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<Review>

Stomatal Response by Ozone

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오존에 대한 식물 기공 반응 고찰

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ABSTRACT

Stomatal closing by ozone and water stress could reduce further ozone injury by inhibition of ozone influx to the tissue. Direct effect of ozone on stomata can be explained from two aspects which are a stimulation of stomatal closing and an inhibition of stomatal opening. An increase of Ca^{2+} influx into cytoplasm by ozone could stimulate potassium efflux ion channel and inhibits inward potassium ion channels. By this mechanism ozone could induce stomatal closing. On the other hand, ozone could inhibit stomatal opening by affecting the activity of H^+ dependent ATPase of the membrane in guard cells. This would inhibit proton efflux which precede stomatal opening. It is also possible that ozone could reduce the activity of photosynthesis in guard cells which lead to affect the production of osmotically active sugars and energy.

Indirect effect of ozone to stomata is through the effect of CO_2 elevation as a result of damage of the photosynthetic machinery. This indirect effect is slower than the direct effect.

Key words: Stomatal closing, Ozone, Photosynthesis.

INTRODUCTION

Pollution of ambient air by ozone occurs frequently and is widespread in North America, Europe and other industrialized regions of the world (Reich *et al.* 1985). Hewitt *et al.* (1990) reported that typical mid-latitude ozone concentrations have more than doubled in the past 100 years. Because of such an increase of ozone concentration, ozone, alone or in combination with sulfur dioxide (SO_2) and or nitrogen dioxide (NO_2), is responsible for up to 90% of crop losses caused in the United States by air pollutants (Heck *et al.* 1982). The effect of O_3 on grain yeild can be linked to a decline in photosynthesis (Reich and Amundson 1985). One of the major controlling factors influencing levels of carbon fixation is stomatal conductance. Stomata play a critical role in the regulation of plant uptake of not only CO_2, but ozone and other gaseous pollutants.

Question remains what is the role of the stomata between crop losses and ozone exposure. This review suggests a possible role of stomata between crop losses and ozone exposure. This paper suggests a possible role of stomata and a hypothesis how stomata close in response to ozone.

Uptake of ozone

Access of ozone into the leaf is mainly through the stomata. Moist surfaces within the leaves(e.g. the extracellular fluid of mesophyll tissues) allow ozone to dissolve and diffuse down a concentration gradient similar to that of carbon dioxide. Ozone is approximately one-third as soluble as carbon dioxide. This means that the mesophyll tissue is the primary site of deposition of less soluble gas. It is estimated that over 70% of total cells the stomata may be less sensitive to O_3 and this may cause the mesophyll cells to be attacked first(Moldau *et al.* 1990). The rate of deposition could be influenced by solubility, rate of decomposition and pH of the medium(Wellburn 1987).

Cuticular uptake of O_3 appears to take place partially. Especially when stomata is closed, the rate of deposition through cuticular epidermis will be increased. Recent application of carbon isotope discrimination method have identified that gas phase conductance under chronic O_3 exposure is affected more than that of the liquid phase(Greitner and Winner 1988, Martin *et al.* 1988). This means that O_3 is decomposed in stomatal pores or substomatal cavities. The rapid decomposition of O_3 damages cell walls and plasmalemma. This may cause increased uptake of O_3 into heavily injured epidermis.

Stomatal closing by ozone

At concentrations below 200 ppb ozone, a diversity of stomatal response depending on species has been reported, wide opening in two species, closure in four species and no changes in a further species(Darrall 1989). Darrall(1989) reported that the response of stomatal conductance to ozone below 200 ppb is highly varible and appears to be both species- and cultivar-dependent. Most of recent reports demonstrate the reduction of stomatal conductance in long term exposure at less than 100 ppb ozone(Sanders *et al.* 1992, Saxe and Murali 1989) and at 160 ppb ozone(Moldau *et al.* 1990).

