsun (Fig. 2). The maximal value of v_g of control plants was considerably higher in cv. BelW3 than in cv. Samsun, being quite low in the latter. Following ozone exposure, maximal v_g increased significantly in ozone-tolerant plants, but there was only a slight decrease in v_g of ozone-sensitive plants (Table 1, Fig. 2).

The rate of stomatal closure was probably determined only by the rate of pressure potential decrease in guard cells when the maximal rate of stomatal closure was already achieved, because

$$v_g = c_1 \times (dP_g/g_s dt)$$

if $P_c = 0$. It seems, that dP_g/dt changes proportionally to g_s (and transpiration) in ozone-tolerant plants. As v_g was constant, stomatal closure was described by one exponent (as we initially proposed) in ozone-tolerant cultivar, but the constant of exponent was increased by ozone. In ozone-sensitive plants dP_g/dt was initially very high but decreased more rapidly than transpiration. The mechanism of stomatal closure is not clear. Differences may occur in abscisic acid pools, cell membranes and/or cell walls in different cultivars.

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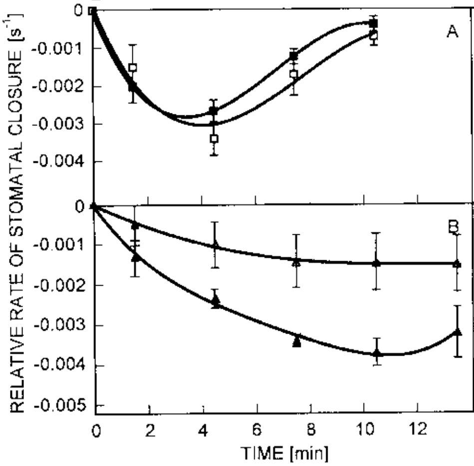

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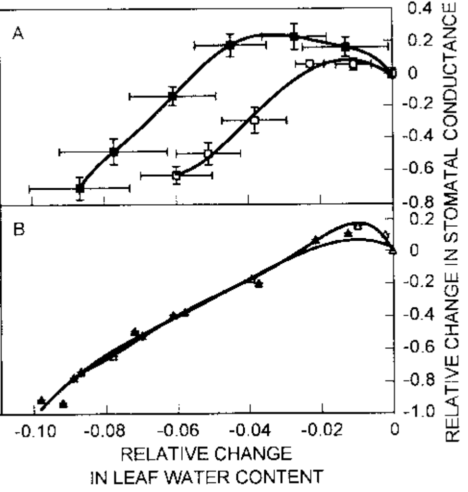

Fig. 2. Time courses of the relative rate of stomatal closure. Closure started at t = 0. Symbols as in Fig. 1.

Fig. 3. Dependence of the relative water loss from the leaves on the relative change in stomatal conductance. Symbols as in Fig.1. As the curves in ozone-tolerant cultivar (*B*) are very similar, SE are not shown.

The drop in leaf water content during stomatal closure was more pronounced in ozonated compared to control plants in cv. BelW3. In cv. Samsun, however, the drop in leaf water content of ozonated and control plants was similar (Fig. 3*B*) and similar to that in ozonated BelW3 plants.

The differences in the ability of stomata to regulate leaf water content (Fig. 3) may be explained by above mentioned formulas for interdependence of changes in stomatal conductance and leaf water content. Water loss from the leaf should be more pronounced if v_g was lower and E_{max}/x_0 was higher.

It was found that water loss from the leaves of cv. Samsun was quite large (Fig. 3*B*). As stomata closed slowly after leaf detachment in control plants of this cultivar, and as leaf water content of intact leaves increased with increasing transpiration under ozone treatment, it is probable that leaf water content was not regulated by stomata in this cultivar. Rapid elastic or osmotic adjustment in mesophyll cells seems to be the case. Stomatal regulation proved evidently more important only after ozone exposure (Fig. 3*B*) when E_{max}/x_0 ratio was higher. Evidently we measured large changes in leaf water content during stomatal closure only because detached plants were used. Leaf water loss should not be large if water stress arises in intact plants, because water content of cells should be rised due to osmotic adjustment if additional water from the roots can flow to the leaf.

In contrast, it can be supposed that rapid stomatal closure is important in regulation of leaf water content in ozone-sensitive plants. Loss of water from the leaves of control ozone-sensitive plants was not large (Fig. 3*A*). More water was lost under ozone exposure mainly due to the longer transient opening of stomata. This points out the effect of ozone on epidermal cells in ozone-sensitive cultivar.

In conclusion we suppose, that plant tolerance to ozone can be predicted from stomatal regulation of water loss. We hypothesize that leaf water content is regulated mainly by osmotic (or elastic) adjustment in ozone-tolerant plants, but mainly by stomata in ozone-sensitive plants.

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