Damage to Plants Caused by Anthropogenic Ozone

Abstract

The ozone concentration of the lower troposphere is increasing as a result of the emmission of additional hydrocarbons and oxides of nitrogen by industry and transport. The protective systems which have formed in higher plants in the process of evolution are not capable of detoxifying the increasing amounts of oxidizing substances which form in living cells after the diffusion of ozone into the plant leaves through the open stomata. Data are presented, which show that stomata tend to close under ozone attack, thus decreasing ozone influx to leaves. Under a given ozone influx, the photosynthesis of kidney bean plants grown in soil is shown to be less suppressed by ozone than that of plants grown in sand. The possible role of endogenous ascorbic acid in protecting the cell interior against ozone attack is discussed,

Ozone and its effects

Ozone, a simple triatomic oxygen molecule (O_3) is well known as an effective stratospheric protectant of life on Earth, due to its ability to absorb the Sun's harmful ultraviolet radiation before it reaches the troposphere. In contrast, the same compound in the lower troposphere is potentially toxic to man, animals and especially to plants, due to its destructive action on many of the components of living cells (Guderian *et al.*, 1987; Heath, 1988).

In general, O_3 is formed by the reaction of atomic oxygen (O) with molecular oxygen (O_2). In the stratospheric region of the defensive O_3-layer the source of O is photolysis of O_2. In the lower troposphere O_3 formation depends mainly on the photodissociation of nitrogen dioxide (NO_2) as a source of O. Some organic compounds, like hydrocarbons, may also act as precursors for O_3 formation (Altshuller, 1988).

Under natural concentrations of its precursors an O_3 equilibrium concentration of 0.02 to 0.04 ppm (40 to 80 gO_3m^{-3}) is typical in the lower

troposphere during the summer months at mid-latitudes. These concentrations are harmless to man and, as a rule, do not cause damage to plant cells. However, additional amounts of O_3 are formed by the action of sunlight on the oxides of nitrogen and on hydrocarbons, both of which are being increasingly emitted by industry and transport. Data from air monitoring networks have revealed O_3 concentrations in downwind plumes from industrial, urban, and intense traffic areas of up to 0.1 ppm, episodically to 0.2 ppm, with short peaks to 0.3 ppm (Eversman and Sigal, 1987). Because the precursors of O_3 are obviously stable, elevated O_3 concentrations may occur many miles from the source of the primary pollutant emissions. Such concentrations may cause disturbances in most of the plant physiological processes; they can retard the growth of many species and induce visual symptoms of injury. Nowadays O_3, as a secondary pollutant, is considered to cause the greatest amount of damage to agricultural vegetation of any gaseous primary pollutant of anthropogenic origin (Adams *et al.*, 1986). Recent estimations indicate that elevated ambient $=_3$ concentrations are costing U.S. agricultural producers and consumers between 1.2 and 2.4 billion dollars annually (Tingey, 1986). Elevated O_3 is now also considered to be one of the principal factors in recent forest decline (Prinz, 1987). In consideration of the serious economic and ecological implications, air quality standards for O_3 have been set in many countries (U.S. Federal Register, 1979).

The reason why O_3 is potentially highly harmful to plant cells lies in its high oxidizing ability. After penetrating the living cell, O_3 reacts with the protein and fatty acid constituents of the cell membrane, including the membrane-bound enzymes responsible for solute transport and osmoregulation (for a comprehensive review see Hearth, 1988). In the process of O_3 interactions with an aqueous solvent, other highly oxydative agents (peroxyl-radical, superoxide) are formed which, in turn, attack the membrane components. The result is an altered membrane function, the leakage of solutes between cell components, and a suppression of physiological processes. This ultimately causes stunted growth, a decreased harvest and reduced economic yield.

At the same time, most terrestial plants possess protective systems capable, to some extent, of detoxifying oxidative agents. These systems have developed in the process of evolution to resist the oxidizing power of molecular oxygen, generated into the atmosphere by plants themselves during the evolution of photosynthesis. These protective systems include scavenger substrates and enzymes which are, in principal, present in all the cell components, including the chloroplasts (Salin, 1987). In addition, plants have the ability to repair membrane perturbations and to restore their activity (Sutton and Ting, 1977).

