

Effects of Gaseous Hydrogen Fluoride on Oxidative Enzymes of *Pelargonium zonale* Leaves

B. W. Poovaiah and H. H. Wiebe

Department of Botany, Utah State University, Logan 84321. Present address of senior author: Department of Horticulture, Michigan State University, East Lansing 48823.

Supported in part by Public Health Service Grant APO0276-05 from the National Air Pollution Control Administration. Utah Agricultural Experiment Station Journal Paper No. 861.

ABSTRACT

Changes in peroxidase and cytochrome oxidase enzymes were established histochemically in hydrogen fluoride fumigated leaves of *Pelargonium zonale*. Highest peroxidase and cytochrome oxidase activ-

ities were localized near the injured areas of fumigated leaves, and the greatest increase was observed in the phloem region. *Phytopathology* 61:1277-1279.

The primary mechanism by which atmospheric fluoride causes injury to plants is unknown. Many workers have suggested that fluoride inhibits respiration (specifically, enolase) (5). Others have reported that fluoride accelerates oxygen uptake (2, 6, 7), even though growth may be depressed. Fluoride fumigation caused increased cytochrome oxidase and peroxidase activities in soybean leaf extracts (4). We now report histochemical studies on the influence of fluoride on the activity of these enzymes within leaves.

The variegated geranium (*Pelargonium zonale* Ait.) was used. The edges of these leaves are particularly suitable for histochemical studies because there are no chloroplasts to interfere with the observation of colors produced in the reactions. Potted plants were fumigated in chambers of polyvinylite plastic (6) continuously at 20-25 ppb hydrogen fluoride. Leaf samples were taken after necrosis became visible, and were sectioned fresh at 30 μ with a rotary microtome, using carrot root as support. Sections were cut to include necrotic, injured, and apparently healthy tissues, and were incubated in the appropriate reaction solutions. The sections were then washed in distilled water, mounted in glycerine, and observed microscopically.

Reagents for the localization of peroxidase were (1, 3): 1% H_2O_2 with 70-95% ethanol solution of 0.1 M benzidine. The sections were incubated for 1-2 min, and photographs taken within 60 sec from the time of substrate application. As a control, 0.1 M potassium cyanide was added to the reaction mixture.

For the localization of cytochrome oxidase (3), the reaction mixture consisted of 25 ml 0.05 M phosphate buffer at pH 7.2-7.6, 1 ml of 1% alpha-naphthol in 40% alcohol, and 1 ml of 1% *N,N*-dimethyl-*p*-phenylenediamine. Sections were incubated for 10-15 min, rinsed in distilled water, and mounted in glycerine. As a control, 0.05 M sodium azide was added to the reaction mixture.

Peroxidase activity was intense throughout the phloem area of both fumigated and control leaves. No

reaction was noted in the xylem. Occasional epidermal cells, some palisade and spongy parenchyma cells, and glandular hairs showed peroxidase activity. In fumigated leaves, regions next to the necrotic zone showed an enhanced benzidine blue coloration in the phloem (Fig. 1-3), with a decreased color or its complete absence in the palisade parenchyma. Even after surrounding mesophyll cells had collapsed, the enzyme activity was clearly evident in phloem (Fig. 2-3).

Cytochrome oxidase activity was present in both mesophyll and epidermal cells of nonfumigated leaves, with the strongest reaction in the phloem. In fumigated leaves, cells next to the necrotic areas showed a deep blue color, indicating increased enzyme activity in that region (Fig. 4). Again, enzyme activity was present in the phloem region even after collapse of nearby cells. The inhibitors, sodium azide and potassium cyanide, prevented color formation in both tests.

Parallel experiments were conducted in which leaf discs were infiltrated with sodium fluoride solutions. The effects of fumigation and solution infiltration on enzymes were similar in most cases. In these also, the cytochrome oxidase activity was not decreased by fluoride, and the activity was stimulated by fluoride. Peroxidase activity, however, was inhibited by fluoride levels higher than 0.01 M in the medium bathing the tissue for 6 hr.

These in vivo results agree with the in vitro observations of Lee et al. (4). Increased oxygen uptake has been reported in plants injured by fluoride fumigation (2, 6, 7). The increased peroxidase and cytochrome oxidase activity in fumigated plants (Fig. 1-4) is consistent with an increased oxygen uptake. Our histochemical observations failed to show positive reaction for both enzymes in lignified plant tissues. The spiral thickening of xylem vessels were always colorless.

