Antioxidant and Antitranspirant Protection of Apple Foliage against Ozone Injury

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Numerous studies have dealt with the deleterious effects of air pollutants, particularly ozone, on cultured plants and the factors which affect their susceptibility (ORMROD and ADEDIPE 1974). A number of investigators have reported on the use of various compounds to protect plants against ozone injury (KENDRICK et al. 1962; TOMLINSON and RICH 1973; REINERT and SPURR 1972; KENDER et al. 1973; MANNING et al 1974; SEEM et al. 1973). The antioxidant diphenylamine has been shown to be an effective agent for the protection of several plants, including apples, against visible foliar injury caused by ozone (WALKER 1967; GILBERT et al. 1975). In the work reported, it has been shown that diphenylamine (DPA) and an antitranspirant Wilt Pruf (Formula NCF, Nursery Specialty Products, Greenwich, Conn.), which is an aqueous formulation of beta pinene, protects apple foliage against histological damage from ozone as well as visible injury.

EXPERIMENTAL

'Northern Spy' apple seedlings (Malus pumila P. Miller) were used. The plants were grown in pots and at 3 months of age were exposed to zone in the large chamber previously described (GILBERT et al. 1975). The conditions of exposure included 30 pphm ozone, 16 hours per day, for 6 days. Light with a flux density at canopy height of 300 $\mu E/M^2$ sec (400-700 µm) was also provided only during the daily, 16-hour ozone exposure period.

Seven replicated plants were used per treatment. The treatments included 1) untreated controls, and plants sprayed to runoff with 2) a 10% aqueous solution of Wilt Pruf, 3) 1000 ppm DPA in a 0.1% aqueous solution of Triton X-100 and 4) 1000 ppm DPA in a 10% aqueous solution of Wilt Pruf. At the end of the 6-day exposure period the extent of injury was rated by an

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adaptation (GILBERT 1975) of the method of Kendrick et al. (1962). Leaves visibly ranging in appearance from normal to those showing typical ozone injury were then sampled from the various treatments for subsequent sectioning and microscopic examination.

RESULTS AND DISCUSSION

The extent of visible injury was in the order: untreated controls >Wilt Pruf > DPA > DPA plus Wilt Pruf.

Figures 1, 2 and 3 illustrate the appearance of normal and ozone-damaged foliar tissue upon microscopic examination in plants to which no protective agents were applied.

The lower epidermis was peeled off to examine tissue of normal cells in unexposed (control) plant foliage and that of ozone-exposed plants. Figures 1 and 2 show corresponding sections of normal and ozone-injured palisade and spongy mesophyll cells. In the ozone exposed foliage both upper and lower epidermal and guard cells appeared to be unaffected. In contrast, palisade cells located in the visibly yellow leaf areas were dead and collapsed with protoplasmic contents completely disintegrated. Some normal-appearing green palisade cells were, however, scattered within these patches of necrotic tissue. Adjacent to this necrotic palisade tissue, some cells of the spongy mesophyll were in stages of disintegration, a few completely dead and colorless. Similarly damage to palisade and mesophyll cells from ozone exposure has been reported in bean leaves and pine needles (EVANS and TING 1974; EVANS and MILLER 1972). The single network layer of mesophyll cells which clings to the lower epidermis when it is peeled off appeared not to be affected or possibly only slightly so. Parenchyma cells adjacent to the veins, where the tissue is more compactly arranged, did not appear damaged, but remained conspicuously green to the naked eye in an otherwise mottled leaf.

In Figure 3 the upper surface of ozone-exposed leaves showed isolated patches of yellowish or pale green tissue located in vein islets, presumably either lacking chlorophyll or in possible stages of disintegration. The under surface of these leaves showed the same subepidermal areas of yellowish tissue in the vein islets separated by a network of dark green tissue. Stomata in the lower epidermis were very numerous and close together but injured areas seemed to be unrelated to stomatal distribution.

