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PHLOEM LOADING AND THE CONTROL OF
SOLUTE TRANSPORT IN RICINUS COMMUNIS L.

by

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A thesis submitted to the Faculty of Science
in the University of Glasgow
for the degree of
Doctor of Philosophy

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ABBREVIATIONS, SYMBOLS AND UNITS

Abbreviations

The following abbreviations are used throughout the text:

ABA	abscisic acid	rpm	revolutions per minute
C	degrees Celsius	SMT	specific mass transfer
DW	dry weight	TDV	turgor displacement volume
FW	fresh weight	TLC	thin-layer chromatography
GLC	gas-liquid chromatography		
IAA	indole-3-acetic acid		
MES	2-(<i>N</i> -morpholino)ethane sulphonic acid		

Symbols

A	area	Φ	net flux
J_w	water flow	Ψ	water potential
K_m	Michaelis-Menten constant	Ψ_m	matric potential
L_p	hydraulic conductance	Ψ_p	pressure potential
V	volume	Ψ_s	solute potential
ϵ	volumetric elastic modulus	ψ	membrane potential
σ	reflection coefficient		

Units

Système International (SI) units are used throughout the text, together with standard prefixes. The SI unit of volume is the m^3 which is related to the litre thus:

$$\begin{aligned}10^{-3}m^3 &= 1 \text{ l} \\10^{-6}m^3 &= 1 \text{ ml} \\10^{-9}m^3 &= 1 \text{ }\mu\text{l}\end{aligned}$$

The unit of concentration is the mol, which is equivalent to molarity as follows:

$$\begin{aligned}\text{kmol m}^{-3} &= \text{M} \\ \text{mol m}^{-3} &= \text{mM} \\ \text{mmol m}^{-3} &= \text{ }\mu\text{M}\end{aligned}$$

SUMMARY

This thesis presents the results of an investigation into the control of phloem loading in Ricinus communis L. The metabolic relationship between solute accumulation in the leaves and the long-distance transport pathways was first investigated to assess the significance of phloem-sap composition. The characteristics of phloem-sap exudation from bark incisions were then studied in terms of the osmotic properties of the transport system.

Comparison of plant growth on a NO_3^- -N versus NH_4^+ -N water-culture medium showed that there were marked differences in metabolism. Malate accumulation in the leaves of NO_3^- -N plants accounted for nearly 70% of the total anion balance, whereas malate levels were negligible in NH_4^+ -N plants. However, the NH_4^+ -N plants possessed much higher levels of organic N in the shoots. Despite these differences in solute accumulation, the organic C and organic N content of the phloem sap in the two groups of plants were very similar. Excess OH^- (from the NO_3^- -N plants) and H^+ (from the NH_4^+ -N plants) generated during N assimilation in the roots was excreted to the root medium, but phloem transport did not constitute a quantitatively important mechanism for removal of excess OH^- or H^+ from the shoots. The differences in metabolism in these plants were considered in relation to control of intracellular pH during growth and solute accumulation.

The bulk solute concentration of phloem sap exuding from bark incisions remained relatively constant over prolonged periods in plants maintained under controlled environmental conditions. Exudation rate increased immediately in response to fresh incisions, and these changes were directly related to alterations in solute flux. Calculation of the total capacity of the sieve tubes in the shoot indicated that prolonged exudation was maintained by phloem loading. Fully expanded leaves were the main sources of assimilates for transport. The kinetics of changes in exudation rate indicated that flow occurred through a low-resistance pathway and was influenced by the water relations of the vascular system.

Solute flux through the phloem was found to increase when water deficits were imposed on the shoot, which suggested that loading was affected by changes in phloem turgor rather than sap concentration. Under conditions of continuous darkness, a rapid fall in sucrose levels in the phloem was associated with an increase in K^+ concentration.

This tended partially to maintain phloem turgor, and implied that the mechanism of solute transfer into the tissue was directly involved in osmoregulation.

The sites of phloem loading in source leaves were examined by transmission electron microscopy, and the sieve element-companion cell complex was typical of that found in other species. Multiple branched plasmodesmata were associated with wall swellings in this cell junction; they may have served to allow high rates of symplastic transport into the sieve tubes. The osmotic characteristics of sucrose loading were also studied using a leaf-disc system. The results suggested that phloem turgor rather than total water potential affected solute uptake, but the system has a limited bearing on the question of solute loading in the intact plant.

The manner in which the solute content of the phloem is controlled supports the concept that the sap constitutes a symplastic phase. In addition, the effects of changes in the osmotic relations of the phloem on solute flux suggested that loading was turgor-pressure dependent. It is proposed that this may represent the mechanism by which phloem transport responds to alterations in the source-sink balance in intact plants.

1. GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

The solute content of plant cells and tissues is largely determined by the transport processes that maintain a supply of nutrients from the environment. Metabolic activity within the cell may affect the state of these substances, but constancy of internal solute levels during growth and development can be achieved only by the integration of metabolism and transport. This requires that aspects of the transport processes must be controlled if the plant is to be at least partially buffered against changes in nutrient availability. At the same time, there may be important distinctions between those responses observed in the process concerned that are passive and correlative as opposed to those that are specific and regulatory.

Phloem transport in higher plants provides developing tissues and organs with a supply of assimilates and ions, these being used in metabolism or the generation of turgor. However, the nutrient demands of the various organs of terrestrial plants must in turn be geared to the supply from long-distance transport. In this thesis, I shall try to examine how these interrelationships influence solute accumulation in the plant and, more specifically, how phloem transport is controlled. An underlying concern is that, although emphasis may be legitimately placed on the phloem in a descriptive sense, it is essential to determine precisely how the tissue is related to other transport systems in the plant if its function is to be properly assessed. Consequently, one important consideration is the extent to which certain characteristics of phloem transport are interpretable in terms of the constraints that operate on transport processes at other levels. Ultimately, these issues bear upon the mechanism of solute transfer into the tissue, which can be called 'phloem loading', and the nature of the signals to which it may respond.

This approach has involved establishing the nature of the solutes transported in the phloem and the manner in which they are influenced by whole-plant metabolism. I have then considered some of the functional characteristics of phloem transport in relation to the water balance of the plant. The work has all been conducted on Ricinus communis because it is important to evaluate how the biochemical and physiological characteristics of phloem transport interact in a single species in the control of the process as a whole. If sufficient quantitative information on the solute content and water status

of the tissues can be obtained, this would itself allow for an appraisal of the extent to which relative growth rate is determined by long-distance transport.

During the course of the following introductory section, I shall attempt broadly to establish the significance of the various transport processes in higher plants. The methods available for studying xylem and phloem transport will then be discussed together with certain fundamental characteristics of the transport pathways. The different hypotheses concerning the mechanisms of longitudinal flow in the phloem have not been dealt with at any length, for two reasons. Firstly, this aspect of the subject has been so extensively reviewed in recent years that there is little to add by way of summary or conjecture: the need instead is for more extensive empirical data in this field. Secondly, a large part of the work on possible mechanisms does not directly contribute to an understanding of how the transport process as a whole responds to changes in demand. In the final sections, I have thus concentrated on the evidence suggesting that solute flux in the phloem may be regulated and the implications this may have for the maintenance of turgor.

1.1 The significance of long-distance transport

A prerequisite for an understanding of the various types of transport processes found in plants is a knowledge of the structural characteristics of the transport pathways. It is axiomatic that all the material contained within a plant cell must have entered the organism by passage at some stage across a cell membrane. In addition to 'membrane transport', it is useful to distinguish three types of transport processes that occur at the supracellular level. These can be described in terms of the following types of anatomical differentiation:

- (1) *gas-filled intercellular air spaces* - these constitute a continuum from the schizogenous system of the plant through the stomata (in herbaceous tissue) and lenticels (in lignified tissue) to the atmosphere (Haberlandt, 1914; Corner, 1964; Slatyer, 1967; Raven, 1970; Armstrong, 1974);
- (2) *the apoplast* - this comprises the water-filled spaces exterior to the plasmalemma (Münch, 1930), and thus includes cell walls, water-filled intercellular spaces and water-filled

dead cells (Clarkson, 1974; Läuchli, 1976); and

- (3) *the symplast* - this comprises the living protoplasm of cells linked through the cell wall by plasmodesmata (Münch, 1930), and is usually regarded as being bounded by the plasmalemma and tonoplast, delimiting it from the cell wall and vacuole, respectively (Clarkson, 1974; Robards, 1975; Gunning and Robards, 1976; Spanswick, 1976).

Of these, the earliest structural modifications seem to have occurred in the symplast, given the presence of plasmodesmata in the chlorophyte and charophyte algae thought to have been progenitors of vascular land plants (see Raven, 1977a).

A considerable degree of intercellular specialization is found in aquatic multicellular algae, despite the relative homogeneity of the environment in terms of the supply of energy and nutrients. Multicellular representatives of the chlorophyte and charophyte green algae possess plasmodesmata, non-green vegetative cells, localized growing points ('meristems') and parenchymatous construction (Stewart and Mattox, 1975; Pickett-Heaps, 1975; Gunning and Robards, 1976; Raven, 1976a). It is only in the large phaeophyte and rhodophyte algae that the symplast is elaborated into a long-distance transport system (Eschrich, 1970; Schmitz and Lobban, 1976), although cytoplasmic streaming can increase symplastic flux rates in giant cells of the green algae (Hope and Walker, 1975).

With the evolution of photolithotrophic plants bearing part of the plant body above the aqueous medium, the selective pressure for the development of thermodynamically efficient pathways for water conduction would have become considerably stronger. The increasing structural complexity of the plant would also have given rise to more efficient symplastic transport pathways. Both these features can be regarded as fundamental characteristics of homoiohydric land plants (Walter and Stadelmann, 1968). The elaboration of intercellular spaces and the control of transport in the gas phase are, furthermore, essential aspects of the maintenance of a balance between CO₂ and H₂O exchange. As a preface to a more detailed consideration of the functional aspects of the transport pathways, it may be worth discussing briefly in what respects these processes can be distinguished from those at the cellular level. This will also serve to introduce certain concepts that underlie the quantitative analysis of various transport phenomena.

The primary requirement of these long-distance transport pathways is that they should be able to accommodate significantly higher rates of flux than can be attained by direct cell-to-cell transfer over an equivalent path length. This applies both to the movement of water and of solutes. Water movement through the transport system can be described in terms of the driving force for flow and the associated resistance to flow thus:

$$J_w = \frac{\Delta\Psi}{r_w} \quad (1.1)$$

where J_w is the flux of water ($\text{m}^3 \text{m}^{-2} \text{s}^{-1}$), $\Delta\Psi$ is the difference in water potential between the ends of the pathway (MPa) and r_w is the resistance to water flow through the system (MPa s m^{-1}). The use of the term Ψ allows the driving forces to be related to the chemical potential of water or its partial molal Gibbs free energy (μ_w , in J mol^{-1}), and provides a convenient and rational basis for the description of the state of water in plant tissues (Slatyer and Taylor, 1960; Dainty, 1963). The water potential is defined, in units of pressure (MPa), by the equation:

$$\Psi = \frac{\mu_w - \mu_w^0}{\bar{V}_w} \quad (1.2)$$

where \bar{V}_w is the partial molal volume of water ($\text{m}^3 \text{mol}^{-1}$) and μ_w^0 is the chemical potential of pure, free water at the reference temperature and pressure and a gravitational position taken to be zero.

The flux of water is also frequently considered in terms of the equation:

$$J_w = L_p \Delta\Psi \quad (1.3)$$

where L_p is the hydraulic conductance ($\text{m s}^{-1} \text{MPa}^{-1}$), and is simply the reciprocal of r_w . This relationship is derived from the basic equations of irreversible thermodynamics describing transport across membranes (Kedem and Katchalsky, 1958; Dainty, 1963). Similar principles can be applied to the analysis of solute flux (see Walker, 1976a). The term L_p essentially measures the permeability of the material to pressure-driven volume flow and can be used in cases where the nature of the driving force gradient is unknown. In point of fact, L_p is invariably referred to as the 'hydraulic conductivity' (cf. Dainty, 1976), its value depending, as does that of r_w , on the dimensions of the material under consideration. If the Ohm's-law

analogy is to be adhered to, however, it is better to reserve this term for reference to the intrinsic ('specific') conductivity, which is designated L_{pp} ($m^2 s^{-1} MPa^{-1}$), the 'hydraulic conductivity coefficient' sensu Tyree (1968). This latter term is the more appropriate for a consideration of flux through long-distance transport pathways, assuming that reasonable estimates can be obtained of the dimensions of the conducting channels.

High flux rates may thus be achieved by having a large driving force or a low resistance to flow, or a combination of the two. These components are especially important in the control of flux in the gas phase. The aerial parts of vascular land plants transmit large amounts of water, yet only a small part of this is used directly in growth. Net assimilation of atmospheric CO_2 requires a diffusion pathway for CO_2 between the atmosphere and the sites of carboxylation. The presence of intercellular air spaces in the photosynthetic organ provides sufficiently high surface area to volume ratios (Haberlandt, 1914) to permit CO_2 fluxes of the order needed for net carbon fixation (Raven, 1970; 1977a). However, the inevitable consequence of photosynthesis is that the rate of water loss (on a tissue volume basis) is very considerable, since there is a large water vapour concentration difference between the cell walls of the photosynthetic tissue and the bulk air. The flux of water into the gas phase is, in fact, about 10^3 higher than the corresponding rate of CO_2 exchange (Boyer, 1977). This represents, for a C_3 -photosynthetic plant, the loss of about 100 times as much water in transpiration as is retained within the plant during growth (Black, 1973; Raschke, 1976).

The water lost in transpiration must be replaced by uptake of water from the soil medium and transport through the plant axis to the sites of evaporation. Further, this water-conducting pathway has to be of low resistance if very low water potentials are not to develop in the photosynthetic cells. The xylem meets all the requirements of such a transport pathway in vascular land plants, consisting of dead, elongated cells with various degrees of lignification and degradation of end walls (Esau, 1965; Zimmermann, 1971). There is a mass flow of water through the lumina of these elements along a gradient of water potential by evaporation at the transpirational termini. The xylem is thus in essence a modification of the apoplast, and the structural equivalent in endohydric bryophytes is the hadrom (Watson, 1971).

The stem resistance to water flow can under some circumstances account for a significant proportion of that presented by the whole plant (Tyree, Caldwell and Dainty, 1974; Hellkvist, Richards and Jarvis, 1974). In comparison with alternative transport pathways, however, the xylem can be regarded functionally as a relatively low-resistance pathway. Taking a range of values for xylem L_{pp} from $0.1 \text{ m}^2 \text{ s}^{-1} \text{ MPa}^{-1}$ for conifers containing only tracheids to $5.0 \text{ m}^2 \text{ s}^{-1} \text{ MPa}^{-1}$ for ring-porous dicotyledons with vessels (Briggs, 1967; Zimmermann, 1971), the conductivity of the xylem for longitudinal water flow can be shown to be of the order of 10^6 to 10^8 times higher than the corresponding conductivity of parenchymatous tissue (Raven, 1977a; Tyree, 1970).

In addition to its function in supplying water, the xylem is also largely responsible for satisfying the demands of the shoots for minerals. The relationship between the transport of ions and water in the xylem is well established (Sachs, 1887; Crafts and Broyer, 1938; Bollard, 1960; Kramer, 1969), although the mechanism of radial ion transfer across the root into the xylem elements remains the subject of controversy (Läuchli, 1972; Clarkson, 1974; Bowling, 1976; Pitman, 1977). The quantity of ions transported to the shoot is dependent on the volume flow in the xylem (which is closely related to transpiration) and the ion concentration in the sap (which is a function of transport into the xylem in the roots and removal during transport to the shoot) (Sutcliffe, 1976). However, it is the developing organs such as meristems and fruits which have the highest net requirement for these ions, yet they also have relatively low rates of transpiration. Thus, to meet the nutritional demands for growth, such organs must also receive ions from the symplast.

The major pathway for the long-distance transport of organic assimilates in vascular plants is the phloem (Hartig, 1837; Mason and Maskell, 1928a; Schumacher, 1930; Zimmermann, 1971), and the tissue may be regarded as an extension of the symplasm (Crafts, 1961; Arisz, 1969; Crafts and Crisp, 1971; Gunning and Steer, 1975). Although the phloem consists of living cells, they are modified at maturity in having lost the tonoplast and most of the organelles through autolysis. Furthermore, the end walls are perforated to form sieve areas (in the gymnosperm sieve cells) or sieve plates (in the angiosperm sieve-tube

elements) (Hartig, 1837; Münch, 1930; Weatherley and Johnson, 1968; Esau, 1969; Parthasarathy, 1975; Evert, 1977). These sieve pores arise from secondary modifications of the plasmodesmata formed in the cell walls at cytokinesis (Northcote and Wooding, 1968; Jones, 1976), but their ultrastructure in transporting sieve elements remains uncertain (Evert, 1977; Spanner, 1978). The important point is that these elements constitute a symplastic continuum for longitudinal conduction in which the resistance to flow (Milburn, 1975; Watson, 1976) is much lower than that presented over equivalent distances by the plasmodesmatal connections between individual cells.

The information available on phloem ontogeny (Parthasarathy, 1975; Evert, 1977), the solute content of the sieve elements (MacRobbie, 1971, Ziegler, 1975) and the metabolic characteristics of the sieve element-companion cell complex (Ziegler, 1974; Eschrich and Heyser, 1975) all contributes to the view that the phloem contents are essentially cytoplasmic (Raven, 1977b). However, the fluxes will be constrained ipso facto by the nature of the energy-coupling reactions that catalyze transport across the cell membranes at the ends of the symplastic pathway. The longitudinal flux of sucrose through the sieve tubes in angiosperms is, nonetheless, of the order of $10\text{-}100\text{ mmol m}^{-2}\text{ s}^{-1}$ (MacRobbie, 1971), which is at least 10^5 times higher than the range of transmembrane fluxes regarded as common in plant cells (MacRobbie, 1970; Anderson, 1974). It is reasonable to infer, therefore, that there must be a very large lateral surface area (relative to the cross-sectional area of the conducting cells) involved in the transfer of solutes into and out of the phloem. In bryophytes, the symplast is modified to form the leptom, which has been shown to conduct assimilates and ions (Eschrich and Steiner, 1968; Trachtenberg and Zamski, 1978). However, the gametophyte of bryophytes is not homologous to the sporophyte of tracheophytes and many differences exist between their conducting elements.

As well as the difficulties involved in quantifying the resistance to flow in the phloem, there are also uncertainties concerning the nature of the driving force for long-distance transport (see MacRobbie, 1971; Canny, 1973; Wardlaw, 1974; Aronoff, Dainty, Gorham, Srivastava and Swanson, 1975; Zimmermann and Milburn, 1975). The hypothesis proposed by Münch (1926, 1930) considers mass flow to occur along an osmotically-generated gradient of hydrostatic pressure; in the other suggested schemes, the driving force for flow is provided by an energy

input along the length of the pathway. In fact, a large part of the controversy over this issue centres on whether the observed osmotic-potential gradients along the pathway can account for the calculated driving force required for flow. This value is usually derived from the Hagen-Poiseuille equation applied to laminar flow through the lumina and sieve pores (Weatherley and Johnson, 1968). The sieve-plate pores certainly present the main resistance to flow, as may also hold under certain conditions (discussed by Walker, 1976*b*) in the equivalent case of intercellular transport via plasmodesmata (Tyree, 1970; Tyree and Tammes, 1975).

An important consequence of this approach is that the values computed from the Hagen-Poiseuille equation are critically dependent on the figures taken from the sieve-pore radius, particularly as this term is raised to the fourth power in the formula. It may therefore be unrealistic to expect that the pressure-flow hypothesis can be rigorously tested by this means, at least until there is more definitive information on the extent to which callose, proteinaceous material and other cytoplasmic contents occlude the functioning sieve pores. There is also little information on the extent to which the preparative procedures used in electron microscopy may affect the ultrastructure of the sieve pores.

Whatever the mechanism, the maintenance of longitudinal flow depends ultimately on the processes by which substances are transported into and out of the tissue. The system therefore has to operate within the limits set by these 'loading' and 'unloading' fluxes, and their properties must be understood before the transport process as a whole can be described in quantitative terms.

As for its rôle in nutrition, long-distance transport in the phloem provides both organic and inorganic substances to developing tissues. The organic assimilates are derived from the products of photosynthetic carbon fixation and the metabolism of inorganic nitrogen, the leaves constituting the principal source of the former. The predominant fluxes in the phloem are to the effectively heterotrophic sinks in the subterranean parts of the plant and to the mainly heterotrophic sinks in the aerial parts, such as meristems and fruits (Pate, 1975; Moorby, 1977). Ion transport in the phloem can, in effect, be viewed as a process of secondary redistribution of material derived from the xylem, although at the same time this transfer can be extremely selective (Sutcliffe, 1976). Indeed, there is now substantial

evidence that phloem transport is largely responsible for providing developing organs with the inorganic nutrients needed for growth (Milthorpe and Moorby, 1969; Zimmermann, 1969; Crafts and Crisp, 1971; Lauchli, 1972; Pate, 1975). As mentioned earlier, this is related to the low rates of transpiration of such organs and the fact that the undifferentiated tissues can be some distance removed from the nearest functional vascular elements: the supply of nutrients must consequently be of symplastic origin. In the most general terms, the transport system can be said to match the demand for substances required in metabolism or the generation of turgor. Yet how this integration of activity at the levels of long-distance transport and cell metabolism is brought about is still not properly understood.

Long-distance solute transport in vascular land plants thus involves both the xylem and the phloem. These pathways can be regarded, respectively, as highly specialized modifications of the apoplast and symplast, the xylem occupying about 5% and the phloem 1% of the plant volume (Metcalf and Chalk, 1950; Zimmermann, 1971; Milburn, 1972; Raven, 1977a). In addition it can be calculated that of the order of 50 times as much water moves in the xylem as in the phloem during growth (Black, 1973; Raven, 1977b). The phloem is, in other words, a very efficient pathway for the movement of organic carbon and nitrogen relative to the dimensions and water relations of the whole plant. The reasonable constancy of ion levels in the shoot is also maintained by phloem transport, a corrolary of which is that both nutrient supply from the soil and the exchange of solutes between the xylem and phloem are likely to be controlled (Williams, 1955; Pate, 1975; Pitman and Cram, 1977; Thornley, 1977).

The maintenance of solute balance in the shoot also requires that there are means of coping with excess levels of certain materials and preventing their accumulation to toxic levels. Since the shoot possesses no large extracellular sink equivalent to the soil solution, solute stress is avoided by three main strategies:

- (1) *selectivity of xylem loading* - e.g. salt exclusion in halophytes (Atkinson, Findlay, Hope, Pitman, Saddler and West, 1967) and control of nitrogen content of the xylem in terms of pH regulation (Raven and Smith, 1976a);
- (2) *direct excretion from aerial parts by specialized glands* - e.g. salt excretion by halophytes (Luttge, 1975);

Hill and Hill, 1976) and disposal of secondary metabolites (Lüttge and Schnepf, 1976); and

- (3) *transport from the shoot to the roots in the phloem* followed by excretion to the soil solution - e.g. limited transport of H^+ and OH^- in pH regulation (Raven and Smith, 1976a) and possibly salt transport in halophytes (von Willert, 1968).

The importance of phloem transport in the prevention of solute stress seems to be limited by the cytoplasmic nature of the transport pathway (Raven, 1977b). Nevertheless, the significance of this mechanism has been very little investigated compared with the other transport processes, and it will be discussed later in more quantitative terms.

These physiological aspects of long-distance transport may consequently be regarded as features associated with the evolutionary modification of transfer processes at the cellular level to accommodate high flux rates at the supracellular level. In terms of ontogeny, xylem and phloem transport are largely responsible for the maintenance of the water and solute balance of the whole plant. Thus, the significance of these pathways must ultimately be determined by their bearing on the control of both water status and whole-plant metabolism.

Although this introductory treatment has been extremely cursory, I have tried to outline in the most basic sense what is functionally required of the long-distance transport processes and why these functions are important. The aim of this thesis is to examine the means by which phloem transport is controlled; this implicitly includes not only longitudinal transport but also the processes by which substances are transported into and out of the tissue. I shall therefore discuss xylem transport only in so far as it seems directly to impinge on these aspects of phloem physiology and biochemistry, and not in the more explicit sense of how flow through this pathway is regulated. The following sections attempt to establish how these issues can be approached.

1.2 Methods of studying xylem and phloem transport

Investigations into long-distance transport in plants must initially establish four things about the processes concerned. Firstly, it is important to determine the exact pathway by which the substances move. Secondly, the nature of the transported substances must be ascertained

by physical and chemical analysis. Thirdly, the flux through the pathway should be quantified. And fourthly, the driving force for transport and the resistance to flow through the pathway must be elucidated.

