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Uptake and Accumulation of the Herbicide Bentazon by Cultured Plant Cells[1]

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ABSTRACT

Cellular absorption of the herbicide bentazon, a weak acid with pK_a 3.45, was investigated using suspension-cultured cells of velvetleaf (*Abutilon theophrasti* Medic.). Bentazon accumulated rapidly to concentrations approximately four times that of the external medium. Bentazon accumulation against a concentration gradient was not due to its conversion to metabolites, partitioning into lipids, or binding onto cellular constituents. Bentazon uptake was related linearly to the external bentazon concentration, implying that movement of the herbicide into cells was not carrier-mediated. Bentazon was able to diffuse freely and extensively out of the cells, indicating that bentazon can readily diffuse across cell membranes. Potassium cyanide and carbonyl cyanide *m*-chlorophenyl hydrazone inhibited bentazon accumulation as did nitrogen gas when bubbled through the uptake medium. Absorption was pH-dependent with the greatest amount of bentazon accumulating at acidic external pH. Calculations indicated that conversion of uncharged bentazon to bentazon anion in the cytoplasm accounts for cellular accumulation of bentazon. These results provide evidence that bentazon is absorbed across membranes via simple diffusion and that bentazon accumulates in plant cells via an energy-dependent, ion-trapping mechanism which results in bentazon accumulation in the cytoplasm.

A herbicide must be absorbed by plant cells and accumulate at the site of action before it causes phytotoxicity. In general, nonfacilitated diffusion is the primary mechanism by which herbicides move across plant cell membranes (10), although active or carrier-mediated transport of a few herbicides into plant cells has been documented (5, 17). Natural, plant growth regulating chemicals (1) and secondary plant products (13) are transported across membranes by a combination of passive and active processes. Knowledge of the mechanisms involved in the assymetric distribution of herbicides across membranes may explain relationships between herbicide concentration and phytotoxicity.

Bentazon[3], a photosynthetic inhibitor used for postemergence control of broadleaf weeds (15), must penetrate both the plasma membrane and the thylakoid membrane to reach its herbicidal site of action. Bentazon accumulated against concentration gradients in both velvetleaf (*Abutilon theophrasti* Medic.) suspension cells and hypocotyl sections without accumulation of bentazon metabolites (20). The objective of this study was to determine the mechanism for bentazon uptake and accumulation by velvetleaf cells. The characteristics of bentazon accumulation investigated included: (a) binding to cellular constituents, (b) energy dependence, (c) carrier mediation, and (d) ion trapping.

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[3] Abbreviations: bentazon, 3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; KCN, potassium cyanide; LSS, liquid scintillation spectrometry; picloram, 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid.

MATERIALS AND METHODS

Cell Culture

Cell suspension cultures of soybean (*Glycine max* [L.] Merr. cv Corsoy 79) and velvetleaf (*Abutilon theophrasti* Medic.) were established and cultured in a modified Gamborg B5 (9) medium (pH 5.6) supplemented with 0.3, 2.0, and 0.6 mg/L of kinetin, IAA, and picloram, respectively, and 5 g/L sucrose.

Bentazon Uptake and Metabolism

For all uptake studies, except otherwise noted, velvetleaf cells (0.5–1.0 g cells in 20 mL fresh medium in a 50-mL Erlenmeyer flask) were incubated on a rotary shaker (125 rpm) at room temperature under standard room fluorescent lighting with 2.82 μCi/L phenyl-[U^{14}C]bentazon (10.5 μCi/μmol) and [^{12}C]bentazon to give concentrations of 1 μM bentazon and 0.1% (v/v) methanol in the final cell suspension. Cell suspension pH was maintained without adjustment at pH 5.6 ± 0.1. After incubation, cells were collected by vacuum filtration on glass fiber filter discs (Whatman GF/A), rinsed by flowing 4 mL of ice-cold culture medium containing no bentazon through the cells to remove extraprotoplasmic radiolabel, and weighed. The rinsed cells were counted by LSS to determine cellular radioactive content (20). Cellular bentazon uptake was calculated as nmole bentazon per gram fresh weight of cells.

