The effects of ozone on the plant cell

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Abstract

The article presents current views on the effects of ozone on the ultrastructure of plant cells, changes in winter hardiness and on some of the resistance responses of cells to the toxicity of this gas and its derivatives.

Key words: ozone, ultrastructure, winter hardiness, cellular resistance responses

INTRODUCTION

Ozone (O_3) which is part of the ozonosphere, that is, a layer of the atmosphere at a distance of over 20 km from the earth's surface, protects living organisms from ultraviolet radiation. Unfortunately, the amount of ozone in this layer is decreasing (the so-called "holes" in the ozone layer). An international convention was signed in 1987 in Montreal ("Montreal Protocol on Substances that Deplete the Ozone Layer"). Under the Protocol, a 50-per-cent cut will start on July 1, 1998 of the production and the emission of substances that deplete the ozone layer. This is certainly not enough — the emission of freons (chlorofluorocarbons), the destructive compounds, should be halted immediately. The wider the ozone hole, the more ultraviolet radiation reaches the biosphere. This is related not only to increased incidences of skin cancer in humans, but also with far reaching changes in microorganisms, especially marine forms.

Ozone is beneficial for living organisms only when it is tens of kilometers from the earth, forming the ozone layer. At close range it can be harmful and pollutes the environment.

In recent years, ozone has become the most well-studied of the three major gases (SO_2 , NO_x and O_3) polluting the atmosphere over highly industrialized areas.

Ozone and PAN (peroxyacetyl nitrate) are secondary pollutants which form in an atmosphere from NO_2 as the product of photochemical reactions.

Ozone can also react with NO and NO_2 is formed again:

Thus, when the atmosphere is polluted by these two compounds, the ozone level will not be very high. It increases, however, when a third pollution compound is present — gaseous hydrocarbons. They can transform NO to NO_2 , and further to PAN without using up the ozone. When NO disappears under certain conditions (the energy of sunlight), ozone accumulates. From the 1950's the concentrations of ozone in the atmosphere over Europe have been increasing, mainly due to the increase in the emission of hydrocarbons and nitrogen oxides by automobiles.

The lower parts of the atmosphere, far from large emission sources contain $40\text{--}80 \mu\text{g m}^{-3}$ ozone ($0.02\text{--}0.04 \text{ ppm}^{-1}$). In cities the concentration of ozone generated by photochemical reactions may periodically reach levels of $300\text{--}600 \mu\text{g m}^{-3}$ ($0.15\text{--}0.3 \text{ ppm}$) (Lendzian and Unsworth 1983).

Ozone is removed from the atmosphere mainly by gaseous uptake by soil and plants and by enters into reactions with other compounds (e.g. with the already mentioned NO).

Ozone enters plants through the stomata, which in many species, respond

¹ ppm — parts per million; 10^6

to ozone by partially closing (Hill and Littlefield 1969, Rich and Turner 1972). Such species are more resistant to ozone (Rich and Turner 1972, Adedipe et al. 1973, Knudson et al. 1979).

THE SYMPTOMS OF OZONE INJURY

After one hour of ozone treatment the first macroscopic symptom of injury are water-logged flecks on the adaxial surface of the leaves. After 3 hours fumigation, irregularly shaped, yellow-green spots appear within the water-logged regions of leaves, and later (after 7 hours) the leaves are slightly wilted and partly browned (Miyake et al. 1984).

In spinach leaves similarly as in radish leaves, the palisade and spongy parenchyma cells were almost equally ozone-sensitive (Athanassious 1980, Miyake et al. 1984). In other species, however, the cells of the palisade parenchyma were more sensitive (Heath 1980).

In the leaves of 3-week-old radish seedlings, the paravenial cells (cells bordering the vascular tissue) reacted almost immediately after mild ozone treatments (≤ 0.5 ppm O_3 , 2-4 h, 5.2 l min^{-1}) by exhibiting plasmolysis. Somewhat later (2-4 h), the vesiculation of chloroplast thylakoids and granulation of stroma were evident and the more distant mesophyll cells had undergone plasmolysis (Athanassious 1980). The cisternae of the Golgi bodies in the yellow-green regions of the leaves of 4-week-old spinach seedlings were swollen after 3 hours of fumigation. Swelling of the cisternae of the endoplasmic reticulum and nuclear envelope also occurred after a similar period of time. The mitochondria exhibited the shrinkage of the cristae. Later (after 5 hr), the entire chloroplasts were deformed (Miyake et al. 1984).