There is extensive evidence that the xylem and phloem constitute the main routes for long-distance transport of water and solutes, although more generally there is still considerable uncertainty over the precise pathways of short-distance transport within the apoplast and symplast. In a number of species, there have also been detailed studies of the types of substances transported in the xylem and phloem. It is on the last two points that information is most seriously lacking, and this largely reflects the inherent difficulties of examining the functioning system directly. Only when a causal relationship between changes in the driving force for (or resistance to) transport and the observed change in flux has been established can the question of 'control' be properly discussed. This also determines the criteria by which the correlative and passive responses can be distinguished from those that are specific and regulatory. I shall thus briefly consider the ways in which xylem and phloem transport can be studied, before concentrating on an assessment of the data obtained by these techniques and their implications.

i) *Xylem transport*

Demonstrations of water movement through the xylem have made extensive use of dyes that are carried in streams of water and under certain conditions seem not to penetrate the cytoplasm (see Strugger, 1939; Greenidge, 1957). The significance of the apoplast pathway for water transport in roots and leaves has also been investigated by similar means with sols of noble metals as markers (e.g. Strugger and Peveling, 1961; Gaff, Chambers and Markus, 1964), or with certain heavy metal salts of ethylenediaminetetraacetic acid (EDTA) that are water soluble (Tanton and Crowdy, 1972*a,b*). However, these techniques do not permit a quantitative evaluation of water flux through the apoplast. Indeed, although the mesophyll cell walls seem to form the major pathway for water movement in leaves (Weatherley, 1970), the extent of symplastic (as opposed to vacuolar) transfer of water has yet to be determined.

The first studies on solute transport through the xylem involving radioisotopes were those of Stout and Hoagland (1939), since when radiotracer techniques have been used extensively in various forms (see Bollard, 1960; Läuchli, 1972). Ion localization at the

microscopic level can be achieved by microautoradiography and electron microprobe analysis (Lüttge, 1972). Moreover, it is also possible to determine the intracellular location of a number of ions, such as Ca^{2+} , Cl^- and SO_4^{2-} , by electron microscopy after their precipitation in situ (Läuchli, Stelzer, Guggenheim and Henning, 1974).

These techniques have established that the xylem is the main pathway for both water and solute transport from the roots to the shoot. Estimates of the amounts of substances in the xylem have come from the direct analysis of xylem sap, but the rates of solute transfer are more difficult to ascertain. Two methods have been used for the recovery of xylem fluids (Pate, 1975). One involves the extraction of 'tracheal' sap from stem segments by centrifugation, liquid displacement or the application of mild suction to one end of a solid shoot or woody twig (see Bollard, 1960; Pate, 1976). The other makes use of the exudation of xylem sap by root pressure from plants cut near the base of the shoot, and is applicable principally to herbaceous species (see Pate, 1975). Sap can also be obtained from other parts of some shoots, for example from cut petioles (Pate, Wallace and Die, 1964) and cut branches or boreholes in the trunk of deciduous trees (Zimmermann, 1961).

Sources of error must be considered in determining the solutes present in the sap and their rates of transfer, and of these contamination is one of the most serious. The main disadvantage with the vacuum-displacement method is that substances can be released from tissues other than the xylem (e.g. Morrison, 1965; Hardy and Possingham, 1969). With the collection of root-pressure sap, some contamination can be avoided by prior removal of the cortex or bark from around the central cylinder of xylem. Microbial activity also has to be prevented and short collection times help to minimize starvation reactions in root cells (e.g. Pate and Greig, 1964).

Rates of transport in the intact plant are difficult to estimate from xylem-sap exudation because of the absence of a transpirational driving force for water flow. In fact, solute levels in the root-pressure sap are invariably higher than those in tracheal sap from the same plant (Kaufmann, 1976; Pate, 1976). The measurements are also referable only to the site of collection, since marked gradients in xylem solute concentrations can occur along the length of the stem (e.g. Klepper and Kaufmann, 1966; Zimmermann, 1971). These are caused both by ion exchange and adsorption onto the cell walls (Bell and Biddulph, 1963; Jacoby, 1966) and by exchange with surrounding tissues (discussed by Pate, 1975).

Root-pressure sap is thus likely to provide a qualitatively representative sample of the transpiration stream in short-term studies with respect to the major solutes in the xylem. The usefulness of extrapolating from the measured fluxes to values for transfer rates in the intact transpiring plant is limited. Nonetheless, the technique constitutes the only direct method available for investigations of this sort. With sufficiently careful standardization of growing conditions, nutrient status and experimental procedure, extensive information can be obtained about the transport pathway.

ii) *Phloem transport*

In many respects, the methods that have been used to study phloem transport are equivalent to those discussed for the xylem. The concept of the sieve tubes being the pathway for long-distance transport of assimilates originates from the investigations of Hartig (1837), but Schumacher's experiments (1930, 1933) with dyes were the first to provide experimental evidence to support this notion (see Mason and Phillis, 1937). A number of radiotracer techniques have been brought to bear on the nature of the conducting channels (discussed by Crafts and Crisp, 1971). The occurrence of exogenously applied radioisotopes in the phloem, however, does not distinguish between mobile and accumulated material. Microautoradiographic studies have shown that a number of isotopically-labelled substances can be readily transported in the phloem (Biddulph, 1956; Ringoet, Sauer and Gielink, 1968; Heyser, Fritz and Eschrich, 1969). It has also been demonstrated that export of labelled assimilates following $^{14}\text{CO}_2$ feeding to mature leaves is associated with the sieve elements (e.g. Schmitz and Willenbrink, 1967). In general, transport seems to be confined to mature sieve elements (Crafts and Crisp, 1971; Wooding, 1974).

Direct analyses of the phloem contents have similarly depended on the collection of sap. The sensitivity of the tissue is such that, on wounding, the sieve plates seem rapidly to become blocked with proteinaceous material and callose. Sap can only be obtained from certain species, but a widely used technique has been that of making direct incisions into the phloem. Since the experiments of Hartig (1858, 1860), phloem-sap exudation has been studied in over 400 species of (mainly deciduous) trees (Münch, 1930; Zimmermann, 1960; Crafts and Crisp, 1971; Ziegler, 1974), a considerable number of arborescent monocotyledons (Die and Tammes, 1975) and a few herbaceous dicotyledons

(Crafts and Crisp, 1971; Milburn, 1975; Pate, 1976). In some cases, sap will exude immediately after wounding, and will continue to do so if the wound is periodically renewed; in others, exudation may take a few days to start, or may depend on regular kneading or pounding of the plant part prior to wounding (Die and Tammes, 1975). Phloem sap can also be obtained after complete excision of an organ, e.g. inflorescence stalks, fruit tips and petioles (Die and Tammes, 1975; Pate, 1976), either from the cut sieve tubes of the plant or the detached part. Amongst the tracheophytes, however, these techniques are applicable only to the angiosperms, although the commercial tapping of phloem sap in the tropics is of considerable economic importance (see Die and Tammes, 1975).

Another method of obtaining phloem sap involves the use of feeding aphids. These insects tap the solute stream by inserting their stylets into sieve elements and sap can, in certain cases, exude from severed stylets for periods of hours (Kennedy and Mittler, 1953; Weatherley, Peel and Hill, 1959; Zimmermann, 1961). Use of the method is restricted to those species that can support colonies of relatively large aphids, and to those parts of the plant on which the aphids feed (Canny, 1973; Dixon, 1975). A number of similar studies have also been made on the honeydew excreted by whole aphids (see Peel, 1975).

Experiments on phloem-sap exudation must also be accompanied by evidence that the sap is representative of the transport stream and a consideration of the sources of error inherent in the techniques. The demonstration that the sap is of phloem origin is usually based on a mixture of direct and circumstantial evidence, but the question of artefacts can often be resolved by reference to a particular system. For example, the displacement of normally non-mobile constituents due to pressure release on incision may account for the high protein levels in sap from cucurbits (Crafts and Crisp, 1971); lateral influx of water with a fall in turgor may explain the progressive dilution of sap sometimes found during exudation (Tingley, 1944; Zimmermann, 1957); and blockage of sieve plates by insoluble contents of the phloem is probably responsible for the impedence of transport in many cases (see Canny, 1973). In such instances, the inference is that the particular feature of sap exudation has been the result of disturbance to the system on incision and does not represent a normal aspect of the transport process in the intact plant.

In other circumstances, the sources of error cannot be determined by analysis of the exuding sap alone. The extent to which solute exchange with other tissues contaminates the phloem sap during exudation (Pate, 1976), and the changes in the source of solutes for transport that can be caused by incision (e.g. Die and Tammes, 1966), are both cases in which the errors can only be assessed by other experimental investigations. The usefulness of the aphid-stylet methods is also qualified because of the range of wounding responses elicited in the plant tissue (see Dixon, 1975). Furthermore, the volumes of sap obtained can be two orders of magnitude lower than those in direct-incision experiments, so that analytical techniques of greater sensitivity are required to give equivalent levels of accuracy.

Taken together, the work on sap exudation and transport of radioactively-labelled assimilates has provided a large body of information on certain characteristics of phloem transport. Most of these results have come from investigations into the pathway of transport, the composition of the phloem sap and kinetic aspects of the transport process. The difficulties of establishing the exact dimensions of the conducting pathway have meant that there are fewer estimates of flux. This has also been influenced by the fact that rates of flux through a pathway cannot be adduced from tracer experiments unless it has been proven that the label has equilibrated with the equivalent pool(s) of the endogenous substance and thus attained a constant ('steady-state') specific activity. This criterion is difficult to meet, but in two cases the approach has been applied to flux through the phloem with some success (Geiger, Saunders and Cataldo, 1969; Housley and Fisher, 1977).

One final point concerning the application of these techniques is that they have also provided a substantial amount of information on the exchange of solutes between the xylem and phloem. The analysis of bulk samples of sap can only provide an inventory of xylem and phloem contents, and the next step must be to establish the origins of the solutes present. Kinetic data obtained in labelling studies have been especially important in examining the nature of solute exchange between the tissues (reviewed by Pate, 1976). These processes, of course, play a direct part in determining xylem and phloem composition and are thus part of the 'loading' mechanism. The implications of this for phloem transport in particular will be considered later (see sections 1.3 and 1.6).

In spite of the number of studies on xylem and phloem transport relatively few investigations have been conducted with the aim of clarifying the interrelationships between the two pathways for a single species. The most extensive work in this respect has been on the nitrogen economy of legume fruits (Pate, Wallace and Die, 1964; Pate, Sharkey and Lewis, 1974, 1975; Sharkey and Pate, 1975). In the present study, the existing knowledge of xylem- and phloem-sap composition in Ricinus (Hall, Baker and Milburn, 1971; Hall and Baker, 1972) has been extended in terms of the osmotically-important constituents of the sap. In contrast with the legume work, the intention was not directly to examine the nutritional connections between phloem transport and the metabolism of the recipient organs, but was rather to ask the more basic question: what determines the content of the phloem? Since this is a fundamental aspect of how phloem transport is controlled, in the following section I shall discuss the significance of the biochemical characteristics of the xylem and phloem in the species for which data are available.

1.3 Biochemical aspects of xylem and phloem transport

The identification and quantification of endogenous substances in plant tissues and extracts is subject to limitations imposed by the analytical procedures. Analysis of the osmotically-important solutes in bulk sap samples from the xylem and phloem has not in itself posed substantial difficulties, but the information on the substances present only at low concentrations (including hormones) is still rather sparse. Rigorous characterization of an atomic or molecular species requires the application of either successive-approximation analysis (to account for the selectivity of the methods in relation to sample concentration) or information-content theory (by which an 'accuracy probability term' with respect to the technique can be determined) (Reeve and Crozier, 1979). The importance of such analyses lies in their bearing on the relationship between long-distance transport and the metabolic activity of the whole plant.

i) *Xylem-sap composition*

Xylem fluids obtained as described previously from plant tissues are somewhat acidic (pH 5.2 - 6.5) and have a low dry-matter content (0.05 - 2.0% w/v) (Bollard, 1960; Fife, Price and Fife, 1962; Sheldrake and Northcote, 1968; Hall et al., 1971; Pate, 1975). Inorganic ions make up a large proportion of the dry weight,

the major cations being K^+ , Ca^{2+} , Mg^{2+} and Na^+ , and the principal anions $H_2PO_4^-$, Cl^- , SO_4^{2-} and (in certain cases) NO_3^- . Phosphorus is usually present as inorganic phosphate (Bieleski, 1973), although in some species it can occur in an organic form, such as phosphorylcholine (Tolbert and Wiebe, 1955; Maizel, Benson and Tolbert, 1956). Similarly, a part of the sulphur may be organic, in the form of methionine, cysteine and glutathione (Bollard, 1960; Pate, 1965). Small amounts of iron, manganese, zinc, copper and boron are also normal constituents of the xylem sap (see Pate, 1975).

The predominant organic solutes in the xylem are usually nitrogenous compounds. A single compound often occurs at relatively high concentrations, but the principal solute depends on the particular species: it may be an amide, amino acid, ureide, alkaloid, or non-protein amino acid (Pate, 1973, 1976). Organic acids are commonly found in xylem sap of herbaceous species, and can be regarded as products of root metabolism or, in a few instances, as evidence for carbon cycling through the roots from the phloem (see Kursanov, 1963; Pate, 1976). Carbohydrates, on the other hand, are generally found in quantity only in the xylem of arborescent plants (see Pate, 1976); in some species, e.g. Acer saccharum, they can reach levels of 100 mol m^{-3} (Taylor, 1956; Sauter, Iten and Zimmermann, 1973). A variety of substances exhibiting auxin-, gibberellin-, cytokinin- and inhibitor-like activity have been detected in xylem sap (see Pate, 1975; King, 1976), and indole-3-acetic acid (IAA) has been identified in Ricinus root-pressure sap by combined gas chromatography-mass spectrometry (GC-MS) (Hall and Medlow, 1974).

Apart from the manifest differences in sap composition found between species, the solute content of the xylem is markedly influenced by the nature of the rooting medium, the nutritional status of the roots and by developmental and seasonal effects on metabolism (e.g. Bollard, 1953; Hofstra, 1964; Avundzhyan, 1965; Collins and Reilly, 1968; Hardy and Possingham, 1969; Olofinboba, 1969). The need to consider these conditions in assessing the significance of sap composition has been forcefully pointed out by Pate (1976). Quantitatively the most important case is the variation in nitrogen composition of the sap: this is largely determined by transport and metabolism in the root, but is also affected by the nitrogen source in the rooting medium. In plants assimilating ammonium-nitrogen, the organic nitrogen compounds in the xylem are predominantly the

dicarboxylic amino acids and their amides (e.g. Pate and Wallace, 1964; Wallace and Pate, 1967); this is associated with the large part of ammonium assimilation occurring in the roots (Yoneyama and Kumazawa, 1974; Ivanko and Ingversen, 1971a, b). There may, however, be a greater proportion of basic amino acids (e.g. Ivanko and Ingversen, 1971b) or some free ammonium (Weissmann, 1964) in the sap.

With nitrate as the N-source, the exact xylem-sap composition depends on the sites of assimilation, since nitrate assimilation can occur in either the root or the shoot, or both (see Pate, 1973). Plants in which nitrate reduction takes place largely in the roots have high levels of organic N in the sap relative to free nitrate or ammonium, e.g. Vicia, Pisum, Lupinus and Raphanus (Pate, 1973; see Raven and Smith, 1976a). Nitrate reduction in the shoots, on the other hand, is associated with nitrate as the principal N form in the sap, e.g. Xanthium, Stellaria, Trifolium, Lycopersicon, Atriplex and Gossypium (Pate, 1973; Minshall, 1964; Osmond, 1967; Radin, 1977), and in Xanthium (Wallace and Pate, 1967) nitrate assimilation seems to occur almost solely in the shoots (but see Radin, 1974). It is important also to note that the proportion of nitrate assimilation carried out between root and shoot is affected by the concentrations of nitrate in the rooting medium (Wallace and Pate, 1967; Kirkby and Knight, 1977; see also Raven and Smith, 1976a).

Of the solutes found in the xylem, a number are transported direct from the rooting medium to the xylem without chemical modification; radioisotope experiments have shown that the majority of inorganic ions fall into this category. The origins of the remaining solutes are more difficult to determine, largely because of problems in the interpretation of kinetic data from feeding experiments. Nevertheless, it is clear that, following uptake from the medium, some substances are metabolized before release to the xylem. This is particularly important in the case of nitrogen assimilation in roots, which ¹⁵N experiments have shown to give rise to the organic compounds found in root-pressure sap (Ingversen and Ivanko, 1971; Ivanko and Ingversen, 1971a; Oghoghorie and Pate, 1972). The carbon required in the synthesis of the organic-nitrogen compounds seems to be supplied by the shoot via the phloem, at least in herbaceous plants (Pate, 1962). The organic phosphorus and organic sulphur compounds found in the xylem similarly arise from the metabolism of absorbed phosphate (Morrison, 1965) and sulphate (Pate, 1965), respectively. Apart from these sources, substances may also appear in the xylem under certain conditions after

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mobilization from other pools of solutes within the root, as well as by direct transfer from the phloem (discussed by Pate, 1975).

ii) *Phloem-sap composition*

Studies on the solute content of bulk samples of exuded phloem sap have revealed features which seem to be common to samples from a large number of species, as well as extensive similarities in the composition of aphid-stylet exudate and sap obtained from direct incisions (Mittler, 1953; Mittler, 1958; Peel and Weatherley, 1959; Crafts and Crisp, 1971; MacRobbie, 1971; Ziegler, 1975). The following is not a complete account of phloem contents, but is intended merely to highlight some aspects of sap composition that seem to be of particular physiological significance.

The pH of phloem exudates appears to vary under different conditions, during ontogeny and between species; the values are generally in the range pH 7.2 to 8.5 (see Ziegler, 1975), the significance of which is discussed later (p. 22). The dry-matter content (5-30% w/v) is considerably higher than that of the xylem, and soluble carbohydrates account for 80-90% of this total (Pate, 1975). Sieve-tube exudate from over 500 species has been examined in terms of its sugar content (see Zimmermann and Ziegler, 1975), but broadly speaking the species can be sorted into three groups (see Crafts and Crisp, 1971; Zimmermann, 1971; Ziegler, 1974), viz. those in which:

- (1) *sucrose is the predominant sugar* - this incorporates the majority of species studied (especially herbaceous species), and includes all the monocotyledons, gymnosperms (see Ziegler, 1975) and the only pteridophyte (Hamilton and Canny, 1960) studied to date;
- (2) *sucrose occurs together with considerable amounts of oligosaccharides* - the oligosaccharides belong to the raffinose family (see Zimmermann, 1971), and have been found in 15 families of dicotyledons thus far (see Ziegler, 1975); or
- (3) *significant quantities of sugar alcohols are found together with the above sugars* - e.g. mannitol is found in the Oleaceae and some Combretaceae, sorbitol in 3 subfamilies of the Rosaceae (see Ziegler, 1975; Pate, 1976).

Free hexoses occur in phloem sap, if at all, only at very low concentrations, and it is a characteristic of those sugars transported in any quantity that they are all nonreducing forms (e.g. Trip, Nelson and Krotkov, 1965; see Ziegler, 1975).

In most species, nitrogenous compounds occur at lower concentrations than the carbohydrates, but they are nevertheless solutes of considerable osmotic importance. A limited number of compounds are used for N transport and there is marked species specificity in the principal form, which may be an amide, amino acid or ureide (Pate, 1976; Mifflin and Lea, 1977). Glutamine is often present at high concentrations, e.g. Yucca (Tammes and Die, 1964), asparagine is predominant in leguminous species (see Lewis and Pate, 1973; Pate *et al.*, 1974), and arginine may also be important (e.g. Tromp and Ovaas, 1973). In many cases, the major nitrogenous solute in the phloem is the same as that in the xylem, e.g. importance of glutamine in Oryza (Yoneyama and Kumazawa, 1974, 1975) and Lycopersicon (Lorenz, 1976a, b). Citrulline, canavanine, allantoin and allantoic acid can also occur in substantial amounts in a number of species (see Ziegler, 1975; Pate, 1976). Individual amino acids can be up to 50 times more concentrated in the phloem than the xylem, glutamic and aspartic acids often being the prevalent forms, as in Ricinus (Hall and Baker, 1972; Baker, Hall and Thorpe, 1978). The general occurrence of relatively high levels of valine, serine, threonine, glycine and alanine seems to be related to pathways of carbon and nitrogen assimilation in the photosynthetic tissues. Protein levels in phloem sap are usually in the range 0.5 to 2.5% (w/v), but in some species of Cucurbitaceae they can be substantially higher (see Ziegler, 1975): this includes the specialized 'P-protein' and enzymic material not thought to be transported under normal conditions (see MacRobbie, 1971; Eschrich and Heyser, 1975). However, this level is significantly lower than the protein content of the cytoplasm, which is around 20% (w/v) (Srere, 1967).

As with the xylem constituents, the carbon and nitrogen content of the phloem is profoundly influenced by diurnal variations, ontogeny and seasonal changes (see Ziegler, 1956; Zimmermann, 1971, Pitman, 1975; Pate, 1976). The organic-acid content of the phloem may also be affected in similar ways, although the information on this subject is rather sparse. Malic, citric and oxalic acids appear to be the predominant forms, constituting in some instances quantitatively the most important anionic species in the sap (e.g. Hall and Baker, 1972);

various other acids are found in small amounts (see Ziegler, 1975). A wide range of nucleosides and nucleotides are also found in the phloem sap, ATP having been recorded at relatively high concentrations in a number of cases (see Ziegler, 1975).

Inorganic ions occur at high levels in the phloem sap, K^+ being the principal cation (up to 100 mol m^{-3}) in all species so far examined. The other quantitatively important cations are Mg^{2+} and Na^+ (which are both found up to 10 mol m^{-3}) and Ca^{2+} (which is invariably less than 3 mol m^{-3}). The exudates thus possess high $K^+ : Na^+$ and $Mg^{2+} : Ca^{2+}$ ratios, and this may be significantly greater than in other parts of the plant (Ehrhardt, 1965; MacRobbie, 1971; Pitman, 1975); low levels of total Na^+ and Ca^{2+} (and free H^+) nonetheless characterize 'normal' cytoplasm (Raven, 1977b). Other cations occurring in the sap in ionic, bound or chelated form may be classified as trace elements (see Ziegler, 1975), although their 'mobility' must be defined in terms of the relation between the transport process and the physiological requirement for the particular solute (see Raven, 1977b; cf. Epstein, 1972; Ziegler, 1975; Loneragan, Snowball and Robson, 1976).

Chloride and phosphate are the major inorganic anions in the phloem, but part of the total phosphorus in the phloem is organic (Bieleski, 1969, 1973). Bicarbonate, sulphate and nitrate are present only in small amounts (usually less than 2 mol m^{-3}), the phloem carrying sulphur and nitrogen almost exclusively in a reduced form (see Ziegler, 1975; Pate, 1976). The pH of the sap and the phosphate levels effectively restrict the occurrence of cations forming phosphates with low solubility products, and calcium and manganese, for example, can be present close to the limit of their solubility (Goor and Wiersma, 1974). Ziegler (1975) attributes the slightly alkaline pH of the sap to the predominance of potassium and phosphate amongst the ionic species. Further, it has been calculated (Raven, 1977b) that the buffer capacity of the sieve-tube contents in the range pH 7 to 8 is probably about $10 \text{ mmol H}^+ (10^{-3} \text{ m}^3)^{-1} (\text{pH unit})^{-1}$, i.e. around half that of cell cytoplasm (Raven and Smith, 1976b), the large part of this capacity being due to organic and inorganic phosphorus.

In broad terms, therefore, specific elements can be at considerably higher concentrations in the phloem than the xylem, but Ca^{2+} (and possibly Na^+), NO_3^- and SO_4^{2-} are important exceptions. In addition, a number of hormone-like substances, sterols and vitamins have been identified in phloem sap from a variety of plants (see Crafts and

Crisp; Ziegler, 1975; Pate, 1976; King, 1976). These may possess a fundamental rôle in the integration of root and shoot activity during growth and development, as (for instance) in the presumed association of the floral stimulus with transport in the phloem (see Evans, 1971). The only identifications based on reasonably selective and accurate physicochemical techniques pertain to the detection of IAA in Ricinus phloem sap (Hall and Medlow, 1974) and of abscisic acid (ABA) in Salix (Lenton, Bowen and Saunders, 1968) and Ricinus (Hoad, 1973).

The general argument for regarding the phloem as a cytoplasmic phase has been discussed by Raven (1977b). There are two features of the osmotically-important solutes occurring in the sap that are of particular importance in this context. The first is that the high levels of organic C and organic N found in the phloem sap reflect the predominance of C and N fluxes at the cell membrane. Cell growth requires a carbon source in the form of CO_2 or HCO_3^- (photosynthetic assimilation) or carbohydrate (heterotrophy), as well as a nitrogen source, which is usually inorganic (NO_3^- or NH_4^+). Membrane transport of these solutes involves by far the largest fluxes across the plasmalemma in nutrient uptake by plant cells (see Raven, 1977c). The nature of the N-source has direct consequences for pH regulation, for the maintenance of cytoplasmic pH requires the excretion or neutralization of excess H^+ (during NH_4^+ assimilation) or excess OH^- (during NO_3^- assimilation) (see Raven and Smith, 1973, 1974; Smith and Raven 1976). Values of cytoplasmic pH have been hard to obtain, but would seem to be slightly above neutrality (around pH 7.3 to 7.5) under most conditions (see Walker and Smith, 1975; Raven and Smith, 1976b; Spanswick and Miller, 1977). The majority of pH measurements on phloem sap have given higher values than this (see Ziegler, 1975), although values close to neutrality are extremely sensitive to changes in the partial pressure of carbon dioxide in the sample. Given that this will be markedly different in the extracted sap in vitro compared with that in vivo, there seems to be as yet no reason for assuming that the phloem pH is significantly higher than the cytoplasmic value.

