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Macroscopic and Microscopic Responses of Gossypium hirsutum L. to Hydrogen Fluoride Fumigation

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Macroscopic and Microscopic Response of Gossypium hirsutum L. to Hydrogen Fluoride Fumigation[1]

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INTRODUCTION

Hydrogen fluoride is one of the more important gaseous air pollutants and its phytotoxic effects have been well established. The principal sources of atmospheric fluorides are producers of phosphate fertilizers, ceramics, and certain metals. Stomatal penetration and subsequent accumulation of fluorides may variously affect the growth rate, yield, and macroscopic appearance of exposed plants (DAINES **et al.** 1952). Wide interspecific and intraspecific differences in susceptibility to macroscopic injury by hydrogen fluoride have been reported (Thomas and Hendricks, 1956).

Microscopic study of fluoride injury in plants has been limited. Investigations by SOLBERG **et al.** (1955) and THRESHOW (1956) revealed microscopic damage only when macroscopic injury was evident and then only in tissues immediately adjacent to macroscopically damaged areas. Other fluoride fumigation studies (SOLBERG and ADAMS 1956) showed microscopic injury to appear first in abaxial mesophyll and lower epidermis. Little apparent damage was found in vascular tissue of exposed leaves. THRESHOW (1956) found epidermal cells less sensitive to damage than underlying mesophyll cells. Investigations by MOHAMED (1968) and MOHAMED **et al.** (1966) have shown that fluorides may also inflict chromosomal injury.

The present study was prompted by the sparsity of available information on microscopic effects of gaseous fluorides in plants, particularly in fluoride resistant plants such as **Gossypium hirsutum** L. Research objectives were to: (1) determine the macroscopic response of **G. hirsutum** to fluorides in a range of exposure levels, (2) compare fluoride injury symptoms to those reported for more sensitive species, and (3) assess microscopic damage in mesophyll of treated plants.

MATERIALS AND METHODS

Plants (**Gossypium hirsutum** L. Acala glandless var.) were grown and exposed to fluoride in environmental chambers under the following conditions: (1) Light — 1800 foot candles from fluorescent tubes and 200 foot candles from incandescent bulbs, as measured at a level 60 cm above the chamber floor; (2) Photoperiod — 14 hours light and 10 hours darkness; (3) Temperature — 30 ± 2 C during light periods and 24 ± 2 C during dark periods; (4) Air exchange — flow of 12 m^3 per minute in each of the 3 m^3 chambers, using fresh carbon-filtered air; (5) Relative humidity — 50 ± 5%; (6) Available moisture and nutrients — all plants were grown in a half-peat, half-perlite medium in 6-inch standard plastic pots and watered daily using a water-soluble 15-15-15 fertilizer at the rate of 1.2 grams per liter of distilled water; (7) Fumigation — fumigant was injected continuously into the treatment chamber at a constant rate of 2100 cm^3/minute, according to the method of HILL **et al.** (1959), modified to maintain the hydrogen fluoride generator at 22 ± 2C. Aqueous HF concentrations of 10%, 5%, and 2.5% were used in the generator during this study and these will subsequently be referred to as "high," "medium," and "low" fumigant levels. Most experiments reported here used high fumigant levels which resulted in HF concentrations of 2.3 to 3.2 $\mu g/m^3$ of air in the treatment chamber.

Initial studies utilized 12-day old plants (post-emergence) which were exposed continuously for the next 14 days to high fumigant levels. Subsequent experiments using low and medium fumigant levels were conducted on plants ranging in age from 0 to 56 days after emergence. All fumigations were repeated five times, using 12 plants per chamber. To minimize variations in stomatal penetration of the fumigant, all exposures were initiated during the early parts of photoperiods.

Leaf samples from treated and control plants were collected repeatedly during each fumigation run and fresh hand sections examined by the phase microscope. Representative samples were also fixed in FAA and later processed into permanent slides. Samples were taken from treated plants exhibiting all types and degrees of macroscopic foliar damage. Hand sections were mounted in isotonic or slightly hypotonic sucrose. The plasmolytic response of cells was studied by varying the sucrose concentration in the mounting medium.

