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Apoplastic distribution patterns of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], glyphosate [N-(phosphonomethyl)-glycine], the naturally occurring apoplastic leaf solutes, and the apoplastic dye PTS (trisodium 3-hydroxy-5,8,10-pyrenetrisulfonate) were compared. Aliquots of sap were expressed from intact sunflower leaves in a pressure chamber over intervals of 0.2 to 0.4 bars. Three distinct fractions were detected in the expressed sap volume; these were tentatively identified as a petiole-midrib fraction, a minor vein-cell wall fraction, and a third fraction comprised of a decreasing minor vein-cell wall fraction and an increasing fraction of membrane-filtered cell sap.

Atrazine and the naturally occurring apoplastic solutes were in a slightly higher concentration in the petiole-midrib fraction than in the minor vein-cell wall fraction. The concentration of apoplastic solutes in the third fraction was decreased with each increment of pressure to a value close to deionized water. The concentration of atrazine in the third fraction was the same as the second fraction, indicating that the plasmalemma was not a significant barrier

to atrazine movement.

The apoplastic distribution of PTS and glyphosate were very similar to each other, showing a marked accumulation in the minor vein-cell wall fraction. Both compounds were diluted with each successive pressure increment to a low value in the third fraction, indicating a resistance to movement into or out of the symplast. The total concentration of atrazine in the apoplast was much lower than glyphosate. Both compounds were in similar concentrations in the symplast. The apparent octanol:water partitioning coefficient for atrazine and glyphosate was determined. These values were comparable to the quotients of the symplastic and apoplastic concentrations for both atrazine and glyphosate. It was suggested that the polar nature and high water solubility of glyphosate were responsible for its slower rate of penetration into the symplast and resulting accumulation in the apoplast. These results support the intermediate permeability hypothesis; that compounds which display the apoplastic transport pattern are freely mobile between the apoplast and symplast. Compounds which are not weak acids and exhibit the symplastic transport pattern penetrate the symplast slowly, but once absorbed, are retained for long-distance transport.

APOPLASTIC DISTRIBUTION OF ATRAZINE AND GLYPHOSATE IN INTACT SUNFLOWER LEAVES

by

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APOPLASTIC DISTRIBUTION OF ATRAZINE AND GLYPHOSATE IN INTACT SUNFLOWER LEAVES

INTRODUCTION

The translocation pattern of a herbicide within a plant depends on the site of application of the compound and also on its ability to penetrate the plasmalemma. Two major patterns of herbicide transport have been extensively described (2, 19), namely the apoplastic pattern and the symplastic pattern. The concept of the apoplast and the symplast were originally introduced by Munch (1930) and have been defined by Crafts and Crisp (1971). The symplast as defined is "The total mass of living cells of a plant constitute a continuum, the individual protoplasts being intimately connected throughout the plant by the plasmodesmata . . . it follows that the mesoplasm is continuous from cell to cell." In contrast, the apoplast "constitutes the total non-living cell wall continuum that surrounds the symplast" (6). Movement in the apoplast occurs mainly via the xylem and cell walls while movement in the symplast occurs via the interconnected protoplasts of the parenchyma cells and the phloem. Herbicides which move in the symplastic transport pattern, when applied to the leaves, stems, or roots of plants, must initially move into and through the apoplast. These materials are then in position to penetrate the plasmalemma-bound symplast where they are available for mobilization and transport, via the phloem, to the sites of active metabolism. Herbicides which move in the apoplastic transport pattern, when applied to the leaves, are not exported. When these materials are applied to stems or roots, they apparently move into

the apoplast and are carried in the transpiration stream to the leaves. There is virtually no symplastic movement of these compounds out of the leaves (19). Chemicals which move in both patterns are termed ambimobile (13).

The question of why some chemicals are transported and others are not is of considerable importance, and the resolution of this problem may provide the information necessary to predict the systemic properties of pesticidal organic molecules. At the tissue level, clarification would appear to depend upon an understanding of the penetration of the compound from the water-filled apoplast into the membrane-bound symplast.

Currently, there are two hypotheses which explain the observed transport patterns in plants. Both assume that the potential for a compound to penetrate the symplast, and for the symplast to retain that compound, can be correlated with the overall pattern of transport it achieves in the plant as a whole. The first hypothesis is the historical view. Earlier references indicate that compounds which display the apoplastic transport pattern do not move readily into the protoplasts of plant cells, and thus are essentially confined to the apoplast, moving with the transpiration stream (see review in 2). Herbicides which move in the symplastic transport pattern were thought to be preferentially absorbed into the symplast, and subject to long distance transport in the phloem (2).

More recent experimental evidence has demonstrated that several compounds which display the apoplastic transport pattern easily

penetrate plant cell membranes (7, 8, 11, 27, 29-31, 39, 40, 41, 60, 62).

Peterson and Edgington (29) suggested that chemicals which penetrate into the symplast but display an apoplastic transport pattern be called pseudoapoplastic. They suggest that since transport in the transpiration stream is much more rapid than transport in the symplast, and since the plasmalemma is extremely permeable to pseudoapoplastic compounds, the symplast cannot retain these compounds for long without their being leached into the apoplast and carried away with the transpiration stream. This theory has resulted in the development of the intermediate permeability hypothesis (50), a complementary theory for symplastic and ambimobile compounds which penetrate the plasmalemma by passive diffusion and do not act like weak acids (e.g. 2,4-D).

According to the intermediate permeability mechanism, a substance which can penetrate the symplast, but to which the plasmalemma is not so permeable that the substance can easily leak out, will be retained in the symplast long enough to be transported to the carbohydrate sinks. A herbicide would display ambimobile translocation if, as symplastic transport occurs, the compound continuously leaks out of the symplast into the apoplast to be recirculated in the xylem. The degree of ambimobility would depend on the rate of leakage from the symplast, with symplastic transport representing a high membrane resistance to leakage, and apoplast transport representing a very low membrane resistance to leakage.

These two theories of transport predict very different inter-

cellular environments. The historical view predicts higher concentrations of apoplastically transported compounds in the cell walls and xylem, with a lower concentration of these materials within the leaf protoplasts. Conversely, there should be a lower concentration of symplastically transported compounds in the apoplast than in the symplast. The intermediate permeability hypothesis predicts the opposite situation, with ambimobile and symplastically transported compounds existing in higher concentrations in the apoplast than compounds which follow the apoplastic transport pattern.

The objective of this study was to determine the distribution of two model herbicides, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and glyphosate [N-(phosphonomethyl)glycine], in the apoplast of intact sunflower leaves. Results of this study were used to distinguish between the historical and intermediate permeability transport theories for herbicide translocation. Atrazine and glyphosate were chosen for study because both compounds have been shown to move into cells by passive diffusion (14, 17, 29, 30). However, atrazine follows the apoplastic transport patterns (2), while glyphosate follows the symplastic transport pattern with a tendency to be slightly ambimobile (9, 17). The apoplastic distribution of these compounds was compared to the apoplastic distribution of naturally occurring leaf solutes, and the fluorescent dye, PTS (trisodium 3-hydroxy-5,8,10-pyrenetrisulfonate). PTS is known to be confined to the apoplast of the plant (12, 45).

MATERIALS AND METHODS

Plant Material. Sunflower plants (Helianthus annus L. '894') were grown in 1-L containers for 21 d in a mixture of 33% sand, 33% peat moss, and 33% clay loam soil. Plants were watered with Long Ashton's Solution (20) every 2 d for the first 14 d and daily for the remaining 7. The plants were maintained in a growth chamber with a 14-h photoperiod, 27°C day temperature, and 10-h dark period at 21°C. Photon flux density at the leaf blade level was 800 $\mu E\ m^{-2}s^{-1}$ (cool white fluorescent plus incandescent light). Leaf midrib lengths of the first true leaf were measured daily. All experiments were performed on leaves 2 to 3 d after midrib elongation ceased.

Pressure-Volume Determinations. The setup and general procedure for the pressure-volume experiments were similar to those described by Scholander et al. (1964, 1965), Tyree and Hammel (1972), and Tyree and Dainty (1973); potential sources of error have been discussed by Cheung et al. (1976).

Leaves were sampled before dawn in the growth chamber from whole plants which had been kept overnight in a saturated atmosphere. To minimize both leaf temperature fluctuations during pressure alterations, and tissue water loss (57), the leaf to be sampled was excised and immediately placed in a small plastic bag which had been prehumidified by breathing into it until moisture condensed on the sides, and which contained a small amount of water (<2 ml) in the bottom. The bag was then wrapped around the leaf such that air was expressed and the opening could be sealed by the latex gasket in the pressure vessel lid. The bag was then tightly wrapped in a second bag in a

similar manner and placed in the pressure vessel (Soil Moisture Equipment Corp., P.O. Box 30025, Santa Barbara, Calif.). The petiole length in all experiments was 5 cm. After the leaf was placed in the pressure vessel, a small amount of water (<1 ml) was placed at the base of the petiole, and the pressure in the vessel was increased to 0.2 bars. The vessel lid was then tightened to the point where no gas bubbles escaped from around the petiole base, after which the water was removed by blotting with tissue paper. This measure insured an air-tight seal with a minimum of mechanical compression of the petiole. A gas mixture of 98% nitrogen and 2% oxygen was used throughout the study.

For clarification, the term "balancing pressure" is defined as the pressure required to bring the water in the leaf back to the surface of the cut petiole after the leaf was detached from the plant. As such, it approximately represents the total leaf water potential of the leaf. Small increases in pressure after the balance pressure had been attained caused the leaf water potential to become greater than zero and water to flow out of the living cells. This caused sap to flow out of the leaf, through the cut petiole, in response to a water potential gradient.

After an initial balance pressure was determined, the pressure in the chamber was increased in steps of 1 to 2 bars over the previous balance pressure. A period of 15 min was sufficient for collecting a suitable volume of expressed sap, after which a new balance pressure was established for the leaf. Expressed sap was absorbed by a preweighed collecting device that consisted of a 10-cm

long glass tube filled with tissue paper. The inside tube diameter was slightly larger than the petiole diameter. The collecting device was weighed immediately after each collection.

The relative water content (RWC) was computed from the following formula:

$$RWC = \frac{Wo - Wd - Ve}{Wo-Wd} (10^2)$$

where Wo is the fresh weight of the leaf at full hydration ($\Psi = 0$) obtained by the extrapolation method of Ladiges (1975), Wd is the dry weight of the leaf after oven drying at 60°C for 24 h, and Ve is the weight of sap expressed from the sample at various balance pressures. At the initiation of each experiment, leaf Ψ was typically between -1.2 and -2.0 bars. At the end of each experiment, the final weight of the leaf was measured. When complete pressure-volume (P-V) curves were developed, the total weight of sap expressed from the leaf, Ve (total) differed by less than 5% from the initial fresh weight of the leaf minus the final fresh weight; in subsequent experiments, where sap was expressed to -5 bars Ψ, the difference was less than 2%.

Data were plotted as $1/\Psi$ versus RWC. A linear regression was performed on the four or five points of the P-V curve that, by inspection, appeared to be in the linear region. From this linear regression, the apoplastic water content of the leaf was determined as the intercept at $1/\Psi = 0$ on the RWC-axis. P-V analysis was done for five plants, one leaf each.

Apoplastic Distribution of Soluble Solutes. Because natural distribution of the model herbicides in the leaf was important in this study, nondisruptive techniques to sample the midrib and cell

wall apoplastic sap from intact leaves were developed. The Scholander pressure vessel was found suitable for this use. The apoplastic concentrations of naturally occurring leaf solutes, PTS, atrazine, and glyphosate were studied by analyzing xylem exudate expressed from the leaves as they dehydrated under pressure.

Leaves were sampled, prepared, and placed in the pressure chamber in the same manner used in the pressure-volume determinations. Before a leaf was placed in the pressure chamber, the cut end of the petiole was rinsed for 30 s in distilled water. After an initial balance pressure was determined, pressure in the chamber was increased in steps of 0.2 to 0.6 bars over the previous balance pressure. A period of 3 to 5 min was sufficient for collecting a suitable sample of expressed sap, after which a new balance pressure was established for the leaf.

In determining the new balance pressure, the chamber must be depressurized just to the point where sap has receded from the cut xylem surface; this was 0.1 to 0.2 bars below the new balance pressure. Greater changes will cause internal mixing of the xylem sap and preclude an accurate determination of the distribution of the apoplastic solutes. Expressed sap was absorbed on a 0.32 cm^2 filter paper disk (Wescor, Inc., 459 S. Main Street, Logan, Utah), which had been placed on the cut end of the petiole protruding from the chamber, until the disk was fully saturated. At saturation, the disk held approx. 8.0 µl of sap. A small vial packed with saturated tissue paper at the bottom was inverted over the petiole and disk to form a humidified chamber and retard evaporation from the filter

paper disk. Upon full saturation of the filter paper disk, the disk was immediately transferred to the sample chamber of a vapor pressure osmometer (Wescor Inc., model 5110C) and a reading was taken. Slight volumetric errors in estimating full saturation of the sample disk did not reduce measurement accuracy (±2 mOs/kg), as the osmometer accommodated sample volume variations in excess of 10% without noticeable variation in the indicated osmolality. The distribution of apoplastic solutes with applied pressure was determined for five plants, one leaf each.

Transpiration Inhibition by Atrazine and Glyphosate. Plants were grown as above. Before plants were sampled, they were kept in a darkened, humidified growth chamber for 12 h at 21°C. At the end of the dark period, plants were removed from the growth chamber and all but the fully expanded first true leaves were removed. The plants were then excised under water at the soil surface. Shoots were transferred to flasks containing 100 µM $Ca(NO_3)_2$ and placed in the dark growth chamber for 90 min. Shoots and flasks were then weighed and placed in a lighted growth chamber (300 µE $m^{-2}s^{-1}$ at leaf blade level). After 90 min. shoots, were transferred to flasks containing 100 µM $Ca(NO_3)_2$ plus 100 µM atrazine or glyphosate, or herbicide-free 100 µM $Ca(NO_3)_2$ solution for 9 h. The total weight of the shoot and flask was recorded every 1.0 or 1.5 h. Leaf area was measured by the end of the experiment, using a Li-Cor area meter (model Li-3000, Lambda Instrument Corp., Lincoln, Nebraska). Transpiration was measured by dividing the change in weight of the flask and shoot by the total leaf area of the shoot. The experiment was conducted as a

completely randomized design with three replications per treatment.

Distribution of PTS, Atrazine, and Glyphosate. Sunflower shoots were prepared as in the transpiration inhibition study. After a 90-min equilibration period in a dark chamber, followed by a 90-min equilibration in a lighted growth chamber, excised shoots were transferred to vials containing 5 ml of 100 μM $Ca(NO_3)_2$ plus either 100 μM [U-ring-^{14}C] atrazine, 100 μM [methyl-^{14}C] glyphosate, or 200 mg/L PTS. Shoots and vials were weighed, and plants were maintained in the lighted growth chamber to allow absorption of the treatment solution.

Following a 90-min exposure, shoots and vials were reweighed to determine the amount of uptake, sealed in pre-humidified plastic bags, and placed in a dark growth chamber. After 1, 4, 7, or 10 h, shoots and vials were removed from the plastic bags and reweighed to determine any further uptake. One leaf was then excised, and xylem sap was expressed and collected in a similar manner as in the soluble solute study. The remaining leaf and stem were immediately mounted on blotter paper and frozen between sheets of dry ice. Xylem sap from the excised leaf was expressed for 5 min so that each successive balance pressure was 0.2 to 0.4 bars greater than the previous balance pressure. Xylem sap was collected on a filter paper disk under a humidified chamber as described previously, after which the filter paper was sealed in a scintillation vial. To determine Ve, the filter paper disk and vial were weighed before and immediately after collection of xylem sap. After sap was expressed to -5 bars, the pressure in the chamber was released, the petiole was quickly

excised, and weights of the petiole and leaf blade were recorded. Leaf parts were then mounted on blotter paper and frozen between sheets of dry ice. Plant parts were subsequently lyophilized and autoradiographs were made from the leaves of each treatment, one set per experimental compound examined. When the shoots were treated with PTS, photographs of the leaves under UV light were made. Results from the efflux analysis of expressed sap after 1, 4, 7, or 10 h dark equilibration were pooled unless significantly different at the 5% level.

Dried shoots were analyzed in parts, i.e., stem below the leaves, growing point above the leaves, petioles, leaves, and a strip of tissue 2 mm wide adjacent to the main veins of the leaf. Radioactivity in the leaf and tissue strips were combined for analysis of the distribution of label among plant parts. Each plant part was weighed and stem parts, petioles, and leaves were ground in a Wiley Mill fitted with a 20-mesh screen. Radioactivity in the plant parts was determined by combustion in a Packard 305 sample oxidizer with subsequent counting of the liberated $^{14}CO_2$ by scintillation spectrometry. Samples combusted comprised greater than 30% of the leaves and greater than 50% of the other plant parts; all of the excised leaf tissue strips were combusted.

Herbicides in the expressed xylem sap were extracted from the filter paper disks in the scintillation vial with 1 ml of methanol or water (for atrazine and glyphosate, respectively) for 24 h. The radioactivity for each sample was determined by scintillation spectrometry. When the treatment solution contained PTS, the samples

were first extracted with 2 ml water, and the concentration of PTS was measured using a Varian Cary 219 spectrophotometer at a wavelength of 404 nm. The concentration of experimental material in the apoplast was determined graphically from the expressed sap efflux curves between 3 and 4 bars applied pressure. The concentration of experimental material in the symplast was calculated from the excised tissue adjacent to the main veins of the opposite leaves of the same shoot which had not had xylem sap expressed, using the following equation:

$$\frac{\text{moles of material in tissue - moles in apoplast}}{\text{total water in leaf strip in grams - \% water in apoplast}}$$

The total weight of water in the leaf strip sample was calculated as:

$$\text{water in sample} = \frac{\text{sample dry weight}}{\text{Wd}} \text{ (leaf fresh weight - Wd)}$$

A symplast-apoplast partitioning quotient for atrazine and glyphosate was calculated by dividing the concentration of material in the symplast by the concentration in the apoplast.

Experiments consisted of one shoot per treatment and were repeated four times for atrazine and three times for glyphosate and PTS.

Identity of Transported Molecules. Thin-layer chromatography was used to verify that the ^{14}C-atrazine was intact atrazine. The leaves of shoots which had been allowed to take up ^{14}C-atrazine for 90 min, sealed in prehumidified plastic bags, and incubated in a dark growth chamber for 10 h were used. Leaves were extracted by homogenization in 10 volumes (approx. 20 ml) of 80% (v/v) methanol:water [10:1 (v/w) ratio of 80% methanol to fresh weight leaves]. The

homogenate was filtered through Whatman No. 1 filter paper. The residue was filtered twice more with the same volume of methanol. The three methanol extracts were combined. Methanol was removed, and the aqueous extract was reduced in volume to 5 ml, in vacuo by rotary evaporation at 40°C. The extract was frozen and stored at -70°C until used.

Aliquots (1 ml) of the extract were lyophilized, and the residue was resuspended in 150 µl of water for spotting onto 20 by 20 cm silica gel thin-layer plates. Two different solvent systems were used: (a) benzene:acetic acid:water (60:40:3, v/v/v), air-dried for 30 min at room temperature, and (b) n-butanol:acetic acid:water (120:30:50, v/v/v). Zones containing radioactivity were located by autoradiography, scraped into scintillation vials, and the radioactivity associated with each zone was determined. This procedure follows that of Frear and Swanson (15), and resulting Rf values of ^{14}C-atrazine and radioactive metabolites were compared to their values. Thin-layer chromatograms with only ^{14}C-atrazine also were prepared for comparison. This analysis was performed for three sets of two leaves each.

