Influence of Hydrogen Fluoride Fumigation on the Water Economy of Soybean Plants

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ABSTRACT

The influence of hydrogen fluoride fumigation on water economy was studied using soybean (Glycine max [L.] Merr.). Fumigation caused partial stomatal closure in 1 hour and practically complete closure within 4 hours. The transpiration rate was greatly reduced by fumigation, while the leaf temperature was increased. Water potential increased after 1 day but fell drastically when necrosis occurred. Effects of interrupted fumigation during the day were somewhat less severe in all respects than those of continuous fumigation; nighttime fumigation caused only minor effects. Fluoride uptake was also much less from nighttime than from daytime fumigations.

Atmospheric fluorides influence the growth of plants, as well as many enzymes and metabolic processes. Their influence on the water balance, however, has received relatively less attention. Navara and Kozinka (8) found stomatal closure and reduced transpiration in several fluoride-fumigated species. Smog is also reported to cause stomatal closure and reduced transpiration (4). While using solution-grown plants in other experiments, we noticed that the fluoride-fumigated plants consistently used less water.

This study reports the influence of fumigation on water economy and the related phenomena of stomatal aperture and leaf temperature.

MATERIALS AND METHODS

Plant Material. Soybean (Glycine max [L.] Merr.) seeds were germinated in vermiculite, transferred after 1 week to a balanced nutrient solution in 1-liter plastic containers, and grown at 28 C in growth cabinets under fluorescent and incandescent light (12-hr photoperiods). In some experiments, plants were grown in soil in the same growth cabinets. The plants were subjected to fumigation when they were about 3 weeks old and the third trifoliate leaf was about half-grown.

Fumigation. Plants were fumigated in chambers of polyvinylite plastic (6, 11) that were installed in growth rooms maintained at 28.5 ± 0.5 C. The 12-hr photoperiod light of 800 ft-c intensity was provided by fluorescent and incandescent light. Air was bubbled through a polyethylene bottle containing 10% hydrogen fluoride at 0 to 1 C and then introduced into the main air stream entering the chamber at the rate of about one change per minute. Rates of bubbling and air flow were adjusted to maintain the atmospheric fluoride concentration of the chamber at 15 to 20 nl/liter, as analyzed by the ion exchange technique (9). Control plants were kept in chambers that were identical in all respects except that no fluoride was added to the air stream.

In one series of experiments that ran for 4 to 5 days, fumigated plants were transferred periodically from the fumigation chamber to an identical control chamber. All plants were illuminated from 0800 to 2000 hr; daytime-fumigated plants were kept in the fumigation chamber from 1200 to 1800 hr; nighttime-fumigated plants were in the fumigation chamber from 0 to 0600 hr. Other plants were fumigated continuously, and the responses to all three treatments were compared to those of unfumigated control plants.

Analysis of Tissue Fluoride. Leaf samples were washed in deionized water, dried at 100 C for 24 hr, and ground by mortar and pestle. A sample (0.5–0.05 g depending on the extent of damage) was mixed with calcium oxide (1:5, calcium to leaf) in a platinum dish and ignited on a low flame and then at 600 C in a furnace (Thermolyne type 2000) until it was completely ashed. The ash was dissolved in a minimal volume of 0.1 N HCl, brought to a 50-m1 volume with deionized water, and passed through anion exchange resin (Dowex 1-X8, 200–400 mesh). The fluoride was removed from the resin by stepwise elution with increasing concentrations of sodium acetate (9). Finally, the absorbance was measured at 527.5 nm with a Hitachi Perkin-Elmer UV-VIS spectrophotometer, model 139. Control reagent blanks were also run. The procedure was verified by analysis of plant samples containing known amounts of fluoride obtained from the Agricultural Division, Geneva Steel Division, United States Steel Corporation, Provo, Utah.

Determination of Stomatal Opening. Stomatal opening was measured with a diffusion porometer (14) which gives the leaf resistance to water vapor in mm cm-1. In some cases, conductivity was calculated as the reciprocal of resistance and was expressed as cm min-1. Nighttime measurements were made with the help of a flashlight.

Transpiration Measurements. Transpiration was measured by weighing the plants four times a day (0600, 1200, 1800, and 2400 hr) on an analytical precision balance.

Leaf Temperature Measurements. Leaf temperatures were measured with a precision radiation thermometer (Barnes Engineering Company model PRT-5). Air temperature was measured with a temperature potentiometer and was found to remain at 28.5 C (± 0.5 C), both day and night.

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Determination of Water and Osmotic Potentials. The water potentials of leaves were measured with Peltier cooled thermocouple psychrometers. Osmotic potentials were determined on the same tissues after freezing and thawing (15).

RESULTS

Visible Injury Symptoms. Leaves of solution-grown soybean plants subjected to continuous fumigation showed marginal yellowing, followed by necrosis that became very severe after about 4 days. Primary and oldest trifoliate leaves were always injured earlier and more severely than the younger trifoliate leaves. Plants that were fumigated for only 6 hr daily, during the daytime, were also severely injured, but less so than continuously fumigated plants. Fumigation given at night only caused slight injury after 4 days.