The second facet of these cytoplasmic characteristics of direct concern is that the C and N compounds that occur are all known to show relatively small charge interactions with proteins. The term 'compatible solute' was first used by Brown and Simpson (1972) to describe low molecular weight compounds synthesized for purposes of osmoregulation, these being assumed to maintain the osmotic balance

of the cytoplasm with respect to the organelles during periods of environmental stress. The distinguishing characteristic of these solutes is that they do not interfere with enzyme activity, even when present at very high concentrations, and they probably serve to protect the proteins against damage from high levels of inorganic salts (Santarius, 1969; Borowitzka and Brown, 1974; Wyn-Jones, Storey, Leigh, Ahmad and Pollard, 1977). Neutral carbohydrates and amino acids accumulate in sugar-tolerant yeasts, moderately salt-tolerant bacteria and salt-tolerant algae during water stress (see references in Hellebust, 1976a). In higher plants, accumulation of glycinebetaine in halophytes is restricted to the cytoplasm (Wyn-Jones *et al.*, 1977; Hall, Harvey and Flowers, 1978), and certain other quaternary ammonium compounds, the amino acid proline and various polyols are also thought to function as compatible solutes (Flowers, Troke and Yeo, 1977; Storey and Wyn-Jones, 1977).

The principal carbohydrates transported in the phloem are all found as compatible solutes in cell cytoplasm in various microorganisms, e.g. sucrose in Scenedesmus (Wetherell, 1963) and Chlorella (Hiller and Greenway, 1968), and mannitol in Platymonas (Kirst, 1977; Hellebust, 1976b). At the same time, organic N can occur in the phloem at up to about 200 mol m^{-3} , although the organic C:N ratio may be as high as 50:1 (Raven, 1977b). The predominant N forms are usually the amides glutamine and asparagine (see Pate, 1976), with the notable exception of proline in Tilia and Robinia (cited in Ziegler, 1974), and these molecules too are effectively uncharged at pH values close to neutrality. Amino acids are also used in osmoregulation in a number of algae (see Cram, 1976a; Hellebust, 1976a).

An additional point concerns the prevalence of sucrose as the transported sugar in the phloem, rather than hexose equivalents. This has been discussed by Arnold (1968) in terms of the non-reducing sugars (including the raffinose sugars and sugar alcohols) being 'protected derivatives' of glucose. The hexoses would presumably be more susceptible to metabolism in a cytoplasmic environment, and hexose accumulation is probably restricted to the vacuoles (see Gayler and Glasziou, 1972; McNeil, 1976); sieve-tube sap, on the other hand, does not show invertase activity (Eschrich and Heyser, 1975). Utilization of sucrose in anabolic processes could also be energetically more efficient than the use of free hexoses, because the residues bound to the fructose molecule can be transglycosylated without prior

activation by nucleotide phosphates (Ziegler, 1975). Moreover, sucrose may be a preferred solute on osmotic grounds (pace Peel, 1974), since about twice as much organic C can be transported for a given level of phloem turgor than with monosaccharides (also see Crafts and Crisp, 1971; Baker, 1978).

By way of a final comment on sap composition, it must be emphasized that the solute content of the phloem is to a large extent a qualitative reflection of properties of the loading process. As discussed above, the selectivity of phloem loading and the constraints on metabolism within the sieve tubes can be related to the cytoplasmic nature of the contents. The characteristics of the loading mechanism, however, must also be considered in terms of the origins of the substances that are transported; as with the xylem constituents, these are not always easy to determine. Labelling studies have been especially important in this respect (see Pate, 1976), particularly in elucidating the metabolic relationships between the phloem solutes and the first-formed products of photosynthesis (e.g. Kursanov, 1963; Pate, 1975). Other assimilatory processes in the shoot are responsible for the conversion of inorganic solutes supplied by the xylem into organic form prior to phloem loading, this being true for nitrogen and to some extent for sulphur (Pate et al., 1975; Pate, 1976). At the same time, certain solutes occur in substantial quantities in both the xylem and phloem and seem to be transferred directly between the tissues without metabolism: this applies to a number of inorganic ions and in many cases to the predominant N compounds, such as the amides (see Pate, 1975). In feeding experiments, though, the physiological significance of the concentration, form and mode by which the exogenous substance is applied must be carefully assessed (Pate, 1976). Superimposed on this, developmental changes can have pronounced effects on the solute content of the phloem (particularly the mineral elements and amino acids), as, for example, during leaf maturation, flowering and senescence (see Pate, 1975; Moorby, 1977; Pitman and Cram, 1977). The ramifications of these issues pervade all studies on phloem-sap exudation.

1.4 Physiological characteristics of phloem transport

The considerations of the previous section have shown that the chemical composition of the xylem and phloem is determined by the uptake processes supplying solutes to the tissues and by certain aspects of metabolism. Compared with that of the apoplast, the solute content of the symplast has to be closely controlled so that biochemical reactions are not impaired; these constraints can be interpreted in terms of the regulation of transport and metabolism at the cellular level. As pointed out in section 1.1, however, the functions of the phloem have to be considered at the supracellular level, and I shall now concentrate on properties of the transport process in relation to the growth of tissues and organs. This requires a consideration of what is known about patterns of phloem transport in the whole plant, to which is appended a note on aspects of solute recirculation in the plant.

One of the most frequently discussed facets of long-distance transport in the phloem is the nature of the relationship between 'sources' and 'sinks'. These terms are used in various senses (see Warren Wilson, 1972), and the difficulties of formulating a quantitative description of the concepts involved have been discussed by Thornley (1977). For practical purposes, it is convenient to be able to use the terms in a general sense, such as that of Moorby (1977), who defines 'a source as a region of the plant which is supplying materials of any kind to the transport system, and a sink as a region where the material is being removed or lost from the system'. At the same time, the use of such a definition must not obscure the fact that the terms may strictly apply only to cells or groups of cells, and to particular substances. For example, changes from net import to net export do not occur synchronously throughout a developing leaf, either spatially or with respect to the various solutes (e.g. Jones, Martin and Porter, 1959; Doodson, Manners and Myers, 1964; Hopkinson, 1964; Thrower, 1967; Larson and Gordon, 1969; Turgeon and Webb, 1973; Fellows and Geiger, 1974; Turgeon and Webb, 1975; Ho and Shaw, 1977).

Net export of carbon occurs predominantly from photosynthetic tissues, although at certain stages storage organs are also major sources. All heterotrophic tissues, on the other hand, are sinks for the net import of organic C: these include apical and vascular meristems, root tissues, non-photosynthetic stem tissue, and organs such as flowers, fruits and seeds. The bulk transport of organic materials always occurs from source to sink, and this concept has been

applied extensively in describing patterns of phloem transport (Mason and Maskell, 1928*b*; Mason and Phillis, 1937; Swanson, 1959; Wardlaw, 1968; Crafts and Crisp, 1971). A central concern, however, is what rôle the transport pathway plays in these interactions; this is pertinent to the mechanism of phloem transport and the control of the transport system as a whole. But it has proved particularly difficult to assess, because the phloem itself is a sink with varying demands for assimilates (Müller and Leopold, 1966; Wardlaw, 1974). Further, the extent to which transport and growth must be integrated means that a change in one part of the system can elicit complex responses throughout the whole.

A direct consequence of the nature of the transport pathway is that supply patterns are delimited by orthostichy and the extent of vascular interconnection (Wardlaw, 1968; Canny, 1973). The first quantitative work on the experimental restriction of the transport pathway was by Mason and Maskell (1928*b*), who showed that the resulting reduction in transport was much less than the reduction in cross-sectional area of the pathway. Compensation of this form has been commonly observed in subsequent work in response to an effective increase in sink demand (in terms of flux per unit area of conducting tissue), but there have been fewer studies on the transport pathway itself. Fischer and Wilson (1975) and Wardlaw and Moncur (1976) could find no evidence for restrictions on grain yield in cereals by the vascular system (see also Bremner and Rawson, 1978); it also appears unlikely that the transport system in potato imposes any restriction on rates of tuber growth (Moorby and Milthorpe, 1975). In those cases where a proportionality has been found between phloem cross-sectional area and mass transfer through the pathway (Geiger *et al.*, 1969; Evans, Dunstone, Rawson and Williams, 1970), changes in dimensions of the pathway may be a correlative but not a causal factor in the different rates of flux.

Thus, although there is little information on cases such as transport to the shoot apex (cf. McIntyre, 1977) or developing cereal grains (cf. Jenner, 1976), it seems that under a range of environmental conditions the phloem has a carrying capacity in excess of that required to maintain normal growth (Milthorpe and Moorby, 1969; Evans, 1976; Wardlaw, 1976; Moorby, 1977). An important inference from this conclusion, if it proves to be of general applicability, is that the mechanism of longitudinal flow through the phloem is unlikely to explain how the transport process as a whole is controlled. This

point underlies the need to consider the control of transport in terms of the transfer processes into and out of the tissue.

If, then, assimilate distribution is determined largely by events at the physiological sources and sinks, how are the patterns of transport established? In a variety of studies using radiotracer techniques, it has been found that the lower leaves of a plant tend to export primarily to the roots, that the upper leaves tend to serve the shoot apex, and that intermediate leaves export in both directions. This was established largely by Russian workers for Glycine and Gossypium, and has also been examined extensively in the Gramineae and other plants (e.g. Thaine, Ovenden and Turner, 1959; Thrower, 1962; see Wardlaw, 1968; Crafts and Crisp, 1971). However, the factors which determine the strength of a sink have not been easy to elucidate.

Warren Wilson (1972) has described 'sink strength' as the product of sink size (i.e. dry weight) and its relative growth rate. In some cases, certainly, sink size is of over-riding importance, as shown by the work of Peel and Ho (1970) on the relationships between aphid colonies of different sizes as artificial sinks for assimilates. The importance of this aspect has also been demonstrated by Cook and Evans (1976), although their results further suggest that the distance between sources and sinks may be critical, particularly over short pathlengths (see Canny, 1973). In particular, labelling studies have shown that foliar organs are heavily committed to supplying organic C to fruits that they subtend (Carr and Wardlaw, 1965; Hansen, 1967; Brown, 1968; Flinn and Pate, 1970; Kipps and Boulter, 1973).

A requirement for a metabolically-active sink in whole-plant studies has been demonstrated on many occasions (e.g. Crafts and Yamaguchi, 1958; Nelson, 1962; Stoy, 1963; Edelman, Jefford and Singh, 1969; Lovell, Oo and Sagar, 1972; Walker and Ho, 1976; Walker, Ho and Baker, 1978; see also Crafts and Crisp, 1971). However, the studies on sap exudation have shown that solution flow can occur for prolonged periods to an artificial sink, implying that it is not metabolism per se but solute removal from the phloem that maintains transport. The concept of sink strength is of limited applicability in the definition of Warren Wilson (1972), since it assumes that relative growth rate is not limited by nutrient availability (Wareing and Patrick, 1975). This is probably only rarely the case. Moreover, as discussed by Evans (1975), there is a need to explain how

the stem apex is usually the dominant sink for assimilates and yet is both small and often has a lower relative growth rate than other organs.

Patterns of assimilate distribution have been extensively studied by experimental manipulation of sink tissues, and radiotracer methods have again contributed especially in this area (see Wardlaw, 1968). The reservations about the use of radioisotopes mentioned earlier (p.15) nevertheless still hold, for in many instances there has been no evidence presented to show that net flux through the phloem can properly be inferred from the movement of applied tracer. Treatments such as shading, pruning, defoliation and excision of organs have shown that distribution patterns are markedly influenced by perturbations to the source-sink balance (see Crafts and Crisp, 1971). Indeed, under certain conditions this balance can be reversed (Leonard, Bayer and Glenn, 1966; Tammes, Vonk and Die, 1967; Quinlan and Weaver, 1969; Wu and Thrower, 1973; Walker and Ho, 1976). Some extremely pertinent studies in this line have also concerned the changes in assimilate distribution related to ontogeny, e.g. from dormancy to budbreak (Hill, 1962), during development of mycorrhizae (Nelson, 1964), on the relative demand of root nodules (Lawrie and Wheeler, 1974; Gordon and Wheeler, 1978), during flowering and fruiting (e.g. Tollenaar, 1977) and with senescence (Thrower, 1967). The hormonal control of sink activity may be a fundamental aspect of these developmental effects on transport (Thrower, 1967; Moorby, 1968; Peel, 1974; Phillips, 1975; Patrick, 1976; Moorby, 1977). The evidence that hormones might directly affect the transport pathway (rather than the loading and unloading processes) is no more than circumstantial (see Patrick, 1976; Patrick and Wareing, 1978), and certain results weigh against this possibility (e.g. Wardlaw and Moncur, 1976).

It is clear that by whatever means a change in effective sink strength is brought about, this can elicit some sort of response in transport and metabolism at the source. In point of fact, if such a response could be demonstrated in circumstances where a change in sink metabolism quite clearly had no effect on the transport pathway, this would be very strong evidence that the control of transport resided in the source-sink relations. At any rate, an increase in sink demand can change the pattern of assimilate distribution, decrease temporary storage around the conducting tissue and increase the speed of movement of assimilates through the vascular system (Thrower, 1962; Wardlaw, 1965, 1974; Bremner and Rawson, 1972; Marshall and Wardlaw, 1973;

Moorby, Troughton and Currie, 1974; Wardlaw and Moncur, 1976). In those cases where a net increase in assimilate transport occurs, rather than just a redistribution of solutes, this must correspond to an increase in the rate at which substances are loaded into the phloem.

Various responses are observed in the source with alterations in demand, but it remains unclear which are most closely related to a change in transport. There is evidence that transport is able to increase in proportion to photosynthesis (Plaut and Reinhold, 1969; Habeshaw, 1969; Liu, Wallace and Ozbun, 1973; Lush and Evans, 1974; Servaites and Geiger, 1974; Heyser, Leonard, Heyser, Fritz and Eschrich, 1975; Christy and Swanson, 1976; Ho, 1976), although the rate of vein loading may be more directly related to the concentration of sucrose in the 'transport pool' in the source leaf (Husain and Spanner, 1966; Geiger and Batey, 1967; Christy and Swanson, 1976; Troughton, Currie and Chang, 1977). Furthermore, there are indications that under some conditions the supply of assimilates may limit sink activity, e.g. nitrogen fixation by root nodules (Hardy and Havelka, 1976; Gordon and Wheeler, 1978). Conversely, there are many instances in which the rate of photosynthesis shows correlations with changes in either sink demand (e.g. King, Wardlaw and Evans, 1967; Habeshaw, 1973; see Moorby, 1977) or assimilate levels in the source leaves (e.g. Habeshaw, 1973; Upmeyer and Koller, 1973), but the case for the direct control of photosynthesis by this means remains to be proven (Neales and Incoll, 1968; Geiger, 1976a).

In practice, it is important to decide on which processes in the leaf are primarily affected by these interactions. Changes in net photosynthesis occur with either changes in leaf resistance or intracellular resistance, or a combination of the two (see Pearson, 1974; Thorne and Koller, 1974; Kriedemann, Loveys, Possingham and Satoh, 1976; Satoh, Kriedemann and Loveys, 1977; Hall and Milthorpe, 1978). A further complication is that there are marked differences in the carbon assimilation and distribution characteristics between different species. One good example of this is the apparent association between high growth rates, rapid transport, efficient vein loading and high photosynthetic rates in C_4 - compared with C_3 -species (Hofstra and Nelson, 1969a, b; Lush and Evans, 1974; Moorby, Troughton and Currie, 1974; Gallaher, Ashley and Brown, 1975; Lush, 1976). At the same time, the nature of the responses will be influenced by the environment and by genetic differences between varieties (Nelson, 1963;

Liu et al., 1973; Wardlaw, 1976; Tollenaar, 1977).

Despite these complexities, radiotracer experiments and computer simulations suggest that the kinetics of export of assimilate can be described in terms of events in the source leaf (Evans, Ebert and Moorby, 1963; Fisher, 1970a, b, c; Troughton, Moorby and Currie, 1974; Fisher, 1975; Troughton et al., 1977; Ferrier and Tyree, 1976). However, the extent of lateral transfer and exchange with stem reserves can seriously complicate interpretation of transport profiles (Hoad and Peel, 1965a; Geiger et al., 1969; Ho and Peel, 1969; Fisher, 1970a; Canny, 1971, 1973; Cataldo, Christy, Coulson and Ferrier, 1972), as does the degree of compartmentation within the source leaf (Geiger, Giaquinta, Sovonick and Fellows, 1973; Outlaw and Fisher, 1975; Outlaw, Fisher and Christy, 1975; Swanson, Hoddinott and Sij, 1976).

Source-leaf metabolism may thus determine the limits within which the transport system operates, but there is a need to clarify how sink demand interacts with the supply of assimilates. In particular, it would be useful to know if the relationship between photosynthesis and export holds only in cases where sink growth is not restricted by other environmental constraints. The buffering capacity of the transport system is possibly one of its inherent characteristics: even in the absence of correlations between carbon fixation and transport, the rate of export can be maintained by breakdown of reserve material (see Ho, 1976, 1978; Moorby, 1977).

As a final comment on the subject of assimilate distribution, it is worth emphasizing that the patterns of solute transport in the whole plant cannot be explained in terms of the phloem alone. If bulk flow of solution occurs in the sieve tubes, there is likely to be a degree of water recirculation back into the xylem, possibly of the order of 5% (Münch, 1927 ; see Anderson, 1974). Moreover, the characteristics of nutrient exchange between the xylem and phloem (section 1.3) suggest that solute recirculation is an integral part of the long-distance transport processes. Various aspects of the subject have been covered by Mason and Maskell (1931), Biddulph, Biddulph, Cory and Koontz (1958), Pate (1975), Pitman (1975), and Pitman and Cram (1977), as has the significance of nutrient recycling by foliar absorption and leaching of ions from leaves by Tukey (1970) and Pitman (1975). For the present, I shall consider just one facet of this topic.

Little information exists on the extent to which the solute balance of the shoot can be maintained by exporting surplus ions to the root (see Pitman, 1975; Pitman and Cram, 1977; Raven, 1977b). As discussed earlier, this may have important consequences for pH regulation in higher plants and impose constraints on the sites at which N assimilation can occur (Raven and Smith, 1976a). Now, for most plants, anion uptake exceeds cation uptake, and electroneutrality is maintained by organic-anion metabolism (and net H^+ or OH^- excretion) (Pierce and Appelman, 1943; Wit, Dijkshoorn and Noggle, 1963). It has been proposed by Dijkshoorn (1958, 1969), and subsequently by Ben Zioni, Vaadia and Lips (1970, 1971), that if the shoot is the principal site of NO_3^- assimilation, carboxylate anions formed can be transported with K^+ in the phloem to the root; excretion of OH^- after metabolism of the carboxylate anion would then allow uptake of NO_3^- by exchange, which is in turn transported up the xylem with the remaining K^+ . This putative rôle for K^+ recirculation has not been studied as yet with specific reference to the transport systems (see discussions by Kirkby, 1974; Raven and Smith, 1976a; Kirkby and Knight, 1977; Raven, 1977b), and will be considered in more detail subsequently (section 3.5). This is simply one instance in which an appraisal of the physiological characteristics of the transport system may be important in assessing its function as regards the nutrition of the whole plant.

1.5 Phloem water relations and the control of transport

Most of the evidence on patterns of assimilate distribution seems to support the contention that transport is largely controlled by source-sink relationships. These studies have highlighted the diversity and complexity of the responses that occur to changes in supply and demand, although as yet there is no clear understanding of how these responses are brought about. In some way, the mechanism of transport has to be reconciled with the functions of the phloem. But it is in this sense that the apparent characteristics of longitudinal flow through the sieve tubes may not be very helpful in determining how the system as a whole is controlled. If we accept that the pathway itself does not usually limit transport, then we should be looking more closely at the source and the sink, that is, at the loading and unloading processes.

Before turning to the question of how flux into and out of the tissue might be regulated, it is useful to consider what fundamental

properties may be expected of an osmotic system such as the phloem, and how experimental work on longitudinal transport has contributed to this issue. The studies on phloem-sap exudation, and current evidence in general, tends to support the concept of a mass flow of solution in the phloem (MacRobbie, 1971; Die and Tammes, 1975; Milburn, 1975; Weatherley, 1975; Ziegler, 1975), although subscription to this view is not universal (see reviews by Wardlaw, 1974; MacRobbie, 1975). Nonetheless, it is important to be aware of the extent to which phloem transport may be interpretable in terms of the water relations of the vascular system.

The definition of water potential, Ψ , given earlier (equation 1.2, p.4) forms a starting point for the analysis of the water relations of plant cells and tissues. Thermodynamic considerations show that Ψ can be expressed in terms of the following components (Dainty, 1963; Slatyer, 1967; House, 1974):

$$\Psi = P - \pi - \tau \quad (1.4)$$

where P is the hydrostatic pressure above (or below) atmospheric, π is the osmotic pressure (due to dissolved solutes), and τ is the matric pressure (due to the retention of water by surface forces such as capillarity, adsorption and hydration). In this thesis, however, I shall follow the notation of Taylor and Slatyer (1962), which serves to emphasize that the pressure, solute and matric terms are component potentials, thus:

$$\Psi = \Psi_p + \Psi_s + \Psi_m \quad (1.5)$$

where Ψ_p , Ψ_s and Ψ_m are the (turgor) pressure potential, solute (or 'osmotic') potential and matric potential, respectively. These components all possess units of pressure (MPa). In plant cells, Ψ_p is usually positive, but Ψ_s and Ψ_m are always negative, since the solutes and matrix reduce the capacity of the water to do work. Ψ_m is commonly omitted on the basis of being small or constant, but may be important where there are significant amounts of colloidal material present, as in the cytoplasm; it can also be implicitly included in the Ψ_s term (Wiebe, 1966; Weatherley, 1970). A gravitational component, Ψ_z , is sometimes included in addition, but is of negligible importance in all cases except tall trees. The total water potential, Ψ , is usually negative or zero, only reaching positive values during phenomena such as guttation.

An expression for water flow, J_w , across a membrane can now be

derived using the above terms (Kedem and Katchalsky, 1958). By combining equations 1.3 and 1.4, and excluding the matric term, the expression is

$$J_w = L_p (\Psi_p - \sigma \Delta \Psi_s) \quad (1.6)$$

where L_p has its usual meaning, and the term in brackets is the overall driving force for water flow: Ψ_p is the hydrostatic pressure potential inside the cell, σ is the reflection coefficient, and $\Delta \Psi_s$ is the solute-potential difference across the membrane permeable to the solute. The osmotic efficiency of the solute is described by σ , which is 1 for a solute to which the membrane is ideally impermeable and 0 for one that is indistinguishable from the solvent. The equation assumes that the water moves solely in response to physical forces, i.e. that there is no active transport of water (Slayman's (1977) 'Central Dogma of Osmoregulation'). It is furthermore only valid if water flow is equal to the volume flow, in other words, when the contribution of solute flow to volume flow is negligible.

Equation 1.6 emphasizes that both pressure and solute terms affect flow across the membrane; moreover, the terms L_p and σ reflect membrane properties. Movement of water across the cell membrane, however, is also controlled by the elastic properties of the cell wall, since shrinkage or swelling of the cell will decrease or increase, respectively, the tension in the wall, thus altering the hydrostatic pressure potential (i.e. cell turgor). A differential pressure change ($d\Psi_p$) is related to the corresponding fractional change in cell volume (dV/V) by the equation (Philip, 1958)

$$\varepsilon = V \frac{d\Psi_p}{dV} \approx V \frac{\Delta \Psi_p}{\Delta V} \quad (1.7)$$

where ε is the volumetric elastic modulus and has units of pressure (MPa). The problems involved in the measurement of these quantities have been extensively discussed (Slatyer, 1967; Boyer, 1969; Dainty, 1976; Zimmermann, 1978; Zimmermann and Steudle, 1978).

One consequence of these properties is that the osmotic status of the phloem is likely to be directly influenced by water availability. Xylem Ψ will be of critical importance in this respect, especially in cases where the tissues are separated by a few cells: this has been clearly shown in work on sap exudation (Weatherley et al., 1959; Peel and Weatherley, 1963; Hall and Milburn, 1973). A large number

of studies on assimilate transport in whole plants have also indicated that water (deficit) stress affects transport (see Crafts, 1968; Crafts and Crisp, 1971; Wardlaw, 1974; Reinhold, 1975). What is not clear is which part of the transport system is directly affected, or indeed on what basis the effects on transport should be assessed. There have been few estimates of net flux, but Wardlaw (1967, 1969) found that grain filling and assimilate movement in Triticum and Lolium were unaffected by water stress. In fact, when rates of carbon assimilation are taken into account, it is arguable that water deficits have little effect on transport that cannot be attributed to a decrease in photosynthesis and assimilate available for export (Munns and Pearson, 1974; Moorby, Munns and Walcott, 1975; cf. Plaut and Reinhold, 1965). Furthermore, in cases where moisture stress does seem to inhibit transport more severely than photosynthesis (e.g. Hartt, 1967), this could be the result of decreased sink demand, due to reduced expansion growth in the leaves and roots (see Iljin, 1957; Wardlaw, 1968).

