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Mechanisms of Herbicide Absorption Across Plant Membranes and Accumulation in Plant Cells[1]

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Abstract. **In most cases, a herbicide must traverse the cell wall, the plasma membrane, and organellar membranes of a plant cell to reach its site of action where accumulation causes phytotoxicity. The physicochemical characteristics of the herbicide molecule including lipophilicity and acidity, the plant cell membranes, and the electrochemical potential in the plant cell control herbicide absorption and accumulation. Most herbicides move across plant membranes via nonfacilitated diffusion because the membrane's lipid bilayer is permeable to neutral, lipophilic xenobiotics. Passive absorption of lipophilic, ionic herbicides or weak acids can be mediated by an ion-trapping mechanism where the less lipophilic, anionic form accumulates in alkaline compartments of the plant cell. A model that includes the pH and electrical gradients across plant cell membranes better predicts accumulation concentrations in plant cells of weak acid herbicides compared to a model that uses pH only. Herbicides also may accumulate in plant cells by conversion to nonphytotoxic metabolites, binding to cellular constituents, or partitioning into lipids. Evidence exists for herbicide transport across cell membranes via carrier-mediated processes where herbicide accumulation is energy dependent; absorption is saturable and slowed by metabolic inhibitors and compounds of similar structure.**

Additional index words: **Active transport, carrier-mediated, herbicide transport, herbicide uptake, ion trapping, passive diffusion, passive absorption, weak acids, #[3] ABUTH, AEGCY, BROTE, CASOB, CYNDA, HELTU, IPOHG, LEMNI, LOLMU, SETFA, SETVI, SETVP.**

INTRODUCTION

Prior to phytotoxic action, a herbicide must be absorbed by the plant and reach its site of action, usually located within an organelle in the plant cell. Once at the active site, the herbicide may accumulate to concentrations that will elicit a phytotoxic response. Several factors can influence the final herbicide concentration at sites of action, including root or shoot absorption, translocation from sites of absorption to sites of action, or metabolism of the herbicide to nonphytotoxic compounds (38, 40). Once the herbicide reaches the plant cell, it must traverse the cell wall and the plasma membrane to enter the cytoplasm, and in many cases traverse organellar membranes to reach sites of action. This review will focus on movement of herbicides into plant cells.

Research examining mechanisms of herbicide absorption across plant membranes is limited; however, over the past decade, numerous studies have explained the mechanisms of herbicide absorption at the cellular level. To obtain relatively accurate measurements of herbicide concentration in plant cells, researchers have used either excised tissues such as roots (24, 26, 49, 73), tuber slices (4, 58, 68, 97), cotyledon (33, 44) or leaf tissues (27, 55, 77, 96), suspension-cultured plant cells (15, 21, 34, 81), protoplasts (6, 19, 22, 54), plasma membrane vesicles (80), or algal cells (62, 100). The use of excised tissues reduces the confounding of translocation which continually reduces herbicide concentrations at the site of absorption (73). Suspension-cultured cells are more simply organized compared to tissue sections allowing for accurate estimates of cellular herbicide concentrations. Use of protoplasts (plant cells lacking cell walls) or plasma membrane vesicles allows for direct exposure of the plasma membrane to the external solution (19, 80). Generally, radiolabeled herbicides are used to detect final herbicide concentrations and patterns of herbicide absorption in these tissues. This review is limited to research conducted on such tissue, where tissue was bathed in medium containing nontoxic concentrations of herbicide and no metabolism of the herbicide was detected unless stated otherwise.

An understanding of the principles of solute transport in plants is essential for describing mechanisms of herbicide absorption by plant cells; these principles are discussed by Briskin (10) in this review series. Physicochemical characteristics of the herbicide molecule, the plant cell membrane, and the electrochemical potential across the plant cell membrane control herbicide absorption across plant membranes and subsequent accumulation in the cell. In general, mechanistic studies have demonstrated that herbicides are absorbed across plant membranes by either passive or active processes. This review will discuss the various mechanisms by which specific herbicides are absorbed across plant membranes, and in certain cases how herbicides are accumulated within plant cells. The chemical names of herbicides discussed in the text are listed in Table 1.

PASSIVE ABSORPTION

Most herbicides are thought to move across plant membranes via nonfacilitated diffusion (40). Nonfacilitated diffusion is solute movement down an electrochemical gradient (63). Herbicides absorbed via passive diffusion can be separated into two classes depending on the physicochemical characteristics of the herbicide molecule: a lipophilic, neutral molecule or a lipophilic molecule with a functional group sensitive to pH which can

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 West University Ave., Champaign, IL 61821-3133.

Table 1. Chemical names of herbicides and herbicide metabolites mentioned in the text.

Ametryn	*N*-ethyl-*N*′-(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine
Amitrole	1*H*-1,2,4-triazol-3-amine
Atratone	2-methoxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine
Atrazine	6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine
Bensulfuron	2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoic acid
Bentazon	3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
Chloramben	3-amino-2,5-dichlorobenzoic acid
Chlorbromuron	3-(4-bromo-3-chlorophenyl)-1-methoxy-1-methyl-urea
Chlorimuron	2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid
Chlorpropham	1-methylethyl 3-chlorophenylcarbamate
Chlorsulfuron	2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide
Chlortoluron	*N*′-(3-chloro-4-methylphenyl)-*N*,*N*-dimethylurea
Clopyralid	3,6-dichloro-2-pyridinecarboxylic acid
Cyanazine	2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile
Dalapon	2,2-dichloropropanoic acid
Diclofop	(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid
Dinoseb	2-(1-methylpropyl)-4,6-dinitrophenol
Diuron	*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea
EPTC	*S*-ethyl dipropyl carbamothioate
Fenuron	*N*,*N*-dimethyl-*N*′-phenylurea
Fluorodifen	*p*-nitrophenyl-α,α,α-trifluoro-2-nitro-*p*-tolyl
Fluometuron	*N*,*N*-dimethyl-*N*′-[3-(trifluoromethyl)phenyl]urea
Glyphosate	*N*-(phosphonomethyl)glycine
Haloxyfop	2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid
Hydroxyatrazine	2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine
Imazapyr	(±)-2-[4,5-dihydro-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid
Imazaquin	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid
Imazethapyr	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid
Ioxynil	4-hydroxy-3,5-diiodobenzonitrile
Linuron	*N*′-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea
MCPA	(4-chloro-2-methylphenoxy)acetic acid
Metribuzin	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one
Metsulfuron	2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoic acid
MH	1,2-dihydro-3,6-pyridazinedione
Monuron	*N*′-(4-chlorophenyl)-*N*,*N*-dimethylurea
Napropamide	*N*,*N*-diethyl-2-(1-naphthalenyloxy)propanamide
Norflurazon	4-chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2*H*)-pyridazinone
Oryzalin	4-(dipropylamino)-3,5-dinitrobenzenesulfonamide
Paraquat	1,1′-dimethyl-4,4′-bipyridinium ion
Picloram	4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid
Propazine	6-chloro-*N*,*N*′-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine
Sethoxydim	2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexene-1-one
Simazine	6-chloro-*N*,*N*′-diethyl-1,3,5-triazine-2,4-diamine
Sulfometuron	2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid
Thifensulfuron	3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid
Trifluralin	2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine
2,4-D	(2,4-dichlorophenoxy)acetic acid
2,4,5-T	(2,4,5-trichlorophenoxy)acetic acid

dissociate into a less lipophilic ion. The most important physicochemical properties of a herbicide molecule in terms of absorption by plant cells are lipophilicity and acidity (12). Lipophilicity or polarity is assessed by the octan-1-ol water^{-1} partition coefficient (K_{ow})[4] of the herbicide molecule, either by direct measurement or estimation using water solubility values. The dissociation constant (pK_a)[4], a measure of acidity, determines the relative proportion of the acid and its conjugate base present at a particular pH and can be measured or estimated. The importance of these physicochemical characteristics for whole plant translocation are discussed in many reviews (12, 23, 25) and will not be addressed here.

Lipophilic, neutral herbicides. Lipophilic, neutral herbicide molecules are thought to passively diffuse into plant cells. The lipophilic nature of these herbicides allows them to diffuse rapidly across the lipid bilayer of plant membranes down their concentration gradient to reach equilibrium concentrations between external solutions and the cell interior (Figure 1a). Several mechanistic studies strongly support this hypothesis for amitrole (49, 88), monuron (26), norflurazon (56), oryzalin (99), and triazines (73, 87). In addition, a variety of other studies add supplemental support for this hypothesis. Although amitrole is amphoteric[5] and the triazines are weak bases, they will be included in this section because they behave, in terms of cellular absorption at physiological pH, as lipophilic, neutral herbicides. Experimental criteria supporting passive diffusion of herbicides include saturation of uptake over time to equilibrium concentrations, herbicide absorption rates related linearly to external herbicide concentrations, rapid herbicide efflux, temperature coefficients (Q_{10}) of absorption which are less than 2, and insensitivity of absorption to metabolic inhibitors.

In general, passive diffusion of herbicides into plant tissues and cells to reach equilibrium concentrations occurs rapidly. Amitrole absorption by bean (*Phaseolus vulgaris* L.) roots was biphasic with an initial rapid phase through 4 h followed by a slower absorption phase through 24 h where equilibrium concentrations were nearly reached (49). Oryzalin absorption by corn (*Zea mays* L.) root apices plateaued after 1 h (99). Another dinitroaniline, trifluralin, and a diphenyl ether, fluorodifen, were absorbed by isolated *Zinnia elegans* Jacq. leaf cells or tomato (*Lycopersicon esculentum* Mill.) immature fruit protoplasts within 2 h (6). Norflurazon absorption by sicklepod (*Cassia obtusifolia* L. #[3] CASOB) and cotton (*Gossypium hirsutum* L.) root segments reached equilibrium concentrations after 10 min (56). Simazine absorption by barley (*Hordeum vulgare* L.) roots was complete in 5 min (87). Similarly, diuron reached equilibrium concentrations after only 5 min in unicellular microalgae (*Ankistrodesmus braunii* Naegeli) (62), whereas monuron absorption by barley roots was complete after 1 h (26). In contrast, other phenylurea herbicides, fluometuron, and chlorbromuron were not absorbed by isolated *Zinnia* leaf cells or tomato immature fruit protoplasts although these results may be due to the extensive rinsing techniques causing efflux of any absorbed herbicide (6). Atrazine rapidly diffused into excised roots of velvetleaf (*Abutilon theophrasti* Medic. # ABUTH) (73, 75), corn (20, 75), oat (*Avena sativa* L.) (75), potato tuber slices (*Solanum tuberosum* L.) (68, 75), and microalgae cells (62) reaching concentrations equal to external concentrations after 10 to 30 min. Atrazine reached equilibrium concentrations in oat root segments within 10 min (Figure 2a). In contrast, atrazine absorption by corn root protoplasts (20) and diclofop-methyl absorption by oat seedling protoplasts (98) was complete in 10 and 5 s, respectively, suggesting that the complex organization