Bentazon metabolism was determined using HPLC and LSS. Radiolabeled compounds were extracted from the cells by homogenization in 100% methanol as described previously (20). Following centrifugation to remove cell debris, cell extracts were concentrated individually, filtered, and injected on to a C_{18} HPLC column. The radioactive content of each fraction eluting from the column was quantitated by LSS. The radiolabel extracted from velvetleaf cells coeluted with bentazon as described previously (20).

Concentration Dependence

Cells were incubated for 4 h in solutions containing 2.82 μCi/L phenyl-[U^{14}C]bentazon and [^{12}C]bentazon to give final bentazon concentrations of 0.1 to 100 μM. Radiolabeled compounds were extracted and quantitated as described in the previous subsection.

Metabolic Inhibitors

Aqueous solutions of CCCP, a proton ionophore, and KCN, a respiratory inhibitor, were added to cell suspensions to reach final concentrations of 10 and 100 μM, respectively. The potassium ionophore valinomycin, which was dissolved in ethanol, was added to cell suspensions to reach final concentrations of 25 μM and 1% (v/v) ethanol. Bentazon was added immediately after the inhibitors and cells were incubated in the presence of both for 1 h. Ethanol (1%, v/v) had no effect on bentazon uptake, so bentazon uptake for each treatment was compared to bentazon uptake by the aqueous control.

For experiments in which bentazon uptake in the presence or absence of oxygen was evaluated, either air or nitrogen gas was bubbled through cell suspensions for 10 min after which bentazon was added. Flasks were immediately capped with rubber stoppers to reduce gas exchange and cells were incubated for an additional 1 h.

pH Dependence

Cell suspensions (about 5 g/200 mL) were brought to pH 4.6, 5.6, or 6.6 with HCl or KOH. After addition of bentazon, aliquots (20 mL) were removed from the cell suspensions at 5, 10, 15, 30, 45, and 60 min. Cell suspension pH did not change more than 0.1 pH unit during the 1-h incubation.

Cell Lysis

Cells were collected by vacuum filtration and weighed. One-half of these cells was frozen at −70°C for 1 h, lysed by thawing to room temperature, and resuspended in medium. The other half of the cells was resuspended in medium without the freeze/thaw cycle. Lysed and intact cells were incubated with bentazon for 1 h and collected by vacuum filtration on glass fiber filter discs as described earlier. Bentazon uptake by lysed cells was calculated as nmole bentazon per gram fresh weight of cells based on their weight prior to freezing.

Efflux

Cells (about 10 g) were incubated in 150 mL medium containing [^{14}C]bentazon for 2 h. After this loading period, cells were collected on a 74-μm nylon mesh screen and quickly rinsed one time with ice-cold medium (40 mL) which did not contain bentazon. The cells were resuspended in 450 mL medium lacking bentazon. At 14 time points from 15 s to 4 h after resuspension, aliquots of cells (25 mL) were removed from the efflux solution, cells were collected by vacuum filtration, rinsed, and weighed, and their radioactive content was determined by LSS. After 4 h, all remaining cells were collected, rinsed, and resuspended in 300 mL medium for a second efflux period. At six additional time points from 15 s to 2 h after the second resuspension, 40-mL aliquots of cells were removed and radioactive contents of the cells were determined.

Lipid Extraction

Total lipids were extracted from velvetleaf and soybean cells according to the method of Folch *et al.* (8). Cells were homogenized with 20 volumes of chloroform:methanol (2:1, v/v) using a Ten-Broeck homogenizer. The homogenate was vacuum-filtered through glass fiber filter discs. The filtrate was partitioned three times against 0.2 volume of 0.15 μM $CaCl_2$. The chloroform:methanol phase was collected in glass vials, evaporated to dryness under nitrogen gas, and weighed. Extracted lipids were incubated in 1 μM [^{14}C]bentazon medium at pH 5.6 in glass vials (1:20, w/v, lipid/medium). Radiolabel remaining in the aqueous phase was quantitated after 48 h. There was no measurable binding of bentazon to the glass vials in the absence of extracted lipids.

