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Cellular Alterations Resulting from Foliar Applications of HOE-39866[1]

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Abstract. **Formulated and technical grade HOE-39866 [ammonium-(3-amino-3-carboxypropyl)methylphosphinate] at concentrations of 10^{-1}, 10^{-2}, and 10^{-3}M were applied to leaf blade tissues of nonreproductive adult redroot pigweed (*Amaranthus retroflexus* L. #[3] AMARE) and fall panicum (*Panicum dichotomiflorum* Michx. # PANDI). Tissues were sampled at regular intervals after treatment and prepared for light microscopic examination. The major response of both species involved rupture and contortion of the interveinal mesophyll cells with concomitant disorganization** of the bundle sheath cells. Rapid epidermal collapse occurred in redroot pigweed but not in fall panicum. The **absence of adjuvants resulted in nonuniform symptom expression as herbicide droplets accumulated in depressions and along leaf margins. No other adjuvant-specific effect was observed. Herbicide concentration did not alter the final response but the time-to-expression increased as concentration decreased.**

Additional index words. **Herbicide-induced cytological damage,** *Amaranthus retroflexus, Panicum dichotomiflorum,* **AMARE, PANDI.**

INTRODUCTION

The effects of herbicides on cellular morphology within leaves are not often investigated, yet histological examination of treated tissue can identify probable areas of compound-specific activity. Mode of action studies, determination of species selectivity, and development of crop protectants would be facilitated with preliminary anatomical studies. For example, Morrison et al. (14), using histological techniques, found that the methyl ester formulation of flamprop [*N*-benzoyl-*N*-(3-chloro-4-fluorophenyl)-*DL*-alanine] interfered with normal differentiation of vascular tissues in wild oats (*Avena fatua* L. # AVEFA), and ultimately led to an inhibition of growth and development. Yamasue et al. (18), using similar techniques, showed that benefin [*N*-butyl-*N*-ethyl-2,6-dinitro-4-(trifluoromethyl)benzenamine] vapors caused cellular alterations in the leaf primordia and shoot apices of tobacco (*Nicotiana tabacum* L.).

Abu-Irmaileh and Jordan (1) have reported that light microscopy examination of purple nutsedge (*Cyperus rotundus* L. # CYPRO) leaves revealed abnormally swollen or clumped groups of chloroplasts 48 h after treatments with 5 mM glyphosate [*N*-(phosphonomethyl)glycine]. Following extended exposures, the plasmalemma had a constricted appearance (1). Glyphosate has been reported to cause numerous ultrastructural alterations in leaf mesophyll cells of quackgrass [*Agropyron repens* (L.) Beauv. # AGRRE] (7), white mustard (*Sinapis alba* L. # SINAL) (15), velvet mesquite [*Prosopis juliflora* var. *velutina* (Woot.) Sarg. # PROJV] (7), and duckweed (*Lemna gibba* L.)[4]. All of these species exhibited varying degrees of chloroplast swelling, grana disorganization, and chloroplast rupture.

HOE-39866 has provided control of several weed species, which was comparable or superior to the control achieved with glyphosate, but symptom response to HOE-39866 applications occurred within 36 h, whereas response to glyphosate was observed after 4 to 7 days (3, 9). Field studies with HOE-39866 and glyphosate indicated that the pattern of plant responses to the two herbicides was distinctly different (3). Due to the structural similarity of HOE-39866 and glyphosate, a study was performed to determine if the cellular changes caused by HOE-39866 were similar to those reported for glyphosate. This research examined the effects of foliar applications of formulated and analytical grade HOE-39866 on structural alterations in the cellular morphology of two C-4 species, redroot pigweed and fall panicum.

MATERIALS AND METHODS

Plant material. Seeds of redroot pigweed and fall panicum, collected in September 1982 at the Virginia Truck and Ornamentals Research Station, Painter, were germinated in horticultural grade perlite. When seedlings reached the second true-leaf stage, four plants of uniform size were transplanted into 23- by 10-cm styrofoam flats containing a 1.5:1.1 (v/v/v) mix of clay loam soil, sand, and vermiculite. All plants were fertilized weekly with a nutrient solution containing 475 mg/L nitrogen, 209 mg/L phosphorus, and 418 mg/L potassium. Plants of both species reached maturity in 6 to 10 weeks following transplanting when greenhouse temperatures were maintained at a constant 22 C day and 18 C night. Through the use of incandescent lamps, a 15-h photoperiod was maintained to promote only vegetative growth in redroot pigweed in particular, which is a quantitative short-day species (2). Fall panicum did not flower under this light regime.

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[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

[4] Hoagland, R. E. and R. Paul. 1978. Ultrastructural effects of glyphosate on *Lemna gibba* L. Abstr. Weed Sci. Soc. Am. Page 78.

Herbicide formulations. Treatment solutions of 10^{-1}, 10^{-2}, and 10^{-3} M concentrations of active ingredient were made from experimentally available formulated HOE-39866 (0.2 kg ai/L). To verify that cellular changes were not influenced by adjuvants in the formulation, a second series of studies was conducted using technical grade HOE-39866 (96% pure) at identical concentrations. Additionally, leaves were treated with a 1% solution of the formulation blank used in the formulated HOE-39866. New solutions were made before the initiation of each study.