These difficulties in interpretation show, if nothing else, that the transport system cannot be regarded in isolation, but must be considered, as far as its functions are concerned, in terms of the interactions between sources and sinks as well. Of equal importance is the question of how the transport processes might adapt to conditions of water stress, on which there is hardly any information at all. In the context of a pressure-flow mechanism, the maintenance of transport will depend on the preservation of a gradient of Ψ_p along the pathway, rather than of a particular value of Ψ_p at any point in the system. If energy is required in addition along the pathway to drive longitudinal transport, the effects of water potential on the system may be far more complex. Thus, although the phloem will show osmotic responses to water availability, these have to be distinguished from specific effects on the driving force for transport.

Another related issue is the nature of water movement into and out of the phloem. As MacRobbie (1971) and Anderson (1974) have pointed out, providing the sieve tubes are bounded by a semi-permeable membrane and xylem Ψ is not unduly low, there will inevitably be an osmotic movement of water as a result of sucrose transport into the phloem, as well as water loss from the system in regions where sucrose moves out of the tissue. All the other proposed mechanisms of longitudinal transport tacitly assume that loading and unloading

occur in a form similar to that envisaged by Münch (1930). The question is whether subsidiary mechanisms are required along the length of the pathway to drive transport at the observed rates (see p.8). Rough estimates of the energy requirements of these subsidiary mechanisms show that in some cases (especially that of electroosmosis) the demands would be far in excess of the energy available (see MacRobbie, 1971; Wardlaw, 1974). Studies on the effects of low temperatures and metabolic inhibitors on the transport pathway have given very varied results with different species (see Crafts and Crisp, 1971; Wardlaw, 1974; Geiger and Sovonick, 1975; Reinhold, 1975; Watson, 1975). However, with the usual reservations about site and specificity of action, the fact that some conducting systems seem to function adequately at low temperatures, and that there is in general no consistent effect of metabolic inhibitors on transport, seems to argue against the requirement for an energy input along the pathway to drive transport.

Rather than being a question of 'reconciliation', it may therefore be more appropriate to view the issue of mechanism from a different side. That is, the mechanism of transport must implicitly provide for the means by which the process as a whole is controlled. The preceding considerations suggest that some form of mass (bulk) flow through the sieve tubes is almost inevitable. With the evidence implying that the metabolic activity of the pathway is not an essential part of the driving force for transport, the important question becomes that of how the loading and unloading processes are regulated.

1.6 Phloem loading and unloading: regulation of flux

Given that source-sink relationships seem to be predominant in determining the activity of the transport system, considerable importance is attached to the properties of the loading and unloading process in the phloem. For if the sieve-tube contents are to be regarded as an extension of the symplast (section 1.3), transport within the aqueous phase must be described in terms of diffusion or bulk flow (see Walker, 1976b). As mentioned earlier, current opinion tends to favour the latter mechanism, and the evidence that the sieve plates exercise metabolic control over longitudinal transport is not strong (sections 1.4 and 1.5). Consequently, the membrane transport processes at the ends of the symplastic pathway may have a crucial rôle in determining rates of net flux.

The presumed semi-permeability of the sieve element-companion

cell membrane means that transport of solute across the membrane will be followed by an osmotic movement of water in the same direction. This water flux is regarded as being passive, and therefore not under the direct influence of metabolism. If these processes can quantitatively account for the bulk flow of solution at the source and sink, the responses of the transport system to changes in demand must be considered primarily as properties of the mechanism of solute transport.

In the original formulation of the pressure-flow hypothesis (Münch, 1930), it was postulated that sugar moved from the chloroplasts to the phloem in the leaf by diffusion. However, Mason and Maskell (1928b), and subsequently Curtis and Scofield (1933) and Roeckl (1949), recognized that phloem loading often occurs against a net solute-potential gradient. It was thus proposed (see Crafts, 1951; Wanner, 1952; Bauer, 1953) that solute loading included a thermodynamically active step by which the particular solute(s) would be concentrated in the sieve elements against a concentration gradient. The equivalent events associated with unloading from the phloem are less clear, since the sink tissues are not easily accessible to experimental analysis and the regions of unloading can be hard to define. In most cases, unloading probably proceeds down a net solute potential gradient, so that membrane transport is not required on energetic grounds for the same purposes as in the loading process. There is, in fact, good evidence that unloading may be entirely symplastic (Sacher, 1966; Jenner, 1974; Chin and Weston, 1975; Dick and ap Rees, 1975; Giaquinta, 1977c), although whether invertase activity is an important part of the process may depend on the species (Hatch, Sacher and Glasziou, 1963; Glasziou and Gayler, 1972; Gunning, Pate, Minchin and Marks, 1974; see McNeil, 1976; Giaquinta, 1977c, 1978; Walker *et al.*, 1978).

From these considerations, it is obvious that flux through the phloem is ultimately related to the rate of loading. The process can certainly respond to some changes in transport activity (section 1.4), but the way in which loading may be regulated is a different matter. This issue can only be properly discussed with respect to a particular solute, of which the major sugar species (in terms of generation of phloem Ψ_s) will be the most important. It now seems established that sucrose loading does involve active transport (see Geiger, 1975a, 1976b; Giaquinta, 1977a, b; Komor, Rotter and Tanner, 1977; Malek and Baker, 1977; Hutchings, 1978a, b), yet the site of the 'loading membrane' remains uncertain. If sucrose uptake from the apoplast is part of the loading process, the membrane would correspond to the

plasmalemma of the sieve element-companion cell complex (see Kursanov and Brovchenko, 1970; Geiger, 1975a). Alternatively, following sucrose synthesis in the cytoplasm (see Heldt and Sauer, 1971; Kelly, Latzko and Gibbs, 1976; Pontis, 1977), transport to the phloem could be confined to the symplast, in which case the loading membrane might be associated with the endoplasmic reticulum or plasmodesmata (see Fellows and Geiger, 1974; Morr  and Mullenhauer, 1976; Gunning, 1976). Both these pathways may turn out to be operative under certain conditions (Gunning, 1976; Moorby, 1977).

Phloem loading has been defined by Geiger (1975a) as 'the process by which the major translocated substances are selectively and actively delivered to the sieve tubes in the source region prior to translocation'. (cf. Geiger, 1975b, 1976b). However, because loading can be regarded as an osmotic event, it is perhaps more appropriate to use the term to include the movement of all solutes into the phloem, this being closer to the broad nature of the 'source' and 'sink' concepts (p.25). Moreover, there is simply not enough information on the

characteristics of loading (except for the major sugars) even to conjecture about which solutes are actively and which passively transported. On comparative grounds, a priori reasoning may not be especially helpful either: in C_4 species, for example, the net solute potential gradient from the bundle-sheath cells to the phloem may be downhill, not uphill. The fact remains that an understanding of how phloem transport is controlled implicitly requires a knowledge of the means by which phloem loading is regulated.

The experiments described in this thesis attempt to define some of the ways in which phloem transport is controlled in Ricinus. The significance of phloem-sap composition is considered firstly in the context of whole-plant metabolism. This aspect of the work centres on a comparison of the effects of $NO_3^- -N$ versus $NH_4^+ -N$ nutrition on solute accumulation in the leaves, and the way in which this is related to long-distance transport in the xylem and phloem. The osmotic characteristics of phloem transport are then studied by examining the response of sap exudation to bark incisions under controlled environmental conditions. Quantitative considerations show that these responses must be associated with alterations in the rate of solute loading. The effects of water deficits and continuous

darkness on phloem transport are also investigated, and suggest that the loading process is affected by the water relations of the tissue in a way that tends to maintain phloem turgor. The osmotic effects on sucrose uptake by leaf discs further suggest that that this response is associated with changes in phloem Ψ_p rather than Ψ . On the basis of these results it is proposed that phloem loading is turgor-pressure dependent, and that this may account for the mechanism by which transport responds to changes in sink demand in the intact plant.

2. MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 Plant material

The experimental plant used in these studies was Ricinus communis L. var. gibsonii Nichols. (the castor-bean). The genus belongs to the family Euphorbiaceae and contains only one species, R. communis, which is probably native of tropical Africa (see Narain, 1974). In the tropics, the plant is a tree up to 13 m, but when grown as an annual is a shrub or large herb of 1-5 m at maturity. The leaves are up to 600 mm long, being peltate and palmately 5- to 9-fid, with lanceolate to ovate-lanceolate, acuminate, irregularly dentate lobes; these leaves are arranged alternately (with a phyllotaxis of 2/5), developing after the cotyledons and first opposite leaves (see Plate 2.1). The plant is monoecious with paniculate flowers, the female (pistillate) above, male (staminate) below. Projections of various forms occur on the fruits; the seeds are 9-20 mm and are smooth, reddish-brown to black, with various markings and a large caruncle. At the varietal level, the plants are distinguished by seed markings, the size and processes of the capsule, cotyledon shape, the colour of the stem and leaves, and the morphology of the extrafloral nectaries on the petiole (Bailey, 1949). R. communis L. var. gibsonii Nichols. is of relatively small form, possessing reddish leaves with a metallic lustre.

Ricinus possesses a number of attributes that render it convenient material for physiological investigations, the following points being based on those invoked by J.D.B. Weyers (personal communication):

- (1) Ricinus is easily and quickly grown: the seeds germinate readily, the seedlings are more or less uniform, and plants under glasshouse conditions will fruit within two or three months;
- (2) the general morphology is useful for experimental purposes: the stem is robust, the leaves are large and the plant is not excessively waxy or hirsute;
- (3) the plant is glycophytic (or 'mesophytic') and shows the usual characteristics of C₃ photosynthetic carbon metabolism;
- (4) secondary thickening gives rise to a 'woody' stem within about two months under glasshouse conditions: this allows the bark to be separated from the xylem, facilitating the collection of

Plate 2.1 Nine-week-old plant of Ricinus communis grown in water culture. The plant has 6 alternate leaves, and one pair of opposite leaves (the cotyledons having abscised). Magnification X0.13.



root-pressure sap (section 1.2) and permitting studies on phloem transport by interruption of the pathway in ring-girdling experiments;

- (5) Ricinus shows the phenomenon of phloem-sap exudation from bast incisions (Milburn, 1970, 1971): exudation can continue for a period of hours, and provides a means of studying directly the transport system; and
- (6) the genus is of considerable economic importance: the castor oil extracted from the seed is in widespread medicinal use, although the majority of the crop is now taken up for industrial purposes, the oil being an important processing agent.

Seed of R. communis (Daggs Ltd., Glasgow, U.K.) was stored in the dark at 4 C prior to use. The plants of Beta vulgaris L. cv. Sharpes Klein E (monogerm) referred to in section 6.3 were also grown from seed (R.K.Gemmel and Co., Glasgow, U.K.) stored under similar conditions.

2.2 Experimental conditions

i) Plant growth and culture conditions

Ricinus plants were grown for experimental purposes on a simple nutrient solution (Long Ashton Formula) using a water-culture system. The standard solution contained NO_3^- as the N-source. Transport and metabolism in plants grown on this medium were compared with that in plants grown on an equivalent solution containing a NH_4^+ -N source in section 3, but in all the other work the NO_3^- -N solution was used as standard. The media were based on those described by Hewitt and Smith (1975) and Hewitt (1966), their composition being given in Table 2.1. The macronutrient levels (including NO_3^-) in the Long Ashton solution are similar to those in other commonly used culture media; however, the Cl^- concentration is eleven times that in the medium of Arnon and Hoagland (1940), and the solution contains Co^{2+} . Concentrated stock solutions of the components were stored at 4 C in the dark and made up to volume in combination as required.

The seeds were sown in moist vermiculite (Hoben Davis Ltd., Newcastle, Staffordshire, U.K.) in rectangular polypropylene boxes (252 x 172 x 105 mm), approximately 20 mm below the surface and usually 20 seeds per box. Before sowing, the vermiculite was washed through with three volumes of tap water followed by two volumes of nutrient

Table 2.1 Composition of Long Ashton solutions based on nitrate-
or ammonium-nitrogen

Solution type	Compound	kg m ⁻³	mol m ⁻³
Macronutrients: NO ₃ ⁻ -N solution	KNO ₃	0.404	4.0
	Ca(NO ₃) ₂ anhydrous	0.656	4.0
	MgSO ₄ .7H ₂ O	0.368	1.5
	NaH ₂ PO ₄ .2H ₂ O	0.208	1.3
Macronutrients: NH ₄ ⁺ -N solution	K ₂ SO ₄	0.348	2.0
	CaCl ₂ anhydrous	0.444	4.0
	MgSO ₄ .7H ₂ O	0.368	1.5
	Na ₂ HPO ₄ .12H ₂ O	0.478	1.3
	(NH ₄) ₂ SO ₄	0.528	4.0
Micronutrients: common to both solutions	Fe citrate.5H ₂ O	0.335	0.10
	MnSO ₄ .4H ₂ O	0.00223	0.01
	ZnSO ₄ .7H ₂ O	0.00029	0.001
	CuSO ₄ .5H ₂ O	0.00025	0.001
	H ₃ BO ₃	0.00310	0.05
	Na ₂ MoO ₄ .2H ₂ O	0.00012	0.0005
	NaCl	0.00585	0.10
	CoSO ₄ .7H ₂ O	0.000056	0.0002

solution, which served to lower the pH of the material. The seedlings were then raised in an Autogrow P40 Thermostatic Propagating Tray (Autogrow, Blyth, Northumberland, U.K.) at 25 ± 2 C, relative humidity (RH) 95-100% in a growth room. The vermiculite was further moistened as necessary, the seeds germinating after about 3 d.

Nine to 10 d after sowing, when the cotyledons were fully unfolded, the seedlings were transferred to two-thirds strength (NO_3^- -N or NH_4^+ -N) nutrient solution. The root systems were gently washed free of vermiculite using tap water pre-equilibrated to ambient temperature, the seedlings then being transferred to plastic pots of volume 10^{-3}m^3 containing the diluted Long Ashton solution also at ambient temperature. To minimize the effects of irradiation on the temperature of the culture solution, the pots were covered with an undercoat of oil-based matt black and a topcoat of white-gloss polyurethane paint. Plants were supported in holes of diameter 18 mm in the lids of the pots by split rubber tubing with cotton-wool padding around the base of the stems. A system of plastic T-pieces and interconnecting polyvinyl tubing (Portex Ltd., Hythe, Kent, U.K.) allowed the solution in each pot to be aerated through an aquarium stone (Petcraft: Thomas's, Halifax, West Yorkshire, U.K.) for 10 min in every hour using a Reciprotor electromagnetic piston pump Type 506F (Reciprotor A/S, Copenhagen, Denmark). The plants were grown under greenhouse conditions (see below). After 2 weeks, the pots were emptied and refilled with full-strength solution; subsequently, the pots were topped up with fresh solution as necessary, and were rinsed out with hot water once a week. The arrangement of the water culture system is shown in Plates 2.2 and 2.3.

Soil-grown Ricinus plants were used in a few experiments (referred to specifically in the text). These were grown in compost mixture (Lindsey and Kesteven Fertilizers Ltd., Glasgow, U.K.) of the proportions sand:loam:peat, 1:1:1 (v/v) in plastic flower-pots of diameter 120, 130 and 135 mm (successively) in the greenhouse. The Beta plants were raised in this way also. Infection of Ricinus by Tetranychus urticae Koch (the red spider mite) was effectively prevented by the use about once quarterly of 'Morestan' smoke generators (Bayer Agrochem Ltd., Bury St. Edmunds, Suffolk, U.K.), which provide a vaporized insecticide containing 20% (w/w) quinomethionate.

ii) Greenhouse conditions

Experimental plants were grown in a heated greenhouse with solar

Plate 2.2 Root system of eight-week-old plant grown on NO_3^- -N long Ashton solution. Magnification X 0.57.

Plate 2.3 Arrangement of water-culture system under glasshouse conditions. Magnification X 0.06.



irradiation supplemented by artificial lighting on a photoperiod of 16 h. The artificial irradiation was provided by Atlas 400 W MBFR/U Kolorlux high-pressure mercury-vapour lamps suspended 1.06 m above the greenhouse bench and spaced 0.81 m apart; the irradiance at upper-leaf height was 18-33 W m⁻² in the range 400-1000 nm from this source, as measured with a Model 40X Opto-meter with foot-candle diffuser (United Detector Technology, Inc., Santa Monica, California, U.S.A.). The temperature range was 12 C (night minimum) to 36 C (day maximum) and the RH 60 to 100%, measured using a thermohygrograph (Casella, London, U.K.).

iii) Growth-room conditions

Three d before experimentation, plant material was transferred from the greenhouse to a growth-room (2.30 x 2.12 x 2.14 m) with forced ventilation and thermostatically controlled temperature. A bank of five fluorescent tubes (Omega 65/80 W White, West Germany), 0.71 m above the bench surface, spaced 0.01 m apart, supplied a photoperiod of 16 h; the irradiance at upper-leaf height was 22-28 W m⁻² in the range 400-1000 nm (Model 40X Opto-meter). The temperature in the growth-room during the light-period was 23.0 ± 1.5 C (mercury-bulb thermometer) and the RH 65-100% (hair hygrometer, Fischer, G.D.R.).

For experiments in which plants were subject to prolonged periods of darkness (section 4.3), the material was transferred at the requisite time to a growth-room of identical design but without lighting. The temperature and RH were kept within the range of values in the control growth-room. Plant material was handled in the dark-room under physiologically 'safe' green light. This was provided by an Atlas 65/80 W warm-white fluorescent tube covered with one layer of orange (No. 5) Cinemoid (Rank Strand Electric Ltd., London, U.K.) and two layers of primary green (No. 39) Cinemoid. Beehive lamps with tungsten-filament bulbs were also used, these being covered with the above filters as well as a layer of dark blue (No. 19) Cinemoid because of the higher irradiation in the red region of the spectrum. The spectral characteristics of these light sources is given by White (1973).

Aeration was provided in growth-room experiments by Hyflo Model B pumps (Medcalf Bros. Ltd., Potters Bar, Hertfordshire, U.K.) in the same way as in the greenhouse.

iv) Chemicals and glassware

Chemicals were of 'AnalaR' grade or equivalent, and unless stated otherwise were supplied by B.D.H. Chemicals Ltd., Poole, Dorset, U.K. The details of the radioisotopes used are given in Table 2.2. Stock solutions of the radiochemicals were made up by dilution of the source material with distilled water; these and the working solutions were stored in the dark at -15 C.

Glassware used in microanalytical studies was washed first in hot tap water and then twice in $\sim 2 \text{ kmol m}^{-3}$ HCl, rinsed six times with tap water, and finally rinsed three times with glass-distilled water.

2.3. General methods

i) Growth studies

Plant growth was recorded by measurement of fresh weight, dry weight and leaf area. Fresh weights of intact plants were obtained using a Mettler Pl63N top-loading balance with the nutrient solution emptied from the plant pots, the weight of the pot plus air-tubes being subtracted subsequently. Dry weights were obtained after drying the plant material in a Gallenkamp Hotbox Oven OV-100 at 90 C for 24 h to constant weight. Areas of alternate leaves were calculated from the linear relationship between leaf (length x breadth) and leaf area using a standard correlation curve computed from measurements from a representative sample of plants (see Hanada and Uchida, 1974; Dadoo, 1978). Areas were calculated from the equation (J.D.B. Weyers, unpublished observations)

$$y = 1.369x + 471.2 \quad (r = 0.991, P < 0.01)$$

where $y = \text{leaf area}(\text{mm}^2)$ and $x = \text{leaf (length x breadth)}(\text{mm}^2)$, determined for water-cultured Ricinus plants.

ii) Collection of sap: methods and strategies

The analysis of leaf-sap, xylem-sap and phloem-sap composition provided the basis for the study of solute accumulation in leaf tissue and its relation to the long-distance transport pathways. Sap was collected from eight-week-old plants using established techniques, the methods being described more fully in the relevant references.

Extraction of leaf-cell sap was carried out by freeze-disruption of the leaf tissue (Ehlig, 1962; Barrs, 1968; Slavik, 1974). A leaf lamina was excised from the plant at the point of insertion of the

Table 2.2 Radiochemical characteristics of isotopes (source material)

Radiochemical	Specific activity	Code/batch number	Source
U- ¹⁴ C-sucrose	318.2 GBq mol ⁻¹	CFB. 4/56	The Radiochemical Centre, Amersham, U.K.
	370.0 GBq mol ⁻¹	CFB. 4/57	"
6,6' (n) - ³ H-sucrose	44.40 TBq mol ⁻¹	TRA. 332/6	"
D-U- ¹⁴ C-glucose	111.0 GBq mol ⁻¹	CFB. 2/214	"
(±) -G- ³ H-ABA	17.17 TBq mol ⁻¹	TRQ. 702	"
5- ³ H-IAA	869.5 TBq mol ⁻¹	TMM-92-B	Commissariat à l'Énergie Atomique, France

petiole, after which the lamina was tightly sealed in a polyethylene bag and placed in a freezer at -17 C for 2.0 h. The tissue was then allowed to thaw at room temperature. The major veins were dissected out from the lamina and a sample of the interveinal tissue centrifuged at 2700 rpm for 10 min (in a Gallenkamp Universal Centrifuge, Gallenkamp, London, U.K.) through a layer of filter paper (Whatman No. 1) in a $2 \times 10^{-6} \text{ m}^3$ plastic syringe (B-D Plastipak: Becton, Dickinson and Co. Ltd., Ireland) into a pyrex centrifuge tube (see Dodoo, 1978). This sap was used for chemical analysis.

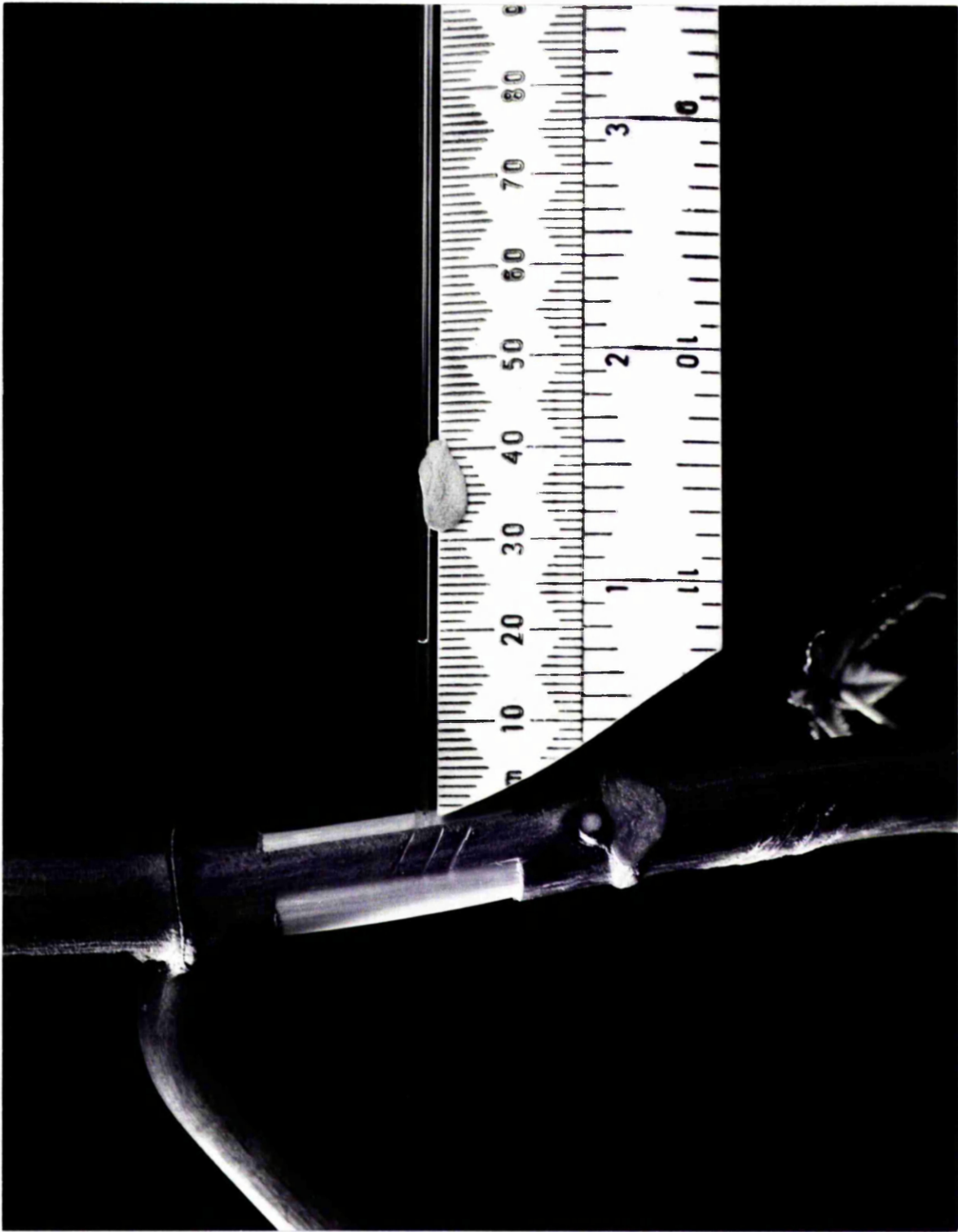
Samples of xylem sap were obtained by collection of root-pressure exudate (Hall et al., 1971). About 1 h prior to excision of the shoot, the base of the stem was ring-girdled by removing a length of 30-40 mm of bark: this was to avoid contamination from the phloem during collection but also gave the cut sieve elements time to seal beforehand. The exposed tissues were then rinsed with distilled water and dried, and the shoot was excised with a sharp, clean razor-blade in the middle of this exposed length. Sap was collected in a short piece of polyvinyl tubing with a glass pipette attached vertically (Plate 2.4), and the sap removed as soon as sufficient volume was available for analysis.

