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Ozone and Sulfur Dioxide Effects on the Ultrastructure of the Chloroplasts of Hybrid Poplar Leaves

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The effects of ozone on the ultrastructure of leaves from herbaceous crop plants have been documented by the works of Thomson et al. 1966; Wellburn et al. 1972; Swanson and Thomson 1973; Rufner et al. 1975; Pell and Weissberger 1976; and Mitchell et al. 1979. Fewer studies have dealt with gaseous sulfur dioxide effects on plant ultrastructure: Wellburn et al. 1972; Malhotra 1976; and Wong et al. 1977. This report is concerned with the effects of ozone and sulfur dioxide, alone and in combination, on leaf chloroplasts of a deciduous woody plant. Populus deltoides represents one of the few deciduous plants to be studied for the effects of these two common air pollutants. Other reports using the same or similar experimental fumigation regime have been recently presented. Scanning electron microscopy (SEM) was used to describe microtopographical changes of interior surfaces of fractured leaves (Krause and Jensen 1978) and exterior surfaces of leaves (Krause and Jensen 1979). Physiological data relative to leaf growth and abscission has been reported by Noble and Jensen (1980). In each of these studies the same species of plant was used as well as near identical conditions of treatment with ozone and sulfur dioxide individually and in combination.

This report details the results of these pollutants on hybrid poplar leaf mesophyll cells under similar experimental conditions as above, but processed for conventional transmission electron microscopy. A portion of this work has appeared in abbreviated form (Noble, Pechak, and Jenson 1980.

MATERIALS AND METHODS

Hybrid poplar cuttings (Populus deltoidies Bartr.x P. trichocarpa, Troy and Gray) were rooted and greenhouse grown for six weeks. Plants were randomly divided into

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four groups and placed in separate constant environment chambers at the USDA Forest Service Laboratories at Delaware, Ohio with a fumigation system as described by Jensen and Bender (1977). One group consisted of control plants on a 12 hr photoperiod at 25 C in which all air was filtered through charcoal. The experimental plants consisted of three treatments; one group was fumigated at 0.5ppm sulfur dioxide, a second group was fumigated at 0.25ppm ozone, and a third group was fumigated with a combination of sulfur dioxide and ozone at the same concentrations as above. All fumigation was run for twelve hours per day for 21 days. Light and humidity were the same for all treatments: 1,500-1,700 ft-c at plant height and relative humidity of 45+5%. Details of fumigation monitoring is described elsewhere (Noble and Jensen 1980). Leaves were rated on the basis of visual injury on a scale from L-0 (no visible injury) to L-4 (necrotic spots covering more than 50% of the leaf surface. Interveinal leaf samples were prepared for transmission electron microscopy (TEM) from various aged leaves. Leaves were designated such that the first leaf from the apex which exceeded 20mm in length was defined as leaf and node zero. Subsequent leaves and positions followed sequentially down the stem with leaves 5,9, and 12 used in this study since these have been reported to be the most susceptible and represent leaves maturing during the 21 day fumigation period (Craker and Starbuck 1973; Townsend and Dochinger 1974).

The interveinal leaf samples were fixed for 2 hours at 0-4 C in 3% glutaraldehyde in 0.2M Sorensen's phosphate buffer with 2.5% sucrose added. Postfixation with 1% OsO_4 in the above buffer was for 1 hour at 0-4 C. Dehydration by means of an acetone gradient series was followed by infiltration and embedment in low viscosity embedding medium (Spurr 1969). Thin sections for TEM observation were obtained by cutting the material perpendicular to the leaf surface such that both the upper and lower epidermis were included. Sections were stained with either 2% aqueous uranyl acetate or 10% methanolic uranyl acetate followed by lead citrate (Reynolds 1963). Observations were primarily confined to cells of the palisade layer and accomplished with either an Hitachi HS-8F-1 or JEOL 100S transmission electron microscope.

RESULTS AND DISCUSSION

Chloroplasts of control plants were normal in appearance with slight thylakoid separation in all age groups (Fig. 1). Electron dense bodies increased in size from the youngest (node 5) to the oldest (node 12) leaves while starch grains decreased in numbers and size from youngest to the oldest leaves. Sulfur dioxide fumigated material showed considerable thylakoid separation at all three age groups and the thylakoids often appeared irregularly wavy in nature (Fig. 2). This condition was less prevalent in ozone or ozone plus sulfur dioxide treated material and was observed only occasionally in control material. The electron dense bodies of node 9 and 12 leaves were smaller than in control material of comparable age and appeared similar in size and number as in the youngest material. Starch grains were large and numerous as in intermediate aged (node 5) leaves of control plants.