Sampling procedures. The sampling procedures and tissue preparation techniques reported here are the result of numerous preliminary trials to determine the best fixative, vacuum infiltration conditions, and dehydration schedules. Tissue response to all procedural steps varied with species and age of the sampled tissues. A glass atomizer, delivering an even spray covering at a rate of 10 ml/min, was used for application. Aliquots (1 ml) of the herbicide solutions were applied to entire adaxial surfaces of single leaves on nonreproductive adult fall panicum and redroot pigweed plants. The first fully expanded leaf below the redroot pigweed apex or the leaf of the fourth node in fall panicum was chosen for treatment. Herbicide application began at 0900 h with sampling occurring every 2 h during a 24-h period. Untreated control plants were sampled according to the same schedule. At the predetermined time intervals after treatment, 1.0-cm sections were cut from the proximal end of the redroot pigweed leaf blade and from the center of the treated fall panicum leaf. All tissue sections were immediately placed in plastic mesh cassettes, submerged in Bouin's fixative (15:5:1 saturated aqueous picric acid, formalin, glacial acetic acid) (5), and subjected to a vacuum of 41 kPa for 3 min. Sections were then transferred to individual holding vials of the same fixative.

Tissue preparation. An automatic tissue processor was used for the dehydration and paraffin infiltration of the sampled leaf material. Each sample was placed first in a protective embedding bag to prevent curling during processing, and then in a plastic cassette. Tissue was dehydrated for 25, 10, 10, 25, 10, and 20 minutes in 70, 80, 95, 100, 100, and 100% ethanol solutions, respectively, and then placed in three xylene rinses for 10, 15, and 15 min. Following removal from xylene, the tissue was infiltrated twice in paraffin for 20 and 50 min, respectively. Processed samples were subsequently removed from the protective bags and immediately embedded in paraffin blocks.

Sectioning and staining. Preliminary experiments indicated that fall panicum leaves possessed well-developed vascular bundle fibers which caused tearing during sectioning. Therefore, samples of this species were softened (10) before sectioning. Sectioning was done at a thickness of 10 μm on a rotary microtome. Sections were mounted on glass slides and subsequently stained by a standard safranin (60 min)—fast green (15-s) sequence (5). The sectioned plant material was examined with a light microscope.

Environmental conditions during sampling periods. The first series of treatments, using formulated HOE-39866, was conducted from January to April. Two days before each treatment period, greenhouse temperatures were raised to 27 C day and 21 C night. Photosynthetic photon flux density (PPFD) from natural sunlight and supplementary incandescent lamps averaged 800 to 1200 $\mu E \cdot m^{-2} \cdot s^{-1}$ throughout the daytime portion of the 24-h sampling periods. Additional work with formulated HOE-39866 and all work with technical grade HOE-39866 was done during the months of July and August, eliminating the need for supplemental lighting and elevating temperatures before treatment. PPFD during the latter periods averaged 1200 to 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$. Although the greenhouse was shaded during the summer months, temperatures generally averaged 30 C day and 26 C night.

The experimental design was completely randomized. A single plant constituted a replication and 4 to 5 plants were sampled at each 2-h interval for each of the herbicide treatments and untreated controls. As a result of the repeated studies, the observations reported here are based on a survey of more than 400 individual slides.

RESULTS

Herbicide-induced injury did not differ as a result of concentrations or formulations. While both species responded similarly with regard to changing concentration and formulation, redroot pigweed is presented in Figures 1 to 3 to pictorially demonstrate the phenomena observed. In the case of concentration, degree of injury was less with decreasing concentration of HOE-39866 (Figure 1), and at a single concentration (10^{-2} M) symptom progression was evident (Figure 2). Evaporative drying confounded the interpretation because, as water was removed from the leaf surface, the active ingredient became more concentrated, making it difficult to determine the actual concentration at the time of injury. Formulation altered herbicide distribution and, subsequently, the uniformity of symptom expression. Surface 'puddling' of the technical grade solutions revealed localized areas of internal injury that in themselves were not different from the more extensive areas of injury observed with formulated HOE-39866 (Figure 3). No other adjuvant-specific effects were detected. Since concentration and formulation did not alter actual cellular response, and to avoid the difficulties involved with nonuniform herbicide distribution, the remaining discussion will be based upon photomicrographs of leaves following application of the 10^{-2} M solutions of formulated HOE-39866.

Symptom expression is discussed here with the understanding that not all cells of a leaf sample exhibited treatment effects nor did all affected cells show the same magnitude of response. The frequency with which specific cellular alterations occur, however, is sufficient to attribute them to the specific herbicide treatments. The lack of simultaneous, uniform cellular response to herbicides is not unusual and has been observed in glyphosate-treated quackgrass (6) and