The phenomenon of stomatal closure caused by long-term ozone exposure at low concentration(chronic stress) could be the result of the stress-avoidance, the reduction of net assimilation and accelerated aging. Stomatal closure of this type may be a stress-avoidance mechanism which prevents further ozone uptake by the plant(Takemoto *et al.* 1988). This stress-avoidance mechanism can be explained as a defensive mechanism to maintain homeostasis. Otherwise, it could be the result of the decrease of the net assimilation and thereby increase internal CO_2 concentration in intercellular spaces to cause stomata to close.

Reich *et al.*(1985) reported that abaxial conductance increased as leaves aged from 4 to 10 days and then declined with further aging. Likewise, ozone accelerates leaf aging in poplar leaves(Reich 1984). The phenomenon of plant aging includes general decline in the responsiveness of the stomata. The earlier decline in carbon uptake rate with increasing O_3 stress can be related to the accelerating effect of O_3 on leaf senescence(Grandjean and Fahrer 1989), which involves a reduction in the amount and activity of Rubisco(Lehnherr *et al.* 1987, Dann and Pell 1989). Thus, the effect of O_3 on CO_2 assimilation was accelerated by O_3, but significant treatment effects only occurred in older leaves during senescence. It could be speculated that the inhibition of photosynthesis by O_3 is due to metabolic changes during leaf senescence rather than to direct damage of O_3 to thylakoid membranes(Grandjean and Fuhrer 1992). This indicate that in young leaves, O_3 has a direct negative effect on the stomatal opening, whereas in older leaves, this effect is overruled by decreased CO_2 concentrations in the mesophyll.

Above 200 ppb, stomatal closure was found to occur in majority of species(Darrall 1989). Saxe and Murali(1989) found that inhibition rate of transpiration of Norway spruce was greater than 50% when the tree was exposed to ozone over 300 ppb for 14 hours. Olszyk and Tibbitts(1981) also found over 50% inhibition of stomatal opening when plants were exposed to ozone at 270 ppb for 2 hours. Hill and

Littlefield(1969) reported that stomatal width of oats plants exposed to 600 ppb ozone was immediately decreased to near zero.

Water stress reduce ozone injury through stomatal closing

Reduced ozone sensitivity was observed with increasing moisture stress(Adedipe *et al.* 1973, Reich *et al.* 1985, Temple 1986, Tingey and Hogsett 1985). Reduced sensitivity to ozone can be explained in two ways. Firstly, water stress causes a decrease in the water potential of leaf and leads stomata to close. This closing will inhibit ozone uptake to the tissue. Otherwise, it could reduce ozone sensitivity through physiological, anatomical and biochemical change which is unknown. Schulze *et al.*(1980) reported that soil water stress modified diurnal varience in leaf conductance and gas exchange and decreased photosynthetic metabolism in leaf.

In field studies, O_3 injury was greater on plants grown in moist soil than those grown in dryer soils (Dean and Davies 1967, Taylor *et al.* 1960, Walker and Vickery 1961) and the injury intensity was proportional to the amount of irrigation water applied (Dean and Davies 1967, Walker and Vickery 1961). Plants that were water stressed just prior to O_3 exposure showed little or no foliar injury compared to well-watered plants (Harkov and Brennan 1980, Khatamian *et al.* 1973, Markowski and Grzesiak 1974, Olszyk and Tibbitts 1981). Only a few days of water stress were sufficient to protect plants from O_3 injury (Taylor *et al.* 1960, Tinget *et al.* 1982). When water stress was eliminated, plants rapidly regained their O_3 sensitivity(Dean and Davies 1967, Tingey *et al.* 1982). Stomata of water stressed plants opened to a smaller degree, closed earlier during the day, and also closed more rapidly in the presence of O_3(Dean and Davies 1967, Rich and Turmer 1972). The results cited above indicate that water stress treatments reduced plant injuries to O_3 probably through partial stomatal closure. Tingey and Hogsett(1985) also examined whether water-stress induced reduction in O_3 sensitivity resulted from short term physiological alterations within the plant or from reductions in stomatal aperture. They used abscisic acid and fusicoccin for artificial aperture controls. They found that both water stress and abscisic acid induced stomatal closing and reduced ozone injury. In water stressed plants, fusicoccin induced stomatal opening and those plants were as sensitive to ozone as were the non-water-stressed plants. They suggested that water stress protects plants from ozone injury mainly through its influence on stomatal aperture rather than through biochemical or anatomical changes. However, Heck *et al.* (1977) suggested that water stress during plant growth (prior to O_3 exposure) reduced plant sensitivity to O_3 through physiological changes within the plant, while water stress during exposure reduced plant response through stomatal closure. It is possible that water stress may induce changes in plant anatomy and thereby influence O_3 sensitivity. However, it is more likely that the primary means by which water stress protects plants against O_3 injury is through stomatal closure rather than through physiologic changes within the plant.