Higher ozone concentrations (0.5-1.0 ppm for 2-4 h) caused damage to cell membranes, however, the outer membranes of both chloroplasts and mitochondria remained intact. The destruction of cell membranes led to the collapse of the vacuole, due to which organelles and cytoplasm collected in the center of the cell. The organelles which were found inside the partially coagulated mass had relatively intact membranes. Damage to the radish outer chloroplast membrane became apparent 24 hrs. after fumigation. The final stage of the damage was the complete coagulation of the cell's contents (Athanassious 1980, Miyake et al. 1984).

The process of changes in the cell's structure can be divided into two stages, early (reversible) — characterized primarily by changes in chloroplast ultra-structure and the second stage (irreversible) characterized by the breakdown of cellular membranes (Athanassious 1980). The reversible stage of ozone-damage is regarded as a symptom of water deficit (Thomson et al. 1966, Swanson et al. 1973, Miyake et al. 1984). The irreversible stage is most probably the result of the direct action of ozone on membranes (Athanassious 1980).

sious 1980). Ozone can easily be decomposed in the air and in water. When ozone breaks down in water, it is thought that large amounts of hydroxyl ion, hydroxyl radical and hydrogen peroxide are formed (Heath 1979). Hydroxyl radical is particularly dangerous to cells since they belong to the most reactive species of oxygen and there are no known protective mechanisms within the cell. One of the substances intercepting free radicals is vitamin E (tocopherol) (Boguth 1968, Tappel 1972).

Ozone attacks mainly unsaturated fatty acids in the cell (Criegee 1975) as well as compounds containing sulfhydryl groups (Heath et al. 1974) and ring structures (Mudd et al. 1969). All three types of compounds are constituents of the plasmalemma. The permeability of the plasmalemma for K^+ was drastically increased after introduction of ozone into a culture of *Chlorella* cells (Chimiklis and Heath 1975, Heath 1975, Heath and Frederick 1980). The permeability of bean leaf cell membranes was also enhanced by ozone (Evans and Ting 1973, Perchorowicz and Ting 1974). These changes were rapidly reversible when ozone was removed from the medium. Prolonged incubation with ozone, however, leads to irreversible changes in permeability. Ozone also weakened the mechanical properties of the plasmalemma (Cailoux et al. 1978) and disturbed the water potential of the cell. A net water loss was observed immediately after exposure to ozone (Evans and Ting 1973, Elkiey and Ormrod 1979).

The negative effects of ozone were less severe in the cells of leaf vascular bundles than in the cells lying between the bundles. Some of the vascular parenchyma cells, especially in the main bundles, remained intact even when the cells between the bundles were almost completely necrosed at this time (Miyake et al. 1984).

Most of the injuries caused by ozone were similar to those caused by sulphur dioxide and the herbicide, paraquat — swelling of the thylakoids (Harvey and Fraser 1980), and breakdown of cell membranes (Baur et al. 1969, Harris and Dodge 1972). Both ozone and paraquat are sources of hydroxyl and other radicals in the plant cell (Dodge 1975, Hoigne and Bader 1975). They cause the peroxidation of lipids, increased permeability of cell membranes (Pauls and Thompson 1980) and disruption of membranes (Dodge 1975). For this reason the swelling of thylakoids and shrinkage of cristae in the early stages of ozone injury are considered to be related to changes in membrane permeability. The breakdown of the organelles, however, may be caused by the upset of the osmotic balance and/or the release of hydrolytic enzymes due to the disruption of the tonoplast and plasmalemma (Harris and Dodge 1972).

THE EFFECTS OF OZONE IN THE DARK AND IN LIGHT

Light is an important factor influencing the phytotoxicity of ozone. The leaves of peas and tomatoes showed more necroses after exposition to ozone in

the light than in the dark (Olszyk and Tingey 1984). The increase in ozone toxicity in the light was related, at least partially, to increased O_3 uptake (open stomata). When peas were pre-treated with fusicoccin (a fungal metabolite that induces stomatal opening in light or dark) ozone was also toxic in the dark (Fig. 1). At an ozone concentration of 0.1 and 0.2 $\mu l l^{-1}$ the plants treated with fusicoccin showed more injury in the light than in darkness. This was, at least partially, connected with the higher ozone uptake in the light (Olszyk and Tingey 1984).