Phloem sap was obtained by direct incision into the bast tissue with a clean, previously unused razor-blade (Milburn, 1970, 1971; see Milburn, 1975; Milburn and Zimmermann, 1977b). The usual procedure was to make successive incisions up the stem in an acropetal direction; the incisions were 4-6 mm long, at an angle of about 45° to the vertical. The first drop of exudate was taken up with absorbent tissue (Kleenex Tissue: Kimberly-Clark Ltd., Maidstone, Kent, U.K.) and discarded, because of the release of contents of cells other than the sieve elements. The sap exuding subsequently was collected directly in glass capillaries ('Microcap': Drummond Scientific Co., U.S.A.) of volume 10, 25, 50 or $100 \times 10^{-9} \text{ m}^3$ (Plate 2.5). Exudation rate was recorded by mounting the micro capillaries on a ruler with Bostik Blu-Tack (Bostik Ltd., Leicester, U.K.) and converting the scale reading recorded at timed intervals to absolute volumes. In section 4, phloem-sap exudation studies were carried out with plants in which a longitudinal strip of bark 4-5 mm wide and 30-50 mm long had been isolated by removing the bark tissue from the remainder of the stem circumference. This arrangement is shown in Plate 2.5 and served to ensure that (a) sap collection was from the same cross-sectional area of tissue with each incision in a series, and (b) the phloem sap originated, from the

Plate 2.4 Collection of xylem sap (root-pressure exudate)
Magnification X1.16.



Plate 2.5 Collection of phloem sap from bark incisions. Sap is being collected from 3rd incision in series; note ring-girdling of stem to isolate bark strip. Magnification X 1.56.



second incision onwards, solely from the upper part of the shoot.

iii) Studies on transport of foliar-applied radioisotopes

In the experiments described in section 4.3, the appearance of radioactivity in exuding phloem sap was studied following the application of radiochemicals to a leaf surface. Of the methods described by Hocking (1973), the most efficient for the feeding of isotopes was adapted in the following way.

Healthy plants 6 to 8 weeks old were selected and 100 mm² of the adaxial surface of fully expanded leaf was gently abraded with moist 'Aloxite' No. 50 Optical Smoothing Powder (The Carborundum Co. Ltd., Manchester, U.K.). The abraded surface was rinsed with 1.0 mol m⁻³ CaCl₂ and subsequently blotted dry with absorbent tissue. A volume of 50 x 10⁻⁹ m³ of an aqueous solution of 10 mol m⁻³ 2-(N-morpholino) ethanesulphonic acid (MES) buffer at pH 5.5 containing the radioactive compound(s) was then supplied by micropipette to the abraded area. A small polyethylene square was immediately placed over this area to reduce evaporation of the solution. Collection of 10, 25 or 100 x 10⁻⁹ m³ samples of phloem sap from bast incisions in the stem internodes was started at the same time. Analysis of the radioactivity in these samples was carried out by liquid scintillation spectrometry.

iv) Sugar uptake by leaf discs

Sucrose and glucose uptake by leaf discs was studied using U-¹⁴C-sucrose and U-¹⁴C-glucose as tracers, respectively (section 6.3). The method was based on that of Giaquinta (1977b).

Areas of the adaxial surface of fully expanded Ricinus or Beta leaves were gently abraded with moist 'Aloxite' 50. Discs of area 40.8 mm² were cut with a clean, sharp cork-borer; the discs were rinsed with and then floated on 1.0 mol m⁻³ CaCl₂, adaxial surface downwards, for 0.75 h in a pyrex dish. After this time, groups of 6, 12 or 18 discs were randomly sorted from the total for incubation on different osmotica, the discs being quickly blotted on absorbent tissue and transferred with fine forceps to the wells of plastic incubation trays (Linbro Six-Well Multi-dishes: Linbro Scientific Co. Inc., Hamden, U.S.A.) containing the osmoticum minus tracer for a pre-incubation period of 0.50 h (floating adaxial surface downwards). The discs were then transferred in the same way to the incubation media (adaxial surface downwards). The incubation media consisted of the following: 1 mol m⁻³ CaCl₂, 20 mol m⁻³ MES buffer, sucrose or glucose

at 30 mol m^{-3} , and sorbitol or ethylene glycol at a concentration depending upon the required Ψ_s value of the medium; the medium was adjusted to a pH of 6.0, this giving a K^+ concentration of 10.7 mol m^{-3} . Each well of the trays contained $2.00 \times 10^{-6} \text{ m}^3$ osmoticum plus $50 \times 10^{-9} \text{ m}^3$ tracer of specific activity 370 or 463 GBq m^{-3} (^{14}C -sucrose) or 607 GBq m^{-3} (^{14}C -glucose); the difference between the Ψ_s of this medium and that used for pre-incubation was thus less than 2.5%. The trays were placed on a mechanical shaker (Gallenkamp) in the growth room for the required period at a temperature of 23 C and irradiance $16\text{-}19 \text{ W m}^{-2}$ in the range 400-1000 nm.

After incubation the leaf discs were washed three times (10 min each) in $1.0 \text{ mol m}^{-3} \text{ CaCl}_2$. The discs were then transferred to plastic scintillation vials (supplied by A. and J. Beveridge Ltd., Edinburgh, U.K.) (one disc per vial) containing $100 \times 10^{-9} \text{ m}^3$ of 30% (v/v) hydrogen peroxide plus $100 \times 10^{-9} \text{ m}^3$ of 70% (v/v) perchloric acid. The vials were tightly capped and heated in a water-bath (Grant Instruments (Cambridge) Ltd., Cambridge, U.K.) at 63 C for 0.50 h with occasional agitation: this completely digests and decolorizes the leaf material, and results in no loss of ^{14}C activity (Gianquinta, 1977b). The vials were finally allowed to cool and analysis of the radioactivity carried out by liquid scintillation spectrometry.

The purity of the ^{14}C -sucrose used in these experiments was checked by thin-layer chromatography (TLC) using 200 x 50 mm Camlab pre-coated glass TLC plates (SIL G-25: Macherey-Nagel and Co., Düren, F.R.G.), the chromatograms being developed in a solvent system of ethyl acetate: glacial acetic acid:water (10:5:2, v/v). The distribution of the radioactivity on the plates was determined with a Panax Thin Layer Scanner RFLS-1A (Panax Equipment Ltd., Redhill, Surrey, U.K.). A flow of propane:argon (1:49, v/v) (British Oxygen Company Ltd., Glasgow, U.K.) passed through the detection chamber at a pressure of 34.5 kPa, the scanning attachment was set at a time constant of 10 s and a slit width of 2 mm, and the output was recorded on a potentiometric chart recorder (Servoscribe RE 511.20). The purity of the ^{14}C -sucrose was > 95% as quantified by peak area.

2.4 Analytical techniques

i) Determination of plant water status

Plant water relations were investigated in terms of the free-energy status of the water of the tissues and extracts (Barrs, 1968; Boyer,

1969; Nobel, 1974). The notation introduced in section 1,1 is used throughout the text. The symbols, however, represent a form of abbreviation only: it is implicitly accepted that the terms Ψ_s and Ψ_p represent the components of the water potential due to dissolved solutes and hydrostatic pressure, respectively, and also that they have thermodynamic equivalents in energetic and entropic terms (Kramer, Knipling and Miller, 1966; Spanner, 1973). After the determination of Ψ and Ψ_s , the algebraic difference, $\Psi - \Psi_s$, gave an estimate of Ψ_p , assuming the matric component of the water potential to be numerically small or constant (see Gardner and Ehlig, 1965; Wiebe, 1966; Boyer, 1967b; Warren Wilson, 1967a).

Xylem water potential was measured by using a Scholander-Hammel pressure bomb (Scholander, Hammel, Hemmingsen and Bradstreet, 1964; Scholander, Hammel, Bradstreet and Hemmingsen, 1965) to obtain a value for xylem Ψ_p and adding the value of xylem Ψ_s determined independently. Gas pressure was supplied from a compressed-air cylinder to a Standard Water-Potential Apparatus (Chas. W. Cook and Sons, Perry Barr, Birmingham, U.K.) fitted with a Budenberg Standard Test Gauge (Budenberg Gauge Co.Ltd., Broadheath, Cheshire, U.K.). The water potential was measured after excising the leaf from the plant by cutting through the petiole about 25 mm from the lamina with a clean, sharp razor-blade. The petiole was fitted into a rubber bung containing a central hole; inserting a cork-borer of an appropriate size into the hole beforehand from the opposite side of the bung allowed the petiole to be positioned without damage to the tissues. The cork-borer was removed and the bung inserted into the cover plate of the pressure bomb, which left 5 mm of the petiole protruding from the top side of the bung: this excluded portion was not subject to the same pressure as the tissue within the chamber, but its length was considered short enough to avoid significant errors from this source (see Millar and Hansen, 1975). A polyethylene bag was placed loosely around the lamina to minimize water loss during measurement (De Roo, 1969; Gee, Liu, Olvang and Janes, 1974; Wenkert, Lemon and Sinclair, 1978), the cover plate finally being screwed back onto the top of the chamber. The petiole was at no stage recut after excision from the plant (Scholander *et al.*, 1964). The pressure in the bomb was increased gradually, at a rate of 0.2 - 0.3 MPa min⁻¹, to reduce air-temperature changes in the chamber associated with the pressure increments (see Waring and Cleary, 1967; Puritch and Turner, 1973; Tyree, Dainty and Hunter, 1974; Tyree, MacGregor, Petrov and Upenieks, 1978). The pressure was recorded at which xylem-sap

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exudation from the end of the petiole was just detectable. This (positive) balance pressure was taken as equal and opposite to the (negative) xylem Ψ_p (Scholander et al., 1964; Tyree and Hammel, 1972; Tyree, Dainty and Benis, 1973); a second measurement was also made to check this value after partially venting the bomb (Boyer, 1967a; Boyer and Ghorashy, 1971; Ike, Thurtell and Stevenson, 1978).

The value of xylem Ψ_s was determined separately by osmometry or refractometry (see below) on the first 10 to 25 x 10⁻⁹ m³ of sap extruded beyond the balance pressure from the cut petiole. Xylem Ψ was thence given by summation of xylem Ψ_s and xylem Ψ_p (Boyer, 1967a). To obtain a value for leaf Ψ , the experimental leaf was sealed in a polyethylene bag on the plant for 2.0 h prior to excision: this greatly reduced transpiration by the leaf and thereby allowed the leaf water to come to equilibrium with that in the xylem. Xylem Ψ was subsequently determined in the normal way.

Solute potentials of leaf-cell sap, xylem sap and phloem sap were determined cryoscopically using an Osmometer (Knauer Electronic Temperature Measuring Instrument with a Thermoelectric Cooling Instrument: KG Dr.-Ing. Herbert Knauer and Co. GmbH, Berlin, F.R.G.). The instrument was calibrated against standard solutions of NaCl and distilled water before and during series of measurements. Samples of 100 x 10⁻⁹ m³ were used directly after collection, the precautions involved in the operating procedures having been described by Dodoo (1978).

Where values for a large number of samples were required, the refractive index (RI) of the sap was measured by refractometry (Barrs, 1968; Slavík, 1974) using an Abbe refractometer No. 302 (Atago Optical Works Co. Ltd., Japan) with the prism thermostatically maintained at 30.0 C. The RI is proportional to solute concentration, and readings could be converted to values of Ψ_s using a calibration curve determined for sap samples measured cryoscopically. The method can be used with samples as small as 10 x 10⁻⁹ m³, which proved preferable to diluting samples of this size to 100 x 10⁻⁹ m³ for osmometry.

ii) Chemical analysis

Carbohydrates

Sugar levels were measured by the colorimetric method of Nelson (1944) developed for the assay of reducing sugars. The sap samples were divided into two fractions: one fraction was assayed directly to give a figure for total reducing sugars; the other was first incubated with

invertase or HCl (see below) to convert sucrose to hexoses, the assay of this fraction giving a figure for total reducing sugars plus the hexoses derived from sucrose. The concentration of sucrose was represented by the difference between the two fractions.

The standard assay for reducing sugars involved boiling 10^{-6} m^3 of sample (diluted x 1000 by volume with water) with an equal volume of copper reagent made up according to Somogyi (1952). After the sample had cooled, 10^{-6} m^3 of arsenomolybdate reagent (Nelson, 1944) were added, this being the colour-forming reagent; care was taken to ensure that the Cu_2O dissolved completely. The mixture was diluted to 10 or 25 x 10^{-6} m^3 with water and the colour measured spectrophotometrically using a Pye Unicam SP8000 Ultraviolet Recording Spectrophotometer (Pye Unicam Ltd., Cambridge, U.K.) with silica-glass cuvettes. Readings were taken at 741 nm (the absorbance maximum) for the greatest sensitivity of response, or at 550 nm (Nelson, 1944) if a larger concentration range had to be assayed. Calibration curves showed that there was no difference in the linearity of the response at different wavelengths across the spectrum. The dilution factors were incorporated in such a way as to give an absorbance of less than half full-scale deflection. Readings were converted to sugar concentrations by means of standard curves constructed during each experiment. Equal concentrations of glucose and fructose produced identical responses, and the colour of the complex formed in the reaction was shown to persist unaltered for 24 h.

For the assay of sucrose levels, $5 \times 10^{-6} \text{ m}^3$ of the diluted sap was first incubated with 10^{-6} m^3 of 2% (v/v) invertase solution (ex invertase concentrate from yeast, stabilized with glycerol) in $2 \times 10^{-6} \text{ m}^3$ of 100 mol m^{-3} potassium hydrogen phthalate buffer at pH 4.50 (with NaOH) in a waterbath with shaking tray for 24 h at 35 C. The reaction was stopped and the solution deproteinized by addition of 10^{-6} m^3 of 150 mol m^{-3} $\text{Ba}(\text{OH})_2$ followed by an equal volume of approximately 170 mol m^{-3} ZnSO_4 standardized according to Somogyi (1945). The sample was then centrifuged at 4500 rpm for 10 min in a BTL Bench Centrifuge (Baird and Tatlock (London) Ltd., Chadwell Heath, Essex, U.K.) and 10^{-6} m^3 of the supernatant taken for the Nelson-Somogyi assay.

Dilute acid was used latterly instead of invertase for sucrose hydrolysis in phloem samples prior to reducing-sugar assay; Ricinus phloem sap contains no other non-reducing sugar besides sucrose (Hall and Baker, 1972), and thus no specificity was lost in the assay. Two x 10^{-6} m^3 of diluted sample was added to 10^{-6} m^3 of 10 mol m^{-3} HCl and

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incubated in a waterbath at 90 C for 0.3 h. The sample was neutralized with 10^{-6} m^3 of $10 \text{ mol m}^{-3} \text{ NaOH}$ and the colorimetric assay conducted as usual. Furthermore, the reducing-sugar levels in phloem samples were always negligible, so that only those samples incubated with HCl were assayed. Duplicate analyses were carried out for each sample, and the assay was sensitive to changes of less than 0.5% in the sucrose levels in the samples.

Inorganic cations

Determinations of K^+ , Na^+ , Mg^{2+} and Ca^{2+} levels in the sap were carried out by atomic-absorption spectrophotometry. Samples were diluted by up to X1000 by volume with distilled water and analyzed using an EEL Atomic Absorption Spectrophotometer Mark 2 (Evans Electroelenium Ltd., Halstead, Essex, U.K.) with the appropriate hollow cathode lamp (Activion Special Products Division, Halstead, Essex, U.K.). The fuel was acetylene (BOC Ltd.) supplied at a pressure of 100 kPa, and the support gas was air at 100 kPa (EEL Model 349 Compressor). The dilution factors incorporated brought the cation levels in the samples into the range of optimum sensitivity for the particular lamp (generally $10 - 100 \text{ mmol m}^{-3}$). Calibration curves were constructed for standard solutions during each series of measurements; blank values for distilled water were usually negligible.

The possible enhancement of K^+ readings by Na^+ and PO_4^{3-} present in the sample was examined with combinations of standard solutions and samples using LaCl_3 as a complexing agent. Interference was insignificant in terms of the levels of Na^+ and PO_4^{3-} found in the samples, so that addition of LaCl_3 was unnecessary. Emission spectrophotometry for K^+ and Na^+ analyses ^{was} not found to offer any advantage compared with absorption measurements.

Inorganic anions

Nitrate analyses were carried out by the method of Wood, Armstrong and Richards (1967) using sap samples diluted X2000 or X4000 by volume with water. The samples were treated with 100 mol m^{-3} tetrasodium EDTA and passed through a column of copperized cadmium filings. The nitrite thus produced was quantified by diazotization with sulphanilamide and coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a deep-red azo dye. The absorbance was measured spectrophotometrically at 543 nm, and the nitrate concentration calculated from standard curves constructed during the assay. This

reduction method was used in preference to the phenoldisulphonic-acid assay because of the sensitivity and accuracy of the diazotization method for the quantification of nitrite.

Chloride activities were measured in samples diluted X 10 by volume with water using a chloride-sensitive electrode (EIL Chloride Electrode Model 8004-2: Electronics Instruments Ltd., Chertsey, Surrey, U.K.) with a mercury-mercurous sulphate reference electrode (EIL Part No. 1370-230). Each sample was diluted with an 'ionic-strength and pH-adjustment buffer' (recommended by EIL) in the proportion 1 part buffer:10 parts samples by volume; the buffer was ammonium acetate-acetic acid ($57 \times 10^{-6} \text{ m}^3$ of acetic acid plus $27 \times 10^{-6} \text{ m}^3$ of 0.88 (w/v) ammonia per 10^{-3} m^3). A pH meter (EIL Laboratory pH Meter Model 7050) was calibrated for the ion-sensitive electrode measurements using standard solutions containing the buffer.

Phosphate determinations were carried out colorimetrically by the method of Murphy and Riley (1962). Samples diluted X 300 (for phloem sap) or X 1000 (for leaf sap) by volume with water were allowed to react at room temperature with the composite reagent containing molybdic acid, ascorbic acid and trivalent antimony. The resulting blue-coloured complex was measured spectrophotometrically at 653 nm. Calibration curves were made up for standard solutions, and the readings were corrected for reagent and turbidity blanks.

Organic acids

Preliminary analysis of organic acids was carried out by TLC. The samples were mixed with $10 \times 10^{-6} \text{ m}^3$ of 80% (v/v) ethanol and any insoluble material removed by centrifugation (Hall and Baker, 1972). The supernatant was taken to dryness in a rotary evaporator (Büchi Rotavapor-R, Switzerland) under reduced pressure at 42 C and the residue redissolved in $0.5 \times 10^{-6} \text{ m}^3$ of 95% (v/v) ethanol. Samples were run on TLC plates coated with silica-gel using a solvent system of ethanol:water:ammonia (8:1:1, v/v) (Hall and Baker, 1972) or benzene:methanol:acetic acid (79:14:7, v/v) (Harborne, 1973). Organic acids were located by spraying the developed plates with alkaline 0.4% (w/v) bromocresol green (0.4 g bromocresol green in $100 \times 10^{-6} \text{ m}^3$ of 10 mol m^{-3} NaOH). Rf values were compared with those of standards run on the same plates.

For quantitative analysis, the organic acids in aqueous sap samples were either purified by ion-exchange chromatography and analyzed by GLC, or were directly quantified in diluted samples by enzymic assay.

Ion-exchange chromatography was carried out using glass columns (120 x 13 mm) approximately two-thirds full with ion-exchange resin. The cation-exchange resin was Dowex 50W-X8 (20-50 mesh): this was converted to the H^+ form by eluting with 1.5 kmol m^{-3} HCl. The anion-exchange resin was Dowex 1-X8 (20 - 50 mesh): this was converted to the acetate form by eluting with 1.0 kmol m^{-3} sodium acetate followed by 0.1 kmol m^{-3} acetic acid (ap Rees and Beevers, 1960). For the samples used, the exchange capacities of the resins were in excess of that required for quantitative recovery.

Each sample was diluted to $10 \times 10^{-6} \text{ m}^3$ with water and poured onto the cation-exchange resin, followed by elution with about $50 \times 10^{-6} \text{ m}^3$ water; this separated the amino acids (which bound to the column) from the neutral and acidic fractions. The complete eluant from this column was then poured onto the anion-exchange column and eluted with about $50 \times 10^{-6} \text{ m}^3$ water to wash through the neutral fraction (mainly sugars and sugar phosphates). The acidic fraction, containing the organic acids, was displaced from the resin by eluting with $25 \times 10^{-6} \text{ m}^3$ of 8.0 kmol m^{-3} formic acid followed by about $60 \times 10^{-6} \text{ m}^3$ water, and this fraction was evaporated to dryness under reduced pressure at 42 C. To remove residual formic acid, the sample was redissolved in $10 \times 10^{-6} \text{ m}^3$ water and reduced to dryness, this being performed twice. The sample was finally redissolved in $10 \times 10^{-6} \text{ m}^3$ water and titrated with 10 mol m^{-3} NaOH to pH 7.00 to give a value for total titratable acidity.

For analysis by GLC, the organic acids were separated by ion-exchange chromatography and reduced to dryness with two washes as above. Trimethylsilyl (TMSi) derivatives of the acids were prepared and analyzed by a procedure based on that of Phillips and Jennings (1976a) and Rutter, Johnston and Willmer (1977), as modified from Clark (1969) and Nierhaus and Kinzel (1971). Dried samples were dissolved in $200 \times 10^{-9} \text{ m}^3$ of anhydrous pyridine, being shaken and allowed to stand for 10-15 min at room temperature. The acids were silylated by addition of $200 \times 10^{-9} \text{ m}^3$ of *N,O*-bis-(trimethylsilyl)acetamide (BSA) (Sigma London Chemical Co., Poole, Dorset, U.K.); the mixture was left to stand for 20-30 min at room temperature before use. BSA derivatives are stable for at least 56 h (Phillips and Jennings, 1976a) but the analyses were completed within 24 h. In later experiments, silyl derivatives were prepared using Sylon BFT (Supelco, Inc., Bellefonte, Pennsylvania, U.S.A.; supplied by Chromatography Services Ltd., Wirral, Merseyside, U.K.), which contained a mixture of

bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS).

Samples of volume 2 to $5 \times 10^{-9} \text{ m}^3$ of the TMSi derivatives were injected into a Pye Series 104 Chromatograph (Pye Unicam Ltd.) fitted with either a standard glass column (1.52 m long) containing 3.0% (w/w) SP-2250 (the stationary phase) on Supelcoport 100/120 mesh (the solid support), or a 2.13 m column containing 1.5% (w/w) QF-1 on Chromosorb W 80/100 mesh. SP-2250 is methylphenylpolysiloxane, with 50% methyl, 50% phenyl by proportion and is equivalent to OV-17; it is similar to the SE-30 (100% methyl) and SE-52 (90% methyl, 10% phenyl) stationary phases used by Phillips and Jennings (1976a). QF-1 is fluorinated alkyl silicone, and is equivalent to OV-210. The carrier gas was di-nitrogen (BOC Ltd.) at a flow rate of $60 \times 10^{-6} \text{ m}^3 \text{ min}^{-1}$. The temperature programme was 100 to 200 C at 8 C min^{-1} , the minimum and maximum temperatures being held for 5 min and 2, 5 or 10 min, respectively. Output from the flame-ionization detector and amplifier was recorded on a chart recorder (Servoscribe RE 541.20). Detector response to derivatives of organic-acid standards was calibrated by peak area for individual compounds, since detector response changes with temperature and there are different degrees of irreversible adsorption of the compounds to the stationary phase (Knights, 1967).

Malic acid was also quantified by direct enzymic assay of sap samples, this method being used for the most accurate estimates of absolute concentration because of the number of steps involved in purification and conversion for GLC analysis. Measurements were made on samples diluted X 50 (leaf sap), X 11 (xylem sap) or X 10 or X 20 (phloem sap) by volume with water using the method of Möllering (1974), except that the buffer (adjusted to pH 10.0) was 600 mol m^{-3} glycylglycine. The assay involved the enzymic coupling of two reactions in situ in silica-glass spectrophotometer cuvettes: malate was first converted to oxaloacetate (OAA) by malate dehydrogenase (Boehringer Mannheim GmbH, F.R.G.), with the associated reduction of NAD^+ (Sigma London Chemical Co.) to NADH; this was followed by the conversion of OAA in the presence of glutamate to 2-oxoglutarate and aspartate by glutamate-oxaloacetate transaminase (Boehringer Mannheim GmbH), this second reaction pulling the first to completion. The formation of NADH was measured spectrophotometrically by the absorption change at 340 nm. Malic acid concentration was calculated from this reading (see Möllering, 1974) and corrected on the basis of values obtained for standards during the series of measurements.