Is stomatal closing by ozone related to the reduction of photosynthesis?

Ozone may affect stomatal behavior in three ways. Firstly, ozone exposure may cause direct damage to guard cell function, reducing the biological control of stomatal conductance at a whole leaf level. Secondarily, ozone exposure may indirectly alter guard cell responses as a result of secondary effects from damage to the photosynthetic machinery in the leaf mesophyll(Jones 1983). Thirdly, ozone may affect both guard cell and mesophyll functions simultaneously. The third possibility has been shown by the studies of Furukawa *et al.*(1984), who found that ozone-induced reductions in net photosynthesis(*Pn*) and transpiration rate in sunflower and two popular species(hybrids) were linearly related and comparable

in magnitude.

However, Sanders *et al.*(1992) reported that stomatal conductance and to a lesser extent *Pn* were depressed during ozone exposure. There are many supporting reports that the stomatal conductance of *P. vulgaris* was inhibited earlier, and to a greater extent than was *Pn* following the onset of ozone exposure (Coyne and Bingham 1978, Heath 1980, Hill and Littlefield 1969, Moldau *et al.* 1991, Takemoto *et al.* 1988). Under these conditions, the net CO_2 fixation rate remains unchanged initially. This causes decrease of leaf internal CO_2 concentration, which indicates that the initial response of stomata under high concentrations of O_3 is not mediated by leaf internal CO_2 concentration but is caused by a direct effect of the pollutant to the cells of the stomatal complex (Moldau *et al.* 1991, Roper and Williams 1989).

Visible symptoms of ozone injury or significant physiological changes are clearly depend on the concentrations and the period of ozone treatment. For example, ozone exposures of <100 ppb for 7 hours per day(45~99 days) have been shown to no major changes in the chlorophyll and carotenoid pigments level, but considerable cellular disruption (Sanders *et al.* 1992), an inhibition of photosynthesis (Saxe and Murali 1989, Reich and Amundson 1985), reductions in the amount and activity of ribulose-bisphosphate carboxylase(Pell and Pearson 1983, Lehnherr *et al.* 1987, Dann and Pell 1989). Ozone exposures of >200 ppb for a few hours per day have been shown induce pigment breakdown(Sakaki *et al.* 1983, Smith *et al.* 1990, Rufner *et al.* 1975), an inhibition of photosynthesis(Black *et al.* 1982, Saxe and Murali 1989), membrane rupture in mesophyll cells(Pell and Weissberger 1976), and changes in the protein content of leaves(Price *et al.* 1990), tonoplast rupture(Harris and Dodge 1972). Such responses are indicative of cellular and tissue death, and fall within the definition of acute ozone stress(Heath 1980). From the above reports mentioned it is clear that ozone causes serious damage to photosynthetic apparatus depending on exposure concentration and period. The measurements of chlorophyll fluorescence also showed that even though there was no serious damage to photosystem Ⅱ, a generally reduced rise in fluorescence was observed(Barnes *et al.* 1990, Farage *et al.* 1992, Grandjean and Fuhrer 1989, Reich *et al.* 1985). Hill and Littlefield(1969) reported simultaneous reduction in apparent photosynthesis and transpiration and stomatal closure in response to O_3 in a variety of plant species. Certainly, a general correlation exists among light intensity, mesophyll assimilation and conductance(Morison 1987). Since C_i declines as assimilation increases, and since conductance in many cases increases with decreasing C_i, it has been supposed that assimilation controls conductance by affecting changes in C_i(Raschke 1976). Stomatal closure with pollutant exposure was correlated with the extent of leaf injury in some studies(Amiro and Gillespie 1985, Buttler and Tibbitts 1979, Rosen *et al.* 1978, Olszyk and Tibbitts 1981, Sanders *et al.* 1992, Temple 1986).