A glyphosate metabolism study was not conducted because numerous studies have shown that glyphosate metabolism does not occur during very short-term experiments (16, 23, 33, 36, 58, 59).

Partitioning Coefficient. Labeled atrazine or glyphosate was added to 22-ml scintillation vials containing 5 ml of 2-octanol and buffer. Buffer (Hydrion, Micro Essential Laboratory, Brooklyn, N.Y.) solutions ranged from pH 3 to pH 12 in pH units of 1.0. The vials

were placed in a reciprocating shaker for 8 h and the aqueous and organic phases were sampled 24 h later. After correcting for counting efficiency, the partitioning coefficient was calculated by dividing the number of dpm's in the organic phase by those in the aqueous phase. The experiment was conducted as a completely randomized design with five replications per treatment.

RESULTS AND DISCUSSION

Pressure-Volume Analysis. A typical pressure-volume curve for a fully expanded sunflower leaf is shown in Figure 1. Such curves show the relationship between the inverse of the balance pressure ($1/\Psi$) and the cumulative weight of sap expressed (Ve) or the relative water content (RWC) of the leaf (49). Curves of this type typically exhibit an initial rapid linear decline and a subsequent asymptotic approach to linearity followed by a slow linear decline. The initiation of the curved portion of the pressure-volume relationship, following the initial rapid linear decline, is the point where some of the cells in the leaf begin to reach incipient plasmolysis; the curve begins to slow linear decline after all the cells in the leaf have lost their turgidity (5).

Initiation of incipient plasmolysis for sunflower leaves, grown under the conditions described, occurred at 4.76 ± 0.41 bars (Point A, Fig. 1). Extrapolating the second linear portion of the pressure-volume curve to the abscissa (Point B, Fig. 1) estimates the volume which would be expressed at infinite balance pressure. This point is interpreted as the fraction of osmotically inactive water or the apoplastic water volume (4, 49). For the first fully expanded true leaves of sunflowers, the apoplastic water volume was found to be 11.16 ± 0.93% RWC.

Soluble Solute Distribution. The concentration of soluble solutes was determined for exudate samples which had been collected on filter paper disks at balance pressures increasing in steps of 0.2 to 0.4 bars as leaves dehydrated under pressure (Fig. 2). The efflux

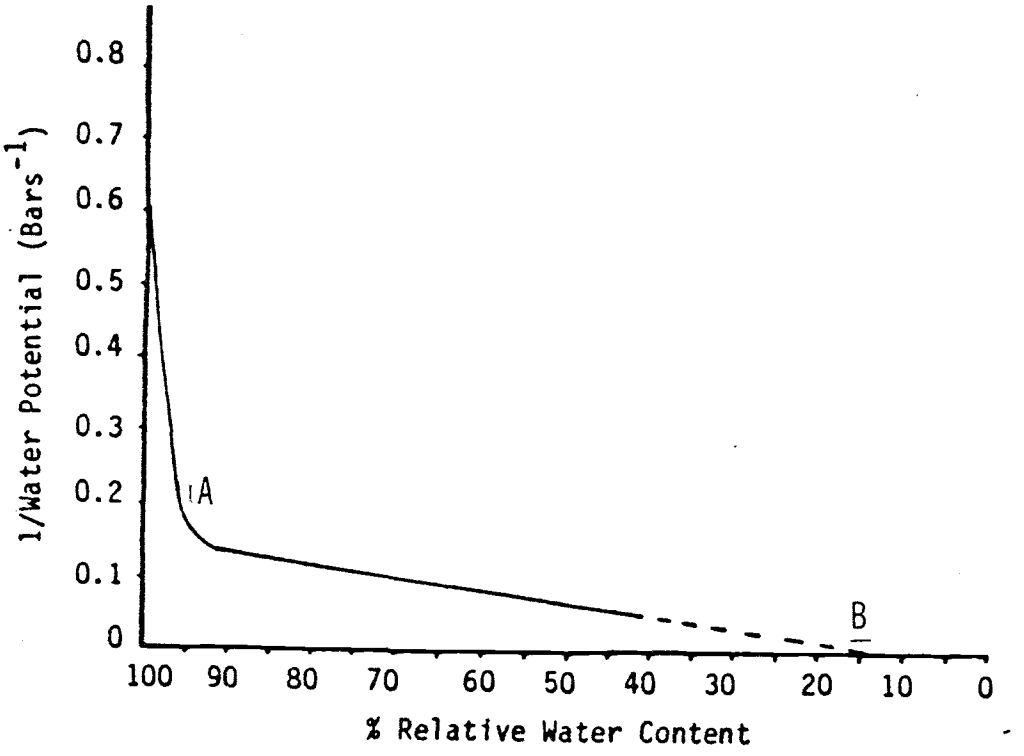


FIGURE 1. A typical pressure-volume curve for a single fully expanded sunflower leaf. Error bars for points A and B represent the standard error for five plants, one leaf each.

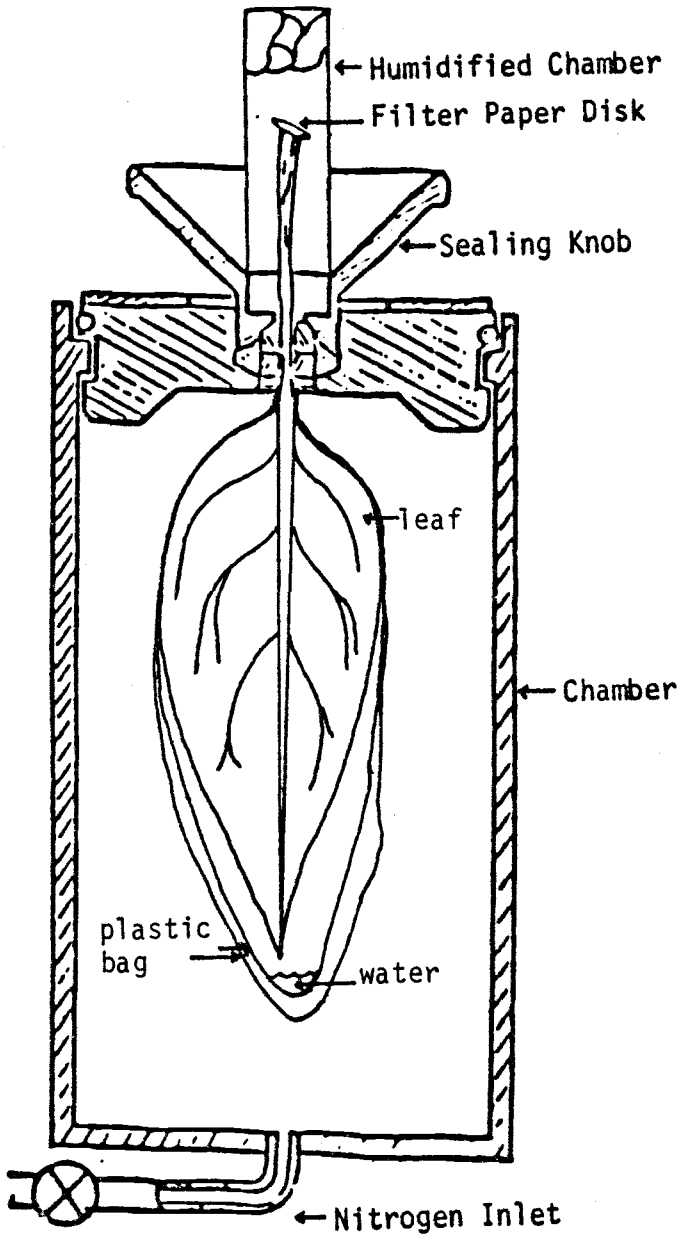


FIGURE 2. Pressure bomb apparatus used to obtain sap efflux from leaves under pressure.

of solutes showed a biphasic pattern, with two distinct plateaus, with respect to applied pressure (Fig. 3). The first plateau was at a concentration of 20.5 ± 3.7 mOs/kg and extended from the initial balance pressure to 2.4 ± 0.2 bars of applied pressure. The volume of sap expressed from the petiole alone after it had been excised with two simultaneous cuts from the stem and leaf and subjected to a pressure of 1 bar, accounted for about 20% of the total volume of the first plateau. The first plateau was followed by a decline in osmolarity to a second plateau at 12.0 ± 3.4 mOs/kg, and which extended from 3.3 ± 0.2 to 4.2 ± 0.2 bars applied pressure. The concentration of xylem sap expressed following the second plateau was successively diluted with each increment of applied pressure to a value close to that of distilled or deionized water (3.3 ± 2.0 mOs/kg).

The pathway of water movement through mesophyll leaf tissue is thought to be through the cell walls (25, 46, 54, 55). While this may be the predominant pathway during transpiring conditions, the path of water movement in the pressure chamber may be different. Tyree and Cheung (5) and Strochine et al. (44) have suggested that, after a pressure increment, water driven out of living cells would likely flow from cell to cell, across the membranes and the thin part of the common cell walls, to the nearest vascular bundle, and then out of the leaf through the vascular elements. This would have the effect of flushing solutes out of the cell walls adjacent to the minor veins as water is forced out of living cells under pressure. Since the plasmalemma is believed to be a near-perfect semipermeable barrier (32), water forced out of living cells should have the

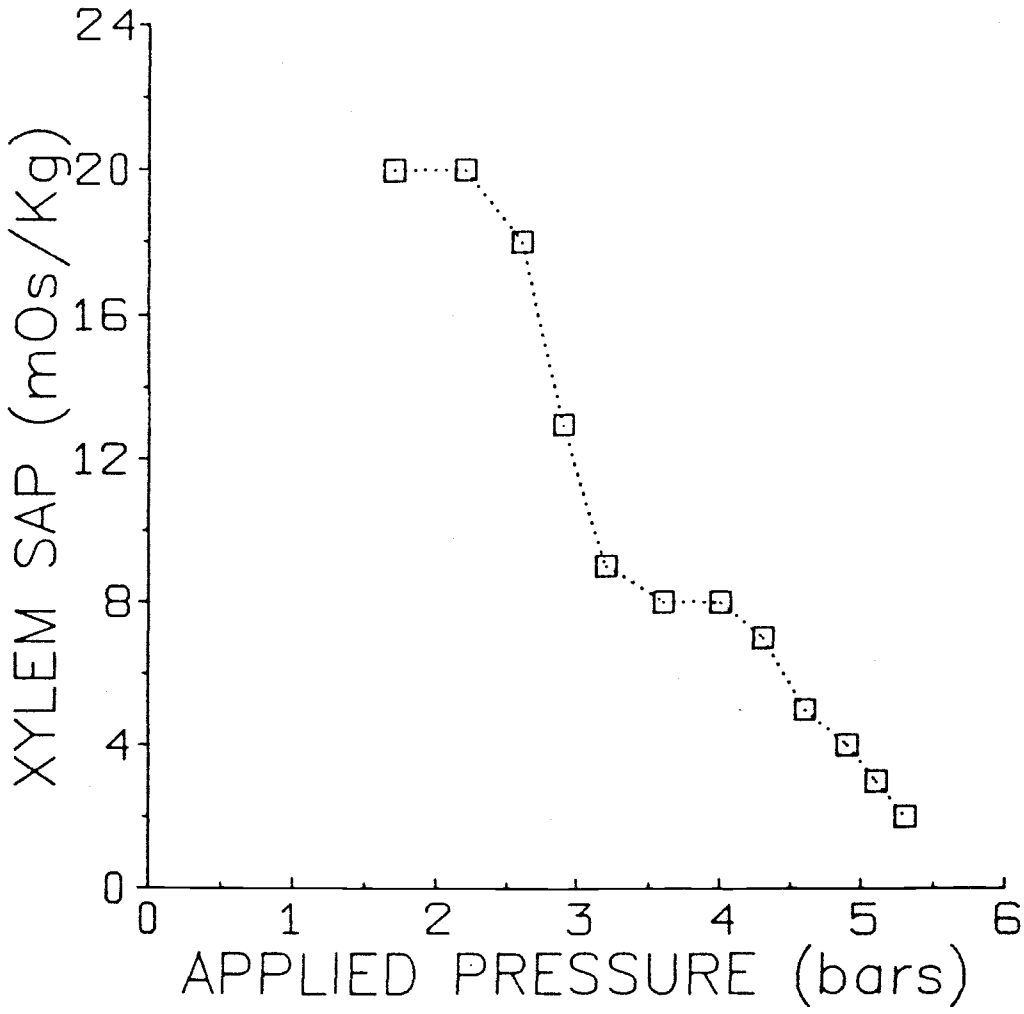


FIGURE 3. Natural apoplastic solutes in exudates of sunflower leaves subjected to pressure dehydration. The pressure in the chamber was increased in steps of 0.2 to 0.6 bars over the previous balance pressure to obtain exudates. Samples were collected over a period of 3 to 5 min. Data for one replication only.

composition of distilled or deionized water. As water is moved from the mesophyll cells and into the xylem elements of the minor veins, the sap in the minor veins would be displaced and forced into increasingly larger vascular elements and ultimately out of the petiole to the atmosphere, following a path of decreasing resistance. Stroshine et al. (44), modeling water movement out of wheat leaves under pressure, showed that the larger vascular bundles represent paths of low resistance through which water can travel rapidly through the leaf, while the smaller intermediates have a larger resistance. Based on calculations of the expected velocities needed to express sap from xylem at observed experimental rates, Stroshine et al. (44) calculated a Reynolds number for flow through the xylem to be 0.068. This value is well within the range of nonturbulent or laminar flow, and thus very little mixing between expressed volumes should occur as sap is expressed under pressure. This model of water movement out of a leaf under pressure suggests that the first fraction of sap expressed from sunflower leaves, to approximately 2.4 bars applied pressure, would contain solutes from the petiole and midrib of the leaf. This would be followed by a second fraction of water from the minor veins and cell walls. This sap fraction is represented by the second plateau of the soluble solute efflux curve (Fig. 3). Further expression of plasmalemma filtered cell sap should result in a reduction of the solute concentration of the expressed sap, approaching that of deionized water. These expected results have been observed in this study, and support the concept of differential permeability of the plasmalemma to water and solutes.

These data are consistent with the observations of Scholander et al. (34) who measured the freezing point depression of expressed sap in 26 species of flowering plants. In that study, Scholander et al. (34) noted that "The first portion that comes out is contaminated with normal xylem sap." A similar observation was made by Ackerson (1), studying ABA movement in leaves. He noted that the initial volume of expressed sap contained approximately ten times as much ABA as sap obtained during later stages of dehydration, and that the osmotic potential of the expressed sap depended on the balance pressure at which it was collected.

The solute composition of the xylem sap is largely made up of mineral ions, though many other substances occur (24). Figure 3 indicates that the concentration of solutes in the leaf is highest in the petiole-midrib fraction and that the concentration in the minor vein-cell wall fraction is significantly less. This is the distribution of ions which would be expected if the xylem stream were depleted by active and facilitated removal of ions from the apoplast by the living cells (28). This view of ionic distribution in the apoplast is supported by the observation that if sunflower plants remained in conditions which allowed no transpiration for 48 h (100% humidity in the dark) prior to sampling, the concentration of the first plateau was reduced to approximately 12 mOs/kg and the second plateau did not occur (data not shown). This indicates that under conditions of no light and no transpiration, the leaf cells can deplete the apoplast of solutes almost entirely.

PTS Distribution. Shoots were xylem-fed 200 mg/L PTS for 90 min and allowed to equilibrate in the dark at 100% humidity for 1, 4, 7, or 10 h. Photographs of the non-expressed leaves which were opposite those used for subsequent xylem sap analysis indicated that PTS is in highest concentration in the major and minor veins of the leaf (Fig. 4a). PTS was distributed throughout the leaf. This pattern of distribution did not change over the course of the experiment.

Two concentration plateaus were observed in the efflux analysis of PTS in exudates collected during the dehydration of sunflower leaves under pressure (Fig. 5). The first plateau was at 215 ± 16 mg/L and extended from the initial balance pressure to 2.2 ± 0.4 bars of applied pressure. The second plateau extended from 3.1 ± 0.3 to 3.4 ± 0.3 bars applied pressure and averaged 906 ± 267 mg/L.

While the PTS efflux curves had plateaus at approximately the same positions as the soluble solute efflux curve, the pattern of PTS efflux from the leaf was quite different. The first fraction expressed was approximately the same concentration as the original experimental solution applied to the excised shoots. This indicates that none of the PTS was removed from the transpiration stream as the treatment solution moved into the leaves. This is consistent with the work of Strugger (1949) and Dybing and Currier (1961), who showed that PTS is completely excluded from the symplast.

The second plateau is approximately 5 times more concentrated than the first plateau. As more sap was expressed, the concentration of PTS in the xylem sap was successively diluted with each increment of applied pressure to a low value. There were no significant

FIGURE 4. Sunflower leaves after 90-min uptake of PTS, ^{14}C-atrazine, or ^{14}C-glyphosate treatment solution followed by 10 h dark equilibration in 100% R.H. (a) PTS xylem-fed at 200 mg/L; photographed under u.v. light. (b) Autoradiograph, 100 µM xylem-fed ^{14}C-atrazine. (c) Autoradiograph, 100 µM xylem-fed ^{14}C-glyphosate.