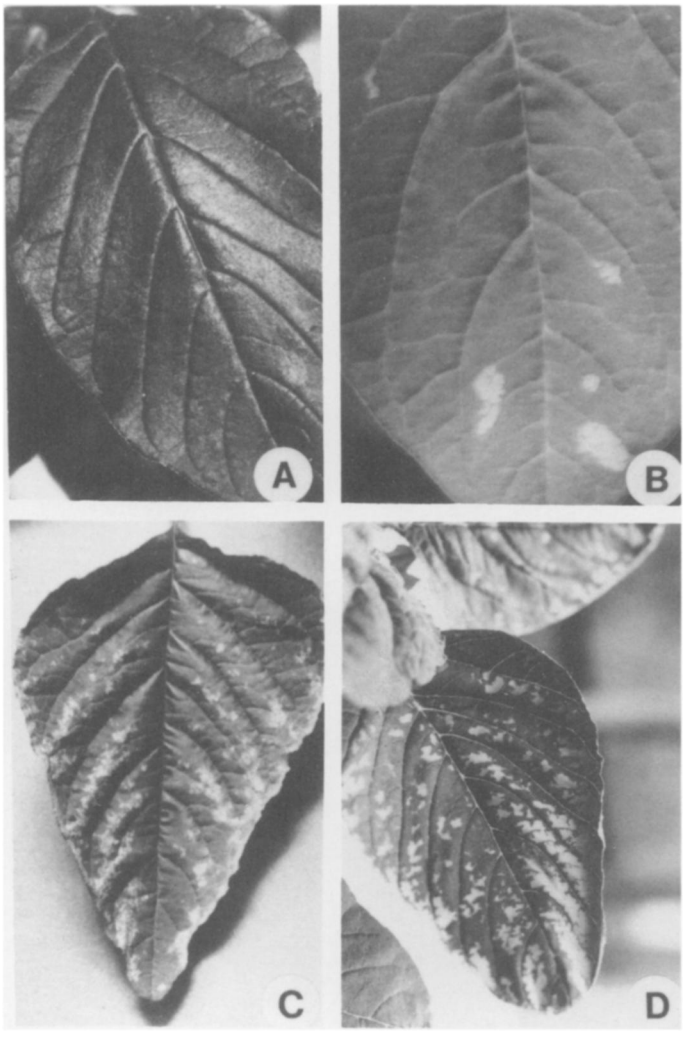


Figure 1. Injury symptoms appearing on redroot pigweed leaf surfaces 10 h after treatment with 1% formulation blank (A), and 10^{-3} M (B), 10^{-2} M (C), and 10^{-1} M (D) concentrations of formulated HOE-39866.

triazine-treated wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.)[5].

Fall panicum. When compared to the untreated control leaves, HOE-39866 caused 'pearling' (Figure 4), a term used here to describe a string-of-beads effect seen in leaf cross-sections. Evidence of dessication was apparent 6 h after treatment. Initially, HOE-39866 caused interveinal mesophyll cells to constrict and occasionally rupture. Bundle sheath cells shrank and the normally centrifugal arrangement of the bundle sheath cell chloroplasts became dis-