It can be suggested that ozone induces stomata to close by direct effect on guard cells. Initial stomatal closing by ozone belong to this response. However, if the longer ozone exposure last or the higher ozone concentration is treated, serious damages of photosynthetic mechanism couldn't be avoided and this would increase intercellular CO_2 concentration. Stomatal closing by the elevation of CO_2 could be explained as an indirect effect of ozone on stomata.

Stomatal closing by ozone inhibit photosynthesis

Photosynthetic rate could be reduced as a result of O_3-induced stomatal closure. There have been controversial reports about the differential effect of O_3 on carboxylation and CO_2 uptake. The relatively stronger effect of O_3 on carboxylation could explain the reduction in water use efficiency(Farage *et al.* 1991, Matyssek *et al.* 1991, Reich and Lassoie 1984, Reich *et al.* 1985). Water Use Efficiency(WUE) is defined as CO_2 assimilation (mg) per H_2O lost (g). Temple (1986) reported that conductance was more sensitive than transpiration to inhibition by O_3. This means that water use efficiency is low. The facts that stoma-

tal conductance was inhibited earlier than was net photosynthesis indicate that stomatal closing by ozone inhibit photosynthesis by reducing CO_2 uptake. On the contrary, there was no consistent correlation between O_3-induced reductions in WUE and in photosynthesis, indicating that the effects on WUE and photosynthesis were not directly related and that O_3 probably affects each in distinct ways(Reich and Lassoie 1984). Lehnerr *et al.*(1988) also reported that activity of ribulose-bisphosphate carboxylase is more sensitive to O_3 than CO_2 diffusion. The O_3-induced reduction in photosynthesis was not due to an increase of both an increase in gas-phase resistance to CO_2 and the impairment of photosynthetic mechinery in mesophyll.

Possible stomatal closing mechanism by ozone

It is not well understood how stomata close in response to ozone, but mechanisms of ozone damage to stomatal control will involve several components of guard cells and mesophyll cells.

1. pH changes of guard cell wall and an increase of membrane permeability to K^+ efflux.
2. An possible increase of guard cell cytosolic calcium concentration.
3. The production of stress ethylene.
4. Inhibition of photosynthesis of guard cells and mesophyll cells.
5. Inhibition of guard cell membrane H^+-dependent ATPase.

The first sites of initial O_3 attack to stomatal control will take place in epidermal and guard cell walls. Once in solution, ozone immediately forms other derivatives which show varing degree of reactivity. Amongst these include hydroxyl radicals, monatomic oxygen, hydrogen peroxide or superoxide radicals($O_2^{\cdot -}$). Initial response of O_3 attack in cell wall is the change of the ionic environment, including H^+. A greater pH change(release of hydroxyl ion or uptake of protons) is observed upon O_3 solubilization and subsequent reaction at lower pH, suggesting that a protonation of O_3 upon water solubilization releases a net hydroxyl ion to the solution(Selm 1959, Gurol and Singer 1982). Alkalization by releases of a net hydroxyl ion could affect stomatal aperture. Some information about the pathway taken by the ions moving across the stomatal complex in *Commelina* was obtained by measuring the activity of K^+ and Cl^- in the apoplast(Bowling 1987). Bowling(1987) found that when stomata opened there was a rise in ionic activity in the wall of the epidermal cells. The release of ions into the apoplast by the epidermal cells as stomata open suggests that there is a signal originating in the guard cells which passes to the epidermal cells(Smith and Bowling 1990). Edwards *et al.*(1988) suggested that this signal was the wave of pH change which moves across the stomatal complex when the stomata are stimulated to open. Smith and Bowling (1990) put forward a hypothesis in which acidification of the epidermal cell apoplast results in the release of potassium which subsequently moves to the guard cell to be accumulated as part of process of stomatal opening.