Under normal background O_3 concentrations these systems are more or less able to cope with O_3-induced perturbations. Under rising anthropogenic O_3 levels the protective capacities of plants may become exhausted. Irreversible changes in the physiological process, visual injury and serious loss of productivity are the result.

Measurements show that the threshold aereal O_3 concentrations for perturbations and injury range from slightly above the background values (0.04-0.06 ppm) in O_3-indicator plants (morning glory, tobacco variety BEL W3) to 0.2-1.0 ppm in more resistant species and cultivars (Nouchi and Aoki, 1979 ; Skärby *et al.*, 1979). This wide range of O_3 sensitivity, however, is not necessarily indicative of the different scavenger and repair capacities of these plants, because the threshold values are, as a rule, given in relation to the O_3 concentrations outside the leaves. The actually perturbing quantity, the absorbed O_3 influx, depends on both the above-leaf O_3 concentration and, more especially, the stomatal openings - or the ports through which O_3 primarily enters the leaves (Rich *et al.*, 1970 ; Laisk *et al.*, 1989). As is known, the stomatal openings vary considerably between species and among cultivars (Körner *et al.*, 1979), they depend also, to a great extent, on environmental conditions (Willmer, 1988). For example, under water stress when the stomata are nearly closed, plants are able to tolerate much higher aereal O_3 concentrations (King, 1988). Moreover, the stomatal cells may respond rather rapidly to O_3 itself by closing the stomatal openings and thus reducing the O_3 influx to the leaf. Evidently the absorbed O_3 influx is the more relevant parameter in investigations of the leaf's internal sensitivity to O_3. Unfortunately, this parameter has, so far, only been used in a very few cases (Reich, 1987).

Results and discussion

We are currently using two related parameters, the « O_3 absorption rate » (Q) and the « absorbed O_3 total » (AOT) to investigate the sensitivity of stomatal and internal leaf cells to sudden O_3 attacks. The first parameter characterizes the amount of O_3 absorbed per unit leaf area per unit of time, the second is the total amount of O_3 absorbed per unit leaf area over the period of ozonation (Moldau *et al.*, 1990).

Stomatal opening during ozonation is characterized by « stomatal conductance » (g_s) and the photosynthetic ability of the internal leaf cells (mesophyll) by « mesophyll conductance » (g_m). Both parameters are widely used in investigations of the water vapour and carbon dioxide exchange of leaves (Schulze, 1986).

Table 1 illustrates the results of our experiments where a sudden onset of O_3-polluted air on a kidney bean plant (*Phaseolus vulgaris* L. var. Oregon)

was simulated under laboratory conditions. A rapid and distinct closure of stomatal openings (decrease in g_s) after the start of ozonation could be seen, suggesting that the stomatal cells were easily affected. At the same time g_m remained virtually unchanged over the whole 30 min ozonation period (up to values of AOT = 930 ng/cm^2). Apparently the photosynthetic process in the chloroplasts was maintained, due to the cellular defence systems. In addition, a reduction of O_3 attack on the internal cells was subsequently cheived by a decrease in Q due to stomatal closure under O_3 attack.

TABLE 1. — THE RESPONSE OF STOMATAL (g_s) AND MESOPHYLL (g_m) CONDUCTANCE OF KIDNEY BEAN LEAVES EXPOSED TO AN O_3 CONCENTRATION OF 0.30 PPM FOR 30 MIN. Q IS O_3 ABSORPTION RATE., *AOT* IS ABSORBED O_3 TOTAL. THE PLANTS WERE GROWN FOR TWO WEEKS IN A SAND + NUTRIENT SOLUTION UNDER PHOTOSYNTHETICALLY ACTIVE RADIATION 75 W/m^2 AND WITH A LIGHT/DARK PERIOD 14/10 h. FOR DETAILS SEE (MOLDAU *et al.*, 1990).

