A MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION OF LEAF BURN INDUCED FROM FOLIAR APPLIED NUTRIENTS

by

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A B S T R A C T

Foliar applied nutrients have been used to overcome nutrient deficiencies on a variety of crops; however, leaf burn is often a problem. Zea mays plants were treated with 12% N from urea to study the leaf burn phenomenon. A 4 µl drop applied to the adaxial leaf surface was observed at 2 hr. intervals for 8 hrs. Samples were studied with a dissecting, compound, and scanning electron microscope for changes resulting from the application of foliar applied fertilizer salts. Damage, observed under the dissecting microscope after 2 hrs., consisted of a darkening in the epidermal cells. After 8 hrs., the epidermis was desiccated, sunken, and discolored forming a lesion on the leaf surface. Slides of leaf sections showed wrinkling and collapse of epidermal cells at 4, 6, and 8 hrs. Disorganization of mesophyll was observed after 8 hrs. SEM micrographs revealed collapsed and wrinkled epidermal cells with sunken stomates after 2 hrs. Events associated with visual damage appear to be related to water loss since epidermal and mesophyll cells become desiccated.

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REVIEW OF THE LITERATURE

Foliar applications of nitrogen, phosphorus, potassium, calcium, sulfur, iron, and zinc have been sucessfully used on a variety of crops (1). Emino, Storey, and Smith (2) found zinc uptake to be enhanced by foliar applications of nitrogen zinc nitrate solution on container grown shrubs. The advantages of foliar fertilization include the rapid absorption and efficient utilization of nutrients (8, 12). In addition, this practice can often be combined with disease and insect spray programs, therefore saving time and labor (12).

One of the main problems encountered with foliar sprays is that of leaf burn. Neumann and Prinz (7) report that such burn damage can lead to leaf drop, and in the case of fruits can considerably reduce their market value because of their poor appearance. Syverud, Walsh, and Oplinger (11) found that foliar applications of N, P, K, and S caused damage to the surface of soybean leaves. They hypothesized that the spots of necrotic or dead tissue were caused by the desiccation of leaf cells when the solution droplets dried on the leaves. Since the cuticle of leaves and fruits is generally a waxy water repellent layer, aqueous sprays tend to form into droplets on cuticular surfaces. As droplets of spray solutions dry out, the relative concentration of solute increases rapidly and diffusion through the cuticle can lead to osmotic or toxic damage to underlying cells (7).

The development of leaf burn resulting from foliar sprays is not clearly understood; however, leaf burn due to acid rain and air pollutants such as ozone and sulfur dioxide has been studied extensively. Haines and Stefani (5) subjected 8 plant species to artificial acid rains ranging in pH from 2.5 to 0.5. Droplets of pH 2.0 produced brown necrotic spots on 7 of the 8 species, while droplets of pH 1.0 produced necroses on leaves of

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This thesis is consistent with the style and format of the Journal of the American Society for Horticultural Science.

all species examined. In addition, the size of the necrotic spots increased with increasing acidity. Evans et al. (3) subjected Phaseolus vulgaris and Helianthus annus to daily exposure to simulated sulfate acid rain, pH 2.7. Injury was in the form of lesion development on the adaxial leaf surface which ranged from shallow, circular depressions in the leaf surface less than 0.25 mm in diameter to a large, irregularly shaped lesion greater than 2 mm in length in severe cases.

Varshney and Garg (3) worked with plant responses to air pollution and concluded that plants grown in polluted air showed visual leaf injury such as necrotic patches, chlorosis, dead interveinal tissues, and enrolled leaf margins. Swiecki et al. (10) subjected bean leaves to exposure to gaseous hydrogen chloride, and observed injury in all leaves studied. Visible necrotic lesions, often in the form of "pits", were seen in 0.060 N HCl exposures. The "pits" appeared as indented, roughly circular lesions usually 1 mm or less in diameter. Swiecki et al. hypothesize that some portion of the gaseous HCl condenses on the leaf surface, producing an aqueous acid solution that promotes cellular injury. They further speculate that the acid acts on the plasmalemma, making it "leaky", resulting in an array of symptoms that stem from disruption of membrane function and resultant water loss from affected cells. Evans and Miller (4) exposed Pinus ponderosa to fumigations of 0.45 ppm ozone for 12 hours per day. Chlorotic mottling occured on the pine needles, being the most intense on the older needles. The current-year needles exhibited mottling which developed from the tip to the base of the needle. Complete chlorosis and needle-tip dieback followed.