Fig. 1. Leaf injury to +fusicoccin (+FC) peas exposed to O_3 for 2 h in the light or dark, and leaf injury to control plants. (According to Olszyk and Tingey 1984 — modified)

OZONE AND WINTER HARDINESS

The widespread decline of conifers and broadleaved trees in Europe and the U.S.A. is gaining in extensiveness and intensity (Blank 1985, Krause et al. 1986). The caused of this phenomenon are unknown, although it is commonly attributed to a complex of stresses including compounds polluting the air (Krause et al. 1983, Mc Laughlin 1985). In Europe, the most important of these compounds seems to be ozone. Its strong toxicity is well known and, besides that, a correlation exists between ozone formation and distribution in the atmosphere and the decline of forests (Arndt et al. 1982, Prinz et al. 1982). The ozone concentrations in the FRG sometimes reach peak values of over 600 $\mu g m^{-3}$ (Krause et al. 1986). Such concentrations are high enough to cause acute injury to sensitive plant species (Roberts 1984,

Skärby and Selldén 1984). However, in relatively resistant plants (spruce, fir), controlled fumigation did not evoke symptoms comparable with those in declining forests. In particular, it was not possible to produce injury on older needles without having a similar effect on younger needles (Krause et al. 1983, Skeffington and Roberts 1985). At that point, several authors have suggested that the decline of tress, although involving air pollution, may be triggered by frost or drought (Rehfuess 1986, Rehfuess and Ziegler 1986). There is little experimental evidence in support of this claim. Recently some has come from an experiment in which clones of 3-year-old *Picea alba* trees were fumigated with ozone in the summer ($> 204 \mu\text{g O}_3 \text{ m}^{-1} = 0.1 \text{ ppm}$) and inadvertently exposed to a sudden early frost (Brown et al. 1987). Although there were no unfrosted control trees, the severe injury that developed only on fumigated previous year's needles indicated that exposure to ozone might have indirect effects many weeks later, most probably through reducing the plant's resistance to low temperatures.

In order to investigate whether ozone affects freezing resistance, pea, which is very convenient model plant, was recently used (Davison et al. 1988). The fluorescence kinetics of chlorophyll was also examined in the experiment, since it allows the early detection of ozone injury. Frost reduced the maximal rate of rise of induced fluorescence while in the plants exposed to ozone, it was almost entirely eliminated. This effect was associated with an increase in leakage of electrolytes from leaf discs (Fig. 2) and a drop in the number of individuals surviving low temperatures (Fig. 3). Similar experiments were conducted on stem segments of cloned 3-year-old spruces (Barnes and Davison 1988). No visible injury was found on the current year's needles during the fumigation, hardening period as well as from low temperatures. Low temperatures did cause extensive visible injury (in the form of severe, uniform necrosis) to the previous year's needles only to some of the ozone-treated spruce clones. The affected needles began to fall prematurely. Ozone also significantly reduced the induced fluorescence of chlorophyll in the clones that showed frost injury. The authors suggest that O_3 -induced membrane injury increased the sensitivity of the needles to freezing. One of the clones did not show any effect of ozone on chlorophyll fluorescence kinetics, but showed severe ozone-induced injury of the previous year's needles. The injury developing about 40 days after fumigation independent of the temperature. These needles were characterized by increased cuticular transpiration which allows it to be concluded that ozone may have induced gradual desiccation of the needles. Ozone could have reacted with unsaturated fatty acids (Heath 1980) which are common constituents of the epicuticular waxes (e.g. in spruces and firs — Beri and Lemon 1969) and could have increased the structural degradation of the waxes (Barnes et al. 1988). Water loss through the cuticle was increased due to the degradation of the epicuticular waxes. It should be noted here that the trees showing symptoms of decline were characterized by

a particularly distinct degradation of waxes on the older needles (Sauter and Vogl 1986) and the ozone-damage was restricted to the older spruce needles (Barnes and Davison 1988). There is also the possibility that in certain concentrations, ozone inhibited stomatal closure or damaged epidermal cells (Black and Black 1979).