Fluoride undeniably caused profound changes in the activities of the enzymes studied, and therefore, logically also modifies the metabolism and physiology of the tissues.

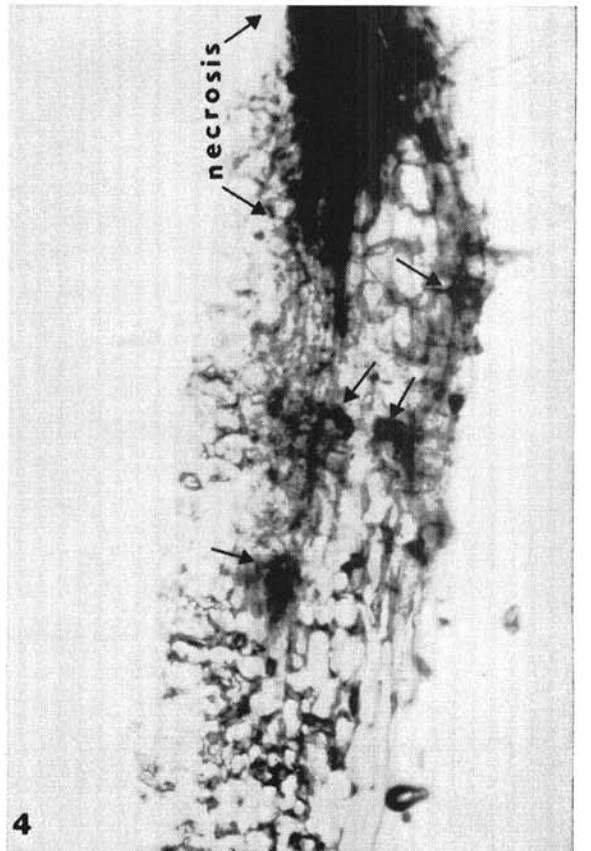
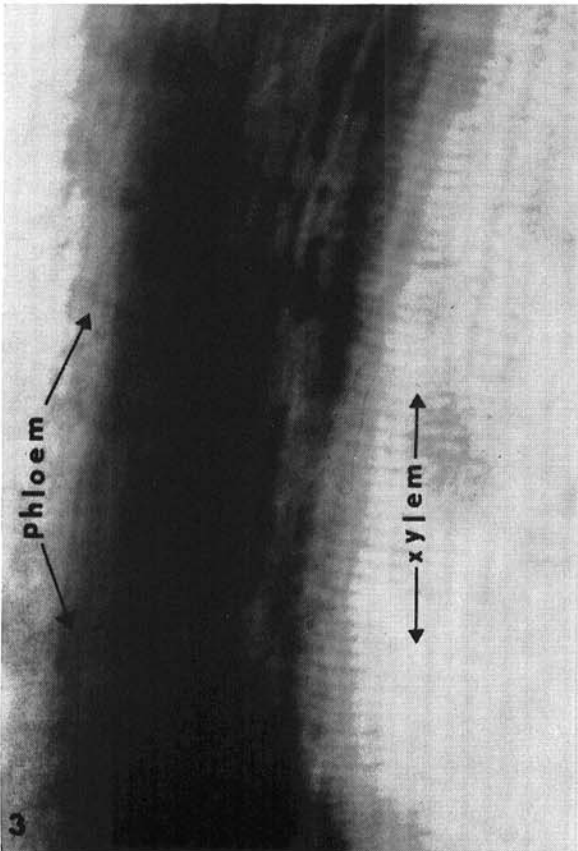
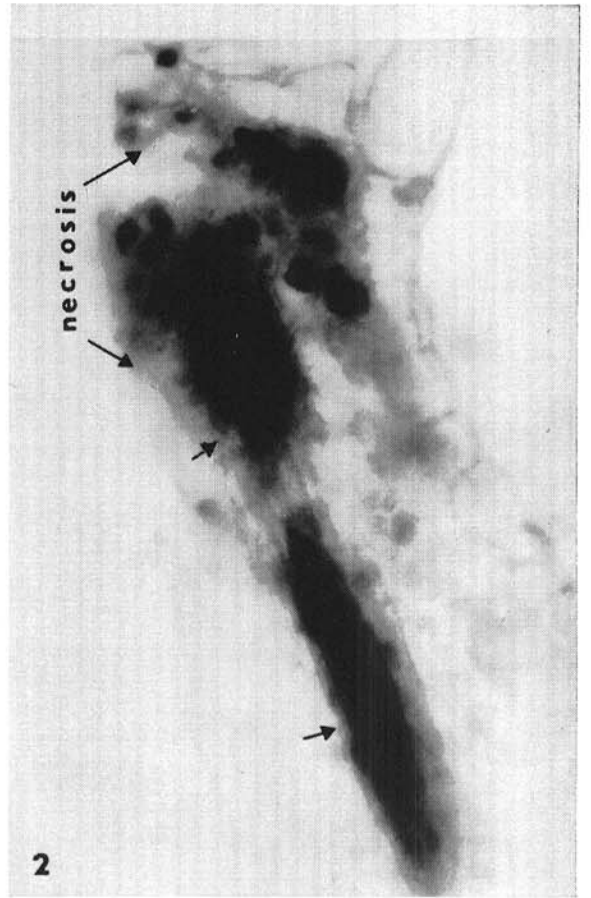
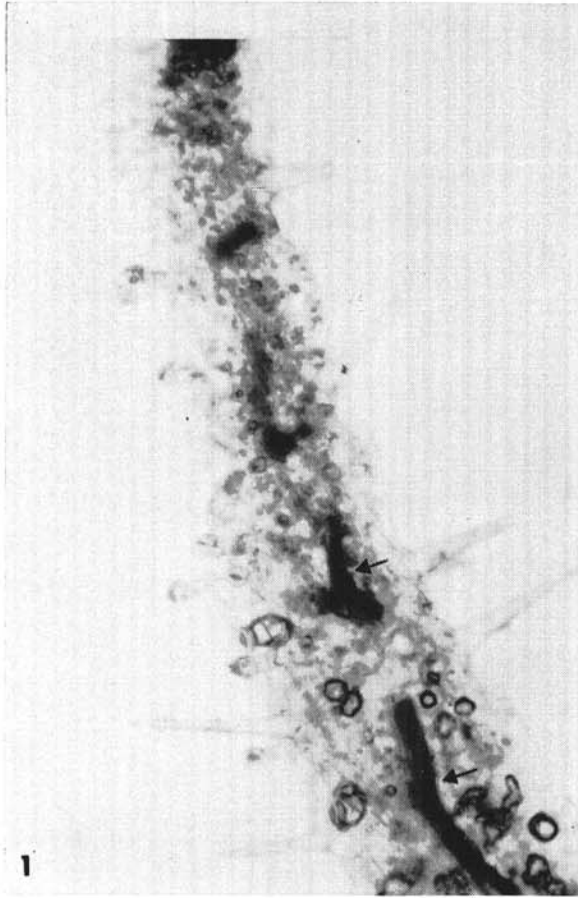


