TRANSLOCATION AND METABOLIC EFFECTS OF FLUORIDES IN GLADIOLUS LEAVES[1]

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The literature dealing with the effect of fluoride air pollutants upon plants includes many references to gladiolus (1, 3, 4, 5, 6, 8). Gladiolus plants are of interest in this connection as indicators of the presence of atmospheric fluoride owing to the high degree of sensitivity to the toxicant. The most characteristic effect of fluorides is a marginal and tip necrosis commonly referred to as leaf scorch. There have been repeated efforts to determine whether the obvious injury represents the full extent of the damage by fluoride fumigation (2, 3, 7) without satisfactory resolution of the question.

The work to be reported here was undertaken for the purpose of learning more about the mode of action of fluorides in their various effects upon the gladiolus leaf.

METHODS

Methods in use for the determination of rates of respiration and photosynthesis and fluoride content as well as for the measurement of the width of stomatal openings are described in another report (6).

Gladiolus plants of Friendship and Orange Gold varieties were grown in eight-inch plastic pots containing virgin Broward fine sand, pH 6.0. Plants were irrigated as required with well water, pH 7.6 containing 750 ppm soluble salts and fertilized weekly with 20-20-20 soluble fertilizer (fluorine-free) containing small amounts of secondary and minor nutrient elements. Calcium chloride was used as the calcium source.

Fumigation of gladiolus plants was accomplished in six Mylar chambers, each of 1000 liter capacity, containing electric fans for air circulation. The source of hydrogen fluoride gas was solutions of hydrofluoric acid of various concentrations which were equilibrated with air by rapid movement of the air in the chambers to produce atmospheres of graded fluoride content. Potted plants were fumigated overnight prior to translocation studies.

Water-conducting elements in the leaves of freshly fumigated plants were severed by cutting notches or disks from the leaves. Plants were placed in the greenhouse for observation of the nature of developing patterns of necrotic areas relative to fluoride translocation.

Detached leaves for metabolic studies were removed from potted plants with the leaf blade being immersed in deionized water at the point of cutting to avoid causing air to be drawn into the transpiration column when tension was released by cutting the leaf blade. Fresh weight change (Table 1) was determined by quickly weighing leaves before and after the two-hour imbibition period.

Friendship variety plants for the illustration of the soil-fluoride toxicity pattern were grown in cypress wood boxes of two square-foot area and seven-inch soil depth. Superphosphate (1.7% fluoride) was applied to Broward soil at the rate of 4250 pounds per acre together with 200 pounds of sulfur per acre.

RESULTS

The normal fluorine leaf scorch pattern is depicted by leaf B in Fig. 1. Leaf C represents the pattern of fluorine scorch from superphosphate applied to the soil in which the plant grew. The tendency was apparent for fluoride taken up by the roots in this instance to scorch the interior rather than the margin of the leaf. Fumigated leaves D and E showed that a cut in the leaf would stop the normal flow of water causing a marked change in the pattern of necrosis as compared to the common pattern illustrated by fumigated leaf B. Fluorine accumulated below the cut causing burn at that point. Water and fluorine both moved from all available directions into the partially dehydrated tissues thereby concentrating fluorine above the cut in the manner depicted in leaves D and E. Non-fumigated leaves failed to develop any necrosis above or below leaf cuts.

Orange Gold variety of gladiolus translocated more fluoride upward than Friendship for a given concentration of hydrofluoric acid (Table 1). There was a marked reduction in photosynthetic efficiency for Orange Gold at the three high