Figs. 2 and 3. Effects of 0.15 ppm ozone on response of pea cv. Alaska to sub-zero temperatures. Fig. 2 — Percentage leakage of electrolytes. Fig. 3 — Survival score 0-10 scale. (According to Davison et al. 1988 — modified)

Thus, the data obtained recently indicates that ozone predisposes plants to freezing injury, winter desiccation and drought, and should therefore be counted among the stress factors contributing to the decline of high-altitude forests in Europe (Barnes and Davison 1988, Barnes et al. 1988, Davison et al. 1988).

RESISTANCE RESPONSES IN PLANTS

The response of plants to stress may be by stress avoidance, e.g. by shedding leaves, closing stomata, or by stress tolerance (Levitt 1972). It seems that resistance is subject to hormonal regulation. One of the consequences of stress factors, including ozone, are changes in the levels of endogenous phytohormones. These changes can be either a signal (or signals) inducing plant responses or only concomitants of plant responses to stress factors. The causal relationship between stress factors, phytohormones and the plant responses to stress are still only little understood (Meyer et al. 1987).

These relationships have been relatively studied the best in respect to ethylene. Ozone, similarly as other stress factors (e.g. SO_2 , heavy metals, phytopathogens) induce a very pronounced ethylene formation (stress ethylene — Åbeles 1973) in many species of plants (Craker 1971, Tingey et al. 1976, Adedipe and Tingey 1978, Lieberman 1979, Tingey 1980, Hogsett et

al. 1981, Stan et al. 1981, Krimmerer and Kozłowski 1982, Stan and Schicker 1982, Rodecap and Tingey 1983, Chappel et al. 1984, Li 1984, Pell and Puente 1986). Stress ethylene synthesis started after a lag-phase of 12-18 minute after the start of ozone fumigation (Hogsett et al. 1981), therefore stress ethylene began to be produced before visible injuries occur and therefore gives a very valuable message of latent pathological changes. When the degree of injurious due to fumigation rose, so did the synthesis of ethylene, after which, when the area covered by necrosis was large, ethylene production rapidly declined (Li 1984). Ethylene can only be synthesized by living cells (Yang and Hoffmann 1984), and the enzymes engaged in this synthesis are associated with the plasmalemma (Mattoo and Lieberman 1977). Interesting results have been obtained by studies on localizing stress ethylene synthesis in plants. After fumigation of intact bean shoots with ozone, an increased ethylene evolution was observed only by the leaves (Tingey et al. 1976, Tingey 1980). However, when the roots were exposed to ozone, an ethylene increase could be observed in the shoots, too (Gentile and Matta 1975, Jackson et al. 1978, Bradford and Yang 1980).

What is the physiological role of stress ethylene? As has already been mentioned, the stimulation of ethylene synthesis is a rapid and a widespread physiological response of plants to a variety of stress factors. The newest data indicate that ethylene is something of a chemical messenger or trigger that subsequently mediates some of the notable changes in carbon gain, in the physiology of stomatal cells and water use (Taylor et al. 1988).

The interaction of ozone with other phytohormones is also known. Abscissic acid (ABA) for example, participates in regulating the stomatal closure (Raschke 1975, 1979). In most cases, ozone fumigation induced stomatal closure (Mohr 1983), while the seedlings of more ozone-resistant rice varieties contained higher levels of endogenous ABA, which supports the view of stress avoidance by endogenous ABA (Jeong et al. 1980). In nearly all of the experiments phytohormone treatment of plants prior to or during fumigation of ozone yielded the plant some kind of protection. The greatest protective effect was found with ABA (it also provided protection from SO_2 and heavy metals). The mode of action of ABA is believed to reduce the uptake of air pollutants by stomatal closure (Kondo and Sugahara 1978, Kondo et al. 1980, Olszyk and Tibbitts 1981a, Tingey and Hogsett 1985).

The protection by cytokinins against ozone (Adedipe and Ormrod 1972, Tomlinson and Rich 1973) is probably due to a chemical reaction between cytokinins and ozone or its reaction products (free radicals), and not on metabolic changes in the cell caused by the cytokinins themselves. They can intercept free radicals (free radical scavengers) (Leshem et al. 1981).