Fig. 1-4. 1) Section of a fumigated leaf showing enhanced peroxidase activity in the phloem (arrows) ($\times 110$). 2) Fumigated leaf section with necrosis of leaf margin at the top, increased peroxidase activity in the phloem near the injured area (arrows), and absence of activity in the palisade and spongy parenchyma ($\times 280$). 3) Enlarged view of the vascular region of fumigated leaf showing peroxidase activity in phloem and its absence in xylem ($\times 940$). 4) Cytochrome oxidase activity in hydrogen fluoride-fumigated leaf section showing necrosis at the top and a marked increase in enzyme activity adjacent to the necrotic area (arrows) ($\times 110$).

←

LITERATURE CITED

1. DE JONG, D. W. 1966. Speculation on the mechanism of ion transport in roots based upon indirect evidence from histochemical studies. *Bot. Gaz.* 127:17-26.
2. HILL, A. C., M. R. PACK, L. G. TRANSTRUM, & W. S. WINTERS. 1959. Effect of atmospheric fluoride and various types of injury on the respiration of leaf tissue. *Plant Physiol.* 34:11-16.
3. JENSEN, W. A. 1962. *Botanical histochemistry*. W. H. Freeman & Co., San Francisco, Calif. 408 p.
4. LEE, C. J., G. W. MILLER, & G. W. WELKIE. 1966. The effect of hydrogen fluoride and wounding on respiratory enzymes in soybean leaves. *Air Water Pollut. Int. J.* 10:169-181.
5. MILLER, G. W. 1958. Properties of enolase in extracts from pea seeds. *Plant Physiol.* 33:199-206.
6. ROSS, C. W., H. H. WIEBE, & G. W. MILLER. 1962. Effect of fluoride on glucose catabolism in plant leaves. *Plant Physiol.* 37:305-309.
7. WEINSTEIN, L. H. 1961. Effect of atmospheric fluoride on metabolic constituents of tomato and bean leaves. *Contrib. Boyce Thompson Inst.* 21:215-231.