Chloroplast frequencies in functional palisade cells of 26-day old plants were determined after one 14-day fumigation run with high fumigant levels. For this purpose, five treated and five control plants were randomly selected at the end of fumigation. The number of chloroplasts was then microscopically determined in each of 30

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palisade cells from three randomly selected sections obtained from the first leaves above the cotyledons on each plant.

The fresh and dry weights of all plant parts above and including cotyledons were determined after five separate fumigations. Each of these fumigations exposed 12-day old plants for the next 14 days to high fumigant levels and utilized 12 control and 12 treated plants. Dry weights were recorded after dehydration for 24 hours at 105 C.

RESULTS AND DISCUSSION

When the concentration and duration of fluoride fumigation is sufficient to induce injury, the macroscopic response of **G. hirsutum** is similar to that reported for more sensitive species. Table I summarizes the chief macroscopic effects produced by fumigating 12-day old plants for the following 14 days with high fumigant levels. Response of plants treated with lower fumigant levels was found to be similar except that injury symptoms appeared later and were less pronounced. Figures 5 - 8 show the injury symptoms most frequently observed in fumigated plants. Typical macroscopic damage to foliage consisted of varying degrees of marginal and intercostal chlorosis or necrosis. Wrinkling and distortion of the lamina was also common in younger leaves. Injury was most extensive in emerging or partially expanded cotyledons and leaves. Treated plants, especially during early stages of growth, increased more slowly in height, fresh weight, and dry weight, than corresponding controls. As shown in Figure 1, the effect of fluoride on fresh and dry weight production was pronounced. Although some variation in response to fluoride was common, many treated plants were observed to increase slightly in height but very little in foliar development over that present at the initiation of fumigation.

Light microscopy revealed a close correlation between the macroscopic and microscopic appearance of leaves. The photomicrograph in Figure 9 shows a transverse hand section of a normal expanded leaf, while Figures 10 and 11 show microscopic injury in leaves of fumigated plants. Except for the observation of fewer chloroplasts in treated mesophyll, microscopic damage was restricted to foliar tissue within 1 mm of areas exhibiting macroscopic effects. Within 0.5 mm of necrotic areas, all cells were typically in varying states of plasmolysis when placed in a medium considered isotonic or slightly hypotonic for control cells. Mesophyll cells from other macroscopically damaged tissues were also found to plasmolyze and undergo cell wall collapse more readily than control cells.

The number of chloroplasts in palisade cells of expanded leaves was determined by randomly selecting tissue samples and then focusing through individual cells with the light microscope. As shown in Figure 2, the mean cellular chloroplast frequency was significantly lower in treated plants than in controls. Whether this lower frequency in treated cells resulted from the degradation of chloroplasts during the fumigation period could not be determined by light microscopy. However, chloroplasts in moderately to severely chlorotic tissues apeared in the light microscope to have decreased pigmentation and frequently exhibited a surface granularity as seen in face view.

Spongy mesophyll cells generally responded to fumigation before palisade cells. This difference in response may be attributable to an increased exposure to the fumigant brought about by comparatively more extensive intercellular air spaces and less intercellular support in the spongy layer. However, in leaves which were nearly fully expanded before fumigation, damage frequently developed in localized intercostal spots, with the collapse of palisade cells and upper epidermis preceding the collapse of abaxial leaf tissue (Figure 10). In advanced stages of injury (Figure 11) a complete collapse of leaf tissue occurred. After dehydration, such tissue would appear as necrotic areas on the leaf (Figure 8).

Cells of the vascular tissue were generally more resistant to cell-wall collapse and appeared less sensitive to plasmolysis than surrounding mesophyll cells. However, these differences in plasmolytic behavior were not so pronounced when all cells, adjacent to cut surfaces, received approximately equal exposure to the fumigating atmosphere.