Amino acids

Analysis of the free amino acids in leaf sap, xylem sap and phloem sap was carried out on diluted samples using an amino-acid autoanalyzer.

Amino acids were quantified with a Jeol JLC-5AH autoanalyzer (Japan Electron Optics Laboratory Co. Ltd., Japan), with separation by column chromatography using a sodium-citrate buffer system (Thompson and Miles, 1964). The samples were diluted X1000 (leaf sap), X33 or X50 (xylem sap), or X100 or X200 (phloem sap) by volume with water and added to the column, being progressively eluted with buffer over the temperature range 45-70 C. Two glass columns were used of length 100 mm and 600 mm containing LCRI Resin: the former was eluted with pH 5.28 buffer (104 min) at a flow rate of $1.22 \times 10^{-6} \text{ m}^3 \text{ min}^{-1}$ (at 45 C), the latter with pH 3.28 buffer (120 min) and pH 4.25 buffer (138 min) at a flow rate of $0.83 \times 10^{-6} \text{ m}^3 \text{ min}^{-1}$ over the range 45 to 70 C at 0.36 C min^{-1} . The composition of the sodium citrate buffers is given in Table 2.3. Separation of proline and glycine was achieved by addition of 2.5% (w/v) methylcellulose (May and Baker Ltd., Dagenham, Essex, U.K.) to the buffer. Amino acids were identified and quantified by their reaction with ninhydrin and comparison with the response to standards.

This method permitted the quantification of all the amino acids except for serine and the amides glutamine and asparagine, the peaks of which overlapped. A Locarte system (Locarte, London, U.K.) was therefore used to separate these compounds (see Kedenburg, 1971). Samples of 10, 20 or $60 \times 10^{-6} \text{ m}^3$ were applied to a glass column (250 mm long) containing Locarte resin and were eluted with a lithium-citrate buffer at pH 2.81 (see Table 2.3) at a flow rate of $0.50 \times 10^{-6} \text{ m}^3 \text{ min}^{-1}$ (with a temperature programme of 37 to 64 C at 0.39 C min^{-1}). This system allowed the quantification of aspartic acid, threonine, serine, asparagine, glutamic acid and glutamine.

pH

The pH of undiluted sap samples was measured with an EIL pH combination electrode or an Activion pH combination microelectrode (Activion Glass Ltd., Kinglassie, Fife, U.K.) connected to a pH meter (EIL Model 7050). Measurements were made immediately the sap sample was available, the meter having been calibrated against pH 4.00, 7.00 and 10.00 buffers referred to potassium hydrogen phthalate standards. The pH of $100 \times 10^{-9} \text{ m}^3$ volumes of sap was recorded for samples placed in

Table 2.3 Composition of buffer solutions used for amino-acid analysis.

Buffer		Composition	
1. Jeol-Formula buffers	sodium citrate	78.8 g	pH 3.28
	HCl	$49.2 \times 10^{-6} \text{m}^3$	
	thiodiglycol	$20.0 \times 10^{-6} \text{m}^3$	
	water	to $4.00 \times 10^{-3} \text{m}^3$	
	sodium citrate	78.8 g	pH 4.25
	HCl	$33.6 \times 10^{-6} \text{m}^3$	
	thiodiglycol	$20.0 \times 10^{-6} \text{m}^3$	
	water	to $4.00 \times 10^{-3} \text{m}^3$	
	sodium citrate	137.2 g	pH 5.28
	HCl	$26.0 \times 10^{-6} \text{m}^3$	
	thiodiglycol	0	
	water	to $4.00 \times 10^{-3} \text{m}^3$	
2. Locarte-system buffer	LiOH	28.8 g	pH 2.81
	citric acid	75.7 g	
	LiCl	23.8 g	
	HCl	$46.8 \times 10^{-6} \text{m}^3$	
	thiodiglycol	$10.0 \times 10^{-6} \text{m}^3$	
	water	to $4.00 \times 10^{-3} \text{m}^3$	

small glass bulbs (used for osmometry), which covered the end of the pH 'microelectrode' (tip diameter about 4 mm). Continuous pH readings were recorded on a chart recorder (Servoscribe RE 541.20).

iii) Radiometry

Radioassay of the low energy β -emitters carbon-14 and tritium was carried out by liquid scintillation spectrometry using a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3380 (Packard Instrument Co. Inc., Illinois, U.S.A.). The samples were counted in plastic scintillation vials (supplied by A. and J. Beveridge Ltd., Edinburgh, U.K.) after addition of $10 \times 10^{-6} \text{ m}^3$ of toluene/Triton X-100 (2:1, v/v) liquid scintillant and equilibration at 4 C; this scintillant is used for emulsion counting of aqueous samples (Patterson and Greene, 1965; Turner, 1968). The toluene (A. and J. Beveridge Ltd.) contained 4.0 kg m^{-3} PPO (2,5-diphenyloxazole: Packard Instrument Co. Inc.), this being mixed two parts to one by volume with Triton X-100 (ex Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire, U.K.). The vials were counted for periods of either 2 x 5 min or 10 min.

The counts recorded were converted to values for absolute radioactivity (Bq) by correction for chemical quenching and colour quenching. Calibration curves were constructed to relate the efficiency of counting to the automatic external standard (AES) ratio using $n-1-^{14}\text{C}$ -hexadecane or $n-1,2(n)-^3\text{H}$ -hexadecane standards (The Radiochemical Centre) of known activity quenched to varying degrees with methanolic extracts. For any sample, the counting efficiency (between 0 and 1.0) could then be computed from the recorded AES ratio; after correcting for background radiation, the absolute radioactivity was calculated from the equation

$$^3\text{H or }^{14}\text{C radioactivity} = \frac{\text{counts} - \text{background}}{\text{efficiency}}$$

For experiments in which both ^{14}C and ^3H were present in the sample, radioactivity was determined by using the channels for double-label counting and marking up calibration curves as above. The radioactivity could then be calculated from the following equations:

$$^{14}\text{C radioactivity} = \frac{^{14}\text{C counts in } ^{14}\text{C}(^3\text{H}) \text{ channel} - \text{background}}{^{14}\text{C efficiency in } ^{14}\text{C}(^3\text{H}) \text{ channel}}$$

$$\text{and } ^3\text{H radioactivity} = \frac{\text{counts in } ^3\text{H}(^{14}\text{C}) \text{ channel} - \text{background}}{^3\text{H efficiency in } ^3\text{H}(^{14}\text{C}) \text{ channel}} \times \frac{^{14}\text{C radioactivity}}{\text{isotope ratio}}$$

$$\text{where isotope ratio} = \frac{^3\text{H efficiency in } ^3\text{H}(^{14}\text{C}) \text{ channel}}{^{14}\text{C efficiency in } ^3\text{H}(^{14}\text{C}) \text{ channel}}$$

Counting efficiencies were in the range 0.84 - 0.87 for ^{14}C , and 0.27 - 0.32 for ^3H ; in the leaf-disc experiments, the efficiency for ^{14}C counting was between 0.79 and 0.82.

iv) Soluble-compound autoradiography

Distribution of radioactivity in leaf discs after uptake of labelled sugars was studied by soluble-compound autoradiography using a method based on that of Itai, Weyers, Hillman, Meidner and Willmer (1978).

Glass microscope slides (76 x 26 mm) (Chance Propper Ltd., Warley, West Midlands, U.K.) were cleaned in chromic acid and prepared for use by 'subbing', the slides being dipped in 5% (w/v) gelatin solution containing 0.5% (w/v) chrome alum at 20 C and allowed to dry. After incubation, the leaf discs were washed as described previously to remove the free-space radioactivity and mounted on the slides, two per slide, adaxial or abaxial surface downwards as required. The tissue was immediately frozen by immersion of the whole slide in liquid di-nitrogen at -196 C. Subsequently, the tissue was freeze-dried (Greaves, 1954) over a period of about 24 h in an Edwards Model B5A Freeze-Drier (Edwards High Vacuum, Crawley, Sussex, U.K.). The material was then allowed to equilibrate with room temperature. In complete darkness, a strip of unexposed Ilford Pan-F film precut to size was appressed to each slide, the emulsion in direct contact with the tissue; a second glass slide was positioned on the other side to hold the film in place, and the whole was tightly bound with Sellotape (Adhesive Tapes Ltd., Borehamwood, Hertfordshire, U.K.). The slides were placed in light-proof boxes containing silica gel, which were then sealed, wrapped in aluminium foil and stored at -17 C for the required period. The approximate time for which the film needed to be exposed, K , was calculated according to the equation $K \approx 4.5 \text{ d Bq mm}^{-2} \text{ tissue}$, for ^{14}C β -emission per Bq mm^{-2} tissue (Weyers, personal communication). Controls were included for positive chemography

(unlabelled tissue plus unexposed film) and negative chemography (labelled tissue plus exposed film) (Rogers, 1973).

After exposure to the tissue for 1 to 3 weeks, the film was allowed to equilibrate with room temperature and was removed from the slides in a dark-room. The film was developed with diluted Ilford PQ Universal Developer (Ilford Ltd., Ilford, Essex, U.K.) (3 min) and Ilford Hypam fixer (5 min) and then washed in flowing water (30 min).

v) Microscopy

Light microscopy

Transverse sections of stem tissue were examined by light microscopy after staining with lacmoid, following the procedure of Cheadle, Gifford and Esau (1953). The tissue was sectioned with a sharpened razor and the material washed in $120 \text{ mol m}^{-3} \text{ NaHCO}_3$ in 25% (v/v) ethanol. The pre-staining stage in tannic acid and ferric chloride used by Cheadle *et al.* (1953) was omitted and the tissue transferred directly to petri dishes containing lacmoid solution (= resorcin blue) (Hopkin and Williams Ltd., London, U.K.): this was 0.25% (w/v) in 30% (v/v) ethanol adjusted to pH 7.5 with $120 \text{ mol m}^{-3} \text{ NaHCO}_3$ in 25% (v/v) ethanol. Sections were stained for 24 h and afterwards washed (3 x 10 min) in the same solution minus lacmoid. The sections were dehydrated in an ethanol series, mounted in Canada balsam and examined using a Leitz microscope (Leitz, Wetzlar, F.R.G.). Callose deposits were stained blue. A camera lucida was used for making drawings of the phloem tissue, from which cross-sectional areas could then be quantified.

Sections of leaf tissue were examined using a Zeiss Photomicroscope II (Carl Zeiss, Oberkochen, F.R.G.). The leaf tissues were fixed, post-fixed, dehydrated, embedded in Epon-araldite resin and sectioned as described below. The sections were stained in 0.05% (w/v) toluidene blue in 1% (w/v) borax and mounted in Epon-Araldite resin plus accelerator. Ilford Pan-F or Kodachrome 64 film (Kodak Ltd., Hemel Hempstead, Hertfordshire, U.K.) was used for photography.

Electron microscopy

Small portions of the leaf laminae of two-month-old plants were processed for transmission electron microscopy by one of two methods.

In the first method, tissue was fixed by floating on 5% (v/v) glutaraldehyde plus 4% (v/v) formaldehyde in 150 mol m^{-3} dibasic sodium phosphate buffer at pH 7.2 for 1 h at room temperature followed by a further 12 h at 4 C. The tissue was then washed three times

(15 min each) in phosphate buffer and post-fixed in 1% (w/v) osmic acid in phosphate buffer for 2 h. After a further wash in phosphate buffer, the material was dehydrated in an acetone series. This was followed by two washes with propylene oxide before infiltration with a propylene oxide: Epon resin mixture (1:1, v/v), the resin finally being hardened by polymerization over 24 h at 60 C.

Fixation of tissue in the second method was carried out by floating the material on 3% (v/v) glutaraldehyde in 50 mol m⁻³ sodium cacodylate buffer at pH 7.4 for 3 h at room temperature followed by a further 12 h at 4 C. The sodium cacodylate buffer was then used to wash the tissue (2 x 1.5 h), which was subsequently post-fixed in 2% (w/v) osmic acid in sodium cacodylate buffer for 3 h. After a further wash in the same buffer (2 x 1.3 h), the material was dehydrated in an acetone series thus: 2 x 20 min in 25% acetone; twice over 12 h in 50%; 2 x 20 min in 75%; 2 x 20 min in 90%; and 3 x 15 min in 100% (all % v/v). For infiltration, the tissue was left in Epon-Araldite resin (15.45 g Epon: 10.00 g Araldite CY 212) for 12 h, and then in pure resin (3 h), pure resin plus DMP 30 accelerator (TAAB Laboratories, Reading, Berkshire, U.K.) (2 x 10 min at 60 C), and finally pure resin + DMP 30 for 24 h at 60 C.

The blocks were trimmed to shape and thin silver sections (60-90 nm thick) cut with a glass knife on an LKB Ultratome III Ultramicrotome (LKB-Produkter AB, Stockholm, Sweden). Sections were floated onto copper grids (3.05 mm diameter, G 50 grid) coated with 2% (w/v) collodion in amyl acetate. They were then stained either with 2% (w/v) uranyl acetate (60 min) and 2% (w/v) lead citrate (30 min), in the first method, or with 7.5% (w/v) uranyl acetate (35 min) and 2% (w/v) lead citrate (25 min), in the second. The specimens were washed and dried, and were examined on an AEI Type EM6B Electron Microscope (AEI Scientific Apparatus Ltd., Harlow, Essex, U.K.) at 80 kV.

2.5 Biometry

The sample mean (\bar{x}) was calculated from the equation

$$\bar{x} = \frac{\Sigma x}{n}$$

where x = the value of each variate, and n = the number of observations.

Standard error (S.E.) of the mean was determined from the relation

$$S.E. = \frac{s}{\sqrt{n}}$$

where s , the estimated standard deviation, was given by the equation

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n - 1}}$$

In the text, S.E. is represented graphically as a vertical bar above and below the point of the mean, equivalent to $\bar{x} \pm S.E.$

For the two-sample t -test, values of t were computed from the equation

$$\text{observed } t = \frac{\bar{x}_1 - \bar{x}_2}{s_x \sqrt{(1/n_1 + 1/n_2)}}$$

where $\bar{x}_1 - \bar{x}_2$ is the difference of means between the two groups of samples containing n_1 and n_2 variates, respectively. The null hypothesis (that the population means from the two groups were equal) was rejected at the conventional levels of probability ($P < 0.05$, 0.01 or 0.001) if the observed value of t from the significance test was greater than that given in tables of the t -distribution (Fisher and Yates, 1963). N.S. denotes that the difference was not significant at these conventional levels of probability.

Regression analysis was carried out by the method of least squares using a Hewlett-Packard HP-67 Programmable Pocket Calculator (Hewlett-Packard Co., California, U.S.A.) to give a value for r , the correlation coefficient. Curves of best fit were computed according to one of the four following functions:

- 1) linear regression, where $y = a + bx$;
- 2) exponential curves, where $y = ae^{bx}$ ($a > 0$);
- 3) logarithmic curves, where $y = a + b \ln x$; or
- 4) power curves, where $y = ax^b$ ($a > 0$).

The levels of significance for r were determined using $n - 2$ degrees of freedom.

3. LONG-DISTANCE TRANSPORT AND WHOLE-PLANT METABOLISM

3. LONG-DISTANCE TRANSPORT AND WHOLE-PLANT METABOLISM

3.1 Introduction

The data available on the composition of phloem exudates indicate that the sieve-tube sap may be considered as a symplastic phase possessing many of the metabolic characteristics of cell cytoplasm. These similarities, however, must be examined not only in a descriptive sense, but also in terms of the specific consequences they may have for the control of transport within the system. The issues discussed in the introductory sections suggested that this question can only be answered with reference to the processes of nutrient transport to the shoot in the xylem and solute accumulation in the leaves. It is the nature of these relationships that is investigated in this chapter, in an attempt to provide an adequate basis for the discussion in the later chapters of other aspects of phloem physiology.

The approach adopted for this purpose was to consider the ways in which the metabolic constraints on the phloem could be changed in an experimentally convenient manner. For photolithotrophic plants carrying out photosynthetic CO_2 fixation and requiring a source of inorganic nitrogen, the uptake of C and N represent the largest solute fluxes across the bounding membranes of the organism (see Raven, 1977a). This is reflected in the predominance of organic C and N (on a dry-matter basis) in the elemental composition of the whole plant, but also extends to the proportion of organic C and N relative to other substances in the phloem. Although in practical terms it is not feasible to change the exogenous carbon source for photosynthesis, a widely used modification in studies on plant nutrition has been the provision of different sources of inorganic N. A large number of investigations have compared the effects of NO_3^- -N, NH_4^+ -N and di-nitrogen assimilation on various aspects of growth and solute accumulation (see Wit et al., 1963; Dijkshoorn, 1969; Kirkby, 1969; Pate, 1973; Osmond, 1976; Raven and Smith, 1976a). The basis of the present experiments with Ricinus was a comparison of the specific effects of NO_3^- -N versus NH_4^+ -N nutrition.

Changes in N-source have far-reaching consequences for the maintenance of the ionic balance of the plant tissues and for the control of intracellular pH. Cations and anions are usually absorbed from the nutrient medium at different rates, but electroneutrality is maintained within the plant tissues: this is achieved by accumulation and degradation of non-volatile organic acids, and by net excretion of

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either H^+ or OH^- from the roots to the nutrient medium (see Kirkby, 1969; Rayen and Smith, 1976a). Although the manifestations of these processes are relatively well understood in terms of solute accumulation in the shoots, the rôle of long-distance transport in the xylem and phloem in metabolism has been little studied. The ability to analyze the transport streams directly in Ricinus means that this relation between transport and metabolism can be investigated.

For the present purposes, it was important to establish firstly how shoot and root growth in Ricinus was influenced by the alternative sources of inorganic N. The consequences of NO_3^- -N versus NH_4^+ -N nutrition for solute accumulation in the leaves, were also examined, and this was followed by determination of xylem- and phloem-sap composition in these plants. Finally, the significance of the transport processes in the control of the solute balance of the shoot is discussed.

3.2 Growth characteristics of plants in water culture

The basic composition of the culture solutions containing either NO_3^- -N or NH_4^+ -N as the nitrogen source was given in Table 2.1. These two Long Ashton solutions were made up in such a way as to provide identical concentrations of K^+ , Mg^{2+} , Ca^{2+} and PO_4^{3-} . The differences in nutrient levels between the solutions arose from two sources. Firstly, the nitrate was supplied as KNO_3 and $Ca(NO_3)_2$, and for the NH_4^+ -N solution these salts were substituted by K_2SO_4 and $CaCl_2$ to give equivalent cation levels; in addition, the ammonium was supplied as $(NH_4)_2SO_4$, which further enhanced the SO_4^{2-} concentration compared with the NO_3^- -N solution. Secondly, the pH of the solutions was standardized to pH 5.50 because of the influence of pH on nutrient availability and uptake by roots (Black, 1968): this required the addition of KOH to the NO_3^- -N solution and of H_2SO_4 to the NH_4^+ -N solution.

A comparison of the resultant levels of the macronutrient ions in the two solutions is given in Table 3.1. The major anion in the NH_4^+ -N solution was SO_4^{2-} , which of the inorganic anions seems to be the one least likely to affect or be affected by the uptake of other ions (Dijkshoorn, 1959; Mengel, 1961). Also, the Cl^- level in this solution was half that of SO_4^{2-} , but was 80 times the Cl^- concentration in the NO_3^- -N solution. The possible consequences of such major differences in the levels of these ions have to be carefully taken into account. Note also that the N level was 50% higher, and the Na^+ level 50% lower, in the NO_3^- -N solution than the NH_4^+ -N solution. Micronutrient levels

Table 3.1 Comparison of levels of major nutrients in NO_3^- -N and NH_4^+ -N Long Ashton solutions at pH 5.50. The figures for K^+ and SO_4^{2-} marked with an asterisk * denote levels after adjustment of solution to pH 5.50 with KOH and H_2SO_4 , respectively.

	concentration / mol m ⁻³ equivalents	
	NO_3^- -N solution	NH_4^+ -N solution
K^+	4.20*	4.00
Na^+	1.33	2.67
Mg^{2+}	3.00	3.00
Ca^{2+}	8.00	8.00
NH_4^+	0	8.00
NO_3^-	12.00	0
SO_4^{2-}	3.00	16.00*
PO_4^{3-}	4.00	4.00
Cl^-	0.10	8.00

were the same in both solutions, except that the trace amounts of NaCl supplied in this form were omitted from the NH_4^+ -N solution. The solute potential of the two solutions was about -0.05 MPa.

To characterize the responses of Ricinus to NO_3^- -N versus NH_4^+ -N nutrition, growth was first compared in terms of changes in leaf area (Hanada and Uchida, 1974; Dodoo, 1978), plant height, fresh weight, dry weight and relative growth rate with time. Plants were raised on vermiculite moistened with NO_3^- -N solution and were transferred 7 d after germination to two-thirds strength NO_3^- -N or NH_4^+ -N Long Ashton solution. Any loss of solution through uptake by the plant was made up with full-strength solution, and after 7 d the plants were all transferred to full-strength NO_3^- -N or NH_4^+ -N solution. Throughout the experimental period, the pots were quickly cleaned and refilled with fresh solution at pH 5.50 once every three days. Nitrate analyses on the NH_4^+ -N solution showed that at the end of such three-day periods there was no detectable nitrate in the medium. The plants were harvested 57 d after germination for the determinations of dry weight. The experiment was performed four times, and the results presented in this section are from one experiment representative of the series.

Measurements of leaf area were made on the cotyledons, opposite leaves and alternate leaves every 3 d throughout the experimental period. The increase in area of successive leaves followed sigmoidal curves of the form described by Dodoo (1978), and the areas of leaves of plants grown on NO_3^- -N solution were throughout greater than those of plants grown on NH_4^+ -N solution. Table 3.2 presents the values for alternate-leaf areas on the plants at the end of the experimental period, as well as plant height from the lid of the water-culture pot to stem apex; at this stage the cotyledons had abscised, together with the opposite leaves on some of the plants. The results show that the areas of all the alternate leaves on the NO_3^- -N plants were significantly greater (although at different levels of significance) than those of the corresponding leaves on the NH_4^+ -N plants. The stature of the NO_3^- -N plants was also greater in terms of their height. This difference in leaf areas was indicative not only of the maximum area attained by the leaves, but represented also a disparity in the developmental stage reached by the plants, i.e. their 'physiological' ages. The relative sizes of the leaves on each of the groups of plants constitute one measure of the contrasting ontogenetic stages.

Table 3.2 Areas of alternate leaves and heights to stem apex
 NO_3^- -N versus NH_4^+ -N Long Ashton solution, 57 d after
germination. Figures are means \pm S.E. for 4 plants,
together with levels of significant differences between the
means.

alternate leaf	alternate-leaf area / mm ²		P
	NO_3^- -N plants	NH_4^+ -N plants	
1st (oldest)	24259 \pm 2810	12621 \pm 3862	< 0.05
2nd	35660 \pm 3032	18149 \pm 2878	< 0.01
3rd	44055 \pm 1364	17303 \pm 1549	< 0.001
4th	44163 \pm 5877	11776 \pm 1203	< 0.01
5th	30573 \pm 7107	3166 \pm 1373	< 0.01
6th	9413 \pm 4084	emerging	-
7th (youngest)	emerging	-	-
height / mm	335 \pm 21	224 \pm 10	< 0.01

A general observation on the morphology of the plants grown in water culture was that they possessed markedly less red anthocyanin pigmentation in the stems and leaves compared with soil-grown plants under identical conditions. During the first few weeks of growth, NO_3^- -N plants in fact had very little surface pigmentation, and NH_4^+ -N plants tended to be intermediate in coloration between these and soil-grown material. Leaves of NH_4^+ -N plants also occasionally developed small areas of chlorosis and necrotic lesions towards the edges of the laminae.

The fresh-weight yields of the plants described in Table 3.2 are plotted against time after germination in Fig.3.1. With increasing time, the difference in yields between the two groups of plants became progressively greater, such that at harvest the fresh weight of the NO_3^- -N plants was 3.6 times that of the NH_4^+ -N plants. In Table 3.3, the results of the analysis of dry-weight yields of these plants are presented. Both the shoot and root dry weights were greater for the NO_3^- -N plants compared with the NH_4^+ -N plants. The fact that the ratio of these yields for NO_3^- -N versus NH_4^+ -N plants is somewhat greater than that for the fresh-weight yields is reflected in the respective percentages of fresh weights to dry weights for the plants,, the NO_3^- -N plants being marginally less succulent (cf. Craven, Mott and Steward, 1972). There is also a suggestion that root dry-weight yield in NH_4^+ -N plants is impaired relatively less than shoot yield, as compared with the proportions in NO_3^- -N plants, although the shoot:root ratios were not significantly different in this experiment.