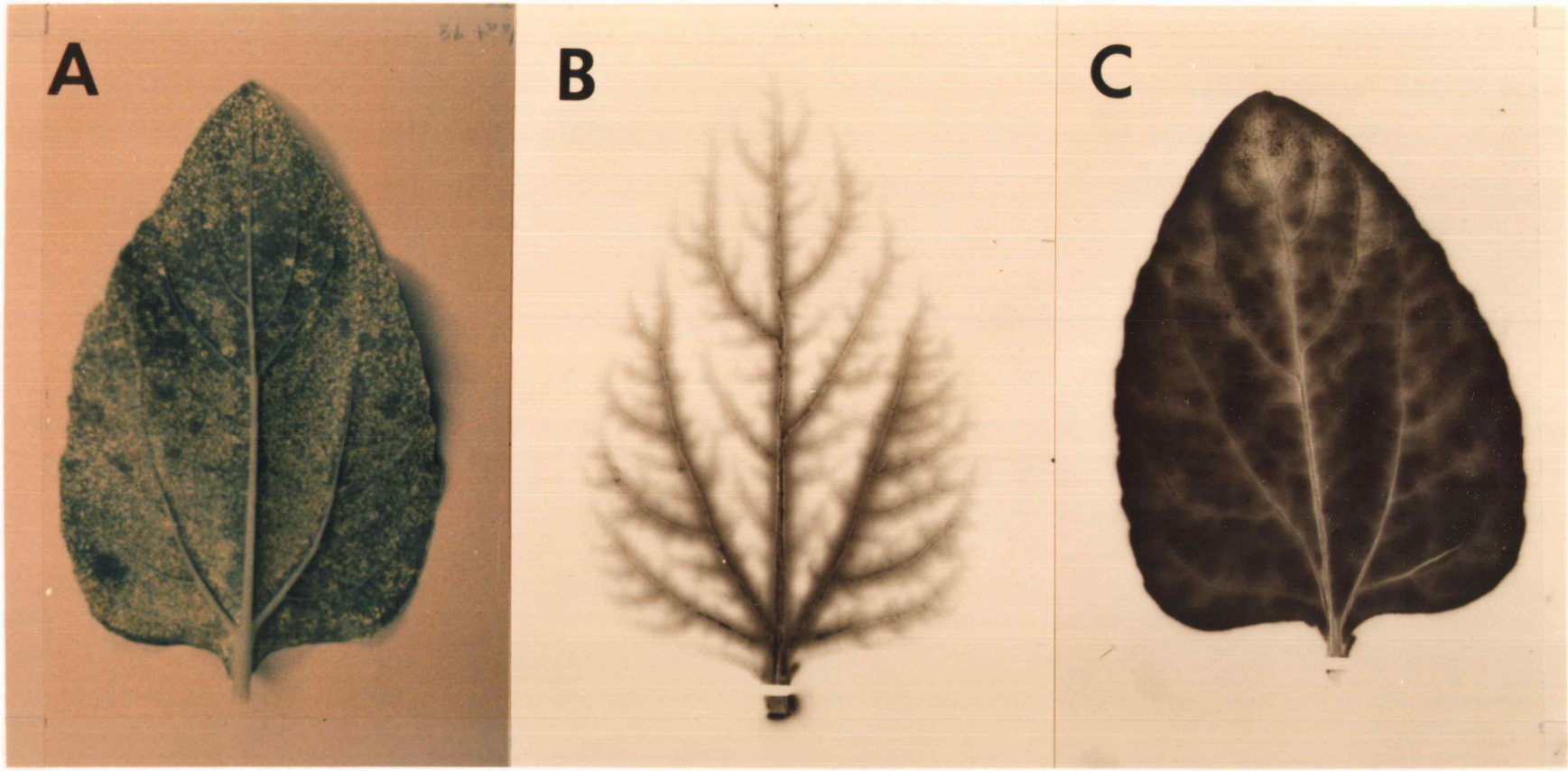


FIGURE 4

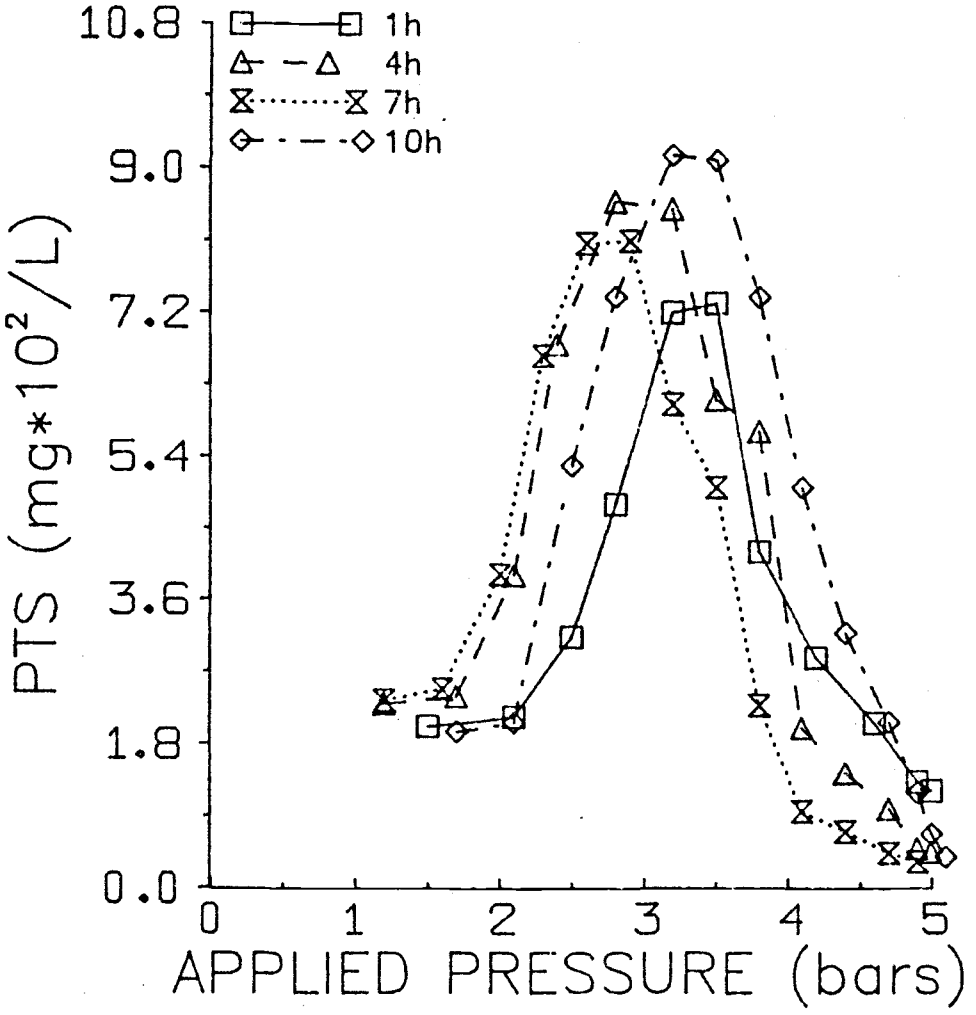


FIGURE 5. PTS in exudates of sunflower leaves subjected to pressure dehydration. The pressure in the chamber was increased in steps of 0.2 to 0.4 bars over the previous balance pressure. Samples were collected over a period of 5 min. Data from one replication.

differences in efflux patterns or plateau concentrations between shoots allowed to equilibrate in the dark for 1, 4, 7, or 10 h (Fig. 5).

These results suggest an accumulation of material in the minor vein-cell wall fraction due to an inability of this material to penetrate the plasmalemma. The rapid dilution following the second plateau is interpreted as the result of plasmalemma-filtered cell sap displacing the minor vein-cell wall solution as sap is expressed from the leaf. These results also support the contention that this technique can be used to collect three distinct fractions of expressed sap, i.e., an initial petiole-midrib fraction, followed by a minor vein-cell wall fraction, and a third combined fraction which is comprised of a decreasing minor vein-cell wall component and an increasing component of membrane-filtered symplastic sap.

Inhibition of Transpiration by Atrazine and Glyphosate. Since uptake and translocation of herbicides can be affected by the dose applied (6, 52), it was important to determine if the dose received by excised sunflower shoots was in the physiologically active range. Atrazine is known to inhibit photosynthesis (3, 61), which leads to a buildup of CO_2 within the leaf and, in turn, causes closure of the stomates, thus inhibiting transpiration (42). The transpirational response of sunflower to glyphosate has been shown to be one of the earliest indications that leaf tissue has received a lethal dose of the herbicide (37, 38). The site of transpiration inhibition by glyphosate may be in the guard cells (37). Both atrazine and glyphosate caused an inhibition of transpiration at the concentration and

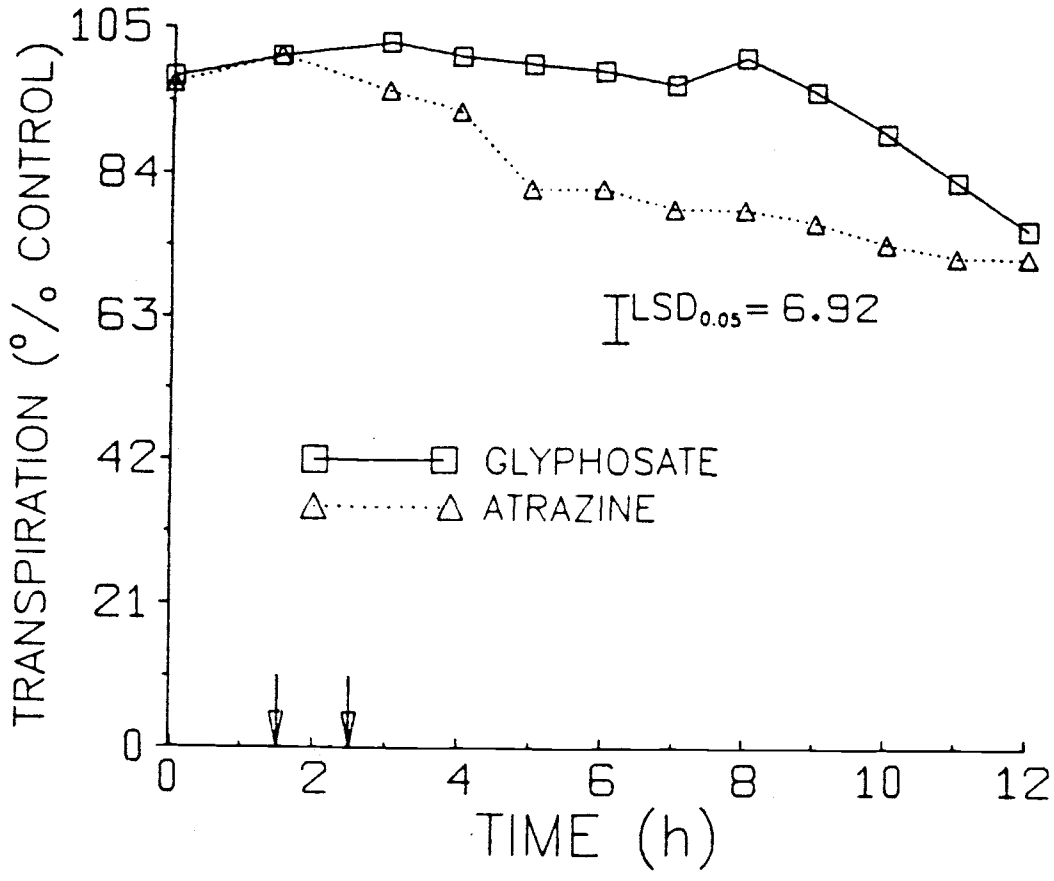


FIGURE 6. Time course of transpiration following a 90-min pulse of either 100 µM glyphosate or atrazine. First arrow on time axis represents transfer of shoots from 100 µM $Ca(NO_3)_2$ to $Ca(NO_3)_2$ plus 100 µM herbicide. Second arrow on time axis represents transfer of shoots from herbicide solution to herbicide-free 100 µM $Ca(NO_3)_2$.

period of exposure utilized in this experiment (Fig. 6). The time course for initiation of transpiration inhibition by atrazine and glyphosate was similar to that reported for corn (21) and sunflower (37), respectively.

Distribution of Atrazine and Glyphosate in the Shoot. Shoots were xylem fed 100 µM atrazine or glyphosate for 90 min and allowed to equilibrate in the dark at 100% humidity for 1, 4, 7, or 10 h. Both atrazine and glyphosate were absorbed and translocated throughout the shoots. However, the distribution of these herbicides in the shoots was quite different (Fig. 7 and 8). Most of the atrazine in the shoot (Fig. 7) was found in the stems below the leaves (60 to 70%); very little atrazine was translocated into the portion of stem above the leaves (2.5 to 4%). The petioles and leaves contained approximately equal amounts of atrazine (14 to 19% and 10 to 17%, respectively). No significant differences in atrazine distribution occurred with longer dark equilibration periods.

Accumulation of ^{14}C-atrazine in the tissues resulted from accumulation of atrazine rather than metabolites of atrazine. Two radioactive compounds were found on thin-layer plates with Rf values of 6.6 and 9.8. The first compound, at Rf 6.6, was hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-s-triazine] and represented 3% of the extracted radioactivity. The second compound at Rf 9.8, was intact atrazine which co-chromatographed with the atrazine standard and represented 97% of the extracted radioactivity. These Rf values agree with those of Frear and Swanson (15). Because only a

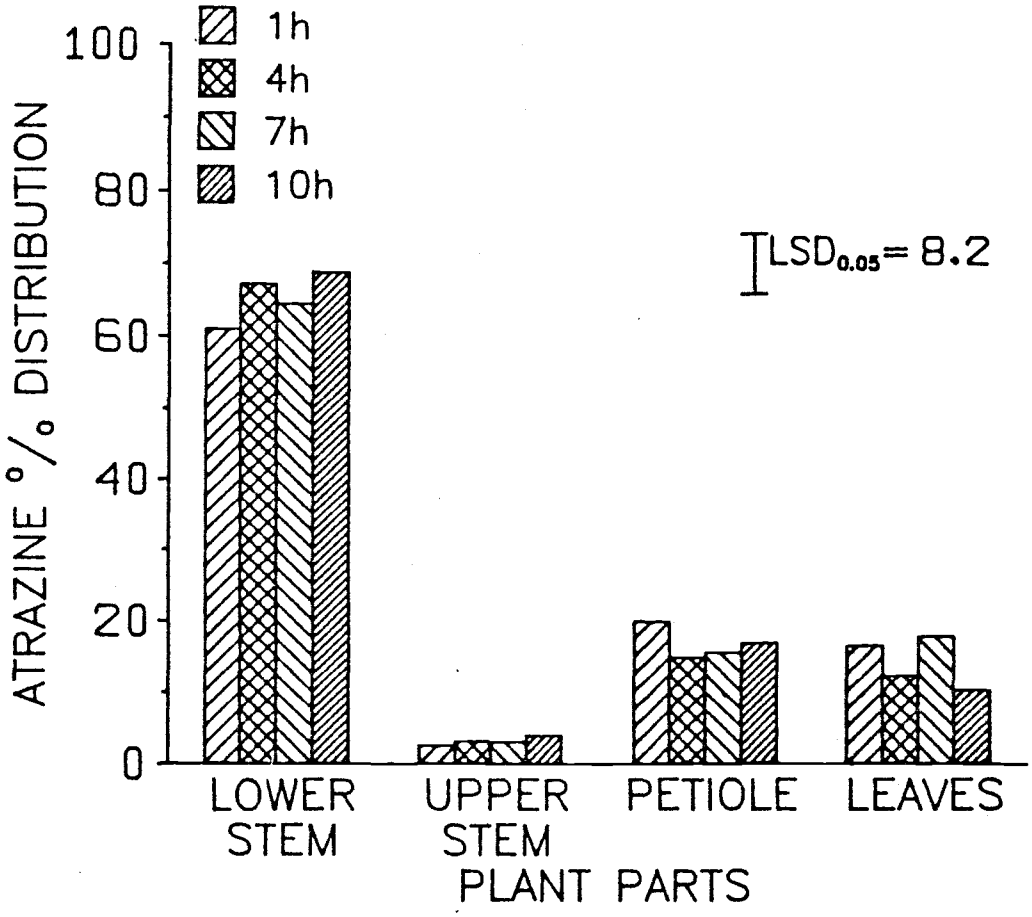


FIGURE 7. Distribution of atrazine in excised sunflower shoots. Values represent average of four experiments. Recovery of total absorbed radioactivity per plant was greater than 96%.

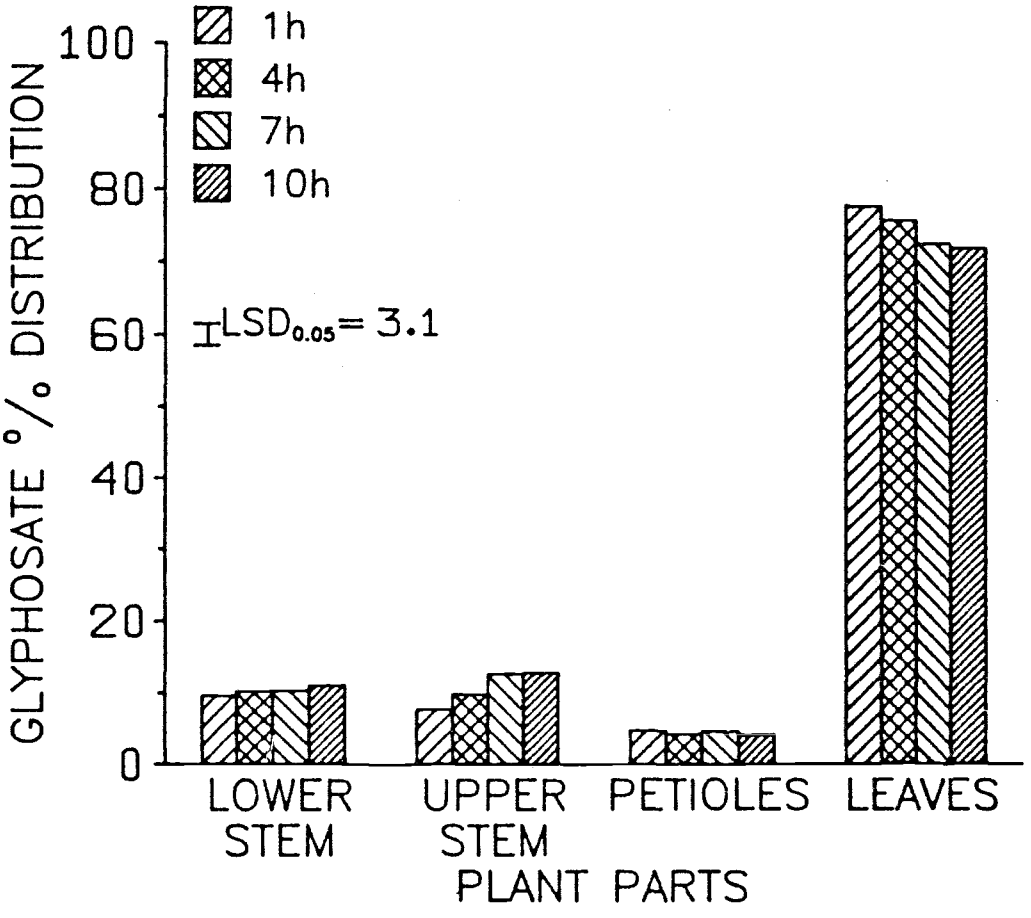


FIGURE 8. Distribution of glyphosate in excised sunflower shoots. Values represent average of three experiments. Recovery of total absorbed radioactivity per plant was greater than 97%.

small amount of atrazine was metabolized, ^{14}C-label accumulated in the tissue primarily as unaltered atrazine.

These results are consistent with the observations of Graham and Buchholtz (18) who reported that when roots of intact plants were exposed to atrazine solution and subsequently placed in herbicide-free solution, the roots and stem acted as a reservoir for continued atrazine movement to the leaves. This pattern of distribution, in which a large fraction of the herbicide is retained in the stem tissue below the transpiring leaves could be due to the adsorption of atrazine in the stem apoplast. However, atrazine has been shown not to bind to the cell walls of potato tuber tissue (29), oat roots (30), setaria roots (27), or purified cellulose (53). Thus, the large fraction of atrazine retained in the stem tissue may be explained by penetration of the molecule into the symplast. Atrazine has been demonstrated to freely and reversibly penetrate the symplast of potato tuber tissue (29), corn root tissue and protoplasts (7), barley roots (39, 41), and velvet leaf (Abutilon theophrasti) roots (30, 31). Simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] was shown to resemble tritiated water in moving rapidly across cell membranes and vacuoles (41).

Van Bel (51), investigating the apparent free space of the xylem translocation pathway of the tomato stem, estimated that the cross-section of tissue actually involved in the apoplastic longitudinal flow of organic molecules to be at least 2.5 times the cross-section of the xylem vessels. This indicates that materials transported in the xylem move freely in a lateral manner into the apoplasmic

compartments of the phloem and medulla parenchyma cells, and that a rapid, reversible transfer of organic molecules from the xylem to symplast is possible via an apoplastic route. This explanation also suggests that the small fraction of atrazine translocated to the shoot tip is due to the inability of the stem symplast to retain atrazine as water in the transpiration stream moves into the leaves.