Auxin (IAA) provided only a very limited protection in ozone-fumigated radish seedlings (*in vitro* IAA is inactivated by ozone) (Adedipe and Ormrod 1972).

In summary, it may be said that phytohormones are a part of the plant cell's responses to air pollution, but their exact role and their mode of action in these processes still needs further studies.

The possible pathways for metabolism of ozone in both the light and darkness are presented on Fig. 4. In general, either ozone itself or ozone-induced very active oxy and peroxy radicals react with membrane components or are detoxified by scavenger enzymes and reducing agents in cells (Lee and Bennett 1982, Tingey and Taylor Jr. 1982). In order that a plant cell can be resistant to oxidative stress, it must have an effective superoxide dismutase (SOD) and a hydrogen peroxide decomposing system. SOD catalyzes the dismutation of peroxide anions (O_2^-) to H_2O_2 and O_2 (Asada et al. 1974, 1977). This enzyme plays an essential role in scavenging superoxide radicals and protects cells against O_2 or oxy-radical reaction products (Lee and Bennett 1982). H_2O_2 is finally reduced to H_2O in a series of reactions in which glutathione reductase participates catalysing the reduction of glutathione with NADPH as the donor (Nakano and Asada 1980, Jablonski and Anderson 1982, Anderson et al. 1983, Asada and Badger 1984).

Fig. 4. Possible pathways for metabolism of ozone. (According to Olszyk and Tingey 1984 — modified)

It was reported in the early 1980's that a certain phenylurea derivative, N-/2-(2-oxo-1-imidazolidinyl)ethyl/-N'-phenylurea (EDU), increases the ozone resistance of sensitive plants (Lee et al. 1981a and b, Lee and Bennett 1982). The mechanism of its action still has not been worked out to the end, but it is known that the increased ozone resistance in leaf cells evoked by EDU is correlated with increases in SOD and catalase activities. In control plants the SOD activity decreased from high values in young tertiary trifoliates through to values found in fully expanded mature trifoliates and primary leaves. Maximum injuries in the most sensitive leaves corresponded to SOD levels below 150 units per mg protein (Lee and Bennett 1982). In EDU-treated

(tolerant) plants, the activity of SOD in all of the developmental stages was equivalent to those found in the youngest (most tolerant), tertiary leaves of the control plants. Electrophoresis of proteins from leaves of EDU-treated and untreated (control) plants did not show any qualitative differences between them. In comparison with the control the leaves from EDU-treated plants had more proteins with molecular weights of 32 and 16 kilodaltons (kDa). This corresponds to the molecular weights of the undissociated dimer (32 kDa) and dissociated subunit (16 kDa) of the commercial SOD separated electrophoretically (Lee and Bennett 1982). It may thus be accepted that EDU-enhanced plant tolerance to ozone by inducing SOD activity.

An increase in the activities of SOD and ascorbate peroxidase were also noted when plants were fumigated with low concentrations of ozone which caused no visible injury (Tanaka et al. 1985). Another enzyme which protects the cell from the effects of ozone is glutathione reductase (GR). It was found, for example, that ozonization of spinach plants (4 days, 0.07 ppm) by which treatment leaves did not show any visible symptoms, caused a 2.5-fold rise in GR activity (Fig. 5). The enzyme was synthesized *de novo* and/or underwent decomposition more slowly since its amount increased as the duration of ozonization was extended (Tanaka et al. 1988). The plants which tolerated ozone were also found to have higher levels of ascorbate than susceptible plants (Lee et al. 1984).

For this reason it seems that the general strategy of the cell for increasing ozone tolerance is increasing the synthesis of antioxidants and the synthesis and/or activity of antioxidation enzymes (SOD, GR and ascorbate peroxidase). However, it still remains unknown what triggers the synthesis of antioxidative enzymes, and at which steps of their biosynthesis their level in the cell is controlled.

Fig. 5. Effect of a low concentration of ozone on glutathione reductase (GR) activity in spinach leaves. (According to Tanaka et al. 1988 — modified)

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Działanie ozonu na komórkę roślinną

Streszczenie

Przedstawiono aktualne poglądy na wpływ ozonu na ultrastrukturę komórki roślinnej, zmianę wrażliwości na niskie temperatury oraz niektóre reakcje obronne komórki na toksyczne działanie tego gazu lub jego pochodnych.