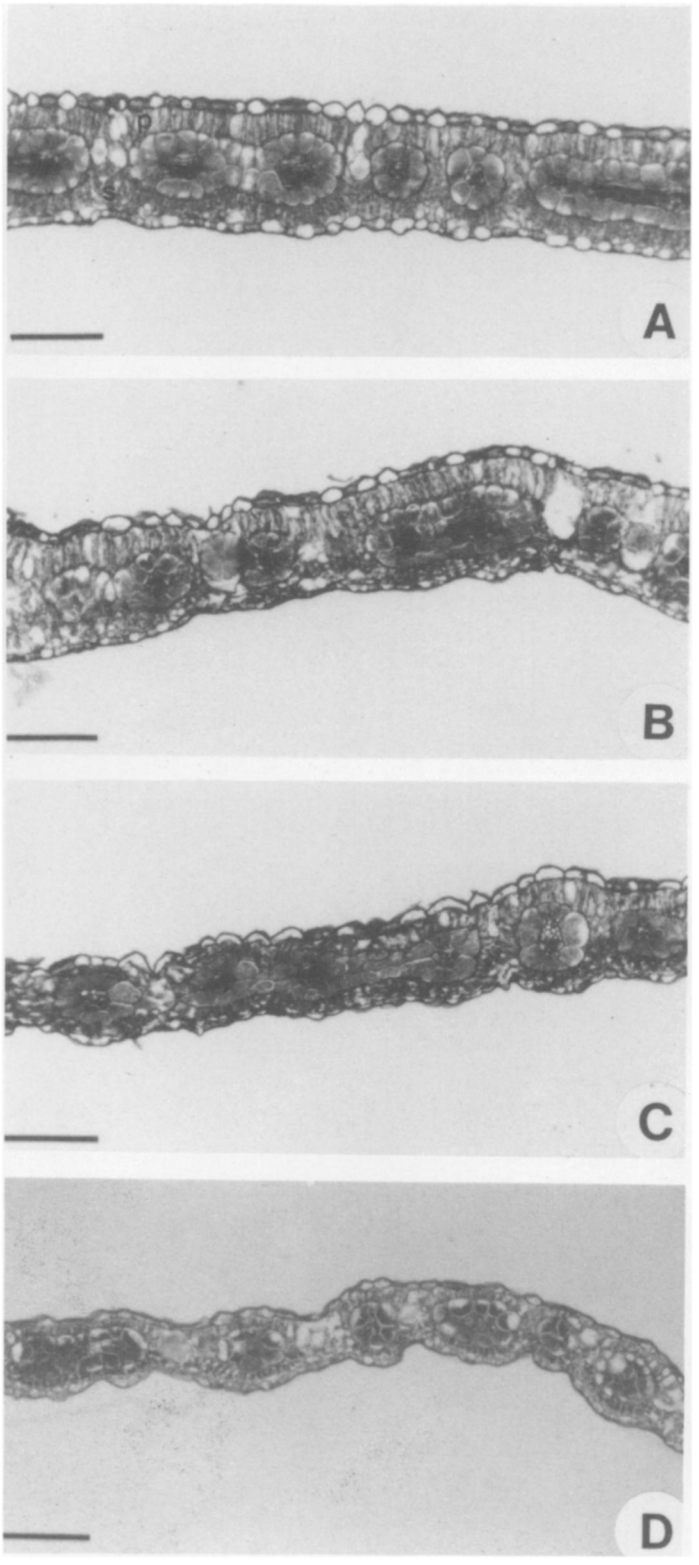
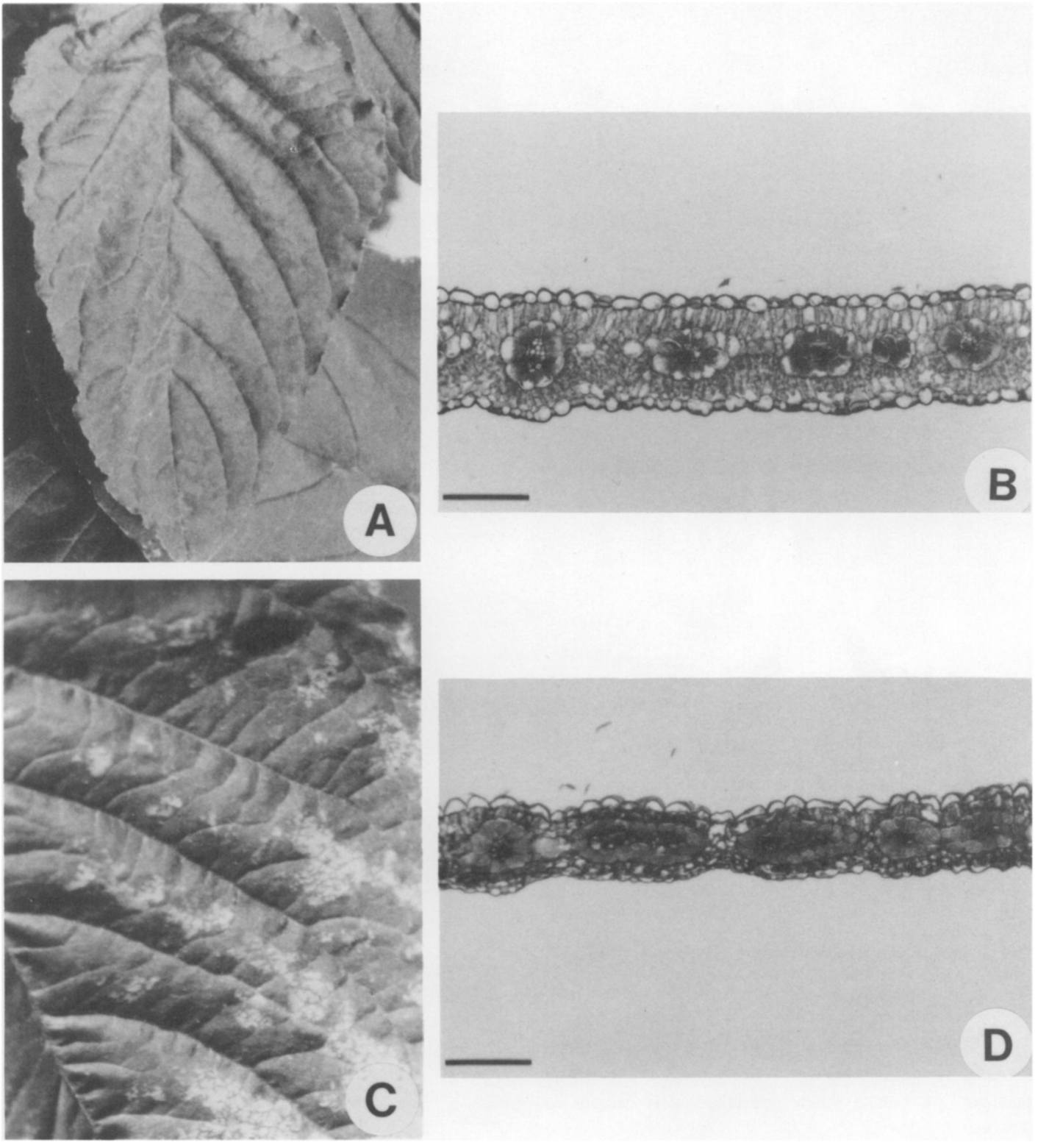


Figure 2. Symptom development in transverse sections of redroot pigweed leaves treated with 10^{-2} M HOE-39866: (A) control, (B) 6 h, (C) 12 h, and (D) 18 h after treatment. Note the progressive collapse of the spongy mesophyll cells and the palisade layer. p = palisade layer and s = spongy mesophyll layer in A. Bars = 100 μm.

[5] Campbell, W. F. and J. O. Evans. 1971. Influence of some new triazine herbicides on wheat and alfalfa chloroplasts ultrastructure. Abstr. Weed Sci. Soc. Am. Page 53.