Statistical Analysis

All experiments were conducted twice with three or four replications each. In experiments in which large batches of cells were used (*i.e.* pH and efflux experiments), each batch was a separate replication. Experiments were designed as randomized complete blocks. The time course experiment comparing bentazon uptake by cells incubated in solutions at different pHs was designed and analyzed as a randomized complete block with factorial (pH by time) arrangement of treatments. Analyses of variance were performed on data expressed as nmole bentazon per gram fresh weight. Means were compared using Fisher's LSD Test at the 0.05 level of significance (19). Standard regression analysis was performed using least square procedures (19).

RESULTS

Bentazon Uptake

Bentazon uptake by suspension-cultured velvetleaf cells was rapid during the first hour of exposure to [^{14}C]bentazon and reached a maximum cellular concentration by 3 h (Fig. 1). The cellular concentration of ^{14}C was fourfold greater than the external concentration after 3 h (assuming 1 g fresh weight of cells equals 1 mL of volume [14]). Bentazon accumulation against a concentration gradient was not due to rapid binding of bentazon to the cells because very little bentazon was associated with the cells at 15 s. Also, in other experiments bentazon accumulated gradually in velvetleaf cells at incubation times between 0 and 1 h (21). No bentazon metabolites were detected in velvetleaf. Thus, accumulation of [^{14}C]metabolites of bentazon did not account for the accumulation of [^{14}C]bentazon against a concentration gradient.

Binding of Bentazon to Cellular Constituents

Intact velvetleaf cells accumulated over 20 times the [^{14}C]-bentazon accumulated by lysed cells in 1 h (Table I). Therefore, intact membranes appeared necessary for bentazon ac-

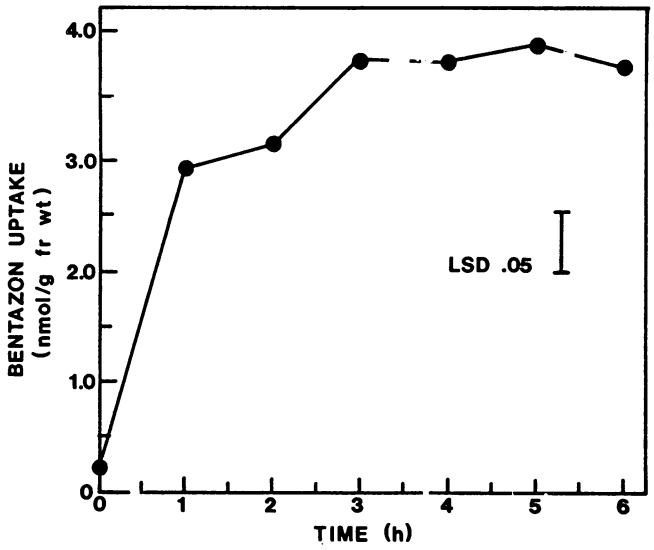

Figure 1. Time course of bentazon uptake by velvetleaf cells from 15 s to 6 h. The uptake solution was a modified Gamborg B5 medium (pH 5.6) (20) and contained 1 μM bentazon. The suspension-cultured cells were in the log phase of their culture growth cycle. The bar indicates the Fisher's LSD value for mean (n = 8) comparison at the 5% level of significance.

Table I. *Bentazon Uptake by Intact or Lysed Velvetleaf Cells*

Cells were lysed by freezing at −70°C for 1 h and thawing to room temperature. Lysed and intact cells were incubated for 1 h in a modified Gamborg B5 medium (pH 5.6) (20) containing 1.0 μM bentazon.

Condition	Bentazon Uptake
	nmol/g fresh wt·h
Intact	2.18
Lysed	0.10
F-value	**[a]

[a] The *F*-value of the means (n = 6) differed at the 1% level of significance.

cumulation. When [^{14}C]bentazon was extracted from velvetleaf with absolute methanol, only about 5% of the ^{14}C remained in the methanol insoluble fraction (data not shown). These results provide evidence that the accumulated bentazon was not associated with or bound to cellular constituents.