SUMMARY

The macroscopic and microscopic effects of chronic exposure of **G. hirsutum** to hydrogen fluoride were studied. Macroscopic foliar injury included wilting, marginal and intercostal chlorosis and necrosis, and distorted expansion of leaves. The rapidity with which damage became evident and its extent were inversely correlated with plant age and directly correlated with fumigant concentrations and durations. Visible growth retardation in exposed plants was further evidenced by lower fresh and dry weight production. Light microscope study revealed altered plasmolytic behavior and fewer chloroplasts in mesophyll cells of fumigated plants.

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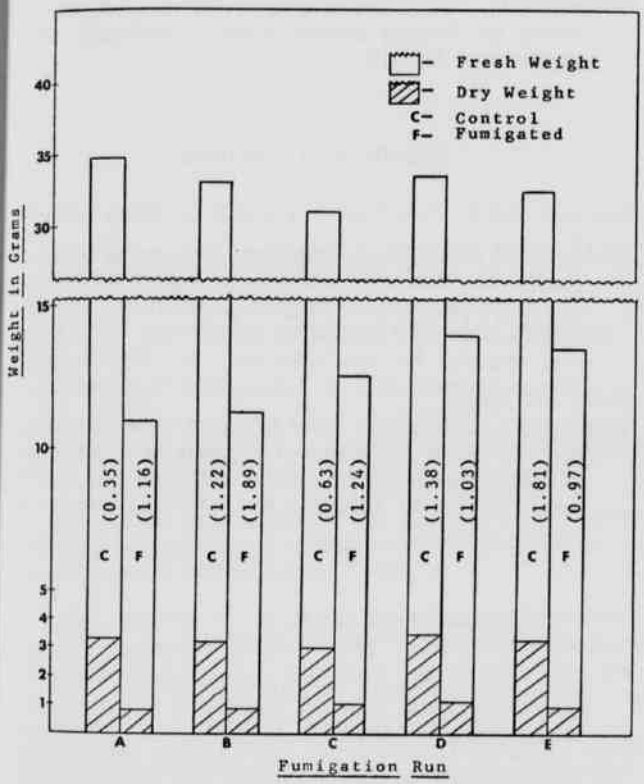
FIGURE 1

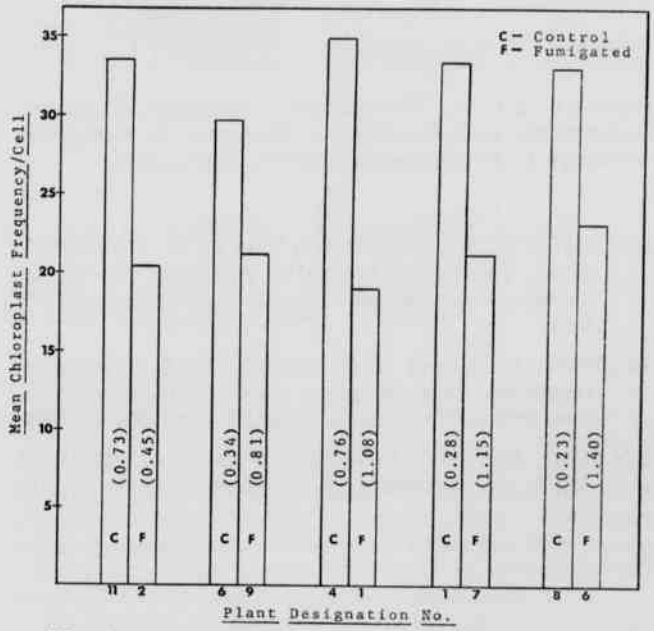
Mean* Fresh and Dry Weights of 26-Day Gossypium hirsutum L. Exposed For Preceding 14 Days to High Fumigant Concentrations - Versus Control Plants



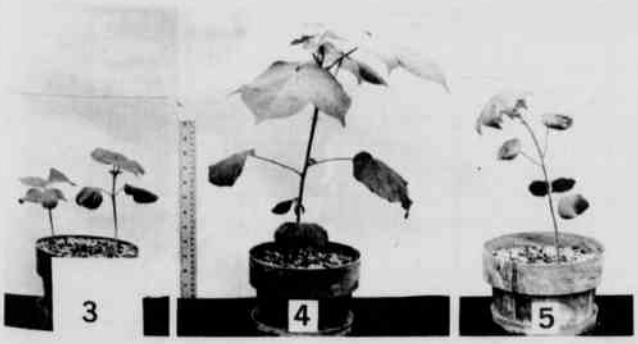
*Mean fresh and dry weights based on 12 treated and 12 control plants for each fumigation run. Standard error of the mean for fresh weights shown in parentheses.