Thus alkalization of the epidermal cell walls by ozone attack could inhibit a release of potassium from cytoplasm to apoplast, thereby reducing potassium influx to guard cells. It is known that pH change of the wall can influence transport of ions and sugars(Lefebvre and Giller 1973, Tromballa 1974, Schwab and Komor 1978). Stomata open wider in low pH and a rise in pH, similar to the effects of O_3 alters these transport properties(Heath 1988). Thus the changes of pH in the cell wall could inhibit full stomatal opening.

The main factors which cause changes of membrane structure are both of ozonolysis and peroxidation. By these mechanisms, hydrocarbons are divided and alternate double bonds and degradation to single bonds are produced. Both mechanisms also produce radical($OH^{\cdot}$) and organic radicals. The radicals can attack biological materials such as protein or lipids of membrane. Alternately, other radicals such as hydroxyl($OH^{\cdot}$) and peroxyl($OH_2^{\cdot}$) can be produced by interactions with an aqueous solvent. The reaction of hydroxyl radical with unsaturated

fatty acids and amino acids in cell membrane damages the cellular permeability barrier and the reduction of its binding capacity. The increased permeability causes the loss of intracellular K^+ levels would be expected to displace Ca^{2+} and that the depletion of extracellular Ca^{2+} further increases the membrane permeability to K^+. It is clear that ozone-induced stomatal closing is partly obtained by the efflux of K^+ ion. However, it is unclear that how ozone trigger K^+ ion to leak from the membrane and how K^+ ion can displace Ca^{2+}.

Normally, opposite responses happen when stomata close. Namely, stimulus-induced increases in $[Ca^{2+}]$ cyt mediate stomatal closing. The Ca^{2+} entering guard cells then acts as a second messenger to regulate the ion fluxes that determine guard cell turgor (McAinsh *et al.* 1990). Schwartz (1985) reported that the inwardly conducting K^+ channel was inhibited by 0.1μ M concentrations of cytosolic calcium. This report provides a link between stimulus-induced increases in $[Ca^{2+}]$ cyt and the inhibition of stomatal opening. Ca^{2+} stimulate potassium efflux ion channel and inhibits inward potassium ion channels, thereby inducing stomatal closure. Stomatal closure, on the other hand, may be initiated by the calcium-mediated release of anions and cations from the vacuole (Mansfield *et al.* 1990). The concurrent opening of calcium-activated chloride channels may depolarize the plasma membrane sufficiently to activate the outwardly conducting K^+ channel (Schroeder 1988). This would provide a mechanism whereby a stimulus-induced increase in $[Ca^{2+}]$ cyt could initiate stomatal closure (Schroeder and Hedrich 1989). This mechanism could happen in ozone-induced stomatal closing.

Accumulated evidence over recent years has shown that perturbation of calcium homeostasis is an important event in the cytotoxicity of oxidising stress to animal cells (Bellomo *et al.* 1984, Cotterrill *et al.* 1988, Jewell *et al.* 1982). In hepatocytes, oxidative stress causes an increase in cytosolic calcium (Jewell *et al.* 1982). The oxidation of glutathione precedes a flux of calcium into the cytoplasm from internal and external sources (Orrenius and Bellomo 1989). Price (1990) showed that similar events occurred in the detached epidermis taken from leaves of *Commelina communis*. He found that paraquat (10^{-4} M) and hydrogen peroxide (10^{-3} M) caused a marked reduction in stomatal aperture when incubated in the incubation medium of illuminated eqidermal strips. Castillo and Heath (1990) also reported that ozone stimulted a higher influx of Ca^{2+} and lost the ability of extruding Ca^{2+} out of the cell.