The possibility that bentazon preferentially partitioned into cellular lipids of velvetleaf or soybean was evaluated. In 1 h both velvetleaf and soybean cells accumulated [^{14}C]bentazon to concentrations greater than the external bentazon concentration (Table II). Compared to soybean, velvetleaf cells accumulated 1.4-fold more ^{14}C and reached a bentazon concentration similar to the equilibrium concentration observed with longer incubation times (Fig. 1). No bentazon metabolites were detected in velvetleaf. However, approximately 25% of the [^{14}C]bentazon absorbed by soybean was metabolized to the glucose conjugates of OH-bentazon (21). Therefore, accumulation of bentazon metabolites contributed to the accumulation of [^{14}C]bentazon by soybean but not by velvetleaf cells.

Table II. *Bentazon Partitioning into Lipids Extracted from Soybean and Velvetleaf Cells*

Total lipids were extracted according to Folch *et al.* (8). See "Materials and Methods" for additional details. Extracted lipids were incubated in glass vials containing 1 μM [^{14}C]bentazon in medium at pH 5.6 (1:20, w/v, lipid/medium). Radiolabel remaining in the aqueous phase was quantitated after 48 h. Units for bentazon distribution coefficient are ([dpm·mg^{-1} lipid) ÷ (dpm·mg^{-1} solution]). Soybean and velvetleaf cells were incubated for 1 h with 1 μM bentazon. Additional details are in Figure 1.

Species	Lipid Content	Bentazon Distribution Coefficient	Cellular Bentazon Uptake
	mg/g fresh wt	K_{1w}	*nmol/g fresh wt·h*
Soybean	4.85	0.130	2.28
Velvetleaf	4.33	0.120	3.12
F-value	NS[a]	NS	**[b]

[a] Means (n = 6) were not significantly different. [b] The *F*-value of the means (n = 6) differed at the 1% level of significance.

The quantity of lipid extracted in the chloroform:methanol fraction was the same for velvetleaf and soybean cells (Table II). Extracted lipid was approximately 0.5% of cell fresh weight, a value in close agreement with the lipid content (0.7%) measured in sycamore (*Acer pseudoplatanus* L.) suspension cells (3). In addition to similar lipid content in velvetleaf and soybean cells, [^{14}C]bentazon partitioned similarly into the lipids extracted from the two species (Table II). The distribution coefficient, K_{1w}, of bentazon between the extracted lipid and the aqueous solution was the same in both species. Apparently, the greater accumulation of bentazon by velvetleaf than soybean was due neither to different lipid quantities being present in the two species nor to differential partitioning of bentazon into lipids of velvetleaf compared to soybean. The quantities of different types of lipid were not determined in this experiment. If bentazon were more soluble in particular lipids which were preferentially present in velvetleaf, bentazon could accumulate to higher concentrations in velvetleaf lipids than in soybean lipids.

Metabolic Inhibitors

To determine if metabolic energy was required for bentazon uptake, velvetleaf cells were treated either by inclusion of the respiratory inhibitor KCN in the medium or by bubbling nitrogen gas through the cell suspension. KCN reduced bentazon uptake by 21% (Table III). Bentazon uptake was also reduced 20% when nitrogen was bubbled through the uptake medium (Table IV). These results indicated that cellular metabolic energy enhanced bentazon uptake.

A proton ionophore, CCCP, and a potassium ionophore, valinomycin, were used to determine if maintenance of proton or potassium gradients across cell membranes was necessary for bentazon uptake. Bentazon uptake was reduced 61% by CCCP but was not affected by valinomycin (Table III). These results indicated that proton gradients, but not potassium gradients, across membranes might be involved with bentazon uptake.

Table III. *Effect of Metabolic Inhibitors on Bentazon Uptake by Velvetleaf Cells*

Cells were incubated at 1 h in solutions containing 1 μM bentazon and desired inhibitor at pH 5.6. See Figure 1 for additional details.