When glyphosate was xylem-fed to sunflower shoots, 71 to 78% of the compound was found in the leaves, 9 to 11% was in the lower stem, 7 to 13% in the shoot tip, and 4 to 5% was in the petioles (Fig. 8). Glyphosate was redistributed from the leaves to the shoot tips during the 10-h dark equilibration period (Fig. 8). This indicates that the shoot system employed in this study was not a static system and that transport from the leaves to the shoot tips in a source-to-sink manner was occurring. These results are consistent with the work of Gougler and Geiger (17) who demonstrated that glyphosate was readily transported in the phloem to sink leaves in the first several hours when applied to source leaves of sugarbeet plants. The amount of glyphosate in the lower stem and petioles did not significantly change with larger dark equilibration periods. While glyphosate can bind to organic matter in soils, it does not bind to ethyl cellulose (43). This suggests that the glyphosate associated with lower stem tissue was not bound in the apoplast. The large differences in the amount of atrazine and glyphosate in the lower stem tissue suggest that these two compounds, which were xylem-fed in equal molar concentrations, penetrated different volumes of the tissue. These data indicate that atrazine was able to diffuse throughout the stem

tissue. Glyphosate appeared to have limited access to the stem symplast, a large fraction remaining in the apoplast for subsequent transport to the leaves. Glyphosate which did penetrate the lower stem symplast was retained and transported past the leaves to the shoot tips. These observations are supported by the work of Dewey and Appleby (9) in Ipomoea purpurea. In that study, glyphosate was shown to exhibit significant apoplastic distribution into all transpiring tissues following a stem application. In addition, some glyphosate entered the symplast of the stem and moved via the phloem in a source to sink pattern. Glyphosate moved from the xylem to the phloem in Ipomoea stems; however, movement from phloem to xylem was quite limited, which suggested glyphosate retention in the phloem.

Distribution of Atrazine and Glyphosate in the Leaf. The distribution of atrazine and glyphosate in the non-expressed leaves which were opposite those used for xylem sap analysis was determined by autoradiography. In each case, radioactivity was found in the leaf; however, the distribution of atrazine and glyphosate in the leaf was markedly different (Fig. 4b and c). Atrazine was associated with the leaf vascular system and the tissues adjacent to the veins. Movement of atrazine through the tissues adjacent to the veins was evident during the 10-h dark equilibration period. This distribution pattern is consistent with the triazine translocation patterns reported in several studies (see review in 19). In contrast, glyphosate was distributed throughout the leaf, in a similar manner to PTS (Fig. 4). Vein loading of glyphosate was observed after 4 h dark equilibration.

Earlier observations on atrazine distribution in whole shoots indicated that the transpiration stream can be depleted of atrazine by diffusion into surrounding tissues. This suggests that the observed pattern of atrazine distribution in leaves was due to the diffusion of atrazine out of the xylem vessels and into the adjacent tissues, depleting the transpiration stream of atrazine before it was distributed throughout the minor vein system of the leaf. Conversely, we suggest that glyphosate and PTS both achieved rapid distribution throughout the leaf because of limited or no depletion of the transpiration stream by lateral diffusion into the symplast. Since PTS was found in the xylem sap in the same concentration as in the uptake solution, we assumed that none of this material was depleted from the transpiration stream in the leaf.

This interpretation is supported by the results of Darmstadt and Balke (7) in a study of triazine absorption by excised corn root tissue and isolated corn root protoplasts. In that study, they observed that atrazine concentration in isolated protoplasts reached that of the external solution in 10 s, and that the atrazine concentration in excised tissue reached that of the external solution in 10 min. These results indicate that the plasma membrane is freely permeable to atrazine. Darmstadt and Balke (7) also observed that hydroxyatrazine absorption by protoplasts did not plateau until 5 min, and the concentration of hydroxyatrazine in excised tissue reached only 15% of the external concentration by 30 min. Hydroxyatrazine is similar to both glyphosate and PTS in its high water solubility. The limited penetration of these compounds may be

explained by the strong negative correlation of membrane permeability with the water solubility of a solute (10).

When sap was expressed under pressure from the leaves of the shoots which had been xylem-fed atrazine, two concentration plateaus were observed (Fig. 9). The first plateau was at $(3.7 \pm 0.6) \times 10^{-7}$ M and extended from the initial balance pressure to 3.0 ± 0.6 bars of applied pressure. This was followed by a second plateau which extended through the remaining measurements and averaged $(2.0 \pm 0.5) \times 10^{-7}$ M. There were no significant differences in efflux patterns or concentrations between shoots allowed to equilibrate in the dark at 100 R.H. for 1, 4, 7, or 10 h (Fig. 10).

At less than 4 bars applied pressure, the atrazine efflux pattern was similar in form to that obtained for the natural leaf apoplastic solutes (Fig. 3). The first petiole-midrib fraction was slightly more concentrated than the second minor vein-cell wall fraction. This indicates that atrazine was not accumulated in the apoplast.

The pattern of atrazine efflux after 4 bars applied pressure was quite different from that obtained for the leaf apoplastic solutes. Previous studies of the efflux pattern of leaf apoplastic solutes and PTS have indicated that at approximately 4 bars of applied pressure, expressed sap is comprised of a decreasing minor vein-cell wall fraction and an increasing fraction symplastic membrane-filtered cell sap. Since there was no decrease in atrazine concentration following 4 bars applied pressure, then atrazine must be moving across the plasmalemma with the water as the cells lose volume under pressure.

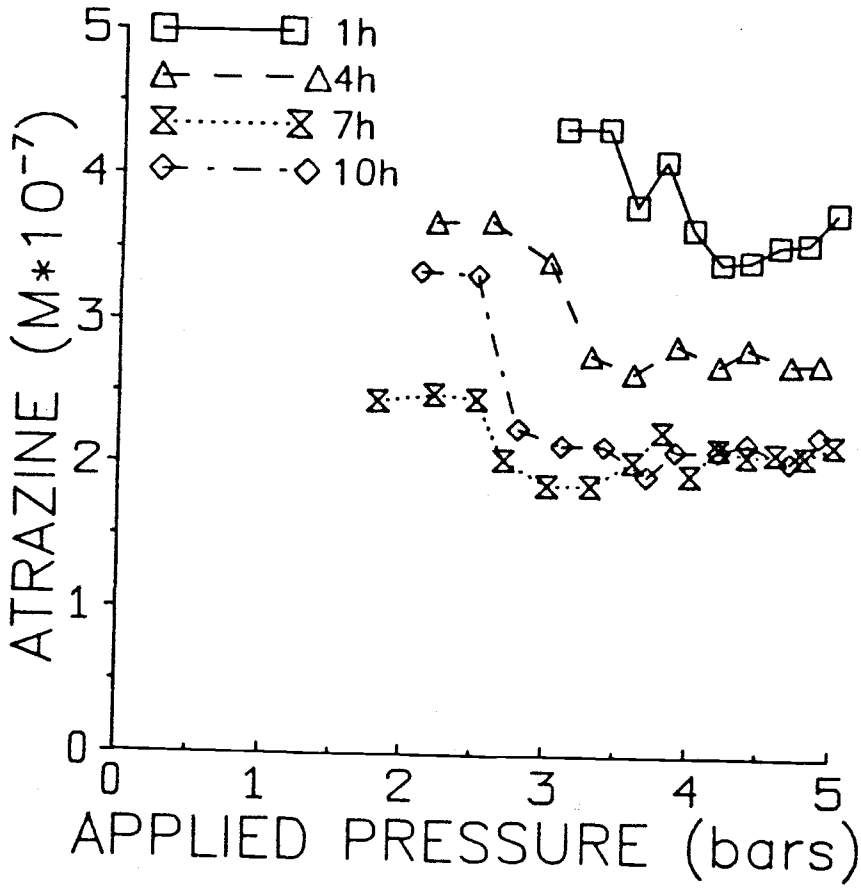


FIGURE 9. Atrazine in exudates of sunflower leaves subjected to pressure dehydration. The pressure in the chamber was increased in steps of 0.2 to 0.4 bars over the previous balance pressure. Samples were collected over a period of 5 min. Data for one replication only.

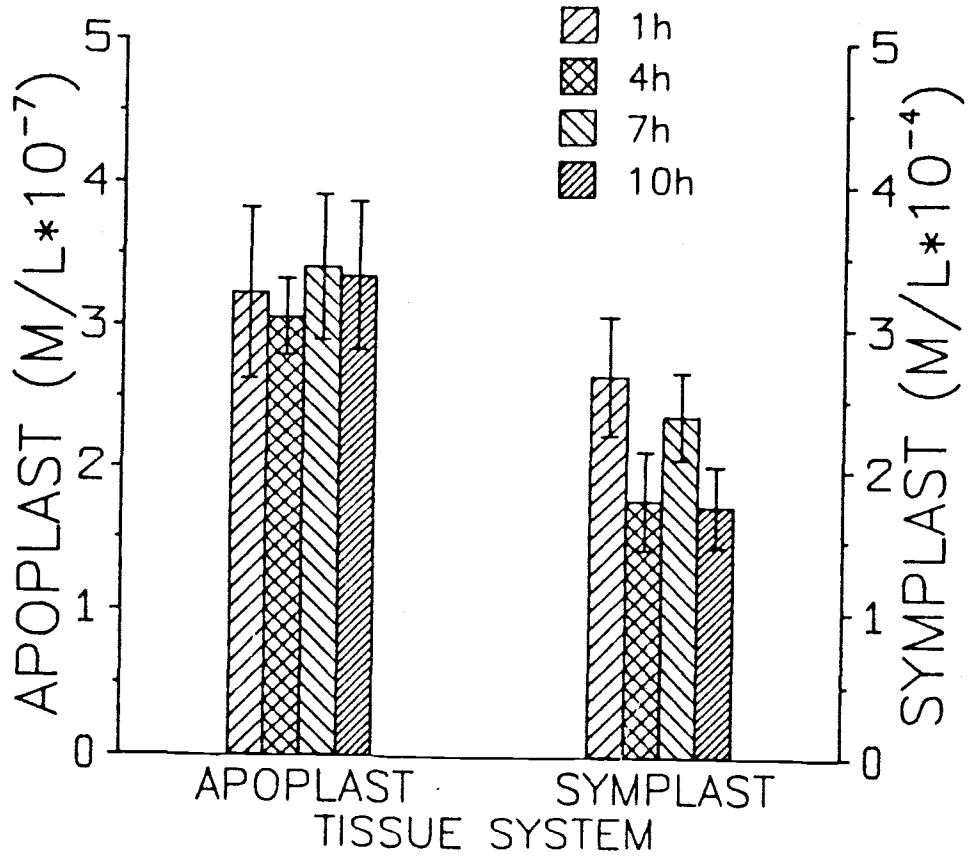


FIGURE 10. Distribution of atrazine between the apoplast and symplast of intact sunflower leaves. Concentration of atrazine in the apoplast was determined graphically from expressed sap efflux curves between 3 and 4 bars applied pressure. Concentration of symplast was calculated from excised tissue samples adjacent to the main veins of the leaf, as described in "Materials and Methods". Vertical bars represent the standard error of each plotted mean.

This indicates that the plasmalemma is not a significant barrier to atrazine movement in sunflower.

When sap was expressed under pressure from the leaves of shoots which had been xylem-fed glyphosate, an efflux pattern very similar to that obtained for PTS was obtained (Fig. 11). Two concentration plateaus were observed. The first plateau extended from the initial balance pressure to 2.2 ± 0.3 bars applied pressure and averaged (1.5 ± 0.3) x 10^{-6} M. This was followed by a second plateau which extended from 3.2 ± 0.3 bars to 3.5 ± 0.3 bars. The concentration of the second plateau was reduced significantly with increasing dark equilibration period (Fig. 12), from (7.3 ± 0.6) x 10^{-6} M at 1 h dark equilibration to (5.1 ± 0.8) x 10^{-6} M at 10 h dark equilibration.

The glyphosate and PTS curves differ in that the amount of glyphosate in the initial fraction expressed from the leaf was less than that in the uptake solution (Figs. 5 and 11). This was probably due to movement into the symplast and transport to the shoot tip. However, in all other respects, the efflux patterns of glyphosate (Fig. 11) and PTS (Fig. 5) are very similar. Both compounds show an accumulation of material in the second minor vein-cell wall phase of the efflux curve. This indicates a resistance to movement into the symplast compared to atrazine (Fig. 9) and the natural apoplastic solutes (Fig. 3).

The second plateau glyphosate concentration was reduced with increasing dark equilibration time (Fig. 12). A regression analysis conducted to compare concentrations of the minor vein-cell wall fraction (Fig. 12) and total glyphosate concentration in the same

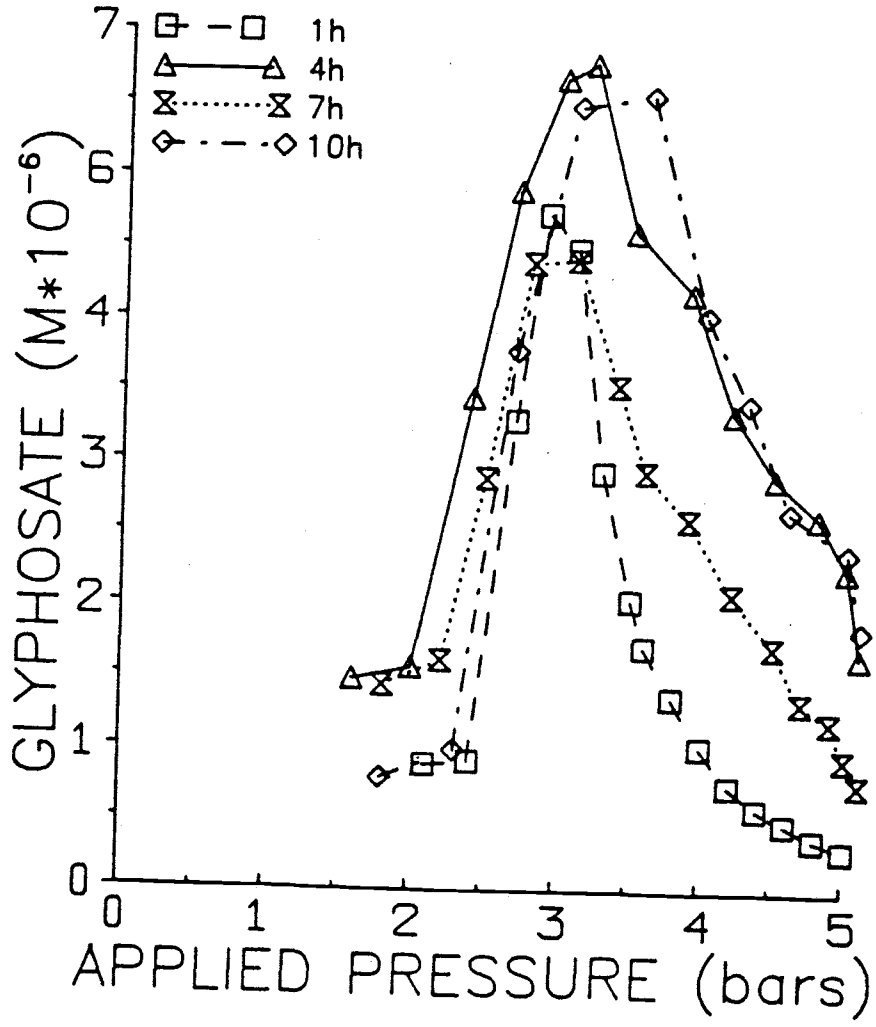


FIGURE 11. Glyphosate in exudates of sunflower leaves subjected to pressure dehydration. The pressure in the chamber was increased in steps of 0.2 to 0.4 bars over the previous balance pressure. Samples were collected over a period of 5 min. Data for one replication only.

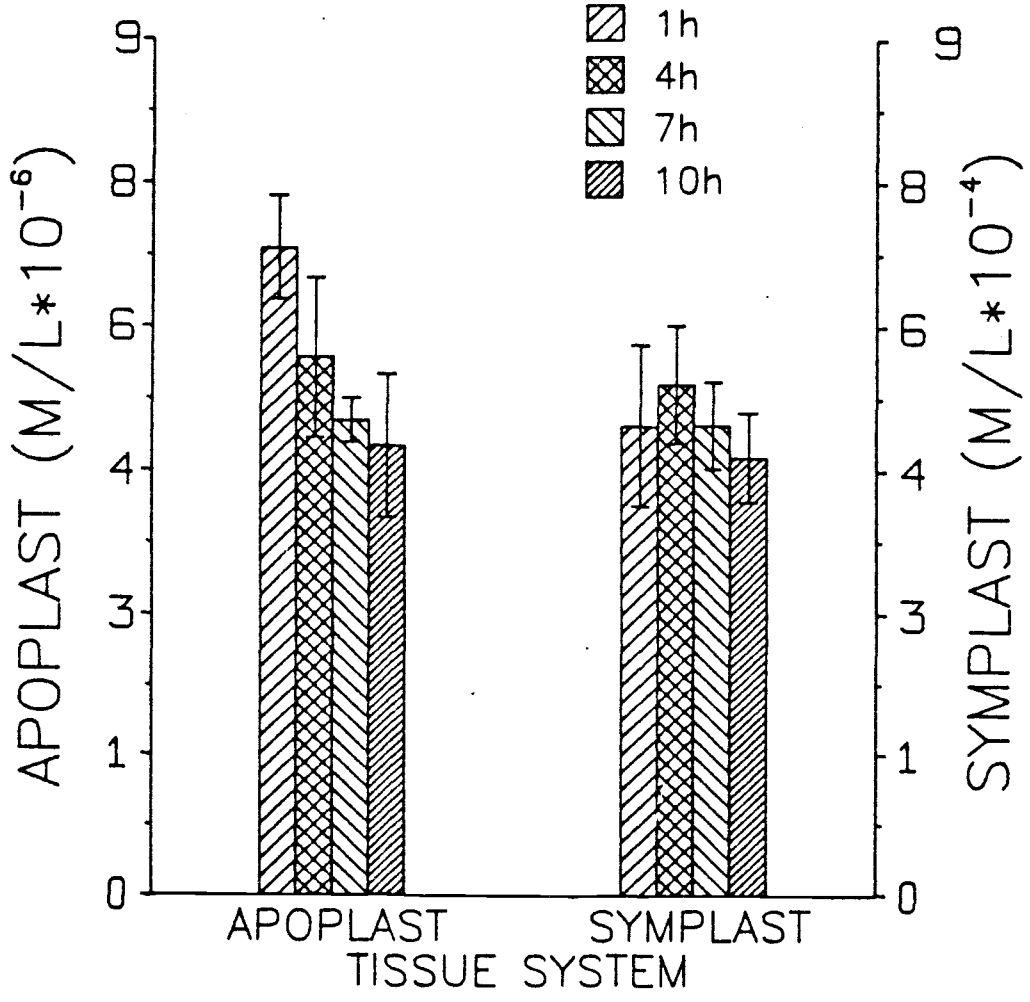


FIGURE 12. Distribution of glyphosate between the apoplast and symplast of intact sunflower leaves. Concentration of glyphosate was determined graphically from expressed sap efflux curves between 3 and 4 bars applied pressure. Concentration of symplast was calculated from tissue samples adjacent to the main vein of the leaf, as described in "Materials and Methods." Vertical bars represent the standard error of each plotted mean.

leaf (Fig. 8) was conducted. Reduction in the glyphosate concentration of the second plateau was highly correlated (r^2 = 0.949) with export of glyphosate from the leaf. These data indicate that glyphosate was moving from the apoplast into the leaf symplast, and was subsequently transported to the shoot tip via the symplast.

Glyphosate and PTS both demonstrated a rapid decrease in concentration with each increment of applied pressure following the second plateau. This trend indicates that glyphosate in the leaf symplast is unable to quickly penetrate the plasmalemma, and is retained as the cells lose volume under pressure.