Syverud et al. (11) suggested that the damage caused to the adaxial leaf surface was caused by the desiccation of leaf cells when the solution droplets dried on the leaves. If this hypothesis is correct, lesion devel-

opment should be characterized by a desiccation of the epidermal cells followed by plasmolysis of mesophyll and vascular tissue. The experiment described here explains how foliar applied nutrients influence the leaf structure when leaf burn occurs by looking at the surface morphology and cross-sectional anatomy of the leaf. Detailed observations of macro- and microscopic injury symptoms in leaves exposed to foliar applications of urea are reported.

MATERIALS AND METHODS

The third fully expanded leaf of corn (Zea mays L. var. Dekalb XL55A), grown from seed in a controlled greenhouse environment was used in the experiment. Plants were grown in 10-cm pots with 3 seeds planted per pot. The soil medium consisted of equal parts of peat moss and vermiculite. The plants were hand watered regularly and fertilized with 200 ppm N, 88 ppm P, and 166 ppm K at each watering.

Leaves of corn plants were exposed to treatments 14 days after seeding. All experiments were carried out in the laboratory under room temperature of 21^{o} C and a relative humidity of approximately 68. The following treatments were employed: 1) 12% N in the form of urea, pH 6.3. 2) Distilled water, pH 6.8, as a control. The treatments were applied as a 4 μl drop to the adaxial leaf surface. The treated plants were placed under artificial light. Treated leaves of all plants were observed for visible injury and sampled for light and scanning electron microscopy at 2 hr. intervals for 8 hrs. after the start of the treatments as shown in Table 1.

TABLE 1. Time Course Study Procedure

Treatment Number	Time in Hours	Number of Plants
1	0	6 → 2 for surface observation
	2	
	4	2 for SEM
2	6	
	8	2 for compound microscopy

For surface observation, live plant material which had been treated was observed under a dissecting microscope, where notes were recorded and photomicrographs taken. For light and scanning electron microscopy, leaves were excised and tissue samples were cut from the lamina with razor blades. Samples were taken from the treated area, approximately in the center of the leaf. For light microscopy, the tissue was killed and fixed in formalin-acetic acid (FAA) under reduced pressure. The samples were prepared using standard histological techniques. The samples were dehydrated in a graded ethyl alcohol series. After dehydration, the tissue was infiltrated and embedded in paraffin and allowed to solidify. Sections were cut at 10 μm on a rotary microtome and stained in safranin-fast green. The slides were observed under the compound microscope where photomicrographs were taken. Leaf tissue for SEM examination was killed and fixed in FAA under reduced pressure. The samples were dehydrated using ethanol, transferred to iso-amyl acetate, critical point dried, mounted on an aluminum stub with double stick tape, and coated with 200 Å gold/paladium. Specimens were examined with a JEOL JSM-25SII SEM operating at 15 KV.

RESULTS

After exposure to urea at 12% N, the leaves of 14-day-old corn plants exhibited inconsistent damage at the various sampling times. The drop of urea exhibited 1 of 3 fates as observed under the dissecting microscope. Rapid drying of the urea caused salt crystal formation on the leaf surface with no apparent injury. This was seen at all sampling times. Complete absorption of the urea was observed after 4 hrs. This was associated with desiccation of the epidermal cells in the treated area along with a slight color change in the epidermis. The third fate of the urea treatment was the formation of a hydroscopic gel-like deposit on the adaxial leaf surface. This was seen after 4 hrs., and was associated with a darkening in the epidermal cells followed by collapse and desiccation of the epidermis forming a lesion on the leaf surface. The damage observed from the urea treatment was random among the plants sampled. Where injury was observed, the 8 hr. observations differed from the 4 hr. observations by minor increases in visible injury. No injury was seen in the leaves treated with distilled water.