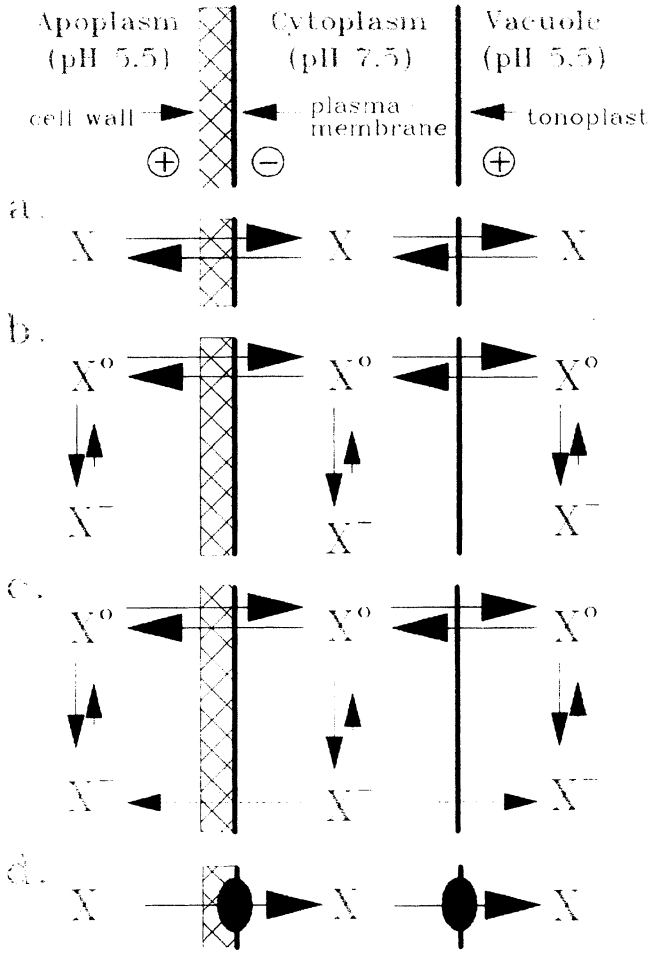


Figure 1. Models of herbicide absorption and accumulation by plant cells: a) passive diffusion of lipophilic, neutral herbicides, X; b) passive diffusion of lipophilic, ionic herbicides (X^o, nondissociated acid; X^-, anionic species) and accumulation of anionic species in cell compartments due to inability to diffuse across the lipid bilayer of plant membranes; c) passive diffusion of lipophilic, ionic herbicides (X^o, nondissociated acid; X^-, anionic species) and diffusion of anionic species across membranes; and d) carrier-mediated transport of herbicide, X. Membrane potentials across the plasma membrane and tonoplast are equal to ca. –120 and –90 mV, respectively.

[4]Abbreviations: A^-, conjugate base or anionic species; DNP, dinitrophenol; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; DCMU, 3-(3,4-dichlorophenyl)-1-1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; HA, undissociated acid; H^+-ATPase, proton-ATPase; K_{ow}, octan-1-ol water^{-1} partition coefficient; PCIB, *p*-chlorophenoxyisobutyric acid; pK_a, dissociation constant.

[5]J. Baron, Rhone-Poulenc Agric. Co., Res. Triangle Park, NC. 1993. Personal communication.

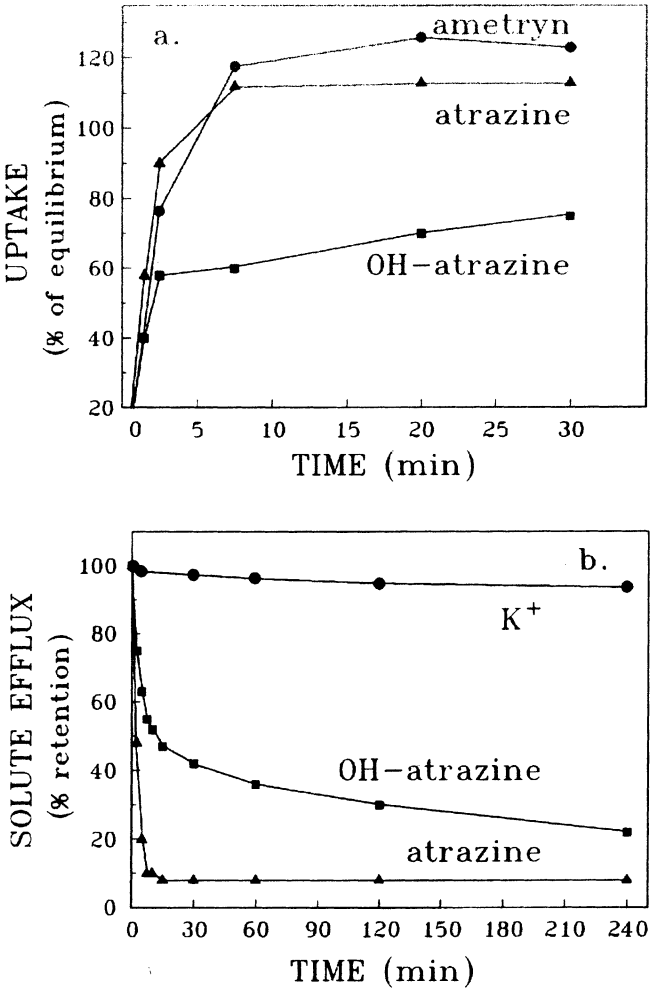


Figure 2. Time course of: a) ametryn, atrazine, and hydroxyatrazine influx into and b) atrazine, hydroxyatrazine, and K^+ ($^{86}Rb^+$) efflux from oat root segments. From Balke and Price (1).

of tissue sections compared to individual protoplasts or cells results in slower penetration. Also, these results suggest the plasma membrane is not a barrier to lipophilic, neutral herbicide absorption.

Absorption of lipophilic, neutral herbicides by plant cells is related to their relative lipophilicities. Absorption of nonelectrolytes by *Chara* internodal cells was positively correlated with partition coefficients of the chemicals between olive oil and water (16). Initial rates of triazine (atrazine, atratone, hydroxyatrazine, simazine) and phenylurea (diuron) herbicide absorption correlated positively with their partition coefficients in olive oil and water or in *n*-dodecane and water (87). Diuron and atrazine partition coefficients were independent of pH (62, 87), suggesting the neutral, nonpolar form of atrazine predominates at physiological pH. Atrazine and ametryn were rapidly absorbed by excised corn (20) and oat (1) roots reaching equilibrium concentrations within 10 min in contrast to slower absorption of hydroxyatrazine, a less lipophilic molecule approximately the same size as atrazine (Figure 2a). Log K_{ow} values measured for ametryn, atrazine, and hydroxyatrazine were 2.9, 2.5, and 0.8, respectively (1). Diclofop-methyl uptake by bean and maize chloroplasts (41) and oat protoplasts (98) was 8 and 10 times greater than the less lipophilic molecule diclofop, respectively. These results suggest that although the plasma membrane is not a barrier to triazine or diclofop-methyl absorption, the membrane is a barrier to less lipophilic molecules such as hydroxyatrazine and diclofop, respectively. In whole plant studies, root uptake of nonionized chemicals was determined primarily by the lipophilicity of the chemicals and was largely independent of plant species (8).

Further evidence that cell membranes do not present an appreciable barrier to herbicide molecules is the ability of these molecules to efflux out of cells or tissues that contain the herbicide. Norflurazon efflux from corn and sicklepod root segments occurred in two phases: a rapid phase where about 70% of the radiolabel was lost within 30 min, and a slow phase, through 6 h, where an additional 10 to 20% diffused out of the tissues. Atrazine diffused rapidly out of excised corn (20, 75), foxtail (*Setaria faberi* Herrm. # SETFA) (66), and oat (1, 75) roots, potato tuber discs (68) and slices (75), and soybean [*Glycine max* (L.) Merrill] (59) and velvetleaf (73, 74, 75) roots. For all these studies, 50 to 90% of the initial atrazine present in tissues effluxed within 30 min. When atrazine efflux from oat roots was compared to efflux of the less lipophilic molecules, hydroxyatrazine and the mineral ion K^+ (as tested using $^{86}Rb^+$), atrazine efflux was nearly complete after 30 min followed by hydroxyatrazine and K^+ with 60% and less than 1% eluted after 30 min, respectively (Figure 2b). The pools from where the herbicide eluted could not be identified; these pools, as proposed for mineral ions using compartmental analysis of efflux data, include the free space, cytoplasm, and vacuole of the tissue (1, 20). These results support further that cell membranes are not barriers to either influx or efflux of lipophilic, neutral herbicides.

Additional evidence supports absorption of lipophilic, neutral herbicides by simple diffusion and not by an energy-dependent process such as carrier-mediated transport. A linear increase in herbicide absorption with increasing external herbicide concentrations indicates absorption is not saturating at higher concentrations and absorption is not carrier mediated. Absorption of amitrole (49, 88), atrazine (54, 62, 73), diclofop-methyl (98), metribuzin (13), monuron (26), and simazine (86) was linear with external concentrations of each herbicide, suggesting the mechanism by which these herbicides enter plant cells is simple diffusion. Temperature coefficient (Q_{10}) values for amitrole, atrazine, chlorpropham, EPTC, linuron, and napropamide ranged from 1.1 to 1.85 (3, 49, 59, 73, 88). These values are similar to Q_{10} values near 1 for physical or passive processes while coefficients of 2 or greater indicate metabolic processes are involved (63). Amitrole (88), oryzalin (99), and triazine (66) absorption by plant tissues was insensitive to metabolic inhibitors, suggesting absorption did not require energy; however, evidence for amitrole (88) and atrazine (73) was less conclusive, suggesting some energy requirement.

Thus, uptake of many lipophilic, neutral herbicides is a passive process where the neutral herbicide is able to freely diffuse across cell membranes, reaching equilibrium concentrations with the external solution or tissue apoplasm. Accumulation of these herbicides to concentrations greater than the external concentrations are discussed below.