These results support the intermediate permeability hypothesis (50); that compounds which display the apoplastic transport pattern are freely mobile between the apoplast and symplast. Compounds exhibiting the symplastic transport pattern penetrate the symplast slowly, but once absorbed, are retained for long-distance transport.

Atrazine and Glyphosate Partitioning. The apparent partitioning coefficient, a measure of the lipid solubility and charge characteristic of a molecule, was determined for atrazine (Fig. 13a) and glyphosate (Fig. 13b) in a 2-octanol/water system. These data indicate that atrazine partitions into the lipid phase of the system to a much greater extent than glyphosate. The apparent partitioning coefficient for both atrazine and glyphosate remained essentially unchanged from pH 4 to 8, the range of physiologically significant pH. These partitioning coefficients strongly reflect the water solubility of the two herbicides; water solubility for atrazine and glyphosate is 33 ppm and 12,000 ppm, respectively (56).

FIGURE 13. Apparent octanol/water partitioning coefficient as a function of pH of the aqueous phase for (A) atrazine and (B) glyphosate. Vertical bars represent the standard error of each plotted mean.

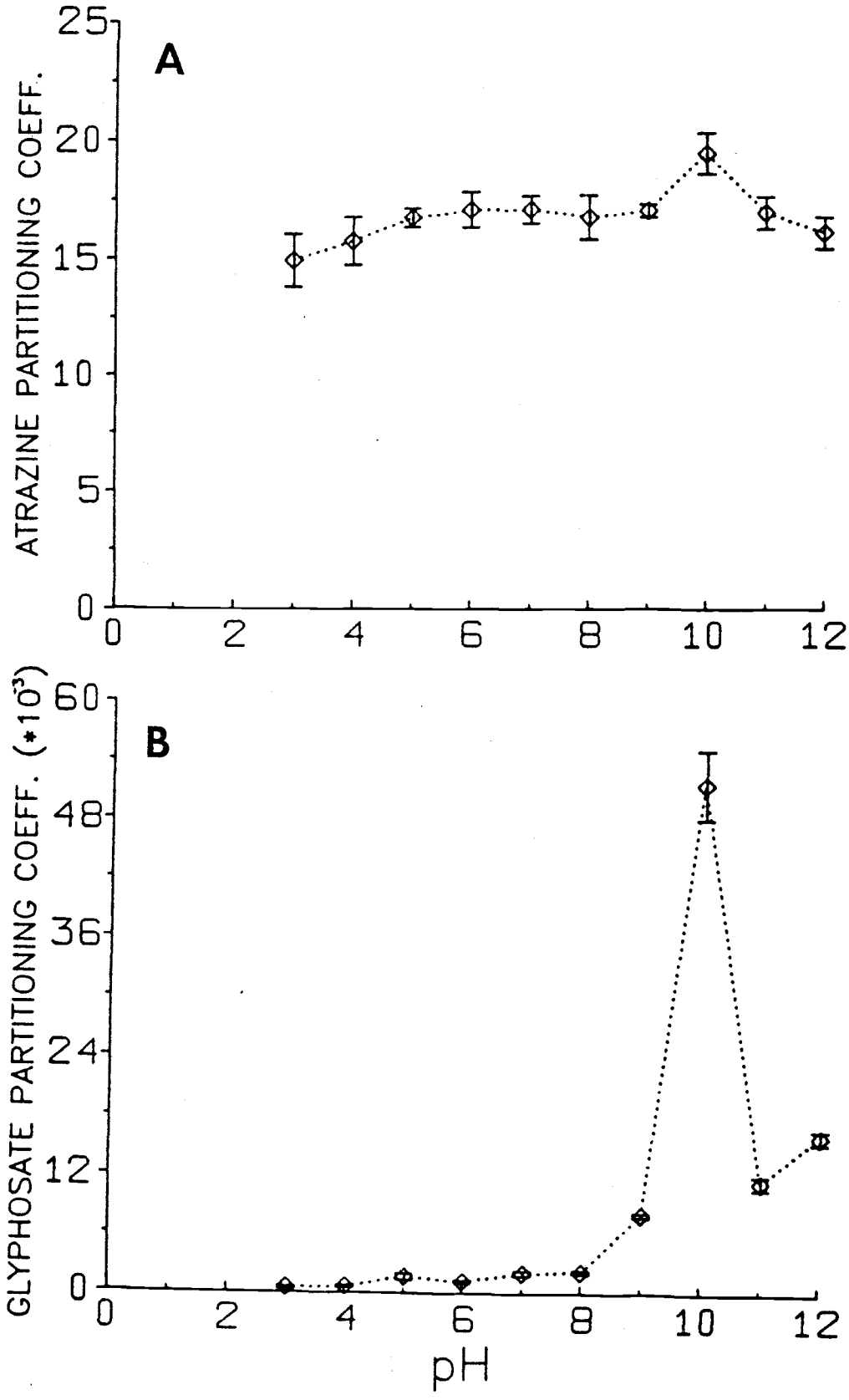

A
ATRAZINE PARTITIONING COEFF.
25
20
15
10
5
0
0
2
4
6
8
10
12
B
GLYPHOSATE PARTITIONING COEFF. (*10³)
60
48
36
24
12
0
0
2
4
6
8
10
12
pH

FIGURE 13

TABLE 1. Symplast/apoplast partitioning quotients[a] for atrazine and glyphosate.

Dark equilibration time	Partitioning quotient	
	Atrazine	Glyphosate
h		
1	533 ± 268	76 ± 34
4	544 ± 287	96 ± 25
7	710 ± 62	99 ± 21
10	569 ± 278	102 ± 26

[a]Apoplast concentrations were determined graphically from expressed sap efflux curves between 3 and 4 bars applied pressure. Symplast concentrations were calculated from excised tissue samples adjacaent to the main veins of the leaf as described in "Materials and Methods." Data are expressed as the mean ± SE for 4 experiments for atrazine and 3 experiments for glyphosate.

FIGURE 14. Fully expanded first true leaf of sunflower. Tissue sections adjacent to main veins were excised and used to calculate symplast concentration of atrazine and glyphosate as described in "Materials and Methods."

The symplast/apoplast partitioning quotients of atrazine and glyphosate (Table 1) in tissue adjacent to the main veins of the leaf (Fig. 14) were determined. Since glyphosate and atrazine were present in the whole leaf in such different concentrations (Figs. 7 and 8) and were distributed differently (Fig. 4), the symplastic concentration was determined only for the tissue near the veins, as these tissues contained somewhat comparable concentrations of the herbicides. The tissue strips contained $(4.1 \pm 1.0) \times 10^{-6}$ mol/g dry wt of glyphosate and $(2.3 \pm 1.3) \times 10^{-6}$ mol/g dry wt of atrazine.

The partitioning quotients for atrazine for a 1, 4, 7, or 10 h dark equilibration period were approximately 7 times greater than those for glyphosate. These data indicate that atrazine partitions into the symplasm to a greater extent than glyphosate. These data reflect the approximately equal amounts of glyphosate and atrazine which were present in the symplast, and that atrazine was found in a much lower concentration in the apoplast than was glyphosate (Fig. 10 and 12).

Since the rate limiting step in membrane penetration is passage through the water-membrane interface (10), the symplast/apoplast partitioning quotients are comparable to the partitioning coefficients for atrazine and glyphosate. The activation energy for crossing this interface will be determined by the same intermolecular forces which govern lipid:water partitioning coefficients, namely, the forces between solute and water which must be broken and the forces between solute and lipid, or membrane, which must be gained. Solute-water forces have been shown to be considerably stronger than solute:

lipid forces (10) and dominate both partitioning coefficients and partitioning quotients. This suggests that glyphosate's polar nature and resulting high water solubility are primarily responsible for its slower rate of penetration into the symplast and resulting accumulation in the apoplast.

BIBLIOGRAPHY

1. Ackerson RC 1982 Synthesis and movement of abscisic acid in water-stressed cotton leaves. Plant Physiol. 69:609-613
2. Ashton FM, AS Crafts 1973 Mode of Action of Herbicides. John Wiley and Sons, New York, 504 pp
3. Ashton FM, G Zweig, G Mason 1960 The effect of certain triazines on $^{14}CO_2$ fixation in red kidney beans. Weeds 8:448-451
4. Campbell GS, RI Papendick, E Rabie, AJ Shayo-Ngowi 1979 A comparison of osmotic potential, elastic modulus, and apoplastic water in leaves of dryland winter wheat. Agron. J. 72:31-36
5. Cheung YNS, MT Tyree, J Dainty 1976 Some possible sources of error in determining bulk elastic moduli and other parameters from pressure volume curves of shoots and leaves. Can. J. Bot. 53:1345-1346
6. Crafts AS, D Crisp 1971 Phloem Transport in Plant. WH Freeman and Co., San Francisco, 480 pp
7. Darmstadt GL, NE Balke 1982 Triazine absorption by isolated corn root protoplasts and excised corn root tissue. Proc. Weed Sci. Soc. Am. p. 81
8. Darmstadt GL, TP Price, NE Balke 1983 Triazine absorption by excised corn root tissue and isolated corn root protoplasts. Pest. Biochem. Physiol. (in press)
9. Dewey SA, AP Appleby 1983 A comparison between glyphosate and assimilate translocation patterns in tall morningglory (Ipomoea purpurea). Weed Sci. 31:308-314

10. Diamond JM, EM Wright 1969 Biological membranes: the physical basis of ion and nonelectrolyte selectivity. Annu. Rev. Physiol. 31:581-646

11. Donaldson TW, DE Bayer, OA Leonard 1973 Absorption of 2,4-dichlorophenoxyacetic acid and 3-(p-chlorophenyl)-1,1-dimethylurea (monuron) by barley roots. Plant Physiol. 52:638-645

12. Dybing DC, HB Currier 1961 Foliar penetration by chemicals. Plant Physiol. 36:169-174

13. Edgington LV, CA Peterson 1977 Systemic fungicides: theory, uptake and translocation. In MP Siegel, HD Sisler, eds, Antifungal Compounds, Vol. 2, Interactions in Biological and Ecological Systems. Marcel Dekker, New York

14. Fernandez CH 1978 Absorption of glyphosate [N-phosphonomethyl)-glycine] by leaf sections of tobacco Nicotiana tabacum 'Xanthi.' Ph.D. dissertation. Univ. of California, Davis

15. Frear DS, HR Swanson 1970 The biosynthesis of S-(4-ethylamino-6-isopropylamino-2-s-triazine) glutathione: partial purification and properties of a glutathione S-transferase from corn. Phytochemistry 9:2123-2132

16. Gottrup O, PA O'Sullivan, RJ Schraa, WH Vanden Born 1976 Uptake, translocation, metabolism, and selectivity of glyphosate. Weed Res. 16:197-201

17. Gougler JA, DR Geiger 1981 Uptake and distribution of N-phosphono-methylglycine in sugarbeet plants. Plant Physiol. 68:668-672

18. Graham JC, K Buchholtz 1968 Alteration of transpiration and dry matter with atrazine. Weed Sci. 16:389-392

19. Hay JR 1976 Herbicide transport in plants. In L.J. Audus, ed, Herbicides--Physiology, Biochemistry, Ecology. Vol. 2, Academic Press, New York

20. Hewitt EJ 1966 Sand and water culture methods used in the study of plant nutrition. Tech. Comm. No. 22, 2nd ed. Common. Agric. Bureaus, London. 547 pp

21. Jachetta JJ, SR Radosevich 1981 Enhanced degradation of atrazine by corn (Zea mays L.). Weed Sci. 29:37-44

22. Ladiges PY 1975 Some aspects of tissue water relations in three populations of Eucalyptus viminalis Labill. New Phytol. 75:53-62

23. Marquis LY, RD Comes, CP Yang 1979 Selectivity of glyphosate in creeping red fescue and reed canarygrass. Weed Res. 19:335-342

24. Mengel K, EA Kirkby 1982 Principles of plant nutrition. International Potash institute, Worblaufen-Bern, Switzerland. 655 pp

25. Molz FT, EI Ikenberry 1974 Water transport through plant cells and cell walls: theoretical development. Proc. Soil Sci. Soc. Am. 38:699-704

26. Munch E 1930 Die Stoffbewegung in der Pflanze. Gustav, Fischer, Jena, Germany. p. 73

27. Orwick PL, MM Schreiber, TK Hodges 1976 Absorption and Efflux of chloro-s-triazines by Setaria roots. Weed Res. 16:139-143

28. Pate JS 1975 Exchange of solutes between phloem and xylem and circulation in the whole plant. In MH Zimmerman, JA Milburn,

eds, Encyclopedia of Plant Physiology. Transport in Plant I: Phloem Transport. Springer-Verlag, New York

29. Peterson CA, LV Edgington 1976 Entry of pesticides into the plant symplast as measured by their loss from ambient solution. Pestic. Sci. 7:483-491

30. Price TP, NE Balke 1982 Characterization of rapid atrazine absorption by excised velvet leaf (Abutilon theophrasti) roots. Weed Sci. 30:633-639

31. Price TP, NE Balka 1983 Characterization of atrazine accumulation by excised velvetleaf (Abutilon theophrasti) roots. Weed Sci. 31:14-19

32. Salisbury FB, CW Ross 1978 Plant Physiology. Wadsworth Publishing Co., Inc., Belmont, California. 422 pp

33. Sandberg CL, WF Meggitt, D Penner 1980 Absorption, translocation and metabolism of ^{14}C-glyphosate in several weed species. Weed Res. 20:195-200

34. Scholander PF, HT Hammel, DE Bradstreet, EA Hemmingsen 1965 Sap pressure in vascular plants. Science (Wash.) 148:339-346

35. Scholander PF, HT Hammel, EA Hemmingsen, ED Bradstreet 1964 Hydrostatic pressure and osmotic potentials in leaves of mangroves and some other plants. Proc. Nat. Acad. Sci. U.S.A. 51:119-125

36. Schultz ME, OC Burnside 1980 Absorption, translocation, and metabolism of 2,4-D and glyphosate in hemp dogbane (Apocynum cannabinum). Weed Sci. 28:13-20

37. Shaner DL 1978 Effect of glyphosate on transpiration. Weed Sci. 26:513-516

38. Shaner DL, SL Lyon 1980 Interaction of glyphosate with aromatic amino acids in transpiration in Phaseolus vulgaris. Weed Sci. 28:31-35

39. Shone MGT, BO Bartlet, AV Wood 1974 A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide by barley. II. Relationship between uptake by roots and translocation by shoots. J. Exp. Bot. 25:401-409

40. Shone MGT, DT Clarkson, J Sanderson, AV Wood 1973 A comparison of the uptake and translocation of some organic molecules and ions in higher plants. In WA Anderson, ed, Ion Transport in Plants. Academic Press, New York. pp. 571-582

41. Shone MGT, AV Wood 1974 A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide by barley. I. Absorption in relation to physiochemical properties. J. Exp. Bot. 25:390-400

42. Smith D, KP Buchholtz 1964 Modification of plant transpiration rate with chemicals. Plant Physiol. 39:572-578

43. Sprankle P, WF Meggitt, D Penner 1975 Absorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 23:229-234

44. Stroshine RL, JR Cooke, RH Rand, JM Cutler, JF Chabot 1976 Mathematical analysis of pressure chamber efflux curves. Proc. Amer. Soc. Agric. Eng. Paper No 79-4585 Ithaca, New York

45. Strugger S 1949 Praktikum der Zell- und Gewebephysiology der Pflanze. Springer, Berlin

46. Tanton TW, SH Crowdy 1972 Water pathways in higher plants: III. The transpiration stream within leaves. J. Exp. Bot. 23:619-625

47. Tyree MT, YNS Cheung 1977 Resistance to water flow in Fagus grandifolia leaves. Can. J. Bot. 55:2591-2599

48. Tyree MT, J Dainty 1973 The water relations of hemlock (Tsuga canadensis). II. The kinetics of water exchange between the symplast and apoplast. Can. J. Bot. 51:1481-1489

49. Tyree MT, HT Hammel 1972 The measurement of turgor pressure and the water relations of plants by the pressure bomb technique. J. Exp. Bot. 23:267-282

50. Tyree MT, CA Peterson, LV Edgington 1979 A simple theory regarding ambimobility of xenobiotics with special reference to the nematicide, oxamyl. Plant Physiol. 63:367-374

51. Van Bel AJE 1978 The free space of the xylem translocation pathway of the tomato stem. J. Exp. Bot. 29:295-303

52. Vostral HJ, KP Buchholtz, CA Kust 1970 Effect of root temperature on absorption and translocation of atrazine in soybeans. Weed Sci. 18:115-117

53. Ward TM, K Holly 1966 The sorption of s-triazines by model nucleophiles as related to their partitioning between water and cyclohexane. J. Colloid Interface Sci. 22:221-230

54. Weatherly PE 1963 The pathway of water movement across the root cortex and leaf mesophyll of transpiring plants. In AJ Rutter,

ed, Symposium of the British Ecological Society, John Wiley and Sons, New York

55. Weatherly PE 1965 The state and movement of water in the leaf. Symposia of the Soc. for Exp. Biol. 10:157-184

56. Weed Science Society of America 1983 Herbicide Handbook of the Weed Science Society of America, 5th ed. WSSA, Champaign, Illinois

57. Wenkert W, ER Lemon, TR Sinclair 1978 Changes in water potential during pressure bomb measurement. Agron. J. 70:353-355

58. Wyrill JB III, OC Burnside 1976 Absorption, translocation, and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. Weed Sci. 24:557-566

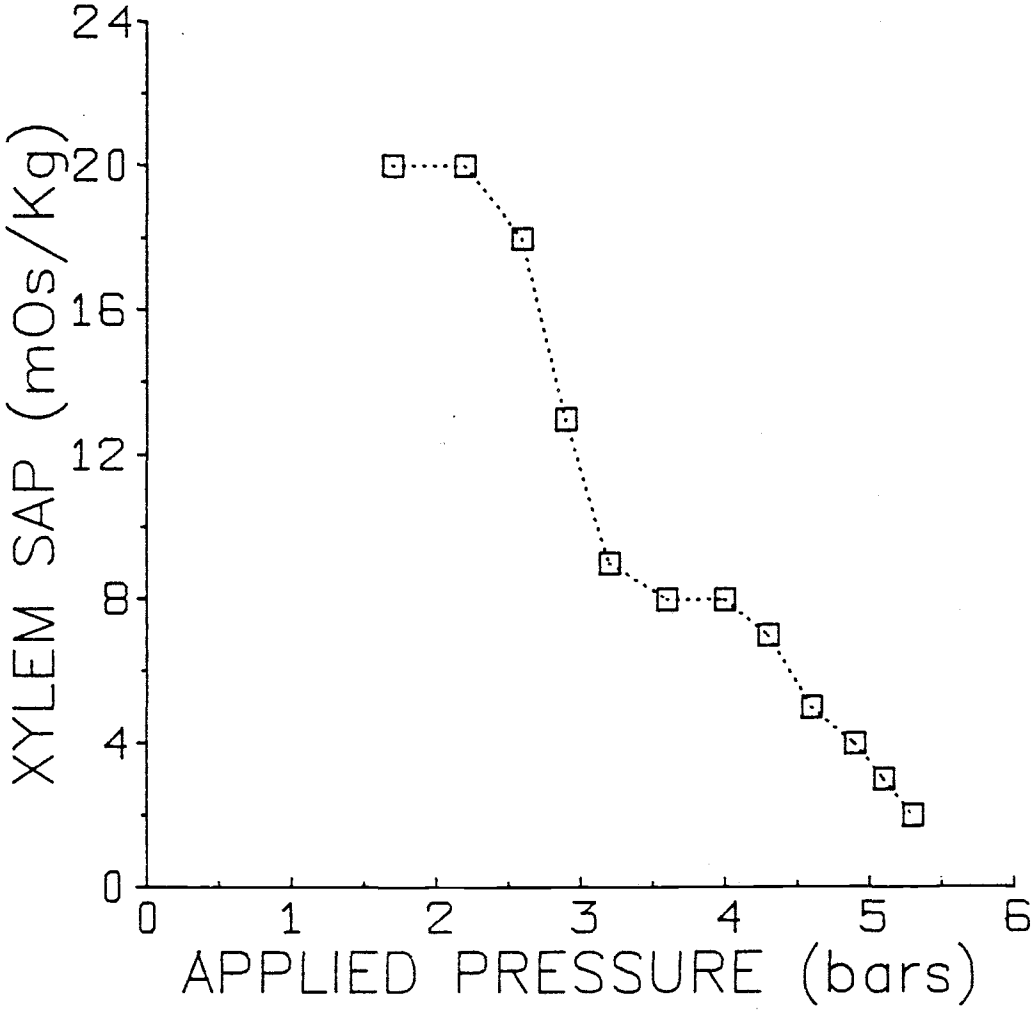
59. Zandstra BH, RK Nishimoto 1977 Movement and activity of glyphosate in purple nutsedge. Weed Sci. 25:268-274