In longitudinal and cross-sectional views of leaves treated with distilled water, no injury was observed at any time interval. The upper and lower epidermis of the control exhibited large turgid cells and the mesophyll was compact with few intercellular spaces. Vascular bundles were scattered throughout the mesophyll and were surrounded by bundle sheath cells with thick cell walls (Fig. 1a, c, and e). Cellular injury was first observed in a longitudinal section of a leaf treated with urea after 6 hrs. Damage consisted of collapse and wrinkling of both the adaxial and abaxial epidermal cells. The mesophyll cells plasmolyzed creating large intercellular spaces. Longitudinal sections of leaves treated with 12% N from urea

after 8 hrs. showed similar results. Adaxial and abaxial epidermal cells were distorted in shape due to collapse and severe wrinkling. Epidermal collapse was followed by plasmolysis of mesophyll cells resulting in the formation of large intercellular spaces causing the mesophyll to appear disorganized (Fig. 1d). Cross-sectional views of leaves sampled 8 hrs. after treatment with 12% N from urea appeared similarly. The epidermis in the treated area was collapsed and wrinkled causing it to appear distorted. The vascular bundle looked disorganized as compared with that of the control. Large intercellular spaces were present in the mesophyll due to plasmolysis (Fig. 1b and f).

Scanning electron micrographs showed similar results. Leaves treated with distilled water were not injured and illustrated the large raised epidermal cells of an undamaged leaf (Fig. 2a and e). Injury was observed in leaves sampled 2 hrs. after treatment with 12% N from urea. Damage appeared to involve wrinkling of the adaxial epidermal cells. Several damaged areas were evident (Fig. 2c). Injury was further characterized by collapse of the epidermis in the treated area along with severe wrinkling as seen after 2 and 4 hrs. (Fig 2b and d). After 4 hrs., in addition to collapse and wrinkling of the epidermis, the guard cells appeared somewhat sunken as compared to the distilled water control (Fig. 2f).

Figure 1a-f. Adaxial leaf sections of Zea mays after treatment with distilled water and 12% N from urea. a. Control sampled immediately after treatment with distilled water. 1 bar = 100 μm. b. Leaf section sampled 8 hrs. after treatment with 12% N from urea. Cells of both the adaxial and abaxial epidermis appear collapsed. Mesophyll cells appear plasmolyzed. Vascular bundle shows slight disorganization. 1 bar = 100 μm. c. Leaf section sampled 8 hrs. after treatment with 12% N from urea, taken from an untreated area, illustrating large turgid epidermal cells and compact mesophyll with few intercellular spaces. 1 bar = 100 μm. d. Sampled 8 hrs. after treatment with 12% N from urea. Adaxial and abaxial epidermal cells show plasmolysis and cell wall waviness indicative of incipient collapse. Mesophyll cells have plasmolyzed resulting in large intercellular spaces. 1 bar = 100 μm. e. Control sampled immediately after treatment with distilled water. Mesophyll cells are compact with few intercellular spaces. 1 bar = 50 μm. f. Sampled 8 hrs. after treatment with 12% N from urea. Mesophyll cells have plasmolyzed resulting in the formation of large intercellular spaces. 1 bar = 50 μm.