Inhibitor		Bentazon Uptake
Compound	Concentration	
	μM	*nmol/g fresh wt·h*
Control		1.60
CCCP	10	0.62
KCN	100	1.26
Valinomycin	25	1.51
LSD 0.05		0.12

Table IV. *Uptake of Bentazon by Velvetleaf Cells in the Presence of Air or Nitrogen Gas*

Either air or nitrogen gas was bubbled through the cell suspension for 10 min. Bentazon at 1 μM was then added, flasks were sealed to reduce air exchange, and cells were incubated for an additional 1 h. Additional details are in Figure 1.

Gas Source	Bentazon Uptake
	nmol/g fresh wt·h
Air	3.10
N_2	2.50
F-value	*[a]

[a] The *F*-value of the means (n = 8) differed at the 5% level of significance.

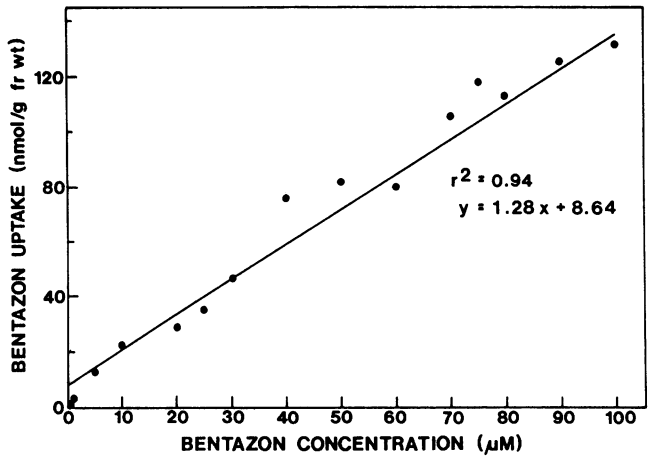

Figure 2. Concentration dependence of bentazon uptake by velvetleaf cells over the range 0.1 to 100 μM bentazon for 4 h. Best fit line was determined by least square linear regression analysis.

Concentration Dependence

Bentazon uptake by velvetleaf was linearly related to the external bentazon concentration from 0.1 to 100 μM bentazon (Fig. 2). The high r^2 value calculated using least square regression analysis indicated that a straight line fit the data very well. There did not appear to be any saturation of bentazon uptake at these bentazon concentrations, indicating the mechanism by which bentazon entered the plant cell was simple diffusion. The cellular bentazon concentration was greater than the external bentazon concentration at each bentazon concentration tested. This result agreed with previous experiments (Fig. 1; Tables I–III) where bentazon also accumulated against a concentration gradient in velvetleaf.

pH Dependence

Bentazon uptake by velvetleaf cells was influenced by external media pH (Fig. 3). Bentazon accumulated to the greatest concentration in cells incubated at pH 4.6 and to the least concentration in cells incubated at pH 6.6.

Efflux Experiments

Bentazon moved out of velvetleaf cells logarithmically after transfer of bentazon-loaded cells to bentazon-free medium (Fig. 4). Efflux of bentazon from the cells was relatively rapid; <30% of the [^{14}C]bentazon present in loaded cells at t = 0 min remained in the eluted cells at t = 30 min. After resuspension of the cells in bentazon-free medium at t = 240 min (see arrow in Fig. 4), bentazon efflux was again rapid. A logical explanation for additional bentazon eluting from cells during the second efflux period is that bentazon had reached equilibrium between the cells and medium during the first efflux period. This conclusion is supported by the bentazon equilibrium ratio being about 6 after the first efflux period, a value close to the bentazon equilibrium ratio of the influx experiment (Fig. 1). Because efflux equilibrium had been reached, compartmental analysis to determine intracellular distribution of bentazon could not be performed. Only 6% of the ^{14}C present in the loaded cells at t = 0 min was present in the eluted cells at t = 360 min. This value is near the amount of ^{14}C associated with lysed velvetleaf cells (5%) and the amount of ^{14}C in the methanol insoluble fraction (5%). Apparently, some bentazon irreversibly associated with cellular constituents that may have included membranes or cell wall material. However, this quantity was not large enough to