In leaves of plants treated with Wilt Pruf alone and showing no visible injury, microscopic examination revealed small isolated necrotic areas in the layer of epidermal cells of the upper leaf surface. Some internal damage in the palisade and

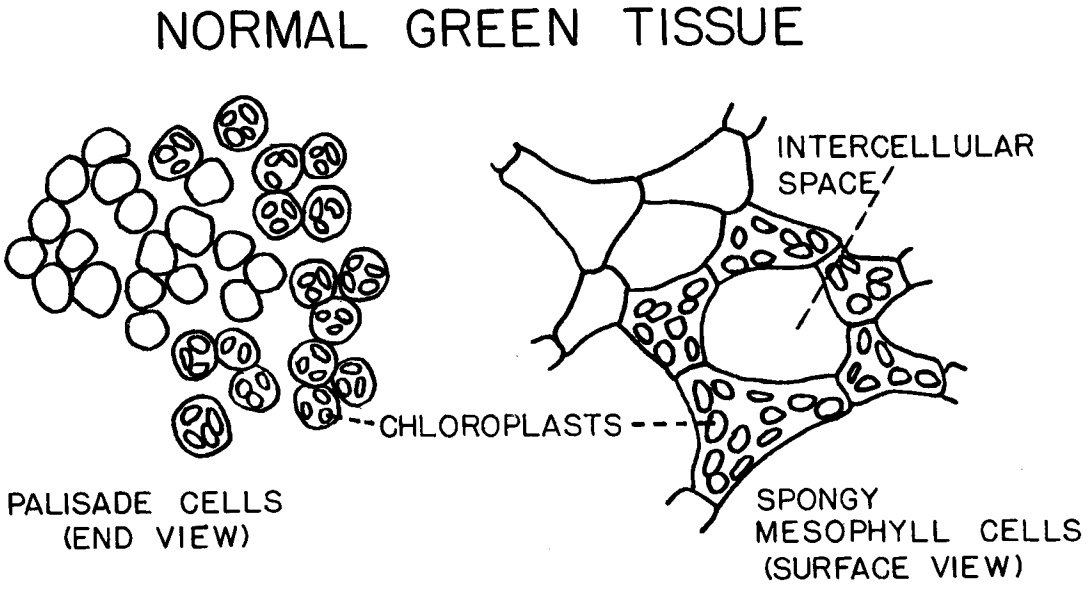

Figure 1. Diagram of normal cells of palisade layers and spongy mesophyll. (No protective agents were used on the plant).