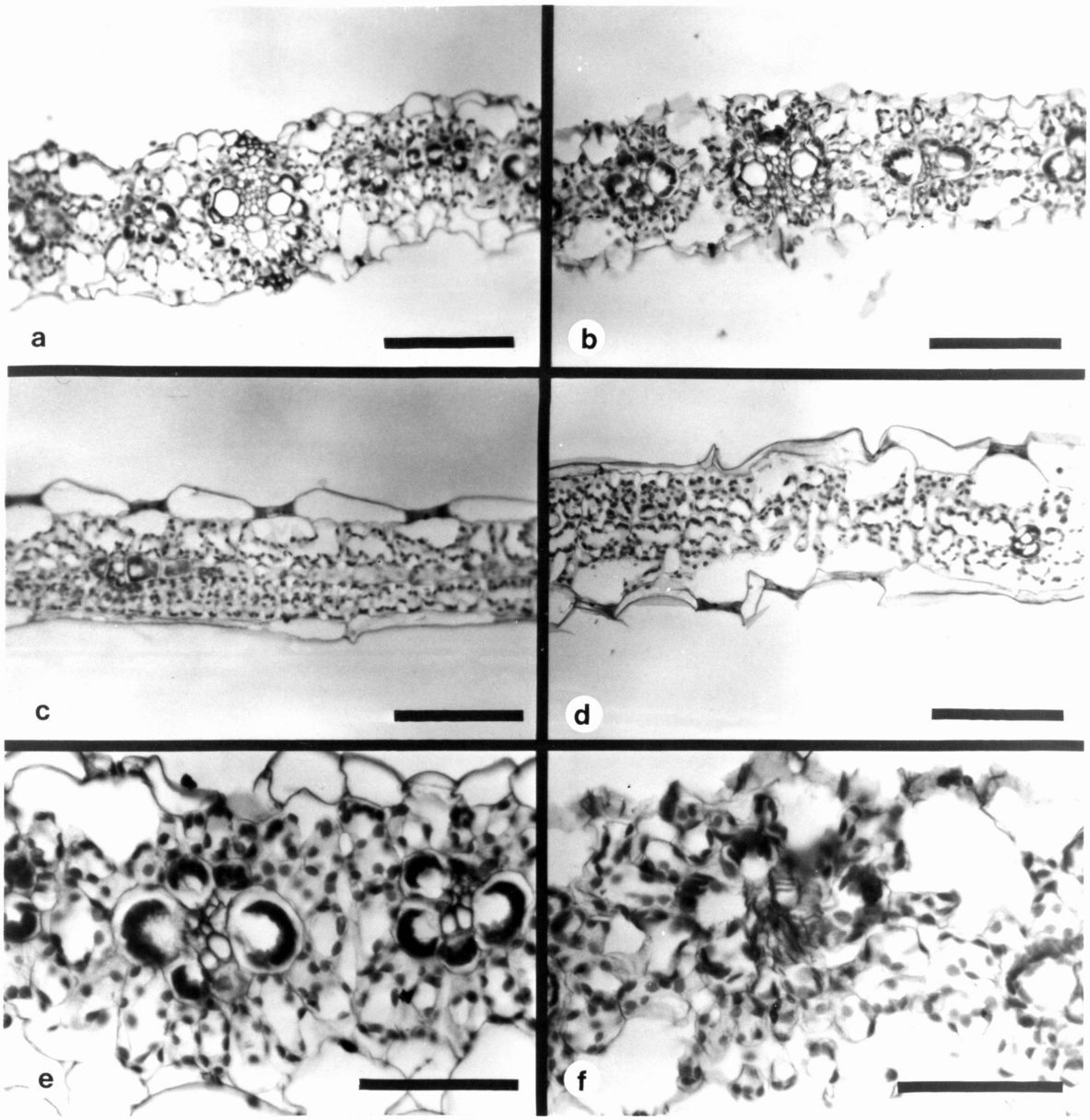
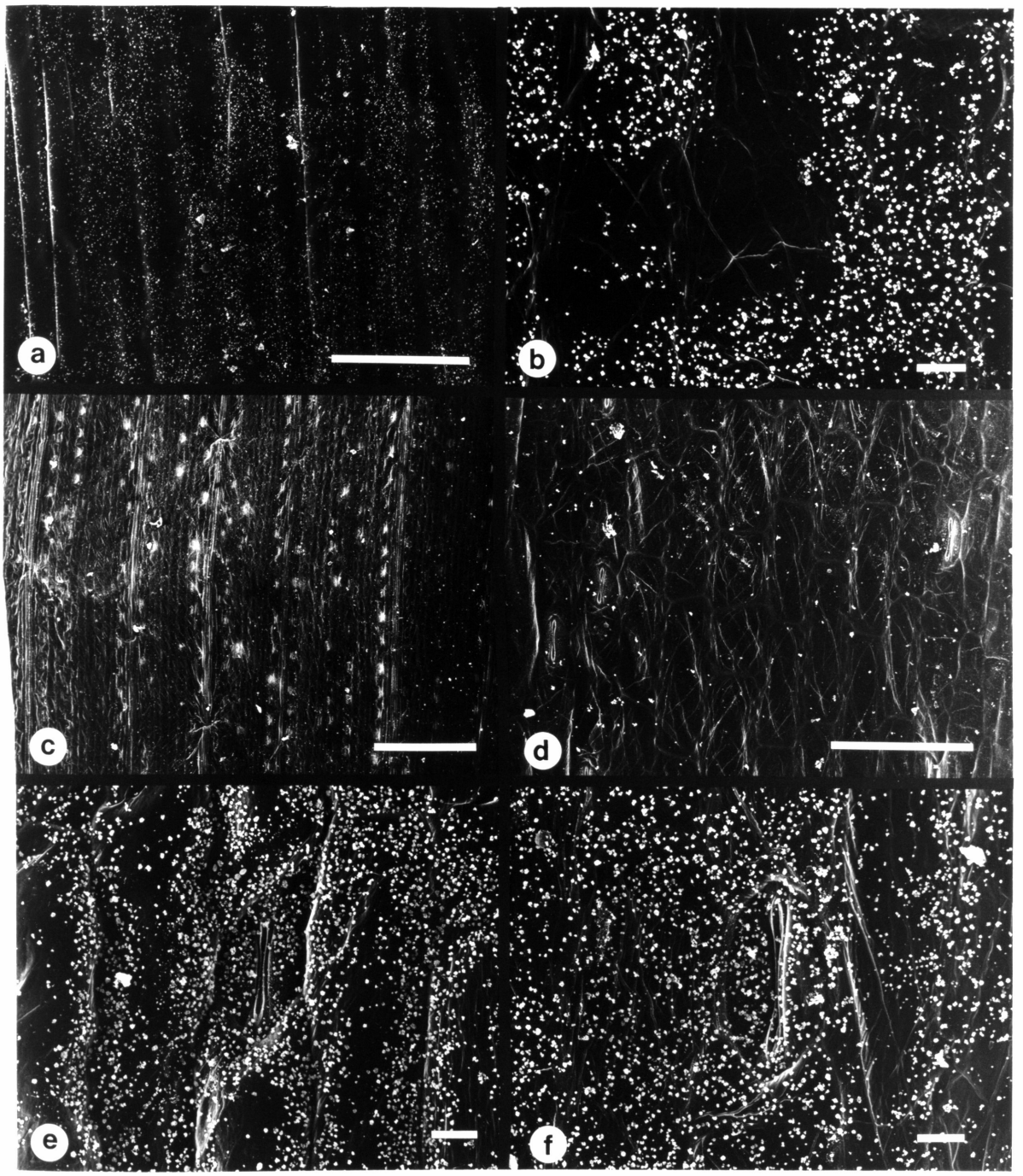