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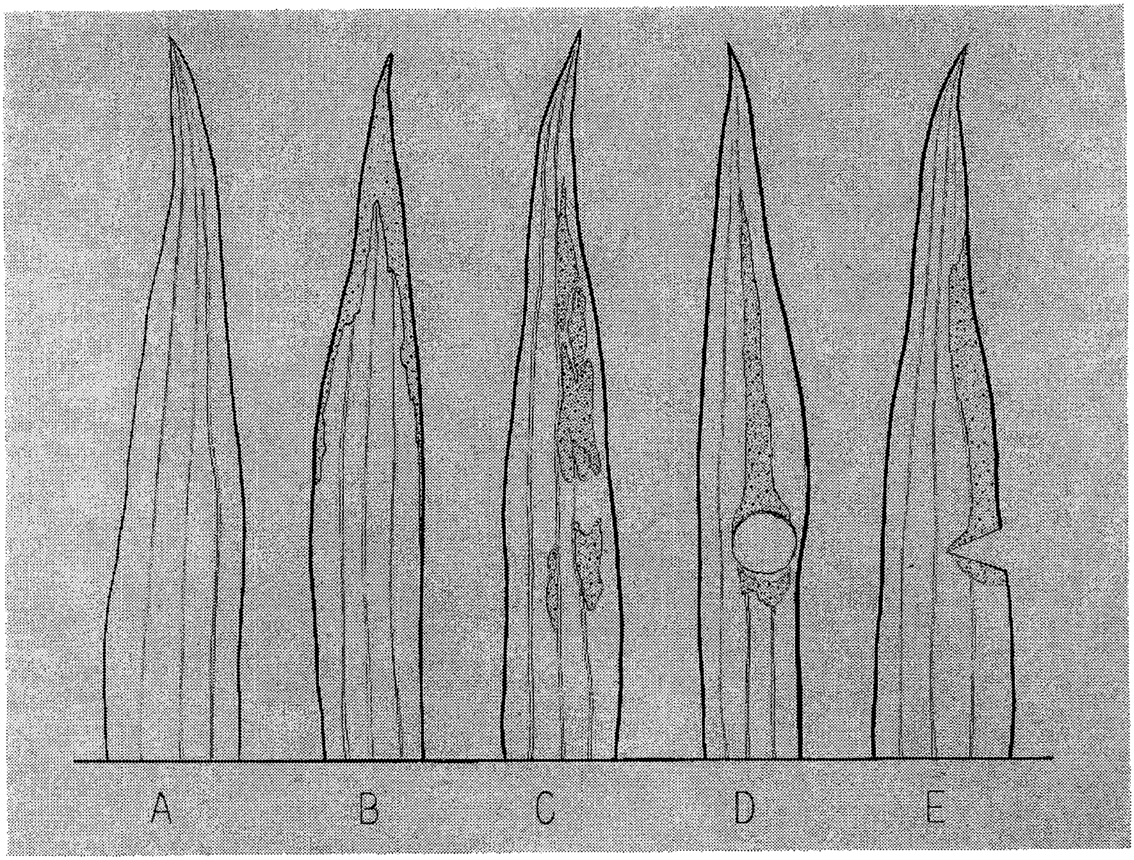

Figure 1.—Illustrations of types of necrotic patterns induced by various treatments of Friendship variety gladiolus: A—control, B—normal fumigation, C—soil-derived fluoride, D—necrosis above and below area from which a disk was removed after fumigation and E—necrosis above and below area from which a notch was removed.

concentrations of hydrofluoric acid. The photosynthetic activity of Friendship did not appear to be similarly affected. The effect of fluoride upon respiration did not show clear-cut trends. Both varieties exhibited greater stomatal opening as fluoride concentration was increased in the imbibed solutions. Orange Gold showed greater response to treatment than Friendship.

Detached Orange Gold gladiolus leaves (Table 2) showed a greater opening of stomata in response to imbibition of 10 ppm fluoride as hydrofluoric acid solution than did the control leaves imbibing water. Data for attached gladiolus leaves are included to depict normal stomatal action in the greenhouse for the same times of measurement. Friendship variety showed a slight response to fluoride but was much less affected than Orange Gold (Table 1). The opening of stomata by the dilute hydrofluoric acid was evident by the end of one-half hour and tended to decrease in magnitude by the time of the four-hour measurement. Stomata of leaves remaining on the plants were nearly closed at the four-hour reading (2 P.M.) even though the pots were well-watered. Under the prevalent greenhouse conditions in late May, Orange Gold had much smaller stomatal apertures than Friendship.

The results of a slightly different imbibitional experiment are shown in Table 3. By the end of 6 hours both Friendship and Orange Gold showed greater stomatal opening due to hydrofluoric acid treatment than at earlier readings. Friendship was, however, affected to a lesser degree than the other variety. Orange Gold, in this experiment, apparently experienced both opening and closing effects due to fluoride in the two and four-hour readings.