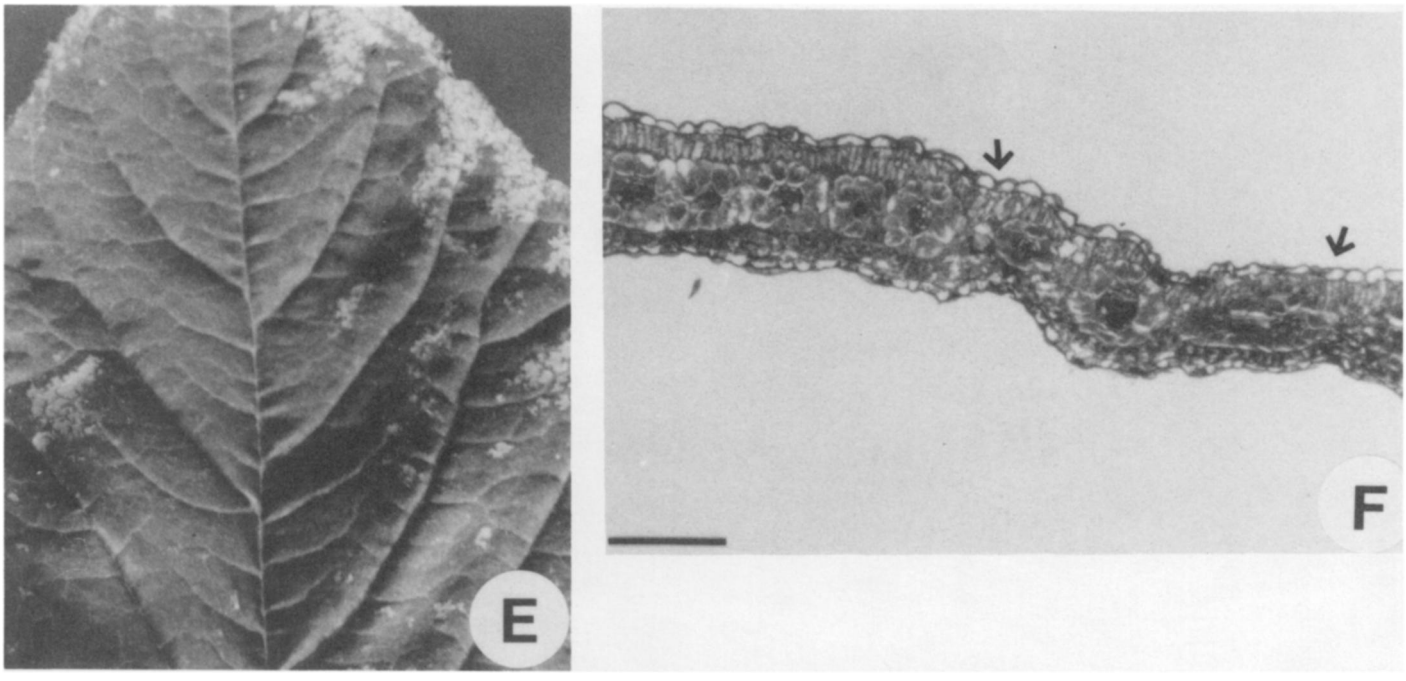


Figure 3. External and internal symptom expression in redroot pigweed leaves 6 h following treatment with 1% formulation blank (A,B), 10^{-2} M formulated (C,D), and 10^{-2} M technical (E,F) HOE-39866. Note that the cellular collapse, which occurs uniformly in D, occurs only between the black arrows in F. Bars = 100 μm.

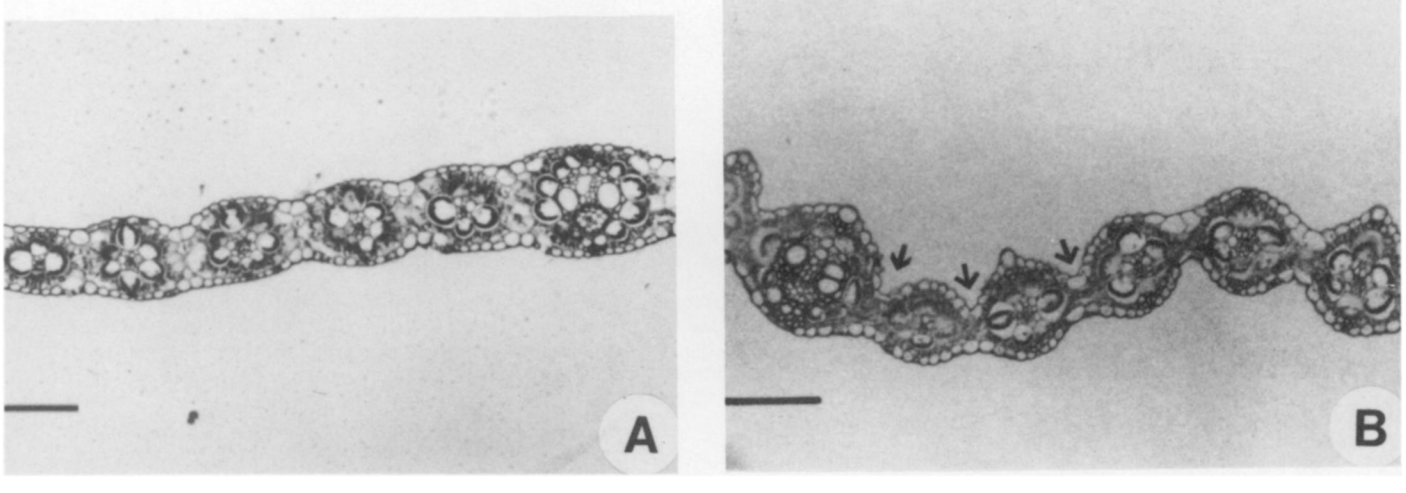


Figure 4. Transverse sections of fall panicum leaves: untreated (A) and 6 h (B) after treatment with 10^{-2} M formulated HOE-39866. Note the centrifugal arrangement of bundle sheath cells in untreated leaves. In treated leaves 'pearling' results, caused by cellular constriction in the interveinal mesophyll regions (black arrows). Bars = 100 μm.

