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

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ABSTRACT

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

1

necrosis
2

Phloem
xylem
3

necrosis
4

Fig. 1-4. **1)** Section of a fumigated leaf showing enhanced peroxidase activity in the phloem (arrows) (×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 (×280). **3)** Enlarged view of the vascular region of fumigated leaf showing peroxidase activity in phloem and its absence in xylem (×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) (×110).

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