Lipophilic, ionic herbicides. Lipophilic, ionic molecules are either weak acids or weak bases. In an aqueous solution, neutral weak acids are in equilibrium with their conjugate base which is charged (equation 1); neutral weak bases are in equilibrium with their conjugate acid which is charged (equation 2). The relative concentrations of the hydrophobic, undissociated acid molecules (HA)[4] or the more polar anions (A^-)[4] depend on solution pH and strength of the acid as described by the Henderson-Hasselbach equation (equation 3). Ability of these molecules to be either neutral or charged greatly affects rates of movement across plant membranes as well as accumulation concentrations.

$$R\text{–}COOH \rightleftharpoons R\text{–}COO^- + H^+ \quad (1)$$

$$R\text{–}NH_2 + H_2O \rightleftharpoons R\text{–}NH_3^+ + OH^- \quad (2)$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad (3)$$

Mechanistic evidence for passive absorption of lipophilic, ionic herbicides and their accumulation by ion trapping as described below exists only for weak acid herbicides such as bentazon (94), 2,4-D (19, 81, 87), clopyralid and chlorsulfuron (24), imidazolinones (34, 77), and sethoxydim (96) (Figure 3). The principles of passive absorption described in the previous section for lipophilic, neutral herbicides apply also to weak acid herbicides which passively diffuse into plant cells. However, in contrast to lipophilic, neutral herbicides, weak acid herbicide absorption saturates over time with the herbicide reaching cellular concentrations greater than external concentrations (19, 24, 34, 94, 96). Weak acid herbicide accumulation in plant cells to concentrations greater than external concentrations can be reduced by metabolic inhibitors or anoxia and is enhanced by acidic pH external to the cell, suggesting accumulation is energy dependent and is mediated by ion trapping. Higher concentrations of the undissociated herbicide exist at acidic rather than neutral pH (equation 3). The undissociated acid diffuses across the plasma membrane because cell membranes are more permeable to undissociated, neutral molecules compared to dissociated, charged molecules (76). Once in the alkaline compartments of the cell, the acid dissociates creating a concentration gradient for further influx of the acid which results in herbicide accumulation as the anion (Figure 1b). Cytoplasm of the plant cell is maintained alkaline by the continual removal of H^+ to the vacuole or to the apoplast by the proton-ATPases (H^+-ATPases)[4] located on the plasma membrane and tonoplast of the plant cell (10); therefore, accumulation of weak acid herbicides requires metabolic energy.

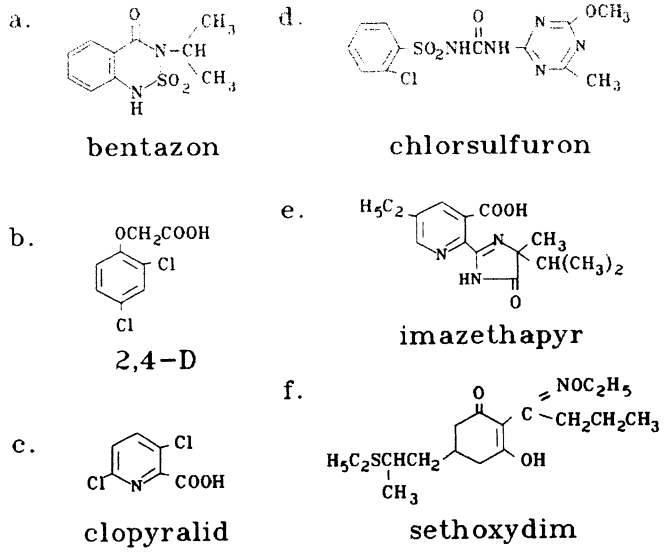


Figure 3. Structures of weak acid herbicides accumulated in plant cells by ion trapping.

Weak acid herbicides are absorbed by simple diffusion. Absence of saturation kinetics indicates that weak acid herbicide transport into plant cells is not carrier mediated and is via nonfacilitated diffusion. Absorption of several weak acid herbicides by various plant species and tissues was linearly related to external herbicide concentration including bentazon (Figure 4), bromoxynil (33), chloramben (95), clopyralid (24), chlorsulfuron (24), 2,4-D (44, 100), imazapyr (77), imazaquin (77), imazethapyr (34, 77), ioxynil (62), dinoseb (62), maleic hydrazide (32), MCPA (31), picloram (21, 43, 97), sethoxydim (91, 96), and the sulfonylureas bensulfuron methyl, chlorimuron ethyl,

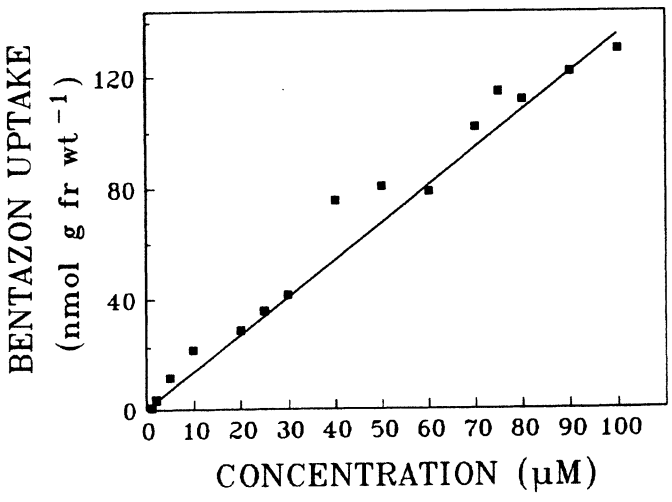


Figure 4. Concentration dependence of bentazon uptake by velvetleaf cells. From Sterling et al. (94).

chlorsulfuron, thifensulfuron methyl, metsulfuron methyl, sulfometuron methyl (65), and chlorimuron ethyl ester (61).

Nonfacilitated movement of weak acid herbicides across plant cell membranes is also supported by the ability of the herbicides to diffuse out of cells into solutions not containing the herbicide. Herbicide efflux from tissues loaded with bentazon (94), bromoxynil (33), 2,4-D (44, 100), clopyralid (24), chlorimuron ethyl ester (61), chlorsulfuron (24, 55), the imidazolinones (77), and picloram (43, 60, 97) was between 75 and 95% of initial herbicide concentrations and was complete within 30 to 90 min. Two phases of efflux were identified for imazaquin elution, suggesting initial elution was from cell wall free space followed by elution from the cytoplasm and vacuole (77). These results suggest the accumulation of weak acid herbicides is not due to irreversible binding and that neither the plasma membrane nor the tonoplast are effective barriers to weak acid herbicide transport. In contrast, frozen potato tuber slices released 2,4-D freely after transfer to a solution not containing 2,4-D; however, nonfrozen tissue released only 3% after 30 min, suggesting the cell membranes were barriers to 2,4-D movement (68). Similarly, efflux from intact compared to freeze-thawed tissue loaded with imazaquin was more rapid, suggesting that imazaquin is retained in the tissue by an intact plasma membrane (39, 77). Overall, rates of efflux for weak acid herbicides were slower than efflux of lipophilic, neutral herbicides discussed above and may be due to lower membrane permeability to weak acid herbicides compared to lipophilic, neutral herbicides.

The feature controlling the transport of weak acid herbicides across membranes is their lipid-soluble, weakly acidic nature. Uptake of 12 imidazolinone analogs by sunflower (*Helianthus annuus* L.) and corn roots was highly correlated to their lipophilicity (51, 52). In addition, imidazolinone analogs required their carboxylic acid group for activity although the acidity and lipophilicity of each was more important for transport than for enzyme inhibition at the site of action (48). Similarly, rates of sulfonylurea analog uptake by velvetleaf cells were governed primarily by the lipophilicity (K_{ow}) of each analog (65). Chlorsulfuron (K_{ow} = 1.3) absorption by excised pea roots was faster than clopyralid (K_{ow} = 0.0018) absorption (24). Lipophilicity (K_{ow}) values are dependent on pH with greater K_{ow} values at lower pH or at pH closer to the pK_a of each herbicide as demonstrated for 2,4-D (87), dinoseb (62), bromoxynil (33), and sethoxydim (96). In addition, rates of imidazolinone absorption were higher at low pH compared to high pH and appeared related to the larger K_{ow} values at lower pH compared to higher pH (Table 2). Membranes are more permeable to the lipophilic, undissociated acid molecules, which predominate at low pH (equation 3) compared to their more polar anions (9). Raven (76) estimated that the permeability of an algal (*Hydrodictyon africanum*) cell membrane to nonionized indole-3-acetic acid (IAA) is approximately 10^{-3} m s^{-1}, about a thousand times greater than the permeability of its anion. Therefore, the undissociated acid diffuses across the plasma membrane because cell membranes are more permeable to undissociated, neutral molecules compared to dissociated, charged molecules. Alternatively, lipophilicity may not be the sole factor determining the behavior of weak acids (9). Although ion trapping explains accumulation of the imidazolinones, this mechanism does not fully explain why rates of uptake of imidazolinone analogs vary greatly with small changes in chemical structure (39). Therefore, other factors besides K_{ow} and pK_a also may regulate uptake of weak acid herbicides.

Table 2. Effect of pH on imidazolinone K_{ow} values and uptake rates. From Reider Van Ellis and Shaner (77).

Herbicide	K_{ow} pH 4	K_{ow} pH 7	Imidazolinone uptake pH 4	Imidazolinone uptake pH 7
			nmol · g dw^{-1} · h^{-1}	
Imazapyr	0.1	0.004	13	3
Imazethapyr	1.4	0.017	24	3
Imazaquin	7.7	0.038	45	3

Although experimental evidence supports simple diffusion as the mechanism of weak acid herbicide transport across cell membranes, cellular metabolism is necessary for their accumulation to concentrations greater than external concentrations. The respiratory inhibitor potassium cyanide (KCN) and low oxygen, as induced by bubbling nitrogen gas (N_2) through the uptake medium, reduced bentazon absorption by velvetleaf cells (94) and chlorimuron ethyl ester absorption by excised velvetleaf roots (61), suggesting that cellular metabolic energy enhanced uptake of weak acid herbicides. The addition of ATP increased sethoxydim uptake by wheat (*Triticum aestivum* L.) leaves, further supporting the need for metabolic energy (Table 3). The proton ionophore, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)[4], reduced absorption of bentazon (94), bromoxynil (33), MCPA (31), maleic hydrazide (32), imidazolinones (77), and sethoxydim (Table 3) indicating that proton gradients across membranes are involved in weak acid herbicide uptake. The proton ionophore, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP)[4], also reduced absorption of imidazolinones (39, 77). The uncoupler, dinitrophenol (DNP)[4], reduced absorption of MCPA (31), maleic hydrazide (32), picloram (43), and

Table 3. Influence of various compounds on sethoxydim uptake by wheat leaf sections. From Couderchet and Retzlaff (17).

Substance added	Sethoxydim uptake
	(% of control)
ATP (0.5 mM)	121.8
ATP (1 mM)	133.3
ATP (2 mM)	234.7
ATP (4 mM)	254.6
ATP (7.6 mM)	255.0
Fusicoccin (0.5 mM)	117.5
CCCP (20 µM)	75.3
Vanadate (100 µM)	34.7
DES (100 µM)	52.0

several imidazolinones (51, 77) probably by disrupting the hydrogen ion concentration gradient and reducing membrane integrity (51). Similarly, imidazolinone uptake was inhibited with sodium azide, sodium cyanide, and 3-(3,4-dichlorophenyl)-1-1-dimethylurea (DCMU)[4] (77); and picloram uptake was inhibited by sodium azide and sodium arsenate (43). In contrast, KCN (100) and DNP (44) did not affect 2,4-D uptake nor did sodium azide affect picloram absorption (60). Uptake of 2,4-D and 2,4,5-T was independent of available energy as tested by addition of CCCP, *N,N'*-dicyclohexylcarbodiimide (DCCD)[4], and lecithin (89). The potassium ionophore, valinomycin, did not affect bentazon absorption (94), suggesting that potassium gradients across cell membranes were not necessary for bentazon uptake.

