PHYSICO-CHEMICAL FACTORS AFFECTING TRANSLOCATION AND DISTRIBUTION OF XENOBIOTICS IN PLANTS

by

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ABSTRACT

An existing technique using Ricinus communis for studying the translocation of xenobiotics in phloem was modified in order to obtain a quantitative description of the factors controlling this translocation as well as the distribution of xenobiotics in the plant. The non-ionised chemicals tested, three O-methylcarbamoyloximes (including aldicarb sulphone and oxamyl) and three phenylureas, spanning a range of lipophilicity of log K_{ow} = -0.57 to +2.27, all entered the phloem freely. However, only the more polar chemicals were sufficiently retained in the phloem for translocation over long distances to occur, indicating that polar chemicals cross cell membranes more slowly than chemicals of intermediate lipophilicity; these findings substantiate the 'intermediate permeability hypothesis' of phloem translocation of non-ionised xenobiotics. However, the amount of chemical reaching or retained in the sink tissues, especially in the root, was very small even for the best phloem-translocated of the non-ionised chemicals tested.

Studies of the phloem translocation of a series of phenoxyacetic acids (pKa $\sim$ 3.0) spanning a range of lipophilicity from log K_{ow} (undissociated acids) 0.06 to 4.5, of flamprop (pKa = 3.7, log K_{ow} = 2.9) and maleic hydrazide (pKa = 5.6, log K_{ow} = -0.6) showed that maleic hydrazide and the phenoxyacetic acids of intermediate lipophilicity (log K_{ow} $\sim$ 2.0) were the compounds best translocated. In contrast to the polar non-ionised chemicals, these acids were strongly accumulated in leaf phloem and were efficiently phloem-translocated from treated leaves to other parts of the plant, although only maleic hydrazide was found in appreciable amounts in roots. The most polar phenoxyacetic acids accumulated in leaf phloem to a lower extent than the phenoxyacetic acids of intermediate lipophilicity; the most lipophilic phenoxyacetic acid studied was strongly retained at the site of application i.e. the hollow petioles of the mature leaves,

due to strong partitioning onto the tissue solids. The extent to which the weak acids accumulated in leaf phloem could be reasonably well explained by the 'ion trap' mechanism of accumulation of weak acids in plant compartments of higher pH. The extent of accumulation varied with the pKa of the acids and with the ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of the acids. This ratio of permeabilities for each acid was estimated in barley root cells after measuring the accumulation of the acids by roots of intact barley plants from buffered nutrient solution and appeared to be related to the lipophilicity of the acid (except for flamprop), with maximal values for the weak acids of intermediate lipophilicity; the value for flamprop was much lower than those for the phenoxyacetic acids of similar lipophilicity, and this was consistent with the much lesser accumulation of flamprop in the leaf phloem of R. communis. These findings indicate that lipophilicity is not the sole factor determining the ratio of the permeabilities of cell membranes to the undissociated and dissociated forms of weak acids, but the extent to which weak acids accumulate in leaf phloem does appear to be explicable in terms of this ratio of permeabilities and the pKa of the acids.

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LUIZ and ODILA

1. GENERAL INTRODUCTION

1.1. Introduction

Although the development of modern synthetic pesticides has provided acceptable solutions for many crop protection problems, there are pests and diseases not yet satisfactorily controlled. Many of the outstanding problems in crop protection involve the control of soil-borne pests and diseases; such control is difficult because, in order to ensure an adequate concentration of the chemical in the root zone, relatively large amounts of the chemical need to be incorporated into the soil in an 'overkill' approach, which can be costly, wasteful and environmentally undesirable. Thus, for controlling these pests and diseases, there are clear advantages in having active compounds which would be phloem translocated downwards in plants and could therefore be applied to the foliage of crops. Quoting Edgington (1981): 'The discovery of fungicides that are translocated downwards in the phloem to control root-invading pathogens is sorely needed'.

The discovery of effective phloem-mobile fungicides, insecticides and nematicides has been a major goal of pesticide research for many years but progress has been slow, probably because of a general lack of understanding of the factors affecting the uptake into phloem and subsequent translocation of xenobiotics, and because of the paucity of experimental techniques."

A number of xenobiotics have been reported to be translocated in phloem, most of which are either polar non-ionised chemicals such as oxamyl and aminotriazole, or weak acids such as phenoxyacetic acid analogues and maleic hydrazide. It has been postulated that these xenobiotics are translocated in phloem as a result of their physico-chemical properties, although this idea has not yet been confirmed and thus has not been unanimously accepted. If this postulate were

confirmed and the physico-chemical properties required of a xenobiotic to be phloem translocated could be identified in detail, the search for new effective phloem-mobile pesticides would be helped.

1.2. Literature review

1.2.1. Pathways of solute translocation in plants

Two main pathways exist for the translocation of solutes in plants. One of these comprises the extra-protoplasmic continuum (apoplast) of the plant, including transfer over short distances through the intercellular spaces and over long distances through the xylem vessels. The second pathway comprises the cytoplasmatic continuum (symplast) of the plant, including cell-to-cell transfer over short distances via plasmodesmata and over long distances through the phloem sieve cells. The predominant direction of flux within this system is usually from photosynthetically active structures, which serve as bulk sources of concentrated solutes such as carbohydrate, to sink regions of various kinds in which these solutes are consumed in growth or in establishment of new reserves (Crafts and Crisp, 1971; Pate, 1975; Moorby, 1981).

The xylem vessels consist of non-living cells, the walls of which form long uninterrupted tubes, which carry the transpiration stream from the roots to the leaves. Solutes are dissolved in the water and are distributed over the plant shoot. The suction effect caused by the transpiration at the leaf surface is the main driving force for xylem transport. It is generally agreed that movement of water and solutes through the epidermis and cortex of the roots takes place primarily by mass flow and diffusion through the apoplast. Apoplastic movement through the endodermis is prevented by the development of the Casparian strip in the walls of the endodermal cells. This strip is formed by the deposition of a band of suberin and lignin in the cell walls and appears to reduce the permeability of the walls to water and

solutes since it forms a barrier on all but the tangential walls. The physiological implication of these structural changes is that they ensure that materials only enter the xylem after movement through the symplast of the roots (Richardson, 1976; Moorby, 1981).

In contrast to xylem transport, the mechanism of phloem transport is still the subject of some controversy. There are many hypotheses about the possible mechanism of transport in this system, but the most widely supported (Richardson, 1976; Christ, 1979) is Munch's mass flow hypothesis. Munch (1930) suggested that there is an osmotically generated flow of solution through the phloem cells. This hypothesis assumes that there is a high concentration of sucrose in the phloem in the source (developed leaves) and that the plasmamembrane of the phloem cells is semipermeable. Water therefore moves osmotically into the phloem cells from the surrounding tissues and, because the cell walls prevent unlimited cell expansion, a high hydrostatic pressure in the phloem cells is established. At the same time, a lowering of the concentration of sucrose in the sink regions (fruits and growing tissues of the shoots and roots), where the assimilates are stored or consumed, results in a lowered hydrostatic pressure. Because of the gradients of hydrostatic pressure so created, there will tend to be a mass flow of solution and dissolved solutes from the source to the sink via the phloem, now accepted to be via the phloem sieve cells.

The search for alternative mechanisms to explain phloem transport has arisen for two reasons. Firstly, the degree to which the sieve plates are porous is questioned and, secondly, certain observations have seemed to invalidate Münch's model (Milburn, 1975). Spanner (1958) suggested an electro-osmotic system, metabolically maintained, to facilitate movement of water and solutes through the sieve pores. Thaine (1961; 1969) proposed a mechanism of phloem transport involving protoplasmatic streaming associated with trans-

cellular strands, these strands being observed to traverse the sieve plates in the phloem of some species. The possible mechanisms of phloem transport have been discussed recently by Moorby (1981).

On the basis of the symplast-apoplast concept for describing the translocation of solutes in plants, Crisp (1972) defined a symplastic pesticide as one that can be absorbed by the plant, pass through the plasmalemma into the living symplast, diffuse from one cell to another through plasmodesmata, and move along with the assimilate stream in the phloem to other areas of the plant. He also defined an apoplastic pesticide as one that can be absorbed by the plant and is almost exclusively transported by the transpiration stream within the apoplast continuum. Peterson and Edgington (1976) gave the term ambimobile to pesticides that can be transported in both systems.

1.2.2. Uptake and translocation of non-ionised chemicals in plants.

Previous studies have established that translocation of non-ionised chemicals to the shoots, following uptake by roots, is affected by the lipophilicity of the chemicals (Crowdy, 1973; Shone and Wood, 1974; Briggs et al., 1976; 1982).

The efficiency of translocation of chemicals to the shoots following uptake by roots from nutrient solution has been conveniently described by the Transpiration Stream Concentration Factor (TSCF) (Russell and Shorrocks, 1959) defined as:

$$TSCF = \frac{\text{Concentration in transpiration stream}}{\text{Concentration in external solution}}$$

These authors introduced this concept to describe the extent to which nutrients are transferred from nutrient solution ~~solution~~ to plant shoots. Shone and Wood (1974) and subsequently other authors have adopted this concept to express the efficiency of translocation of xenobiotics. The concentration in the transpiration stream is usually assessed indirectly from the amount of chemical accumulated in the

shoots for a known volume of water transpired. The TSCF has a maximum value of 1.0 for passive uptake and, as measured by this method, it is independent of time if loss of accumulated chemical due to metabolism is taken into account (Shone and Wood, 1974).

Similarly, Shone and Wood (1974) expressed the extent to which a chemical is accumulated in roots as the Root Concentration Factor (RCF) where:

$$RCF = \frac{\text{Concentration in roots}}{\text{Concentration in external solution}}$$

These authors found that the RCF of some non-ionised herbicides in barley roots were correlated with their partition coefficients in olive oil / water or n-dodecane/water systems and, as with their TSCF's, were greater for lipophilic than for polar chemicals. Both RCF and TSCF were shown to be independent of the external concentration for dilute solutions.

Using series of O-methylcarbamoyloximes and substituted phenylureas spanning a wide range of lipophilicity assessed using log K_{ow} (logarithm of the octan-1-ol/water partition coefficient), Briggs et al. (1982) identified in more detail the effect of lipophilicity of non-ionised chemicals on their accumulation in barley roots and translocation to the shoots. Accumulation in roots was ascribed to two processes:- partitioning of chemicals onto lipophilic root solids and uptake into the aqueous phase in roots contained in the intercellular space and within the root cells. The partitioning process was shown to be dominant for lipophilic chemicals, giving rise to large RCF values. Although the second process was thought to occur for all chemicals, it could be clearly demonstrated only for polar chemicals, which partition weakly to root solids, implying that these chemicals are able to penetrate the cell membranes. Translocation to shoots was maximal for compounds with intermediate lipophilicity i.e. with log

$K_{ow} \sim 2.0$; this was ascribed to the effect of lipophilicity of the chemicals on their permeation rates through the membranes of the endodermal cells, with highest permeation rates for chemicals of intermediate lipophilicity.

Barret and Ashton (1983) monitored the uptake of ^{14}C-napropamide by root segments of maize over a period of 8 h and separated the total influx into an exchangeable fraction (component that eluted from the tissue) and a non-exchangeable fraction. The overall influx was attributed to an initial passive diffusion supplemented with time by binding of napropamide to cellular components. However, this interpretation should be viewed with caution since the authors neglected the effects of metabolism of napropamide, and the samples were simply oxidized to release $^{14}CO_2$ which was trapped and counted. In an earlier paper (1981), the authors reported that, following root uptake for 8 h, about 40% of the total radioactivity in the roots was not extracted and so was not napropamide. Therefore, assuming that the radioactivity not extracted was due to retention of napropamide metabolites in the tissue, the non-exchangeable fraction might have been a result of metabolism of napropamide rather than binding of the parent chemical to cellular components.

Briggs et al. (1983) established relationships between lipophilicity of non-ionised chemicals and their distribution in barley shoots following uptake by roots. The authors ascribed the accumulation of the chemicals in the leaf bases of barley (referred to as the stem) to a reversible partitioning of chemical between the stem tissue and the xylem sap. Constant, maximum concentrations in stems were rapidly attained for chemicals of $\log K_{ow} < 3$, but only more slowly for more lipophilic ones. Only after equilibrium was achieved between chemical entering the stem in the xylem and the whole of the stem was chemical efficiently transported to the leaves. By analogy with the RCF, the authors defined a

Stem Concentration Factor (SCF) as:

$$SCF = \frac{\text{Concentration in stem}}{\text{Concentration in external solution}}$$

"The SCF remained essentially constant for at least 96 h, implying that any loss of chemical by degradation in the stem was made good by rapid equilibration with the xylem sap."

"Uchida (1980) showed that the distribution of dialkyl 1,3-dithiolan-2-ylidenemalonates between the upper and lower parts of the shoots of rice plants was determined by lipophilicity, the more lipophilic chemicals being more strongly retained by the lower parts of the shoots."

"Perfusing stem sections with solutions of fungicides, Barak et al. (1983b) observed that the more polar chemicals broke through before the more lipophilic ones." "Woody stems of older cotton and pepper plants retained the chemicals more strongly than younger ones and these differences were ascribed to the greater lignin content of woody tissues." In previous paper, Barak et al. (1983a) found significant correlation between the extent of the adsorption of the chemicals on the lignins extracted from three different plant species and the log K_{ow} of the compounds.

Hussain et al. (1974) examined the translocation of O,S-dialkyl N-alkyl-phosphoroamidothioates applied on cotton leaf petioles towards the corresponding leaf blades and obtained a parabolic relationship between movement and hydrophilicity assessed as log K_{ow}, although steric effects were also significant for N-alkyl substituents.

The translocation characteristics of several non-ionised pesticides, following foliar application, have been reported.

In early studies, whole plant autoradiography, following application of ^{14}C-labelled chemicals to different plant parts, was used to assess translocation patterns of test compounds (Crafts, 1967; Crafts and Crisp, 1971). Based on his own work using this technique and on the results reported by other workers, Crisp (1972) concluded that organo-

phosphorus and carbamate insecticides should move away from the site of application only by an apoplastic route.

Although the autoradiography technique has provided useful information about translocation patterns of test compounds, it does not give either quantitative data on movement or any information about metabolism of the chemicals, which may lead to misinterpretation of the results. To overcome this difficulty, methods that involve radiochemical analysis of the plant tissues for the parent chemical have been more widely used in translocation studies, though autoradiography remains a useful technique providing the chemical under test is sufficiently stable in the test plant.

Kirby (1972) reviewed the progress made in studying the movement of fungicides in plants and concluded that movement of these compounds is acropetal (apoplastic) and never the reverse. Export of systemic fungicides, via the phloem, from leaves to other organs has usually not been found, and in the few cases of symplastic transport from leaves and cotyledons reported hitherto (Solel et al., 1973; Wallerstein et al., 1976; Martin and Edgington, 1981), the amounts involved were very small.

Although the majority of existing non-ionised pesticides are not translocated in phloem, two polar chemicals, oxamyl and aminotriazole, have been reported to be phloem-mobile.

Studying the translocation of the nematicide oxamyl in potato plants, Peterson et al. (1978) observed some movement of the chemical from treated mature leaflets to the rest of the plant, including apex, roots and other untreated mature leaflets. These results indicated that oxamyl is translocated out of the treated leaf with assimilates, and leakage out of the phloem to the transpiration stream resulted in transport of the chemical to other untreated leaflets. Martin and Edgington (1981) treated young barley and soybean plants with oxamyl and, although phloem transport of oxamyl was confirmed, the results were somewhat

variable between the two species. Oxamyl exhibited primarily apoplastic transport (movement above the treated area within the treated leaf). In barley, from the total amount translocated after 3 days, only 2.4% was found in parts of the plant other than the treated leaf. In soybean, the value was 14.6% but only 3.3% was found in the roots. Wright et al. (1980) found only very small amounts of oxamyl in cucumber roots following foliar application though nematode control was observed, leading these authors to suggest that the oxamyl could not have been uniformly distributed throughout the roots but must have been concentrated at the nematode feeding sites.

Crafts (1967), using an autoradiography technique, reported that aminotriazole (Amitrole) was found in appreciable amounts in roots of young soybean plants following application to cotyledon, epicotyl or primary leaves. However, although Chamberlain et al. (1984) found small amounts of the parent compound in phloem exudate from the stem of R. communis following application of the chemical into the petiole of a mature leaf, only 20% of the total radioactivity measured in the exudate was parent compound (Dr. Keith Chamberlain, personal communication). Furthermore, Massini (1963) observed that 24 h after application of ^{14}C-aminotriazole to a leaf of French dwarf bean or tomato seedlings, most of the radioactivity extracted from the plant was in the form of β (3-amino-1,2,4-triazolyl-1-)∝-alanine; when bean plants were treated with this metabolite by a leaf injection method, the distribution of the radioactivity was comparable to that found in similar experiments with aminotriazole. Thus, translocation studies of aminotriazole in which the plant tissues are not analysed for the parent chemical should be viewed with caution.

1.2.3. Uptake and translocation of weak acids in plants.

1.2.3.1. Uptake of weak acids by cells and plant tissues.

The uptake of weak acids by plant cells has been of great interest because the plant hormone indol-3-yl acetic acid (IAA) as well as several weak acid herbicides have been shown to accumulate in plant cells against a concentration gradient.

There is known to be a passive component in the uptake of IAA and other weak acids (2,4-dichlorophenoxyacetic acid (2,4-D), benzoic acid and naphthylacetic acid) into single-celled algae and/or suspensions of tissue cells, and this has been ascribed to the diffusion of the undissociated acid across the cell membranes (Albaum et al., 1937; Wedding and Erickson, 1957; Rubery and Sheldrake, 1973; Raven, 1975).

Uptake by the diffusion mechanism is linearly related to the concentration of the undissociated acid, which is itself a function of the pKa of the acid and the pH of the surrounding medium. Assuming that undissociated molecules are the only permeant species, the equilibrium distribution of a weak acid between cells and surrounding solution is given by (Davson and Danielli, 1943; Wedding and Erickson, 1957; Rubery and Sheldrake, 1973):

$$\frac{\text{total concentration of acid inside the cells}}{\text{total concentration of acid outside the cells}} = \frac{1+ \text{antilog } (pH_{inside} - pKa)}{1+ \text{antilog } (pH_{outside} - pKa)}$$

When the pH of the surrounding solution is lower than that of the cells, the cells accumulate weak acids; the equation requires that the concentration of the undissociated acid should be the same inside and outside the cells.

Studying the effect of the bathing solution pH over the range from 4 to 7 on the uptake of 2,4-D by cells of the alga Chlorella pyrenoidosa, Wedding and Erickson (1957) found that uptake was not linearly correlated with the proportion of undissociated molecules in the bathing solution. It was suggested that 2,4-D anions also penetrate the cell but at a rate considerably slower than the undissociated molecules. By incorporating a term for the permeability of the cell membrane to 2,4-D anions in the above equation, the authors observed that the uptake

of 2,4-D over the pH range from 4 to 7 agreed well with predicted values, the permeability of the cell membrane to 2,4-D molecules being about 1000 times as great as the permeability to 2,4-D anions.

The protoplasm of plant cells is electrically negative with respect to the bathing solution and so, if the weak acid anion can indeed penetrate through the cell membranes , net anion efflux will occur down the gradient of electrochemical potential thus reducing accumulation of the acid ($AH + A^-$) inside the cells (Raven, 1975). Flux equilibrium is achieved when total influx ($AH + A^-$) is equal to total efflux, and accumulation is described by the following equation (Raven, 1975):

$$\frac{[AH]_i + [A^-]_i}{[AH]_o + [A^-]_o} = \frac{(1+10^{pH_i - pKa})\left(\frac{P_{AH}}{P_{A^-}} + \frac{FE/RT}{1 - e^{-FE/RT}} \cdot 10^{pH_o - pKa}\right)}{(1+10^{pH_o - pKa})\left(\frac{P_{AH}}{P_{A^-}} + \frac{FE/RT}{1 - e^{-FE/RT}} \cdot 10^{pH_i - pKa} \cdot e^{-FE/RT}\right)}$$

where P_{AH} and P_{A^-} are the permeabilities of acid and anion respectively, E is the membrane potential ($E_i - E_o$) in volts, F is the Faraday constant (96479 coulombs $mole^{-1}$), R is the gas constant (8.31 joules $^oK^{-1}$ $mole^{-1}$), and T is the temperature (^oK). The equation is also used to calculate the vacuolar concentrations of AH and A^- by sequential application to the plasmalemma and tonoplast. Studying the uptake of IAA by cells of the alga Hydrodiction from bathing solution, Raven (1975) measured concentrations of the auxin in the vacuole that agreed with calculated concentrations obtained using known values for the vacuolar pH and potentials across the membranes, and assuming the cytoplasm pH equals to 7 and a P_{IAAH}/P_{IAA^-} of 10^3.

The transmembrane transport of IAA and 2,4-D anions by cells of higher plants has been shown to involve also a carrier-mediated component for influx and efflux (Rubery and Sheldrake, 1974; Rubery, 1977; 1978; Raven and Rubery, 1982), which has been suggested to

underlie polar transport of these chemicals. However, the ability of cells to accumulate weak acids is not dependent on an uptake carrier, and kinetic studies provided no evidence for the participation of carriers in the transmembrane transport of benzoic or naphthylacetic acids (Rubery, 1977).

Darmstadt et. al. (1983) studied the absorption of 2,4-D by maize root protoplasts from bathing solution at two pH's. 2,4-D accumulated to twice and 16 times the external concentration at pH 6.5 and 4.5, respectively. Predictions of theoretical 2,4-D concentrations in protoplasts (assuming that only the undissociated molecules permeate through the cell membranes) gave concentrations about twice lower at pH 6.5 and three times higher at pH 4.5 than those observed. It was suggested that deviations between predicted and observed concentrations in the protoplasts were the result of transmembrane movement of 2,4-D anion by diffusion or by a saturable carrier.

Uptake of weak acids by roots and stem segments have also been investigated by a number of authors. Foy and Yamaguchi (1964) found rapid uptake of 2,4-D by excised barley roots over an initial period of 30 minutes, followed by loss back to the solution. It was suggested that accumulation during that period was mainly due to an adsorption process and that, since 2,4-D could be leached out, it was apparently held by rather weak binding forces. Donaldson et. al. (1973) studied the absorption of 2,4-D from nutrient solution by excised barley roots and, by washing the roots for one hour following absorption, divided total absorption into two phases, exchangeable and non-exchangeable. It was suggested that 2,4-D is taken up by the roots by an adsorption mechanism and that energy is required to maintain the integrity of the adsorbing surfaces of the cells. However, 2,4-D was still being lost after 1 h, and the authors suggested that all 2,4-D in the root might be slowly exchangeable and

a single mechanism of uptake might be involved. The studies mentioned above, dealing with uptake of 2,4-D by root systems, did not take into account the effect of the pH of the external nutrient solution.

Studying the uptake of some herbicides by barley roots, Shone and Wood (1974) found that accumulation of 2,4-D in roots is dependent on the pH of the external nutrient solution. Concentrations of 2,4-D in roots were 88 and 8 times higher than that in the nutrient solution at pH 4.0 and 6.5 respectively. Uptake at pH 4.0 was reduced at low temperatures and by sodium azide, which led the authors to suggest that uptake of 2,4-D is influenced by metabolism. No attempt was made to explain the differences in RCF values at pH 4.0 and 6.5 in terms of different permeation rates of dissociated and undissociated forms through cell membranes.

Zsoldos and Haunold (1979) found a similar effect of the nutrient solution pH on the uptake of 2,4-D by excised wheat roots, although the results were not expressed in terms of RCF values. The authors suggested that 2,4-D enters the root cells rapidly at pH 4.0 and mostly as the undissociated molecules; however, the magnitude of the observed accumulation was not further analysed.

Uptake of weak acids by segments of stem has also been shown to be dependent on the pH of the bathing solution. Saunders et al. (1965a) found that stem segments of pea (Pisum sativum) accumulate 2,4-D at concentrations much higher than that in the bathing solution. Accumulation was higher the lower the pH and, based on the work of Wedding and Erickson (1957) mentioned earlier, the authors suggested that the greater permeability of the cell membranes to the undissociated molecules than to the anions of 2,4-D could have accounted for the results obtained. Studying the uptake of a series of chlorinated phenoxyacetic acids by stem segments of cotton (Gossypium hirsutum), Saunders et al. (1965b) found large differences in rates of uptake of these chemicals

from solutions buffered at pH 5.5. It was suggested that the acids which possess pronounced auxin-like properties are accumulated largely by a mechanism which is different from that responsible for the uptake of the less active members of the group. In a subsequent paper from the same laboratory, Venis and Blackman (1966) measured the uptake of benzoic and 2-chlorobenzoic acids by stem segments of pea (P. sativum) over a range of pH from 4.0 to 6.5. Uptake was higher the lower the pH, although uptake was not entirely correlated with the concentration of the undissociated molecules in the bathing solution.

The fact that certain chlorinated phenoxyacetic and benzoic acids accumulate in stem segments led Smith (1972) to investigate the importance of an active transport system for 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) accumulation. Parenchyma tissue from potato tubers was treated with inhibitors of oxidative phosphorylation and of membrane-bound ATP. Accumulation of 2,4-D and 2,4,5-T was shown to be independent of available energy as influenced by these inhibitors. Lecithin-treated tissue accumulated the chemicals to a larger extent, which was ascribed to possible binding of the herbicides to the lecithin moiety. Peterson and Edgington (1976) found that 2,4-D was concentrated about sixteen-fold in potato tuber discs from a bathing solution at pH 5.3, but no attempt was made to explain this result.

1.2.3.2. Translocation in whole plants.

Most investigations into the translocation of weak acid pesticides in whole plants have been carried out following foliar application of the chemicals.

Crafts (1967) investigated the translocation of several ^{14}C-labelled herbicides applied either to a primary leaf, epicotyl or cotyledon of young soybean plants using autoradiography to detect the chemicals. Applied to one cotyledon, 2,4-D moved acropetally through the stem to the expanding trifoliate leaf and shoot tip in moderate

concentration, and through the hypocotyl to the roots in higher concentration. Applied to the epicotyl, 2,4-D moved efficiently both acropetally and basipetally; in both cases, both primary leaves were completely bypassed. Applied to one primary leaf, 2,4-D moved to the trifoliate leaf, the shoot apex and the roots; the opposite primary leaf was bypassed. When maleic hydrazide was applied to one cotyledon, epicotyl or primary leaf, both cotyledons, both primary leaves and all trifoliate leaves were labelled; by autoradiography, the treated organs were labelled intensely, the trifoliate leaves and shoot apices were strongly labelled, and the untreated cotyledons and primary leaves were weakly labelled. It was concluded that 2,4-D is distributed via the symplast exclusively, and maleic hydrazide is distributed principally via the symplast, but a small amount migrates to the xylem and moves in the transpiration stream. When the primary leaf was treated with 2,4-D, the observed darkening of the stem in the autoradiograph led the author to suggest that the chemical is lost along the assimilate path and is strongly retained in the adjacent stem tissue.

It is generally accepted that movement of foliage-applied weak acid herbicides occurs in the phloem along with the assimilates. Radwan et al. (1960) demonstrated by autoradiography that the phloem is the path of movement of foliage-applied ^{14}C-labelled 2,4-D in Vicia faba. Pickering (1965, mentioned by Robertson and Kirkwood, 1970), investigated the foliar penetration pathways of 2,4-D by autoradiography and found that it readily entered the symplast of cotton and bean leaves, where it became highly concentrated in the protoplasm, subsequently leaking into the vacuoles and surrounding apoplast.

According to Robertson and Kirkwood (1970), weak acid herbicides appear to move in phloem as parent compound and not chemically-bound to sugars. The proportion of herbicide molecules ultimately reaching the sink tissues is influenced by the extent of accumulation in parenchyma adjacent to the sieve tubes and also by fixation at, what he called,

other metabolically non-active sites.

Hallmén and Eliasson (1972) studied the uptake, translocation and metabolism of ^{14}C-labelled 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2,4-D in seedlings of wheat. The uptake of the herbicides through the upper surface of the first leaf was slow but was almost complete after nine days. Picloram was translocated into the stem and untreated leaves to a greater extent than 2,4-D. Only small fractions of the activity were recovered from the roots and from the nutrient solution. Picloram and 2,4-D formed water-soluble conjugates in the tissues and 2,4-D was also incorporated into an insoluble fraction comprised of cell walls and proteins. The percentage of the total activity identified as the parent acids in the treated leaf decreased rapidly and was less than 20% after 3 days. The fact that the activity that translocated out of the treated leaf remained unchanged from 1 to 9 days led the authors to suggest that the chemicals were translocated as the parent acids. Nevertheless, no stimulation of translocation as a result of cycloheximine pretreatment was observed in spite of the fact that the level of free 2,4-D was substantially increased in the treated leaves of the test plants.

Long and Basler (1974) studied the translocation of 2,4,5-T and 2,4-D in bean (P. vulgaris) seedlings. ^{14}C-labelled chemicals were injected into the centre of the stem at the cotyledonary node with a 1-µl syringe. Considerable basipetal as well as acropetal translocation of the herbicides was observed from the site of injection. Acropetal translocation of 2,4,5-T to primary leaves was very rapid for the first 2 h after treatment, and during the following hours the ^{14}C disappeared from the primary leaves. Acropetal translocation of 2,4,5-T to the young shoots was slow but continuous up to 24 h after injection. It was suggested that 2,4,5-T rapidly entered the primary leaves via the transpiration stream and was subsequently exported in phloem with the assimilates to the young shoots. The translocation pattern of 2,4-D was very similar to that of 2,4,5-T. From the results presented by

the authors, less than 5% of the injected radioactivity was found in the roots 72 h after application but a considerable fraction (about 30%) was found in the nutrient solution. Unfortunately, however, the radioactivity found in the nutrient solution and in the plant tissues was measured without separation of the parent chemicals from possible metabolites.