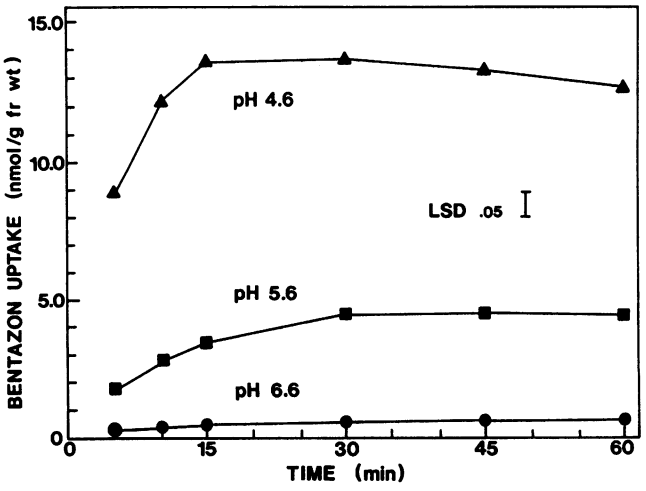

Figure 3. Time course of bentazon uptake by velvetleaf cells at pH 4.6, 5.6, and 6.6. Additional details are in Figure 1.

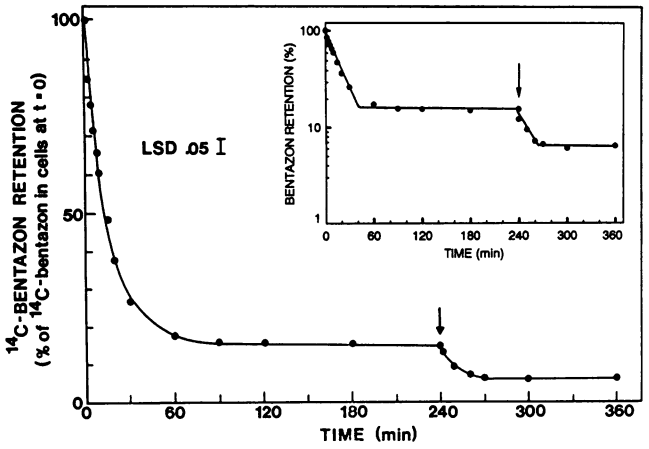

Figure 4. Time course of [^{14}C]bentazon efflux from velvetleaf cells loaded with [^{14}C]bentazon for 2 h. Inset is a semilog plot of the data. Additional details are in "Materials and Methods." At t = 0 min of efflux, the cells contained 2.9 nmol ^{14}C/g fresh weight (100%). Cells were transferred to fresh bentazon-free medium at t = 0 min. The arrow indicates when cells were transferred to new bentazon-free medium a second time. The bar indicates the Fisher's LSD value for mean (n = 6) comparison at the 5% level of significance.

account for the amount of bentazon accumulating in velvetleaf cells above passive equilibrium. Because the ^{14}C associated with the cells after efflux for 3 h was most likely bound to cellular constituents, velvetleaf cell membranes were probably not effective barriers to bentazon efflux.

The possibility that reduced bentazon uptake in the presence of KCN (Table III) was caused by an alteration of velvetleaf cell membrane permeability to bentazon was investigated with efflux experiments. Cells containing bentazon were incubated in media without bentazon but in the presence or absence of 100 μM KCN. The pattern of bentazon efflux and the quantity of bentazon remaining in the cells after a 4-h efflux period were very similar whether or not KCN was present (data not presented). This result indicated that KCN was not altering the permeability of velvetleaf cell membranes to bentazon.