Table 1. Effect of fluoride uptake upon photosynthesis, respiration and stomatal action of detached gladiolus leaves

Fluoride content of HF solution imbibed[1] ppm	Fluoride content of leaf tissue[2] ppm Dry Wt.		% Fresh wt. change in leaves by end of imbibition period		Respiration[3]		NPE[3]		Mean stomatal width, microns, at end of Imbibition	
	F[4]	O[4]	F	O	F	O	F	O	F	O
0	4	3	+0.9	0	10	11	42	55	2.1	0.3
0.6	3	4	+0.7	0	9	13	42	45	3.0	1.3
1.3	7	17	+1.0	-0.2	9	16	43	41	2.2	1.0
2.5	11	26	+1.2	-0.2	10	12	47	9	3.7	2.3
5.0	21	30	+2.4	-0.7	11	11	38	29	3.5	1.9
10.0	34	46	+1.4	-1.0	11	16	50	25	4.0	2.8

[1]Leaves severed under water were placed in indicated solutions, then closed to the atmosphere by polyethylene bags wrapped around the solution containers and the base of the leaves. Period of imbibition in greenhouse was 2 hours.

[2]Leaf portion sampled was similar to that used in Warburg apparatus.

[3]Leaf disks, 12 mm diameter, were used in duplicate determinations, four per vessel. Respiration is expressed as microliters oxygen consumed per hour per cm^2 leaf. NPE = net photosynthetic efficiency expressed as net oxygen evolution during 30 minutes dark and 30 minutes illumination at 1250 foot candles intensity. All manometric measurements were made at 32° C.

[4]F = Friendship; O = Orange Gold variety.

Discussion

The changes in necrotic patterns due to severing conductive tissues in fumigated gladiolus leaves appear to be of significance in indicating that fluorides move quite freely with the water transpiration stream. Gladioli are noted in the literature (4) for the speed with which fluorides are moved toward the tips and margins of leaves. Since this appears to be a relationship that is fairly well supported by experimental evidence, one might extrapolate to say that the transpiration rate and local stomatal action over the gladiolus leaf might well affect the type of damage experienced by gladiolus plants during and following a given fumigation. For example, rapidly transpiring plants should be expected to move fluoride rapidly toward the tips and margins of leaves thus reducing damage to the remainder of the leaf. Hidden injury such as reduction in photosynthetic efficiency would be worse if fluoride were translocated only very slowly toward the extremities of the leaf. Also, in the event of a very severe fumigation followed by a period of retarded transpiration, one would expect much more leaf tissue to be killed by fluoride because of the absence of normal translocation to marginal areas.

The difference in patterns of necrosis from foliar uptake compared to root uptake (Fig. 1) requires some discussion. The path of travel of foliar-acquired fluorides would be expected to be more in the nature of a flow from cell to cell with much less emphasis on the vascular bundles. The foliar fluoride could travel in a sheet arriving at the margins of leaves. Soil-acquired fluoride should travel initially in the vascular bundles radiating into interveinal areas as it travels

Table 2. Effect of fluoride uptake upon degree of stomatal opening of detached gladiolus leaves

	Stomatal width[1], microns									
	Friendship					Orange Gold				
Hours of treatment	1/2	1	2	4	3[2]	1/2	1	2	4	3[2]
Imbibition treatment[3]	Tip of leaf									
Attached	3.8	4.0	4.7	2.8	-	2.0	2.1	2.6	0.6	-
Water	4.0	3.2	4.5	3.9	0.2	0.9	1.7	1.1	1.3	0.1
10 ppm F	4.5	4.4	4.2	4.2	1.1	2.4	2.1	1.1	3.6	0.1
	Middle of leaf									
Attached	3.5	3.7	4.7	1.2	-	1.8	1.6	3.0	0.2	-
Water	1.1	2.7	2.2	3.5	0.5	0.1	0.1	0.1	2.8	0.1
10 ppm F	1.9	2.5	2.3	4.5	0.1	0.9	0.9	0.5	2.5	0.1
	Base of leaf									
Attached	3.3	3.4	4.5	1.0	-	1.8	1.6	2.5	1.0	-
Water	4.1	3.6	4.9	4.9	1.5	0.1	0.3	0.4	4.3	0.1
10 ppm F	1.7	4.7	4.8	4.7	0.1	0.2	2.9	2.4	4.0	0.1
	Mean widths for three leaf locations									
Attached	3.5	3.7	4.6	1.7	-	1.9	1.8	2.8	0.7	-
Water	3.1	3.2	3.9	4.1	0.7	0.4	0.7	0.5	2.8	0.1
10 ppm F	2.7	3.9	3.8	4.5	0.4	1.2	2.0	1.3	3.4	0.1

[1]Data for attached leaves are from single observations; others are averages for two observations.