Energy-dependent absorption is most likely due to the cell expending metabolic energy to maintain a pH gradient across the plasma membrane and the tonoplast; membrane-bound ATPases, which pump H^+ out of the cytosol, require ATP as their substrate (10). Ammonium sulfate-stimulated imazethapyr uptake was sensitive to the plasma membrane-ATPase (PM-ATPase) inhibitors sodium orthovanadate and diethylstilbestrol (DES), to the uncoupler CCCP, and to metabolic inhibitors sodium azide and N_2, suggesting that uptake is dependent on ATP production and a functioning PM-ATPase (34). Similarly, sethoxydim absorption was reduced by vanadate and DES and increased by the PM-ATPase stimulator, fusicoccin (Table 3). In addition, bentazon-inhibited uptake of sethoxydim was probably due to bentazon inhibition of PM-ATPase activity from wheat leaves (18). Overall, cellular metabolism and pH gradients appear necessary for the absorption of weak acid herbicides.

Energy-dependent uptake of weak acid herbicides is supported further by pH-dependent absorption. In addition, pH dependent absorption supports ion trapping as the mechanism for weak acid herbicide accumulation. Because higher concentrations of the undissociated herbicide exist at acidic rather than neutral pH (equation 3), absorption of weak acid herbicides by plant cells and tissues was greater at the pH close to the pK_a of each herbicide such as bentazon (pK_a = 3.45) (50, 78, 94) (Figure 5); bromoxynil (pK_a = 4.10) (33); 2,4-D (pK_a = 2.6 to 3.3) (19, 45, 81, 87, 102); 2,4,5-T (pK_a = 2.6 to 3.1) (5, 90); picloram (pK_a = 3.58) (4, 97); clopyralid (pK_a = 2.33) (24); chlorsulfuron (pK_a = 3.60) (24, 55) and other sulfonylureas (65); MCPA (pK_a = 2.9 to 3.3) (31, 90); haloxyfop (pK_a = 4.76) (42); imidazolinones (pK_a = 3.8 to 4.0) (34, 39, 51, 77); ioxynil (pK_a = 3.96) (62); maleic hydrazide (pK_a = 5.65) (32); and sethoxydim (pK_a = 4.60) (96). In contrast, decreased absorption of 2,4-D (53), picloram (4), or sethoxydim (96) with increasing pH was not totally explained by the concentration of undissociated molecules at changing pH. Possibly, the membrane is permeable to the anionic form also (Figure 1c) which may explain greater accumulation than expected. In addition, the medium pH may be greater than the pH at the uptake site, such as the relatively acidic cell wall region, where more herbicide would be present in its nondissociated form.

Models for ion trapping. Models for absorption and distribution of the weak acid hormone IAA (29, 76, 83) also predict herbicide concentrations in plant cells and plant organelles. Two models calculate the expected equilibrium concentrations of a weak acid in plant cells by either using pH gradients (equation 4) or by using pH and electrical gradients present across cell membranes (equation 5). Equation 4 is essentially two Henderson-Hasselbach equations combined for calculating the pH-dependent distribution of a weak acid in internal and external cellular compartments (83). For one cellular compartment (i) surrounded by a membrane permeable only to the undissociated acid (Figure 1b) and an external medium (o), the concentration expected for a herbicide with a dissociation constant (K) can be calculated using (83):

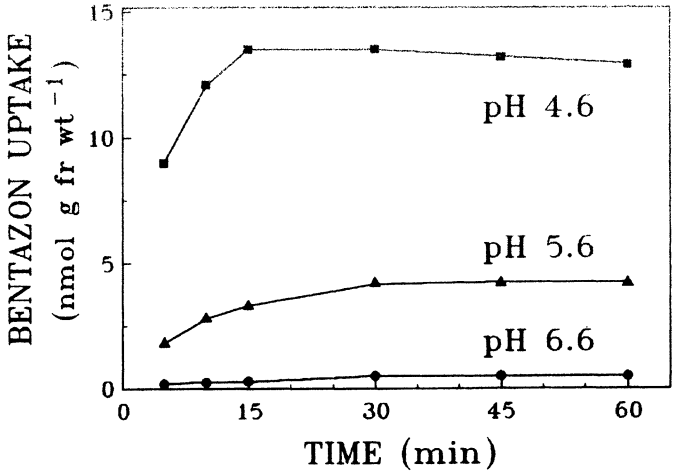


Figure 5. pH-dependent uptake of bentazon by velvetleaf cells over time, From Sterling et al. (94).

$$\frac{[\text{total herbicide}]_i}{[\text{total herbicide}]_o} = \frac{([H^+]_i + K)[H^+]_o}{([H^+]_o + K)[H^+]_i} \quad (4)$$

To support the ion-trapping hypothesis of weak acid herbicide accumulation in plant cells, the predicted concentrations of bentazon in suspension-cultured velvetleaf cells (94) and 2,4-D in corn root protoplasts (19) were calculated. Predicted concentrations of each herbicide in the alkaline cytoplasm were much greater than those predicted in the acidic vacuole (Table 4). Total cellular concentrations predicted with equation 4, assuming 90% of the cell volume was occupied by the vacuole, were about threefold greater than observed values for each herbicide (Table 5). Therefore, the predicted concentrations for bentazon and 2,4-D in plant cells were relatively close to those measured in plant cells, suggesting ion trapping as defined by equation 4 was a valid model for weak acid herbicide accumulation in plant cells. Considerations that may explain the greater predicted compared to observed equilibrium concentrations are that herbicide elution during tissue rinsing reduced internal herbicide concentrations or the membrane was permeable to the ionic form of each herbicide.

Table 4. Calculated equilibrium concentrations of weak acid herbicides in cytoplasm, vacuole, and total cell based on either the Henderson-Hasselbach or Raven (76) equations as modified by Rubery (83).[a]

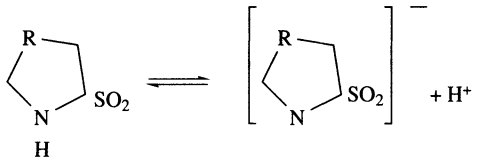
Herbicide	pK_a[b]	External medium[c]		Henderson-Hasselbach			Raven		
				Cytoplasm	Vacuole	Total	Cytoplasm	Vacuole	Total
		pH	μM herbicide	μM			μM		
Clopyralid	2.33	5.4	1.0	148	2	16	11	3	3
2,4-D	2.96	4.5	0.8	797	8	87	86	61	64
Bentazon	3.45	5.6	1.0	79	1	9	24	1	3
Chlorsulfuron	3.60	5.4	1.0	127	1	14	50	1	7
Imazethapyr	3.90	5.8	10.0	484	5	53	264	6	32
Sethoxydim	4.60	5.7	200.0	5857	66	645	4843	2	486

[a]Assumptions: external pH and herbicide concentration were constant; cytoplasm and vacuole occupy 10 and 90% of total cell volume, respectively; cytoplasmic and vacuolar pH values are 7.5 and 5.5, respectively; plasma membrane and tonoplast membrane potential values are −120 and −90 mV, respectively; 25 C; P_{AH} and P_{A^-} are 10^{-2} and 10^{-5} m s^{-1}, respectively, based on values from Raven (76) for P_{IAAH} and P_{IAA^-} for the plasmalemma of *Hydrodictyon africanum.*

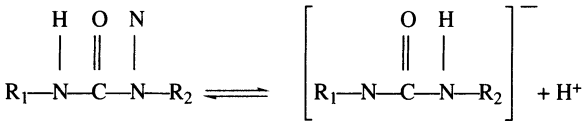
[b]Clopyralid (24); 2,4-D (19); bentazon (94); chlorsulfuron (24); imazethapyr (34); sethoxydim (96); pK_a values are derived from the reaction (refer to Figure 3 for full structures):

$$R\text{—}COOH \rightleftharpoons R\text{—}COO^- + H^+$$

for 2,4-D, clopyralid, and imazethapyr;



for bentazon;



for chlorsulfuron; and

$$R\text{—}OH \rightleftharpoons R\text{—}O^- + H^+$$

for sethoxydim.

[c]Clopyralid (24); 2,4-D (19); bentazon (94); chlorsulfuron (24); imazethapyr (34); sethoxydim (96).

The amount of herbicide accumulating for a given pH gradient depends on whether the undissociated acid is the only permeant species or whether the anion can cross the membrane also (Figure 1c). Net fluxes of the acid and the anion are combined in equation 5 which was established by Raven (76) and modified by Rubery (83):

$$\frac{[\text{total herbicide}]_i}{[\text{total herbicide}]_o} = \frac{([H^+]_i + K)\{P_{AH}[H^+]_o RT(1 - e^{-FE/RT}) + P_{A^-}KFE\}}{([H^+]_o + K)\{P_{AH}[H^+]_i RT(1 - e^{-FE/RT}) + P_{A^-}KFEe^{-FE/RT}\}} \quad (5)$$

where P_{AH} and P_{A^-} are the permeabilities of the undissociated and dissociated species, respectively, R is the universal gas constant, T is the temperature in degrees K, F is the Faraday constant, and E is the membrane potential ($E_i - E_o$). If the membrane is permeable to the anion, the total herbicide accumulation concentration at equilibrium would be lower compared to the equilibrium concentration calculated with equation 4 when the anion is not considered. The alkaline cytoplasm creates an anion concentration gradient with a higher anion concentration inside compared to the external solution. This concentration gradient drives anion efflux from the plant cell. In addition, the electrical potential across the membrane, negative inside, drives the anion down the electrical gradient out of the cell. Application of equation 5 to bentazon and 2,4-D accumulation in plant cells supports the hypothesis that membrane permeability to the anion is important to the ion-trapping model. For each herbicide, predicted concentrations in the cytoplasm were much less using equation 5 compared to equation 4, resulting in a lower predicted concentration for the total cell (Table 4). These calculations suggest the membrane is permeable to anionic species of bentazon and 2,4-D and the diffusion of anions down their electrochemical gradients reduces accumulation concentrations at equilibrium. Total cellular concentrations predicted with equation 5, assuming 90% of the cell volume was occupied by

Table 5. Comparison of observed equilibrium concentrations in cells to predict equilibrium concentrations in cells for various weak acid herbicides.