Comparing the translocation of several xenobiotics in seedlings of soybean and barley following foliar application of the chemicals, Martin and Edgington (1981) found apoplastic and symplastic movement of 2,4-D, although the results varied somewhat between the two species. From the total amount translocated out of the treated area 6 h after application, the proportion moved in the apoplast (above treated area in the treated leaf) in barley and soybean were 34.9 and 4.1% respectively. At that time, most of the 2,4-D that moved in the symplast (below the treated area and remainder of the plant) was found in the lower part of the treated barley leaf and in the stem of soybean, which was attributed to possible binding of 2,4-D to cellular components.

Crisp and Larson (1982) investigated the effect of ring substituents on translocation and metabolism of ^{14}C-labelled phenoxyacetic acid and six analogues in soybean, following application at three different sites:- primary leaf, cotyledon and epicotyl. Translocation was assessed by determining the concentration of the parent acids and metabolites in different parts of the plants. Following application to the primary leaf, the most phloem-mobile compounds were 4-fluoro- and 4-chlorophenoxyacetic acids, though less than 8% of the applied amount was found in roots 48 h after application. 2,4-D and 2,4,5-T were phloem translocated to a lesser extent and there was no significant phloem transport of 2-methyl-4-chlorophenoxyacetic, 2-chlorophenoxyacetic and phenoxyacetic acids, though these acids were almost completely metabolised during the experiments. Similar results were obtained

from cotyledon and epicotyl treatments. It was suggested that mono-para-halo substituted phenoxyacetic acids are more phloem mobile because they are not inactivated by rapid metabolism or by binding to receptor proteins for plant growth regulators.

Translocation of 3,5-D and 2,4-D after foliar application was investigated by Burrell (1982) in potato plants. 3,5-D was translocated to the tubers faster and metabolized less than 2,4-D. When the same amounts of 3,5-D and 2,4-D were applied to leaves, 10% of the applied dose of 3,5-D reached the tubers after 4 days compared with 2% of 2,4-D. The proportion of the applied 3,5-D translocated to the tubers increased 5-fold over the first 8 days of the experiment; this increase paralleled the increase in tuber growth. It was suggested that translocation of 3,5-D to the tubers is most probably associated with the flow of carbohydrate.

The translocation of weak acids in whole plants, following uptake by roots, has been studied to a lesser extent than it has been following foliar application.

Studying the translocation of several herbicides and a systemic fungicide to shoots of barley seedlings following uptake by roots from nutrient solution, Shone and Wood (1974) found that, in contrast to the non-ionised chemicals, the concentration of 2,4-D in the transpiration stream was several times higher than that in the nutrient solution at pH 4.0. The TSCF values at pH 4.0 and 6.5 were, respectively, 3.12 and 0.14. 10^{-3}M sodium azide was observed to reduce the TSCF at pH 4.0 to unity, which suggested that accumulation of 2,4-D was influenced by metabolism.

The translocation pattern of root-absorbed ^{14}C-labelled picloram in young soybean plants was investigated by Gaudiel and Vanden Born (1979). They showed by means of a stem girdling technique that picloram applied to roots was rapidly transported via the xylem, mainly to the

primary leaves. These leaves almost immediately exported most of the chemical again via the phloem, primarily to the young apical leaves. After 4 h uptake from nutrient solution, no change in distribution of radioactivity took place in plants left for periods of up to 2 days. Amounts of root-absorbed picloram translocated from shoots back to the roots in split-stem plants were extremely small. By using a micro-antoradiography technique, O'Donovan and Vanden Born (1981) studied further the distribution of ^{14}C-labelled picloram in soybean following root uptake. The results indicated that the radioactivity retained in the roots appeared to be associated mainly with the protoplasm of the cortical cells. In the stem, radioactivity was present in both the xylem and phloem with comparatively little movement into the other tissues. Considerably more radioactivity was present in the younger apical leaves than in the older primary leaves. In the primary leaves the radioactivity was present only in the xylem whereas in the apical leaves it was present both in phloem and xylem. It was suggested that the apparent absence of ^{14}C-labelled picloram in the phloem of the primary leaves is a result of a rapid loss of the chemical through export to the apical leaves.

1.2.4. Uptake and translocation of cationic and zwitterionic chemicals

Price et al. (1975) studied the xylem and phloem transport of 1-methyl pyridinium chloride and related compounds applied to wheat leaves. The chemicals were applied midway on the leaf blade and the acropetal xylem and basipetal phloem movement were measured. The 1-methyl pyridinium cation and two related cationic analogues had little xylem mobility and moderate phloem mobility, but were strongly retained in the treated area. Retention was suggested to occur inside the cells due to the negative electrochemical potential favouring the cell accumulation of cations. Less retention in the treated area, which resulted in greater phloem and xylem mobility, was achieved by adding a carboxyl

group in the para position or a sulphonic acid group in the meta position of the pyridinium ring. These structures form zwitterions, and their lesser retention inside the cells than 1-methyl pyridinium was ascribed to their zero net charge at physiological pH.

Radioactive glyphosate [N-(phosphonomethyl) glycine] was reported by Sprankle et al. (1975) to be absorbed by leaves of quackgrass (Agropyron repens) and readily phloem translocated to untreated shoots and rhizomes. When the chemical was applied to one leaf of a single shoot, about 60% of the translocated radioactivity was found in the rhizome and in other untreated shoots. Martin and Edgington (1981) observed that glyphosate was very efficiently phloem translocated in barley and potato plants. Its pattern of transport resembled that of sucrose and, from the total amount translocated from the treated leaf, most was found in the roots. Uptake of the chemical by potato tuber tissue was slow while uptake and translocation were rapid, which was ascribed to possible active mechanisms of uptake in intact plants because of the structural similarities of the chemical with aminoacids. Unlike 2,4-D, glyphosate was shown not to accumulate against a concentration gradient in potato tuber tissue. It was suggested that the slow movement through cell membranes, as shown by the uptake of the chemical by potato tuber tissue, might be associated with the retention of glyphosate in phloem and consequent transport with assimilates. It should be noted that in these studies, metabolism of glyphosate was not assessed and the radioactivity measured in untreated parts of the plants was interpreted as being the parent chemical.

1.2.5. Hypotheses describing translocation of xenobiotics in phloem

1.2.5.1. Non-ionised chemicals

As mentioned earlier, apoplastic chemicals are characterised by being transported in the direction of the transpiration stream with long distance transport occurring in the xylem. Chemicals exhibiting

an apoplastic pattern of transport were thought to be unable to penetrate the semi-permeable plasmalemma of the symplast and thus to be confined to the apoplast (Crisp, 1972). However, several independent studies have shown that the symplast is freely accessible to many chemicals, including those that move in the apoplast but not in the symplast.

Donaldson et al. (1973) found that the apoplastic chemical monuron was taken up by barley roots in amounts indicating that the concentration of the chemical inside the cells was similar to that in the bathing solution. Peterson and Edgington (1976) examined the uptake of several apoplastic pesticides by potato tuber slices and found that the chemicals rapidly diffused into and out of the cells, attaining the same concentration inside the cells as in the bathing solution. The question raised from these findings was:- if some apoplastic pesticides are present in the symplast of the plant, why do they not show evidence of symplastic transport? Peterson and Edgington (1976) suggested that it is because they are not retained by the symplast and, since transport in the transpiration stream in the apoplast is much more rapid (and usually occurs in the opposite direction), the pesticide tends to move in the direction of the transpiration stream.

Peterson et al. (1978) explained the translocation of oxamyl from a treated mature leaf to other untreated parts of potato plants (including apex, roots and other mature leaves) as being due to the slow rate of oxamyl movement through cell membranes; this rate was estimated following uptake of oxamyl by potato tuber discs from solution and compared with those obtained with other apoplastic pesticides. It was suggested that once inside the phloem in the treated leaf, the oxamyl is retained in the phloem for a long enough time to be transported out of the treated leaf with the assimilates.

In a subsequent paper, Tyree et al. (1979) termed this idea the intermediate permeability hypothesis and made some theoretical

assumptions to explain the phloem mobility of certain xenobiotics that are not weak acids, based on their slow permeation rates through cell membranes. It was suggested that there is an optimum permeability that permits greatest circulation through the symplast and apoplast (including phloem and xylem) of plants. The optimum permeability was large enough to allow substantial passive permeation into phloem sieve cells in the source leaf and yet was small enough to retain the chemical sufficiently in the phloem to permit phloem transport. The optimum permeability was determined by the velocity of sap flow in sieve cells, the radius of the sieve cell, the overall length of the plant and the length of the source region in the leaf. It was argued that the permeability of cell membranes to oxamyl is near the optimum permeability under some experimental conditions.

Collander (1959), working with cells of the alga Nitella mucronata, concluded that the rate of movement of a compound through cell membranes is correlated with its oil-water partition coefficient . Briggs et al. (1976) found that translocation of non-ionised chemicals to shoots of barley, following uptake by roots from solution, is governed by the lipophilicity of the chemicals, with efficiency of translocation (as TSCF) increasing sharply above log K_{ow} = -0.5 to a broad optimum around log K_{ow} = 1.7 and declining sharply above log K_{ow} = 3. These observations led Peterson et al. (1978) and subsequently Edgington (1981) to suggest that polar non-ionised chemicals other than oxamyl should also have slow permeation rates through membranes and thus satisfy the requirements of the intermediate permeability hypothesis of translocation of xenobiotics in phloem. However, this hypothesis has not yet been fully substantiated and currently there is little information on which to assess its applicability to the phloem translocation of polar non-ionised chemicals in general.

1.2.5.2. Weak acids

Reviewing the literature on the phloem transport of natural assimilates, plant growth regulators and herbicides, Crisp (1972) noted that most chemicals known to be phloem-mobile have in common the carboxyl (-COOH) functional group. He suggested that an effective phloem-mobile chemical must be a weak acid. He also suggested that the incorporation of a carboxyl group into a pesticide molecule should result in phloem mobility; this idea was named the acid activation hypothesis. Supporting evidence for this idea was obtained from studies of the translocation patterns of aminocarb (4-dimethylamino-m-tolyl methylcarbamate) and of an acid analogue, 4-(N-dimethylamino)-m-tolyloxy-acetic acid, in soybean. Autoradiography of whole plants, following application of the chemicals to cotyledons or primary leaves, showed aminocarb to be apoplastic whereas the acid analogue was shown to be symplastic.

Other authors have acknowledged Crisp's observations and findings, which have been referred to as the weak acid hypothesis (Tyree et al., 1979; Edgington, 1981 ; Chamberlain et al., 1984).

Crisp (1972) did not attempt an explanation of the role of the carboxyl group with regard to the phloem mobility of weak acids. He only suggested that the incorporation of a carboxyl group into an insecticide molecule would enhance its ability to permeate cell membranes and to enter the phloem. Surprisingly, however, referring to Crisp's work, Tyree et al. (1979) wrote: 'According to this theory, weak acids which are in the undissociated molecular state at low pH are able to diffuse through the plasmalemma; the higher pH of the phloem causes the molecules to dissociate and the polar anions are unable to diffuse back through the plasmalemma. In this way a weak acid xenobiotic can be loaded into the phloem and transported away'. It appears as if Tyree et al. (1979) had in mind some of the work mentioned earlier on accumulation of weak acids by cells. Goldsmith (1977), based on a personal communi-

cation from A. R. Sheldrake, wrote: 'Accumulation of IAA in sieve tubes could be the result of the usually higher pH of these cells compared to the surrounding ones'.

Crisp and Look (1979) studied the long distance phloem transport in soybean plants of 4-chlorophenoxyacetic acid and six closely related but non-acidic analogues, including ethanol, ethylamine, ketone and acetoxy analogues. Only the acid was phloem-mobile and it was suggested that weak organic acids are actively loaded into the phloem sieve tube-companion cell complex. This suggestion was based on the work by Etherton and Rubinstein(1978), whose data on accumulation of α -amino-isobutyric acid in coleoptile cells of Avena sativa fitted an amino acid $-H^+$ carrier mediated co-transport model, in which transport is controlled by both the membrane potential and proton concentration components of the chemical potential difference of protons across the coleoptile cell membranes.

Edgington (1981), who is a co-author of the paper by Tyree et al. (1979), referred to the work mentioned above and wrote: 'Crisp and Look (1979) attribute loading of acids into the phloem to the ion trap theory. At the pH of the apoplast, about 5.5, weak acids are only partly ionised. The molecular form easily diffuses through the plasmalemma of the phloem sieve tube-companion cell complex where it ionises at the alkaline pH of the phloem. Since the anion cannot easily pass back through the lipophilic membrane, it is trapped. Therefore, weak acids accumulate against a gradient in the phloem'.

Thus, it can be concluded that Crisp (1972) first noted the importance of an acid group as part of a phloem-mobile molecule. Further studies by Crisp and co-authors have supported this idea and have assumed that weak acids enter the phloem cells by a carrier-mediated process able to recognise the acid group. For reasons that are uncertain, Tyree et al. (1979) and Edgington (1981) misinterpreted Crisp's idea about entry of weak acids in phloem.

Despite the misinterpretations, from the evidence shown previously on the accumulation of weak acids by cells (1.2.3.1.), the principle of an 'ion trap' for describing the accumulation of weak acids in phloem appears to be correct. Further support to this idea was given by Jacob and Neumann (1982), who studied the phloem mobility of several xenobiotics following application to cotyledons of Sinapis alba. Carboxylic acids as well as maleic hydrazide, a weak acid with no carboxyl group, were translocated in phloem. The partition coefficients of the compounds mobile in phloem were higher at pH 8.0 than at pH 5.0 in contrast to the partition coefficients of the xylem-mobile chemicals which were similar at both pHs. These authors suggested that weak acids are accumulated in the phloem sieve cells by an ion trapping mechanism resulting in efficient translocation of these chemicals in phloem. This suggestion was further substantiated by Neumann et al. (1985), who studied the phloem mobility of a wider range of xenobiotics in S. alba.

1.2.6. Techniques for determining chemical concentrations in phloem sap

It has long been known that phloem exudates can be obtained from cuts in the bark of many species of trees (Zimmermann, 1960) or from cuts in the inflorescent stalks of Yucca flaccida (van Die, 1968) but, for detailed translocation studies of xenobiotics in phloem, the search has been for a suitable plant which will provide phloem exudation under controlled laboratory conditions. Plants of the family Cucurbitaceae will yield a limited quantity of phloem sap from the end of a cut stem or petiole but the exudate gels rapidly (Hall et al., 1971). Phloem exudates can also be collected after cutting the stylar end of pods of legumes (Pate et al., 1974). Hoad (1980) described a method for assessing the phloem translocation of chemicals using pods of lupin (Lupinus albus), which are excised from the plant and their stalks placed in solution containing the compounds under test. However, the virtual absence of a transpiration stream in the pod makes this system a rather

specialised one, which may not represent the ability of test chemicals to be phloem translocated over long distances in whole plants. Other investigators have used aphid stylets for obtaining phloem exudate but, according to Hall et al., (1971), it is a difficult technique to perform. Nevertheless, by using severed stylets of the aphid Tuberolachnus salignus sited on bark strips of willow, Peel (1972) demonstrated that 2,4-D, 2-methyl-4-chlorophenoxyacetic acid, 2,4,5-T, trichloroacetic acid and maleic hydrazide accumulate in the phloem sieve cells against a concentration gradient, for the radioactivity of the stylet exudate exceeded that of the bathing solution. However, because of the lack of phloem or xylem flow in the bark strips, this system does not provide information about phloem translocation of the chemicals in whole plants.

Milburn (1970) demonstrated that up to 1 ml of phloem sap can be collected from a single incision in the stem bark of castor oil plants (Ricinus communis L.), and there is evidence that this sap represents a true sample of the sieve-cell contents (Hall et al., 1971; Smith and Milburn, 1980). Baker et al. (1980) developed a technique using R.communis to assess the uptake of exogenously applied aminoacids into phloem; in this technique the hollow of a petiole is perfused with a solution of the chemical, whose concentration is then determined in phloem exudate collected from the stem immediately beneath the treated petiole. Recently, Chamberlain et al. (1984) have adapted the technique of Baker et al. (1980) to study the phloem translocation of xenobiotics. A solution containing the ^{14}C-labelled test chemical and ^{3}H-sucrose (used as a reference standard) was perfused for one hour through the hollow petiole of a mature leaf by means of a peristaltic pump, and translocation was monitored over a period of 6 h by collecting phloem exudate from an incision in the stem bark beneath the treated leaf. The blade of the treated leaf was enclosed in a transparent 'Perspex' box to reduce transpiration. The herbicides and plant growth regulators 2,4-D, 3,5-D, IAA, aminotriazole and maleic hydrazide were moderately mobile in

phloem. Small amounts of oxamyl were detected in phloem exudate whilst ethirimol and triadimefon were not translocated in phloem. The distribution of the chemicals in different parts of treated plants was not assessed. The authors pointed out several advantages of this method, of which the most important are:- "(a) the compounds may be applied to the plant via the hollow petiole, thus avoiding the complication of a cuticular barrier, and (b) the radiolabelled compounds in the phloem exudate are measured directly, and metabolic or chemical breakdown can be detected easily." However, the technique requires rather large plants (75 cm tall) and so the concentration of the chemicals in the various parts of the plant cannot be easily measured. Furthermore, the concentration of the test compound in the phloem exudate cannot be compared with the concentration in the treated leaf because the exudate is likely to contain phloem sap both from the treated leaf and from untreated leaves.

1.3. Objectives

The main objectives of the present work were to test current ideas on the translocation of xenobiotics in phloem and to investigate in detail whether and in what manner physico-chemical properties of xenobiotics determine their movement and distribution in plants.

To test the intermediate permeability hypothesis of phloem transport of non-ionised compounds and to confirm that their translocation is a direct consequence of their lipophilicity, the translocation in phloem of a series of non-ionised compounds spanning a range of lipophilicity was investigated in R.communis, using modifications of an existing technique in order to achieve a more quantitative approach.

The 'ion trap' mechanism proposed to explain the accumulation of weak acids in phloem was also tested in R.communis. Knowledge of the relative permeabilities of the cell membranes to the undissociated and dissociated forms of an acid is essential for the understanding of this mechanism. The ratio of these permeabilities for each of several weak acids was obtained from studies of the uptake of the

chemicals by roots and subsequent translocation to shoots of barley. A series of weak acids of differing lipophilicities and pKa's was examined in order to investigate the effect of these physico-chemical properties on the accumulation and translocation of weak acids in phloem.

2. MATERIALS AND METHODS

2.1. Chemicals used in this work

The ^{14}C -labelled chemicals used in this work are given in Table 1. Flamprop, maleic hydrazide and 2,4-dichlorophenoxyacetic acid were obtained from Shell International Co. Ltd., ICN Pharmaceuticals Ltd. and Amersham International, respectively. The other chemicals were synthesised by Drs. Geoffrey G. Briggs, Richard H. Bromilow and Keith Chamberlain (Rothamsted Experimental Station). All the chemicals had a radiochemical purity of at least 96% as assessed by thin layer chromatography.

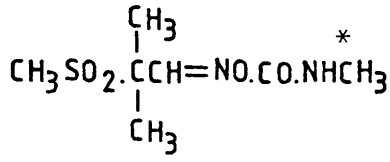
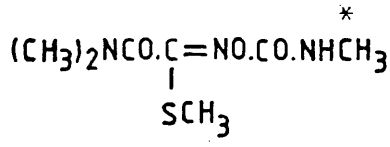
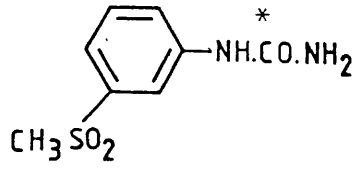
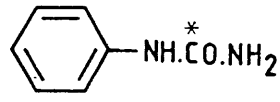
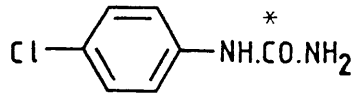
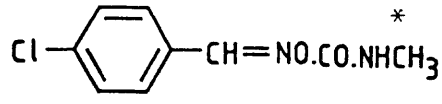
2.2. Measurement of octan-1-ol/water partition coefficients

Octan-1-ol/water partition coefficients (K_{ow}) of the non-ionised chemicals had already been measured (Briggs et al., 1982). Values for the weak acids were obtained by standard procedures (Leo et al., 1971) using 50 gentle inversions of a glass centrifuge tube containing the two phases pre-saturated with each other, followed by centrifuging at 2500 revolutions per minute (700 g). Appropriate volume ratios of the two phases were used according to predicted values of K_{ow} (Dr. Geoffrey G. Briggs, personal communication). 0.5M H_2SO_4 (0.05M for maleic hydrazide) and 0.1M Na_2CO_3 aqueous phases were used for K_{ow} measurements of the undissociated and dissociated forms respectively of the acids. The initial concentration of the chemicals in the aqueous phase varied from 20 to 50 $\mu g\ ml^{-1}$.

^{14}C-labelled maleic hydrazide and 4-mesyl P.O.A. were used in these determinations, concentrations in the aqueous phase before and after partition being measured by liquid scintillation counting; the effect of trace impurities on the measured K_{ow} of these chemicals would be very small as their concentrations in the two phases were very similar.

Table 1. Chemicals used in this work

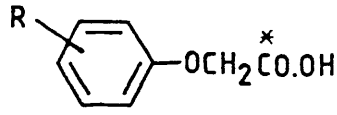
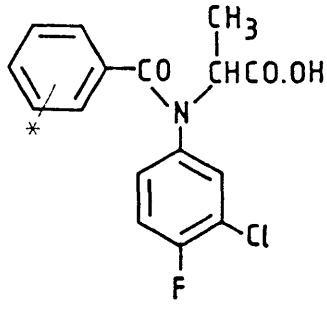
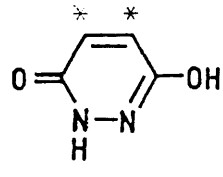
1. Non-ionised chemicals

Chemical[a]		Molecular weight	Log K_{ow}
aldicarb sulphone		222	-0.57
oxamyl		219	-0.47
3-mesylphenylurea		214	-0.13
Phenylurea		136	0.80
4-chlorophenylurea		170.5	1.80
4-chlorobenzaldehyde O-methylcarbamoyloxime		212.5	2.27

a. (*) indicates the position of the ^{14}C-atom

b. K_{ow} = octan-1-ol/water partition coefficient (values from Briggs *et al.*, 1982).

2. Weak acids

Chemical		Molecular weight
phenoxyacetic acids 		
'R' substituents :	Referred to in the text as:	
4-methylsulphonyl	4-mesyl P.O.A.	230
3-methyl-4-methylsulphonyl	3-methyl-4-mesyl P.O.A.	244
4-fluoro	4-F P.O.A.	170
4-chloro	4-Cl P.O.A.	186.5
2,4-dichloro	2,4-D	221
3,5-dichloro	3,5-D	221
4-(2,4-dichlorophenoxy)	4-(2,4-dichlorophenoxy) P.O.A.	313
flamprop 		322
maleic hydrazide 		112

Concentrations of the other more lipophilic acids in the aqueous phase were measured by high-pressure liquid chromatography on a column of 10 μm Spherisorb ODS, with methanol + 0.05M chloroacetic acid in water as eluent in the following proportions: 30 + 70 (3-methyl-4-mesyl P.O.A.), 40 + 60 (4-F P.O.A. and 4-Cl P.O.A.) and 60 + 40 (2,4-D, 3,5-D and flamprop acid). The log K_{ow} for the undissociated form of 4-(2,4-dichlorophenoxy) P.O.A. was estimated from Hansch relationships (Leo et al., 1971) by Dr. Geoffrey G. Briggs (personal communication); the value for the dissociated form of this acid was measured using ^{14}C-labelled chemical.

2.3. Uptake by roots and translocation to shoots of weak acids in barley

2.3.1. Growth of barley seedlings

Seedlings of barley (Hordeum vulgare cv. Georgie), germinated on moist tissue paper, were transferred to aerated half strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Seedlings were individually supported with a piece of foam in holes in an aluminium plate resting on a darkened beaker containing nutrient solution, and grown in a controlled environment chamber with a 16-h day (light intensity 10 klx) at 20°C and 8-h night at 16°C (light intensity ~ 250 μmol photons m^{-2} s^{-1}).

2.3.2. Uptake of chemicals

The experimental procedure was adapted from Briggs et al. (1982). Ten-day-old plants were transferred to nutrient solution containing the chemical under test. Six plants were individually held in rubber bungs and fitted into holes in an aluminium plate resting flush on a 6.5-cm-diameter glass crystallising dish with darkened sides. This dish contained the solution (100 ml) of chemical at an initial concentration of 5 μM (2000 to 5000 dpm ml^{-1}). Transpiration, corrected for evaporation using an identical vessel without plants, was measured by weighing the

vessel and plants at the beginning and end of each test, when the concentration of the chemical remaining in solution was also measured (duplicate 1.0ml aliquots).

Uptake of 2,4-D and 3,5-D was investigated at five pH's (from pH 4 to 8), with two replicates (each of 6 plants) for each pH. Uptake of other weak acids was investigated at pH 4.0 and 7.0 with one replicate for each pH. Solutions were buffered with 0.005M citric acid (pH 4.0 and 5.0), 0.005M sodium dihydrogen orthophosphate (pH 6.0 and 7.0) and 0.01M TRIS [2-amino-2-(hydroxymethyl) propane-1,3-diol] for treatments at pH 8.0. Required pH values were obtained by adding 1.0M NaOH or HCl and after 12, 24 and 36 h the pH's of the solutions were checked and adjusted if necessary by the same method. This procedure provided reasonable pH control, within $\pm$ 0.2 units. At pH 4.0, some of the chemicals were strongly taken up by the roots so that the concentration in the external solution decreased rapidly, and therefore after 2 h the plants were transferred to a fresh solution at the original concentration.

At the end of the uptake period (24 or 48 h), shoots from all the plants in one dish were cut off and combined, and the roots blotted dry, combined and weighed. 20 ml aliquots of the external solution were taken to examine for possible degradation of chemical in the nutrient solution. Plant samples were stored at $-16^{o}C$ until they could be analysed. Typical root weights were 0.1g per plant, and transpiration 1.0 ml per plant per day.

2.3.3. Degradation of chemicals

The rate of degradation of the chemicals in the shoots was estimated using groups of three excised shoots held individually in rubber bungs fitted into 50-ml glass vials containing nutrient solution with the chemical under test (5 μM, 2000 to 5000 dpm ml^{-1}). Roots were cut off under water immediately prior to transferring the excised shoots to treated solution. After 24h, transpiration was measured, and shoots

from one vial were combined and stored. Shoots from the remaining two vials were transferred to nutrient solution without chemical, and combined shoots from one vial were taken for analysis after 24 and 48 h.

2.3.4. Calculation of results

Values of Root Concentration Factor (RCF) and Transpiration Stream Concentration Factor (TSCF) were calculated as follows:

$$RCF = \frac{\text{amount (dpm) in roots x 100}}{\text{wt. of roots (g) x conc. of final solution (dpm ml}^{-1}\text{) x recovery (\%)}}$$

$$TSCF = \frac{\text{amount (dpm) in shoots x 100}}{\text{wt. of water transpired (g) x conc. of solution(dpm ml}^{-1}\text{) x recovery(\%)}}$$

The nutrient solution concentration used in the calculation of TSCF values was the mean of the initial and final concentrations (Briggs et al., 1982). The TSCF value corrected for degradation ($TSCF_o$) was calculated from the experimental value ($TSCF_t$) measured at time (t), using the estimate of the degradation rate (k) of the chemical in shoots assuming a constant rate of uptake and degradation (Briggs et al., 1982):

$$TSCT_o = \frac{TSCT \cdot t \cdot k}{1 - e^{-t.k}}$$

The degradation rate was estimated as follows: the amount of the chemical taken up by excised shoots (see 2.3.3.) prior to transfer to chemical-free solution was calculated from that taken up following the 24-h uptake period, adjusted for the volume of water transpired. From the amounts of parent chemical recovered 24 and 48 h after transfer of the excised shoots to chemical-free solution, the logarithm of the percentage of chemical remaining was plotted against time to obtain the first-order decay constant i.e. degradation rate (k).

2.3.5. Partition of chemicals between macerated roots and water

Roots from10-day old barley seedlings were blotted dry and frozen in liquid nitrogen. The frozen roots were crushed to a powder with a glass rod. Duplicate samples (0.2g) were freeze-dried in

30-ml glass centrifuge tubes, and the root solids shaken for 2 h with a 5 μM solution of the ^{14}C-labelled compound, followed by centrifuging for 10 minutes at 2500 revolutions per minute. Solutions were buffered to the desired pH as previously described (2.3.2.) and the pH after shaking was measured. Appropriate volumes of solution were chosen according to expected values of partition onto macerated roots (Briggs et al., 1982), taking into account the pKa and K_{ow} of the acids and the solution pH. The amount of chemical adsorbed by the root solids was calculated from the change in solution concentration, measured by liquid scintillation counting of duplicate aliquots (0.5 or 1.0 ml). The partition coefficients of the chemicals between macerated roots and water were calculated on the basis of fresh root weights.

2.4. Translocation studies in Ricinus communis

2.4.1. Growth of R. communis

Seeds of Ricinus communis L. var Gibsonii (Nichols) were germinated in moist vermiculite. 5-to 6-day-old seedlings were individually fitted, by using foam rubber pieces, into 8 x 8 cm (two halves 8 x 4 cm with appropriate holes) darkened 'Perspex' pieces resting on a darkened 400-ml beaker containing aerated nutrient solution (half-strength Hoagland's). Plants were grown in a glasshouse at 25-30°C under natural light supplemented (16 h per day) by high pressure mercury lamps (400W), with relative humidity of 80-90%.