[2]Two hours treatment in greenhouse followed by one hour of darkness.

[3]"Attached" signifies comparative data for leaves still on plants. Water = detached leaves imbibing water; 10 ppm F = detached leaves imbibing 10 ppm F as HF.

up the leaf. This type of transport could well result in greater concentrations of fluoride at the middle of the leaf toward the tip than the sheet-like flow of the foliar-acquired fluoride which would always be more available to the leaf margins. Resolution of this question should yield readily to studies using the fluoride radioisotope.

Based on stomatal apertures (Tables 1, 2 and 3) and volumes of solutions imbibed (Table 3) it would seem that Friendship variety has some mechanism for immobilizing fluoride or at any rate that Friendship does not translocate fluoride as well as Orange Gold. This observation is based on the apparently lower transpiration rates of Orange Gold together with its greater fluoride uptake (Table 1). The fresh weight change during the imbibition period (Table 1) was generally positive for Friendship and negative for Orange Gold, indicating that Orange Gold cannot freely accumulate water.

The data (Tables 1-3) indicate that Orange Gold was much more affected by fluoride in terms of damage to photosynthetic processes, and increased stomatal opening widths. Orange Gold was more efficient at taking up fluoride under the conditions of these experiments which may be the sause of the greater sensitivity to fluoride. It has been observed that Orange Gold is more sensitive than most commercial gladiolus varieties to fluorine fumigation in the field.

Table 3. Effect of various concentrations of hydrofluoric acid imbibitional solutions upon stomatal opening of detached gladiolus leaves

Fluoride content of imbibed solutions[3]	Stomatal widths[2], microns					
	Friendship			Orange Gold		
	Hours			Hours		
	2[1]	4	6	2[1]	4	6
0	2.4	5.0	0.7	2.5	2.4	0.2
0.6	3.8	5.0	2.1	1.9	0.5	0.8
2.5	2.7	4.9	3.6	0.1	3.0	1.1
10.0	2.2	5.2	3.9	0.1	1.6	1.5

[1]Readings made at end of 2 hour imbibition period; leaves were then transferred to water.

[2]Means for duplicate measurements.

[3]Total volumes imbibed: Friendship - 11.1 ml.
Orange Gold - 7.4 ml.

The effect of fluorides in causing larger stomatal opening is probably related to the known inhibitory effect of fluoride upon adenosine triphosphatase (ATP-ase). An inhibition of ATP-ase would cause an increase in ATP, respiration and available energy for the enhancement of osmotic pressure in the guard cells. There is also the possibility that fluoride may affect the movement of water through the leaf tissue and the consequent ease of availability to the guard cells to cause opening.

Summary

Evidence is presented to show that fluorides are translocated freely with the transpiration stream in the gladiolus leaf and do not necessarily move to the upper margins of the leaf in the manner commonly noted in fluoride leaf scorch of gladiolus. If the transpiration stream be interrupted by cutting a notch from one side of the leaf, fluoride necrosis resulting from fumigation will be greater on that side of the leaf. Soil-acquired fluoride produced a necrotic pattern involving more of the internal areas of the leaf as opposed to atmosphere-acquired fluoride which affected marginal areas almost entirely.

Fluoride imbibed from solutions by detached leaves reduced photosynthetic efficiency in Orange Gold variety but not in Friendship. The fluoride content of leaves exhibiting impairment in photosynthesis ranged from 26 to 48 ppm on the dry weight basis. Another effect of fluoride so acquired was to cause a pronounced increase in stomatal opening widths. The effects on stomatal opening were more pronounced with Orange Gold than Friendship.

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