As noted by Noble and Jensen (1980) the concentration of sulfur dioxide used may be at the threshold level for leaf injury in this variety. No visible microtopography changes were noted by Krause and Jensen (1978) in the same plant variety when exposed to 0.25ppm sulfur dioxide. When mature Vicia faba leaves were exposed to sulfur dioxide at various concentrations from 0.25-1.0ppm, a similar effect was described (Wellburn et al. 1972). They showed that even with demonstrable thylakoid swelling, the condition could be reversed by removal of the polluting gas. At higher concentrations (2.5ppm) the effects appear to be nonreversible with respect to leaves of Larix (Mlodzianowski and Bialobok 1977), while lower concentrations (0.1ppm) are disruptive for other species such as Pisum (Wong et al. 1977). A common effect of sulfur dioxide on leaves from several plant groups is that its primary activity site is restricted to chloroplast membranes while other cytoplasmic components appear unaffected.

Ozone-treated material showed the greatest structural variation coincident with visual injury. Material which showed no visual surface injury i.e. L-0; at the ultrastructural level had comparable size and number of starch grains as control material. However, the electron dense bodies were larger than in controls and there appeared to be an increase in numbers of thylakoids per granum over that of control material (Fig 3). Leaves which showed moderate levels of visually observable injury i.e. L-2, were observed to have a reduction in size and number of starch grains over those exposed to lower concentrations. Electron dense bodies were less numerous but larger than those observed at L-0, and some thylakoid separation was apparent (Fig. 5).

Leaves which showed the most visual injury (i.e. L-4) exhibited electron dense bodies which were much larger (up to 13 microns diameter, Fig. 6) and were additionally found in the cytoplasm outside the chloroplasts. There was almost a complete lack of starch grains in all leaf nodes. Paracrystalline structures were observed in the stroma of chloroplasts from ozone fumigated material which were not seen with any other treatment. These structures consisted of electron dense particles with an average diameter of 11.6nm and a center to center distance of 17.4nm (Fig. 6). In most sections the paracrystalline structures appeared to be composed of dense spherical particles with electron translucent areas surrounding them; however, several profiles revealed a rod-like structure between rows of the spherical structures.

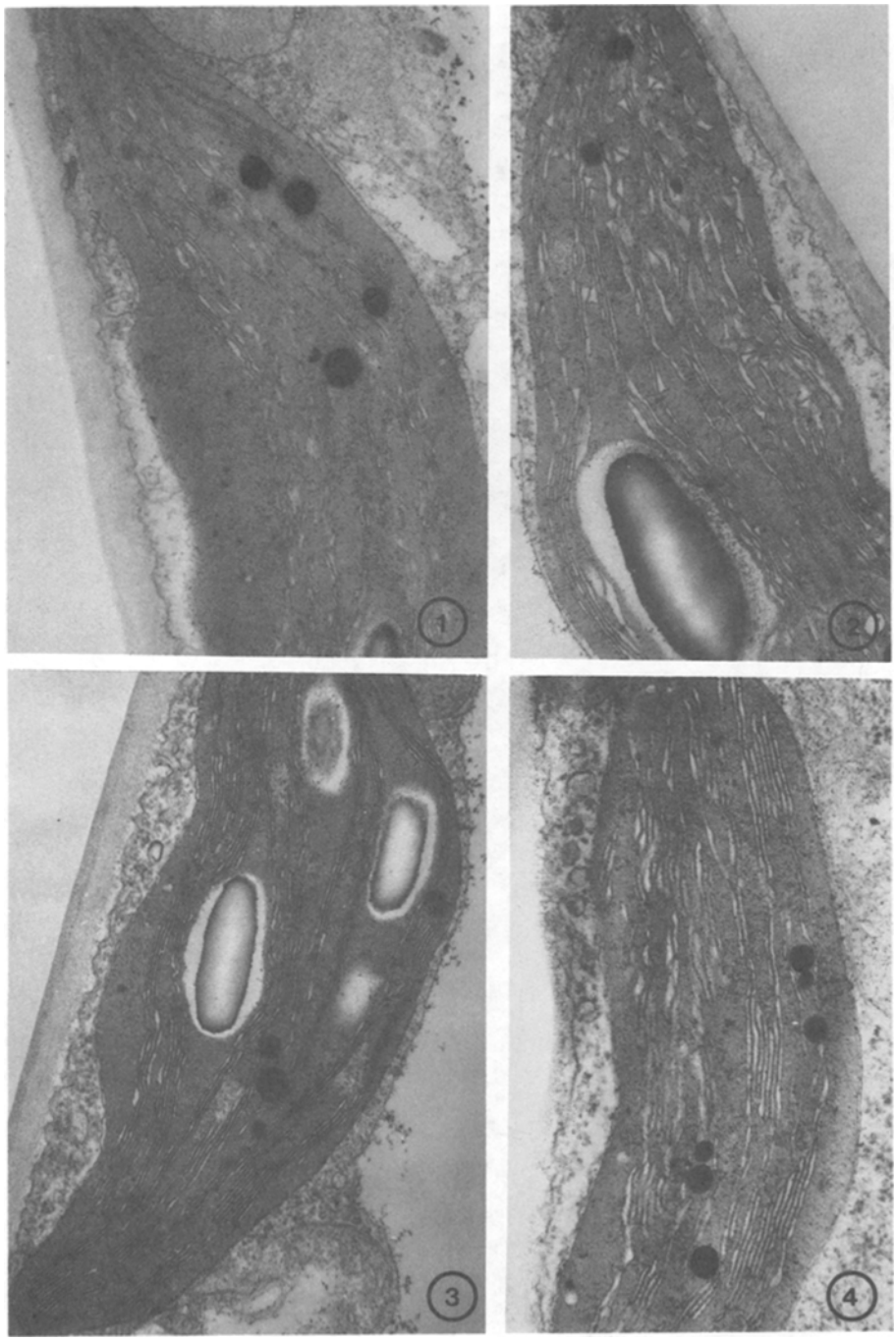
Ozone has a greater influence of leaf ultrastructure (Noble, Pechak, and Jensen 1980; Krause and Jensen 1978, 1979) and physiology (Noble and Jensen 1980) than sulfur dioxide for Populus deltoides as well as other plants (Pell and Weissberger 1976; Rufner et al. 1975; Thomson et al. 1966). For microorganisms, Scott and Lesher (1963) indicate that ozone acts upon the unsaturated fatty acids of membranes, while other studies have not been able to support this contention for higher plant material (Swanson and Thompson 1973). More recently ozone effects on membranes have