The experiments were carried out using 4-to 5-week-old plants, from which the pair of cotyledons and primary leaves had been cut off 2 or 3 days before. Test plants had one pair of developed (mature) leaves and a developing apical leaf whose area was approximately one quarter to one third that of each developed leaf (Figure 1). The average weights ($\pm$ standard error, p = 0.05) of the various plant parts were: the two mature leaves (5.8 $\pm$ 0.5g, comprising 4.5 $\pm$ 0.4g for the

leaf blades and 1.3 ± 0.2g for the petioles), apical leaf (0.9 ± 0.3g), upper half of stem (1.6 ± 0.3g), lower half of the stem (1.9 ± 0.4g) and roots (5.3 ± 0.5g). The transpiration rate of the test plants was typically 45 ± 5 ml per day. Test chemicals were either applied to roots via the nutrient solution or were injected into the hollow petioles of mature leaves. Generally, two replicates (plants) were carried out for each treatment but, if good agreement between the two was not obtained, a third replicate was carried out.



Figure 1. Plant of R. communis as used in the tests.

2.4.2. Uptake of chemicals by roots

In these experiments, test plants each held in a 'Perspex' plate were transferred individually to darkened 400-ml glass beakers containing nutrient solution (360 ml) treated with the chemical under test. As the non-ionised chemicals had low specific activity ($\sim$ 0.25 μCi μmol^{-1}), they were applied at an initial concentration of 10 μM (5000 to 7000 dpm ml^{-1}) in order that the polar chemicals, which were translocated to the shoots to a low extent, could be accurately measured. 4-F P.O.A. and 3,5-D caused wilting symptoms when applied at 10 μM (nutrient solution pH 4.0) but the original specific activity of the weak acids was sufficiently high for them to be applied at an initial concentration of 1 μM (2000 - 3000 dpm ml^{-1}), which did not cause phytotoxic symptoms. Solutions treated with weak acids were buffered as previously described for barley (2.3.2.) and replaced after 3 h by a fresh solution at the original concentration for chemicals strongly taken up by roots. For periods of uptake longer than 48 h, the solution was replaced at this time by a fresh one containing the chemical at the original concentration.

Transpiration was assessed by weighing the beaker and the plant at the beginning and end of each test, when the chemical concentration remaining in solution was also measured (1.0 ml aliquots). The 'Perspex' plate was tightly held onto the beaker with black tape in order to avoid loss of water from the nutrient solution by evaporation.

At the end of the uptake period, after collection of phloem exudate from the upper and lower stem, shoots were divided into leaves (including the petioles and apical leaf, though in some experiments with the non-ionised chemicals and in all experiments with the weak acids the apical leaf was analysed separately) and stem, which was separated into upper and lower halves. The category 'apical leaf' included the uppermost stem tissue above the upper mature leaf, the apical bud

and the apical leaf. After collection of xylem exudate, the roots were blotted dry and stored, together with other plant parts, at -16°C until the samples could be analysed.

The distribution of chemicals was also investigated in plants that, after root uptake for 24 h, were transferred to chemical-free solution for a further period of 24 h. Three hours after transfer, the chemical-free solution was renewed to prevent uptake of chemicals released from the roots.

2.4.3. Injection of the chemicals into the petioles

A solution of each chemical in distilled water was gently injected into the hollow petiole of the mature leaves using a 10-µl microsyringe. Each petiole was injected with equal volumes (6 - 8 µl) of solution. Non-ionised chemicals were used at their original specific activity, concentrations of 10 mM (5000 to 7000 dpm μl^{-1}) being required for these chemicals to be accurately measured in phloem exudates. 4-F P.O.A. was shown to cause slight wilting symptoms when injected at 10 mM and so the weak acids were injected at 2 - 3 mM (5000 to 7000 dpm μl^{-1}). The pH of the solutions was approximately 5.5 (measured by using pH indicator paper) for all chemicals except 4-(2,4-dichlorophenoxy) P.O.A., which was sufficiently soluble only at slightly alkaline pH.

In some experiments, the time-concentration profiles of chemicals in phloem exudate from the upper and lower stem immediately after injection of the chemicals into the petioles were investigated by continuously collecting the exudates over a period of 6 - 8 h, with the exudates collected over each hourly interval being analysed separately.

In most experiments, 24 or 72 h after injection of the chemicals into the petioles, phloem exudate was collected from the upper and lower stem and then the shoots were divided as described previously in the experiments following uptake by roots, except that the petioles were separated from the mature leaf blades. Xylem exudate was generally

collected and duplicate aliquots (100 ml) of the nutrient solution were taken for analysis of chemical.

In studies of the translocation of chemical from one mature leaf to the other, the mature leaf closer to the apical leaf was treated with the chemical via injection into the petiole and the petiole of the opposite non-treated mature leaf was steam-girdled.

2.4.4. Collection of phloem and xylem exudates

To assess the concentration of chemicals in the phloem of treated plants, the contents of phloem sieve cells were obtained by lightly cutting the stem bark using a razor blade. 'V' shaped cuts were made in line with the older petiole. The phloem sieve cell contents (referred to as phloem exudate) were collected, using 50- or 100-µl capillary tubes, from two different regions of the stem i.e. a lower cut (1-2 cm above the stem base) and an upper cut (2-3 cm below the petiole). In Figure 1, the capillary tubes were placed in line with the younger petiole but this was a mistake during the preparation of the photograph. Depending on the size of plant, upper and lower cuts were 10-12 cm apart. Typical rates of exudation from each cut varied from 75 to 100 µl per hour, with a total of 100 - 150 µl being collected from each cut in most experiments except in those set up to investigate the time-concentration profiles of the chemicals in the exudates immediately after injection into the petioles. When new cuts were necessary to maintain satisfactory exudation, they were performed a couple of millimetres above the original cut.

Xylem exudate was obtained after chopping off the stem 2 cm above the roots. The bark on the remaining stump was peeled off and, by using a sleeve of rubber tubing, a glass tube was placed in tight contact with the remaining part of the plant. Xylem exudate was obtained by suction after connecting the other end of the glass tube with a source of negative pressure (30 cm of mercury) and was subsequently

transferred to capillary tubes. Typical rate of exudation was 0.2 - 0.3 ml per hour, but was less for plants having the nutrient solution maintained at pH 4.0.

Phloem and xylem exudates were transferred directly from capillary tubes to a 6.0-cm-wide band on a silica gel 60 GF_{254} thin layer chromatography plate. The water was evaporated under an air stream in a fume cupboard prior to developing the plate in the appropriate solvent system.

2.4.5. Degradation of chemicals

The rate of degradation of the chemicals in R. communis was estimated from the amounts of parent chemical recovered 24 and 72 h after petiole injection. The logarithm of the percentage of chemical remaining was plotted against time to obtain the first-order constant decay constant (k). 4-chlorophenylurea was not sufficiently soluble in water to be injected into the petioles at a concentration comparable to the other non-ionised chemicals, and so the degradation rate was estimated by first accumulating the chemical in shoots over a 24 h period of uptake by roots and then monitoring the subsequent decline 24 and 48 h after transfer of the plants to untreated solution. The amount of the chemical initially present following the 24-h uptake period was calculated from the TSCF.

2.4.6. Uptake of weak acids by segments of stem

Stems from 4-to 5-week-old plants were sliced into 2mm segments using a razor blade. Randomly selected segments (1.0 g) were then transferred to a 50-ml conical flask containing a solution (40 ml) of the ^{14}C-labelled chemical (1 to 2 μM, 2000 to 5000 dpm ml^{-1}), the solution being buffered to the desired pH as previously described (2.3.2.). The flasks were then transferred to a thermostatically controlled water bath maintained at 30 ± 0.5°C.

After 8 h, the segments were separated from the solution by filtration through a cotton wool plug, blotted dry with tissue paper and reweighed. The solution pH at the end of each test was measured, as was the concentration of the chemical remaining in solution (duplicate 1.0 ml aliquots). Experiments were carried out in duplicate. The uptake of each chemical from solution at pH 4.0 was also investigated after 24 h uptake.

2.4.7. Steam girdling of petioles

In some experiments it was desired to prevent the phloem translocation of chemicals from mature leaves and this was accomplished by steam girdling the petioles. The steam girdling technique was adapted from that used by Gaudiel and Vanden Born (1979) to girdle the leaf petiole or stem of young soybean plants. One hour prior to root treatment, each petiole was girdled with a 10 second burst (5 seconds to each side) of steam from a Pasteur pipette connected to a steam source. The pipette tip was brought to a distance of about 1 cm from the petiole and the length of petiole beyond the treated area (1 cm long) was protected by plastic tubing. Such treatment was shown to kill the tissue in the treated area, but the transpiration rate and turgidity of treated leaves were not drastically affected over a period of 48 h. However, girdled petioles were unable to support the weight of the leaf blade, so that treated leaves had to be supported by a wire frame.

2.4.8. Autoradiography

Distribution patterns of chemicals within the mature leaf blades following uptake by roots or petiole injection were assessed by autoradiography. The petioles were injected as described previously (2.4.3.) except for the volume injected, which was doubled for the weak acids in order to increase the amount of ^{14}C-labelled chemicals trans-

located to the leaf blades. For the same reason, concentrations of 4-F P.O.A., 3,5-D, maleic hydrazide and aldicarb sulphone in the nutrient solution were double those described previously (2.4.2.); the concentrations of 4-chlorophenylurea and flamprop were unchanged. Leaf samples were collected following root uptake for 24 h (nutrient solution at pH 4.0 for the weak acids), or 24 h after petiole injection.

The leaf blades were separated from the petioles, rested flat on a 'Perspex' plate (25 x 19 cm) and then covered with a sheet of cellophane. An X-ray film (Kodak^{R} X-Omat S; 24 x 18 cm) was placed, facing the upper surface of the leaf blades, onto the cellophane sheet. The film was kept in close and uniform contact with the leaves by using a second 'Perspex' sheet on top of the film. The two plates were firmly held to each other by using sellotape along the edges, which prevented the leaf tissue from drying out over the periods of storage in the deep freeze (3 to 8 months) until the films could be developed. The plates were wrapped with dark paper to protect the film against the light. A mature leaf containing an estimated radioactivity of 30,000 dpm required at least 3 months of exposure to give a reasonable definition.

2.5. Analytical methods

2.5.1. Extraction procedures

Root or shoot samples of barley plants were macerated with 5 to 10 ml portions of acetone in a glass mortar and pestle, and the extracts were filtered through cotton wool into 100-ml volumetric flasks. Maleic hydrazide, 4-mesyl P.O.A. and 3-methyl-4-mesyl P.O.A. were not efficiently extracted using acetone and methanol was used instead. Extraction was repeated several times and the flask contents were then made up to volume. Duplicate 20 ml aliquots were rotary evaporated to dryness at a bath temperature not exceeding 45^{o}C (room temperature for 4-mesyl P.O.A., as loss of radioactivity was observed

at higher temperatures). R. communis samples were extracted similarly but, because of the large amount of co-extracted material from the mature leaf blade and root samples, smaller aliquots (10 ml) were taken from the volumetric flasks containing these samples in order to avoid overloading of t.l.c. plates. During the initial stage of the studies with R. communis, duplicate aliquots of the extracts were taken but, due to the large number of samples during the later stage, single aliquots were usually taken.

Chemicals were extracted from nutrient solution by partitioning solution aliquots with dichloromethane (three times, equal volumes). Extraction of weak acids was preceded by acidification of the solution to pH < 1.0 with concentrated HCl . The organic phase was filtered without drying (Na_2SO_4 was shown to retain some of the weak acids) and the combined organic phases were rotary evaporated to dryness. Maleic hydrazide could not be efficiently extracted by the organic solvents tried and so 20 ml aliquots of the nutrient solution were rotary evaporated to dryness at a bath temperature of around 80°C.

2.5.2. Thin layer chromatography (t.l.c.)

Following rotary evaporation of the extracts, the residues were transferred in a little acetone (methanol for chemicals extracted with methanol) to a 4.0-cm-wide band on a silica gel 60 GF_{254} t.l.c. plate (6.0-cm-wide band for residues from leaves of R. communis). Pasteur pipettes were used to transfer the residues onto t.l.c. plates. Unlabelled compounds were used as markers. Plates containing extracts from leaves were developed initially in diethyl ether for the weak acids, in order to move most of interfering coloured co-extracts far away from the compounds. The solvent systems used are listed in Table 2. Bands of silica gel containing the parent chemical were observed under an ultraviolet lamp and scraped into 10 ml aliquots of scintillation

Table 2. Thin layer chromatography of the chemicals.

Chemicals	R_F values in solvent system[a] indicated			
	A	B	C	D
4-chlorobenzaldehyde O-methylcarbamoyloxime	0.72			
3-mesylphenylurea		0.18		
oxamyl		0.33		
aldicarb sulphone		0.38		
phenylurea		0.40		
4-chlorophenylurea		0.45		
flamprop			0.43	
2,4-D			0.54	
3,5-D			0.54	
4-F P.O.A.			0.45	
4-Cl P.O.A.			0.50	
4-(2,4-dichloro-phenoxy) P.O.A.			0.47	
4-mesyl P.O.A.				0.30
3-methyl-4-mesyl P.O.A.				0.35
maleic hydrazide				0.42

a. Solvent systems:

A. diethyl ether.

B. diethyl ether: acetone (4:1).

C. hexane: ethyl acetate: acetic acid (9:4:1).

D. ethyl acetate: methanol: acetic acid (30:6:1).

cocktail for radioactivity counting. Aldicarb sulphone could not be located on t.l.c. plates under the U.V. lamp, and so oxamyl was used as a reference to assist in locating the chemical. The retention factor (R_F) of aldicarb sulphone in relation to oxamyl's was assessed by spraying reference plates with 0.5% 2,6-dibromo-p-benzoquinone-4-chlorimine in cyclohexane followed by heating to 110°C for 15 minutes, when the compounds appear as brown spots on a pale background (Briggs et al., 1982).

2.5.3. Liquid scintillation counting.

The scintillation cocktail used during the initial part of this work was prepared by mixing one part of Triton X-100 with two parts of a toluene solution of butyl-PBD (8g l^{-1}) and PBBO (0.5g l^{-1}). Subsequently a commercial cocktail (Scintran^R - cocktail T; BDH Chemical Ltd.) was used. Ten ml of cocktail were generally used in samples containing aqueous solution (1.0 ml) or silica, and the samples were counted in a Kontron-Betamatic scintillation counter. Efficiency of counting was determined by the external standard method and the results were expressed in disintegrations per minute (dpm).

2.5.4. Efficiencies of the analytical procedures

Efficiencies of the analytical procedures were assessed by adding known amounts of ^{14}C-labelled chemicals to duplicate samples of untreated plant tissue, nutrient solution and artificial phloem sap (10% sucrose in distilled water) and were generally better than 80% (73% for maleic hydrazide from leaves of R. communis). All the results were corrected for the efficiency of recovery.

3. RESULTS AND DISCUSSION

3.1. Translocation of non-ionised chemicals in R. communis

3.1.1. Translocation following uptake by roots

Concentrations of the chemicals in the various plant parts, in phloem exudate from the upper and lower parts of the stem, and in the xylem following uptake by roots are shown in Figure 2. The concentrations are expressed relative to those in the nutrient solution; the concentration in the nutrient solution at the end of the uptake period was used to express the concentration in roots, as the chemical in roots equilibrates rapidly with the external solution, and the average concentration in the external solution, which did not differ more than 10% from those at the beginning and end of the uptake period, was used to express the concentrations in the shoot parts, phloem exudates and xylem. The concentrations in xylem assessed from the amounts of chemical accumulated in the shoots, corrected for degradation, for a measured amount of transpiration were used rather than those measured in the xylem sap collected from root stumps, as the latter were higher especially for the more polar chemicals. The flow rate of the xylem sap collected from root stumps was about eight times lower than the normal rate in intact plants (the latter being about 45 ml per day), and so it is assumed that this allowed more time for the chemicals to cross the root endodermis and enter the xylem vessels, thus giving rise to concentrations of the chemicals in the xylem exudates higher than those in the xylem of intact plants. The concentrations in roots and in xylem expressed as described above represent respectively the RCF and TSCF values which, together with the ratio of chemical concentration in phloem exudate from the upper stem to that in the xylem, are given in Table 3.

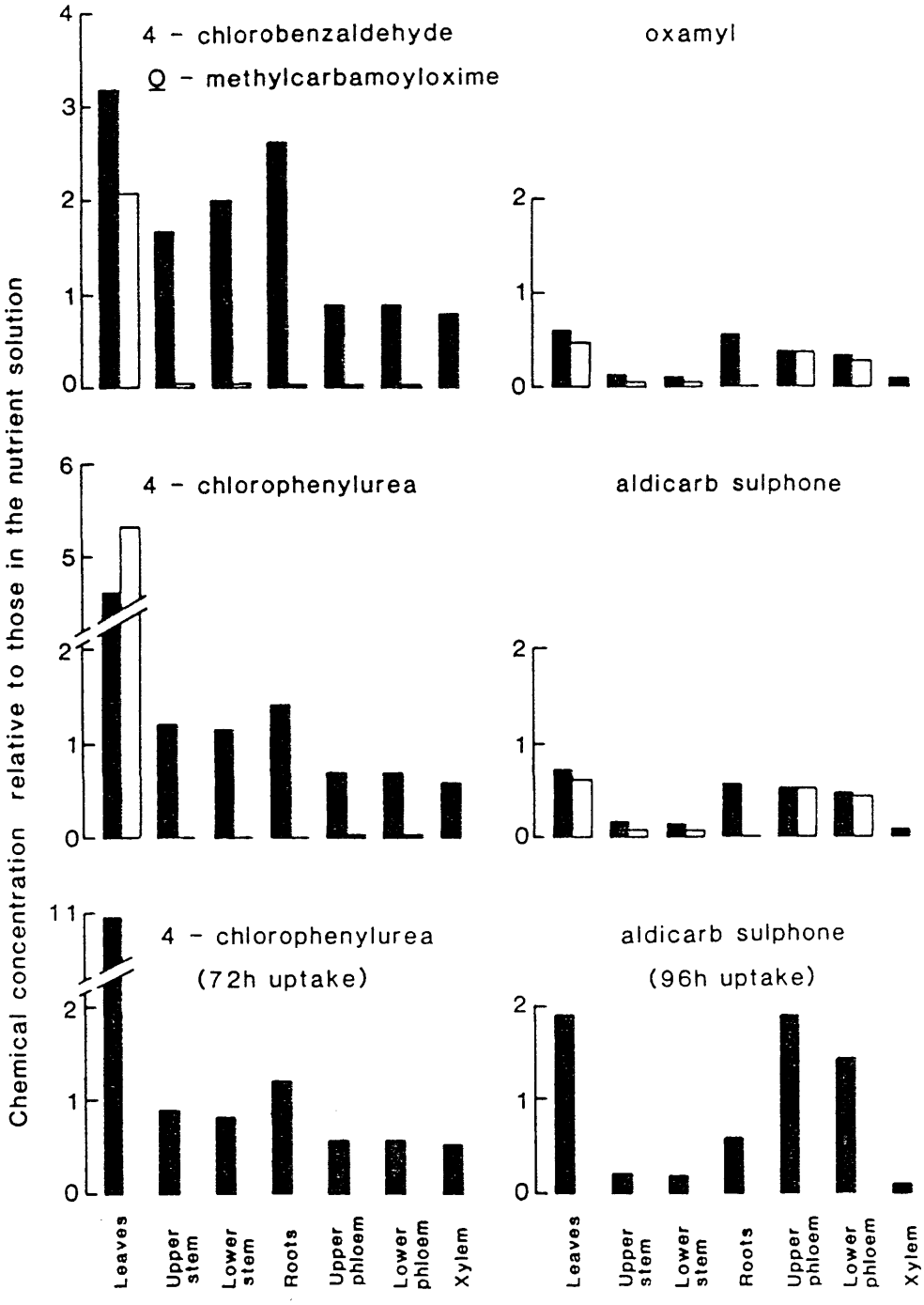


Figure 2. Concentrations of non-ionised chemicals in the various parts of R. communis plants after (unless otherwise stated) 24 h uptake by roots from nutrient solution (shaded areas) and following subsequent transfer for 24 h to chemical-free solution (unshaded areas). The category 'leaves' includes the petioles and the apical leaf.

Table 3. Uptake by roots (as RCF), translocation into the shoots (as TSCF), and distribution of non-ionised chemicals in phloem and xylem of R. communis.

Chemical	k[a] (per day)	Time (h)	RCF	TSCF	Phloem[b]/Xylem concentration ratio
aldicarb sulphone $\log K_{ow} = -0.57$	0.07	24	0.55	0.08	6.4
		48	0.57	0.10	7.7
		96	0.58	0.10	20.0
oxamyl $\log K_{ow} = -0.47$	0.39	24	0.54	0.09	3.8
4-chlorophenylurea $\log K_{ow} = 1.80$	0.07	24	1.40	0.58	1.2
		72	1.43	0.52	1.1
4-chlorobenzaldehyde O-methylcarbamoyl--oxime $\log K_{ow} = 2.27$	0.96	24	2.61	0.74	1.1
		72	2.53	0.71	1.1

a. Degradation rate of the chemicals in the plant shoots

b. Concentration in phloem exudate from the upper stem

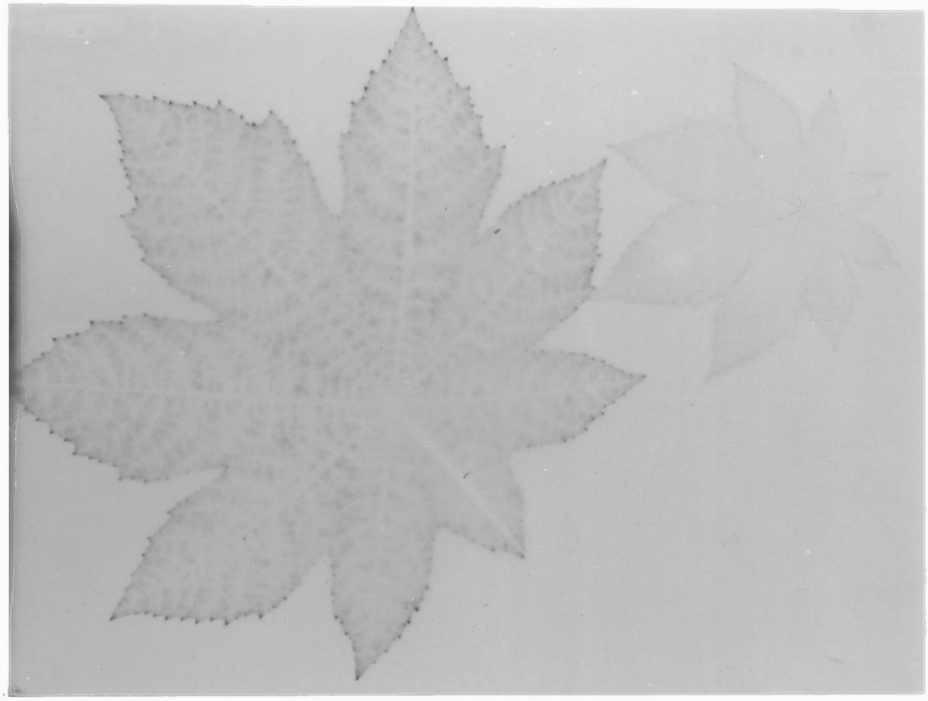
The RCF and TSCF values of the chemicals in R. communis were constant with time and comparable to those obtained by Briggs et al. (1982) in barley, which is in agreement with the suggestion by these authors that the role of lipophilicity of non-ionised chemicals on their uptake by roots and translocation to shoots, as described on page 18, is of general application to many plant species.

All chemicals tested were found in the phloem exudates following uptake by roots, indicating that the phloem sieve cells are freely accessible to both polar chemicals and to those of intermediate lipophilicity. The polar chemicals aldicarb sulphone ($\log K_{ow} = -0.57$) and oxamyl ($\log K_{ow} = -0.47$) were present in phloem exudate from the upper stem at much higher concentrations than in the xylem, the concentration of aldicarb sulphone in the phloem exudate increasing with time and reaching 20 times the concentration in the xylem after 96 h of uptake. This increase in concentration of aldicarb sulphone in the phloem exudate with time paralleled the increase in concentration in the leaves, the concentration in the stem increasing only slightly (see Figure 2), indicating that the polar chemicals are phloem translocated from the leaves into the stem. In contrast, the two chemicals of intermediate lipophilicity 4-chlorophenylurea ($\log K_{ow} = 1.80$) and 4-chlorobenzaldehyde O-methylcarbamoyloxime ($\log K_{ow} = 2.27$) were found at the same concentrations in the phloem exudate and in the xylem at all sampling times (Table 3), indicating that these chemicals are not phloem translocated from leaves and that they enter the phloem in the stem only directly from the adjacent tissue.

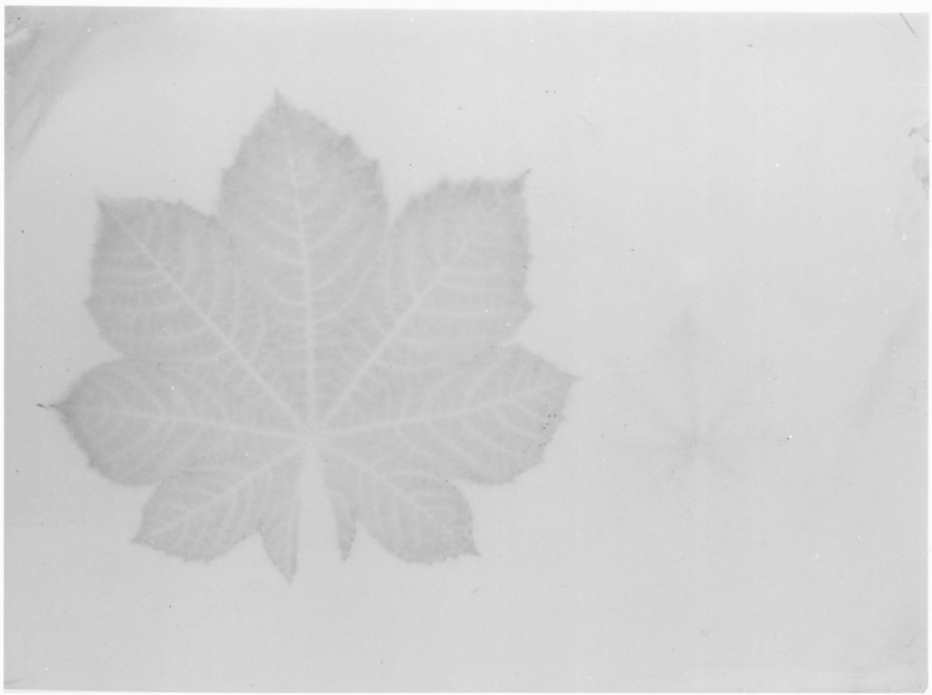
In order to confirm that only the polar chemicals are phloem translocated from leaves, the concentrations of the chemicals in the phloem exudates from plants treated for 24 h were compared with their concentrations in the exudates from plants that, after uptake for 24 h, were transferred to chemical-free solution for a subsequent period of

24 h. As shown in Figure 2, following transfer to chemical-free solution, the concentrations of the two polar chemicals aldicarb sulphone and oxamyl in the phloem exudates were similar to those in the exudates from plants prior to transfer; the concentrations in leaves following transfer diminished slightly, presumably due to degradation, and the amounts in the stem were reduced by more than half. In contrast, very little of the two chemicals of intermediate lipophilicity were found in stems or in the phloem exudates after transfer, although concentrations in leaves remained high, demonstrating that they are only xylem-mobile and not phloem-mobile.

Autoradiographs of the mature leaf blades following uptake of aldicarb sulphone and 4-chlorophenylurea by roots for 24 h (Figure 3) showed that both chemicals were rather uniformly distributed in the leaf blades, with less chemical being present in and adjacent to the veins than in the interveinal spaces. This indicates that these chemicals leave and re-enter the transpiration stream during passage through the leaf and so move to the terminii of the minor xylem vessels i.e. the interveinal spaces in dicots, the lower concentrations in and adjacent to the veins being close to that in the transpiration stream. In monocots, where the main veins in the leaves are parallel and very close together, predominantly xylem-mobile chemicals move to the tip of the lamina e.g. fenarimol, benomyl and oxamyl in barley (Martin and Edgington, 1981).



aldicarb sulphone



4-chlorophenylurea

Figure 3. Autoradiographs showing the distribution of aldicarb sulphone and 4-chlorophenylurea within the leaf blades of R. communis after 24 h uptake by roots from nutrient solution.

3.1.2. Translocation following injection into petioles

The role of lipophilicity of non-ionised chemicals in determining their translocation in phloem was better demonstrated by experiments in which the chemicals were injected into the petioles of the mature leaves.