60. Zhirmanskaya NM, SA Koltsova 1973 Investigation of permeability of barley root cells in relation to the herbicide atrazine. Fiziologiya Rastenii 20:151-157

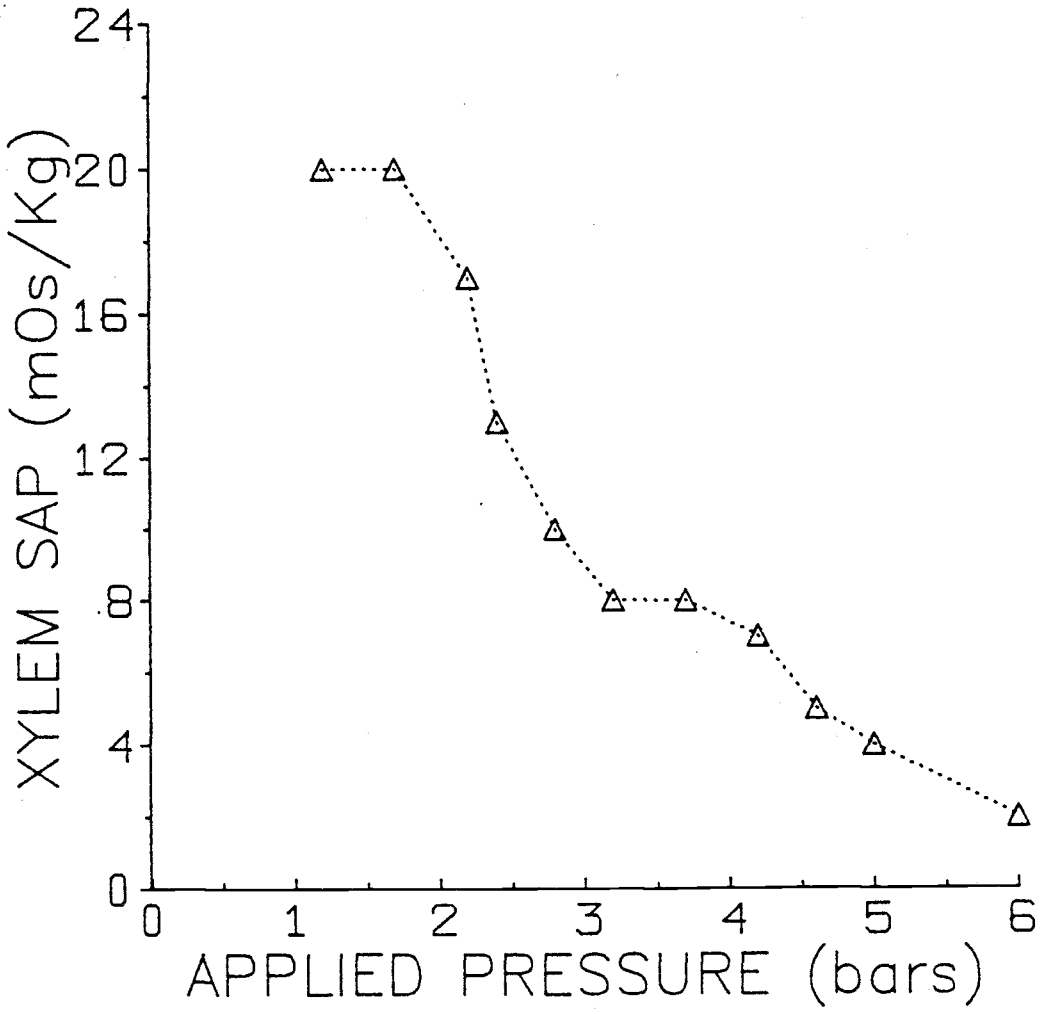
61. Zweig G, FM Ashton 1962 The effect of 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (atrazine) on distribution of ^{14}C compounds following $^{14}CO_2$ fixation in excised kidney bean leaves. J. Exp. Bot. 13:5-11

62. Zweig G, E Greenberg 1964 Diffusion studies with photosynthesis inhibitors in Chlorella. Biochem. Biophys. Acta 79:226-333.

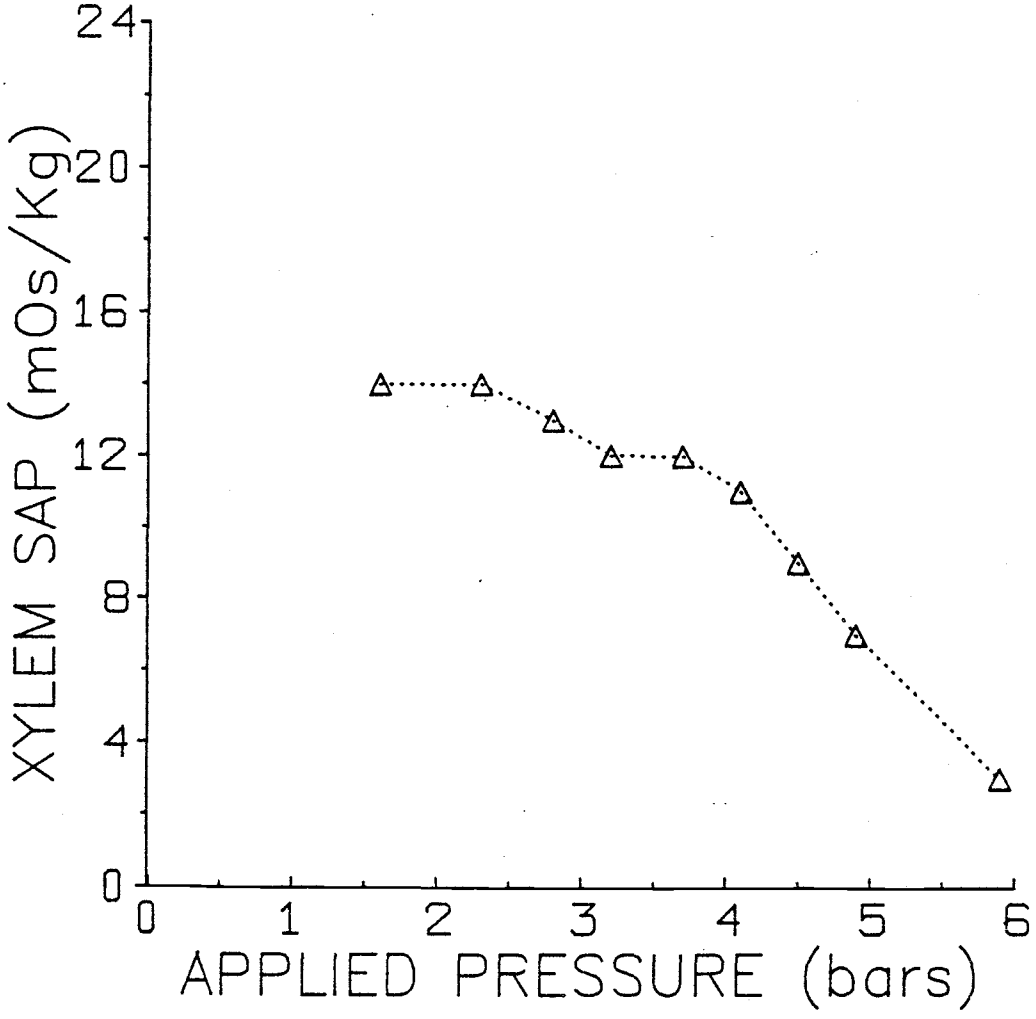
A P P E N D I C E S



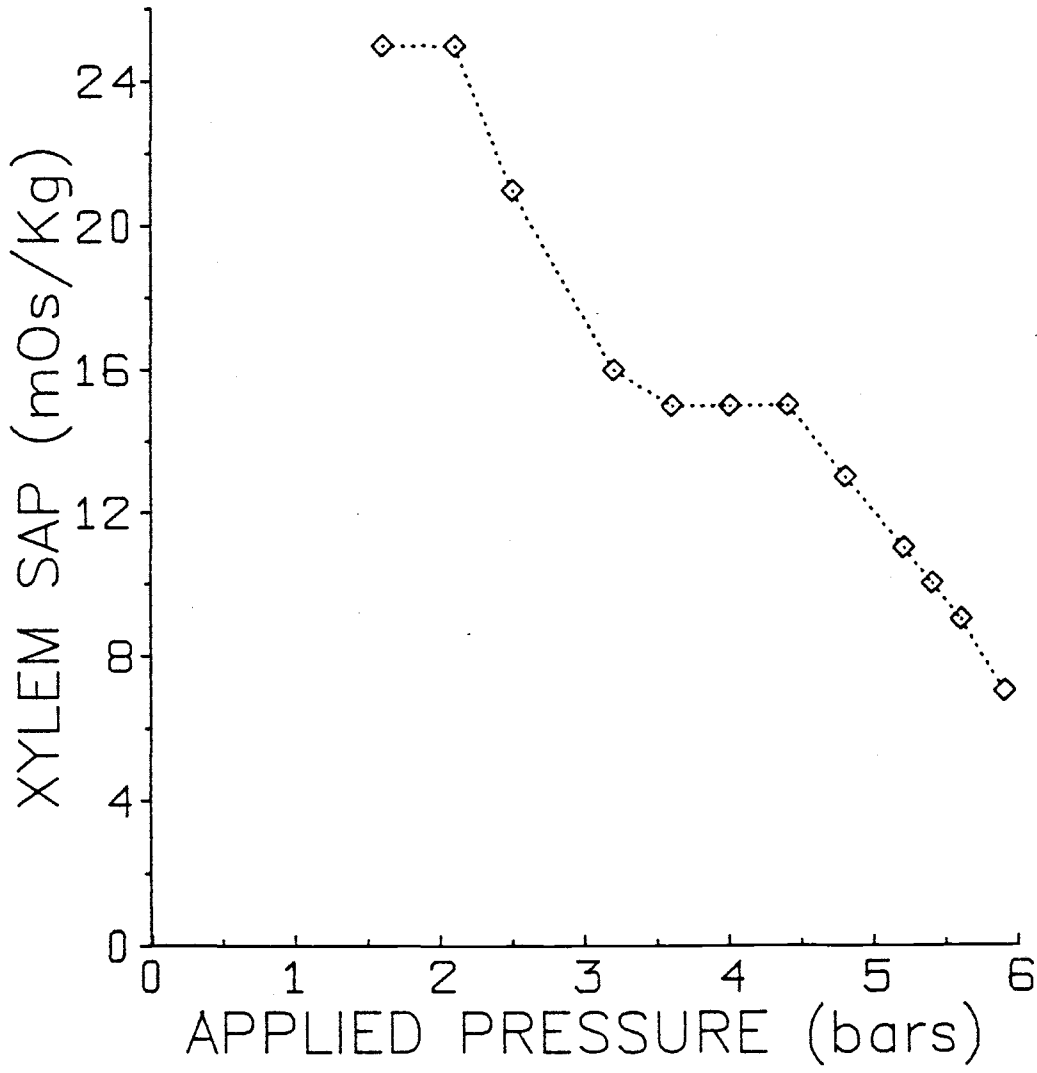
APPENDIX FIGURE 1a. Naturally occurring apoplastic solutes in exudate of sunflower leaf subjected to pressure dehydration. First replicate.



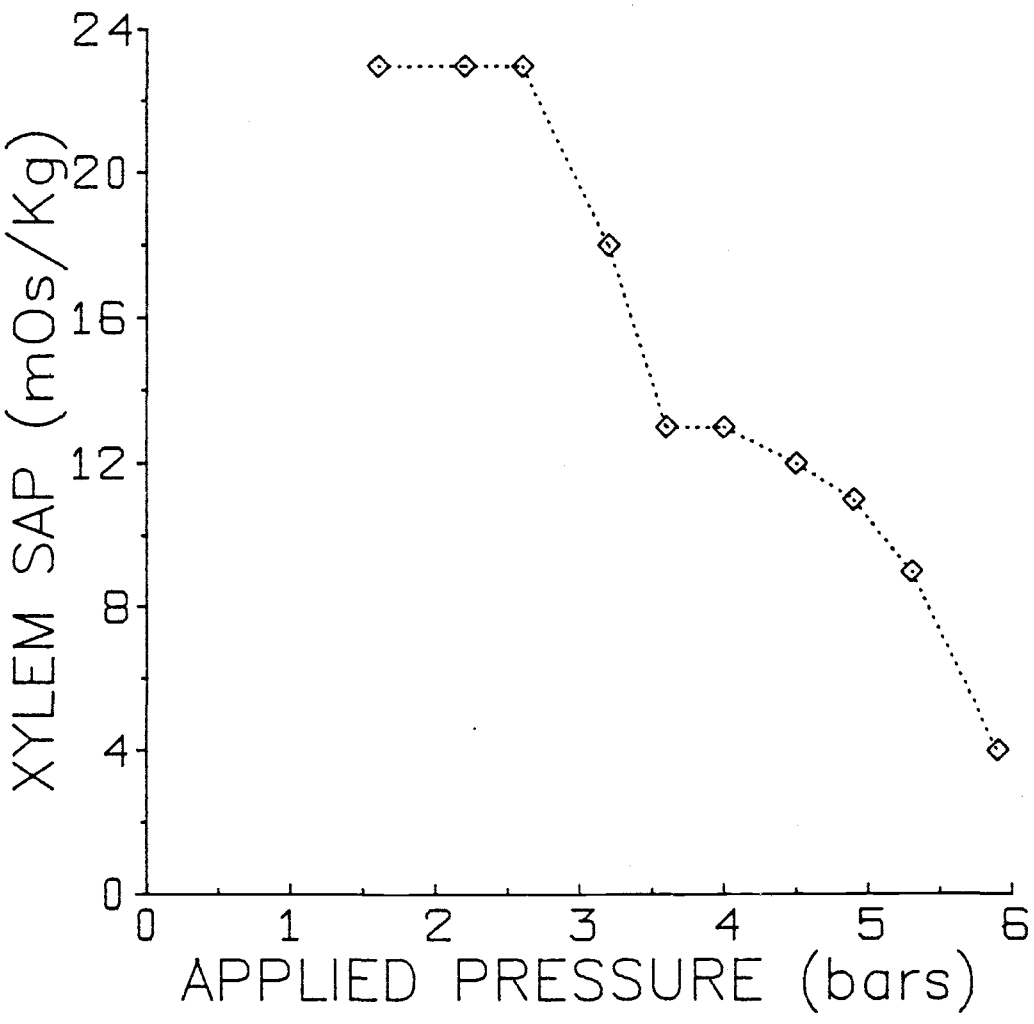
APPENDIX FIGURE 1b. Naturally occurring apoplastic solutes in exudate of sunflower leaf subjected to pressure dehydration. Second replicate.



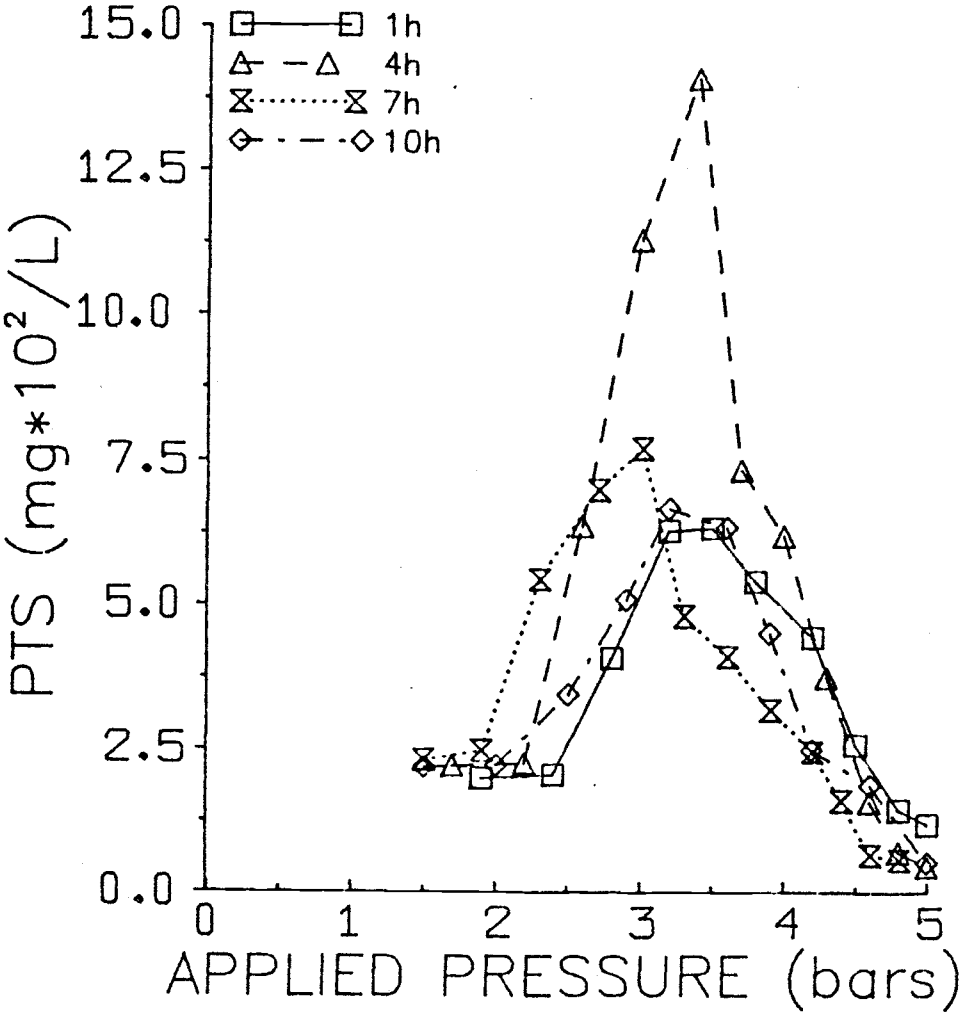
APPENDIX FIGURE 1c. Naturally occurring apoplastic solutes in exudate of sunflower leaf subjected to pressure dehydration. Third replicate.