Time, min		g_s, rel.un		g_m, rel.un	Q, ng/cm^{2}min	AOT ng/cm^2
	cm/s		cm/s			
0	1.70	1.00	0.20	1.00	0	0
5	1.68	0.99	0.20	1.00	36.0	180
10	1.63	0.96	0.21	1.02	34.2	353
15	1.56	0.92	0.20	1.00	33.0	518
20	1.48	0.87	0.20	1.00	31.8	675
25	1.33	0.78	0.20	1.00	28.2	815
30	1.11	0.65	0.20	1.00	23.4	933

Table 2 illustrates the situation for a similar sand-grown plant where the decrese in g_m was achieved by using higher O_3 concentrations above the leaves (0.46 ppm) and correspondingly, higher $=_3$ absorption rates. In addition, the development through time of the potential (CO_2-saturated) photosynthesis (O_s) is presented ; while, data on g_s are omitted. The results are compared with those, obtained for a plant raised in well-fertilized garden soil. Both the photosynthetic parameters decreased in parallel. However, in the sand-grown plant the decrease was about 25 % and in the soil-grown plant less than 5 %. A closer inspection of a broader set of data showed that the different sensitivity was not caused by the somewhat thicker leaves in sand-grown plants (Moldau *et al.*, 1991). Probably the chemical barriers in the plants grown in soil were more effective in preventing O_3 or its degradation products from reaching the photosynthetic units in the cells.

TABLE 2. — DIFFERENTIAL RESPONSE OF MESOPHYLL CONDUCTANCE (g_m) AND OF CO_2-SATURATED PHOTOSYNTHESIS (O_m) IN SAND- AND SOIL-GROWN PLANTS AT SIMILAR ABSORBED QUANTITIES OF, $= {}_3$. Q IS O_3 ABSORPTION RATE, *AOT* IS ABSORBED O_3 TOTAL. FOR DETAILS SEE (MOLDAU *et al.*, 1991).

Time, min	gm, rel.un.		Pm, rel.un.		Q, ng/cm² min	AOT, ng/cm²
	sand	soil	sand	soil		
0	1.00	1.00	1.00	1.00	0	0
5	0.98	0.99	0.97	1.00	57.6	288
10	0.93	0.97	0.92	1.00	54.8	562
15	0.85	0.97	0.86	0.98	51.0	817
20	0.73	0.96	0.75	0.97	49.2	1063

The exact nature of these barriers has not yet been determined, although several mechanisms are suggested. Ascorbic acid and its dissociated form, ascorbate, both of which are also present in cell walls, seem to play a significant role due to their high reaction rate constants with O_3 and with its oxidative reaction products. Ascorbate, or its radical, is effective in removing superoxide and peroxyl radicals from acidic and basic solutions (Heath, 1988). The recent calculations of Chameides (1989), however, indicate that under the physiological concentrations existing in cell walls ascorbate is able to degrade most of the absorbed O_3 directly. He also showed that two plants under the same aereal O_3 concentration and with similar stomatal openings can experience influxes of O_3 to the outer cell membrane of different orders of magnitude if the concentrations of ascorbate in the cell walls differ by only 2-3 times. In this connection it is meaningful to note that Lee *et al.* (1984) has identified an almost 2-fold increase in ascorbate levels in leaves after their exposure to O_3. Thus, ascorbate can act as an effective chemical barrier by simply scavenging O_3 or its oxidative products, before they reach the more vulnerable inner compartments of the cell. By accumulating greater than normal quantities of ascorbate in their cell wall solution, plants also show some signs of active defence against O_3 attack.

Ascorbate is also present in the inner parts of cells including the chloroplasts (Salin, 1987), but its role in protecting the photosynthetic process against O_3-induced oxyradicals in this situation is completely uninvestigated. This is also the case with other endogenous agents which are potentially defensive against oxidative processes. In any case, our data presented above, together with other sources, show that under current anthropogenic O_3 peak concentrations physiological processes in plants may suffer from O_3 attack. The best practical way to decelerate this process is

certainly to reduce the emission of O_3 precursors. The scientific way is to understand the evolutionally evolved protective mechanisms in plants, with an aim to use this knowledge to reduce their susceptibility in relation to growing levels of atmospheric O_3. To achieve this goal, the O_3 sensitivities of different plants under different growth conditions should be compared under comparable absorbed O_3 influxes.

Heino MOLDAU
Institute of Astrophysics and Atmospheric Physics
Estonian Academy of Science
EE - 2444 Tõravere
TARTU, Estonia

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