been studied by freeze fracture electron microscopy (Swanson et al. 1982). Their work indicates that ozone acts upon the hydrophobic bonds of the lipid bilayer in the plasma membrane, thus changing membrane permeability. Such effects would be expected to lead to a general break down of cell processes rather than the more isolated effects of sulfur dioxide on chloroplast membranes.

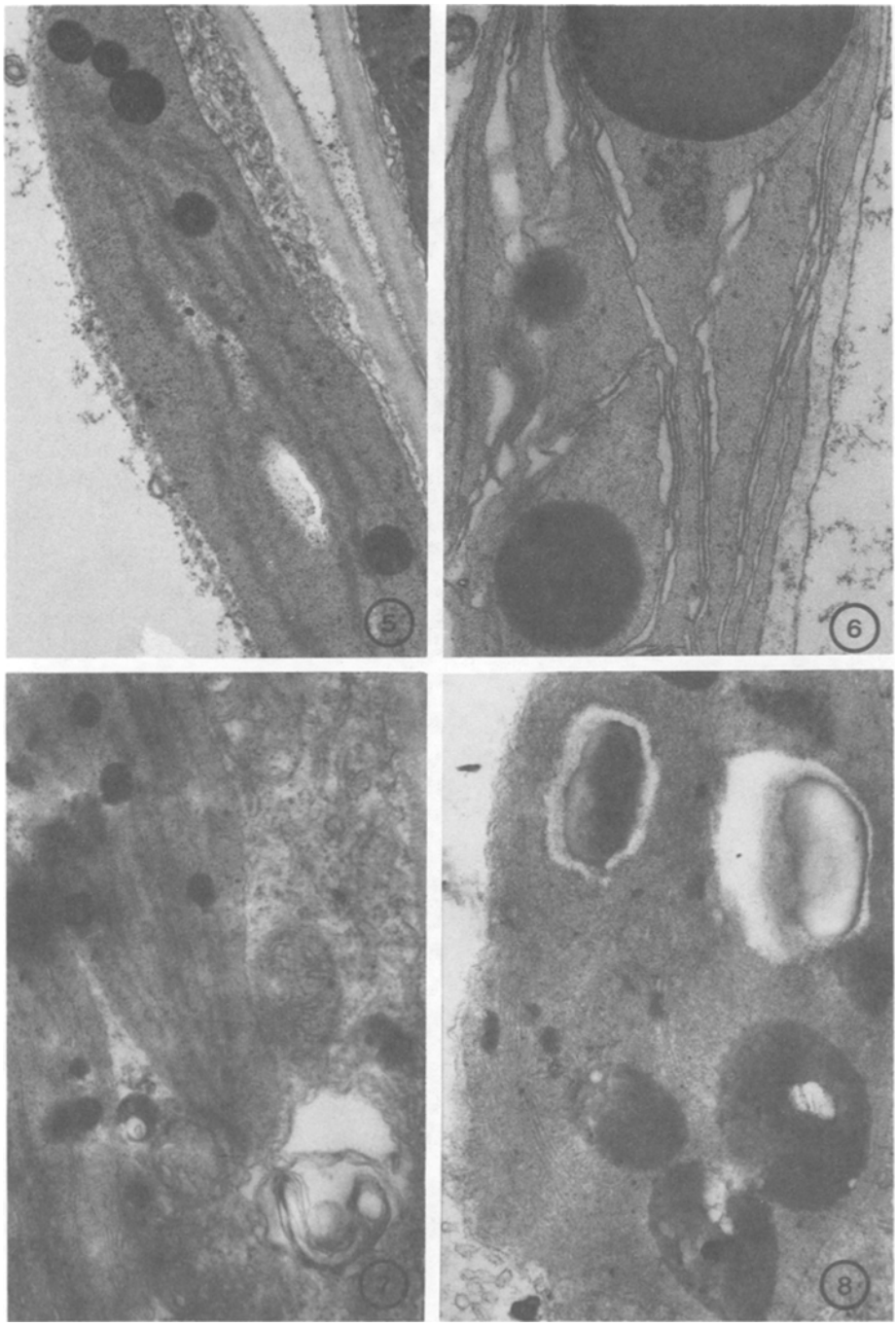
When sulfur dioxide and ozone treatments were combined, the visual injury appeared earlier (i.e. in younger material than with either treatment alone. Very little starch was observed in tissue fumigated with the sulfur dioxide plus ozone in combination, even if the material showed no visual injury i.e. L-0 (Fig. 4). At moderate levels of visual injury (L-2) starch was found to be present but not abundant and the cytoplasm was pulled away from the cell wall to an even greater extent than in combination fumigated L-0 material. Substantial blebbing of membranes into vacuoles was observed, grana membranes were occasionally separated and paracrystalline bodies were prevalent as well as osmiophilic granules (Fig. 7). In material exhibiting visual injury over more than 50% of the surface (level L-4), the thylakoid membranes were disorganized and possibly degraded (Fig. 8). Starch grains were numerous and present as in control material. Membrane blebbing was increased over L-2 material and again paracrystalline bodies were present.