Herbicide	Observed equilibrium concentrations[a]	Predicted equilibrium concentrations: Henderson-Hasselbach (equation 4)	Predicted equilibrium concentrations: Raven (equation 5)	Henderson-Hasselbach (equation 4)	Raven (equation 5)
	μM			predicted/observed	
Clopyralid	3.5	16.1	3.3	4.6	0.9
2,4-D	32.1	86.9	63.5	2.7	2.0
Bentazon	3.0	8.6	3.2	2.9	1.1
Chlorsulfuron	4.5	13.8	7.1	3.1	1.6
Imazethapyr	9.8	53.0	32.0	5.4	3.3
Sethoxydim	200.0	645.0	486.0	3.2	2.4

[a]Clopyralid (24); 2,4-D (19); bentazon (94); chlorsulfuron (24); imazethapyr (34); sethoxydim (96).

the vacuole and the external pH and herbicide concentration were constant, were about one- to twofold greater than observed values for bentazon and 2,4-D, respectively (Table 5). In addition, equation 5 compared to equation 4 was found also to be a better predictor for 2,4-D concentrations in wheat leaf protoplasts (53).

Application of both models to other weak acid herbicides further supports ion trapping as the mechanism for weak acid herbicide accumulation and emphasizes the role of membrane permeability to the anion in influencing accumulation concentrations. Predicted equilibrium concentrations for clopyralid, 2,4-D, bentazon, chlorsulfuron, imazethapyr, and sethoxydim were calculated using the particular experimental conditions from respective references (Table 4). As expected, both equations predicted higher concentrations of each herbicide in the alkaline cytosol compared to the acidic vacuole, and equation 5 compared to equation 4 predicted lower concentrations for accumulation of each weak acid herbicide in the total cell. These predicted accumulation concentrations for each herbicide were compared to the equilibrium concentrations measured for each herbicide under the experimental conditions outlined in each reference (Table 5). Ratios for predicted to observed equilibrium concentrations ranged from 2.7 to 5.4 with equation 4, using pH gradients to predict concentrations. In contrast, with equation 5, using pH and electrical potentials of the cell and anion permeability, predicted values for accumulation were much closer to observed values, with ratios ranging from 1.0 to 3.3. Therefore, ion trapping as defined by equation 5 is a model that closely predicts equilibrium concentrations for weak acid herbicides in plant cells by considering both the pH and electrical gradients present across cell membranes as well as membrane permeability to the anionic species. Discrepancies between observed and predicted equilibrium concentrations may result because assumptions made for each plant tissue were identical. In addition, predicted equilibrium concentrations may be greater than observed if the weak acids released protons in the cytoplasm upon ionization, reducing the cytoplasmic pH and therefore the equilibrium concentration; an unstirred boundary layer surrounding plant cells might reduce the availability of acid for absorption (35); or tissue rinsing may reduce the actual concentrations accumulating in the plant cells.

An exception to ion trapping. Passive absorption of the ionic herbicide glyphosate does not involve ion trapping. Glyphosate exists as both monovalent and divalent anions at physiological pH because its pK_a values range from 2.2 to 2.6 (pK_1), 5.5 to 5.9 (pK_2), and 10.1 to 10.9 (pK_3) (57) (Figure 6). Glyphosate absorption by sugarbeet (*Beta vulgaris* L.) (30) and broad bean (*Vicia faba* L.) (27) leaf discs and by wheat leaf protoplasts (53) showed a linear increase in absorption with increasing external glyphosate concentrations, suggesting that glyphosate was absorbed by passive, nonfacilitated diffusion. Passive diffusion was also supported by efflux kinetics where two phases, apoplastic efflux and cellular efflux, were identified (27, 30), Q_{10} values were between 1.3 to 1.4 (27), and glyphosate absorption into these tissues was insensitive to addition of amino acids or sugars (27) and to phosphate, glyphosine, or glyphosate (53)

The plasma membrane apparently presents a barrier to glyphosate absorption and accumulation as a weak acid. Glyphosate absorption by isolated lambsquarters plasma membrane vesicles was very low relative to absorption of the amino acid glutamic acid and to other herbicides, atrazine and bentazon

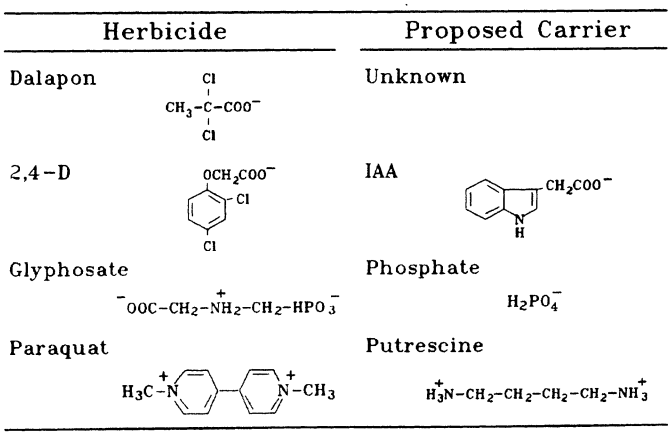


Figure 6. Structures of herbicides absorbed by active transport processes and their proposed carriers.

(80). Glyphosate absorbed by broad bean leaf discs did not freely efflux (27). Internal glyphosate concentrations were less than external concentrations in sugarbeet leaf discs (30), bean mesophyll cells (7), wheat leaf protoplasts (53), and equal to external concentrations in hemp dogbane leaf cells (79), indicating glyphosate was not accumulating in the cytoplasm of these cells. In addition, glyphosate absorption by leaf discs was independent of solution pH from pH 3.5 to 9.7 (27, 30), suggesting weak acid accumulation of glyphosate was not occurring. Although glyphosate absorption by wheat leaf protoplasts was greatest at pH 4.5 and least at pH 5.5, absorption gradually increased as pH increased to 8.5 which is inconsistent with ion trapping, because as pH increases the dianionic form of glyphosate increases (53). Low permeability of the plasma membrane to monovalent and divalent anions (30) (Figure 6), the predominant forms of glyphosate at physiological pH, reduces rates of diffusion across the membrane's lipid bilayer. In addition, the negative electrical potential across the plasma membranes most likely also reduces absorption by repelling glyphosate, reducing its entry into the cytoplasm for accumulation as a weak acid.

Accumulation. In addition to ion trapping as discussed in the previous section, herbicides may accumulate in plant cells to concentrations greater than equilibrium concentrations by other mechanisms including conversion to nonphytotoxic metabolites, binding to cellular constituents, or partitioning into lipids. In contrast to ion trapping, herbicides accumulated by these mechanisms are probably not available for herbicidal activity, particularly when structural changes have occurred as in the case of herbicide metabolism.

Continued absorption of a herbicide to concentrations greater than equilibrium concentrations can be explained, in many cases, by herbicide metabolism. Metabolism acts as a sink by maintaining a concentration gradient for continued absorption over time of the parent herbicide. Herbicide metabolites more polar than their parent molecule accumulate in the cell due to reduced lipophilicity and reduced ability to traverse the membrane while the parent herbicide remains below its equilibrium concentration (1, 93). Atrazine was metabolized to hydroxyatrazine by excised corn roots (75), bentazon was hydroxylated then glucosylated in soybean suspension-cultured cells (93), chloramben was metabolized by velvetleaf and morningglory [*Ipomoea hederecea* (L.) Jacq. # IPOHE] roots (95) and chlortoluron was metabolized by cotton and Italian ryegrass (*Lolium multiflorum* Lam. # LOLMU) suspension-cultured cells (67). These more polar metabolites do not freely diffuse across plasma membranes and therefore remain within the cell (1, a3). Because herbicide metabolism is the primary mechanism of selectivity for most herbicides (38), accumulation in plant cells of herbicides as nonphytotoxic metabolites may be very common.

Alternatively, cellular accumulation of herbicides may be due to binding to cellular constituents such as cell walls or lignin or partitioning into plant lipids. Although the cellular constituents with which herbicides associate are rarely identified, herbicides can accumulate to concentrations greater than equilibrium concentrations by associating irreversibly with these tissues. Slightly greater than equilibrium concentrations of chlorsulfuron and clopyralid were associated with frozen pea root tissue indicating that these herbicides were binding to tissue (24). Excised cotton roots retained up to 60% of absorbed norflurazon after efflux of root tissues into solution not containing norflurazon, suggesting the herbicide was binding to cellular constituents (56). Amitrole was bound to cellular constituents of tobacco (*Nicotiana tabacum* L.) cell cultures but amitrole accumulation by tobacco cell protoplasts suggested that cell walls were not involved (88). Similarly, neither atrazine, dinoseb, nor diuron were adsorbed to isolated cell walls from unicellular microalgae (62). Isolated lignin fractions from 2,4-D-treated soybean and wheat suspension cells contained covalently bound 2,4-D (85). Fluometuron adsorbed more strongly to lignin from pepper (*Capsicum annuum* L.) and cotton stems than to cellulose or bovine serum albumin, suggesting lignin is a sink for herbicide accumulation (2).

Accumulation or binding of herbicides to cellular constituents may involve energy-dependent processes. Napropamide accumulation by corn root tissue to a nonexchangable fraction was nonsaturable, sensitive to anaerobic conditions, and had a Q_{10} value of 1.8, suggesting to the authors that cellular metabolism may be involved in maintenance of napropamide binding sites (3). Similarly, maleic hydrazide binding to corn and pea seedling root material was blocked by metabolic inhibitors, suggesting binding of maleic hydrazide with cell wall fragments was an active process (64). Atrazine accumulation in velvetleaf roots continued through 24 h, was inhibited by DNP, and had a Q_{10} value of 2.5, suggesting velvetleaf roots accumulated atrazine via metabolically dependent processes over long periods of time (74). However, a large temperature coefficient for solute uptake does not necessarily indicate an active process (63).

In addition to association with cell wall material, several investigators suggest herbicides accumulate by partitioning into membrane lipids. Freeze-thaw and efflux experiments suggested bromoxynil was unspecifically bound to cellular constituents such as cutin, lignin, or membrane lipids of *Sinapis* cotyledons and *Cyclamen* conducting tissues (33). Accumulation of atrazine in corn root protoplasts was attributed to the partitioning of atrazine into cellular membranes (19) and into lipid membranes of wheat leaf protoplasts (54). Only a small portion of atrazine accumulation could be attributed to binding at its active site in photosystem II (54). Alternatively, Orwick et al. (66) suggested that the small amount of atrazine remaining in *Setaria* root sections after efflux could be bound as peptide conjugates. Oryzalin accumulation in corn root apices may result from partitioning into membrane lipids (99). Also, metribuzin accumulation in downy brome (*Bromus tectorum* L. # BROTE) and jointed goatgrass (*Aegilops cylindrica* Host # AEGCY) protoplasts indicated the herbicide had partitioned into and/or bound to membranes (13). Therefore, evidence exists for accumulation of herbicides to concentrations greater than equilibrium concentrations due to partitioning into cellular constituents of plant cells; however, the specific interactions between herbicide mole-

cules and cellular constituents are unknown (69). In addition, association of a herbicide with cellular constituents makes availability for phytotoxic action unlikely.