Fluoride Content. The leaves accumulated about 600 μg/g fluoride after continuous exposure to an atmosphere of 15 nl/liter fluoride during a 4-day period (Table I) with lesser amounts in plants fumigated 6 hr day or night. The plants fumigated 6 hr during the day had more than twice as much fluoride as those fumigated at night. The differences in fluoride uptake would account for the differences in injury reported above.

Transpiration. In earlier experiments with solution-grown plants, we noticed that the fumigated plants used much less water than the controls. This was confirmed in the present experiment. On the pretreatment (0) day all plants transpired at essentially the same rate, and during the experiment transpiration of control plants increased slowly, probably because of continued plant growth (Fig. 1, center). Transpiration of continuously fumigated plants was severely depressed. Transpiration rates of plants fumigated for only 6 hr daily were intermediate between those of control and continuously fumigated plants, with higher transpiration among plants fumigated at night than during the day. The full impact of the 6-hr daytime fumigation, which was not begun until the middle of the 1st treatment day, was not apparent until the 2nd day, but the transpiration rate between 1200 and 1800 hr on the 1st day was significantly reduced (Fig. 1), suggesting that fumigation causes stomates to close quickly. Nighttime transpiration of daytime-fumigated plants was somewhat higher than that of other treatments. Peak transpiration of nighttime-fumigated plants definitely occurred in the afternoon, as in the controls, but since the stomates were partially closed all day, the transpiration was lower than that of control plants. In the daytime-fumigated plants, however, transpiration in the afternoon, which was concurrent with fumigation, averaged only slightly greater than transpiration in the morning. Transpiration in the afternoon was apparently reduced because of stomatal closure during fumigation.

Leaf Resistance. Leaf resistance of control plants was low during the day, and high at night, indicating stomatal opening during the day and closure at night. The same diurnal pattern prevailed in fumigated leaves (Fig. 1, bottom), but in continuously fumigated leaves the resistances were much higher during the day, indicating partial stomatal closure. On the 3rd and 4th day of fumigation, their resistances approached those of control leaves at night, indicating practically continuous stomatal closure. A rather surprising result was that the nighttime resistance of fumigated leaves was lower than that of control leaves. This is in agreement with the observed higher nighttime transpiration of fumigated plants mentioned above. We can only speculate as to the cause of the decreased nighttime resistance caused by fumigation. The fluoride may have damaged the stomatal mechanism. An alternate but less likely hypothesis is that the fluoride damaged the cuticle and increased cuticular transpiration. Since soybean leaves have stomates on both surfaces, it was impossible to test the influence of fluoride on cuticular resistance alone.

The resistances of different leaves of control plants were measured separately and are given as their reciprocals or
Table II. Relationship between Leaf Age or Position and Conductivity in Nonfumigated Plants and the Fluoride Contents of Leaves Fumigated for 4 Days

<table>
<thead>
<tr>
<th>Position of the Leaf</th>
<th>Leaf Conductivity</th>
<th>Fluoride Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm min⁻¹</td>
<td>mS/dm² cm⁻¹</td>
</tr>
<tr>
<td></td>
<td>0 hr</td>
<td>0600 hr</td>
</tr>
<tr>
<td>Primary leaf</td>
<td>0.87</td>
<td>1.03</td>
</tr>
<tr>
<td>Second leaf</td>
<td>0.47</td>
<td>0.65</td>
</tr>
<tr>
<td>Third leaf</td>
<td>0.49</td>
<td>0.73</td>
</tr>
<tr>
<td>Youngest leaf (half-grown)</td>
<td>0.66</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of a single 6-hr fluoride fumigation on stomatal opening in soybean leaves. Each point is the average of one leaf from each of three plants.

Table III. Water, φ, and Osmotic, π, Potentials of Continuously Fumigated Leaves

<table>
<thead>
<tr>
<th>Duration of Fumigation</th>
<th>Repl. 1</th>
<th>Repl. 2</th>
<th>Repl. 3</th>
<th>Repl. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>-3.5</td>
<td>-12.8</td>
<td>-5.5</td>
<td>-9.0</td>
</tr>
<tr>
<td>24</td>
<td>-13.5</td>
<td>-12.0</td>
<td>-16.0</td>
<td>-10.5</td>
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<tr>
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<td>-6.5</td>
<td>-4.0</td>
<td>-6.5</td>
</tr>
<tr>
<td>72</td>
<td>-10.0</td>
<td>-11.3</td>
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<tr>
<td>96</td>
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<td>-38.0</td>
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<td>-35.5</td>
<td>-43.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-42.0</td>
</tr>
</tbody>
</table>

conductivities in Table II. The conductivities can be averaged to give mean conductivities for different leaves over a 24-hr period. The highest mean conductivity was found in the oldest trifoliate leaf with conductivities decreasing progressively up the stem with decreasing age of the leaves.