When sulfur dioxide and ozone were combined, Nobel and Jensen (1980) found an antagonistic relationship between the two pollutants such that the effects of ozone were reduced by the presence of sulfur dioxide. In the present study, while thylakoid separation may have been reduced compared to sulfur dioxide alone, all other indications are that the combined pollutants have at least an additive effect on cell structure, particularly in older leaves. Osmiophilic granules were much larger in leaves exposed to the combined treatments and paracrystalline structures which were present in ozone-treated plants were more prevalent in sulfur dioxide plus ozone-treated material. Crystalline-like structures have been reported for plants exposed to ozone (Thomson et al. 1966) as well as in studies involving peroxyacetyl nitrate (Thomson et al. 1965) and ethylene (Toyama 1980). Only in the study by Toyama has the content of such crystals been analyzed by energy dispersive X ray analysis to determine the presence of iron, and thus referred to as phytoferritin. Crystalline structures found by SEM in bundle-sheath extension cells of Populus deltoides exposed to ozone and sulfur dioxide (Krause and Jensen 1978) were much larger then those found in this study. The fact that such structures were not found in plants treated with ozone alone may indicate the structures are not directly comparable to the paracrystalline structures in this study.

The general disruption of the most severely damaged cells in this study correlates with Krause and Jensen's (1978) observation that plants treated with ozone and sulfur dioxide combined show chloroplasts damaged and dispersed within the cell center, while



Figures 1-4. Chloroplasts from the leaf palisade of P. deltoides. 1. Control X19200 2. Sulfur dioxide alone, L-O injury X18300 3. Ozone alone, L-O injury X19200 4. Sulfur dioxide plus ozone, L-O injury X28700



Figures 5-8. Chloroplasts from the leaf palisade of P. deltoides. 5. Ozone alone, L-2 injury X14000 . 6. Ozone alone, L-4 injury X30300 . 7. Sulfur dioxide plus ozone, L-2 injury X20900 . 8. Sulfur dioxide plus ozone, L-4 injury X20900 .

such symptoms were not found in plants treated by either pollutant singly. Using SEM and leaf surface analysis Krause and Jensen (1979) could detect a progressive effect on stomatal morphology when ozone and sulfur dioxide when used in combination but not separately.

Clearly the effects of air pollutants, singly and in combination, on plants is very complex. While these effects have been studied in a wide variety of plants from crop to forest species, there must be an attempt to correlate physiological studies with visual and ultrastructural studies dealing with very closely related if not identical species. Sulfur dioxide and ozone effects on Populus deltoides have been reported using SEM, physiology; and with this report, TEM methods. By combining diverse techniques it is hoped that a fuller understanding of the disruptive mechanism of action of these two air pollutants can be achieved.

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