These data on the basic characteristics of plant growth on the two culture solutions show that growth is more rapid and yield considerably higher on the NO_3^- -N solution than the NH_4^+ -N solution. It is obvious, however, that physiological comparisons between the plants must be related not just to the time course of the experiments but to ontogeny as well. An important index inherent in the growth curves is the absolute growth rate of the plant over a period of time. This can be quantified as the relative growth rate, R_W (Evans, 1972), over a finite period of time, as given by the equation

$$R_W = \frac{1}{W} \cdot \frac{dW}{dt} \quad (3.1)$$

where W = dry weight. Fig. 3.2 shows a comparison of the values of R_W computed for the NO_3^- -N and NH_4^+ -N plants from the results described

Fig. 3.1 Growth curves for plants grown on NO_3^- -N versus NH_4^+ -N Long Ashton solution, expressed as fresh weights of whole plants. Points are means of values for 4 plants, and bars represent 2 x S.E. of the mean.

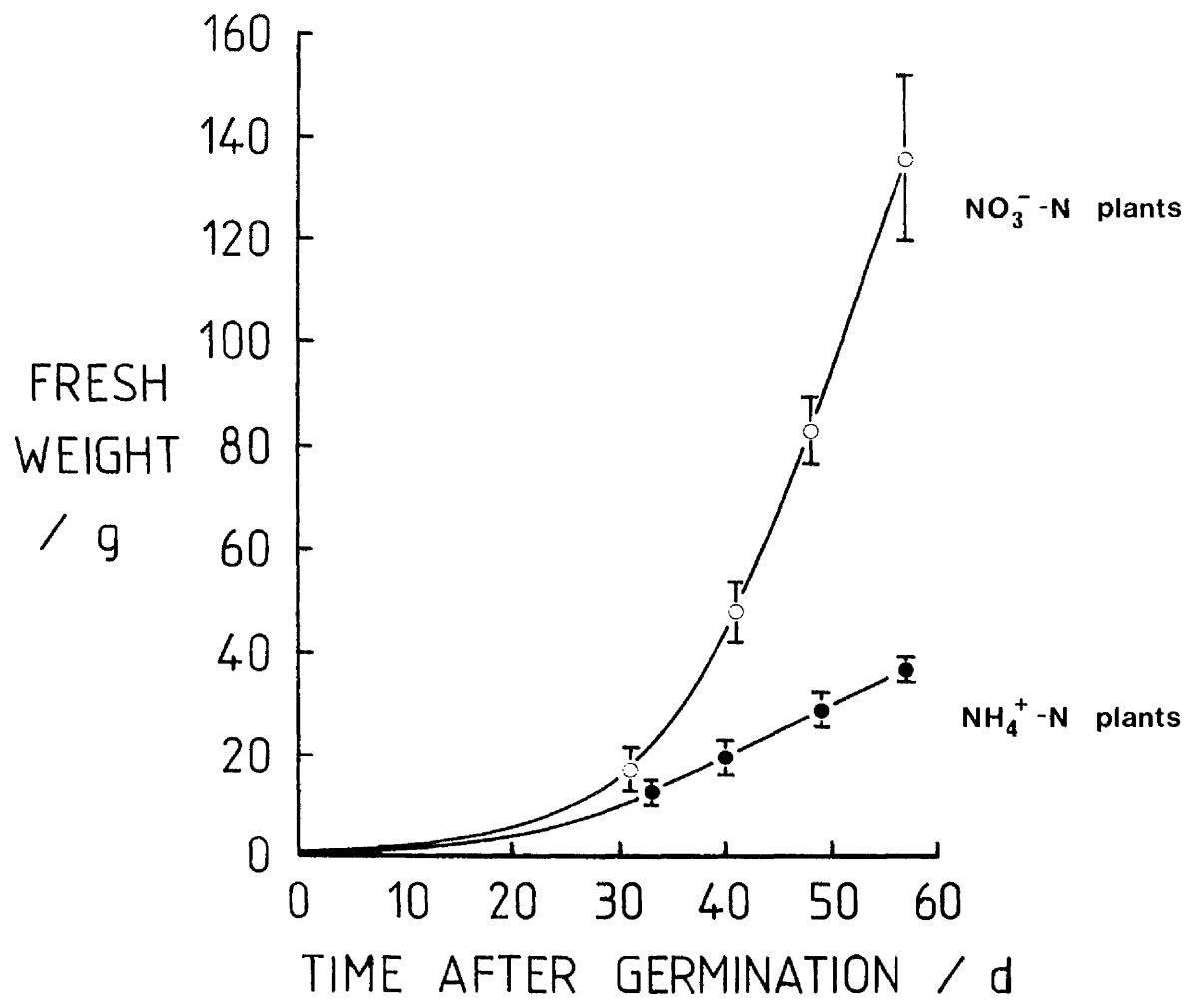
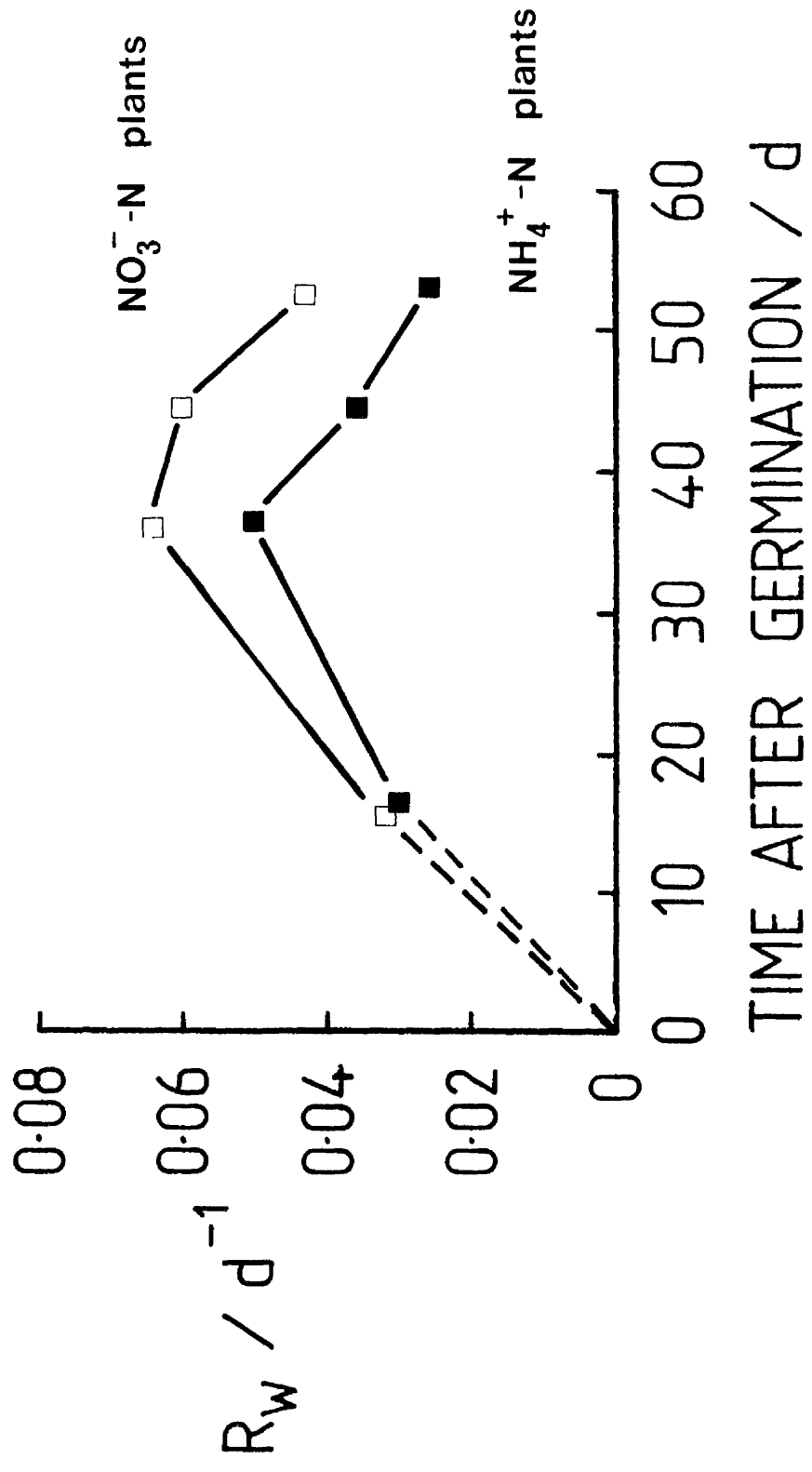


Table 3.3 Dry-weight yields of plants grown on NO_3^- -N versus NH_4^+ -N Long Ashton solution, 57 d after germination. Figures are means \pm S.E. for 4 plants, together with levels of significant differences between the means.

	Dry weight / g			P	Ratio NO_3^- -N: NH_4^+ -N plants
	NO_3^- -N plants	NH_4^+ -N plants			
Shoots	12.42 \pm 1.52	2.72 \pm 0.23	< 0.001	4.6	
Roots	4.33 \pm 0.34	1.10 \pm 0.12	< 0.001	3.9	
Shoot: root ratio	2.87 \pm 0.19	2.47 \pm 0.11	N.S.	-	
% of fresh weight	12.4	10.4	-	-	

Fig. 3.2 Relative growth rates (R_w) of plants grown on NO_3^- -N
versus NH_4^+ -N solution.



above. The broken lines represent those parts of the growth curves for which data were not available; a further assumption was that the ratio of shoot:root dry weights did not change significantly over this time (see Pitman, 1975). The graph demonstrates that, although the NO_3^- -N plants had a higher R_W throughout the experiment, there was in fact a qualitative similarity in the change of R_W during this period between the two groups of plants, R_W starting to decrease about 37 d after germination. Under constant environmental conditions, R_W generally declines throughout ontogeny, largely because of the increasing proportion of non-dividing to dividing cells (see Evans, 1972; Milthorpe and Moorby, 1974). The proportional differences between the R_W values of the NO_3^- -N and NH_4^+ -N plants were less than the equivalent differences in fresh-weight or dry-weight yields.

As a means of assessing the extent to which there were broad similarities in the physiological characteristics of the NO_3^- -N and NH_4^+ -N plants, the water relations of the shoots of eight-week-old plants were investigated in a separate experiment by measurement of leaf Ψ_s . For this purpose, the 5th alternate leaves of the NO_3^- -N plants and the 3rd alternate leaves of the NH_4^+ -N plants were taken as physiologically equivalent. The results of this experiment are shown in Table 3.4. Leaf Ψ_s was not significantly different between the NO_3^- -N and NH_4^+ -N plants, but leaf Ψ was slightly lower in the NH_4^+ -N plants. The estimated values for Ψ_p , however, were not significantly different. Therefore on the basis of the water relations of the bulk leaf tissue, there appeared to be no fundamental qualitative or quantitative differences between the growth characteristics of the NO_3^- -N and NH_4^+ -N plants.

Given the importance of the root-shoot relations in determining growth and yield, it is important to have a physiological measure of condition of the root system as well as that of the shoot. A widely noted phenomenon is that growth on NO_3^- -N solutions brings about an increase in pH of the root medium, and growth on NH_4^+ -N solutions a decrease in pH (see Coïc, Lesaint and Le Roux, 1961; Kirkby and Hughes, 1970). This trend was observed in the course of all the experiments with Ricinus, the pH values after the provision of fresh medium reaching about 7.0 in the NO_3^- -N solution and as low as 3.4 in the NH_4^+ -N solution. By way of an example, Table 3.5 presents the pH values for a group of plants in one experiment, measured about 48 h after the pots were refilled with fresh medium.

Table 3.4 Characteristics of leaf water relations of 5th or 3rd alternate leaf of eight-week-old plants grown on NO_3^- -N or NH_4^+ -N Long Ashton solution, respectively. Figures are means \pm S.E. for 4 plants, together with levels of significance.

	leaf ψ_s / Mpa	leaf ψ / Mpa	estimated leaf ψ_p / MPa
NO_3^- -N plants	-1.67 \pm 0.09	-0.51 \pm 0.03	1.16 \pm 0.10
NH_4^+ -N plants	-1.90 \pm 0.05	-0.60 \pm 0.01	1.29 \pm 0.05
P	N.S.	< 0.05	N.S.

Table 3.5 Summary of pH changes in rooting media, originally at pH 5.50, after about 48 h (eight-week-old plants), Figures are means \pm S.E. for 4 plants together with the level of significance.

pH of rooting medium	
NO_3^- -N plants	6.70 \pm 0.08
NH_4^+ -N plants	3.51 \pm 0.15

P < 0.001

The results show that, from the initial pH of 5.50, there was an increase in the pH of the root medium during growth of the NO_3^- -N plants, and a decrease in pH during growth of the NH_4^+ -N plants. These changes were representative of those recorded in 8 experiments, and constituted a major difference between the growth characteristics of the NO_3^- -N and NH_4^+ -N plants.

The magnitude of these pH changes was investigated in further detail by continuous measurement of the pH of the root media over a period of 24 h. With pH values as low as 3.4, there is likely to be a considerable stress on nutrient-uptake processes and root function in general, the root systems of eight-week-old NH_4^+ -N plants being partly discoloured and in a manifestly poorer condition than those of the NO_3^- -N plants. Younger plants were therefore used for a closer examination of these pH changes, because if the magnitude of the effect was proportional to root biomass, these plants would not have been subject to such severe stress in the three-day periods between changes of solution.

Fig. 3.3a shows the time course of pH changes in the root media of five-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. The plants were transferred from the greenhouse to the growth-room 3 d prior to the start of the experiment to allow for acclimation before making the pH measurements; the solutions were changed daily in the pots during this time. The influence of the aeration cycles on the pH of the solutions, especially around pH 7, served to emphasize the necessity of ensuring that there was adequate equilibration with atmospheric CO_2 . From Fig. 3.3a, it can be seen that the pH changes recorded were within the ranges noted previously. A more direct comparison is shown in

fig. 3.3 (a) Time course of pH changes in root medium of five-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Points are for individual plants in one experiment representative of the series.

(b) Same results plotted as cumulative amounts of net OH^- of H^+ excretion per unit fresh weight (FW) of roots.

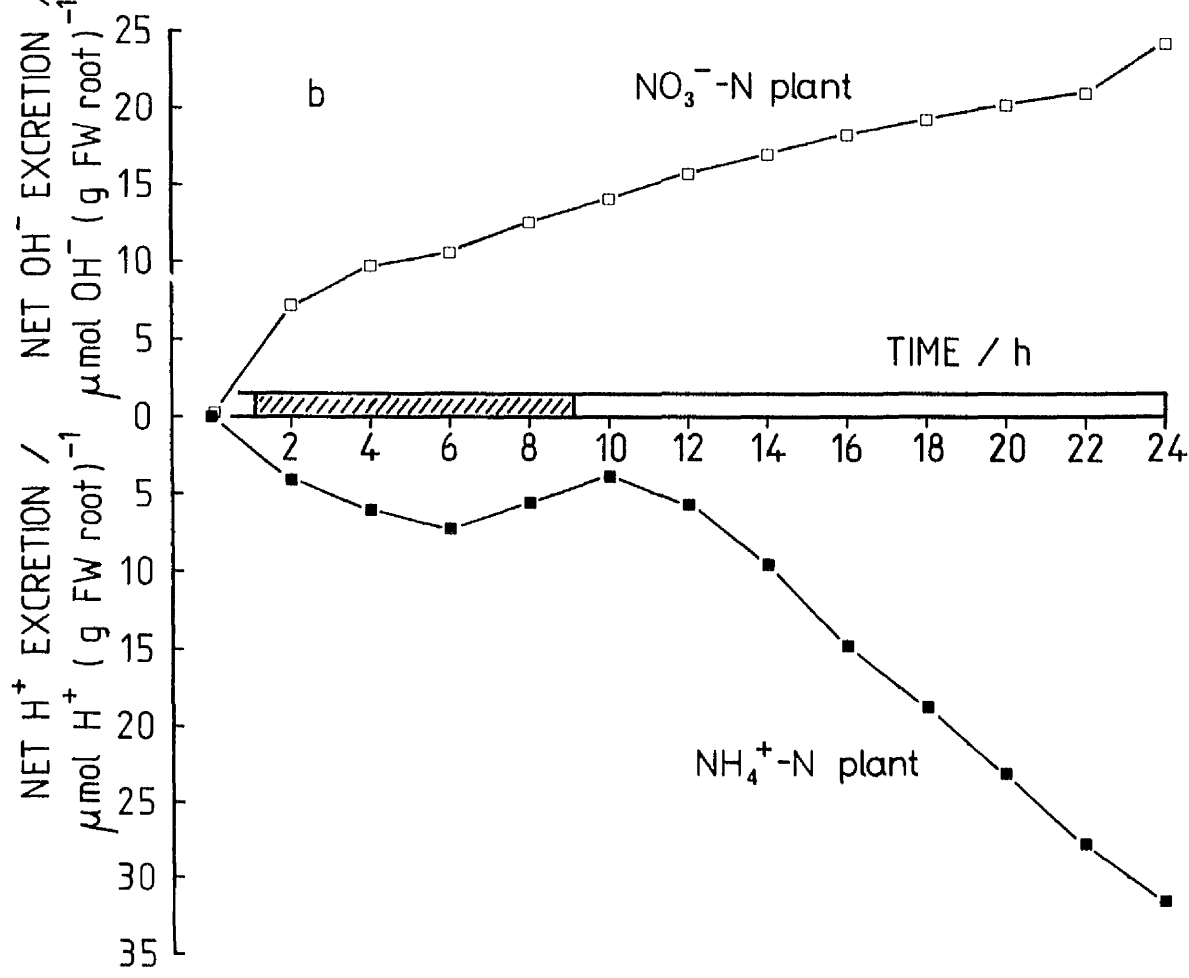
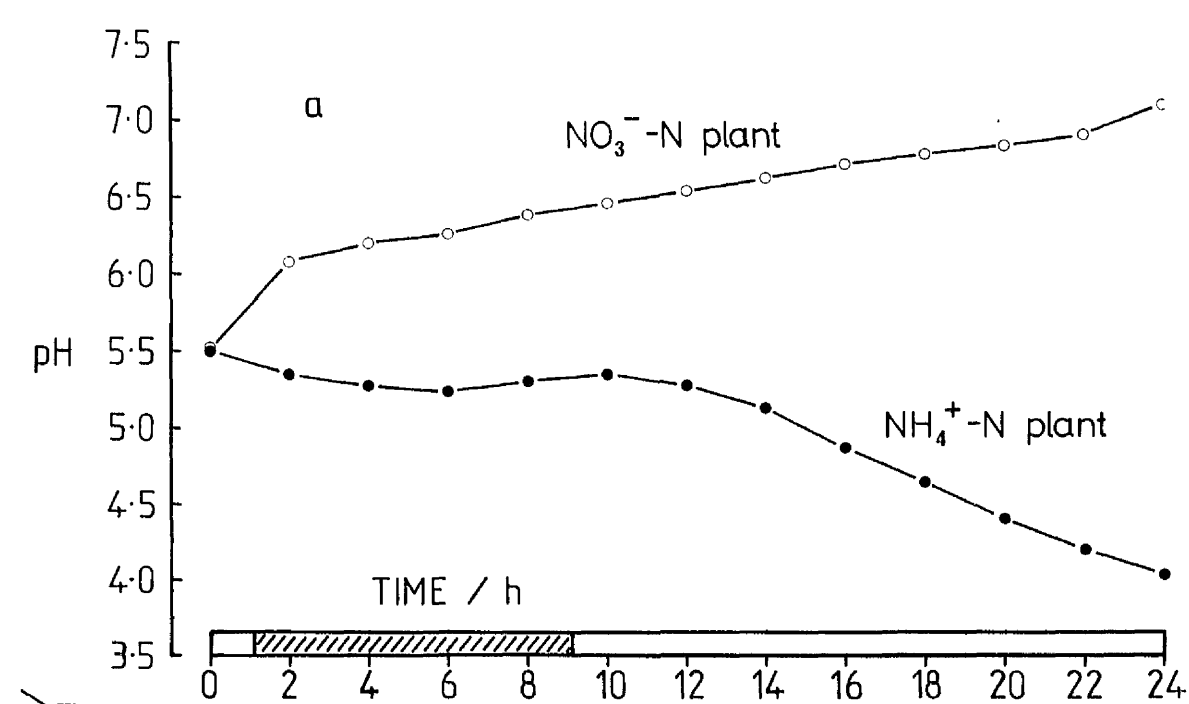


Fig. 3.3b, where the same data are plotted as net OH^- or H^+ excretion per unit fresh weight of root. This conversion required an estimate of the buffering capacity of the two solutions, to which the principal contributions will have been made by citrate⁻/citrate²⁻ ($\text{pK}_a = 4.75$), citrate²⁻/citrate³⁻ ($\text{pK}_a = 5.45$), $\text{H}_2\text{CO}_3/\text{HCO}_3^-$ ($\text{pK}_a = 6.38$) derived largely from respiratory CO_2 , and $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ ($\text{pK}_a = 6.8 - 7.0$). This was obtained by titration (in duplicate) of the same batch of solution used in the particular experiment with standard KOH or H_2SO_4 solution. The net OH^- or H^+ excretion for any part of the observed pH range could then be calculated from the titration curve. Plants were harvested at the end of the experiment for determinations of fresh weight and dry weight.

Fig. 3.3b demonstrates that the cumulative amount of net OH^- or H^+ excretion expressed per unit fresh weight of root of the NO_3^- -N or NH_4^+ -N plant, respectively, was of an equivalent order of magnitude over the 24-h period. These values were corrected to account for the decreasing volume of solution in the pots during the course of the experiment as a result of uptake by the plants. Net H^+ excretion by roots of the NH_4^+ -N plant was in fact greater over this time than OH^- excretion by the NO_3^- -N plant, and appeared also to be more affected by the dark period of the day-night cycle in the growth-room. These data can also be expressed as an integrated rate of net OH^- or H^+ excretion over the 24-h period: this information is presented in terms of both unit fresh weight and unit dry weight of root in Table 3.6.

Table 3.6 Integrated rates of net OH^- or H^+ excretion by roots of five-week old plants over 24 h. Values are for individual plants in Fig. 3.3.

	NO_3^- -N plant	NH_4^+ -N plant
	net OH^- efflux $\text{g}^{-1} \text{h}^{-1}$	net H^+ efflux $\text{g}^{-1} \text{h}^{-1}$
per g FW	1.01	1.32
per g DW	16.21	18.72

These values show that the rate of net OH^- excretion per unit fresh weight of root of the NO_3^- -N plant integrated over 24 h was about 77% of

the equivalent rate of net H^+ excretion by the NH_4^+ -N plant. The corresponding difference between the integrated rates per unit dry weight of root was smaller because of the marginally higher DW:FW ratio of the roots of the NH_4^+ -N plant.

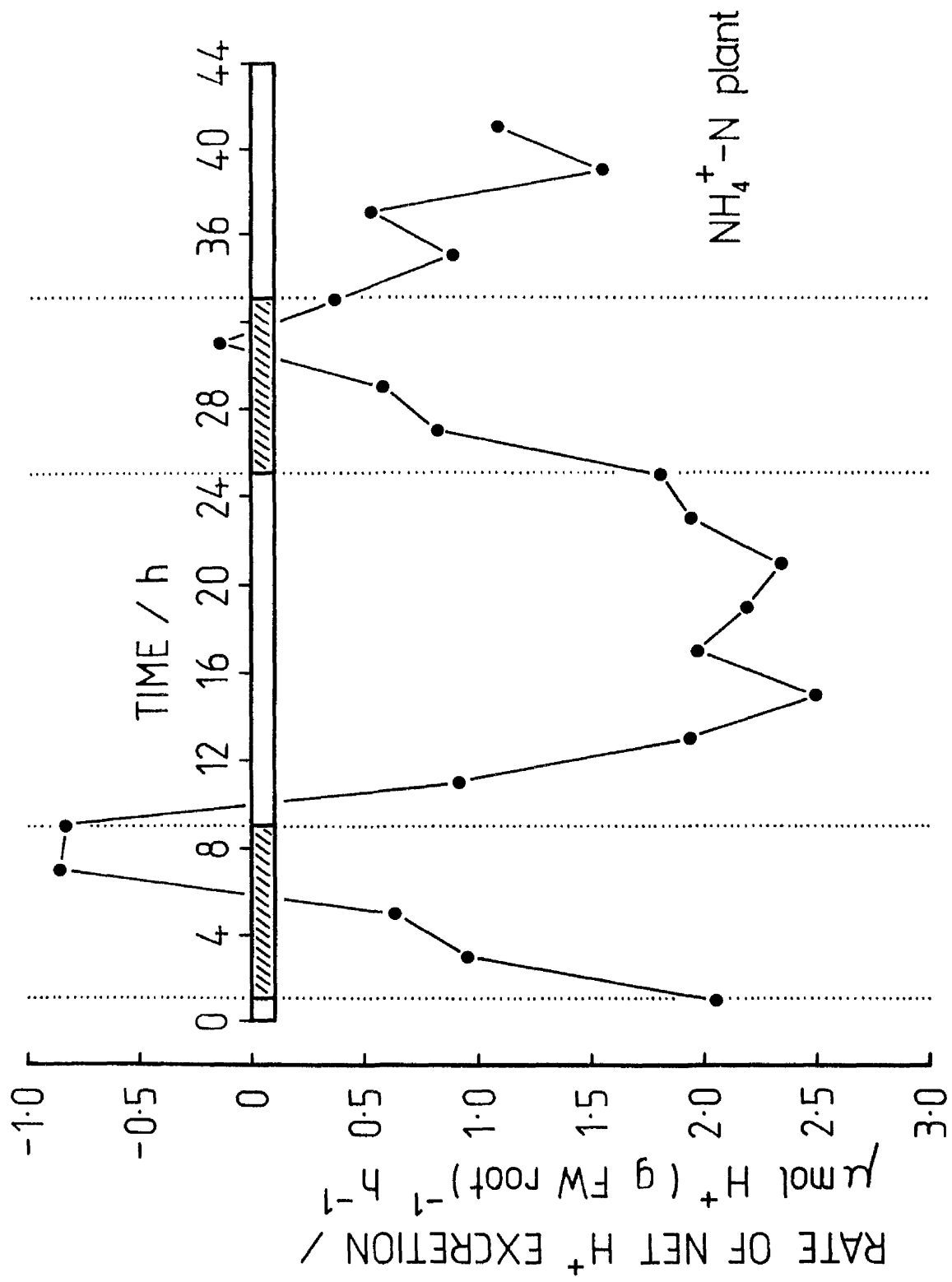
The rates of net H^+ excretion from the roots of the NH_4^+ -N plants were also examined over extended time periods. Fig. 3.4 shows the rate of net H^+ excretion plotted per unit FW per hour over a period of 42 h; these data are simply an extension of the values recorded for the same plant as used in the experiment of Fig. 3.3. Over the experimental period, there were marked changes in the rate of net H^+ excretion. In the two dark periods encompassed, the rates of net H^+ excretion declined, and in both cases there was in fact transient net OH^- excretion towards the end of these periods. The rates of net H^+ excretion increased with the onset of the photoperiod, reaching a maximum value of about $2.6 \mu\text{mol } H^+ (\text{g FW root})^{-1} \text{ h}^{-1}$. Also, the amplitude of the changes in rate appeared to be slightly dampened in the second part of the cycle (24 - 42 h) compared with the first (0 - 24 h). No consistent correlation of the rates of net OH^- excretion by NO_3^- -N plants with the photoperiod was observed.