24 and 72 h after injection, the chemicals were found mostly in the mature leaf blades (Figure 4). Phenylurea and 3-mesylphenylurea were very stable in the plant, with more than 85% of the amounts injected being recovered 24 h after petiole injection; the first-order decay constant (k) of the other chemicals are given on page 63 . Autoradiographs of the mature leaf blades 24 h after injection of aldicarb sulphone and phenylurea into the petioles (Figure 5) showed that these chemicals were rather uniformly distributed in the blades, with higher concentrations in the interveinal spaces. These distributions were similar to those observed following uptake of aldicarb sulphone and 4-chlorophenylurea by roots, save for slightly higher concentrations observed in the leaf margins following injection into petioles. The other chemicals would be expected to be similarly distributed in the leaf blades and, since the stem phloem was shown to be freely accessible to both polar chemicals and to those of intermediate lipophilicity, the concentration of all chemicals in phloem in the leaf blades was expected to be similar to their concentration in the leaf blade itself. Thus, by comparing the concentration of the chemicals in phloem exudate with their concentration in the leaf blades, the retention of the chemicals in phloem could be assessed (Figure 6). The data for 4-chlorophenylurea, which was not sufficiently soluble in water to be injected into the petioles at the same concentration as the other chemicals, were obtained by applying the chemical to the nutrient solution for 24 h followed by 24 h in chemical-free solution to move all the chemical to the leaves (Figure 2).

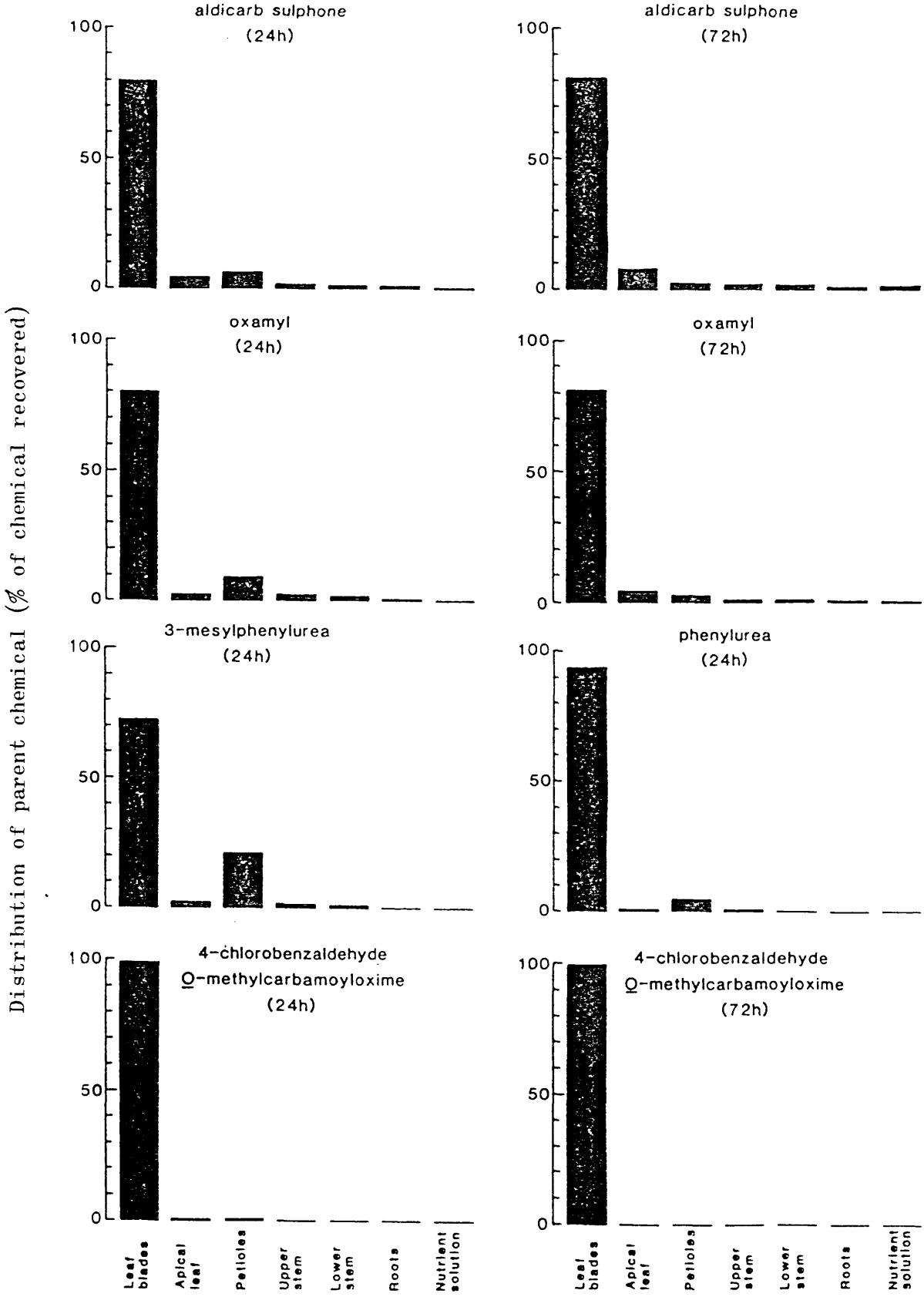
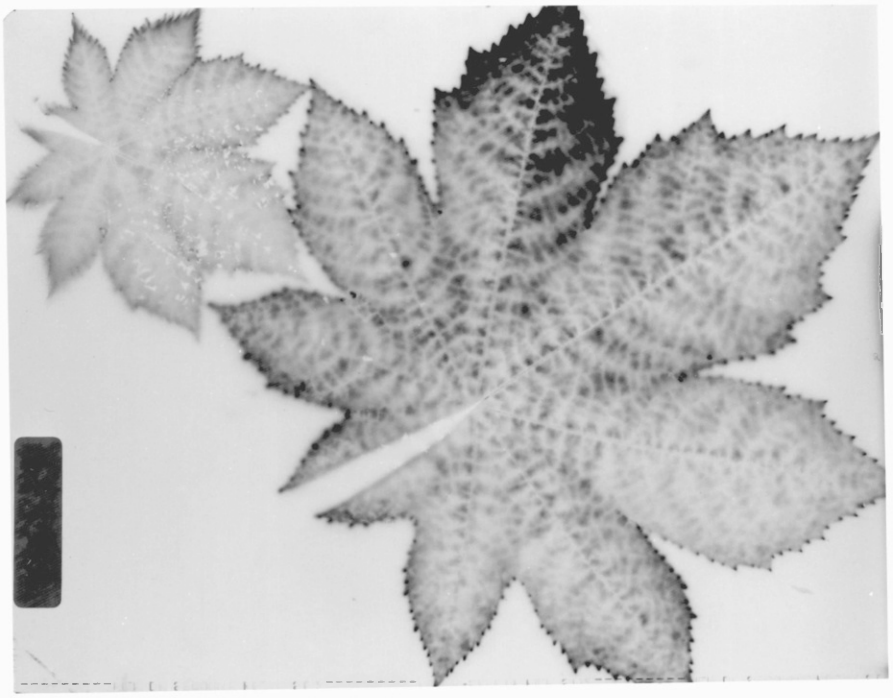


Figure 4. Distribution of non-ionised chemicals in R. communis 24 and 72 h after injection of the chemicals into the petioles.



aldicarb sulphone



phenylurea

Figure 5. Autoradiographs showing the distribution of aldicarb sulphone and phenylurea within the leaf blades of R. communis 24 h after injection of the chemicals into the petioles.

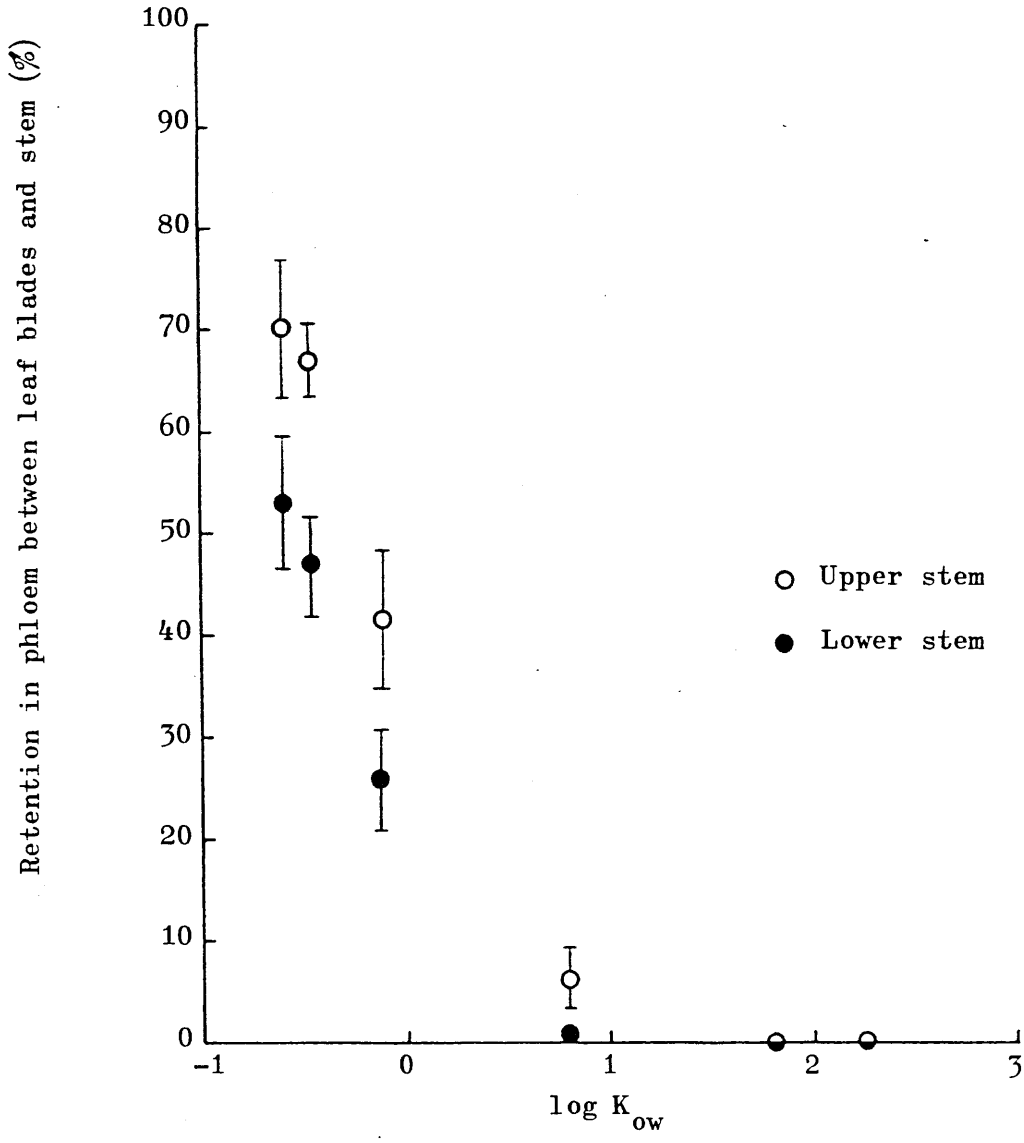


Figure 6. Relationship between the lipophilicity of non-ionised chemicals and their retention in phloem of R. communis. Concentrations in the stem phloem exudates and in the leaf blades were measured 24 h after injection of the chemicals into the petioles (4-chlorophenylurea applied via uptake by roots, see text). Vertical bars indicate the range for the replicates.

Aldicarb sulphone was the chemical best retained in phloem, being present in phloem exudate from the upper cut at 70% of the concentration in the leaf blades, and decreasing to 53% in the exudate from the lower cut. The distance between the leaf blade and the upper cut, along the petiole and part of the upper stem, was similar to that between the upper and lower cuts along the stem (10 - 12 cm) and comparable retention of the chemicals in phloem along the petiole and stem was observed. Retention in phloem decreased as lipophilicity increased and the chemicals of intermediate lipophilicity were lost so quickly that none was found in phloem exudates. The concentration ratios of aldicarb sulphone and oxamyl between phloem exudates and leaf blades 72 h after petiole injection were very similar to those after 24 h.

Briggs et al. (1982) explained the variation of TSCF of non-ionised chemicals in barley as being due to the effect of lipophilicity of the chemicals on their permeation rates through the membranes of the endodermal cells in the roots, with polar chemicals ($\log K_{ow} < 0$) permeating through membranes only slowly and chemicals of intermediate lipophilicity ($\log K_{ow} \sim 2.0$) having the highest permeation rates. Therefore, our findings provide good evidence for the intermediate permeability hypothesis (Tyree et al., 1979) of phloem transport of xenobiotics i.e. most xylem-mobile, non-ionised compounds diffuse freely into the phloem but only compounds that have low permeation rates through cell membranes are sufficiently retained in phloem to be translocated over long distances. In addition, the findings presented here indicate that the ability of non-ionised chemicals to be translocated in phloem is a direct consequence of their lipophilicity, with only polar chemicals ($\log K_{ow} < 0$) being phloem translocated over relatively long distances.

Chemicals of intermediate lipophilicity rapidly escape out of the phloem sieve cells and are swept back to the leaves via the xylem, so that these chemicals are not phloem translocated from the mature leaves into the stem (the xylem flow rate in the test plants was estimated to be about 40 times higher than that in the phloem). The different rates at which the chemicals were shown to leave the phloem supports the basic assumption of the intermediate permeability hypothesis, namely that loss of chemicals from phloem is mainly by diffusion across the plasmalemma and not through the plasmodesmatal connections.

Although the polar chemicals were sufficiently retained in phloem to be phloem translocated from the mature leaves, escape out of the phloem and movement back to the mature leaves via the xylem resulted in very small concentrations of these chemicals in the stem and root tissues (Figure 4). Evidence for this was obtained from another experiment in which aldicarb sulphone was injected into the petiole of one mature leaf and the amount translocated into the other mature leaf, whose petiole was steam-girdled to prevent any phloem transport, was measured. Approximately 10% of the aldicarb sulphone recovered from the whole plant was found in the non-treated mature leaf after 48 h, demonstrating the recycling of the chemical in the whole plant. Assuming that an equal amount also returned to the treated leaf, then together with the amount found in the rest of the plant at least 33% of the aldicarb sulphone in the leaf blade was exported over 48 h.

The time-concentration profile of aldicarb sulphone in the phloem exudates collected continuously over a period of 8 h immediately after injection into petioles showed that aldicarb sulphone appeared in the exudates collected during the first hour and increased slightly in concentration with time, with concentrationsapparently levelling out after about 6 h (Figure 7). After 6 h, the concentrations in the

exudates were about half those in the exudates from other plants 24 h after injection into the petioles. A parallel experiment in which a single plant was sacrificed at 2,4 and 6 h after injection into the petioles showed that the amount of aldicarb sulphone in the leaf blades increased steadily and, after 6 h, the amount of aldicarb sulphone in the leaf blades was about half that 24 h after injection (personal communication, Dr. Richard H. Bromilow and Mr. Mark R. Williams). This indicates that entry of aldicarb sulphone into the phloem occurs predominantly in the leaf blades and not directly in the petioles.

It is worth considering whether concentrations of the chemicals in the phloem exudates represent those in the phloem sieve cells of intact plants. Typically 75 µl of phloem exudate was collected per hour from each incision entering perhaps 30% of the sieve cells around the stem; this rate of flow is probably much greater than in intact plants. Kallarackal and Milburn (1984) reported a much higher specific mass transfer of sucrose in exuding than in intact peduncles of R. communis and attributed the results to release of the turgor pressure in the sieve cells as would be expected by the Münch pressure-flow hypothesis of phloem transport. Enhanced phloem flow rates in our experiments might cause chemical concentrations in the phloem exudates to be reduced by dilution if entry into the leaf phloem is rate-limiting, or alternatively to be increased if entry into the leaf phloem is not rate-limiting but retention in phloem determines transport, as there would be less time for chemicals to escape out of the phloem as flow-rates increased. When 10 x 10 µl sequential aliquots of phloem exudate were collected, 24 h after injection of aldicarb sulphone into petioles, they all contained very similar amounts of the chemical and thus one can be confident that the phloem samples (100 - 150 µl) collected in most of the experiments were representative of those in intact plants. However, in the experiments where phloem exudate was collected continuously for 6 - 8 h,

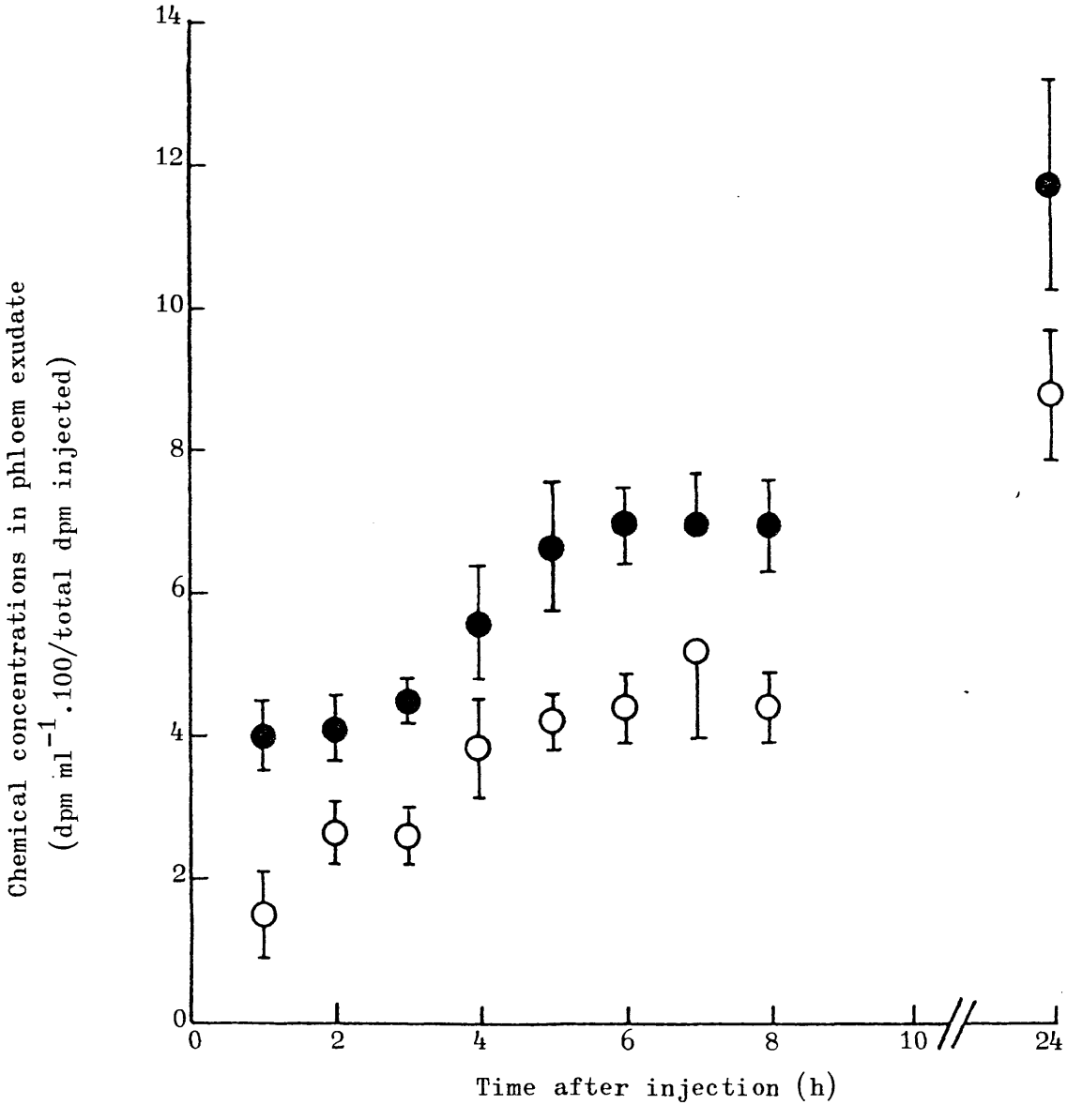


Figure 7. Time-concentration profile of aldicarb sulphone in phloem exudate from the upper (•) and lower (o) stem of R. communis after injection of the chemical into the petioles. Vertical bars indicate the range for the replicates.

it is not certain that the later samples taken during this period are truly representative.

Using an autoradiography technique, Crafts (1967) reported that aminotriazole was found in appreciable amounts in roots of young soybean plants following application to cotyledon, epicotyl or primary leaves. Lichtner (1983) measured the K_{ow} of aminotriazole at various pH's and from the results he obtained the log K_{ow} of aminotriazole is about -0.87 over the pH range 5 to 8. This value of log K_{ow} is not very different from that of aldicarb sulphone (log K_{ow} =-0.57), and so the observation reported here that aldicarb sulphone is found in very small amounts in roots is apparently in contrast to the observation by Crafts (1967) with aminotriazole. However, as discussed previously (page 22), it is likely that aminotriazole is translocated predominantly as an alanine derivative.

Martin and Edgington (1981) reported that following application to a band across the centre of one of the unifoliate leaves of young soybean plants, fenarimol (log K_{ow} = 0.67) was phloem translocated to an extent similar to that of oxamyl, with benomyl (log K_{ow} = -0.21) being phloem translocated to a much less extent. However, the studies did not involve analysis of the plant tissues for the parent chemicals and the given log K_{ow} of benomyl is far lower than that predicted from Hansch relationships i.e. $\sim$ 1.5 (Dr. Geoffrey G. Briggs, personal communication).

Although the literature on translocation of polar non-ionised chemicals is very limited, there has not been any unequivocal report of such chemicals not being translocated in phloem. Moreover, non-ionised chemicals of intermediate lipophilicity have only been found to be mobile in xylem. Thus the findings presented here relating the lipophilicity of the studied chemicals with their ability to be translocated in phloem may be of general application to non-ionised chemicals.

3.2. Uptake by roots and translocation to shoots of weak acids in barley

3.2.1. Octan-1-ol/water partition coefficients (K_{ow}) and pKa's of the weak acids.

The octan-1-ol/water partition coefficients (as log K_{ow}) and pKa's of the weak acids are shown in Table 4. The phenoxyacetic acids (pKa $\sim$ 3.0) spanned a range of lipophilicity, with log K_{ow} values for the undissociated form of these acids varying from 0.06 to 4.5. The undissociated and dissociated forms of flamprop (pKa = 3.7) had log K_{ow} values similar to those of 2,4-D and 3,5-D. Maleic hydrazide was the weakest acid (pKa = 5.65) and also the most polar of the weak acids studied, the undissociated form having log K_{ow} = -0.6. Where measured, the anions were 3.1 to 3.4 log K_{ow} units more polar than the respective undissociated acids. This difference is a little less than the value of 4.0 log K_{ow} units stated by Leo et al. (1971) to occur generally between the undissociated and dissociated forms of weak acids.

3.2.2. Effect of the nutrient solution pH on uptake and translocation of 2,4-D and 3,5-D.

The measured RCF and TSCF values of 2,4-D and 3,5-D following uptake for 24 and 48 h from solution of different pH's are given in Table 5 and Figures 8 and 9. The values represent means of two experiments (6 plants per experiment).

The RCF and TSCF values were markedly affected by the nutrient solution pH, both being greatly increased as the pH decreased. At pH values above 4.0, RCF and TSCF values were fairly similar from 24 to 48 h, indicating that equilibrium was rapidly attained. Further evidence for this was obtained by monitoring the decrease in concentration of the chemicals in the nutrient solution at pH 4.0; in this case, after a rapid decrease in concentration during the first period of 3 h, the

Table 4. Values of log K_{ow} and pKa for the weak acids

Chemical	log K_{ow}		pKa
	undissociated form	dissociated form	
4-mesyl P.O.A.	0.06	-[a]	3.1[c]
3-methyl-4-mesyl P.O.A.	0.54	-	3.0[d]
4-F P.O.A.	1.64	-	3.1[d]
4-Cl P.O.A.	2.25	-	3.1[d]
3,5-D	2.90	-0.24	2.84[e]
2,4-D	2.84	-0.25	2.87[e]
4-(2,4-dichlorophenoxy) P.O.A.	4.5[b]	1.10	3.0[d]
flamprop	2.90	-0.40	3.70[f]
maleic hydrazide	-0.63	-	5.65[g]

a. Missing values indicate that log K_{ow} values were not measured

b. Calculated value (see 2.2.)

c. Measured by titration (Mr. Mark R. Williams, Rothamsted Experimental Station)

d. Calculated values based on the work by Perrin et al., 1981.

e. Kennedy and Stewart (1980).

f. Measured by the solubility method (Dr. Richard H. Bromilow, Rothamsted Experimental Station).

g. Miller and White (1956).

concentration in the nutrient solution changed very little. At pH 4.0, however, both the RCF and TSCF values diminished from 24 to 48 h indicating that the root cells were unable to maintain their internal pH (the effect of the pH inside the cells on uptake of the chemicals is discussed below).

The chemicals behaved similarly, although 2,4-D showed somewhat lower RCF and higher TSCF values than 3,5-D. RCF values obtained by Shone and Wood (1974) for 2,4-D in barley were considerably higher (88.4 and 8.07 at pH 4.0 and 6.5 respectively) than the values obtained in this study. However, Shone and Wood assumed that all the radioactivity found in roots was 2,4-D. Since we found that this chemical is not very stable in shoots of barley, metabolism in roots is also likely to be significant and, if the metabolites are retained in the roots, then measurements of the total radioactivity in roots will lead to an overestimation of RCF values. The TSCF values obtained for 2,4-D in this study (4.1 at pH 4.0 and 0.06 at pH 6.0) were not very different from those of Shone and Wood (3.1 at pH 4.0 and 0.14 at pH 6.5).

The partition coefficients of 2,4-D and 3,5-D between macerated roots and nutrient solution at pH 4.0 were not measurable ($K_{\text{macerated roots/water}} < 0.2$), which was expected since the lipophilic undissociated molecules are present in very small concentration in comparison to that of the polar dissociated molecules (pKa's $\sim$ 3.0). The large accumulation of these chemicals in roots of intact plants is thus not due to partition onto the root solids, as has been shown to explain the large accumulation of lipophilic non-ionised chemicals (Briggs et al., 1982), but must be due to the accumulation within the protoplasm of the cells. This accumulation against a concentration gradient and the resulting values of TSCF greater than unity at pH 4.0 must be an energy requiring process.

As mentioned previously in the literature review, entry of weak

Table 5. Root Concentration Factors (RCF) and Transpiration Stream Concentration Factors (TSCF) for the uptake of 2,4- and 3,5-dichlorophenoxyacetic acids by roots of barley from nutrient solutions of different pH.

pH	time (h)	2,4-D		3,5-D	
		RCF	$TSCF_o$ [a]	RCF	$TSCF_o$ [a]
4.0	24	33.0	4.14	24.5	4.24
	48	24.5	2.78	18.0	3.25
5.0	24	7.10	0.49	6.41	0.72
	48	6.65	0.58	6.14	0.66
6.0	24	2.70	0.06	1.56	0.11
	48	2.16	0.05	1.35	0.12
7.0	24	0.92	0.04	0.60	0.05
	48	1.11	0.04	0.62	0.05
8.0	24	0.71	0.03	0.53	0.04
	48	0.68	0.03	0.54	0.04

a. values corrected for degradation ($k = 0.85$ and $0.68d^{-1}$ for 2,4-D and 3,5-D respectively).

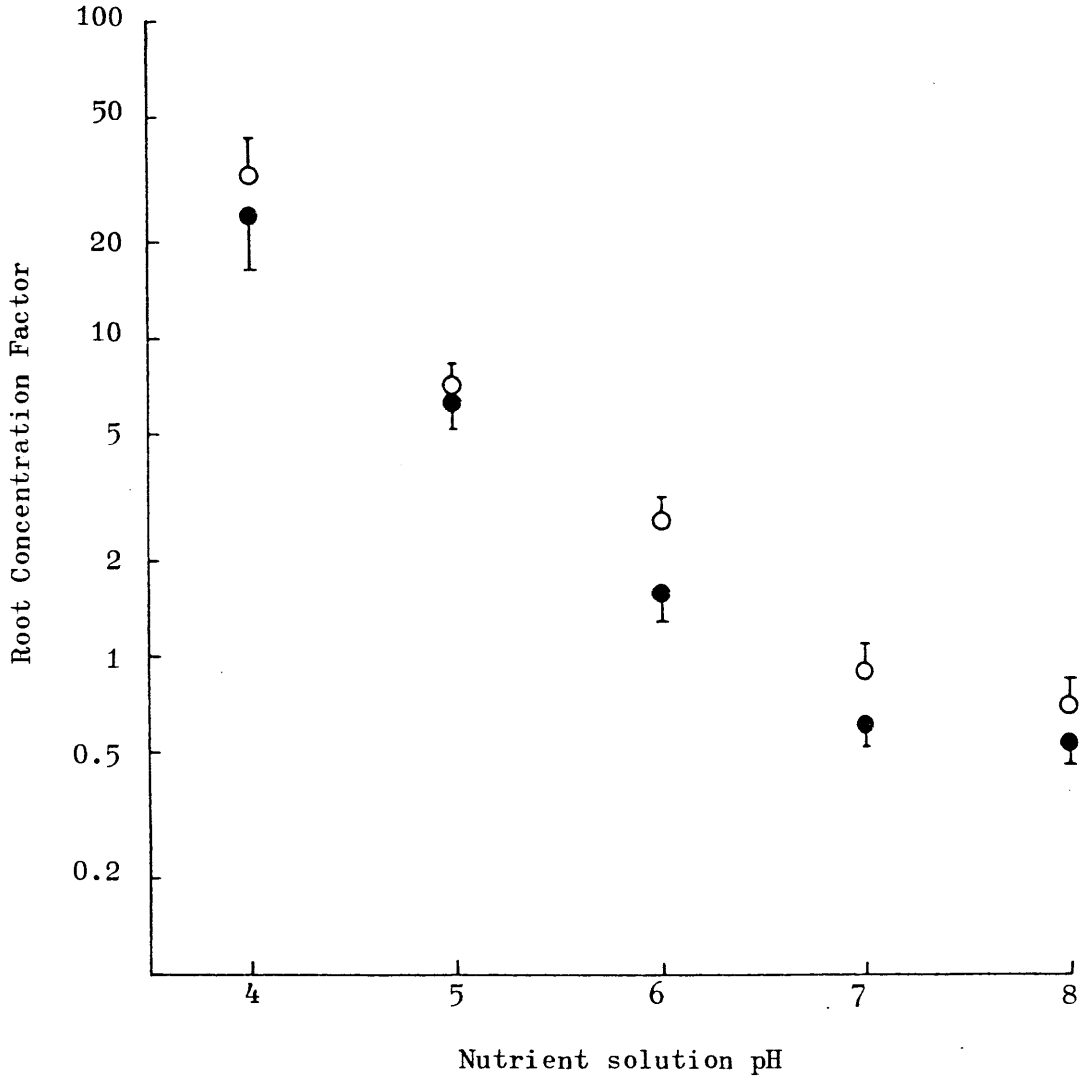


Figure 8. Relationship between the accumulation of 2,4- and 3,5-dichlorophenoxyacetic acids by barley roots (expressed as the Root Concentration Factor after 24 h uptake) and the pH of the nutrient solution. 2,4-D (o) and 3,5-D (•). Vertical bars indicate the range of the replicates.