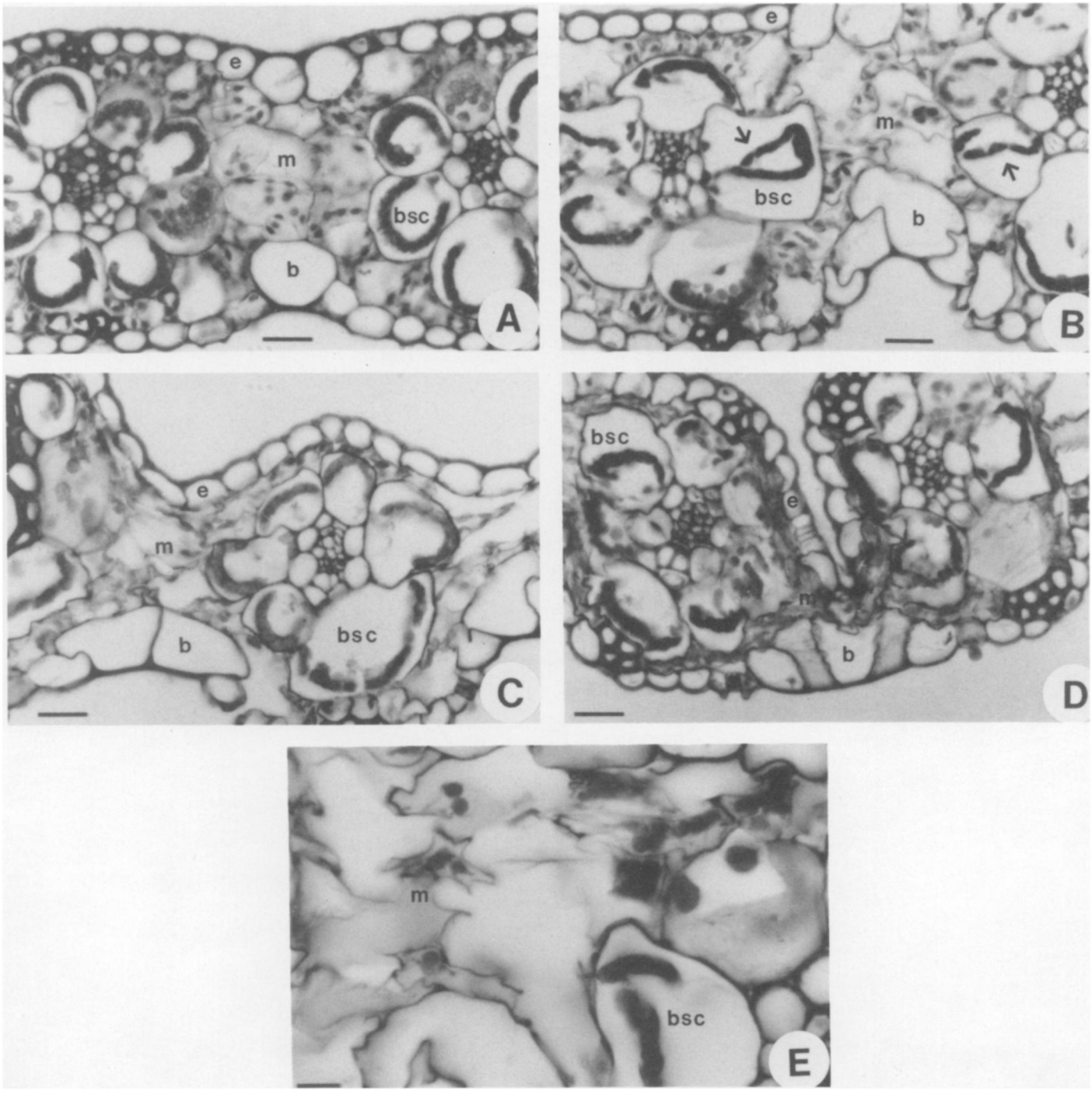


Figure 5. Transverse sections of fall panicum leaves: untreated (A); initial injury (B) in a fall panicum leaf caused by 10^{-2} M formulated HOE-39866 (6 h)—bundle sheath chloroplast disorganization (black arrows) and constriction of interveinal mesophyll cells; advanced injury (C) 12 h; (D) 18 h—interveinal mesophyll cells collapse and severe disorganization of bundle sheath cells; (E) (detail) interveinal mesophyll cell rupture (18 h). b = bulliform cell, bsc = bundle sheath cell, e = epidermis, m = mesophyll cell. Bars = 20 μm (A-D); 10 μm (E).

organized (Figure 5B). Progressive constriction is evident in Figure 5B to D. At 18 h, a large proportion of the interveinal mesophyll cells had lost their integrity and the bundle sheath cell chloroplast was severely disrupted (Figures 5C, D, and E). The edidermal cell layer was the least affected and only after prolonged herbicide exposure did these cells

lose turgor. The bulliform cells on the adaxial surface were the first to exhibit this effect and were also the most heavily damaged.

Redroot pigweed. Massive cellular disruptions resulted from applications of HOE-39866 (Figures 6A to 6D). Interveinal mesophyll cells, particularly spongy mesophyll cells along the abaxial surface, ruptured; and the bundle sheath cells lost turgor and shrank, making the centripetally arranged chloroplasts progressively appear to occupy a greater proportion of the cross-sectional area. Epidermal layers also