DISCUSSION

Bentazon accumulated to cellular bentazon concentrations greater than the external bentazon concentration as reported previously for velvetleaf cells and hypocotyl tissue sections (20). Maximum concentrations of bentazon in velvetleaf cells were reached between 1 and 3 h (Fig. 1) and were maintained for as long as 24 h in the absence of bentazon metabolism (21). The combined results presented here indicate that bentazon enters the cell via a process that does not require a transmembrane carrier and is probably simple diffusion. Passive diffusion as the mechanism for bentazon movement across cell membranes is supported by the nonsaturable uptake kinetics (Fig. 2) and the ability of bentazon to freely diffuse out of cells (Fig. 4). Bentazon accumulation against a concentration gradient was not due to bentazon metabolite accumulation or preferential association with cell constituents. However, cellular accumulation of bentazon was energy-dependent (Tables III and IV) and pH-dependent (Fig. 3) indicating that bentazon accumulation in velvetleaf cells against a concentration gradient might be best explained by an ion-trapping mechanism.

Bentazon movement into velvetleaf cells was via nonfacilitated diffusion as supported by the absence of saturation kinetics (Fig. 2). The mechanism by which most herbicides move across membranes is hypothesized to be diffusion (10). However, absorption of some herbicides into plant cells may be facilitated by their transport on endogenous transport systems that move natural products such as auxin or abscisic acid across membranes (1, 17). 2,4-D transport into crown gall cells was, in part, due to a saturable, carrier-mediated component and was suggested to be via the same transport system that transports IAA (17). Burton (5) suggested that glyphosate was transported across the plasma membrane of potato cells via a phosphate carrier. Evidence supporting this conclusion included saturable uptake systems for both Pi and glyphosate and competitive inhibition of glyphosate uptake by Pi. On the other hand, nonsaturable absorption of the herbicides amitrole and atrazine indicated these herbicides moved across cell membranes by diffusion (14, 18). The absence of saturation kinetics (Fig. 2) indicated that bentazon transport into velvetleaf cells was not carrier-mediated.

Nonfacilitated movement of bentazon across velvetleaf cell membranes was also supported by the ability of bentazon to diffuse rapidly out of the cells during efflux (Fig. 4). The pattern of bentazon efflux from velvetleaf cells was similar to, but slower than, atrazine efflux from velvetleaf root segments (14) and oat (*Avena sativa* L.) root segments (2). Bentazon probably elutes from cells more slowly than atrazine does because of lower membrane permeability to bentazon than to atrazine. Membrane permeability to bentazon was too great to distinguish among bentazon molecules contained in the vacuole, the cytoplasm, and the free space (Fig. 4, inset). Therefore, neither the plasma membrane nor the tonoplast appear to be effective barriers to bentazon efflux from velvetleaf cells.

Although experimental evidence supported simple diffusion as the mechanism of bentazon movement across cell membranes, cellular metabolism was necessary for bentazon accumulation against a concentration gradient (Tables III and IV). Cells expend energy to maintain a pH gradient across the plasma membrane and the tonoplast because membrane-bound ATPases, which pump H^+ out of the cytosol, require ATP as their substrate (22). Therefore, the cell wall and the vacuole are acidic relative to the cytoplasm. This acidification of the cell wall region may be necessary for bentazon accumulation by plant cells. This conclusion is supported by the inhibition of bentazon accumulation in the presence of KCN, nitrogen gas, CCCP, and high pH (Tables III–IV; Fig. 3). Being a respiratory inhibitor, KCN would inhibit ATP synthesis and therefore alter the ATPase-generated pH gradients across the plasma membrane and the tonoplast. Anaerobiosis would also inhibit respiration and alter cellular pH. Such a result was observed in corn root tips where hypoxia reduced the cytoplasmic pH by 0.3 to 0.5 pH units but did not change the vacuolar pH (16). The proton ionophore CCCP may act to reduce bentazon accumulation either by directly collapsing

the proton gradient across the plasma membrane or by uncoupling ATP synthesis in mitochondria. The potassium ionophore valinomycin, which collapses potassium gradients across the plasma membrane, also had no effect on bentazon accumulation. Therefore, the electrical potential across velvetleaf membranes is probably not a primary driving force involved in bentazon accumulation.

Inhibition of ATP hydrolysis and collapse of proton gradients across the plasma membrane could result in either acidification of the cytoplasm or alkalinization of the external surface of the plasma membrane or the cell wall space. In either case, bentazon accumulation would be reduced. This conclusion was supported by pH-dependent bentazon accumulation (Fig. 3). Although pH-dependence of herbicide accumulation might support the involvement of a carrier with herbicide transport across membranes, the absence of saturation kinetics (Fig. 2) argues against bentazon transport being via a carrier.