This suggests an hypothesis that an increase of Ca^{2+} influx into cytoplasm could stimulate potassium efflux ion channel and inhibits inward potassium ion channels, thereby inducing stomatal closure. However, it need more information to be clarified whether this response is common in many plant species.

In water stress, conditions abscisic acid (ABA) produced or imported into leaves, and acting on the outside of the guard cell induces net loss of potassium salts, and hence stomatal closing. ABA-induced efflux transients can occur in very low external Ca^{2+}, but the reduction in the presence of La^{3+} suggests that Ca^{2+} influx is required for the response (MacRobbie 1992). It seems likely that increases in cytoplasmic Ca^{2+}, at least locally, are a universal feature of the ABA response even though there is still argument about the nature and generality of ABA-induced changes in the level of free cytoplasmic Ca^{2+} in guard cells. Therefore, possible involvement of ABA on ozone induced stomatal closing is crucial point in our understanding. However, few studies have been carried out to investigate atmospheric O_3 effects on the level of ABA. Hur (1993) reported that levels of ABA were significantly increased by low level of O_3 fumigation (30 ppb) but significantly decreased by exposure to 80 ppb O_3 on *Azolla pinnata* R. Br. Under 100 ppb O_3 concentrations stomatal response is variable as explained earlier. His results only suggest that ozone could affect the level of ABA in the plant. An important point is whether ozone could affect the level of ABA or not and to conclude the possible involvement of ABA on ozone response, it require further information.

Other possible factor which can affect stomatal ap-

erture in response to ozone is stress ethylene. It has been shown in many plant species that O_3 induced the production of stress ethylene. Investigators have

Finally, even if the role of guard cell chloroplasts is still controversial, ozone could affect photosynthesis in guard cells. This photosynthetic carbon fixation

photosynthesis has to be studied since it could supply energy to fuel proton efflux and osmotically active sugars. It is possible that ozone could damage photosynthetic apparatus including photosynthetic enzymes in guard cells as observed in the mesophyll.

A decline of photosynthetic activity in the mesophyll can be explained as an indirect effect on stomatal closing by ozone since it is known that stomatal responses to CO_2 is a general phenomenon (Morison 1987). This indirect effect could be increased by long term ozone exposure.

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적 요

오존 및 수분스트레스에 의하여 유도되는 기공닫힘은 조직 내로 오존의 유입을 차단함으로써 부가적인 오존 피해를 감소시킬 수 있다. 기공에 작용하는 오존의 직접적인 효과는 기공닫힘의 촉진과 기공열림의 억제라는 두 가지 관점에서 설명될 수 있다. 오존에 의해 유도되는 세포질내 Ca^{2+} 농도의 증가는 칼륨방출 이온채널을 촉진할 수 있으며 또한 칼륨흡수채널을 억제할 수 있다. 이런 기작을 통해 오존은 기공닫힘을 유도할 수 있는 것으로 보인다. 반면에, 오존은 공변세포막에 존재하는 H^+-dependent ATPase의 활성에 영향을 줌으로써 기공열림을 억제할 수 있다. 이 효소의 억제는 기공열림에 선행되는 양성자 방출을 억제할 것이다. 오존은 또한 삼투압에 영향을 주는 당 및 에너지의 합성을 좌우하는 공변세포내 광합성 활성을 감소시킬 수도 있다.

기공에 대한 오존의 간접적인 효과는 광합성 기작에 손상을 초래함으로써 세포내강속 CO_2 농도를 증가시키는 과정을 통해 이루어진다. 이 간접적인 효과는 직접적인 효과보다 느리게 나타난다.

주요어: 기공닫힘, 오존, 광합성

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