a
b
c
d
e
f

Figure 2a-f. SEM views of Zea mays leaf surfaces after treatment with distilled water and 12% N from urea. a. Adaxial leaf surface treated with distilled water at time 0 illustrating large smooth epidermal cells. 1 bar = 100 μm. b. Adaxial leaf surface treated with 12% N from urea after 4 hrs. Epidermal cells show collapse and wrinkling in the treated area. 1 bar = 10 μm. c. Adaxial leaf surface sampled 2 hrs. after treatment with 12% N from urea. Damage involves wrinkling of epidermal cells. Several damaged areas are evident. 1 bar = 500 μm. d. Adaxial leaf surface treated with 12% N from urea after 2 hrs. Collapse and severe wrinkling of epidermal cells have occured. 1 bar = 100 μm. e. Adaxial leaf surface sampled 4 hrs. after treatment with distilled water. Epidermal cells are raised and distinct. 1 bar = 10 μm. f. Adaxial leaf surface sampled 4 hrs. after treatment with 12% N from urea. Collapse and wrinkling of epidermal cells is indicated. Guard cells appear sunken perhaps due to plasmolysis. 1 bar = 10 μm.


a
b
c
d
e
f

DISCUSSION

Leaves of Z. mays treated with 12% N from urea exhibiting damage are clearly the result of the treatment since control plants treated with distilled water had no damage. The mechanism of leaf damage must involve water loss as either a direct result of the damage or an indirect effect resulting from disruption of membrane function as suggested by Swiecki et al. (10).