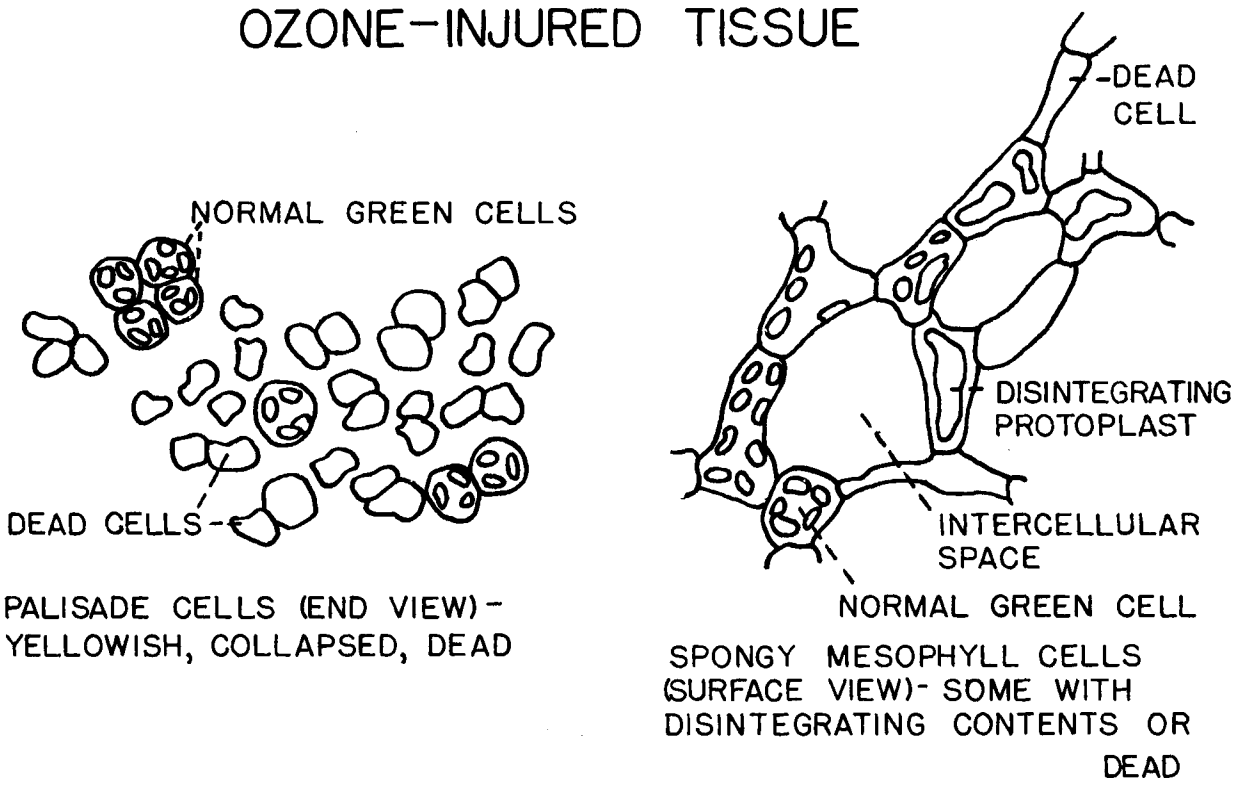

Figure 2. Diagrams of ozone-affected cells of palisade layers and spongy mesophyll. (No protective agents were used on the plant).

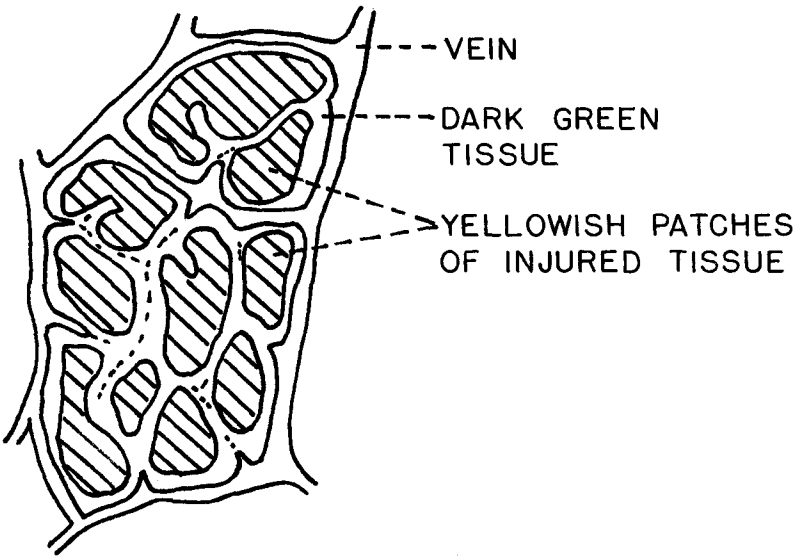

Figure 3. Diagrams of vein islets in leaf of ozone-damaged apple seedling. (No protective agents were used on the plant).

spongy layers was evident. These affected areas showed the typical disintegration of protoplasts and cells with collapsed protoplasmic contents.

Leaves of DPA-treated plants showed less necrosis and collapsed cells than those of the untreated control plants. The areas of collapsed cells were not as widespread as on the controls. In the affected leaves the areas of cell breakdown and mottling were more prevalent near the tips of the leaves while in the control leaves the areas of cell breakdown were more widespread.

Whereas the DPA treatment offered protection against ozone injury the protection was not as complete as in the leaves of plants receiving DPA and Wilt Pruf. Leaves from the latter treatment mostly showed normal internal histology. The palisade cells were normal in appearance and the spongy mesophyll cells were intact with normal distribution of chloroplasts. Clusters of cells with disintegrating chloroplasts were absent. Occasional small necrotic areas were detected on upper leaf surfaces. These appeared to be restricted to the epidermal cells and were likely

caused by the Wilt Pruf since, as was mentioned earlier, some similar spots were detected in leaves which received Wilt Pruf alone. In a few instances some palisade cells adjacent to the epidermis appeared to be affected but this was probably secondary break-down resulting from desiccation as these cells were immediately below the injured epidermal cells.

SUMMARY

Visible injury to apple foliage from exposure to ozone includes brown stippling on lower leaf surfaces and a light yellow mosaic on upper surfaces. Histologically, chlorophyll is destroyed and palisade and spongy mesophyll cells collapse following disintegration of their protoplasm. Foliar spray applications of the antioxidant, diphenylamine (DPA) or the antitranspirant, Wilt Pruf offer protection against this visible and histological injury. Superior protection results from application of both agents in combination.

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