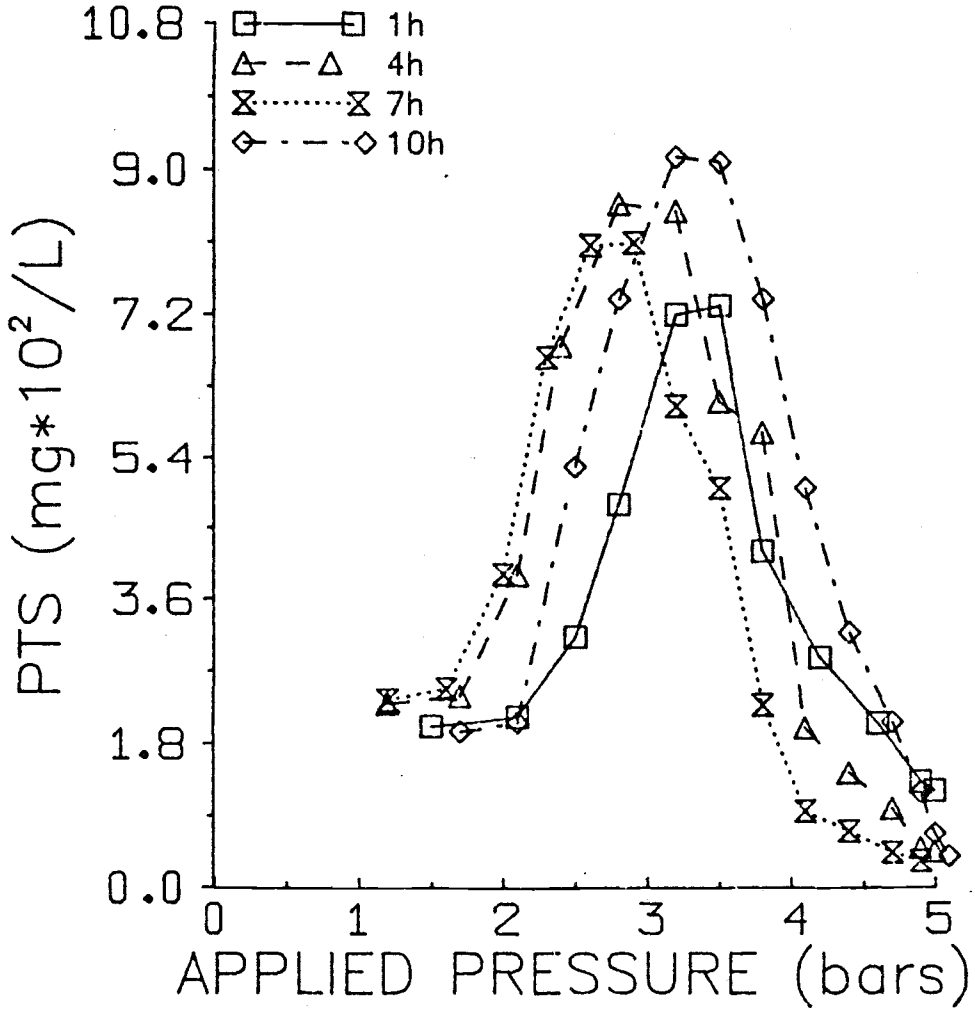
APPENDIX FIGURE 1d. Naturally occurring apoplastic solutes in exudate of sunflower leaf subjected to pressure dehydration. Fourth replicate.



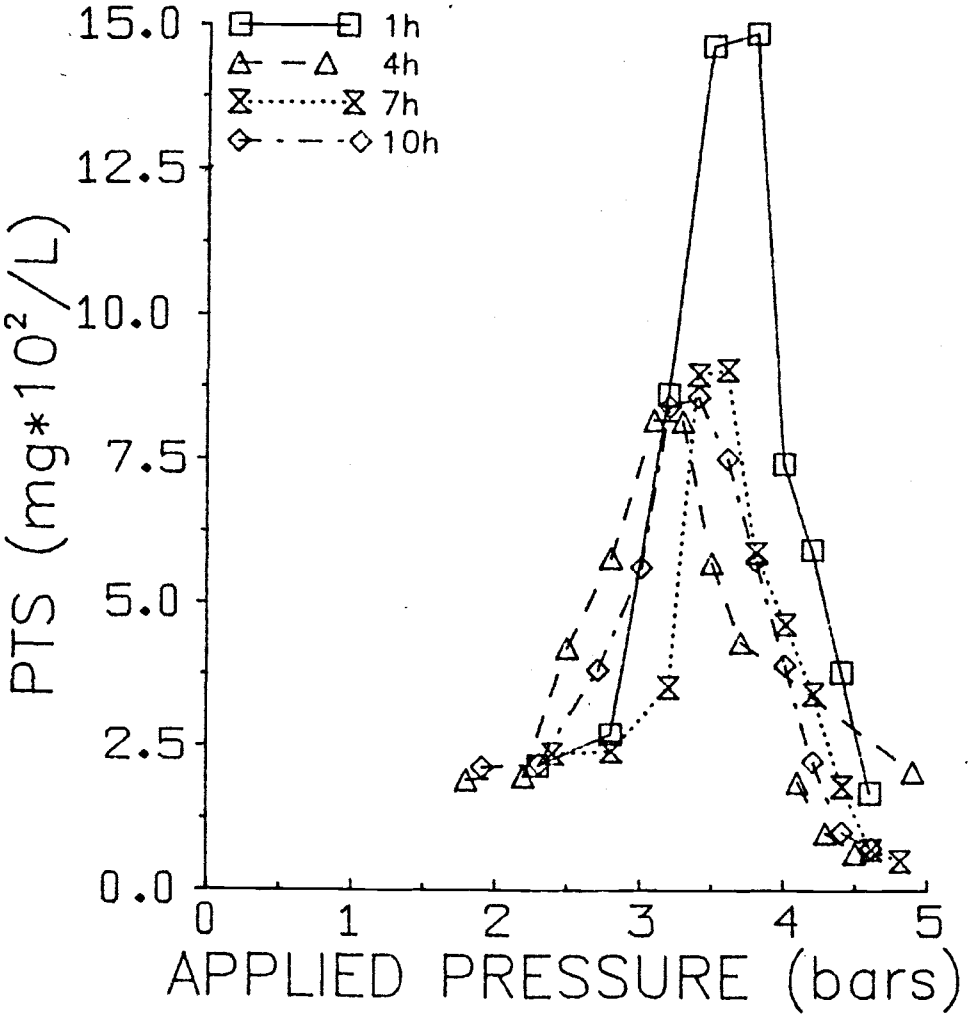
APPENDIX FIGURE 1e. Naturally occurring apoplastic solutes in exudate of sunflower leaf subjected to pressure dehydration. Fifth replicate.



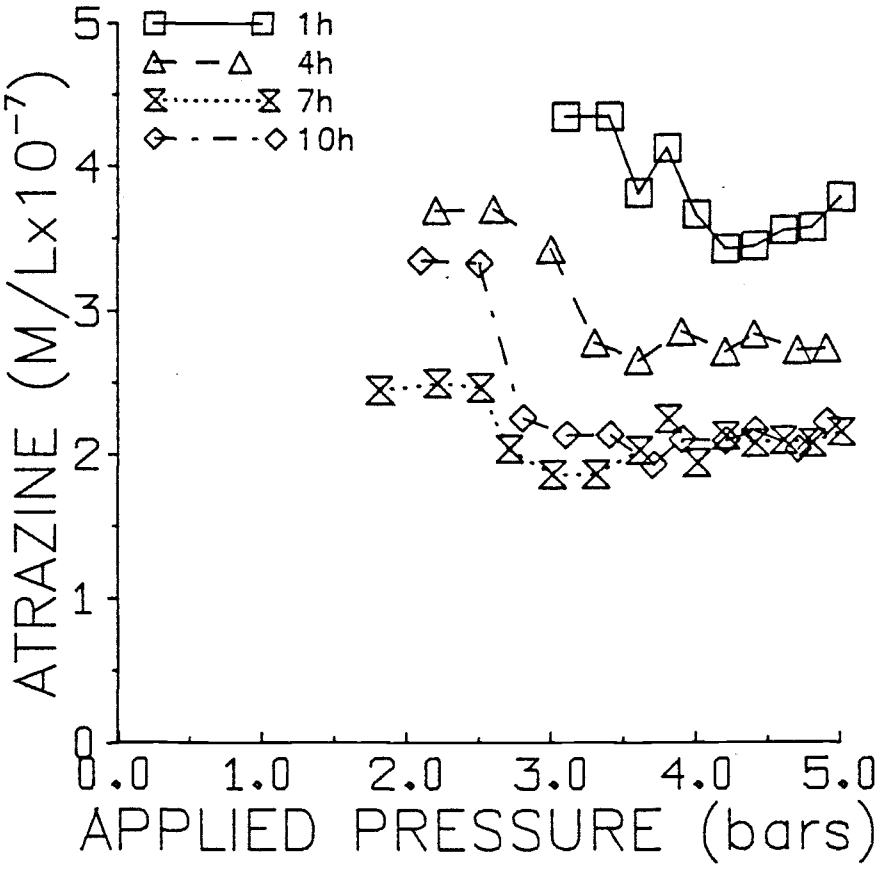
APPENDIX FIGURE 2a. PTS in exudate of sunflower leaf subjected to pressure dehydration. First replicate.



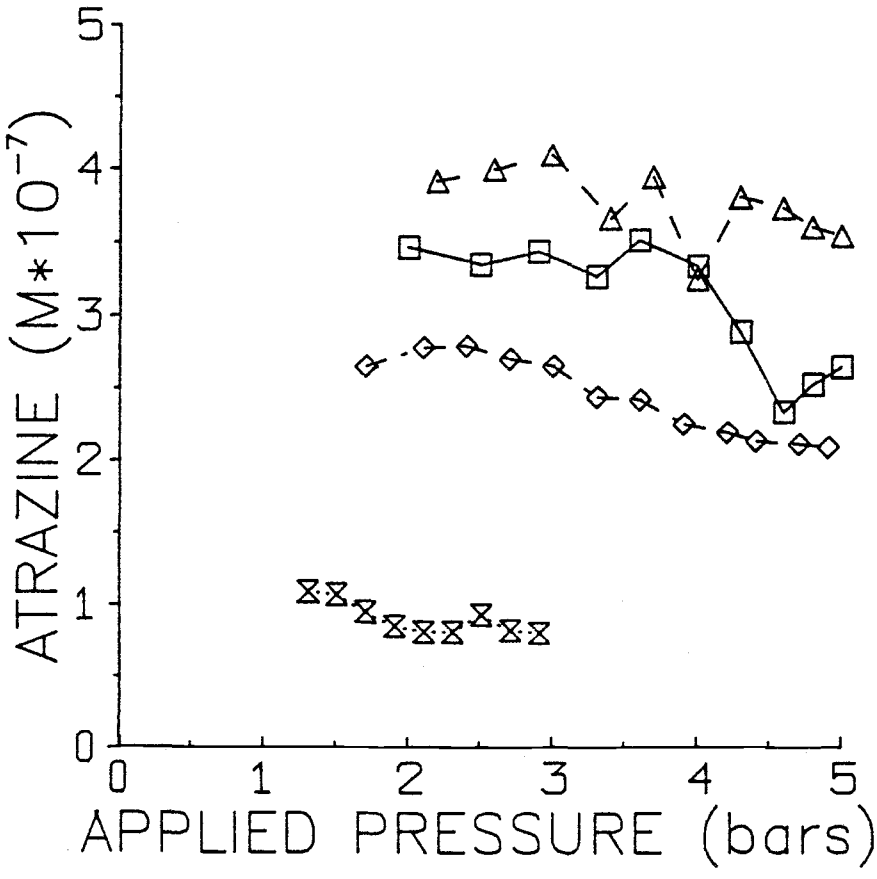
APPENDIX FIGURE 2b. PTS in exudate of sunflower leaf subjected to pressure dehydration. Second replicate.



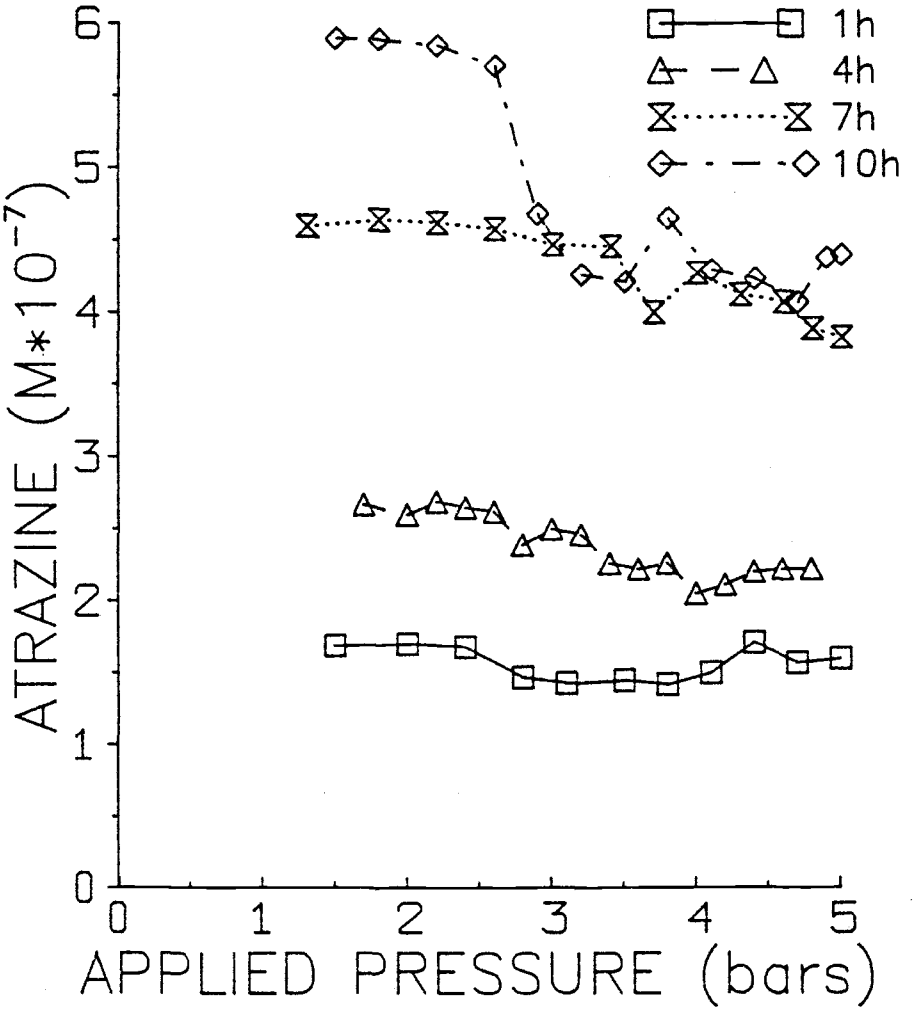
APPENDIX FIGURE 2c. PTS in exudate of sunflower leaf subjected to pressure dehydration. Third replicate.



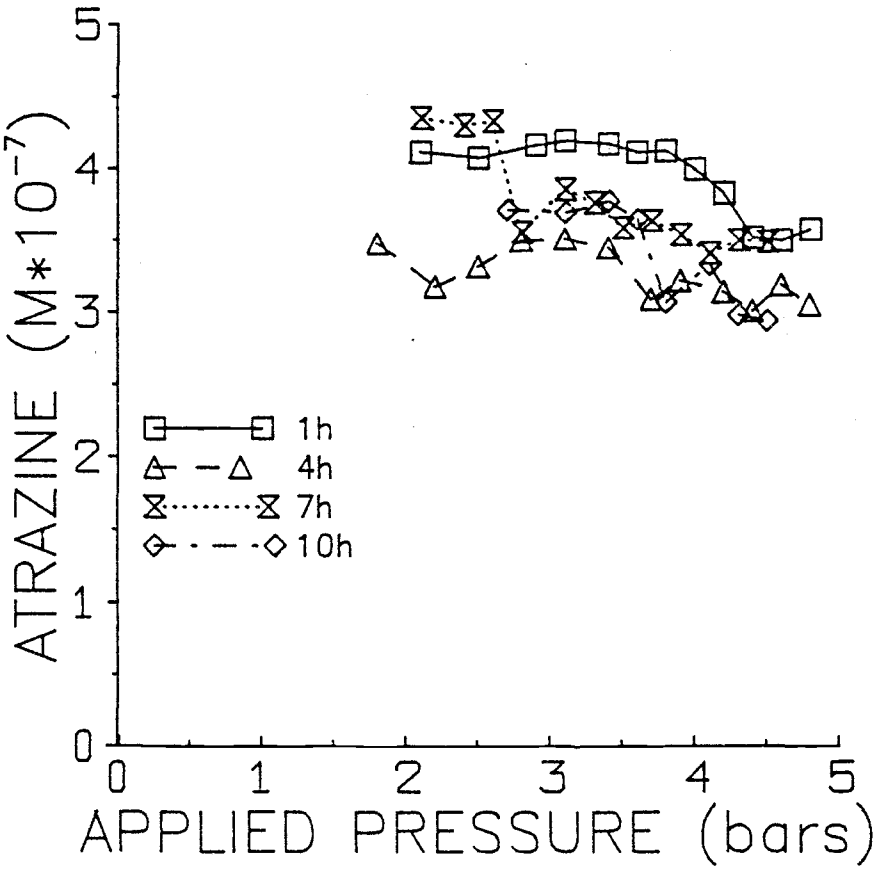
APPENDIX FIGURE 3a. Atrazine in exudate of sunflower leaf subjected to pressure dehydration. First replicate.



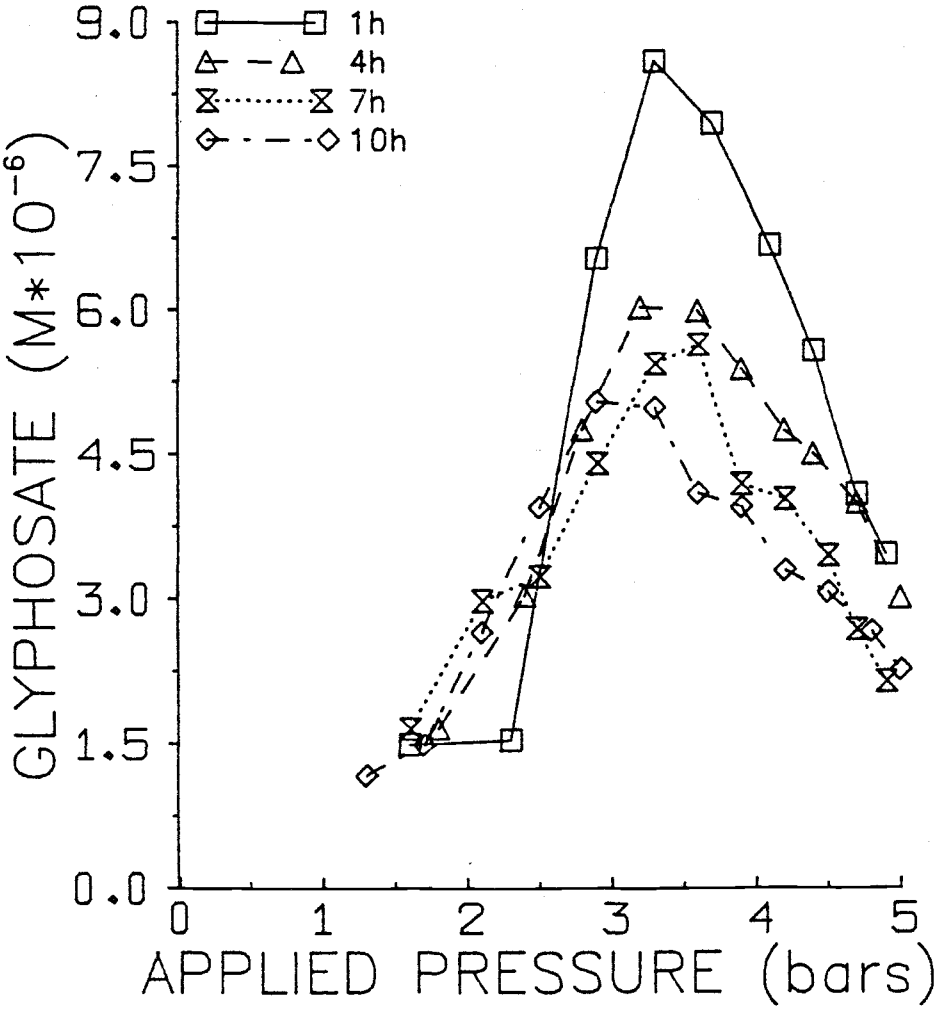
APPENDIX FIGURE 3b. Atrazine in exudate of sunflower leaf subjected to pressure dehydration. Second replicate.