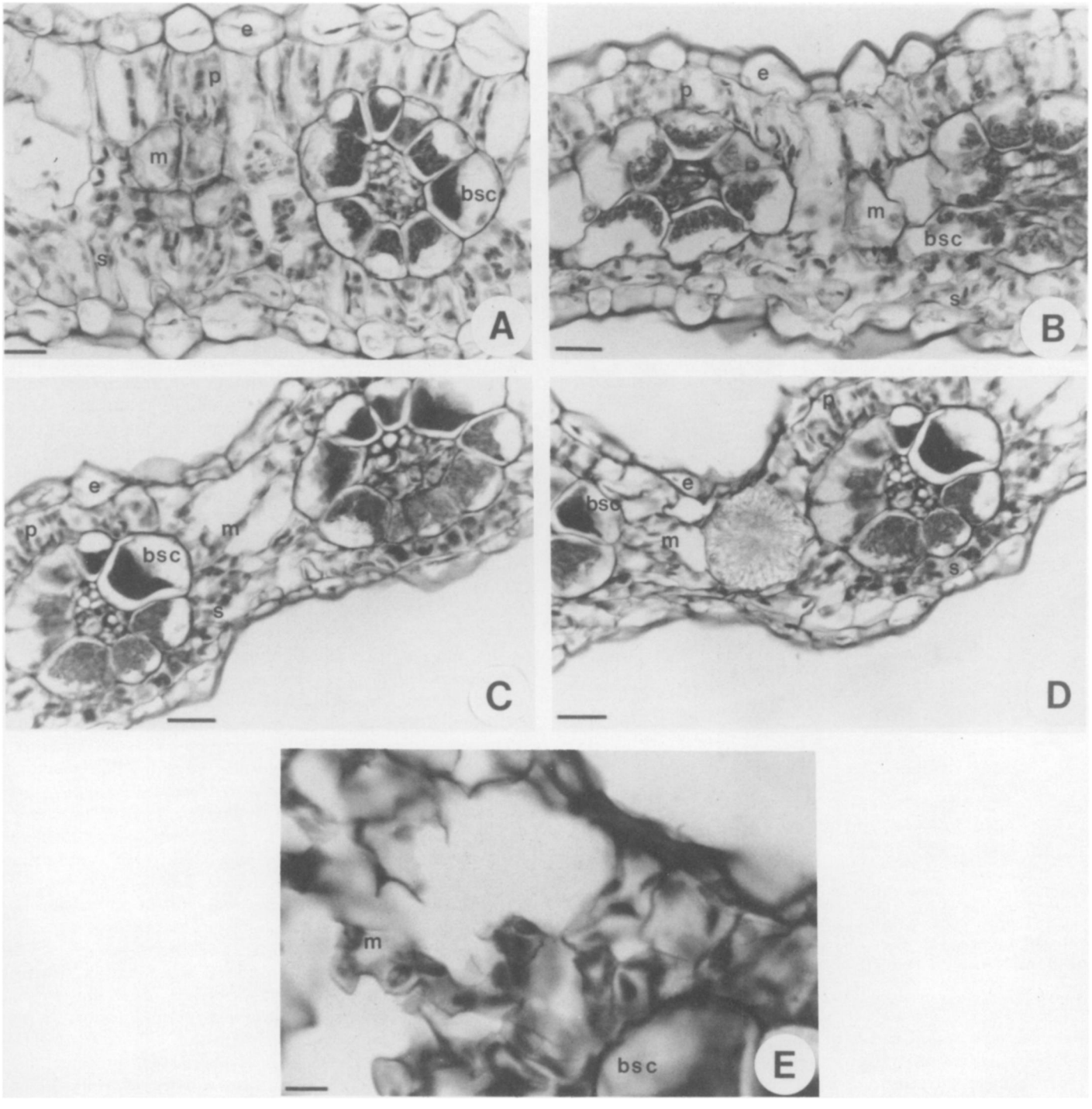


Figure 6. Transverse sections showing cellular organization of untreated (A) and 10^{-2} M formulated HOE-39866-treated redroot pigweed leaves 6 h (B), 12 h (C), and 18 h (D) after application, (E) (detail) interveinal mesophyll cell rupture (18 h). bsc = bundle sheath cell, e = epidermis, m = interveinal mesophyll cell, p = palisade layer, s = spongy mesophyll layer. Bars = 20 μm (A-D); 10 μm (E).