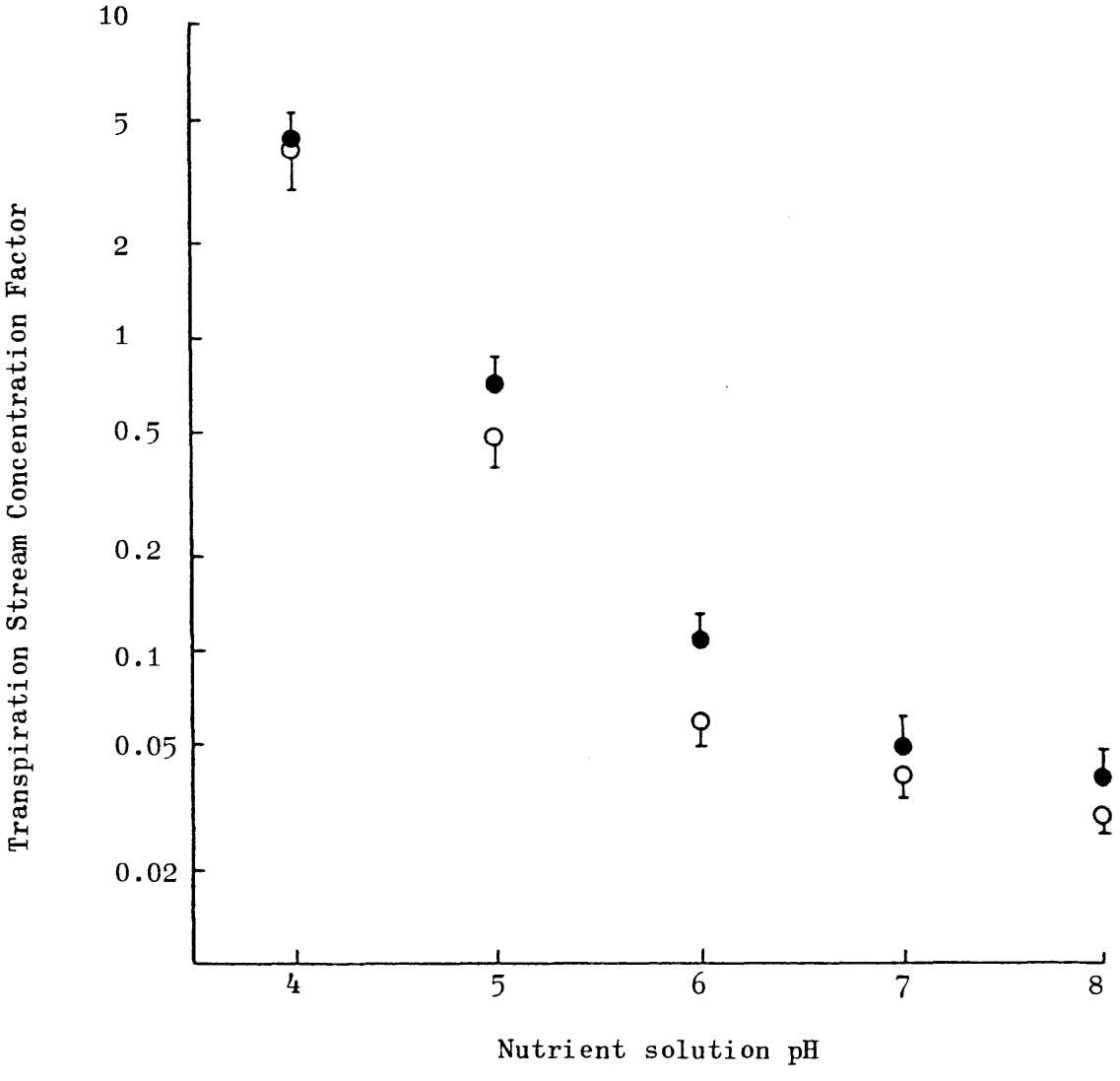


Figure 9. Relationship between the translocation of 2,4- and 3,5-dichlorophenoxyacetic acids to barley shoots following uptake by roots (expressed as the Transpiration Stream Concentration Factor after 24 h uptake) and the pH of the nutrient solution. 2,4-D (o) and 3,5-D (•). Vertical bars indicate the range for the replicates.

acids into plant cells has been shown to be primarily by diffusion of the undissociated acids, which permeate membranes much more rapidly than the respective anions. Once inside the cell, dissociation occurs and the anions are unable to diffuse out again very readily, thus leading to accumulation of weak acids inside the cells when the pH of the bathing solution is lower than that of the protoplasm (see 1.2.3.1). The magnitude of the accumulation by this 'ion trap' mechanism can be predicted knowing the physico-chemical parameters, and this was done using a recent form of the 'ion trap' equation given by Raven (1975).

The predicted accumulations in cells for weak acids of differing pKa's, from a bathing solution of pH 5.0, as a function of the ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of the acids, are shown in Figure 10. The vacuole and cytoplasm were assumed to occupy 90% and 10% of the cellular volume respectively (Sutcliffe, 1979; Darmstadt et al., 1983) and their pH's to be 5.5 and 7.0 respectively (Roberts et al., 1980; Goldsmith et al., 1981). The plasmalemma potential was assumed to be -0.12V (Raven, 1975; Goldsmith et al., 1981). The literature values for the tonoplast potential are rather uncertain but are all low i.e. $_{cyt.}E_{vac.} \sim -0.02$ V (Raven, 1975; Kurkdjian and Barbier-Brygoo, 1983) so that the distribution of the acids between cytoplasm and vacuole was calculated neglecting the tonoplast potential (the equation requires a value $\neq 0$ and -10^{-4}V was used). The effect of a change in the tonoplast potential from -0.02 to -10^{-4}V is to decrease the predicted values of accumulation, the extent being greater the lower the pKa and the lower the ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of the acid (by a factor of 2 for a weak acid of pKa = 3 and $P_{AH}/P_{A^-} = 10^2$ but the effect is less than 20% for acids with $P_{AH}/P_{A^-} \geqslant 10^4$.

From Figure 10, it can be seen that accumulation of an acid of pKa 5.6 e.g. maleic hydrazide is rather insensitive to the permeability

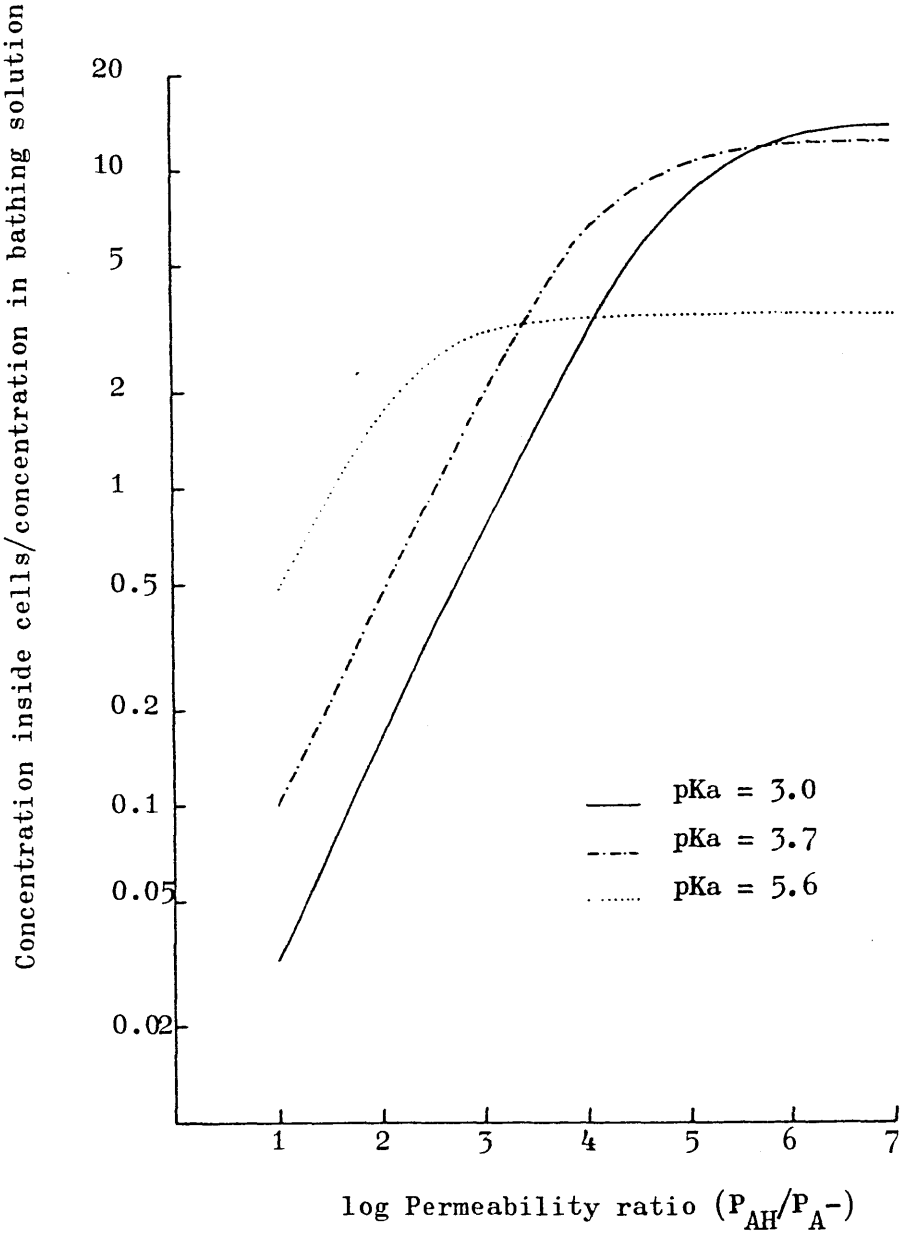


Figure 10. Relationship between theoretical values of accumulation inside cells of weak acids of different pKa's, from a bathing solution of pH 5.0, and the ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of the acids. See text for further details.

ratio, whereas accumulation of acids of pKa ~ 3.0 e.g. 2,4-D and 3,5-D is very sensitive and is very large for permeability ratios above 10^5.

To calculate the ratios of the permeabilities of the membranes to the undissociated and dissociated forms of the acid, it is necessary to know the proportion of accumulated acid that is present in the protoplasm of the root cells. Water in the free space accounts for about 20% of the root weight (Shone and Wood, 1973), and so the chemical in the free space contributes about 0.2 to the RCF at all pH's. Since partition of 2,4-D and 3,5-D onto root solids was shown to be negligible, the amount of chemical in the protoplasm can thus be taken to be the RCF less 0.2. Using this value, taken from the uptake experiments at pH 5.0 at which pH the root cells were assumed to be able to maintain their internal pH, and assuming that the free space was at the pH of the bathing solution, values of the permeability ratios were estimated from Figure 10. The estimated ratios P_{AH}/P_{A^-} for 2,4-D and 3,5-D (pKa~3) were 5.6×10^4 and 4.5×10^4 respectively.

Wedding and Erickson (1957) estimated a value of 1.1×10^3 for 2,4-D in Nitella cells, but these authors did not include the plasmalemma potential in their calculations, consideration of which would have led to a higher value for the permeability ratio. Large permeability ratios have also been found for other weak acids. Assuming a permeability ratio of 10^3, Raven (1975) calculated a concentration of IAA in the vacuole of Hydrodictyon that agreed with experimentally observed values but, based on data produced by other authors on IAA permeation through lipid bilayers in vitro and on measurements of membrane conductance, Raven and Rubery (1982) concluded that the permeability ratio was at least 10^6.

Using the same procedure, the permeability ratios for 2,4-D and 3,5-D were estimated from the observed RCF values at pH 4.0. The estimated values were 1.4×10^4 and 1.1×10^4, respectively. These values are about 5 times lower than those estimated at pH 5.0. It is likely that, even during the first period of 24 h, the external pH 4.0 causes

the internal pH to decrease to a certain extent, which would result in less accumulation and, therefore, the estimated permeability ratio would be less. The RCF values measured at pH's 6.0 and above were higher than those predicted from the permeability ratios estimated from the RCF values at pH 5.0. This may be because the aqueous phase in the free space of the roots was possibly lower than that in the external solution as a result of the proton-pumping mechanism, which maintains the apoplast of rapidly growing tissues at pH levels of about 5.0 (Cleland, 1975).

It is worth mentioning that, whereas literature values of cytoplasm pH are fairly consistent ($\sim$7.0), other authors have given different values for the vacuolar pH and for the proportion of the cellular volume occupied by the vacuole, i.e. pH = 6.0 (Kurkdjian and Barbier-Brygoo, 1983) and 95% of the cellular volume (Goldsmith, 1977). The predicted accumulation of the acids assuming different combinations of vacuolar pH and volumes occupied by vacuole and cytoplasm is shown in Table 6. The assumption of a vacuolar pH = 5.5 (90% of cell volume) gives rise to values of predicted accumulation that are closest to the average of those calculated using the four different combinations and so the choice of a vacuolar pH = 5.5 (90%) is justified.

It seems feasible to conclude that the uptake of 2,4-D and 3,5-D by barley roots can be explained by the 'ion trap' mechanism with no need to invoke the involvement of specific carriers. Shone and Wood (1974) observed that both RCF and TSCF of 2,4-D in barley, following uptake from solution at pH 4.0, were markedly reduced by 10^{-3}M sodium azide and suggested that accumulation of 2,4-D by roots is influenced by metabolism. However, it is likely that sodium azide inhibits the energy-dependent process required for maintenance of the internal pH of the cells rather than a specific carrier mechanism of transport of 2,4-D through cell membranes. The possible role of specific carriers in accumulation by cells and phloem transport of weak acids is discussed

Table 6. Predicted accumulation of weak acids in cells, from a bathing solution of pH 5.0, assuming different values for the vacuolar pH, percentage of the cellular volume occupied by the vacuole and cytoplasm, pKa of the acid and ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of the acid.

	Ratio of permeabilities (P_{AH}/P_{A^-})					
	10^1	10^2	10^3	10^4	10^5	10^6
Vacuole: pH = 5.5 (90%) Cytoplasm: pH = 7.0 (10%)						
pKa = 3.0	0.03	0.17	0.70	2.69	8.82	12.18
pKa = 3.7	0.10	0.48	1.66	6.60	11.26	12.17
pKa = 5.6	0.49	1.68	3.05	3.34	3.37	3.38
Vacuole: pH = 5.5 (95%) Cytoplasm: pH = 7.0 (5%)						
pKa = 3.0	0.03	0.17	0.62	1.87	5.58	7.60
pKa = 3.7	0.10	0.40	1.24	4.91	8.70	9.44
pKa = 5.6	0.16	0.96	1.87	2.06	2.09	2.09
Vacuole: pH = 6.0 (90%) Cytoplasm: pH = 7.0 (10%)						
pKa = 3.0	0.03	0.20	1.22	4.57	13.31	18.05
pKa = 3.7	0.11	0.74	2.98	10.13	16.71	17.99
pKa = 5.6	0.71	2.36	4.18	4.56	4.61	4.61
Vacuole: pH = 6.0 (95%) Cytoplasm: pH = 7.0 (5%)						
pKa = 3.0	0.03	0.20	1.18	3.86	10.32	13.80
pKa = 3.7	0.10	0.72	2.63	7.96	12.80	13.74
pKa = 5.6	0.16	1.96	3.37	3.71	3.71	3.71

in more detail in Section 3.3.2.2.

The increasing concentration of 2,4-D and 3,5-D found in the transpiration stream as the solution pH's decreased paralleled the changes in RCF with pH. The following regression was established for 2,4-D after uptake by roots for 24 h :-

$$RCF = 7.62 \times TSCF + 1.62 \quad (r = 0.99^{**})$$

and for 3,5-D :-

$$RCF = 5.61 \times TSCF + 0.93 \quad (r = 0.99^{**})$$

This proportionality between RCF and TSCF values indicate that these chemicals in the roots were available for transfer to the xylem, though their movement across the endodermis was rather inefficient as concentrations in the transpiration stream were only about 10% of those in the root cortical cells.

3.2.3. Uptake and translocation of other weak acids.

The measured RCF and TSCF values of a series of weak acids and of a non-ionised chemical, 4-chlorophenylurea, after 24 h uptake from nutrient solution of pH's 4.0, 5.0 and 7.0 are shown in Table 7. The extent to which 4-chlorophenylurea was taken up by roots and subsequently translocated to shoots was virtually unaffected by the external solution pH, and the measured RCF and TSCF values were similar to those obtained by Briggs et al. (1982). In contrast, both RCF and TSCF values for the weak acids generally increased as the nutrient solution pH decreased, as observed for 2,4-D and 3,5-D, though the extent of this effect varied considerably amongst the weak acids.

The measured RCF values following uptake from solution of pH 4.0 or 5.0 were highest for the phenoxyacetic acids whose undissociated forms were of intermediate lipophilicity i.e. log $K_{ow} \sim 2.0$ and for the most lipophilic weak acid studied, 4-(2,4-dichlorophenoxy) P.O.A. (log $K_{ow} = 4.5$). The phenoxyacetic acids of intermediate lipophilicity had also the highest

Table 7. Root Concentration Factors and Transpiration Stream Concentration Factors of weak acids and of a non-ionised chemical after 24 h uptake from nutrient solutions of different pH.

Chemical	k[a] (per day)	RCF			$TSCF_o$[b]		
		pH4.0	pH5.0	pH7.0	pH4.0	pH5.0	pH7.0
4-mesyl P.O.A.	0.11	2.62	1.12	-[c]	0.12	0.04	-[c]
3-methyl-4-mesyl P.O.A.	0.08	6.89	1.74	0.86	0.20	0.08	0.04
4-F P.O.A.	0.22	67.9	8.60	0.51	3.95	0.86	0.04
4-Cl P.O.A.	0.31	71.6	11.6	0.58	3.70	0.91	0.05
3,5-D	0.68	24.5	6.41	0.60	4.24	0.72	0.05
2,4-D	0.85	33.3	7.62	0.83	4.14	0.49	0.04
4-(2,4-dichlorophenoxy) P.O.A.	0.56	55.5	26.0	4.80	0.23	0.15	0.04
maleic hydrazide	0.08	2.73	2.14	0.53	0.04	0.13	0.02
flamprop	1.3[d]	5.16	2.20	0.73	d	d	d
4-chlorophenylurea	-[e]	2.11	-[c]	1.96	0.48	-[c]	0.49

a. Degradation rate of chemical in the plant shoots

b. Values corrected for degradation

c. Missing values indicate that they were not measured

d. Degradation of the chemical was very fast, so that the half life, and consequently the TSCF values, could not be properly assessed

e. Degradation rate is sufficiently small in barley shoots to be neglected insofar as correction of the $TSCF_{24}$ is concerned (Briggs et al., 1982).

TSCF values, much higher than those for 4-(2,4-dichlorophenoxy) P.O.A. although their RCF values were comparable. In contrast to the other weak acids, most of the 4-(2,4-dichlorophenoxy) P.O.A. in the roots was strongly partitioned onto the root solids (Figure 11), partition onto macerated roots being only slightly less than uptake by roots of intact plants.

Using the same approach previously discussed for 2,4-D and 3,5-D, the ratios of the permeabilities of the cell membranes to the undissociated and dissociated forms of the weak acids were estimated from the RCF values obtained at pH 5.0 (Table 8). The values of this permeability ratio varied over nearly three orders of magnitude, and was greatest for the acids whose undissociated forms were of intermediate lipophilicity i.e. $\log K_{ow} \sim 2.0$ (Figure 12). In their work on uptake by roots and translocation to the shoots of non-ionised chemicals by barley, Briggs et al. (1982) suggested that chemicals of $\log K_{ow} \sim 2.0$ have the highest permeation rates through cell membranes. Therefore it appears as if the permeability ratio of cell membranes to the undissociated and dissociated forms of weak acids is governed by the permeability to the undissociated acid. The estimated permeability ratio for flamprop was much less than that for 3,5-D, although the two acids have similar $\log K_{ow}$'s. It could be that the extra benzoyl group in the flamprop anion masks the effect of the charge of the molecule and so flamprop anions would permeate the membranes more rapidly than 3,5-D anions. However, this explanation lacks experimental evidence.

The low permeability to the anions appears to be largely due to their charge; for example the anion of 4-(2,4-dichlorophenoxy) P.O.A., despite having a $\log K_{ow}$ of 1.10, did not permeate membranes well as indicated by the low TSCF even at pH 7.0. The ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of this lipophilic acid could not be estimated because, as discussed above, this

chemical was shown to partition strongly onto the root solids (Figure 11) so that the amount of chemical in the protoplasm could not be accurately estimated.

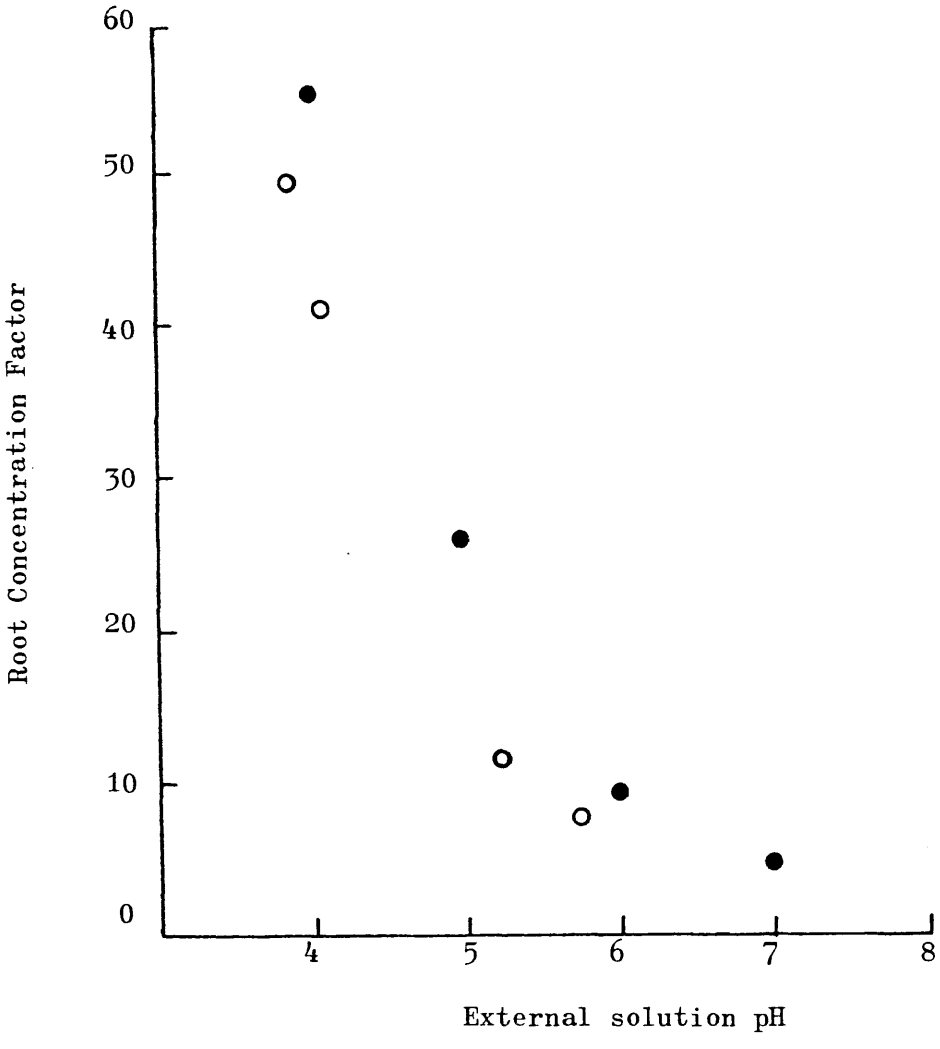


Figure 11. Effect of the bathing solution pH on the uptake of 4-(2,4-dichlorophenoxy) phenoxyacetic acid by roots of barley: (•), roots of intact plants; (o), macerated roots (results expressed on a fresh weight basis).

Table 8. Ratios of the permeabilities of the membranes of barley root cells to the undissociated and dissociated forms of weak acids. Values were calculated from the measured accumulation of the acids inside the cells after 24 h uptake from solution of pH 5.0

Chemical	log K_{ow} (undissoc. form)	Permeability ratio (P_{AH}/P_{A^-})
phenoxyacetic acids ($pK_a \sim 3.0$)		
3-mesyl P.O.A.	0.06	1.4×10^3
3-methyl-4-mesyl P.O.A.	0.54	3.2×10^3
4-F P.O.A.	1.64	1.0×10^5
4-Cl P.O.A.	2.25	4.0×10^5
3,5-D	2.90	4.5×10^4
2,4-D	2.84	5.6×10^4
maleic hydrazide ($pK_a = 5.6$)	-0.63	1.8×10^2
flamprop ($pK_a = 3.7$)	2.90	1.0×10^3

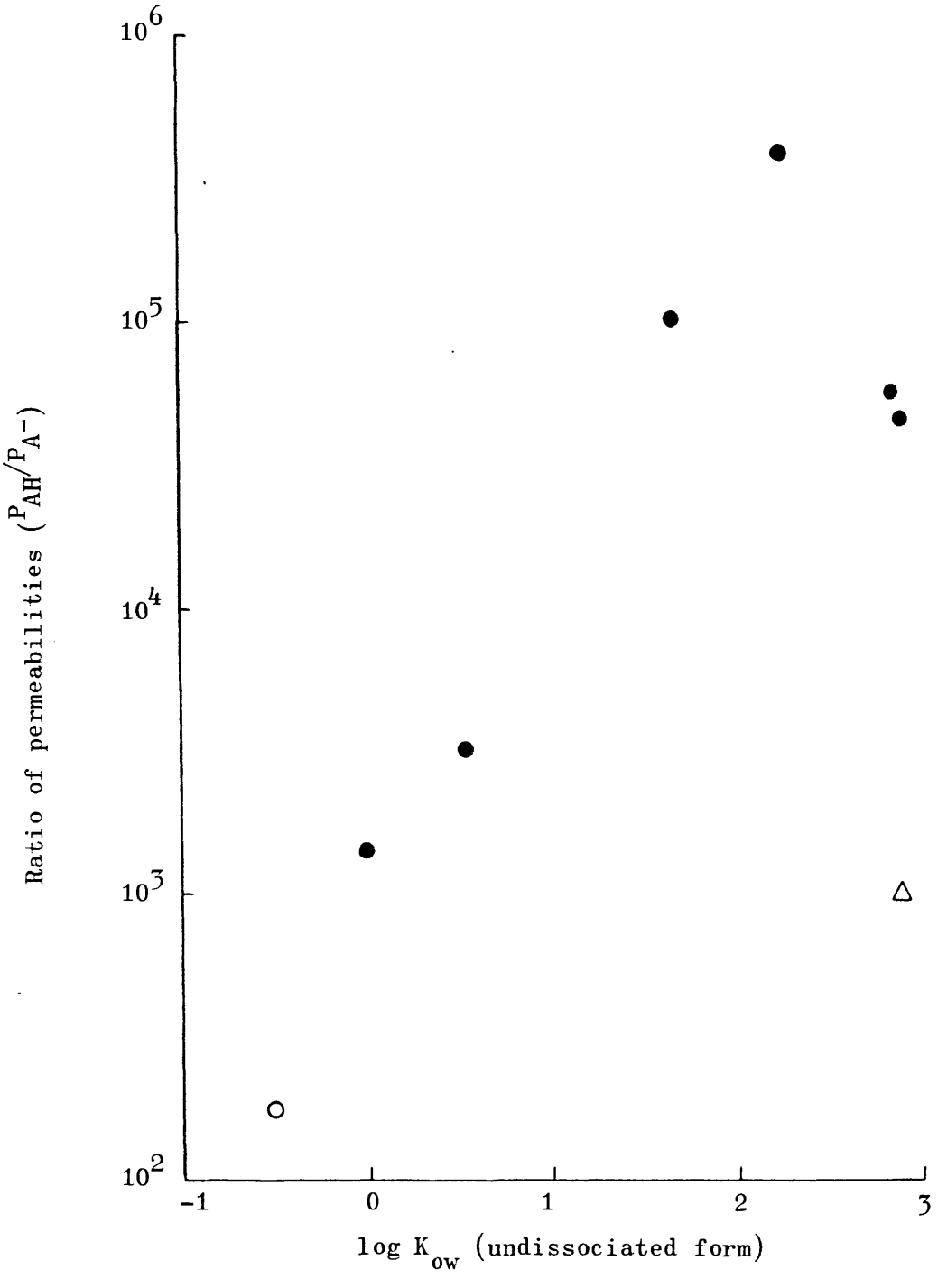


Figure 12. Relationship between lipophilicity of weak acids and the ratio of the permeabilities of the cell membranes to their undissociated and dissociated forms. Phenoxyacetic acids (•), maleic hydrazide (o) and flamprop (Δ).