The observation that young expanding leaves develop symptoms more slowly than mature leaves has been attributed to difference in physiological age (1). Although physiological age may be a factor, the reduced leaf conductivity of the enlarging leaves and the reduced exposure time and dilution effect of leaf enlargement must also be taken into account in explaining relative sensitivity of different age leaves. Fluoride contents were also highest in the oldest trifoliate leaves (Table II). Since the plants were exposed to hydrogen fluoride for only a few days rather than their entire life, fluoride uptake would be expected to be mainly a function of leaf conductivity rather than duration of exposure. The substantially lower fluoride contents of the second and third trifoliate leaves may be due partly to increased resistance, and partly to the dilution of the absorbed fluoride by the expanding leaves which made some growth during the 4-day fumigation. Injury was also most severe on the older leaves, indicating good correlation among leaf resistance, leaf fluoride concentration, and injury.

In our studies, soil-grown plants were consistently more resistant to fumigation injury than solution-grown plants. The consistently higher leaf resistances measured on soil-grown plants would probably account for reduced fluoride uptake and reduced injury.

Speed of Stomatal Response. Measurement of leaf resistance at 6-hr intervals gave no accurate assessment of the promptness of stomatal response. To determine this more accurately, leaf resistance was monitored at 30-min intervals during a single 4-hr fumigation at 15 nl/liter HF. The stomates had clearly begun to close within 1 hr and were nearly closed when the fumigation was terminated after 4 hr and did not open completely the following day (Fig. 2). At night, the leaf resistance of the fumigated leaves seemed slightly lower than that of control leaves, as noted in other experiments. The resistances of control plants, although fluctuating, remained low through the light period, rising sharply when the light went off at 2000 hr. The fluctuations in leaf resistance between 0600 and 0800 hr on the 1st day are associated with transfer from the growth chambers to the fumigation chambers.

Leaf Temperature. All fumigation treatments caused increased nocturnal leaf temperature. The highest temperatures, about a degree above the air temperature of 28.5 C, were observed in continuously fumigated plants (Fig. 1, top). Leaf temperatures of the control plants averaged 2 to 3 C lower than the air temperature.

In both nighttime- and daytime-fumigated plants, leaf temperatures were intermediate between control and continuously fumigated plants and tended to increase as fumigation progressed. This temperature increase agrees closely with the leaf resistance data and also with the transpiration measurements, showing that stomatal closure and reduced evaporative cooling can account for the increased leaf temperature.

Water and Osmotic Potentials. Water potential, ψ, increased (became less negative) after 1 day of continuous fumigation (Table III). This could perhaps be related to stomatal closure, reduced transpiration, and improved water economy of the leaves. Osmotic potential, π, changed little during the 1st day. With continued fumigation, and the eventual necrosis, water and osmotic potentials fell sharply.

It is probable that, with the onset of visible injury, the cell potentials fell sharply.
permeability increased, causing leakage of solution from the cells and waterlogging of the tissue. With the presence of extracellular fluid the water potential measurement would be expected to approximate the osmotic potential. It is also possible that the sharply decreasing osmotic and water potential values resulted from the evaporation of water from the sap that leaks from damaged cells.

**DISCUSSION**

The effects of fumigation in reducing transpiration, raising leaf temperature, and temporarily increasing water potential all appear to be related to the prompt stomatal closure.

To explain the mechanism whereby fluoride induces stomates to close is beyond the scope of this study. As mentioned above, a waterlogged appearance is often associated with fluoride injury, indicating cell collapse and leakage of cell sap into intercellular spaces. If this occurred from guard cells, it would result in loss of turgor and closure. Stomatal opening involves active transfer of K into the guard cell (2, 12). Fluoride could conceivably interfere with this process by increasing the solute permeability so that K leaks out of the guard cell back to the subsidiary epidermal cells. Possibly an interference in Ca function in the membrane is involved (10).

Stomates are sensitive to small changes in internal leaf CO₂ concentration, tending to close partially with any increase in the concentration (3, 7). Fluoride fumigation decreases the rate of photosynthesis (13) and increases the rate of respiration (5, 11). Either factor changing alone, or both concurrently, would certainly cause the CO₂ concentration within a leaf to rise above the level found in healthy, photosynthetic leaves, thereby activating the closing mechanism. It might be argued that the reduced photosynthesis is due to stomate closure, rather than vice versa, but this argument counters the observed increase in respiration. Stomatal closure might thus be brought about directly by fluoride influence on membrane function, or indirectly through an influence on photosynthesis and respiration.

Partial or complete closure of stomates evidently reduces leaf fluoride content and leaf injury. In a study to be reported later, fumigation when stomates are closed during drought has been found to be less harmful than daytime fumigation under optimal soil moisture.

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**LITERATURE CITED**