Dependence of herbicide accumulation on pH of the incubation medium also supports ion trapping as the mechanism for bentazon accumulation (4). Accumulation by plant cells and tissues of herbicides that are weak acids was found to be greater at the pH close to the pK_a of each herbicide (6, 7, 17, 23). Natural compounds which are either weak acids (1) or weak bases (11) have also been shown to accumulate differentially in response to pH, and ion trapping has been suggested to at least partially explain accumulation of those compounds by plant cells. Bentazon uptake was greatest in cells incubated at pH 4.6 (Fig. 3); the cellular bentazon concentration was 13- to 15-fold greater than the external bentazon concentration at this pH. Bentazon accumulation in cells isolated from soybean leaf tissue also increased with decreasing pH (15). These results suggest that bentazon diffuses passively across cell membranes as an undissociated acid since higher concentrations of undissociated bentazon exist at acidic rather than neutral pH. As neutral molecules, undissociated bentazon would distribute across cellular membranes to an equilibrium concentration between cells and external medium. The proportion of bentazon present in the undissociated form would depend on the pH of the external medium. The plasma membrane is probably more permeable to undissociated bentazon than dissociated bentazon, as suggested for other weak acids (4). Once in the cytoplasm, undissociated bentazon would dissociate due to the higher cytoplasmic pH. This phenomenon would allow bentazon to concentrate in the cytoplasm as an anion.

To substantiate the ion trapping hypothesis of bentazon accumulation in velvetleaf cells, the predicted concentration of bentazon in plant cells was calculated (Table V) following the model for absorption and distribution of the weak-acid herbicide 2,4-D (6). Depending on the pH of the external medium (pH 5.6 in the bentazon model), a diffusion equilibrium of undissociated bentazon is established across cell membranes. Values for neutral and anionic bentazon concentrations at each pH were obtained using the Henderson-Hasselbach equation. Cytoplasmic and vacuolar pH were assumed to be 7.5 and 5.5, respectively. The cytoplasmic and vacuolar volumes were assumed to be 10 and 90% of the cell volume (12), respectively. Based on these assumptions, the predicted concentration of bentazon in plant cells is 8.60 μM (Table V). The predicted bentazon concentration in plant cells is relatively close to those measured in velvetleaf cells. In addition, the response of cellular bentazon concentrations to changes in external pH are consistent with the ion-trapping model.

Table V. *Calculated Equilibrium Concentrations of Bentazon in External Medium and Various Cellular Compartments*

Concentration of B^- in each cellular compartment was calculated based on assumed values of pH and volume of that compartment. Assumed values were: cytoplasm pH 7.5 and 10% of total cell volume, vacuole pH 5.5 and 90% of total cell volume. The external medium was pH 5.6.

Bentazon Species[a]	Concentration in			
	External medium	Cytoplasm	Vacuole	Total cell
		μM		
B°	0.007	0.007	0.007	0.007
B^-	0.993	78.874	0.789	8.597
$B^\circ + B^-$	1.000	78.881	0.796	8.604

[a] B°, uncharged bentazon; B^-, anionic bentazon.

Based on these experiments collectively, we conclude that bentazon transport across plant membranes is via simple diffusion and bentazon accumulation in plant cells against concentration gradients is primarily due to ion trapping of bentazon anions in the cytoplasm. Based on the ion trap model, bentazon accumulates to almost 80 μM in the cytoplasm when the ambient concentration is 1 μM. If the pH of the chloroplast is pH 7.5, bentazon would also accumulate in that organelle, within which the herbicidal site of action is located. Accumulation of other weak acid herbicides within the cytoplasm of plant cells may also enhance their phytotoxicity because of the herbicide molecules being concentrated near their potential sites of action. On the other hand, these herbicides may also be concentrated near the enzymes that catalyze their degradation, thus facilitating tolerance to the herbicides via rapid detoxication.

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