ACTIVE ABSORPTION

Evidence for active transport of herbicides across plant membranes is limited to the herbicides dalapon (72), 2,4-D (26, 45, 46, 58, 81), glyphosate (14, 22), and paraquat (37) (Figure 6). Active transport requires energy from metabolic processes to move a molecule across a membrane against its electrochemical potential and usually involves a proteinaceous carrier located on the membrane which transports the molecule into the plant cell (63) (Figure 1d). Experimental criteria supporting active transport are continual, biphasic absorption over time, accumulation against a concentration gradient, Q_{10} values greater than 2, saturation kinetics at increasing external herbicide concentrations, and reduced absorption in the presence of metabolic inhibitors or compounds of similar structure. In most cases, the saturable component of active transport is in addition to passive diffusion of the herbicide into the plant cell.

Active absorption of dalapon by *Lemna minor* L. # LEMNI fronds has been demonstrated (72). Dalapon absorption occurred in two phases: Phase 1 absorption, complete after 30 min, was related linearly to external dalapon concentrations, and Q_{10} values were between 1.2 and 1.4 supporting passive diffusion, while phase 2 absorption through 4 h was sensitive to metabolic inhibitors, rates of uptake were curvilinear with increasing concentration, and Q_{10} values were higher than those in phase 1, suggesting that absorption was via active transport. Pyruvic acid, a molecule of structure similar to dalapon, reduced dalapon absorption but only when present at 300-fold greater concentrations, suggesting absorption of dalapon did not involve pyruvate (Figure 6). Absorption was reduced by cupric nitrate and phenylmercuric nitrate, molecules which react strongly with thiol groups; therefore, dalapon uptake was hypothesized to be mediated by metabolic processes involving thiol groups.

Carrier-mediated transport of the 2,4-D anion has been proposed in addition to the simple, nonionic diffusion of 2,4-D acid discussed above. Absorption of 2,4-D by excised barley roots (26) and suspension-cultured crown gall (*Parthenocissus tricuspidata* Planch.) cells (81, 82) was saturable at increasing external 2,4-D concentrations, suggesting carrier-mediated absorption. In addition, 2,4-D absorption by barley roots was inhibited by metabolic inhibitors and anoxia; however, results are difficult to interpret from this study because pH was not controlled or measured (26). The proposed carrier-mediated component of 2,4-D absorption by crown gall cells (81, 82) and corn protoplasts (46) was pH dependent, consistent with cotransport of the 2,4-D anion with protons (82). Also, 2,4-D absorption was inhibited by the antiauxin *p*-chlorophenoxyisobutyric acid (PCIB)[4] (46) and the endogenous plant hormone IAA, a molecule of structure similar to 2,4-D (Figure 6) (26, 81); these results suggest that 2,4-D and IAA are alternate substrates for the same carrier. Similarly, 2,4-D strongly inhibited absorption of IAA by microsomal vesicles from *Cucurbita pepo* L. hypocotyls and had

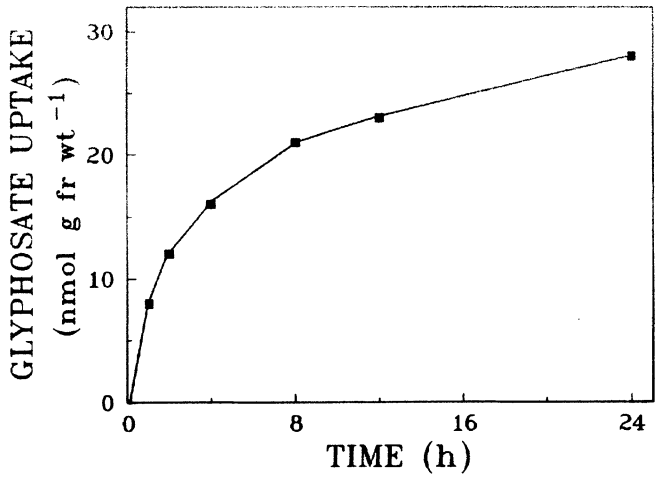


Figure 7. Time course of glyphosate uptake by potato cells. From Burton and Balke (15).

an affinity similar to IAA for the IAA uptake carrier, an auxin anion-H^+ symport (84). Efflux of 2,4-D also possessed a carrier-mediated component (81) although 2,4-D weakly influenced efflux of IAA from microsomal vesicles from *C. pepo* hypocotyls (84). Alternatively, absorption of 2,4-D by Jerusalem artichoke (*Helianthus tuberosus* L. # HELTU) and potato tuber slices was proportional to external 2,4-D concentrations from 10^{-8} to 10^{-4} M, but rates of 2,4-D uptake increased at higher 2,4-D concentrations, suggesting diffusion of the acid may be facilitated by a carrier that changes conformation at certain critical external 2,4-D concentrations (58). Therefore, absorption of 2,4-D may be mediated by an auxin anion-H^+ cotransporter (46, 81) or facilitated diffusion (58), although the carrier-mediated component of 2,4-D absorption is relatively small compared to absorption by simple diffusion of its undissociated acid (81).

In addition to simple, nonfacilitated diffusion, carrier-mediated glyphosate absorption has been characterized in plant cells (14, 22). Glyphosate uptake by bermudagrass [*Cynodon dactylon* (L.) Pers. # CYNDA] leaf tips (101), broad bean leaf protoplasts (22), and suspension-cultured cells of carrot (*Daucus carota* L.) (36), potato (14, 15), tobacco (36), and velvetleaf (57) was nonlinear over time with an initial rapid phase of uptake followed by a slower, continual phase (Figure 7). Internal glyphosate concentrations were less than external concentrations at pH 5.7 even after 72 h, suggesting the lipid bilayer was an effective barrier to diffusion of glyphosate into the cells (15). Glyphosate absorption was inhibited by metabolic inhibitors (14, 22) and Q_{10} value for absorption at 4 and 12 C was 2 (14), further suggesting active processes were involved in glyphosate absorption. Glyphosate uptake was dependent on external concentrations with saturable and nonsaturable phases of uptake resolved for absorption by leaf protoplasts (22) and suspension-cultured cells (14). The saturable component of glyphosate transport was competitively inhibited by phosphate in both systems (14, 22), supporting the involvement of a phosphate carrier which recog-

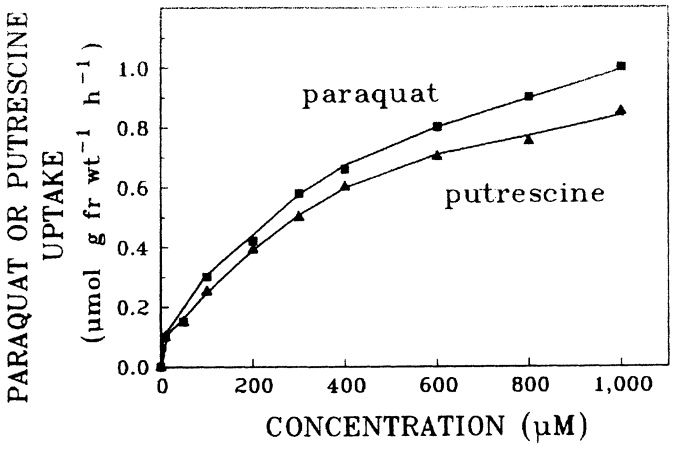


Figure 8. Concentration-dependent kinetics of paraquat and putrescine uptake by intact maize roots. From Hart et al. (37).

nizes the phosphate group on the glyphosate molecule (Figure 6). In addition, phosphonoformic acid, an inhibitor of phosphate transport in animal cells, inhibited glyphosate absorption by leaf protoplasts (22). Furthermore, glyphosate inhibited absorption of phosphate by isolated bean cells (7). Phosphate also inhibited glyphosate uptake by *Arthrobacter* sp. (70, 71) and *Pseudomonas* sp. (28). Therefore, glyphosate uptake by plant cells may be mediated by a phosphate transport system located on the plasma membrane.

Absorption of the divalent cation paraquat also involves active transport (37). Paraquat absorption by intact maize seedlings was dependent on concentration (Figure 8). Uptake kinetics of putrescine, a divalent polyamine with a charge distribution similar to paraquat (Figure 6) were similar to the kinetics of paraquat absorption. Kinetics for both paraquat and putrescine were resolved into a linear component, adsorption due to binding to cell walls, and a saturable component for absorption across the plasma membrane. The slope for putrescine absorption was lower than that for paraquat, suggesting the endogenous putrescine present in the cell wall was competing with exogenously applied putrescine. In addition to putrescine having nearly identical uptake kinetics to paraquat, putrescine competitively inhibited the saturable component of paraquat uptake. Similarly, the diamine cadaverine competitively inhibited paraquat and putrescine absorption although the tetravalent polyamine spermidine noncompetitively inhibited their absorption. Overall, these results suggest that influx of paraquat and putrescine is controlled by a similar system and the saturable nature of uptake suggests it is a protein-mediated process. In addition, this carrier system is most likely needed to transport the polar paraquat molecule across the hydrophobic lipid bilayer although paraquat is moving down its electrical potential to the negative interior of the plant cell.

In summary, examples for active transport of herbicides across plant membranes are limited. Evidence accumulated to date supports the transport of cationic herbicides such as paraquat and the transport of anionic herbicides dalapon, 2,4-D, and glyphosate. In each example, the herbicide appears to be transported by a carrier that recognizes herbicide molecules with structures similar to the endogenous molecules transported by that carrier. The overall importance of active transport to phytotoxicity is unknown.