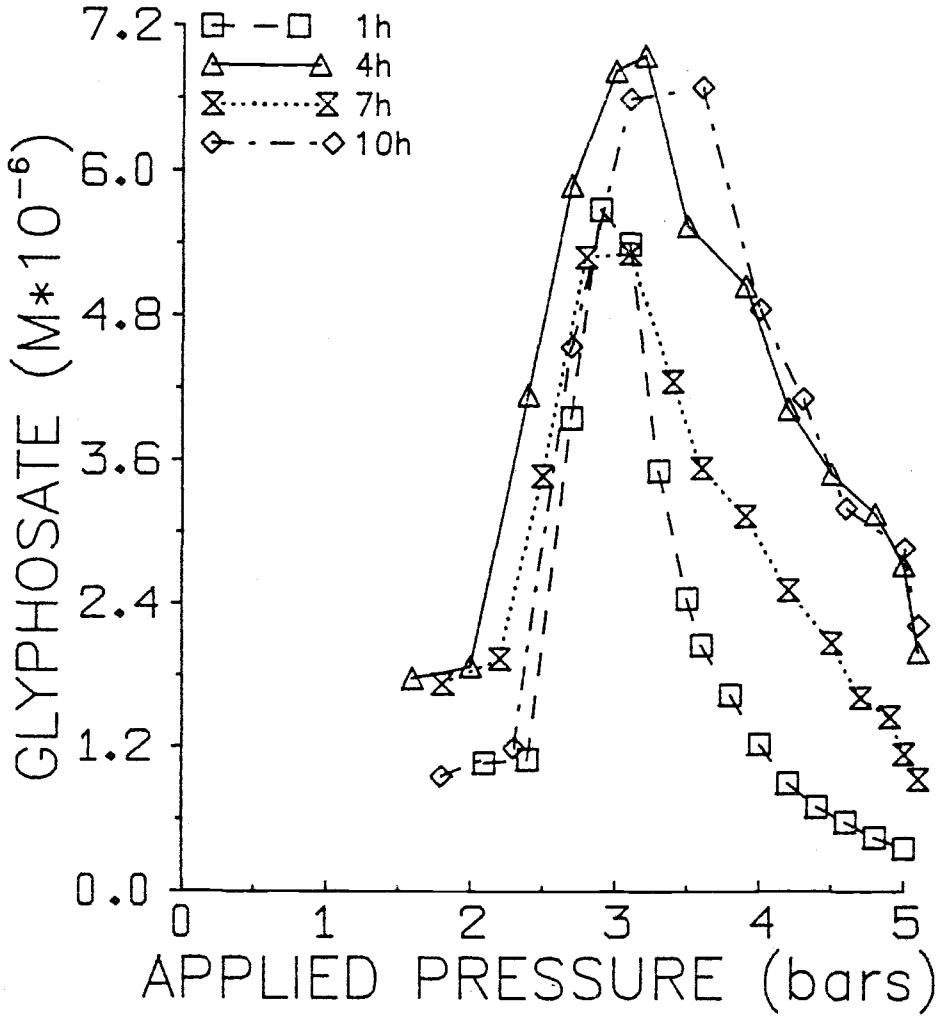
APPENDIX FIGURE 3c. Atrazine in exudate of sunflower leaf subjected to pressure dehydration. Third replicate.



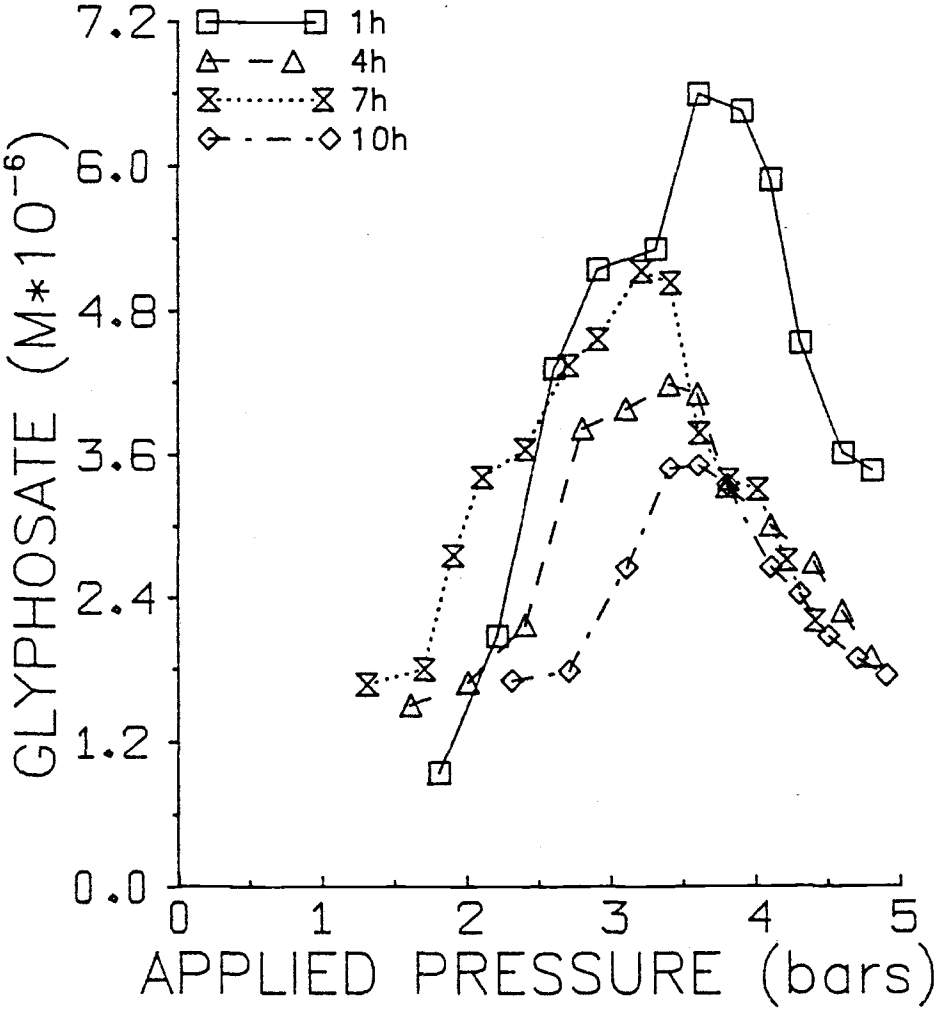
APPENDIX FIGURE 3d. Atrazine in exudate of sunflower leaf subjected to pressure dehydration. Fourth replicate.



APPENDIX FIGURE 4a. Glyphosate in exudate of sunflower leaf subjected to pressure dehydration. First replicate.



APPENDIX FIGURE 4b. Glyphosate in exudate of sunflower leaf subjected to pressure dehydration. Second replicate.



APPENDIX FIGURE 4c. Glyphosate in exudate of sunflower leaf subjected to pressure dehydration. Third replicate.

APPENDIX TABLE 1. Pressure-volume analysis for five fully expanded first true leaves of sunflower. A horizontal line is placed by those values used to calculate a least squares regression fit for the linear portion of the curve.

Leaf 1			Leaf 2			Leaf 3			Leaf 4			Leaf 5		
Ψ	% RWC	$1/\Psi$	Ψ	% RWC	$1/\Psi$	Ψ	% RWC	$1/\Psi$	Ψ	% RWC	$1/\Psi$	Ψ	% RWC	$1/\Psi$
3.0	97.6	0.333	2.0	98.4	0.500	2.0	98.7	0.500	2.4	98.5	0.417	2.3	98.4	0.435
3.7	97.1	0.270	2.5	98.1	0.400	2.5	98.3	0.400	3.1	98.1	0.323	3.0	97.9	0.333
4.6	96.3	0.217	3.0	97.7	0.333	3.0	98.1	0.333	3.7	97.6	0.270	3.7	97.4	0.270
5.5	95.2	0.182	3.6	97.2	0.278	3.6	97.6	0.278	4.5	97.0	0.222	4.5	96.7	0.222
6.5	93.2	0.154	4.4	96.3	0.227	4.5	96.7	0.222	5.4	95.8	0.185	5.4	95.6	0.185
7.2	90.6	0.139	5.3	95.1	0.190	5.3	95.6	0.189	6.2	94.2	0.161	6.2	94.0	0.161
8.2	83.0	0.122	6.3	92.8	0.159	6.3	93.0	0.159	7.0	91.7	0.144	7.1	90.2	0.141
9.8	72.3	0.102	7.5	86.9	0.133	7.5	85.9	0.133	8.0	85.5	0.125	8.3	82.4	0.121
11.4	63.1	0.088	9.1	74.9	0.110	9.1	74.4	0.110	9.6	74.7	0.104	9.8	72.6	0.102
13.1	56.0	0.076	10.7	64.9	0.093	10.7	64.3	0.094	11.7	63.8	0.085	11.8	62.4	0.085
14.5	51.8	0.069	12.6	56.7	0.079	12.3	56.2	0.081	12.7	59.5	0.079	12.9	58.3	0.078
16.4	47.2	0.061	14.3	52.0	0.070	14.5	49.6	0.069	14.1	55.0	0.071	14.4	54.2	0.069
									15.9	50.3	0.063	15.8	51.3	0.063
∞	10.8	0	∞	10.6	0	∞	10.2	0	∞	12.5	0	∞	11.7	0

Least squares regression fit for linear portion of the P-V curve

leaf 1: $1/\Psi = 0.0017 \times -0.0182$ $R^2 = 0.999$
leaf 2: $1/\Psi = 0.0017 \times -0.0181$ $R^2 = 1.000$
leaf 3: $1/\Psi = 0.0017 \times -0.0178$ $R^2 = 0.996$
leaf 4: $1/\Psi = 0.0017 \times -0.0209$ $R^2 = 0.999$
leaf 5: $1/\Psi = 0.0017 \times -0.0195$ $R^2 = 1.000$

APPENDIX TABLE 2. Effect of a 90-min pulse of 100 M atrazine on transpiration (+ and - refer to presence or absence of atrazine, respectively).

Time	Transpiration mg water/cm²/min						% Control				
	R1	R2	R3	CK1	CK2	CK3	R1	R2	R3	Aug	SE
1	247	252	275	250	245	294	99	103	93	98	5
(+) 1.5	272	293	295	264	294	298	103	100	101	101	2
(-) 3	284	272	304	281	284	317	95	98	96	96	2
4	267	262	298	278	285	331	96	92	90	93	3
5	231	223	294	271	275	372	85	81	79	82	3
6	234	214	264	264	268	338	89	80	78	82	6
7	218	207	246	258	262	332	84	79	74	79	5
8	216	202	233	255	256	319	85	79	73	79	6
9	190	198	242	247	250	323	77	80	75	77	3
10	186	183	222	248	248	317	75	76	70	74	3
11	186	166	216	239	241	318	78	71	68	72	5
12	173	163	208	222	234	311	78	70	67	72	6

APPENDIX TABLE 3. Effect of a 90-min pulse of 100 M glyphosate on transpiration (+ and - refer to presence or absence of glyphosate, respectively).

Time	Transpiration mg water/m²/min						% control				
	R1	R2	R3	CK1	CK2	CK3	R1	R2	R3	Aug	SE
1	210	215	208	224	211	218	108	90	89	96	11
(+) 1.5	235	236	235	230	238	235	109	96	99	101	7
(-) 3	248	235	251	232	245	238	107	96	105	103	6
4	251	256	265	232	257	279	108	100	95	101	7
5	270	279	293	267	284	286	101	98	102	100	2
6	256	263	287	249	272	299	103	97	96	99	4
7	215	210	208	211	218	224	102	96	93	97	5
8	205	198	207	192	199	213	107	99	97	101	5
9	193	182	197	193	198	206	100	92	96	96	4
10	187	178	192	204	202	209	90	88	92	90	2
11	170	164	183	208	214	206	82	77	89	83	6
12	158	150	182	207	220	214	76	68	85	76	9

APPENDIX TABLE 4. Atrazine tissue concentrations and symplast/apoplast partitioning quotients.

Dark equilibration time (h)		Symplast concentration ($\times 10^{-4}$)	Apoplast concentration ($\times 10^{-7}$)	Symplast/apoplast partitioning quotient
1	R1	3.07	4.17	735
	R2	2.98	3.87	769
	R3	1.41	3.38	418
	R4	3.16	1.51	209
	Avg.	2.66	3.23	533
	SE	0.83	1.19	268
4	R1	1.13	3.39	334
	R2	2.59	2.77	935
	R3	2.11	3.62	581
	R4	1.27	2.44	325
	Avg.	1.78	3.06	544
	SE	0.69	0.54	287
7	R1	2.83	3.68	769
	R2	1.51	2.05	741
	R3	2.81	3.41	625
	R4	2.38	4.50	704
	Avg.	2.38	3.41	710
	SE	0.62	1.02	62
10	R1	2.35	3.63	645
	R2	2.03	2.18	926
	R3	1.04	2.63	397
	R4	1.52	4.95	306
	Avg.	1.74	3.35	569
	SE	0.58	1.23	278

APPENDIX TABLE 5. Glyphosate tissue concentrations and symplast/apoplast partitioning quotients.

Dark equilibration time (h)		Symplast concentration ($\times 10^{-4}$)	Apoplast concentration ($\times 10^{-6}$)	Symplast/apoplast partitioning quotient
1	R1	7.03	6.59	107
	R2	3.41	8.60	40
	R3	5.48	6.64	83
	Avg.	5.31	7.28	76
	SE.	1.82	1.15	34
4	R1	6.29	5.18	121
	R2	4.24	6.03	70
	R3	6.77	6.96	97
	Avg.	5.77	6.06	96
	SE	1.34	0.89	25
7	R1	5.19	5.13	101
	R2	4.41	5.63	78
	R3	6.35	5.32	119
	Avg.	5.32	5.36	99
	SE	0.98	0.25	21
10	R1	4.62	3.50	132
	R2	4.19	5.04	83
	R3	6.08	6.70	91
	Avg.	4.96	5.08	102
	SE	0.99	1.60	26

APPENDIX TABLE 6. Atrazine 2-octanol/water partitioning coefficients.

pH	R1	R2	$3	R4	R5	Avg.	SE
3	14.39	13.33	15.13	15.70	16.21	14.95	1.13
4	16.08	14.60	15.06	15.87	17.27	15.78	1.03
5	16.47	16.81	16.39	16.86	17.42	16.79	0.41
6	16.58	16.92	16.69	16.92	18.45	17.11	0.76
7	16.69	16.86	16.58	17.92	17.57	17.12	0.59
7.9	15.87	16.45	16.26	16.98	18.32	16.78	0.95
8.8	16.75	17.21	16.78	17.15	17.39	17.06	0.28
9.7	19.31	19.38	18.28	19.80	20.75	19.50	0.89
10.6	16.92	16.98	16.12	16.89	18.05	16.99	0.69
11.4	15.95	16.13	15.95	16.34	16.45	16.16	0.23

APPENDIX TABLE 7. Glyphosate 2-octonal/water partitioning coefficients.

pH	R1	R2	R3	R4	R5	Avg.	SE
3	3.92×10^{-4}	5.03×10^{-4}	6.06×10^{-4}	5.13×10^{-4}	7.25×10^{-4}	5.48×10^{-4}	1.25×10^{-4}
4	6.54×10^{-4}	5.81×10^{-4}	6.13×10^{-4}	6.41×10^{-4}	0.09×10^{-4}	6.80×10^{-4}	1.31×10^{-4}
5	1.53×10^{-3}	1.96×10^{-3}	2.07×10^{-3}	1.75×10^{-3}	1.23×10^{-4}	1.71×10^{-3}	3.38×10^{-4}
6	1.31×10^{-3}	1.31×10^{-3}	1.06×10^{-3}	1.09×10^{-3}	1.17×10^{-3}	1.19×10^{-3}	1.18×10^{-4}
7	2.21×10^{-3}	2.44×10^{-3}	2.16×10^{-3}	1.94×10^{-3}	1.71×10^{-3}	2.09×10^{-3}	2.78×10^{-4}
8	2.28×10^{-3}	2.03×10^{-3}	2.36×10^{-3}	2.35×10^{-3}	1.88×10^{-3}	2.18×10^{-3}	2.14×10^{-4}
9	9.90×10^{-3}	8.47×10^{-3}	8.55×10^{-3}	9.09×10^{-3}	4.29×10^{-3}	8.06×10^{-3}	2.18×10^{-3}
10	5.38×10^{-2}	4.44×10^{-2}	5.21×10^{-2}	4.94×10^{-2}	5.11×10^{-2}	5.02×10^{-2}	3.59×10^{-3}
11	1.08×10^{-2}	1.26×10^{-2}	1.14×10^{-2}	1.10×10^{-2}	1.16×10^{-2}	1.15×10^{-2}	7.01×10^{-4}
12	1.81×10^{-2}	1.75×10^{-2}	1.39×10^{-2}	1.16×10^{-2}	1.99×10^{-2}	1.62×10^{-2}	3.37×10^{-3}

NOTES ON TECHNIQUE

The techniques employed in this study are very precise and exacting. Great patience and care must be used in expressing fluid from leaves in order to prevent equilibration of the expressed fluid with symplastic solutes. The flow of sap out of the leaf must be stopped for no longer than the minimum time possible to obtain the balance pressure for that measurement. Any greater pause in the flow of sap out of the leaf will allow equilibration of the xylem fluid with the surrounding cells. This results in unexpectedly high concentrations of experimental materials in the xylem stream. When studying apoplastic solutes, this effect can cause a saw-toothed pattern in the efflux curves with an endpoint considerably more concentrated than deionized water.

Sunflower leaves were selected for use in this study because of their size and leaf positioning. However, sunflower may not be the best choice for future work. Sunflower leaves have a very open network of hydathodes, a characteristic of many mesic plants. This allowed pressurized gas to be forced through the intercellular spaces of the leaf and out of the petiole. This effect caused drying of the sample disk, and in some cases was actually strong enough to blow the sample disk off of the petiole.

A better choice of plant material would be a more zeric type of plant, such as cotton. Cotton has large leaves with strong petioles and a favorable veination pattern. In addition, the leaves of cotton are very adaptable to pressure-volume analysis; indicating that this would be a superior choice of plant material.