FIGURE 2

Mean* Chloroplast Frequency in Functional Palisade Cells of 26-Day Gossypium hirsutum L. Exposed For Preceding 14 Days to High Concentrations of Fumigant - Versus Controls

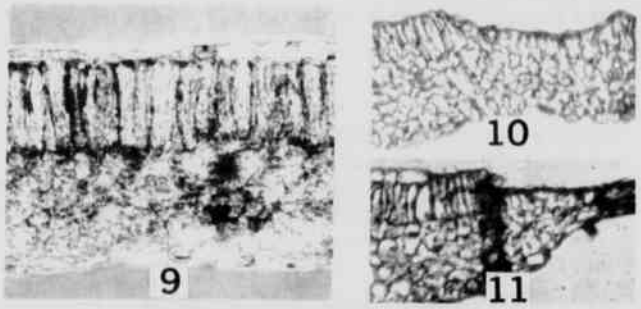


*Mean based on number of chloroplasts in 30 cells from three randomly selected sections per plant. Sections collected from first leaves above cotyledons on five fumigated and five control plants randomly selected and paired at end of fumigation run. Standard errors shown in parentheses.



Figures 3, 4, 5. (Timmermann, Applegate, Engleman.) Macroscopic and Microscopic Response of **Gossypium hirsutum** L. to Hydrogen Fluoride Fumigation.

Figures 6, 7, 8. (Timmermann, Applegate, Engleman.) Macroscopic and Microscopic Response of **Gossypium hirsutum** L. to Hydrogen Fluoride Fumigation.



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LEGENDS FOR FIGURES

Figures 1 and 2. See Figures 1 and 2 for these legends.

Figures 3-5. **Gossypium hirsutum.** Figure 3. Control plants, 12 days after emergence. Figure 4. Control plant, 26 days after emergence. Figure 5. Fluoride treated plant, 26 days after emergence. This plant was exposed for the preceding 14 days to high fumigant levels (2.3 to 3.2µg fluoride/m³ of air).

Figures 6-8. Macroscopic foliar injury in fluoride treated plants. Figure 6. Marginal necrosis in a cotyledon (X0.41). Figure 7. Intercostal chlorosis and wrinkling in a young trifoliate leaf (X 0.31). Figure 8.

TABLE I

Macroscopic Effects Produced by Fumigating 12-Day Old Gossypium hirsutum L. for the Succeeding 14 Days With Atmospheres Containing High Concentrations of Fumigant

Macroscopic Effect	Description and/or Location of Effect	Percentage of Plants Exhibiting Effect After Indicated Days of Exposure*															
		¼	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Water-logged or wilted appearance (Rapidly becoming Necrotic)	Cotyledonary Margins	7	8	23	32	37	52	58	67	72	72	80	83	87	87	87	88
Marginal Chlorosis	First leaves above cotyledons	-	-	-	-	-	-	-	2	5	8	17	22	22	23	30	32
	Other expanding leaves	-	-	-	-	3	7	15	15	42	67	80	87	92	92	95	95
Generalized Intercostal Chlorosis	First leaves above cotyledons	-	-	-	-	-	-	-	-	-	-	2	7	17	17	23	28
	Other expanding leaves	-	-	-	-	-	8	13	37	45	63	75	82	88	90	90	92
Pin-Point Chlorosis	First leaves above cotyledons	-	-	-	-	-	-	-	5	13	18	32	35	42	43	48	55
	Other expanding leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	3	7	7
Marginal Necrosis	First leaves above cotyledons	-	-	-	-	-	-	-	-	-	-	7	13	15	20	23	23
	Other expanding leaves	-	-	-	-	-	-	-	-	10	18	27	43	57	65	72	77
Generalized Intercostal Necrosis	First leaves above cotyledons	-	-	-	-	-	-	-	-	-	-	-	-	8	10	10	15
	Other expanding leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	7	12	13
Pin-Point Necrosis	First leaves above cotyledons	-	-	-	-	-	-	-	-	-	7	17	23	30	33	42	42
	Other expanding leaves	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Wrinkling or Curling of Leaves	First leaves above cotyledons	-	-	-	-	-	-	-	-	-	-	3	8	15	18	27	32
	Other expanding leaves	-	-	-	-	5	17	27	42	53	68	80	87	93	97	97	97
Retardation of Growth	Retarded plant height or decreased foliar development as compared to controls	-	-	-	-	-	22	37	53	73	92	100	100	100	100	100	100