SUMMARY

Plant cells absorb herbicides by either passive or active processes. Research examining mechanisms of herbicide absorption across plant membranes has been conducted primarily using excised tissues, cells, or protoplasts; therefore, research at the plasma membrane, tonoplast, or organelle levels is needed to further knowledge at the membrane level directly. Most herbicides are absorbed by plant cells via nonfacilitated diffusion. Neutral, lipophilic herbicides rapidly diffuse across the lipid bilayer of plant membranes down their concentration gradient to reach concentrations in the cell equal to concentrations in the external medium. Lipophilic, ionic herbicides or weak acids also passively diffuse into plant cells; however, many weak acid herbicides reach cellular concentrations greater than external concentrations due to ion trapping where the less lipophilic anion accumulates in alkaline compartments of the plant cell. However, rates of weak acid absorption are not always explained by the relative lipophilicities of analogs (9, 39) and further work is needed to resolve this anamoly. Weak acid herbicide accumulation by plant cells is energy dependent and can be predicted using a model that includes the pH and electrical gradients across plant cell membranes. Interestingly, weak acid herbicides outnumber weak base herbicides (11). One reason for this may be that weak acid herbicides accumulate in alkaline compartments of the plant cell such as the cytoplasm or organelles, resulting in increased phytotoxicity because the herbicide molecules would be concentrated near their potential sites of action. In contrast, weak base herbicides would accumulate, as do weak base dyes (10, 47), in acidic compartments such as the vacuole where few known sites of action exist. Alternatively, weak acid herbicides may be concentrated near enzymes which degrade them to nonphytotoxic compounds, enhancing tolerance or selectivity by rapid detoxication. Research determining the compartmentation of accumulation is needed and may better relate absorption and accumulation of weak acid herbicides with phytotoxicity. Herbicides can also accumulate in plant cells to concentrations greater than equilibrium concentrations by other mechanisms including metabolism, binding to cellular constituents or partitioning into lipids. In addition, increasing evidence supports active, carrier-mediated transport of herbicides into plant cells. The role these absorption processes play in phytotoxic action should be evaluated further.

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LITERATURE CITED

1. Balke, N. E. and T. P. Price. 1988. Relationship of lipophilicity to influx and efflux of triazine herbicides in oat roots. Pestic. Biochem. Physiol. 30:228–237.
2. Barak, E., A. Dinoor, and B. Jacoby. 1983. Adsorption of systemic fungicides and a herbicide by some components of plant tissues, in relation to some physicochemical properties of the pesticides. Pestic. Sci. 14:213–219.
3. Barrett, M. and F. M. Ashton. 1983. Napropamide fluxes in corn (*Zea mays*) root tissue. Weed Sci. 31:43–48.
4. Baur, J. R. and R. W. Bovey. 1970. The uptake of picloram by potato tuber tissue. Weed Sci. 18:22–24.
5. Baur, J. R., R. W. Bovey, R. D. Baker, and I. Riley. 1971. Absorption and penetration of picloram and 2,4,5-T into detached live oak leaves. Weed Sci. 19:138–141.
6. Boulware, M. A. and N. D. Camper. 1973. Sorption of some ^{14}C-herbicides by isolated plant cells and protoplasts. Weed Sci. 21:145–149.
7. Brecke, B. J. and W. B. Duke. 1980. Effect of glyphosate on intact bean plants (*Phaseolus vulgaris* L.) and isolated cells. Plant Physiol. 66:656–659.
8. Briggs, G. G., R. H. Bromilow, and A. A. Evans. 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pestic. Sci. 13:495–504.
9. Briggs, G. G., R. L. O. Rigitano, and R. H. Bromilow. 1987. Physico-chemical factors affecting uptake by roots and translocation to shoots of weak acids in barley. Pestic. Sci. 19:101–112.
10. Briskin, D. P. 1994. Membranes and transport systems in plants: An overview. Weed Sci. 42:255–262.
11. Bromilow, R. H. and K. Chamberlain. 1991. Pathways and mechanisms of transport of herbicides in plants. Pages 245–284 *in* R. C. Kirkwood, ed. Target Sites for Herbicide Action. Topics in Applied Chemistry (A. R. Katritzky and G. J. Sabongi, series eds.) Plenum Press, New York.
12. Bromilow, R. H., K. Chamberlain, and A. A. Evans. 1990. Physicochemical aspects of phloem translocation of herbicides. Weed Sci. 38:305–314.
13. Buman, R. A., D. R. Gealy, and E. P. Fuerst. 1992. Relationship between temperature and triazinone herbicide activity II. Herbicide absorption by protoplasts and herbicide inhibition of photosynthetic electron transport in thylakoids. Pestic. Biochem. Physiol. 43:29–36.
14. Burton, J. D. and N. E. Balke. 1987. Carrier-mediated transport of glyphosate into plant cells. WSSA Abstr. 27:69.
15. Burton, J. D. and N. E. Balke. 1988. Glyphosate uptake by suspension-cultured potato (*Solanum tuberosum* and *S. brevidens*) cells. Weed Sci. 36:146–153.
16. Collander, R. 1949. The permeability of plant protoplasts to small molecules. Physiol. Plant. 2:300–311.
17. Couderchet, M. and G. Retzlaff. 1990. Bentazone-sethoxydim antagonism: the role of ATP and plasma membrane ATPase. Pestic. Sci. 30:430–433.
18. Couderchet, M. and G. Retzlaff. 1991. The role of the plasma membrane ATPase in bentazone-sethoxydim antagonism. Pestic. Sci. 32:295–306.
19. Darmstadt, G. L., N. E. Balke, and L. E. Schrader. 1983. Use of corn root protoplasts in herbicide absorption studies. Pestic. Biochem. Physiol. 19:172–183.
20. Darmstadt, G. L., N. E. Balke, and T. P. Price. 1984. Triazine absorption by excised corn root tissue and isolated corn root protoplasts. Pestic. Biochem. Physiol. 21:10–21.
21. Davis, F. S., A. Villarreal, J. R. Baur, and I. S. Goldstein. 1972. Herbicidal concentrations of picloram in cell culture and leaf buds. Weed Sci. 20:185–188.
22. Denis, M.-H. and S. Delrot. 1993. Carrier-mediated uptake of glyphosate in broad bean (*Vicia faba*) via a phosphate transporter. Physiol. Plant. 87:569–575.
23. Devine, M. D. 1989. Phloem translocation of herbicides. Rev. Weed Sci. 4:191–213.
24. Devine, M. D., H. D. Bestman, and W. H. Vanden Born. 1987. Uptake and accumulation of the herbicides chlorsulfuron and clopyralid in excised pea root tissue. Plant Physiol. 85:82–86.
25. Devine, M. D. and W. H. Vanden Born. 1991. Absorption and transport in plants. Pages 119–140 *in* R. Grover and A. J. Cessna, eds. Environmental Chemistry of Herbicides. Vol. 2. CRC Press, Inc., Boca Raton, FL.
26. Donaldson, T. W., D. E. Bayer, and O. A. Leonard. 1973. Absorption of 2,4-dichlorophenoxyacetic acid and 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) by barley roots. Plant Physiol. 52:638–645.
27. El Ibaoui, H., S. Delrot, J. Besson, and J. Bonnemain. 1986. Uptake and release of a phloem-mobile (glyphosate) and of a non-phloem-mobile (iprodione) xenobiotic by broadbean leaf tissues. Physiol. Veg. 24:431–442.
28. Fitzgibbon, J. and H. D. Braymer. 1988. Phosphate starvation induces uptake of glyphosate by *Pseudomonas* sp. strain PG2982. Appl. Environ. Microbiol. 54:1886–1888.
29. Goldsmith, M. H. M. and T. H. Goldsmith. 1981. Quantitative predictions for the chemiosmotic uptake of auxin. Planta 153:25–33.
30. Gougler, J. A. and D. R. Geiger. 1981. Uptake and distribution of N-phosphonomethylglycine in sugar beet plants. Plant Physiol. 68:668–672.
31. Grimm, E., S. Neumann, and F. Jacob. 1983. Uptake of sucrose and xenobiotics into conducting tissue of cyclamen. Biochem. Physiol. Pflanz. 178:29–42.
32. Grimm, E., S. Neumann, and F. Jacob. 1985. Transport of xenobiotics in higher plants, II. Absorption of defenuron, carboxyphenylmethylurea, and maleic hydrazide by isolated conducting tissue of cyclamen. Biochem. Physiol. Pflanz. 180:383–392.
33. Grimm, E., S. Neumann, and B. Krug. 1987. Transport of xenobiotics in higher plants, IV. Ambimobility of the acidic compounds bromoxynil and pentachlorophenol. Biochem. Physiol. Pflanz. 182:323–332.
34. Gronwald, J. W., S. W. Jourdan, D. L. Wyse, D. A. Somers, and M. U. Magnusson. 1993. Effect of ammonium sulfate on absorption of imazethapyr by quackgrass (*Elytrigia repens*) and maize (*Zea mays*) cell suspension cultures. Weed Sci. 41:325–334.
35. Gutknecht, J. and A. Walter. 1980. Transport of auxin (indoleacetic acid) through lipid bilayer membranes. J. Membrane Biol. 56:65–72.
36. Haderlie, L. C., J. M. Widholm, and F. W. Slife. 1977. Effect of glyphosate on carrot and tobacco cells. Plant Physiol. 60:40–43.
37. Hart, J. J., J. M. DiTomaso, D. L. Linscott, and L. V. Kochian. 1992. Transport interactions between paraquat and polyamines in roots of intact maize seedlings. Plant Physiol. 99:1400–1405.
38. Hatzios, K. K. 1991 . Biotransformations of herbicides in higher plants. Pages 141–185 *in* R. Grover and A. J. Cessna, eds. Environmental Chemistry of Herbicides. Vol. II. CRC Press, Inc., Boca Raton, FL.
39. Hawkes, T. R. 1989. Studies of herbicides which inhibit branched chain amino acid biosynthesis. Pages 131–138 *in* L. G. Copping, J. Dalziel and A. D. Dodge, eds. Prospects for amino acid biosynthesis inhibitors in crop protection and pharmaceutical chemistry. Proc. Br. Crop Prot. Counc., Monogr. No. 42.
40. Hess, F. D. 1985. Herbicide absorption and translocation and their relationship to plant tolerances and susceptibility. Pages 191–214 *in* S. O. Duke, ed. Weed Physiology. Vol. 2. Herbicide Physiology. CRC Press, Inc., Boca Raton, FL.
41. Hoppe, H. H. and H. Zacher. 1985. Inhibition of fatty acid biosynthesis in isolated bean and maize chloroplasts by herbicidal phenoxy-phenoxypropionic acid derivatives and structurally-related compounds. Pestic. Biochem. Physiol. 24:298–305.
42. Irzyk, G. P., T. T. Bauman, and N. C. Carpita. 1990. Uptake, metabolism, and activity of haloxyfop in liquid cultures of proso millet (*Panicum miliaceum* L. cv Abarr). Weed Sci. 38:484–491.
43. Isensee, A. R., G. E. Jones, and B. C. Turner. 1971. Root absorption and translocation of picloram by oats and soybeans. Weed Sci. 19:727–731.
44. Johnson, M. P. and J. Bonner. 1956. The uptake of auxin by plant tissue. Physiol. Plant. 9:102–118.
45. Kasai, F. and D. E. Bayer. 1991a. Quantitative evaluation of the weak acid hypothesis as the mechanism for 2,4-D absorption by corn root protoplasts. J. Pestic. Sci. 16:163–170.
46. Kasai, F. and D. E. Bayer. 1991b. Relationship between intracellular pH and 2,4-D absorption by corn root protoplasts under the influence of metabolic inhibitors and antiauxins. J. Pestic. Sci. 16:171–177.
47. Kurkdjian, A. and J. Guern. 1989. Intracellular pH: Measurement and importance in cell activity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:271–303.
48. Ladner, D. W. 1990. Structure-activity relationships among the imidazolinone herbicides. Pestic. Sci. 29:317–333.
49. Lichtner, F. T. 1983. Amitrole absorption by bean (*Phaseolus vulgaris* L. cv 'Red Kidney') roots. Plant Physiol. 71:307–312.
50. Liebl, R. A., U. B. Zehr, and R. H. Teyker. 1992. Influence of nitrogen form on extracellular pH and bentazon uptake by cultured soybean (*Glycine max*) cells. Weed Sci. 40:418–423.