3.3. Translocation of weak acids in R. communis

3.3.1. Translocation following uptake by roots

3.3.1.1. Intact plants

The studies of uptake of the weak acids by barley roots from nutrient solutions of different pH showed that the pH gradient across the cell membranes is the driving force for the accumulation of the acids inside the cells. The pH inside the phloem sieve cells (8.0 ± 0.1 according to our own pH measurements of phloem exudate from R. communis) is higher than that in the surrounding tissue and so the observed translocation of some weak acids in phloem has been postulated to be explicable by the 'ion trap' mechanism.

Our initial approach to examining the accumulation and translocation of the weak acids in phloem was to investigate their behaviour in intact plants (i.e. petioles non-girdled) of R. communis following uptake by roots. The accumulation of the weak acids by roots (as RCF values), translocation to the shoots (as TSCF values) and ratio between their concentrations in phloem exudate and xylem are given in Table 9. The concentrations in xylem were assessed from the total amounts of chemical accumulated in the shoots, corrected for degradation, for a measured amount of transpiration. The concentrations of the chemicals in the various plant parts, in phloem exudate from the upper and lower stem and in xylem after 24 h uptake by roots from nutrient solution of pH 4.0, and in some cases following subsequent transfer for 24 h to chemical-free solution, are shown in Figure 13. Similarly, the concentrations after uptake from solution of pH 5.0 are given in Figure 14. The concentrations are expressed relative to those in the nutrient solution as described for the non-ionised chemicals (3.1.1.); the average nutrient solution concentration did not differ by more than 10% from those at the beginning and end of the uptake period.

Table 9. Uptake by roots (as RCF), translocation to shoots (as TSCF), and distribution of weak acids in phloem and xylem of R. communis

Chemical	k[a] (per day)	Nutrient solution pH	Time	RCF	TSCF	Phloem[b]/xylem concentration ratio
3,5-D	0.16	4.0	24	12.3	1.48	10.2
		5.0	24	5.0	0.54	7.0
4-F P.O.A.	0.24	4.0	24	18.2	1.10	8.2
		5.0	24	6.2	0.48	6.7
		5.0	72	5.7	0.43	14.9
maleic hydrazide	0.26	4.0	24	2.7	0.13	32.3
		5.0	24	2.2	0.09	35.0
		5.0	72	2.6	0.12	82.5
flamprop	0.15	4.0	24	4.8	1.10	1.1
		5.0	24	1.9	0.40	1.1

a. Degradation rate of the chemicals in the plant shoots

b. Concentration in phloem exudate from upper stem

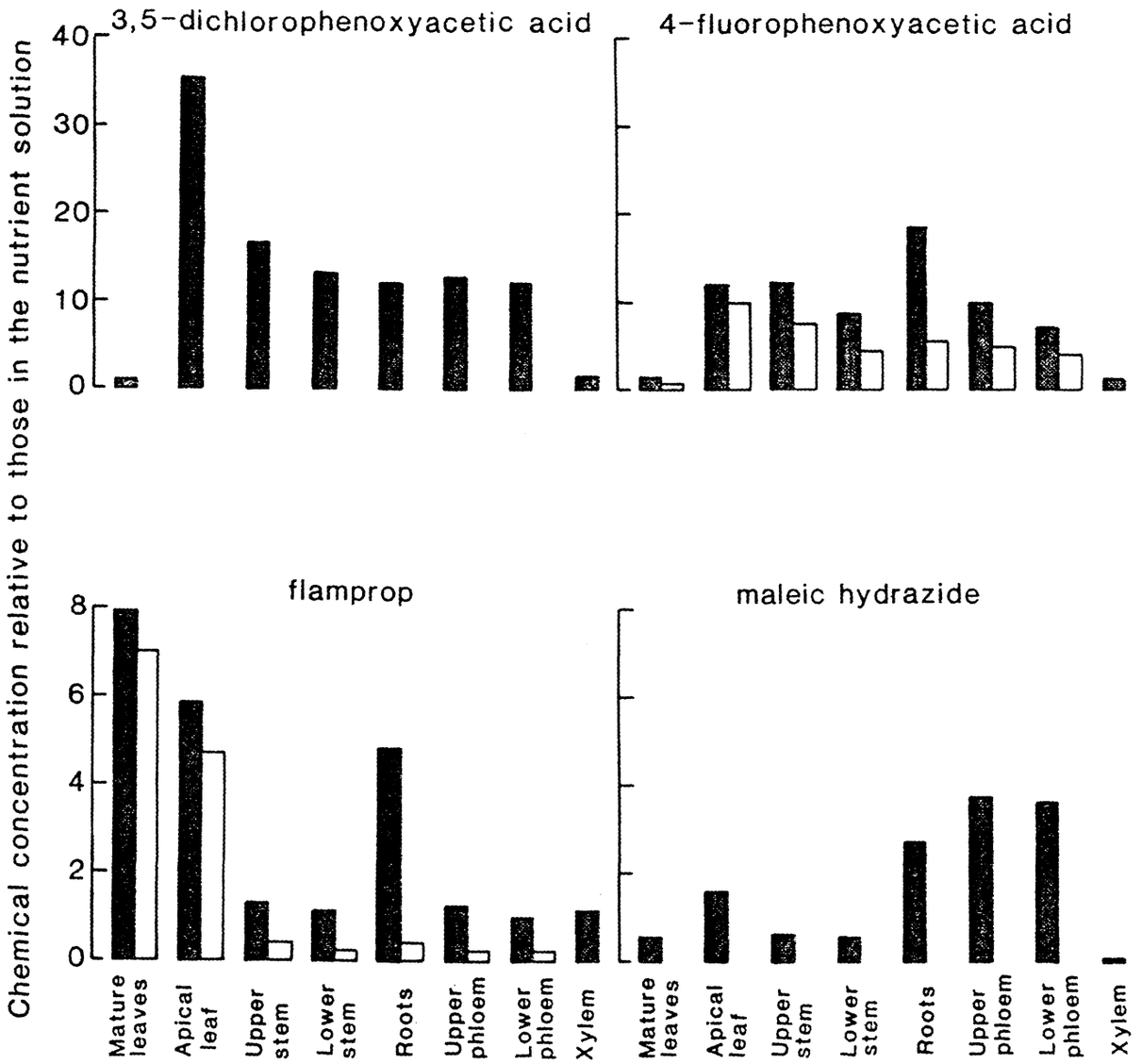


Figure 13. Concentrations of weak acids in the various parts of R. communis plants after 24 h uptake by roots from nutrient solution of pH 4.0 (shaded areas) and following subsequent transfer for 24 h to chemical-free solution (unshaded areas, 4-fluorophenoxyacetic acid and flamprop only). Note the different scale for the phenoxyacetic acids.

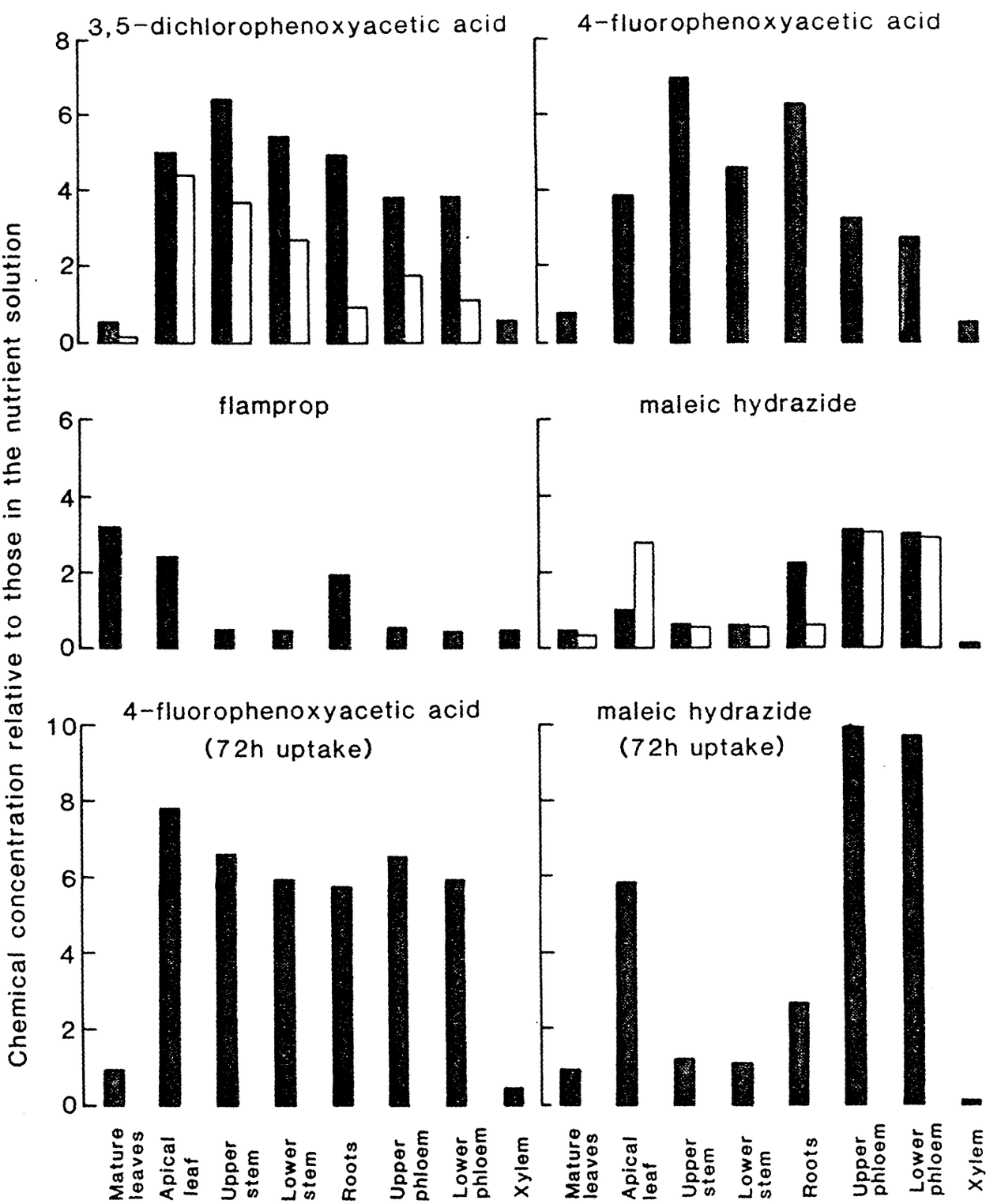


Figure 14. Concentrations of weak acids in the various parts of R. communis plants after (unless stated otherwise) 24 h uptake by roots from solution of pH 5.0 (shaded areas) and following subsequent transfer for 24 h to chemical-free solution (unshaded areas, for 3,5-dichlorophenoxyacetic acid and maleic hydrazide only).

The values of RCF and TSCF of 3,5-D and 4-F P.O.A. at pH 4.0 were considerably lower than those measured in barley but the values were fairly similar at pH 5.0, indicating that the root cells of R. communis are less able to maintain their internal pH than the root cells of barley when the external solution pH is 4.0. The RCF values of flamprop and maleic hydrazide for an external solution of pH 4.0 were not so different from those in barley but these chemicals were less strongly accumulated than 3,5-D and 4-F P.O.A. and so their RCF values at pH 4.0 would be expected to be less sensitive to changes in the internal pH of the cells.

In contrast to its rapid breakdown in barley, flamprop was sufficiently stable in castor oil plants that its translocation to the shoots could be assessed. The TSCF values of flamprop were fairly similar to those of 3,5-D, although the latter accumulated in roots to a greater extent. As suggested previously, flamprop anions may permeate cell membranes more rapidly than do those of other weak acids of comparable lipophilicity. Thus, although 3,5-D is accumulated in roots to a greater extent than flamprop, the greater facility with which flamprop anions permeate cell membranes, in this case the endodermal cells, gives rise to similar TSCF's for the two chemicals.

The concentrations of maleic hydrazide, 4-F P.O.A. and 3,5-D in phloem exudates from the upper stem were much higher than their concentrations in xylem (Table 9). However, these measurements do not distinguish between chemical movement to phloem from the leaves or from the adjacent stem tissue, in which the concentration of the chemicals was in equilibrium with their concentration in xylem. Nevertheless, the phloem/xylem concentration ratios shown in Table 9 do indicate that the ionised form of these chemicals, which is the form almost exclusively present in the phloem sieve cells, permeates the cell membranes sufficiently slowly for the chemicals to be retained in phloem against a concentration gradient with respect to their concentration in xylem. The phloem/xylem

concentration ratios of 4-F P.O.A. and maleic hydrazide increased from 24 to 72 h, which might be attributed to an increase in concentration of these chemicals in the mature leaves. The phloem/xylem concentration ratios of flamprop were close to one, which provides evidence that this chemical does not accumlate in phloem with regard to its concentration in xylem.

The results presented in Figures 13 and 14 show that the concentrations of 3,5-D and 4-F P.O.A. in the apical developing leaf are much higher than in the mature leaves, indicating that, following uptake by roots, these chemicals are transported via the xylem to the mature leaves, from which they are readily phloem translocated to the apical leaf. Evidence for this is presented later.

In the experiments following root uptake for 24 h, a large proportion of the total 3,5-D and 4-F P.O.A. translocated to the shoots was found in the stem, indicating that these chemicals are strongly retained by the stem tissue. When plants treated for 24 h were transferred to chemical-free solution for a further period of 24 h, the concentration of 3,5-D and 4-F P.O.A. in the stem and roots remained high. The concentration of these chemicals in phloem exudate was comparable to or even lower than that in the adjacent stem tissue, in spite of the higher pH inside the phloem sieve cells. These points will be discussed in more detail subsequently (3.3.2.2.), in the light of the results obtained from other experiments with these chemicals.

The distribution of maleic hydrazide indicates that this chemical is also phloem translocated from the mature leaves to the apical developing leaf. In contrast to the phenoxyacetic acids, the concentration of maleic hydrazide in the phloem exudates was much higher than in the adjacent stem tissue.

Flamprop was much less retained by the stem tissue than were

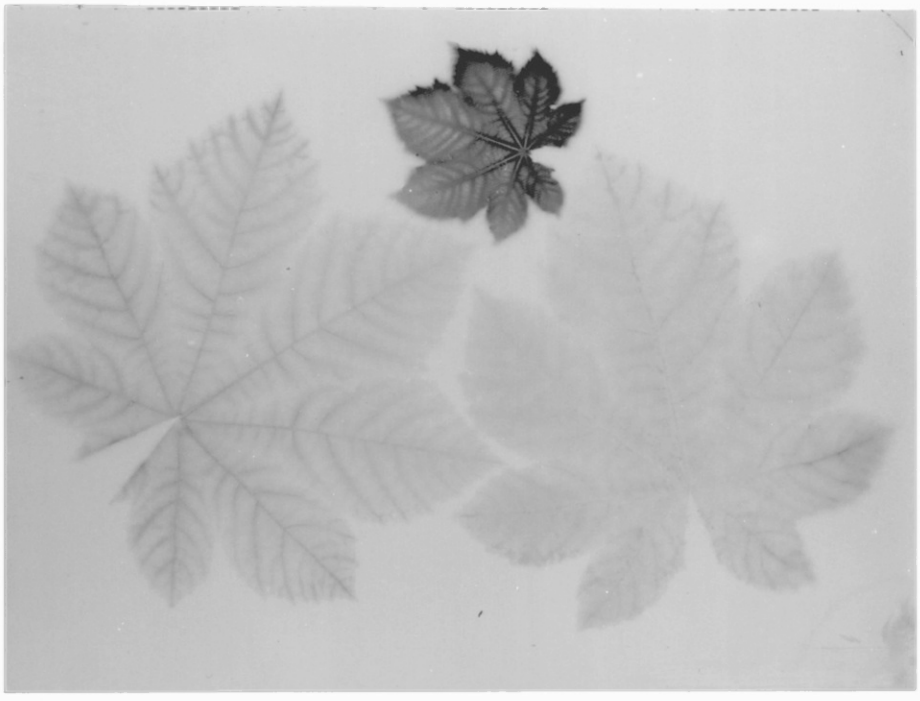
the phenoxyacetic acids and also much less reached the apical developing leaf.

Autoradiographs of the mature leaf blades following uptake of the weak acids by roots for 24 h (Figure 15) showed that, in contrast to the non-ionised chemicals, 4-F P.O.A. was present mainly in and adjacent to the veins, indicating that the same factors determining the retention of 4-F P.O.A. in the stem also determine its retention in the leaf cells. The distribution of 3,5-D was very similar to that of 4-F P.O.A. but the autoradiograph is not shown because the background was too dark. Maleic hydrazide and flamprop were more uniformly distributed in the leaf blades indicating that these chemicals are not as strongly retained by the leaf cells as the two phenoxyacetic acids; the concentration of maleic hydrazide in the interveinal spaces was similar to that in the veins which was probably because of redistribution of maleic hydrazide in the leaves via the phloem.

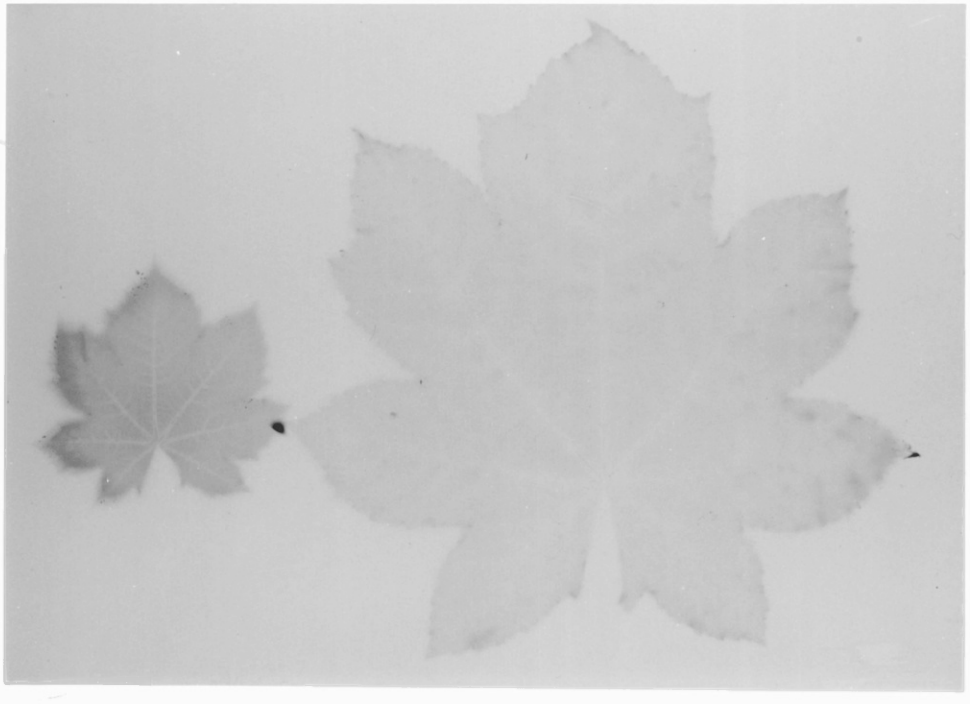
3.3.1.2. Petiole-girdled plants

4-F P.O.A. and 3,5-D were strongly retained in the stem tissue following uptake by roots (see 3.3.1.1.). In order to investigate whether these chemicals accumulate in the stem tissue directly from the transpiration stream, we set up experiments in which the phloem translocation of the chemicals from the mature leaves was stopped by steam girdling the petioles. The distribution of these two phenoxyacetic acids and maleic hydrazide in shoots of the test plants, following uptake by roots for 24 h, is shown in Table 10.

The apical leaf of plants with petiole-girdled mature leaves accumulated much less chemical than that of intact plants. Similar results were obtained by Gaudiel and Vanden Born (1979) who studied the absorption and distribution of root-applied picloram (4-amino-3,5,6-dichloropicolinic acid) in intact soybean plants and in plants whose primary leaf petioles were steam-girdled. The results indicate that



4-fluorophenoxyacetic acid

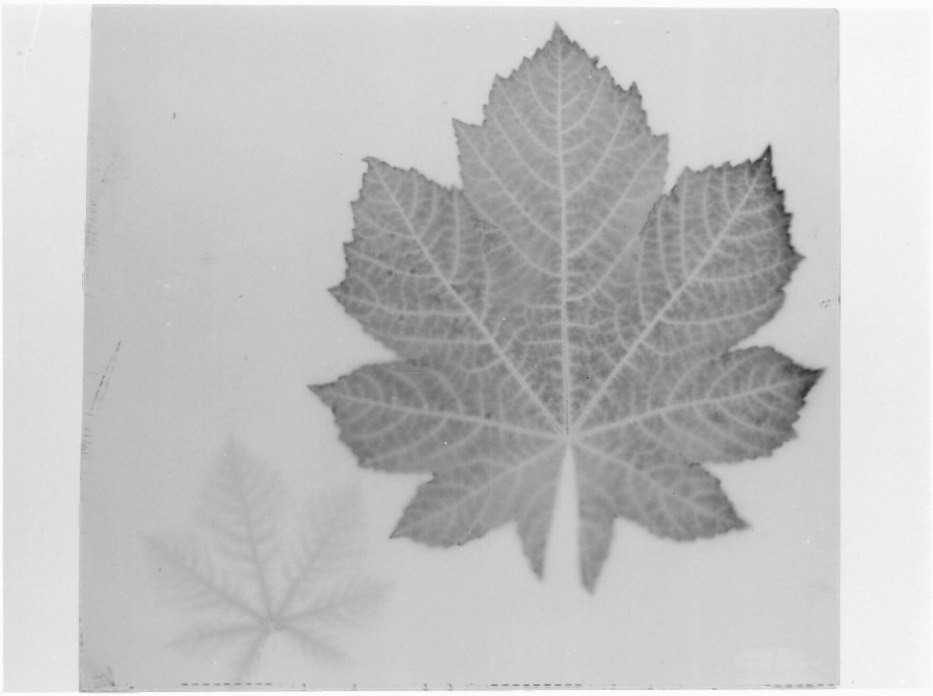


maleic hydrazide

Figure 15. Autoradiographs showing the distribution of 4-fluorophenoxyacetic acid, maleic hydrazide and flamprop within the leaf blades of R. communis after 24 h uptake by roots from nutrient solution of pH 4.0

(continued on the next page)

Figure 15 (contd.)



flamprop

Table 10. Distribution of 3,5-dichlorophenoxyacetic acid, 4-fluorophenoxyacetic acid and maleic hydrazide in petiole-girdled plants of R. communis after 24 h uptake by roots from nutrient solution of pH 4.0 or 5.0.

Chemical	Chemical concentrations relative to those in the nutrient solution				Stem/xylem concentration ratio	
	Mature leaves	Apical leaf	Upper stem	Lower stem	Upper stem	Lower stem
3,5-D (pH 5.0)	1.3	0.8	6.8	6.8	10.7	8.3
4-F P.O.A. (pH 4.0)	3.1	2.1	10.9	7.5	9.3	6.4
Maleic hydrazide (pH 5.0)	0.5	0.3	0.4	0.3	2.4	2.2

most of the 3,5-D, 4-F P.O.A. and maleic hydrazide found in the apical leaf of intact plants after 24 h uptake by roots is a result of phloem transport from the mature leaves. However if, as postulated by Eschrich (1967), sap can flow in opposite directions in parallel longitudinal sieve cells in the stem and transfer of sap between these cells can take place via anastomoses or lateral sieve cells, then some phloem transport of the chemicals to the apical leaf in intact plants of R. communis could occur also directly from the stem, though phloem flow from the mature leaves would still be required for this to occur. Chemical reaching the apical leaf in petiole-girdled plants is likely to have been translocated there exclusively via the xylem; the concentrations of the chemicals were somewhat lower in the apical leaf than in the mature leaves (Table 10), indicating that transpiration per gram of tissue is higher in the mature leaves.

The concentration ratios of the chemicals between stem tissue and xylem, as given in Table 10, show that 4-F P.O.A. and 3,5-D accumulate in the stem tissue to a much higher concentration than in the xylem. Following uptake by roots from a solution of pH 5.0 for 48 h, the stem/xylem concentration ratios of 3,5-D were 11.7 and 7.6, respectively, for the upper and lower stem. These values are very similar to those measured after 24 h (10.7 and 8.3) indicating that equilibrium between the concentration of the chemical in xylem and in the stem tissue is attained within 24 h.

Assuming the concentration of the chemicals and the pH in the stem intercellular space to be the same as those in the xylem sap (measured pH 5.6 $\pm$ 0.1), and the permeability ratio (P_{AH}/P_{A^-}) to be the same as those estimated in barley roots, the predicted stem accumulation of the chemicals by Raven's equation was considerably smaller than the measured values. Thus, if the assumptions made are correct, the

pH in the intercellular space in the stem tissue would have to be lower than that in the xylem sap. The plant hormone indoleacetic acid is capable of stimulating cell enlargement and it is known to activate a plasma-membrane hydrogen-ion pump that reduces the pH of the cell wall from near neutrality in slowly growing tissues to pH 5.0 or less in rapidly growing tissues (Cleland, 1975; McNeil et al., 1984). Therefore, the assumption of a lower pH in the stem apoplast than in the xylem sap is not unrealistic. The required intercellular pH's to match predicted and observed ratios of the concentrations of the chemicals in the stem tissue and in the xylem, respectively for the upper and lower stem, are: 4.8 ± 0.1 and 4.9 ± 0.1 (3,5-D), 4.9 ± 0.1 and 5.1 ± 0.1 (4-F P.O.A.) and 4.4 ± 0.5 and 5.0 ± 0.6 (maleic hydrazide); the pH estimates from the results with maleic hydrazide are very sensitive to small variations in the stem/xylem ratio and are the least reliable values.

Measurements of accumulation of 3,5-D and 4-F P.O.A. by stem segments of R. communis after 8 h uptake from bathing solutions of pH 4.0 and 5.0 showed the following ratios of concentrations respectively in the stem tissue and in the bathing solution: 7.6 and 2.8 for 3,5-D; 9.0 and 3.0 for 4-F P.O.A. Values obtained after 24 h uptake from a solution of pH 4.0 were similar to those obtained after 8 h uptake. Since the average apoplast pH in the stem in intact plants was estimated to be about 5.0, both 3,5-D and 4-F P.O.A. accumulated in stem segments from a bathing solution of pH 5.0 to a much lesser extent than in the stem of intact plants. It is likely that the lower accumulation of these chemicals by stem segments is because the stem cells are no longer able to maintain their normal pH when segments of stem are placed in solution of pH 4.0 or 5.0.

3.3.2. Translocation following injection into petioles

The results from experiments in which the weak acids were applied to the roots via the nutrient solution provided useful inform-

ation about the behaviour of these chemicals. However, the efficiency of phloem transport of these chemicals from the mature leaves to the lower parts of the plant could not be properly assessed. By injecting the chemicals into the hollow petioles of the mature leaves, more information about the translocation of the weak acids in phloem was obtained.

3.3.2.1. Time-concentration profiles of weak acids in stem phloem immediately after injection into petioles.

By monitoring the concentration of the chemicals in phloem exudate over a period of several hours immediately following the injection of the chemicals into the petioles, profiles of chemical concentration in phloem exudate were obtained (Figure 16).

The profiles of 3,5-D and 4-F P.O.A. concentrations in phloem exudate from the upper stem were rather similar and were characterised by a pulse, with highest concentrations occurring 2 to 3 h after injection into the petioles. A parallel experiment in which a single plant was sacrificed each hour for 1 - 6 h after injection into the petioles showed a steady increase in the amount of 3,5-D in the mature leaf blades (Dr. Richard H. Bromilow and Mr. Mark R. Williams, personal communications). This indicates that during the first hours after injection, entry of 3,5-D directly from the petioles into the phloem was important in determining its concentration in phloem in the stem. The concentration of 3,5-D and 4-F P.O.A. in the exudates decreased markedly from the upper to the lower stem, indicating that these chemicals are not well retained in phloem. This observation contradicts the idea that carboxylic weak acids are strongly retained inside the phloem (Crisp, 1972).

With regard to the form in which these acids escape out of the phloem, it is difficult to distinguish between the movement of undissociated and dissociated forms. Although the undissociated molecules of these acids permeate the cell membranes much more readily

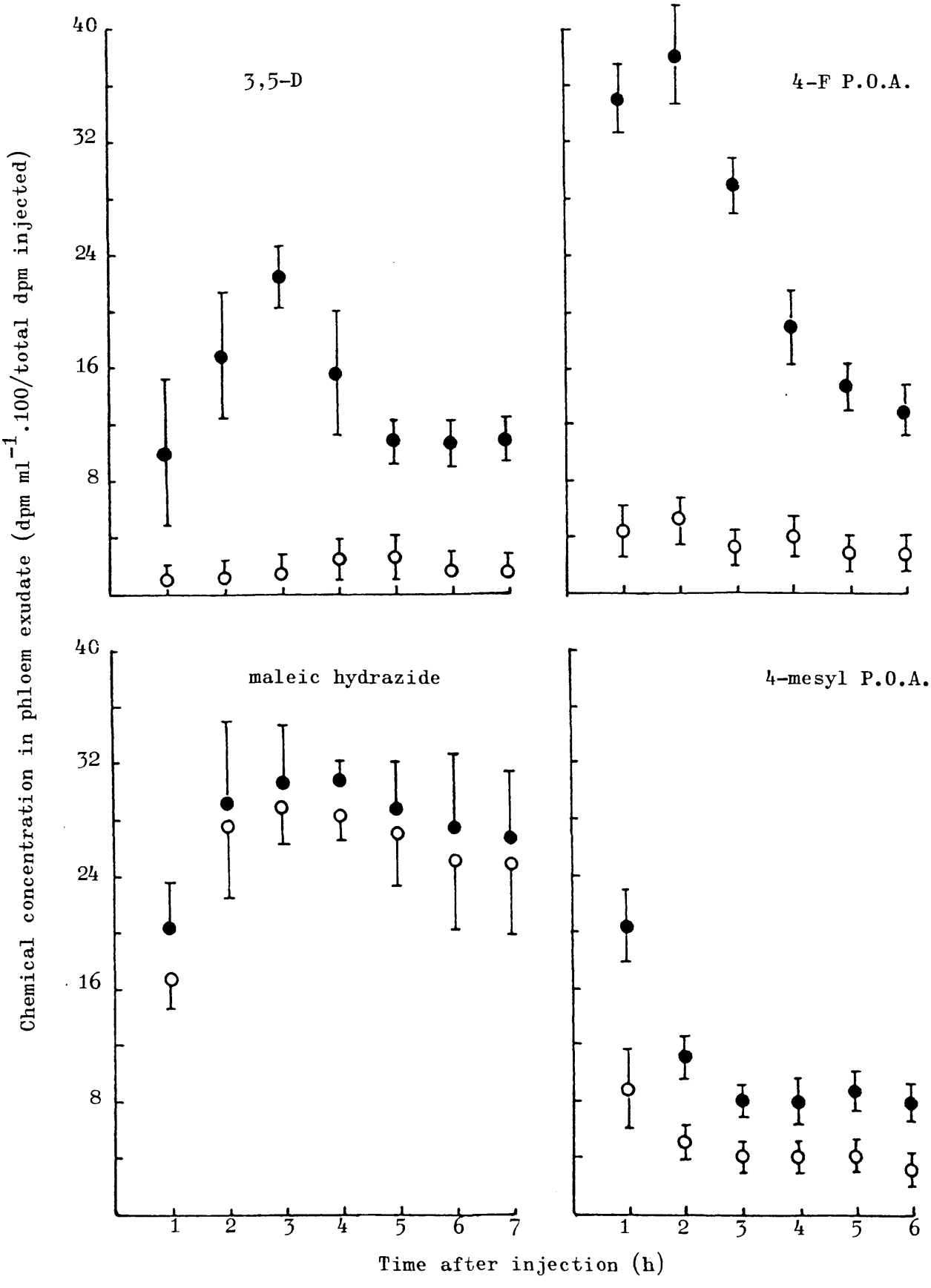


Figure 16. Time-concentration profiles of weak acids in phloem exudate from upper (•) and lower (o) stem of R. communis after injection of the chemicals into the petioles. Vertical bars indicate the range for the replicates.

than the dissociated molecules, at pH 8.0 the concentration of the dissociated molecules is about 10^5 times higher than that of the undissociated molecules, and the effect of the negative electro-chemical potential inside the phloem sieve cells in relation to the adjacent apoplast is to increase the rate with which the dissociated molecules escape out of the phloem. When the concentration gradient of 3,5-D in the phloem exudates along the stem was greatest, it was comparable to that of phenylurea (see 3.1.2.). From the work by Briggs et al. (1982), the undissociated form of 3,5-D is expected to permeate through cell membranes at a similar rate to phenylurea (log K_{ow} values about one unit higher or lower respectively than the optimum value for permeation i.e. ~ 2.0) but 3,5-D is present in phloem sieve cells almost entirely 99.999%) as the dissociated molecule, indicating that the escape of 3,5-D anions probably plays an important role in the loss of 3,5-D from the phloem.

The concentration of 3,5-D and 4-F P.O.A. in phloem exudate from the lower stem did not change significantly with time, despite the decrease in concentration of the chemicals in the exudate from the upper stem. It is possible that, as the concentration in phloem in the upper stem started to decrease, the requirement of chemical by the upper stem tissue to reach equilibrium with the phloem diminished and, as a result, the concentration of the chemicals in phloem no longer decreased so steeply along the stem. If this were the case, despite the decrease in concentration in phloem in the upper stem, the concentration in phloem in the lower stem would remain fairly constant.

The profile of maleic hydrazide was different from those of 4-F P.O.A. and 3,5-D. The concentrations of maleic hydrazide in the exudates from the upper and lower stem were fairly similar at all times, increasing during the first two hours and decreasing very slowly during the subsequent hours. The absence of a gradient in the phloem con-

centration of maleic hydrazide, even during the first hour when presumably the stem tissue was not saturated with the chemical, indicates that maleic hydrazide is well retained in phloem. Retention of maleic hydrazide in phloem is likely to be related to the very low permeation rates through membranes, and consequently very low rates of escape out of the phloem, of both undissociated and dissociated forms of maleic hydrazide (free acid log K_{ow} = -0.6, pKa = 5.6).

The profiles of 3,5-D and maleic hydrazide concentrations in phloem exudate from the upper stem were very similar to those obtained by Chamberlain et al. (1984), who applied the chemicals by perfusion into the petiole of a mature leaf and monitored their concentration in phloem exudate collected from a single point in the stem immediately below the treated petiole.

The profile of a polar phenoxyacetic acid, 4-mesyl P.O.A. (log K_{ow} = 0.06), was also somewhat different from those of phenoxyacetic acids of intermediate lipophilicity. Being a polar chemical, entry into the phloem was expected to be slow, which was confirmed by the relatively low concentration in phloem when expressed as a fraction of the total amount injected. Unexpectedly, however, the concentration in phloem exudate collected during the first hour was higher than those in subsequent hours, which may be ascribed to the typically slower rate of exudation during the first hour, allowing more chemical to enter the phloem per unit of time. A similar effect was not observed with the other chemicals because they enter the phloem more rapidly (3,5-D and 4-F P.O.A. have log K_{ow}'s closer to the optimum value for permeation through membranes and the polar maleic hydrazide has a much higher pKa) and therefore the effect of the phloem flow rate on their concentrations in phloem is likely to be less. The concentration gradient of the 4-mesyl P.O.A. in phloem along the stem was not as steep as it was for

3,5-D and 4-F P.O.A., and the gradient was fairly constant throughout the experiment.

3.3.2.2. Distribution of the weak acids in treated plants.

The distributions of the weak acids in the plants, 24 and 72 h after petiole injection, are shown in Figure 17. The amount of chemical remaining in the petioles varied considerably. The amount found in the petioles 24 h after injection, expressed as the percentage of the total recovered, is plotted in Figure 18 as a function of log K_{ow} of the undissociated form of the acids. Polar phenoxyacetic acids largely remained in the petioles, indicating that these chemicals move from the hollow of the petiole into the vascular tissues (phloem and xylem) only very slowly. As discussed earlier, polar chemicals permeate cell membranes slowly and, because the phenoxyacetic acids were present in the tissue mainly as the dissociated form which would permeate the membranes at a significantly lower rate than the respective dissociated form, the polar phenoxyacetic acids were expected to enter the cells in the petioles (including the phloem sieve cells) very slowly. The slow transfer of these chemicals from the hollow of the petioles into the xylem vessels indicates the presence of a barrier in the apoplastic route between the two compartments, but it is not clear whether this barrier affects the apoplastic movement of some chemicals more than others. Despite being a polar weak acid, maleic hydrazide moved from the petioles more readily than the polar phenoxyacetic acids; this is attributed to the much greater proportion of the hydrazide present in the tissue as the undissociated molecule due to its higher pKa, the cell membranes being much more permeable to the undissociated than to the dissociated form of the acids.

The phenoxyacetic acids of intermediate lipophilicity remained in the petioles to a much lesser extent than the polar phenoxyacetic

acids, indicating that the higher permeation rates through cell membranes of the undissociated form of the former acids result in more efficient transfer into the vascular tissues. Flamprop, another weak acid of intermediate lipophilicity, was also readily translocated from the petioles but, unlike the phenoxyacetic acids, was found largely in the mature leaf blades.

The most lipophilic acid studied, 4-(2,4-dichlorophenoxy) P.O.A., was strongly retained in the petioles, this retention being ascribed to partitioning of the chemical onto the tissue solids as observed for barley roots (page 89). Strong retention of diclofop 2-[4-(2,4-dichlorophenoxy) phenoxy] propionic acid , another very lipophilic weak acid, in the abraded treatment zone of oat and soybean leaves has been reported by Hall et al. (1982), but these authors did not attempt to explain the results.

The extent to which the phenoxyacetic acids were phloem translocated following petiole injection was also controlled by the log K_{ow} of the undissociated form of the acids. The relationship between the percentage of the total chemical recovered that was found in the apical leaf, stem, roots and nutrient solution, 24 h after injection into petioles, and the log K_{ow} of the acids is shown in Figure 19. The phenoxyacetic acids of intermediate lipophilicity are seen to be best phloem translocated, though it is difficult to distinguish between phloem transport from the petioles and from the mature leaf blades. Translocation of these acids to the phloem sinks is favoured both by their ready entry into the phloem from the hollow of the petiole and, as will be discussed later, by their more efficient accumulation in phloem sieve cells in the leaves.

In their study on translocation of a series of phenoxyacetic acids in soybean (see page 30), Crisp and Larson (1982) concluded that phloem translocation of the acids was not correlated with their log K_{ow}

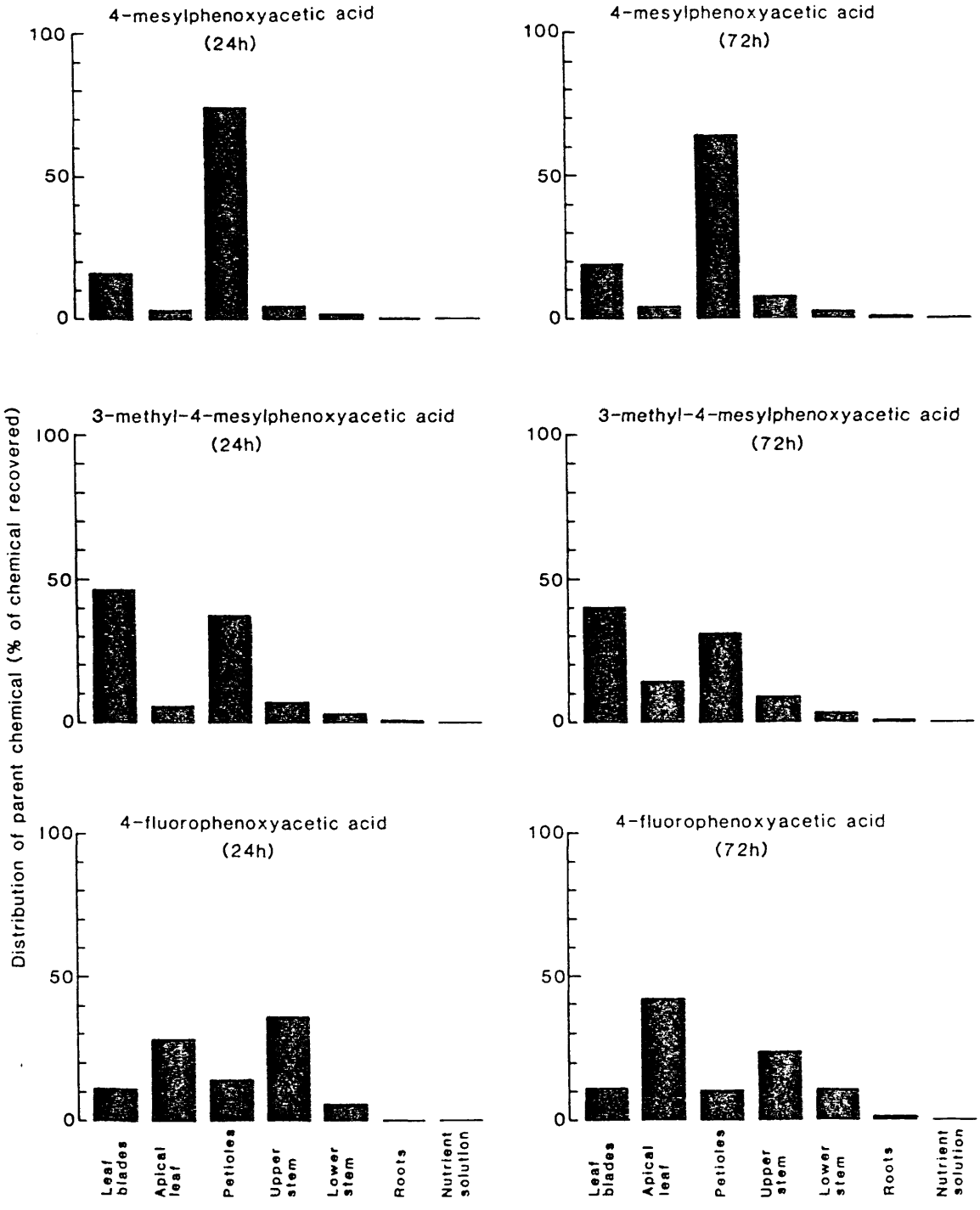


Figure 17. Distribution of weak acids in R. communis 24 and 72 h after injection of the chemicals into the petioles. (Continued on the next two pages).

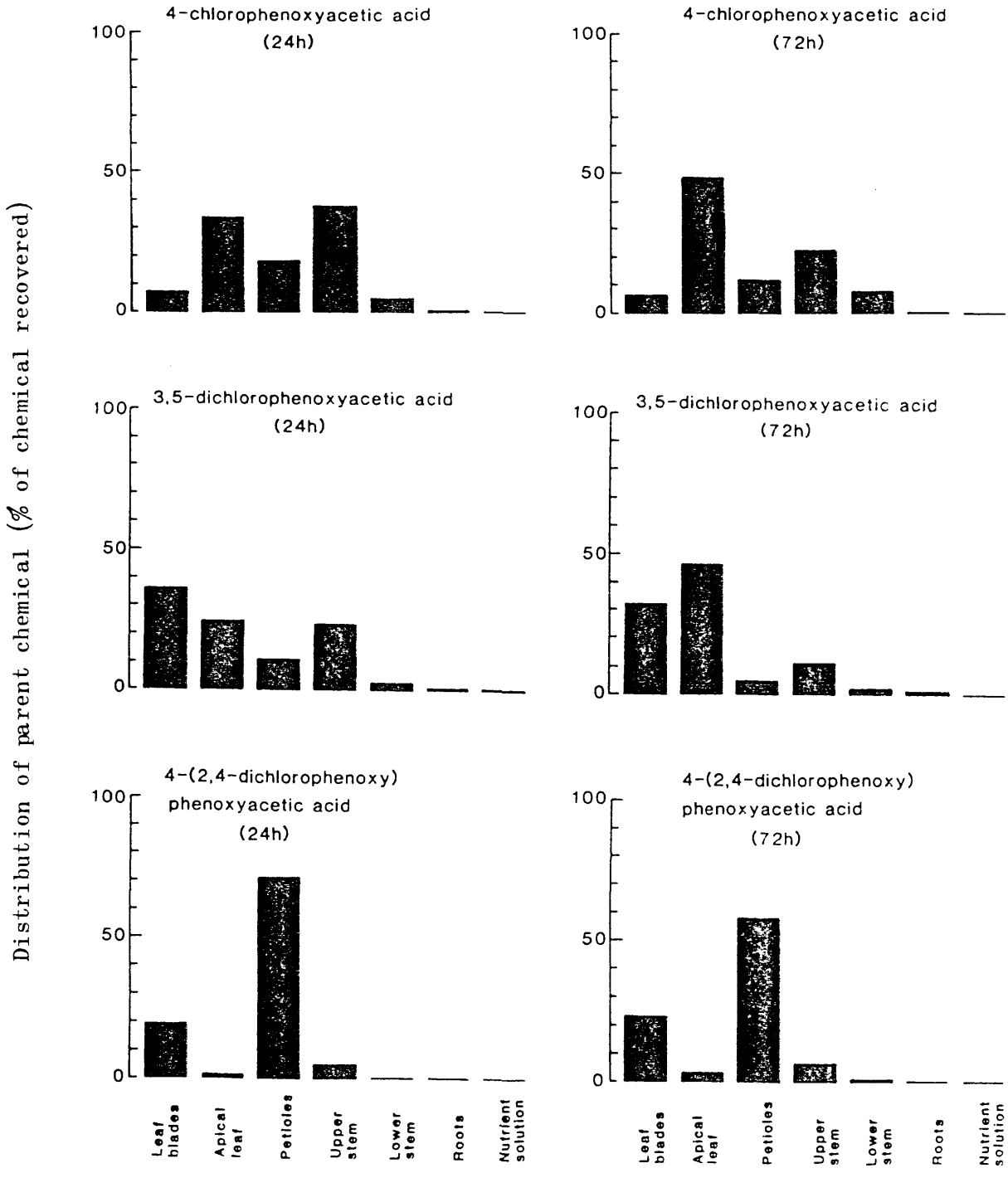


Figure 17. (contd.).

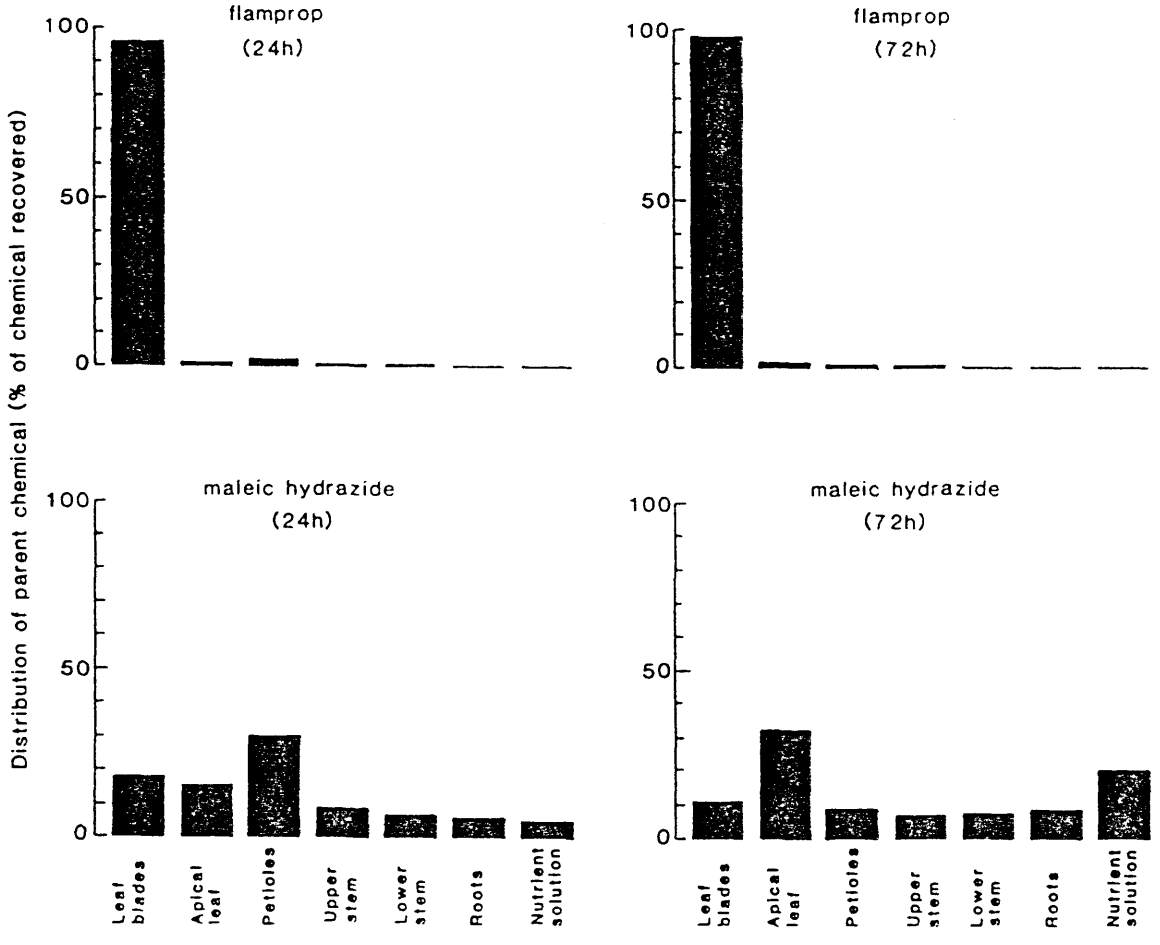


Figure 17. (contd.).

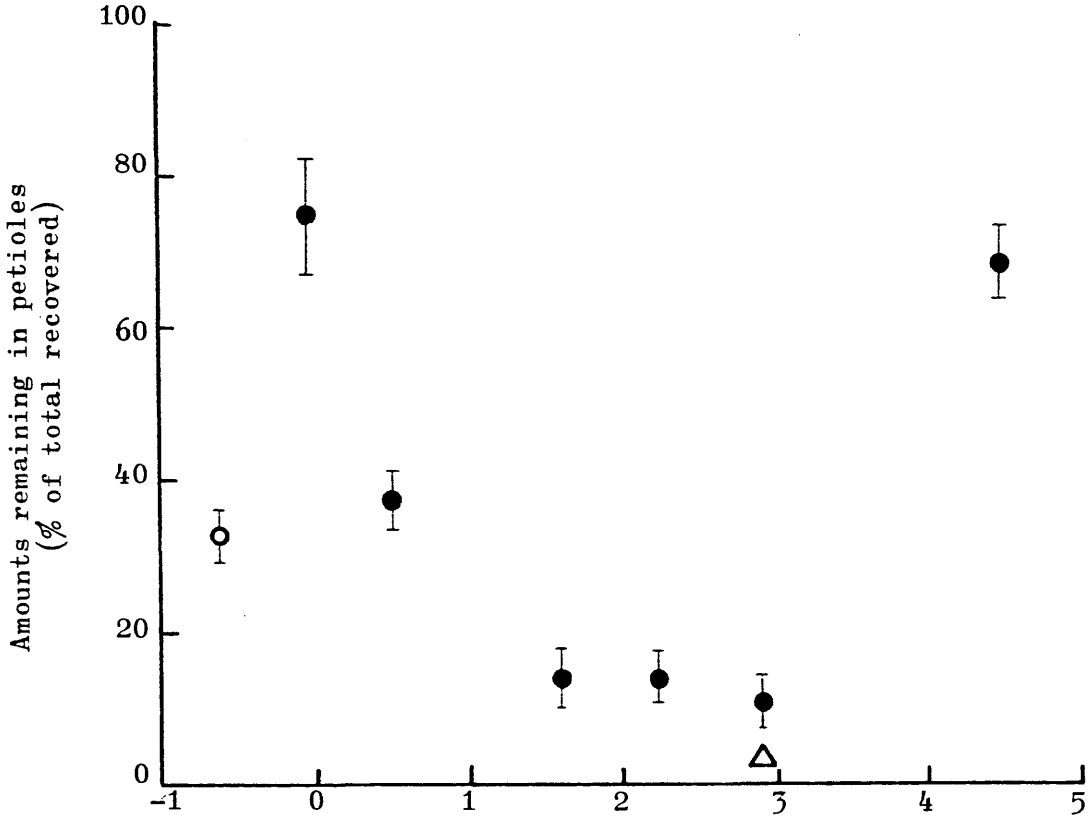


Figure 18. Relationship between lipophilicity of weak acids and their retention in the petioles of R. communis 24 h after injection of the chemicals into the petioles. Phenoxyacetic acids (•), maleic hydrazide (o) and flamprop (△). Vertical bars indicate the range for the replicates.

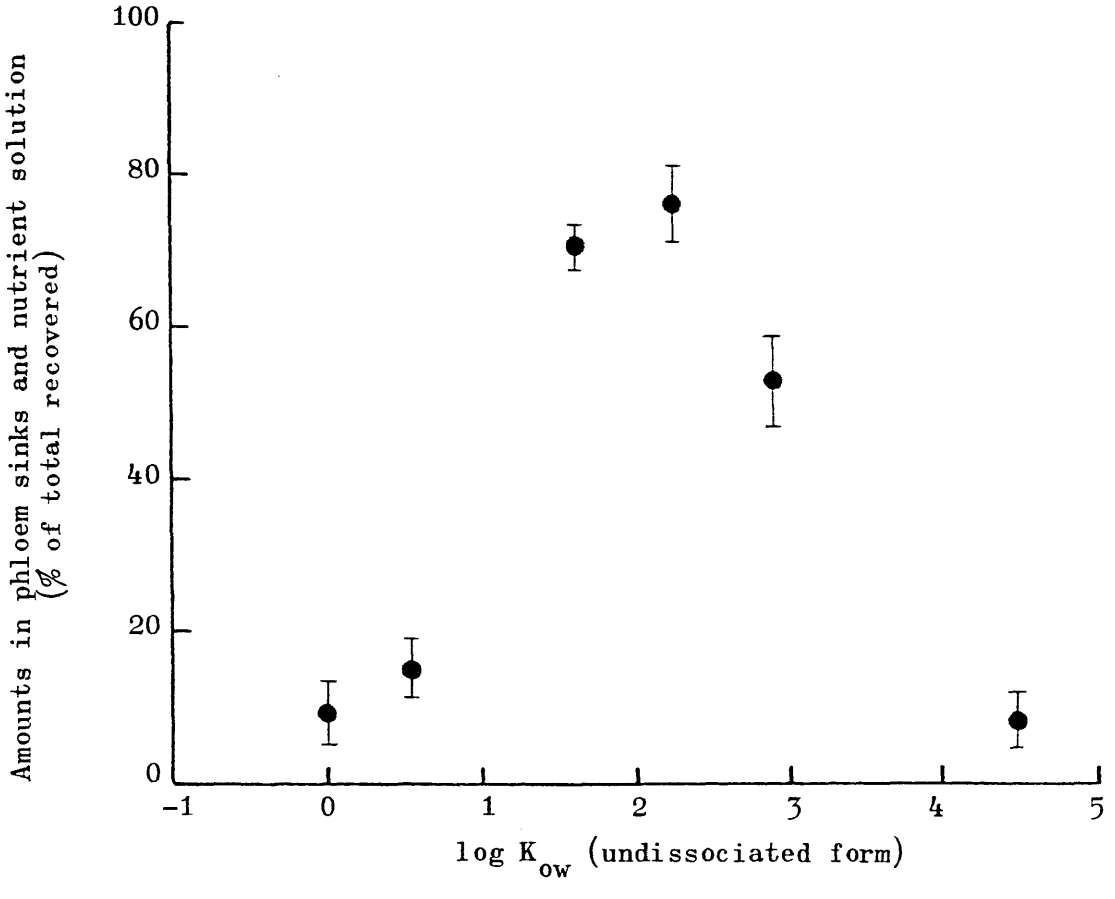


Figure 19. Relationship between the lipophilicity of phenoxyacetic acid analogues and the extent of their phloem translocation from leaves of R. communis 24 h after injection of the chemicals into the petioles. The total amount of chemical found in the phloem sinks (apical leaf, stem, roots) and nutrient solution is expressed as a percentage of the total chemical recovered from the plant. Vertical bars indicate the range for the replicates.

or pKa, the values of which were not given. However, the acids they studied have very similar calculated pKa's ($\sim$3.0, according to Perrin et al., 1981) so that an appreciable effect of the pKa of the acids on their translocation in phloem would not be expected. Furthermore, phenoxyacetic, 2-chloro-phenoxyacetic and 2-methyl-4-chlorophenoxyacetic acids were almost completely metabolised during the experiment so that their behaviour could not be properly assessed. Among the remaining acids, the percentage of the total recovered that was phloem translocated from the treated primary leaf was higher for 4-Cl P.O.A. and 4-F P.O.A. ($\log K_{ow}$'s $\sim$ 2.0) than for 2,4-D ($\log K_{ow}$ = 2.8) and 2,4,5-T (calculated $\log K_{ow}$ = 3.5, Dr. G.G. Briggs, personal communication), which is in agreement with our results.

The distribution patterns of the chemicals in plants 24 and 72 h after injection into the petioles showed that the phenoxyacetic acids of intermediate lipophilicity were phloem translocated mainly to the apical leaf and upper stem (Figure 17). Leakage out of the phloem and retention in the stem tissue resulted in a steep concentration gradient down the stem, and very little of these chemicals was found in roots. The high concentrations of the chemicals in the upper stem tissue probably resulted from their high concentrations in phloem during the first hours after injection, rapid movement from the phloem occurring into the adjacent stem tissue. Although the concentrations in the exudate from the upper stem were shown to decrease sharply after the first hours following injection (3.3.2.1.), concentrations in the upper stem tissue 24 h after injection were still much higher than those in the lower stem tissue. This indicates that, although these chemicals move rapidly from the phloem into the adjacent stem tissue, the return movement from the stem tissue into the phloem is very slow. As will be discussed subsequently, these chemicals are expected, according to the equation

given by Raven (1975), to accumulate in the phloem sieve cells in the mature leaves to concentrations higher than those in the adjacent tissue. However, concentrations in the exudate from the upper stem were indeed lower than those in the adjacent stem tissue (see Table 11). This provides further evidence for the slow release of these chemicals from the upper stem into the descending phloem sap, which contains relatively little of these chemicals coming from the leaves 24 h after injection. The slow release of these chemicals from the stem tissue into the adjacent phloem is in line with the observed lack of phloem accumulation of 3,5-D and 4-F P.O.A., with regard to their concentration in the upper stem tissue, following uptake by roots (3.3.1.1.). Apparently in those experiments, the concentration of the chemicals in the mature leaves was not sufficiently high to result in a concentration in phloem in the upper stem higher than the concentration in the upper stem tissue, the latter being at equilibrium with the concentration in the xylem.

Movement of the phenoxyacetic acids of intermediate lipophilicity from the stem tissue into the mature leaves via the xylem was shown to be also very slow. When 3,5-D was injected into the middle section of the stem of petiole-girdled plants, 88.8% of the total recovered 24 h after injection was still found in the stem (with 9.8% in the mature leaves and 1.4% in the apical leaf). However, the slow release of these chemicals from the stem tissue into the xylem was probably sufficient to reduce the proportion of chemical recovered that was found in the stem at 72 h compared to that at 24 h after petiole injection. Movement of the chemicals out of the apical leaf was not expected since leaves do not export phloem sap until they are approximately one-quarter to one-half of their fully-expanded size (Hopkinson, 1964); as a result of the continuous phloem transport of the chemicals into the apical leaf, the proportion of the total chemical recovered that was found in the apical

leaf increased from 24 to 72 h after injection (Figure 17).

The distribution of the phenoxyacetic acids of intermediate lipophilicity following injection into the petioles resembled that observed by other authors for such chemicals following foliar application to different plant species (see 1.2.3.2.). The observed behaviour does not appear to be restricted to phenoxyacetic acids since picloram (free acid log K_{ow} = 1.1 (calculated value by Dr. Geoffrey G. Briggs, personal communication) and pKa = 3.6 (The Pesticide Manual)) was readily phloem translocated from developed leaves of wheat (Hallmen and Eliasson, 1982) and soybean (Gaudiel and Vanden Born, 1979) but only very little of the chemical was found in roots. Strong retention of these acids by the stem tissue was found but generally no explanation for this retention was offered. Martin and Edgington (1981) suggested that the strong retention of 2,4-D by the stem of soybean plants, following application of the chemical to the primary leaf, is a result of binding of 2,4-D to cellular components. However, the accumulation of 3,5-D and 4-F P.O.A. by the stem of petiole-girdled plants following uptake by roots (3.3.1.2.), as well as their accumulation by roots of barley and R. communis, could be accounted for by the 'ion-trap' mechanism, which does not involve binding of these acids to cellular components nor the participation of carriers in their movement through membranes (the possible existence of membrane carriers for these chemicals is discussed below).

Long and Basler (1974) suggested that the behaviour of 2,4-D and 2,4,5-T is specific for IAA-like herbicides, with their distribution in plants being controlled by carrier-mediated transport of these chemicals through membranes. However, although there is some evidence that the transmembrane transport of IAA and 2,4-D involves also saturable carrier-mediated components for influx and efflux, the extent to which these acids are accumulated by cells is generally agreed to be governed primarily by the 'ion trap' mechanism (Rubery and Sheldrake, 1974; Goldsmith, 1977;

Rubery, 1977; 1978; 1980; Raven and Rubery, 1982). The overall effect of membrane carriers for these acids is suggested to be a reduction in the accumulation by the 'ion trap' mechanism (Rubery, 1980; Raven and Rubery, 1982) and so it is unlikely that the strong retention of the phenoxyacetic acids of intermediate lipophilicity by the stem tissue of R. communis is a result of a carrier-mediated mechanism of transport of these acids through membranes. The rapid efflux of these acids from the phloem sieve cells along the stem is also unlikely to be a result of carrier-mediated transport through membranes because when the concentration of 3,5-D and 4-F P.O.A. in the upper stem exudate after injection into the petioles was maximal (Figure 16), it was over 300 times higher than the concentration of endogenous IAA (0.06 μM) measured in phloem exudate from the stem of R. communis (Hall and Baker, 1972).

Little information is available on the kinetics of the carrier-mediated transport of IAA and 2,4-D through membranes. Studying the uptake of these acids by suspension-cultured crown gall cells, Rubery (1978) reported half-saturation concentrations of about 1 μM for their carrier-mediated uptake from solution at pH 5.0. Kinetics of the carrier-mediated efflux of these acids have not been established. In the studies of uptake by roots and subsequent translocation of 2,4-D and 3,5-D to shoots of barley, the concentration of these acids in the xylem remained proportional to their concentration in the root cells, in which the concentrations varied from 2.5 to 120 μM depending upon the external solution pH (see 3.2.2.); this indicates that efflux of 2,4-D and 3,5-D from the root cells into the xylem is unlikely to be carrier-mediated over this range of concentrations (for comparison, the concentrations of the phenoxyacetic acids of intermediate lipophilicity in the upper stem of R. communis 24 h after injection into the petioles were 4 μM for 3,5-D and 9 μM for 4-F P.O.A. and 4-Cl P.O.A.).

Thus, the observed behaviour of the phenoxyacetic acids of intermediate lipophilicity appears to be determined mainly by the

'ion trap' mechanism, although the rapid loss of these acids from the phloem sieve cells into the adjacent stem tissue and their very slow subsequent movement therefrom to other parts of the plant are not readily explicable.

As discussed previously, the two more polar and the most lipophilic phenoxyacetic acids studied were phloem translocated to a much lesser extent than the phenoxyacetic acids of intermediate lipophilicity. The concentrations of 4-mesyl P.O.A. and 3-methyl-4-mesyl P.O.A., the most polar phenoxyacetic acids, in the phloem exudate from the upper stem 24 and 72 h after petiole injection were not so different from those of phenoxyacetic acids of intermediate lipophilicity (see Table 11). However, the concentrations of the latter acids were much higher during the first hours after injection and thus they were phloem translocated to a much greater extent. Although very little of the polar phenoxyacetic acids was found in roots, the concentration gradient of these acids along the stem, both in the tissue and in the phloem exudates, was not as steep as it was for the phenoxyacetic acids of intermediate lipophilicity, indicating that the polar phenoxyacetic acids are not so rapidly lost from the phloem. 24 h after petiole injection, the concentration of the phenoxyacetic acids in phloem exudate from the upper stem was higher than in the adjacent stem tissue, but the concentrations were rather similar 72 h after injection, indicating that it takes a long time for equilibrium to be attained between the concentration of these chemicals in phloem and in the whole of the adjacent stem tissue (see Table 11). The steep concentration gradient of 4-(2,4-dichlorophenoxy) P.O.A. along the stem, both in the tissue and in phloem exudate, indicates that this chemical is lost from the phloem rapidly and is retained by the adjacent stem tissue due to partitioning onto the tissue solids.

The distribution patterns of maleic hydrazide show that, although maleic hydrazide moved from the petioles slowly, it was readily phloem

translocated from the mature leaves. The concentrations of maleic hydrazide in phloem exudate from the upper and lower stem were similar and were about 7 times greater than that in the stem tissue (see Table 11). As a result, maleic hydrazide was found in appreciable amounts in the roots and leakage out of the roots resulted in appreciable amounts of maleic hydrazide being found also in the external nutrient solution. Efficient phloem translocation of maleic hydrazide to roots, following application to primary leaves of soybean (Crafts, 1967) and of Sinapsis (Jacob and Neumann, 1982), has already been reported.

Flamprop moved readily from the petioles but was found almost entirely in the mature leaf blades indicating inefficient transport in phloem. Although flamprop has log K_{ow} and pKa values comparable to those of 3,5-D, the behaviour of the two chemicals was quite different. As suggested earlier, the dissociated molecules of flamprop appear to permeate the cell membranes more readily than do the dissociated molecules of 3,5-D and so the retention of flamprop anions in phloem is insufficient for the chemical to be efficiently phloem translocated.

Although the phenoxyacetic acids of intermediate lipophilicity were shown to enter the phloem directly from the petioles during the first hours after injection, their concentration in the upper phloem appeared to be correlated with their concentration in the mature leaf blades from 24 h onwards, since the ratio between the concentration of these chemicals in upper phloem and in the mature leaf blades was fairly constant from 24 to 72 h after injection. Such a correlation occurred also for maleic hydrazide (see Table 11).

In contrast to the non-ionised chemicals, the concentration of the phenoxyacetic acids of intermediate lipophilicity and of maleic hydrazide in phloem exudate from the upper stem was several times greater than that in the mature leaf blades, indicating accumulation of these chemicals in the phloem of mature leaves.

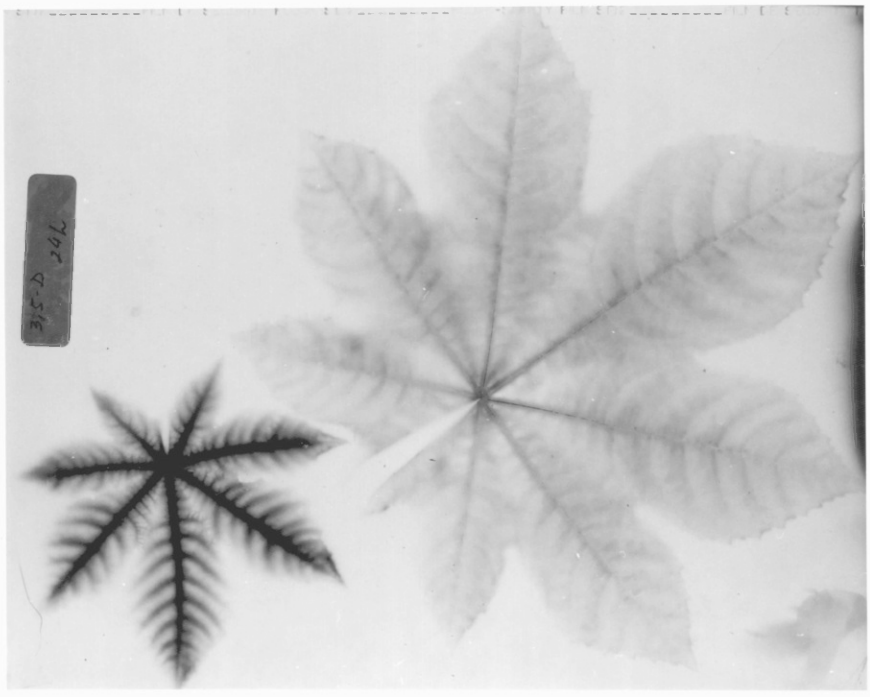
Table 11. Concentrations of the weak acids in phloem exudate from R. communis 24 and 72 h after injection into the petioles. Concentrations are expressed relative to the total amounts in the plant and to the concentrations in plant tissues.

Chemical	k [a] (per day)	Time (h)	Conc. in the exudate (dpm.ml^{-1}).100 / Total chemical recovered (dpm)		Concentration ratios	
			Upper stem exudate	Lower stem exudate	Upper stem exudate / Mature leaf blades	Upper stem exudate / Upper stem tissue
4-mesyl P.O.A.	0.23	24	8.9	4.3	1.3	2.0
		72	4.6	2.7	1.3	1.3
3-methyl-4-mesyl-P.O.A.	0.21	24	10.9	5.3	1.0	2.4
		72	6.0	3.3	0.8	1.0
4-F P.O.A.	0.24	24	19.6	5.9	7.4	0.8
		72	16.4	6.0	7.3	0.9
4-Cl P.O.A.	0.28	24	13.8	3.8	8.9	0.8
		72	12.1	4.7	7.2	1.0
3,5-D	0.16	24	14.4	3.8	2.3	0.9
		72	8.7	3.5	1.7	1.3
4-(2,4-dichlorophenoxy) P.O.A.	0.23	24	1.0	1.1	0.2	0.9
		72	1.4	1.1	0.2	1.3
flamprop	0.15	24	0.4	0.06	0.02	3.4
		72	0.3	0.07	0.02	3.6
maleic hydrazide	0.26	24	43.6	39.3	10.4	8.7
		72	25.2	23.8	10.6	7.3

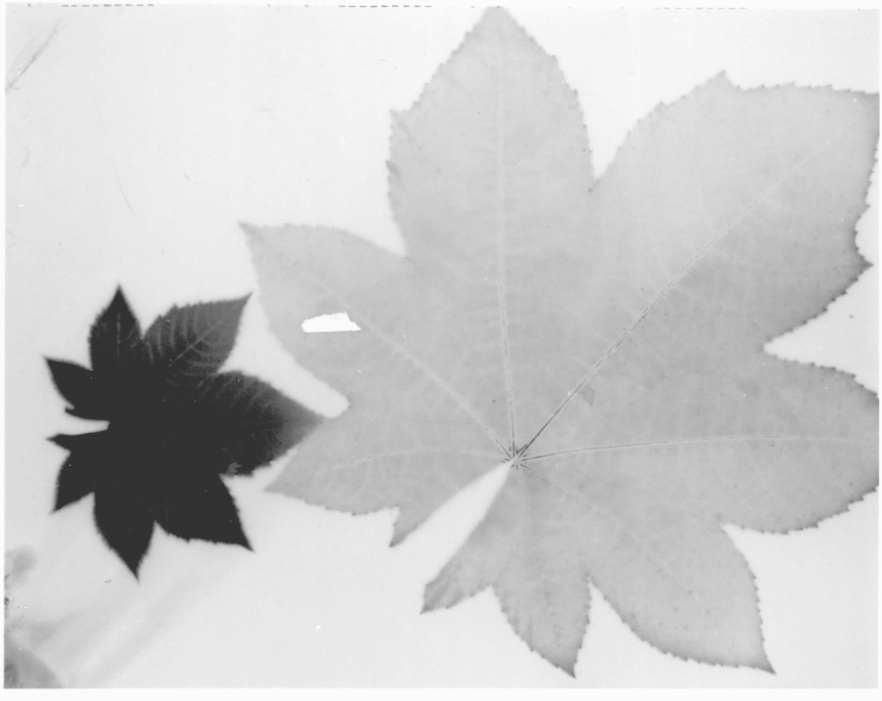
a. Degradation rate of the chemicals in the plant shoots.

The distribution of 3,5-D and maleic hydrazide in the mature leaf blades 24 h after injection of the chemicals into the petioles is shown in Figure 20. 3,5-D was more uniformly distributed in the mature leaf blades following injection into the petioles than following uptake by roots for 24 h, where 3,5-D was present mainly in the tissue adjacent to the veins. Most of the 3,5-D found in the mature leaves following injection into the petioles is likely to have been translocated there during the initial part of the 24 h period, whereas following uptake by roots, most of the 3,5-D was probably translocated to the leaves during the latter part of the 24 h period, after saturation of the stem. Thus, the more uniform distribution of 3,5-D in the mature leaf blades following injection into the petioles indicates that, although the 3,5-D reaching the leaves is initially accumulated by the cells adjacent to the xylem, the chemical is subsequently more uniformly distributed over the leaf blade. The distribution of maleic hydrazide in the leaf blades following injection into the petioles was very similar to that following uptake by roots, with the chemical being uniformly distributed in the mature leaf blades.

By using the equation of Raven, an attempt was made to estimate the accumulation of weak acids in phloem sieve cells with regard to their concentration in the adjacent leaf cells. The ratio of the concentrations of the chemicals between phloem sieve cells and apoplast was calculated assuming the plasmalemma potential of these cells = -0.12V and the phloem sap pH = 8.0. The ratio of the concentrations of the chemicals between leaf cells and apoplast was calculated as for root cells of barley (3.2.2.) and the assumptions made were:- plasmalemma potential = -0.12V, tonoplast potential $=-10^{-4}$V, cytoplasm pH = 7.0 (10% of cellular volume), vacuolar pH = 5.5 (90% of cellular volume). The ratio of the concentrations of the chemicals between phloem sieve cells and leaf cells was calculated by dividing the phloem sieve cells/apoplast ratio and the leaf cells/ apoplast ratio. As would be expected, the ratio calculated this way



3,5-dichlorophenoxyacetic acid



maleic hydrazide

Figure 20. Autoradiographs showing the distribution of 3,5-dichlorophenoxyacetic acid and maleic hydrazide in the leaf blades of R. communis 24 h after injection of the chemicals into the petioles.

was independent of the value taken for the apoplast pH. It is assumed that the specific surface (surface/volume) of contact between phloem sieve cells and bulk of the leaf tissue is sufficiently large for the accumulation of the chemicals in leaves to be independent of both the phloem flow rate and the rate of entry of the chemicals into phloem. Curves in Figure 21 show the predicted values for acids of pKa 3.0, 3.7 and 5.6, for different permeability ratios of the membranes to the undissociated and dissociated forms of the acids. The points on the figure represent the ratio of the chemical concentration in phloem exudate from upper stem to that in the mature leaf blades measured 24 h after injection of the chemicals into the petioles; this ratio being plotted according to the ratio of permeabilities of the cell membranes to the undissociated and dissociated forms of the acid estimated from the measured accumulation of the chemicals in roots of barley after 24 h uptake from solution of pH 5.0 (3.2.3.).

The measured concentration of maleic hydrazide in the upper phloem is likely to be very similar to its concentration in phloem in the leaf blades, since the chemical is well retained in phloem. The measured concentration of maleic hydrazide in the leaf blades is also likely to be a fairly good estimate of its concentration in the leaf cells, since maleic hydrazide was uniformly distributed within the blades as observed by autoradiography (Figure 21). Furthermore, assuming that the leaf cells account for 80% of the leaf weight and that the apoplast (pH = 5.5) occupies the remaining 20%, the equation given by Raven predicts that the concentration of maleic hydrazide in the leaf cells is 85% of the measured concentration in the leaf tissue (the concentration in the phloem sieve cells is neglected because the volume of the leaf blade occupied by these cells is very small). As can be seen on Figure 21, the measured concentration ratio of maleic hydrazide in phloem exudate from the upper stem and in the mature leaf blades agrees reasonably well with the predicted value, indicating that the accumulation of this

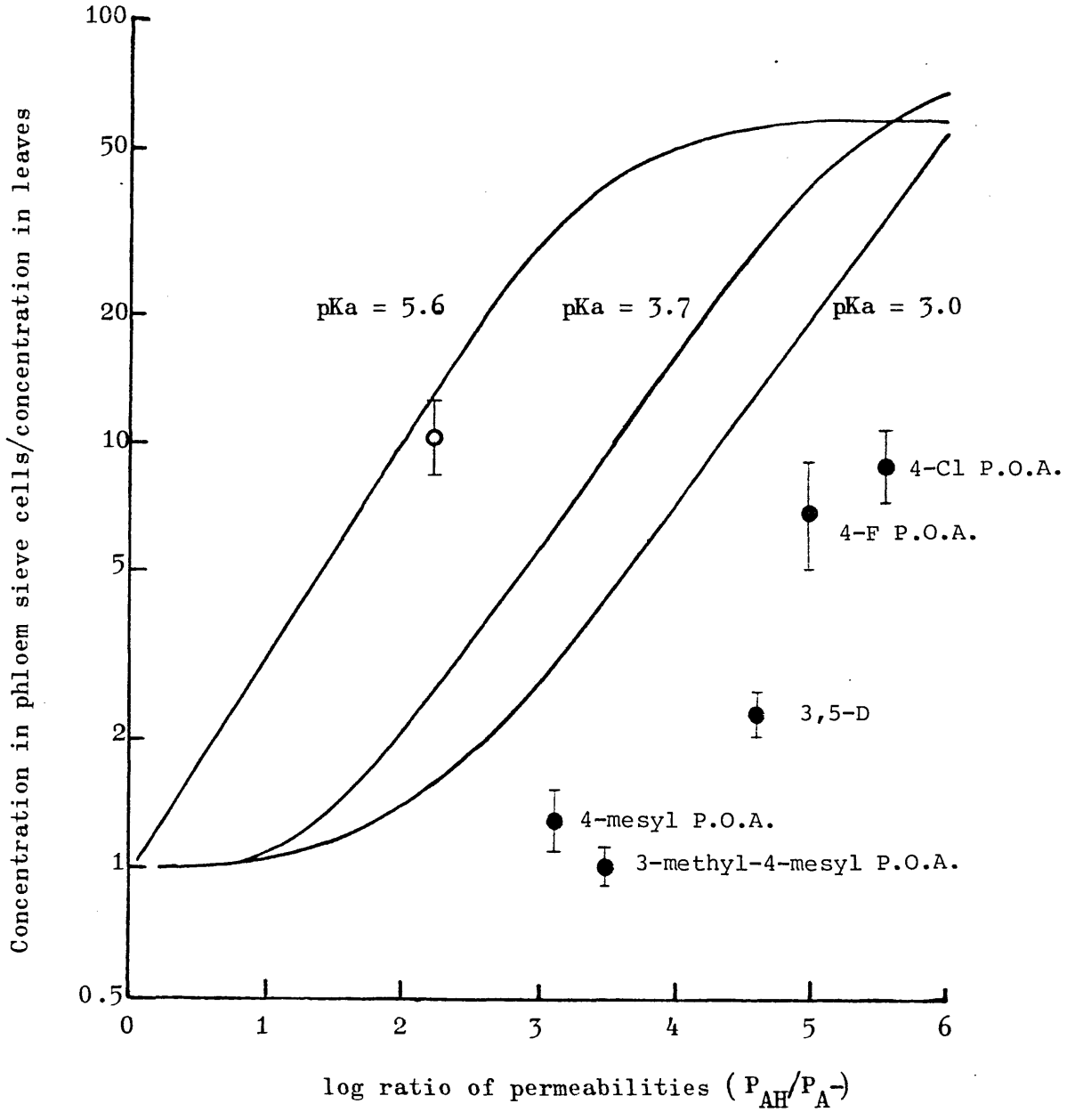


Figure 21. Accumulation of weak acids in phloem sieve cells in leaves of R. communis. Curves show the predicted accumulation for weak acids having different pKa's and the cell membranes having different ratios of permeabilities to the undissociated and dissociated forms of the acids; the points show the observed accumulation of phenoxyacetic acid analogues, pKa ~ 3.0 (•), and of maleic hydrazide, pKa = 5.6 (o), 24 h after injection of the chemicals into the petioles. Vertical bars indicate the range for the replicates. See text for details.

chemical in phloem is well explained by the physico-chemical approach made.

The accumulation of the phenoxyacetic acids in phloem in the leaves is more difficult to interpret because 3,5-D was shown not to be as uniformly distributed in the leaf blades as maleic hydrazide. Furthermore, the phenoxyacetic acids studied were shown not to be well retained in phloem, so that their concentration in phloem exudate from the upper stem is likely to be lower than that in phloem sieve cells in the leaf blades. Nevertheless, the accumulation of the phenoxyacetic acids in phloem in the leaves was both predicted and observed to be highest for the acids of intermediate lipophilicity. Although flamprop was expected to accumulate in phloem in the leaves to about 5 times the concentration in the leaves, the measured concentration of flamprop in phloem exudate from the upper stem was only 0.02 of the leaf concentrations, indicating that flamprop is rapidly lost from the phloem along the petioles.

In contrast to the suggestion by other workers (Crisp, 1972; Crisp and Look, 1979; Crisp and Larson, 1982), the accumulation of the weak acids in phloem in the leaves appears not to be a carrier-mediated mechanism. Carriers have been postulated to participate in the uptake of sucrose (Giaquinta, 1983) and aminoacids (Etherton and Rubinstein, 1978) into the phloem sieve cells. The results support the alternative suggestion by Tyree et al. (1979), further analysed by Jacob and Neumann (1982) and Neumann et al. (1985), that weak acids accumulate in phloem sieve cells by the 'ion trap' mechanism. Thus, entry of weak acids into phloem sieve cells occurs primarily by diffusion of the undissociated acids; at the higher pH of these cells, weak acids are more extensively dissociated and, because the anions diffuse through membranes much more slowly than the respective undissociated acids, weak acids are accumulated inside the phloem sieve cells. The extent to which the weak acids accumulated in phloem in the leaves could be reasonably well explained by the 'ion trap' equation proposed by Raven (1975) and, as expected from

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this equation, the extent of accumulation varied with the pKa of the acids and the ratio of the permeabilities of the cell membranes to the undissociated and dissociated forms of the acids.

The ability of the weak acids to accumulate in phloem in the leaves did not necessarily result in efficient transport of the chemicals to the roots. Although the phenoxyacetic acids of intermediate lipophilicity were readily phloem translocated following petiole injection, very low concentrations of these chemicals were found in the roots. Although the observed strong retention of these chemicals by the stem tissue prevented them from being efficiently translocated to the roots, this was a result of their inability to be well retained in the phloem sieve tubes in the first place. The rate of loss of flamprop from the phloem along the petioles was presumably so rapid that the concentration of this chemical in phloem exudates from the upper stem was only a very small fraction of its expected concentration in phloem in the leaf blades. On the other hand, maleic hydrazide was shown to be well retained in phloem and to be translocated in appreciable amounts to the roots, indicating that efficient phloem transport to the roots, following petiole injection, can only be achieved by weak acids that are well retained in phloem.

Although the above idea appears to be obvious, the explanation of the retention of the weak acids in phloem is not so clear. The results from experiments in which the gradient in phloem concentration of the chemicals was assessed immediately after petiole injection, when presumably the cells adjacent to the phloem were not saturated with the chemicals, showed that maleic hydrazide ($\log K_{ow} = -0.60$) was well retained in phloem. Among the phenoxyacetic acids, 4-mesyl P.O.A. ($\log K_{ow} = 0.06$) showed a lesser gradient during the first hours after injection than did the phenoxyacetic acids of intermediate lipophilicity. These results support the idea that very polar weak acids move out of

the phloem sieve cells very slowly, though the predominant form i.e. undissociated or dissociated, in which weak acids escape out of the phloem sieve cells is uncertain. Further research towards a better understanding of the retention of weak acids in phloem is needed.

3.4. Conclusions

The experimental procedures described using R. communis provide a valuable method for obtaining a quantitative description of the translocation in phloem of xenobiotics and of the factors controlling their distribution in the plant. Stress was laid on the determination of parent compound in the tests, as measurements of radioactivity alone can be misleading especially for rapidly metabolised chemicals. It is expected that the conclusions reached using R. communis as a model plant will be of general application to other plant species, though the additional effects of cuticular penetration must be taken into consideration when chemicals are applied as foliar sprays.

The six non-ionised chemicals tested, three substituted phenylureas and three O-methylcarbamoyloximes spanning a range of lipophilicity from log K_{ow} -0.57 to 2.27, were all freely able to diffuse passively into the phloem of R. communis. Polar compounds (log K_{ow} <0) had the lowest TSCF for uptake by roots and were the best retained in phloem, indicating that these chemicals have the lowest permeation rates through cell membranes. Retention in phloem increased as log K_{ow} decreased in this series of chemicals, and only the polar chemicals were transported in phloem over moderately long distances. These findings substantiate the intermediate permeability hypothesis of translocation in phloem, and additionally confirm that lipophilicity determines permeation rates through cell membranes, with only polar chemicals being sufficiently retained in phloem for transport to occur. However, the usefulness of such polar non-ionised chemicals for pest and disease control in the roots and developing new leaves following symplastic transport from foliar application may be limited, as the amounts of even the best phloem-translocated of our chemicals found in these sink regions were small compared to those achievable with the weak acid maleic hydrazide, though the behaviour of non-ionised chemicals which are

even more polar still requires investigation.

The accumulation of weak acids of different pKa's and of different lipophilicities by roots of barley and R. communis from solutions at different pH's could be accounted for by the 'ion trap' mechanism. Efficiency of translocation to the shoots was proportional to the concentration of the chemicals in the cortical cells of the roots. The estimated ratio of the permeabilities of the root cell membranes to the undissociated and dissociated forms of the acids varied over several orders of magnitude and, amongst a series of phenoxyacetic acids (pKa $\sim$ 3), was greatest for compounds whose undissociated form was of intermediate lipophilicity i.e. log $K_{ow} \sim$ 2. This ratio of permeabilities of the cell membranes to both forms of flamprop, which is not a phenoxyacetic acid, was much lower than that estimated for phenoxyacetic acids of comparable lipophilicity, indicating that it is not possible to predict the value of this parameter from the lipophilicity of the chemical alone.

Studies of the translocation in phloem of the weak acids in R. communis showed that the phenoxyacetic acids of intermediate lipophilicity and maleic hydrazide (pKa = 5.6, log K_{ow}(undissociated acid) = -0.6) were best translocated in phloem. In contrast to the polar non-ionised chemicals, these acids were strongly accumulated in phloem in the leaves and were efficiently phloem translocated from treated leaves to other parts of the plant, although only maleic hydrazide was found in appreciable amounts in roots. The phenoxyacetic acids of intermediate lipophilicity were shown to escape out of the phloem sieve cells rapidly and to be strongly retained by the stem tissue so that very little of these acids was translocated to the roots. The reason for the rapid escape of these acids from the phloem into the stem tissue and very slow subsequent movement therefrom via the xylem or phloem remains unclear and requires further investigation. The more polar

phenoxyacetic acids accumulated in phloem in the leaves to a lower extent than the phenoxyacetic acids of intermediate lipophilicity; the most lipophilic phenoxyacetic acid studied was strongly retained in the site of application i.e. the hollow petioles of the mature leaves, due to strong partition onto the tissue solids.

The extent to which the weak acids were accumulated in phloem in the leaves could be reasonably well explained by the 'ion trap' mechanism, with the pH gradient between phloem sieve cells (pH $\sim$ 8.0) and surrounding tissue being the driving force for the accumulation. The extent of accumulation was shown to vary with the pKa of the acids and with the ratio of the permeabilities of cell membranes to the undissociated and dissociated forms of the acids, this ratio of permeabilities for each acid being estimated in barley root cells. Flamprop accumulated in phloem in the leaves to a much lower extent than the phenoxyacetic acids of comparable lipophilicity, consistent with the much lower ratio of the permeabilities of cell membranes to the undissociated and dissociated forms of flamprop. These findings indicate that lipophilicity is not the sole factor determining the ratio of the permeabilities of cell membranes to the undissociated and dissociated forms of the acids, but the extent to which weak acids accumulate in phloem in the leaves appears to be explicable in terms of this ratio of permeabilities and the pKa of the acid.

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