Where visible injury was observed, it was greatest when a hydroscopic gel-like deposit was present on the leaf surface lending credence to the view that damage results from the desiccation of epidermal cells as water moves from the leaf tissue to the hydrated salt. The damage observed here, a localized lesion on the adaxial leaf surface, was consistent with the description of the burn observed by Syverud et al. (11).

The generalized sequence of injury due to foliar applications of 12% N from urea corresponds closely to injury caused by sulfate acid precipitation (3). Histologically, acid rain injury was characterized by collapse of adaxial epidermal cells followed by plasmolysis and collapse of mesophyll cells. These observations are consistent with the type of injury seen in this study. Evans et al (3) report that even where severe lesion development occured, the vascular tissues and supportive tissues remained distinct possibly due to the rigid cell walls of these tissues. However, in this study, treatment with 12% N from urea after 8 hrs. caused the vascular tissue to appear disorganized and not as clearly defined as that of the control (Fig. 1f).

Swiecki et al. (10) speculate that aqueous HCl acts on the plasmalemma, making it "leaky", which results in an array of symptoms that stem from disruption of membrane function and resultant water loss from affected cells. This could be a possible mechanism for the damage observed from the appli-

cation of foliar applied nutrients since water loss from the affected cells was involved. Plant age has been shown to have a direct affect on the degree of lesion development resulting from acid precipitation and exposure to gaseous HCl. Evans et al. (3) found Helianthus annus more sensitive to acid rain when in the 3-4 leaf stage than when in the 5-6 and 7-8 leaf stages. However, in Phaseolus vulgaris, very young leaves were not as sensitive to acid rain as more mature leaves. Swiecki et al. (10) report that the only differences between leaf age and gaseous HCl injury appeared to be in the number of affected cells and not the type of injury. No correlation between leaf age and degree of lesion development can be drawn from our study since all corn plants used in the experiments were 14 days old and in the 3-4 leaf stage.

It appears that events associated with visual leaf damage resulting from the application of foliar applied fertilizer salts are related to water loss. Injury can be characterized by a darkening of the epidermal cells followed by collapse and desiccation of the epidermis resulting in the formation of a lesion on the adaxial leaf surface. Mesophyll cells exhibit further collapse and plasmolysis. These results are consistent with the hypothesis presented by Syverud et al. (11) that the spots of necrotic tissue are caused by the desiccation of leaf cells when the solution droplets dry on the leaves.

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Appendix 1: Tissue Preparation for Standard Histological Technique.

Jensen, W.A. 1962. Botanical Histochemistry. W.H. Freeman and Co., San Francisco, California.

Kill and Fix Tissue in FAA
(Acetic acid, formaldehyde, alcohol) (v/v 1:2:10)

↓

5% ETOH - 1 to 2 hrs.

↓

10% ETOH - 1 to 2 hrs.

↓

30% ETOH - 1 to 2 hrs.

↓

50% Alcohol - 2 to 4 hrs.
(40 ml 95% ETOH, 50 ml H_2O, 10 ml TBA)

↓

70% Alcohol - 2 to 4 hrs.
(30 ml H_2O, 50 ml 95% ETOH, 20 ml TBA)

↓

85% Alcohol - 2 to 4 hrs.
(15 ml H_2O, 50 ml 95% ETOH, 35 ml TBA)

↓

95% Alcohol - 2 to 4 hrs.
(45 ml 95% ETOH, 55 ml TBA)

↓

100% Alcohol - 2 to 4 hrs.
(25 ml absolute ETOH, 75 ml TBA)