* - Mean Whole Percentages Based on 5 Fumigations With 12 Plants per Fumigation

Severe chlorosis and necrosis in the first leaf above the cotyledons (X 0.31).

Figures 9-11. Photomicrographs of transverse sections of leaves. Figure 9. Fresh hand section of a normal expanded leaf, mounted in isotonic sucrose (X140). Figure 10. Paraffin embedded section showing an initial stage of leaf damage in a fluoride treated plant. Collapse of the upper epidermis and underlying palisade cells is evident (X100). Figure 11. Paraffin embedded section showing an advanced stage of fluoride damage. Progressively increasing degrees of injury can be seen from left to right with complete collapse of leaf tissue evident on the right (X100).

Observations on the Impact of Certain Insecticides On Spider Populations in a Cotton Field

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Insecticide application is the greatest single factor affecting populations of predators which act as biological control agents within cotton fields.

According to a Louisiana Agriculture Experiment Station Bulletin (1967), many bollworm moths are caught in spider webs. The most important spider species effective in control of cotton field insects is the star-bellied orb weaver **Acanthepeira stellata** (Walckenaer), but other large orb weavers **Neoscona sacra** (Walckenaer), and various species of **Araneus** also captured many moths. Certain spiders capture bollworm moths directly, without using webs. **Lycosa rabida** (Walckenaer), **Lycosa helluo** (Walckenaer), **Lycosa carolinensis** (Walckenaer), **Lycosa annexa** (Chamberlin and Ivie), **Schizocosa avida** (Walckenaer) and other hunting wolf spiders attack bollworm moths, especially when they light on the ground.

A few spiders attack large bollworms on the plant. The female green lynx, **Peucetia viridans** (Walckenaer), bites the larva medially and shifts her hold to the head when the larva expresses backward body contortions in response to the initial bite. Large jumping spiders such as **Phidippus audax** (Hentz) will attack even the largest bollworm larva.

Observations of spiders feeding on bollworm eggs showed that jumping spiders accounted for 1.5 per cent destruction of eggs in a local cotton field.

At least 22 species of spiders have been collected from cotton fields and many more from areas adjacent to cotton fields. Spiders appear to be more abundant than any other predators in cotton fields. They also seem to persist better than other predators after applications of certain insecticides such as either 3-5-40 (BHC-DDT-sulfur) or calcium arsenate.

It is a well-known fact that the level of insecticide resistance of boll weevils and bollworms increases as the season progresses and that insecticide application is the greatest single factor affecting populations of predators within cotton fields; therefore, to spray heavily with insecticides drastically reduces predator control by spiders.

In an effort to elucidate the effects of spiders as biological control agents, the writer collected species of spiders from a Clark County, Arkansas cotton field and subjected them to common insecticides to show the reduction in numbers of spiders as insecticides are applied.

Families of spiders such as Salticidae, Oxyopidae, Araneidae, Thomisidae, Lycosidae, and Dictynidae were widely distributed over the fields, and surrounding grasses and wooded areas. Live specimens were taken from the cotton plants, ground, and surrounding vegetation by hand picking and by use of a heavy insect net.

In Clark County, Toxaphene-DDT, Methyl Parathion, and Sevin are the most widely used insecticides for control of insect pests in cotton fields. Standard solutions which are commonly applied to fields were used to determine effects on spiders kept in captivity. The table below shows the results obtained after one day and one application: