

Lecture

Herbicide x Plant Interactions Absorption and Translocation of Herbicides Applied to Foliage

1. Sites of entry (foliar application)

- leaves - most important entry site (see Figure 9-11) (Cross section of a leaf; page 9)

For herbicides applied to aboveground plant parts, the process involves penetration of the waxy cuticle covering epidermal cells → penetration of cell walls of epidermal cells → penetration of plasmalemma of leaf cells → released into cytoplasm within the cell

Penetration of the cuticle and cell wall is by diffusion and penetration of plasmalemma primarily by active energy requiring process (will be discussed later)

ectodesmata - leaves have areas of high concentration of ectodesmata (channels through cell walls; will be discussed later)

trichomes - hairs on leaves; ectodesmata abundant in epidermal cells with trichomes

stomates and guard cells - provide for direct entry through stomate; guard cells have high density of ectodesmata; will be discussed later

cracks in cuticle - provide a direct pathway through the cuticle (bypasses cuticle)

- stems of herbaceous and woody plants - minor for herbaceous plants but important for some woody plants (basal application)
- buds - minimal for systemic herbicides

2. Barriers to herbicide absorption (in leaf)

- cuticle
- cell wall
- plasmalemma (cell membrane)

3. Cuticle as a barrier to absorption

- function - prevents dehydration of the leaf
- sponge-like, cutin matrix with wax filled cavities, not homogeneous in composition - use analogy of a sponge to explain
- epicuticular and embedded wax - nonpolar, oil-like (hydrophobic/lipophilic)

In the cuticle these waxes are C-20 to C-37 hydrocarbon chains of fatty acids

- cutin and pectin - polar (hydrophilic/lipophobic)

Polarity refers to the kind (+ or -) and the strength of electrical charge; chemicals are either polar or nonpolar.

Polar molecules are charged e.g. water and are more hydrophilic; Nonpolar molecules are not charged and are more lipophilic.

In the cuticle, cutins are C-16 to C-18 hydrocarbon chains of fatty acids

If both cutin and waxes are fatty acids, why is cutin more hydrophilic than lipophilic?

Cutin contains free carboxyl (-COOH) and hydroxyl (-OH) groups that can become charged (i.e. -COO^- and -O^- , respectively) making them more compatible with water (hydrophilic)

Pectins are composed of polymers of galacturonic acid and thus has carboxyl groups that can become charged. This explains their hydrophilic nature.

Waxes, cutin, and pectins are all intermingled with one another (not homogeneous). Under good growing conditions the cuticle absorbs or swells with water and becomes hydrated (use analogy of a sponge filled with water)

The degree of hydration of the cuticle can affect how fast or slow a herbicide moves through the cuticle.

The thickness of the cuticle varies depending on environmental conditions. Field grown plants have thicker cuticles than greenhouse grown plants (explain function of the cuticle and differences in weed control studies conducted in the field vs. greenhouse).

- herbicide movement via diffusion and affected by polarity and solubility in water (like dissolves like) - oil and water do not mix
- molecular form (not charged - nonpolar - lipophilic - fat loving) vs. ionic form (charged - polar - hydrophilic - water loving)
- water soluble herbicides move in water filled cavities in cutin and pectin corridors (more water soluble the herbicide the more likely to penetrate)
- importance of hydrated cuticle
- oil soluble herbicides move through waxy portion (more oil soluble the herbicide the more likely to penetrate)
- undissociated herbicide molecule would more easily penetrate cuticle than the ionic form

why? herbicide would more easily move through the waxy portion

- see Figure 6.1 (Simplified scheme of the outer wall of an epidermal cell; page 9)- review components of the cuticle and ectodesmata; point out the water droplet / spreading effect
- surfactants - role in herbicide uptake

Surfactant = **SUR**Face **ACT**ive **Age**NT

A waxy cuticle being lipophilic in nature would tend to repel water droplets.

Hence surfactants are added to spray solution to enhance coverage of the leaf surface by the spray droplet containing herbicide.

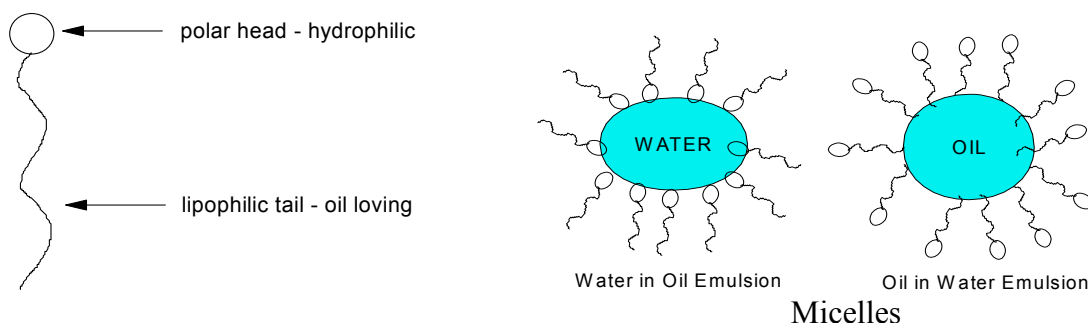
A surfactant decreases the interfacial surface tension of a water droplet causing the droplet to spread over the leaf surface. This increases the exposure sites for uptake of herbicide into the leaf. (See Figure 6.1 - Simplified scheme of the outer wall of an epidermal cell; page 9)



Water droplet on a cuticle

YHow is the interfacial surface tension reduced?

A surfactant chemically has a lipophilic tail (oil loving / nonpolar) and a hydrophilic head (water loving / polar). The decrease in surface tension is accomplished by the lipophilic tail aligning itself with the cuticle and the hydrophilic head with the water droplet forming micelles. This results in the “spreading” of the water droplet on the leaf surface.



4. Cell wall as a barrier to absorption

- function - provide rigidity to the cell and the plant (unlike animals that have skeleton/bones to provide support)
- matrix of interwoven cellulose with water filled interspaces - hydrophilic in nature
- herbicide movement via diffusion and affected by polarity and solubility in water (like dissolves like)
- herbicide in ionic form would more easily penetrate the cell wall
- water soluble herbicides move more rapidly than oil soluble
- ectodesmata submicroscopic channels provide direct path to plasmalemma through cell wall
ectodesmata abundant in guard cells, epidermal cells with trichomes, and leaf veins
- See Figure 6.1 (Simplified scheme of the outer wall of an epidermal cell - show ectodesmata; page 9)

5. Plasmalemma (cell membrane/plasma membrane) as a barrier to absorption

- function - the primary function is containment. It holds things in the cell. It is comprised of lipids/fats and is therefore lipophilic; use analogy of a balloon filled with water.

In respect to herbicide mode of action, Gramoxone Extra (paraquat) is a membrane disrupter. It interacts with the plasma membrane destroying lipids resulting in leakage of the cell contents.

The first symptom is water soaking resulting from leakage of cell contents into the intercellular spaces.

YAll herbicides must enter the cell to be active, therefore, it must cross the cell membrane/plasma membrane.

YHow is the herbicide transported across the plasma membrane?

- passive movement across the plasmalemma by diffusion - common when herbicide concentration is greater outside the cell than inside; no energy is expended by the plant

lipophilic (nonpolar/noncharged) herbicides tend to diffuse more freely across the plasma membrane vs. hydrophilic (polar/charged) **Why?** plasma membrane is lipophilic

- active movement across the plasmalemma via active transport system movement against a concentration gradient

active movement occurs when the concentration is greater or equal inside the cell vs. outside (more common than passive diffusion) i.e herbicides move against a concentration gradient

herbicides that move by this method are mostly weak acids with a carboxylic acid group (R-COO^-) e.g. bentazon, pyridines, sulfonylureas, imidazolinones, sethoxydim, phenoxys, benzoic acids

For this to occur the plant expends energy in the form of ATP, adenosine triphosphate, derived from other energy producing pathways within the plant

process involved:

energy released from conversion of ATP to ADP by ATPase used to pump hydrogen (H^+) ions to the outside of the cell, which affects pH

Cells can therefore maintain a difference in pH across the plasma membrane.

pH outside the cell is lower at 5.0 to 5.5 (higher H^+ concentration) vs. inside the cell at 7.2 to 8.0 (lower H^+ concentration)

weak acid herbicide outside the cell picks up a H^+ and becomes protonated [$\text{R-COO}^- \rightleftharpoons \text{RCOOH}$ (noncharged)] and loses its negative charge. The herbicide is now uncharged (molecular form) making it lipophilic and able to diffuse the lipophilic membrane

See Figure 6.4 (Ion trapping model step 4 showing how weak acid herbicides are transported across the plasma membrane; page **10**)

once inside the cytoplasm where pH is higher (7.2 to 8.0) the hydrogen is stripped from the herbicide ($\text{R-COOH} \rightleftharpoons \text{R-COO}^-$)

the herbicide is now charged and more water soluble

it can not move back across the plasma membrane and becomes trapped in the cytoplasm. uncharged herbicide molecules continue to diffuse into the cell because the concentration of uncharged herbicide molecules is still higher outside the membrane than inside

this is called “ion trapping”

YPreference is for movement of herbicide across the plasma membrane in the molecular form rather than the ionic form

- other mechanisms of active transport (across the plasmalemma)

herbicide transported across plasmalemma by carrier molecules - (commonly accepted mechanism of transport)

carrier molecules (specialized proteins embedded in plasma membrane) pick up the herbicide and transport adsorbed ions or molecules through the plasmalemma releasing them into the cytoplasm e.g. paraquat, 2,4-D, glyphosate

See Figure 6.2 (Hypothetical mechanisms of ion transport across membranes; page 11)

glyphosate has a phosphate molecule as part of its chemistry and crosses membranes via phosphate carriers (discuss example of glyphosate-resistant ryegrass in Australia)

herbicide transported across plasmalemma by invagination of membrane (pinocytosis)

invagination of membrane (pinocytosis) - the herbicide ions align along the outside of the plasma membrane. The membrane invaginates or folds back on itself (inward bulging) to form a balloon-like vesicle (sometimes called membrane vesiculation).

The vesicle then separates from the membrane and moves into the cytoplasm. Ions are now inside the cell.

See Figure 6.2 (Hypothetical mechanisms of ion transport across membranes; page 11)

absorption complete when herbicide released into cytoplasm

herbicide can remain in cytoplasm or in a vacuole, or move symplastically

Yactive transport process allows cells to regulate movement of herbicide - this results in some herbicides being absorbed better than others

6. Points of interest (foliar application)

- foliar applied herbicides must be moved to the site of action within the plant to be toxic
- all herbicides must enter the plant cell to be active
- herbicides may not move at all once absorbed into the cell or may move in the symplast, apoplast, or both
- symplastic movement would result in symptom expression in new growth of terminals
- phloem movement would be upward (acropetal) and downward (basipetal)

- limited apoplastic movement or no movement of herbicide would result in injury primarily to treated tissue
- xylem movement would be upward in the plant (acropetal)
- movement of foliar applied herbicide into the leaf is affected by degree of retention on plant surface and ability to move through cuticle
- penetration of water soluble herbicide more likely to be affected by environmental conditions than penetration by an oil soluble herbicide Why?
- need for surfactant greater for water soluble than for an oil soluble herbicide Why?
- maximum kill of weeds with foliar herbicide expected under warm, humid conditions and adequate soil moisture Why?
- minimum kill of weeds when plants under stress, cool temperature, and low humidity Why?

7. Role of stomata in herbicide absorption

- function is gas exchange with CO₂ in and O₂ out - stomates are openings/pores in the leaf. An additional function is to aid in cooling the plant by the release of water vapor
- guard cells regulate opening and closing - stomates are mostly on the under side of the leaf surface, some are on the top side
- See Figure 9-11 (Cross section of a leaf; page 9)

Guard cells (two) surround the stomatal opening and turgor pressure regulates the opening and closing of the stomate

When turgid the cell would be inflated (full of water) and stomates would be open; if flacid then cells would be water deficient and stomates would be closed

- water release with transpiration (cooling effect)
- guard cells important sites of absorption (dense areas of ectodesmata)
- direct uptake of volatile herbicides when open - not a major site for herbicide absorption
- guard cells mostly located on lower leaf surface
- increased absorption of herbicide by lower leaf surface due to thinner cuticle not presence of more stomata

Electrodyne Sprayer - was developed to enhance coverage of herbicide on the leaf surface. It was hoped that this in turn would increase uptake into the leaf.

Principal involved: The charge on the leaf surface is negative (-). A positive (+) charge was given to spray droplets exiting the nozzle which would encourage attraction to the leaf surface.

This sprayer was sound in theory, but was very expensive to produce and not very practical under field conditions.

This piece of equipment was promoted by ICI (Zeneca), but was never commercialized.

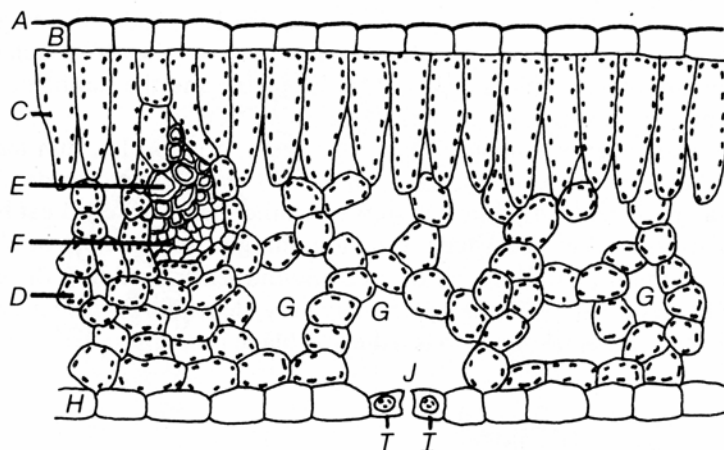


FIGURE 9-11 Cross section of a leaf: A, cuticle; B, upper epidermis; C, palisade cells of mesophyll; D, spongy tissue of mesophyll; E, xylem tissue of leaf vein; F, phloem tissue of leaf vein; G, air spaces; H, lower epidermis; I, guard cells; and J, stoma.

Source: Fuller, H. J. and Donald D. Ritchie, 1967. *General Botany*, 5th Edition, Harper & Row, Barnes & Noble Div., Fig. 10.2, page 66.

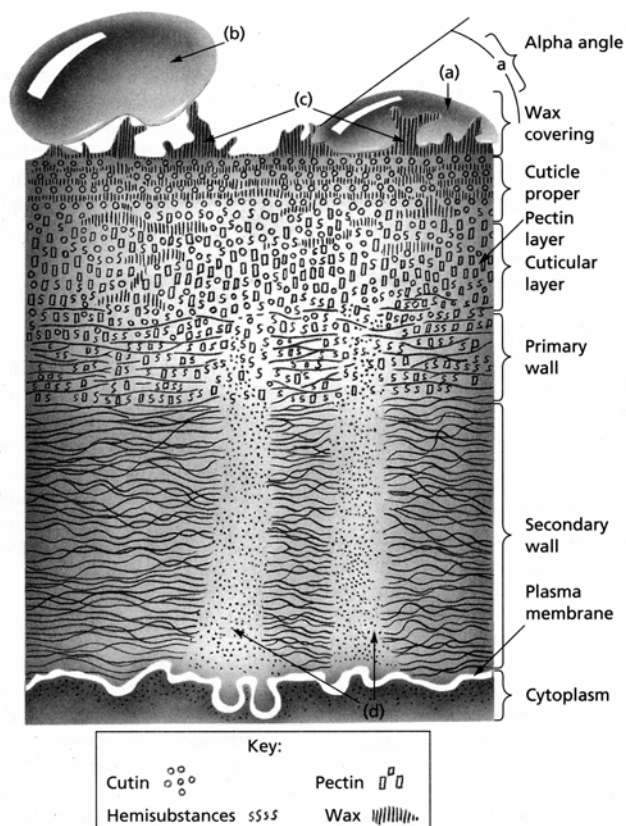


FIGURE 6.1 ■ Simplified scheme of the outer wall of an epidermal cell. (a) Water droplet with detergent, (b) without detergent, (c) wax rodlets, and (d) ectodesmata as nonplasmatic structures.

SOURCE: Redrawn from W. Franke, 1967. Mechanisms of foliar penetration of solutions. *Ann. Rev. Plant Physiol.* 18:284.

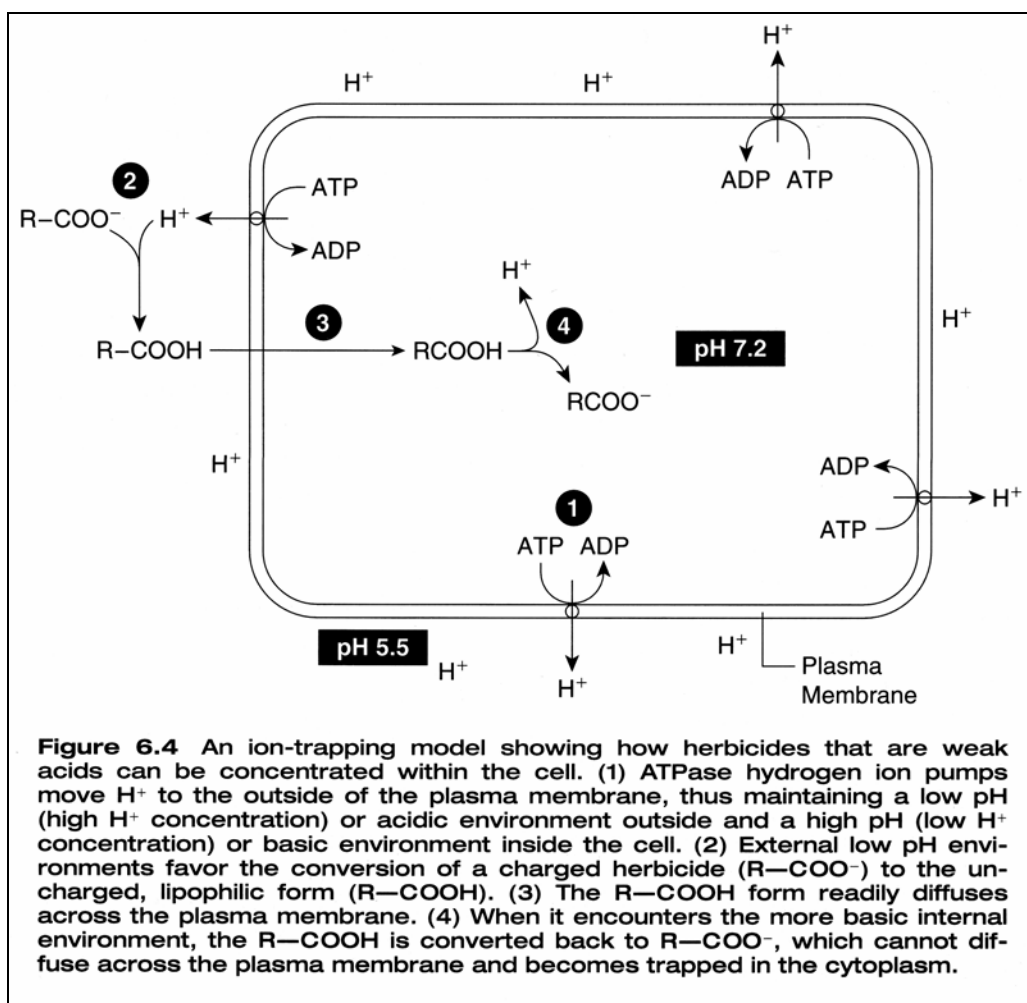


Figure 6.4 An ion-trapping model showing how herbicides that are weak acids can be concentrated within the cell. (1) ATPase hydrogen ion pumps move H^+ to the outside of the plasma membrane, thus maintaining a low pH (high H^+ concentration) or acidic environment outside and a high pH (low H^+ concentration) or basic environment inside the cell. (2) External low pH environments favor the conversion of a charged herbicide ($R-COO^-$) to the uncharged, lipophilic form ($R-COOH$). (3) The $R-COOH$ form readily diffuses across the plasma membrane. (4) When it encounters the more basic internal environment, the $R-COOH$ is converted back to $R-COO^-$, which cannot diffuse across the plasma membrane and becomes trapped in the cytoplasm.

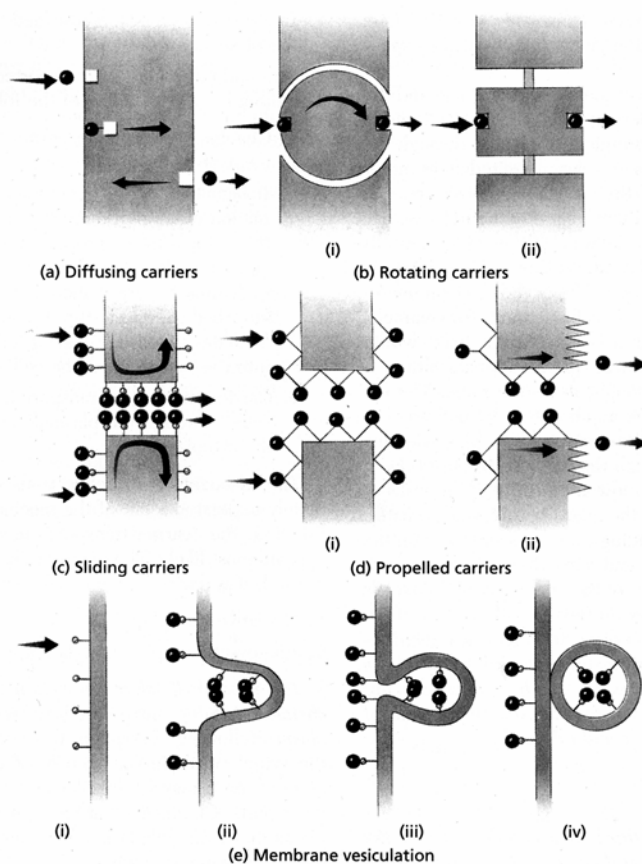


FIGURE 6.2 ■ Hypothetical mechanisms of ion transport across membranes. Ions are indicated by circles. (a) Diffusing carriers. (b) Rotating carriers. (c) Sliding carriers. (d) Propelled carriers, (e) Membrane vesiculation.

SOURCE: Redrawn from J. F. Sutcliffe. 1962. *Mineral salts absorption in plants*, p. 43. New York: Pergamon Press.