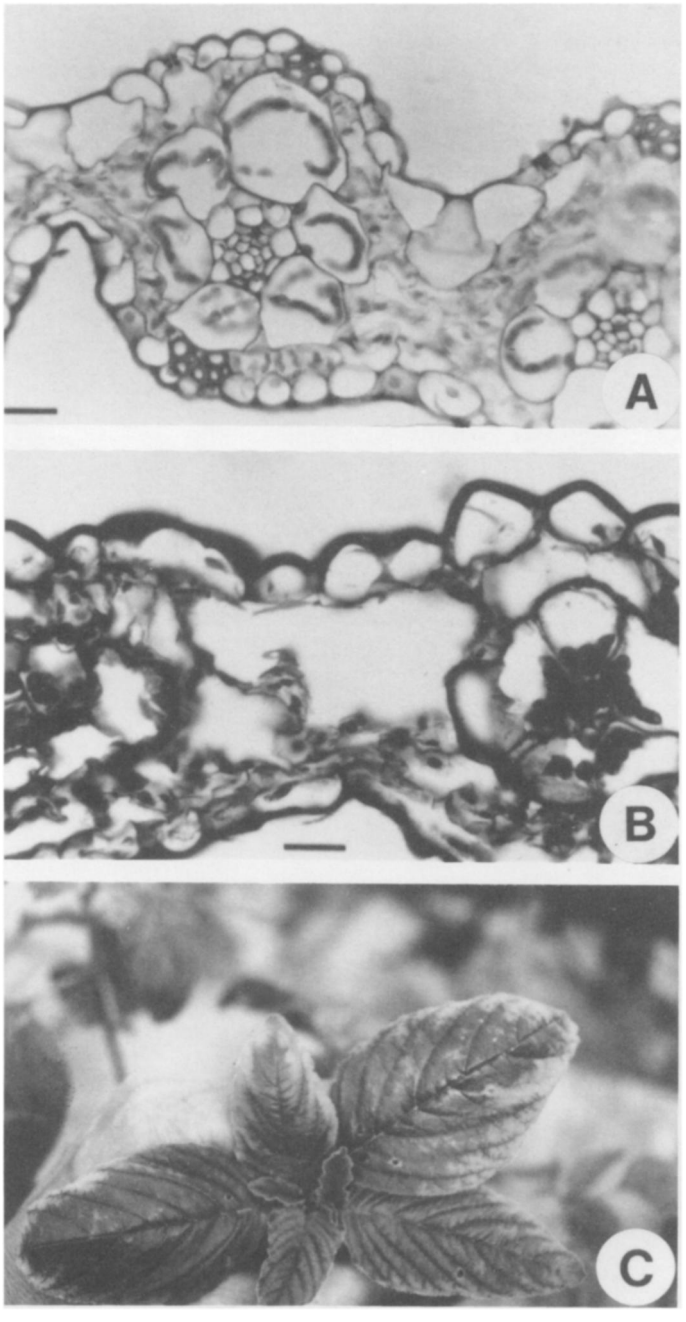


Figure 7. Cellular organization in transverse sections of nontreated leaves above the treated leaf 24 h following application of 10^{-2} M technical HOE-39866 to fall panicum (A) and redroot pigweed (B). (C) Marginal chlorosis occurring on untreated leaves acropetal to the treated leaf on a redroot pigweed plant.

appeared dehydrated early. Palisade cells along the adaxial surface collapsed upon longer exposure resulting in total loss of cellular integrity as well as intercellular space (Figure 6D).

Twenty-four hours after application, marginal chlorosis was visible on untreated apical leaves of treated plants. Frequently these chlorotic areas did not become necrotic until 36 h after treatment. Necrosis did not occur in localized areas, as in treated leaves, but was generalized across the leaf surface. Internally, the same types of cellular aberrations previously demonstrated in treated leaves were also observed in untreated leaves of the treated plants in both species (Figure 7), giving evidence for the translocative potential of HOE-39866. The rapid surface and internal injury observed in treated leaves is most likely due to exposure to the concentrating effect of the evaporation of the spray droplet. However, the symptoms evident in Figure 7 indicate that the herbicide is mobile, moving from the site of application to a nontreated leaf.

DISCUSSION

Cellular changes caused by HOE-39866 occurred quickly and were extensive in both species. Activity at the cellular level appears to be associated with membrane function. This idea is supported by the distinct shrinkage observed in the bundle sheath cells and the loss of integrity in the interveinal mesophyll cells in both redroot pigweed and fall panicum. These cellular changes are most likely secondary events that result from the primary mode of action. Physiological studies have shown what may be a possible explanation for the phenomena observed. Leason et al. (12), Köcher (11), and Wild and Manderscheid (17) have demonstrated that the free acid of HOE-39866, phosphinothricin, caused specific inhibition of glutamine synthetase (GS) in mesophyll cells of peas (*Pisum sativum* L.) and white mustard. Glutamine synthetase is known to be the first enzyme in the assimilation of ammonia in plants (13). Köcher has suggested that through the specific inhibition of GS activity, the primary event, ammonia is allowed to accumulate within cells. He obtained a marked increase in the concentration of ammonia 1 h after treatment with the herbicide, and increased membrane permeability, measured as K^+ leakage, occurred as well (11).

Although the observed cellular damage occurs rapidly and may be the result of accumulation of toxic concentrations of ammonia, there is evidence indicating that ammonia accumulation may be only a partial causative factor. Work with methionine sulfoximine (MSO), a known GS-inhibitor, has shown that a primary effect of MSO was to decrease soluble amino acids and sugars which causes an interruption of the glycolate cycle. This in turn decreases the number of available carbon intermediates required for normal Calvin-cycle function and continued photosynthesis (8, 16).

Although the environmental conditions under which the treatments were applied differed somewhat in temperature and light intensity, the differences were not critical with regard to symptom appearance or progression. Initial symptom expression was observed within 6 h at the highest concentration in all studies. Köcher (11), however, has reported that light enhanced the activity of HOE-39866 when compared to dark-grown plants. Due to the timing of the initial application (0900 h) in the present studies and the speed with which the response begins, this potential

effect of light upon the activity of HOE-39866 cannot be addressed without additional experimentation.

Because this study was performed on two C-4 species, generalization cannot be made to C-3 species. However, in other work by Bellinder et al. (4) using HOE-39866 in delayed light emission studies, it was shown that common pokeweed (*Phytolacca americana* L. # PHTAM) and black nightshade (*Solanum nigrum* L. # SOLNI), two C-3 species, develop spatial and temporal patterns of photosynthetic inhibition which are similar to those observed in redroot pigweed. Therefore, it is likely that cellular responses would also show similarities.

Light-dependent ammonia accumulation and/or reduced photosynthesis, as a result of HOE-39866 applications, have been reported by Köcher (11), Wild and Manderscheid (17), and Bellinder et al. (4). It is unclear at this time whether the uncoupling of photophosphorylation due to increased levels of ammonia or decreased glycolate-cycle activity is of primary importance. It is, however, the former which would impinge most quickly and rapidly upon cell function and integrity. The rapidity of the injury response and the cellular alterations observed in this study tend to support the theory of ammonia accumulation.

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