↓

3 Changes in Tertiary Butyl Alcohol - 8 hrs.

↓

Infiltrate in Paraffin - 2 changes

↓

Embed in Fresh Paraffin

↓

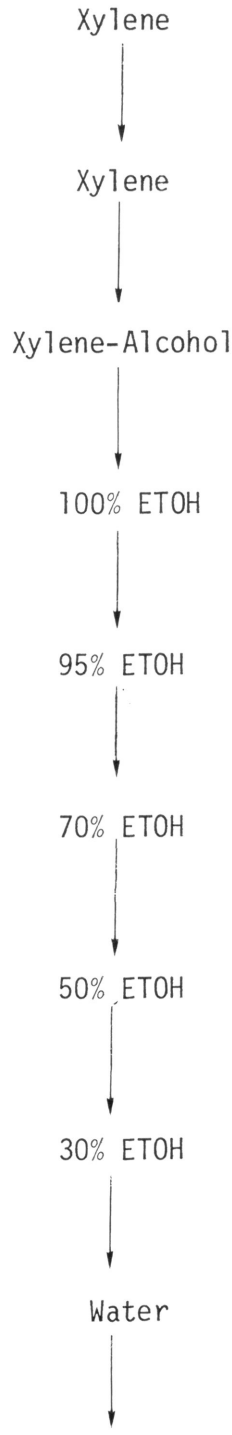
Section 10 μm Thick on a Microtome

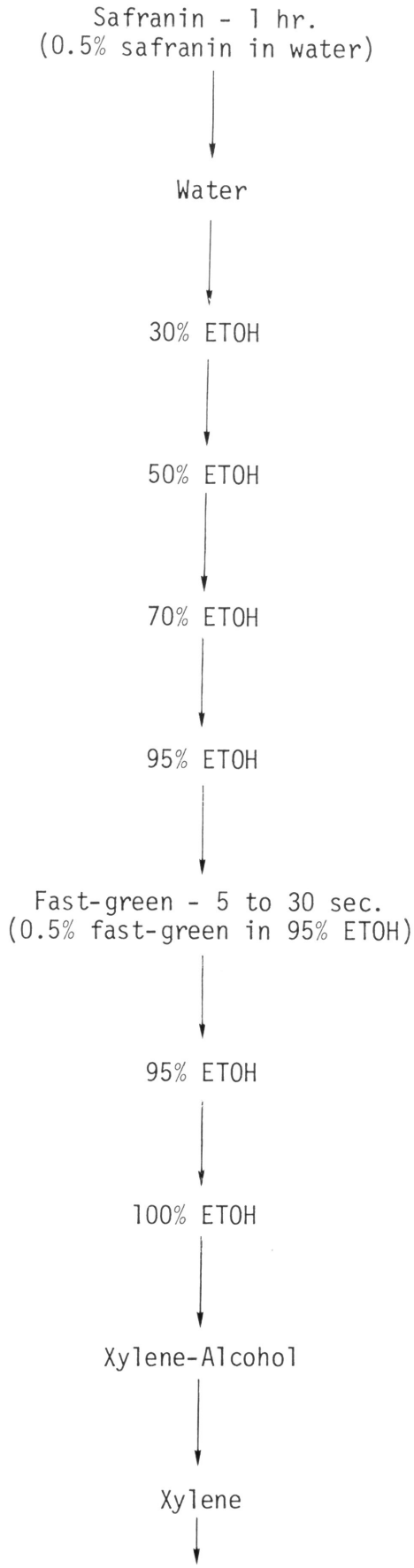
↓

Affix Sections to Slides using Haupt's Adhesive

Appendix 2: Sample Staining Procedure.

Sass, J.E. 1958. Botanical Microtechnique. 3rd. Ed. The Iowa State University Press, Ames Iowa.




Safranin - 1 hr.
(0.5% safranin in water)
Water
30% ETOH
50% ETOH
70% ETOH
95% ETOH
Fast-green - 5 to 30 sec.
(0.5% fast-green in 95% ETOH)
95% ETOH
100% ETOH
Xylene-Alcohol
Xylene

Cover with cover slip held in place with Permount

Appendix 3: SEM Sample Preparation