51. Little, D. L. 1991 . Physico-chemical parameters affecting root absorption and translocation of 5′-substituted pyridine imidazolinones. 165 pp. Ph.D. Diss., Rutgers Univ., New Jersey.
52. Little, D. L. and D. L. Shaner. 1991. Absorption and translocation of the imidazolinone herbicides. Pages 53–69 *in* D. L. Shaner and S. L. O'Connor, eds. The Imidazolinone Herbicides. CRC Press, Boston, MA.
53. McCloskey, W. B. 1991. The absorption of atrazine, 2,4-D and glyphosate by wheat leaf protoplasts. 148 pp. Ph.D. Diss. Univ. of California, Davis.
54. McCloskey, W. B. and D. E. Bayer. 1990. Atrazine absorption and effects on photosynthesis in wheat leaf protoplasts. Pestic. Biochem. Physiol. 37:227–238.
55. Mersie, W. and C. L. Foy. 1987. Influence of pH on the absorption of chlorsulfuron by leaves and excised roots of velvetleaf (*Abutilon theophrasti*). Weed Sci. 35:11–14.
56. Mersie, W. and M. Singh. 1987. Comparison of norflurazon absorption by excised roots of three plant species. Pestic. Biochem. Physiol. 28:114–120.
57. Mervosh, T. L. and N. E. Balke. 1991. Effects of calcium, magnesium, and phosphate on glyphosate absorption by cultured plant cells. Weed Sci. 39:347–353.
58. Minocha, S. C. and P. Nissen. 1985. Uptake of 2,4-dichlorophenoxyacetic acid and indoleacetic acid in tuber slices of Jerusalem artichoke and potato. J. Plant Physiol. 120:351–362.
59. Moody, K., C. A. Kust, and K. P. Buchholtz. 1970. Uptake of herbicides by soybean roots in culture solutions. Weed Sci. 18:642–647.
60. Morrison, I. N. and W. H. Vanden Born. 1975. Uptake of picloram by roots of alfalfa and barley. Can. J. Bot. 53:1774–1785.
61. Nandihalli, U. B. and P. C. Bhowmik. 1989. Chlorimuron absorption by excised velvetleaf (*Abutilon theophrasti*) roots. Weed Sci. 37:29–33.
62. Neumann, W., H. Laasch, and W. Urbach. 1987. Mechanisms of herbicide sorption in microalgae and the influence of environmental factors. Pestic. Biochem. Physiol. 27:189–200.
63. Nobel, P. S. 1991. Solutes. Pages 109–189 *in* Physicochemical and Environmental Plant Physiology. Academic Press, New York.
64. Nooden, L. D. 1970. Metabolism and binding of ^{14}C-maleic hydrazide. Plant Physiol. 45:46–52.
65. Ooka, M. and N. E. Balke. 1991 . Cellular uptake of sulfonylurea analogs: a compartmental analysis, WSSA Abstr. 31:67.
66. Orwick, P. L., M. M. Schreiber, and T. K. Hodges. 1976. Absorption and efflux of chloro-*s*-triazines by *Setaria* roots. Weed Res. 16:139–144.
67. Owen, W. J. and B. Donzel. 1986. Oxidative degradation of chlorotoluron, propiconazole, and metalaxyl in suspension cultures of various crop plants. Pestic. Biochem. Physiol. 26:75–89.
68. Peterson, C. A. and L. V. Edgington. 1976. Entry of pesticides into the plant symplast as measured by their loss from an ambient solution. Pestic. Sci. 7:483–491.
69. Pillmoor, J. B. and T. R. Roberts. 1985. Approaches to the study of non-extractable (bound) pesticide residues in plants. Pages 85–101 *in* D. H. Hutson and T. R. Roberts, eds. Progress in Pesticide Biochemistry and Toxicology. Vol 4. John Wiley and Sons, Ltd.
70. Pipke, R. and N. Amrhein. 1988. Isolation and characterization of a mutant of *Arthrobacter* sp. strain GLP-1 which utilizes the herbicide glyphosate as its sole source of phosphorus and nitrogen. Appl. Environ. Microbiol. 54:2868–2870.
71. Pipke, R., A. Schulz, and N. Amrhein. 1987. Uptake of glyphosate by an *Arthrobacter* sp. Appl. Environ. Microbiol. 53:974–978.
72. Prasad, R. and G. E. Blackman. 1965. Studies in the physiological action of 2,2-dichloropropionic acid, III. Factors affecting the level of accumulation and mode of action. J. Exp. Bot. 16:545–568.
73. Price, T. P. and N. E. Balke. 1982. Characterization of rapid atrazine absorption by excised velvetleaf (*Abutilon theophrasti*) roots. Weed Sci. 30:633–639.
74. Price, T. P. and N. E. Balke. 1983a. Characterization of atrazine accumulation by excised velvetleaf (*Abutilon theophrasti*) roots. Weed Sci. 31:14–19.
75. Price, T. P. and N. E. Balke. 1983b. Comparison of atrazine absorption by underground tissues of several plant species. Weed Sci. 31:482–487.
76. Raven, J. A. 1975. Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. New Phytol. 74:163–172.
77. Reider Van Ellis, M. and D. L. Shaner. 1988. Mechanism of cellular absorption of imidazolinones in soybean (*Glycine max*) leaf discs. Pestic. Sci. 23:25–34.
78. Retzlaff, G., J. L. Hilton, and J. B. St. John. 1979. Inhibition of photosynthesis by bentazon in intact plants and isolated cells in relation to the pH. Z. Naturforsch. 34c:944–947.
79. Richard, E. P., Jr. and F. W. Slife. 1979. In vivo and vitro characterization of the foliar entry of glyphosate in hemp dogbane (*Apocynum cannabinum*). Weed Sci. 27:426–433.
80. Riechers, D. E. 1990. Surfactant effects on the plasma membrane as a mode of action in promoting glyphosate phytotoxicity. 47 pp. M.Sc. Diss. Univ. Illinois, Urbana-Champaign.
81. Rubery, P. H. 1977, The specificity of carrier-mediated auxin transport by suspension-cultured crown gall cells. Planta 135:275–283.
82. Rubery, P. H. 1978. Hydrogen ion dependence of carrier-mediated auxin uptake by suspension-cultured crown gall cells. Planta 142:203–206.
83. Rubery, P. H. 1987. Auxin Transport. Pages 341–362 *in* P. J. Davies, ed. Plant Hormones and Their Role in Plant Growth and Development. Kluwer Academic Publishers, Dordrecht.
84. Sabater, M. and P. H. Rubery. 1987. Auxin carriers in *Cucurbita* vesicles, III. Specificity, with particular reference to 1-naphthylacetic acid. Planta 171:514–518.
85. Scheel, D. and H. Sandermann, Jr. 1981. Metabolism of 2,4-dichlorophenoxyacetic acid in cell suspension cultures of soybean (*Glycine max* L.) and wheat (*Triticum aestivum* L.). II Evidence for incorporation into lignin. Planta 152:253–258.
86. Sheets T. J. 1961. Uptake and distribution of simazine by oat and cotton seedlings. Weeds 9:1–13.
87. Shone, M. G. T. and A. V. Wood. 1974. A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide by barley, I. Absorption in relation to physico-chemical properties. J. Exp. Bot. 25:390–400.
88. Singer, S. and C. N. McDaniel. 1982. Transport of the herbicide 3-amino-1,2,4-triazole by cultured tobacco cells and leaf protoplasts. Plant Physiol. 69:1382–1386.
89. Smith, A. E. 1972. Accumulation of (2,4-dichlorophenoxy)acetic acid and (2,4,5-trichlorophenoxy)acetic acid by parenchyma tissue as influenced by metabolic inhibitors and lecithin. Physiol. Plant. 27:338–341.
90. Smith, A. E. 1989. Degradation, fate, and persistence of phenoxyalkanoic acid herbicides in soil. Rev. Weed Sci. 4:1–24.
91. Smith, A. M. and W. H. Vanden Born. 1992. Ammonium sulfate increases efficacy of sethoxydim through increased absorption and translocation. Weed Sci. 40:351–358.
92. Sterling, T. M. and N. E. Balke. 1988. Use of soybean (*Glycine max*) and velvetleaf (*Abutilon theophrasti*) suspension-cultured cells to study bentazon metabolism. Weed Sci. 36:558–565.
93. Sterling, T. M. and N. E. Balke. 1989. Differential bentazon metabolism and retention of bentazon metabolites by plant cell cultures. Pestic. Biochem. Physiol. 34:39–48.
94. Sterling, T. M., N. E. Balke, and D. S. Silverman. 1990. Uptake and accumulation of the herbicide bentazon by cultured plant cells. Plant Physiol. 92:1121–1127.
95. Stoller, E. W. 1969. The kinetics of amiben absorption and metabolism as related to species sensitivity. Plant Physiol. 44:854–860.
96. Struve, I., B. Golle, and U. Luttge. 1987. Sethoxydim uptake by leaf slices of sethoxydim resistant and sensitive grasses. Z. Naturforsch. 42c:279–282.
97. Swanson, C. R. and J. R. Baur. 1969. Absorption and penetration of picloram in potato tuber discs. Weed Sci. 17:311–314.
98. Tritter, S. A., F. B. Holl, and B. G. Todd. 1987. Diclofop-methyl and diclofop uptake in oat (*Avena sativa* L.) protoplasts. Can. J. Plant Sci. 67:215–223.
99. Upadhyaya, M. K. and L. D. Nooden. 1980. Mode of dinitroaniline herbicide action, II. Characterization of [^{14}C]oryzalin uptake and binding. Plant Physiol. 66:1048–1052.
100. Wedding, R. T. and L. C. Erickson. 1957. The role of pH in the permeability of *Chlorella* to 2,4-D. Plant Physiol. 32:503–512.
101. Whitwell, T., P. Banks, E. Basler, and P. W. Santelmann. 1980. Glyphosate absorption and translocation in bermudagrass (*Cynodon dactylon*) and activity in horsenettle (*Solanum carolinense*). Weed Sci. 28:93–96.
102. Zsoldos, F. and E. Haunold. 1979. Effects of pH changes on ion and 2,4-D uptake of wheat roots. Physiol. Plant. 47:77–80.