3.3 Solute accumulation during growth on NO_3^- -N and NH_4^+ -N solution

The results presented thus far have established that there were major differences in some of the whole-plant characteristics of growth on the water-culture solution containing NO_3^- -N as compared with NH_4^+ -N. Growth measured by leaf area, plant height, fresh weight and dry weight was more rapid, and greater in terms of yield, on the NO_3^- -N solution. Values of R_W showed smaller differences and in fact exhibited similar trends during ontogeny. In terms of the water relations of the shoots there were no gross differences between the two groups of plants, but the qualitatively distinct patterns of net OH^- or H^+ excretion from the roots suggested that there might be fundamental dissimilarities in metabolism. Since shoot growth is directly dependent on processes of solute accumulation in the leaves, it is important to consider how these processes are affected by the biochemical consequences of assimilation of the alternative N-sources. In this section, therefore, the relationships between accumulation of osmotically important solutes in the leaves and whole-plant metabolism are examined in more detail.

Leaf-cell sap was extracted for analysis from freshly harvested

Fig. 3.4 Extended time course showing rate of net H^+ excretion by roots of five-week-old plant grown on NH_4^+ -N solution. Results are from same experiment as described in Fig. 3.3. Dark phase of growth-room day-night cycle corresponded to 20.00 - 04.00 h (BST).



material by the freeze-disruption method described in section 2,3. Samples were taken for the analysis of inorganic cations, organic and inorganic anions, free amino acids and amides and sugars. The experiment was performed four times and the results presented here are from one experiment in the series. Particular groups of results do not necessarily refer to the same experiment in each case, but results from different experiments in the series suggested that the variation was small enough to make the comparisons legitimate.

The levels of inorganic cations in the leaf-cell sap of NO_3^- -N and NH_4^+ -N plants are shown in Table 3.7. Mg^{2+} and Ca^{2+} levels are expressed as mol m^{-3} charge equivalents, and are thus double their concentration in mol m^{-3} ; the total cation content refers to the sum ($\text{K}^+ + \text{Na}^+ + \text{Mg}^{2+} + \text{Ca}^{2+}$), and is also expressed as mol m^{-3} equivalents. None of these four ions was present at significantly different levels in the NO_3^- -N leaves compared with the NH_4^+ -N leaves. K^+ was the predominant ion, followed by Ca^{2+} , Mg^{2+} and then Na^+ , and the ratios of monovalent to divalent cations were comparable in the two cases (1.14 in the NO_3^- -N leaves and 1.53 in the NH_4^+ -N leaves). The total level of inorganic cations was around 300 mol m^{-3} equivalents in both the NO_3^- -N and NH_4^+ -N leaves.

The total content of these four ions is usually taken as effectively equal to the total cation content of the leaf (Wit et al., 1963; Dijkshoorn, 1969). To maintain electrical neutrality within the tissues, there must also be a level of organic and inorganic anions providing the same charge equivalents. However, the chemical nature of these ionic species, and the proportion of organic to inorganic anions, varies greatly between different species and under different conditions (Wit et al., 1963; van Tuil, 1965; Cram, 1976a; Osmond, 1976). Preliminary investigations suggested that malate was the principal anion in leaves of Ricinus plants grown on NO_3^- -N solution, and in the following tables the anionic content of the leaves is presented in quantitative terms.

Table 3.8 shows the results from enzymic analyses of the malate levels in leaves of plants grown on the NO_3^- -N or NH_4^+ -N solutions, and in addition for plants grown on the basic NO_3^- -N solution containing an increased chloride concentration, denoted (NO_3^- -N + Cl^-).

Table 3.7 Levels of inorganic cations in leaf-cell sap from eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are means \pm S.E. for samples from 4 plants together with levels of significance.

	K^+ / mol m ⁻³	Na^+ / mol m ⁻³	Mg^{2+} / mol m ⁻³ equivalents	Ca^{2+} / mol m ⁻³ equivalents	TOTAL mol m ⁻³ equivalents
NO_3^- -N plants	152 \pm 12	6.9 \pm 0.9	51.6 \pm 1.7	87.5 \pm 11.2	298 \pm 18
NH_4^+ -N plants	176 \pm 9	8.8 \pm 0.4	47.5 \pm 2.6	72.9 \pm 10.1	305 \pm 19
P	N.S.	N.S.	N.S.	N.S.	N.S.

Table 3.8 Levels of malate in leaf-cell sap from eight-week-old plants grown on NO_3^- -N solution, NO_3^- -N solution with increased chloride (NO_3^- -N + Cl^-), or NH_4^+ -N solution. Figures are means \pm S.E. for samples from 4 to 7 plants.

	NO_3^- -N plants	(NO_3^- -N + Cl^-) plants	NH_4^+ -N plants
malate concentration/ mol m^{-3}	102 \pm 10	92 \pm 12	0.5 \pm 0.2

The Cl^- was introduced as NaCl in an amount sufficient to raise the Cl^- content of the NO_3^- -N solution up to that of the NH_4^+ -N solution, viz. 8.00 mol m^{-3} . This was to determine whether the availability of Cl^- , rather than the alternative N-source per se, had an effect on anion accumulation in the leaves. As a consequence, the Na^+ concentration in this solution was about 3.5 times higher than in the NH_4^+ -N solution.

The principal finding of these experiments was that the malate levels in the leaves of the NO_3^- -N plants were high (about 100 mol m^{-3}), but were almost negligible in the NH_4^+ -N leaves (0.5 mol m^{-3}) (Table 3.8). A difference of this magnitude was observed in all three experiments in which malate was quantified enzymically. There was no statistically significant difference in the malate content of the (NO_3^- -N+ Cl^-) leaves compared with the NO_3^- -N leaves, although in the two experiments in which the analyses were carried out on (NO_3^- -N + Cl^-) leaves the mean values were slightly lower. This does suggest, however, that high Cl^- levels in the rooting media were not in themselves the cause of the very low malate levels in the NH_4^+ leaves. The malate content alone of the NO_3^- -N leaves accounted for about 68% of the anionic contribution to the charge balance.

With such large differences between the malate levels in the NO_3^- -N and NH_4^+ -N leaves, it was important to determine whether the very low values for the latter (which were only marginally above background) resulted from a qualitative disturbance to or interference with the assay system. This was done by adding known amounts of pure malic acid as an internal standard to the extracts from the NH_4^+ -N leaves prior to analysis. The standards were recovered with exactly the same efficiency as found with pure samples, suggesting that the failure to

70
detect substantial levels of malate in the sap was not due to inhibition of enzyme activity in the assay medium.

As a means of testing these results by a completely distinct method of analysis, the organic-acid content of the leaf-cell sap was also studied by gas-liquid chromatography. This represents a selective and accurate physico-chemical technique for the determination of organic acids in terms of the concentration of the major species present in leaf tissue (Nierhaus and Kinzel, 1971; Phillips and Jennings, 1976a).

The characteristics of the SP-2250 stationary phase were first determined with respect to the trimethylsilyl derivatives of organic-acid standards. In Table 3.9, the retention times of the silyl derivatives prepared using BSA are presented relative to that of malic acid for the operating conditions described in section 2.4. The R_t values recorded by Phillips and Jennings (1976a) using SE-52 are also given for comparison, this stationary phase possessing chemical similarities to the SP-2250. Stationary phases of this composition, however, do not separate citric, isocitric and shikimic acids, which tend to co-chromatograph; derivatization of *cis*-aconitic acid in the present system also appeared to be erratic. The relative R_t values (as well as the absolute retention times) in the leaf samples were often marginally different from those of the pure standards. One peculiarity of this method, as noted by Phillips and Jennings (1976a), is that oxalic acid is lost in the purification procedure. Although the standard silyl derivatives of oxalic acid come off the column rather close to the solvent front, the peak is nevertheless distinct; it appears that oxalic acid is volatile to the extent that it is simply lost during rotary evaporation under reduced pressure (R.D. Phillips, personal communication).

Typical examples of the GLC traces obtained from the TMSi derivatives from leaf extracts of NO_3^- -N and NH_4^+ -N plants are shown in Fig. 3.5. For the NO_3^- -N leaf (Fig. 3.5a) by far the largest single peak as quantified by peak area was that of the malic-acid derivative; in comparison with the response to other organic-acid standards, it was estimated from the traces that this accounted for about 80% of the organic acids recovered in this purification procedure. From the trace for the NH_4^+ -N leaf (Fig. 3.5b), after injection of an equivalent amount of sample, it can be seen that the malic-acid

Table 3.9 Summary of retention times (R_t) expressed relative to malic acid for TMSi derivatives of organic-acid standards on 3% (w/w) SP-2250 analyzed by GLC, and a comparison with those on 2% (w/w) SE-52. The figures in brackets are those not presented as mean values by Phillips and Jennings (1976a) but derived from examples of their GLC traces.

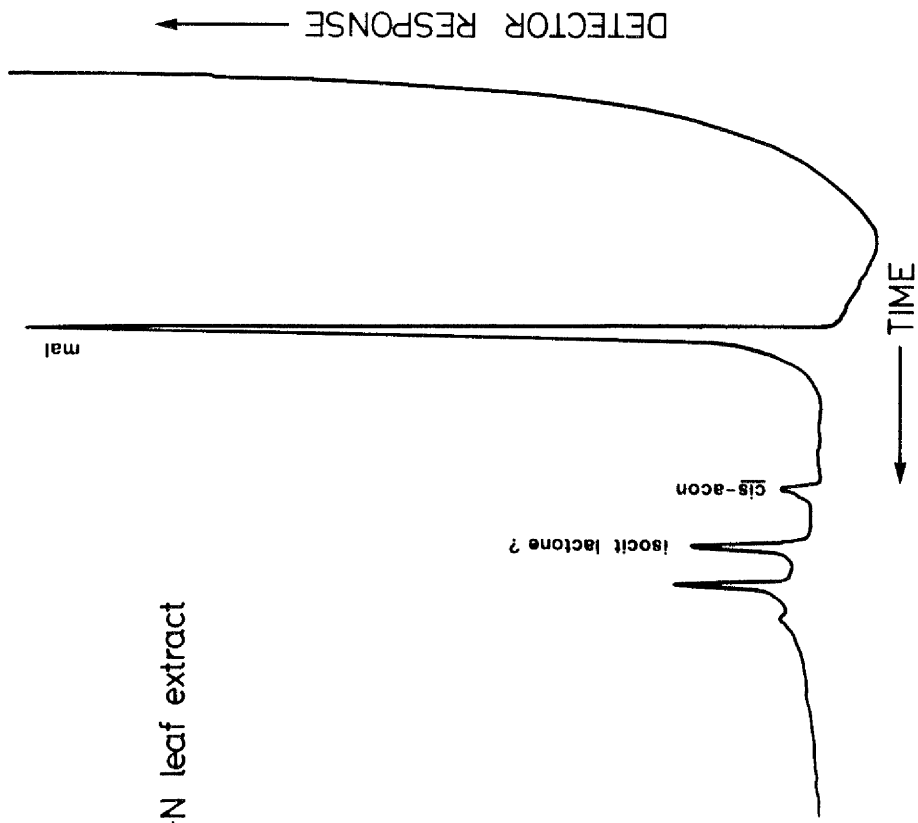
acid standards	R_t relative to malic acid	
	3% (w/w) SP-2250	2% (w/w) SE-52 (Phillips and Jennings, 1976a)
oxalic	0.14	(0.28)
malonic	-	0.51
succinic	0.60	(0.59)
fumaric	0.78	0.69
malic	1.00	1.00
<i>D</i> -tartaric	1.22	(1.32)
isocitric lactone	-	(1.43)
citric	1.30	1.82 (+ isocitric and shikimic)
<i>cis</i> -aconitic	1.51	(1.50)
quinic	-	(>1.82)

Fig. 3.5 GLC traces of separation of TMSi derivatives of organic acids from leaf extracts on 3% (w/w) SP-2250 for (a) NO_3^- -N leaves, and (b) NH_4^+ -N leaves. Abbreviations are as follows:

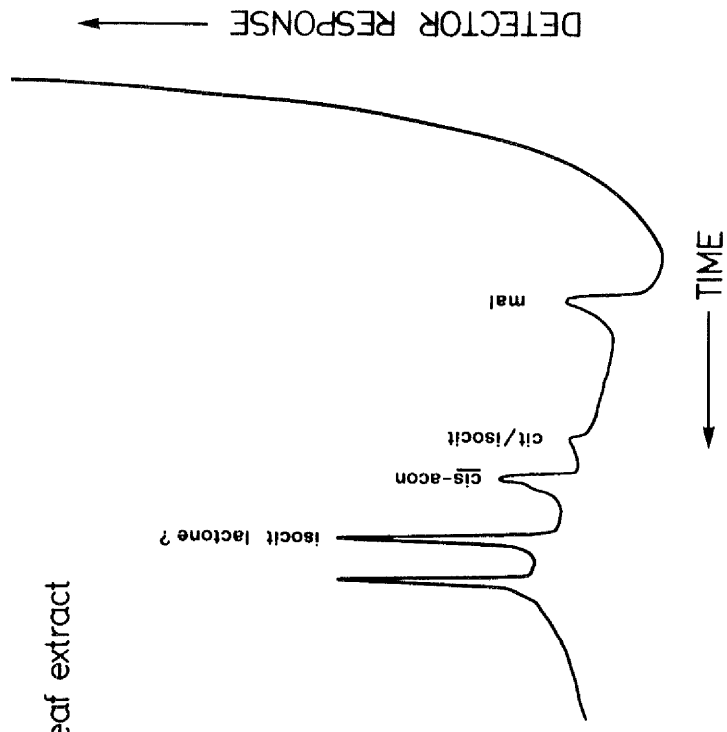
mal	= malic acid
cis-acon	= cis-aconitic acid
cit	= citric acid
isocit	= isocitric acid.

Traces a and b are not directly comparable because of differences in detector response (between different runs) and in quantities of sample injected. The levels of cis-aconitic acid and isocitric lactone (?) were, however, similar in absolute terms in the NO_3^- -N and NH_4^+ -N leaves.

a NO_3^- -N leaf extract



b NH_4^+ -N leaf extract



derivatiye was present in no more than trace amounts. There was much less difference in the quantities of citric/isocitric/shikimic-acid derivatives present in the sample. The other peaks probably represent derivatives of *cis*-aconitic (and possibly *trans*-aconitic and quinic) acids.

Analysis of leaf-cell sap organic acids by GLC thus corroborated the results of the enzymic assays, suggesting that malate occurred only in very small amounts in the NH_4^+ -N levels. Since the total cation levels in leaf-cell sap from the NO_3^- -N and NH_4^+ -N plants were almost identical, some other anion(s) must have been substituting for malate in the leaves of the NH_4^+ -N plants. The two other main groups of free anionic species in plant tissues besides the organic acids are the inorganic anions and the amino acids that bear a net negative charge at the relevant pH.

To take first the inorganic anions, Table 3.10 shows the levels of chloride found in leaf-cell sap from the NO_3^- -N, (NO_3^- -N + Cl^-), and NH_4^+ -N plants. The Cl^- concentration in the NH_4^+ -N leaves (100.5 mol m^{-3}) was nearly nine times that in the NO_3^- -N leaves (11.8 mol m^{-3}). Although the Cl^- level in the (NO_3^- -N + Cl^-) leaves was not significantly higher than in the NO_3^- -N leaves, the mean values were higher for the plants with increased Cl^- in the two experiments in which measurements were made. Also, the higher content of Cl^- in (NO_3^- -N + Cl^-) leaves on the basis of mean values amounted to 17.5 mol m^{-3} , and the malate content of these leaves was about 20 mol m^{-3} equivalents lower (Table 3.8).

The levels of inorganic phosphate in the leaf-cell saps are given in Table 3.11. The difference in H_2PO_4^- concentrations between the NO_3^- -N and NH_4^+ -N leaves was significant ($P < 0.05$), the levels being about 73% higher in the latter based on comparison of the means.

These two sets of results demonstrate that the almost complete absence of malate from the leaf-cell sap of the NH_4^+ -N plants was to some extent compensated for by the high levels of Cl^- and H_2PO_4^- in the tissue. In combination, the two inorganic anions provided nearly four times as many charge equivalents in the NH_4^+ -N leaves compared with the NO_3^- -N leaves. The question of whether the free amino acids made any contribution to the anion balance was next investigated, with a view also to determining the osmotic importance of these compounds in the solute balance of the leaf.

In Table 3.12, the levels of free amino acids and their amides

Table 3.10 Levels of chloride in leaf-cell sap from eight-week-old plants grown on NO_3^- -N solution, NO_3^- -N solution with increased chloride (NO_3^- -N+ Cl^-), or NH_4^+ -N solution. Figures are means \pm S.E. for values from pooled samples in 2 or 3 experiments.

	NO_3^- -N plants	(NO_3^- -N + Cl^-)plants	NH_4^+ -N plants
Cl^- concentration/ mol m^{-3}	11.8 \pm 2.3	29.3 \pm 8.5	100.5 \pm 16.5

Table 3.11 Levels of inorganic phosphate in leaf-cell sap from nine-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are means \pm S.E. for samples from 3 plants.

	NO_3^- -N plants	NH_4^+ -N plants
H_2PO_4^- concentration/ mol m^{-3}	29.0 \pm 4.4	50.3 \pm 5.5

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in leaf-cell sap from the NO_3^- -N and NH_4^+ -N plants are presented both as absolute concentrations (mol m^{-3}) and relative amounts (%). These figures represent values for pooled samples made up from leaves of three plants in each case. Abbreviations for the amino acids and amides follow those given in Biochem. J. 126, 773-780 (1972). Apart from glutamic acid, alanine and methionine, all the amino acids were present in leaf-cell sap from the NH_4^+ -N plants at higher levels than in sap from the NO_3^- -N plants. Similarly, the concentration of the amides glutamine and asparagine combined was about twelve times higher in the NH_4^+ -N leaves. The total concentration of all the amino acids and their amides together in the NH_4^+ -N leaves was about 59 mol m^{-3} and this was over six times the level in the NO_3^- -N leaves. In terms of their concentration, quantitatively the most significant differences between the two groups of plants were in the levels of serine, arginine and histidine. On the basis of their proportions, glutamic acid and alanine were relatively much more important in the NO_3^- -N leaves, as were asparagine and arginine in the NH_4^+ -N leaves. Arginine, in fact, accounted for 38% of the total free amino acids and amides in the leaves of the NH_4^+ -N plants.

Another measure of the significance of this soluble pool of amino acids and amides (as well as the net charge balance) is the total concentration of organic N present in this form. This is more closely related to the contributions of these compounds to the nitrogen economy of the leaves, although it is the concentration in molecular terms (as discussed above) that is more significant on osmotic grounds. The organic nitrogen concentration can be calculated by summation, given that all the amino acids contain one atom of nitrogen per molecule, except for lysine (and the amides glutamine and asparagine) which contains two N per molecule, histidine which contains three, and arginine which has four. The net charge balance can also be determined by inspection of the pI values of the compounds. To a first approximation, it is reasonable to take the effective charge on the amides and amino acids at pH values around or slightly below neutrality as being negligible, except for the charged polar molecules aspartic acid, glutamic acid, histidine, lysine and arginine. Of these, aspartic acid (pI = 2.97) and glutamic acid (pI = 3.22) bear a net negative charge at such pH values, and histidine (pI = 7.58), lysine (pI = 9.74) and arginine (pI = 10.76) carry a net positive charge (values from Mahler and Cordes, 1971).

Table 3.12 Levels of free amino acids and amides in leaf-cell sap from nine-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are for pooled samples from one experiment expressed as concentration (mol m^{-3}) or relative amount (%)

	NO_3^- -N leaf		NH_4^+ -N leaf	
	mol m^{-3}	%	mol m^{-3}	%
Gln	1.29	13.8	8.73	14.9
Glu	1.75	18.7	1.52	2.6
Asn	0.22	2.3	10.04	17.1
Asp	0.82	8.7	1.20	2.1
Ser	1.66	17.7	7.66	13.1
Ala	1.57	16.8	0.99	1.6
Thr	0.52	5.5	1.44	2.5
Pro	0.27	2.8	0.89	1.5
Lys	0.27	2.8	0.64	1.1
Val	0.22	2.4	0.72	1.2
Leu	0.18	1.9	0.23	0.4
Phe	0.13	1.3	0.31	0.5
Ile	0.13	1.3	0.45	0.8
Arg	0.11	1.1	22.50	38.4
Gly	0.09	0.9	0.17	0.3
His	0.08	0.8	0.92	1.6
Tyr	0.06	0.6	0.12	0.2
Met	0.02	0.2	0.02	0.04
Cys	absent	-	absent	-
TOTAL	9.35	100	58.55	100

The information on the organic N content of the leaves derived in this way is presented in Table 3.13. Expressed as the concentration of free organic N, the difference between the leaves of the NO_3^- -N and NH_4^+ -N plants was even more pronounced. The free organic N content of the sap from the NH_4^+ -N leaves was 147.3 mol m^{-3} , and this was nearly thirteen times the level in the NO_3^- -N leaves. The change in proportions between the two groups of leaves was almost entirely caused by the contribution of four N per molecule by arginine in the NH_4^+ -N leaves. For the same reason, whereas the amino acids plus amides in the NO_3^- -N leaves carried a small net negative charge, those in the NH_4^+ -N leaves possessed a substantial net positive charge.

These data broadly account for the solutes commonly regarded as the osmotically important species in leaves of dicotyledonous plants. By collation of the results, it is possible to consider to what extent these concentrations tie in with the Ψ_s values of the leaves recorded in Table 3.4. For this purpose the solutes can be distinguished as those that are ionic (i.e. charged). The uncharged solutes may be taken as the sugars, uncharged amino acids and the amides. The total cation content can be regarded as the sum of ($\text{K}^+ + \text{Na}^+ + \text{Mg}^{2+} + \text{Ca}^{2+}$), to which may be added the positively charged amino acids and NH_4^+ . It can then be assumed that the total cation content is balanced by the same number of charge equivalents of (inorganic + organic) anions. From these figures, the approximate Ψ_s contribution of each group of solutes can be determined from standard tables (Weast, 1971) for the relevant compounds or ones that are chemically similar on a molar or molal concentration basis. Account can also be taken of the proportion of monovalent- to divalent-cation salts.

A balance sheet of this sort has been drawn up in Table 3.14, in which the theoretical contribution of the measured solutes to leaf-cell sap Ψ_s is compared on summation with the values of Ψ_s determined cryoscopically; this proportion is expressed as a percentage relative to the mean, values of leaf Ψ_s recorded in all the experiments combined. The data are derived from the results in Tables 3.7 and 3.12. Non-reducing sugars were quantified as sucrose equivalents, the values being 113 mol m^{-3} for NO_3^- -N and 311 mol m^{-3} for NH_4^+ -N leaf-cell sap; reducing sugars were estimated as glucose and fructose equivalents, the levels being 174 and 475 mol m^{-3} respectively. The concentration of free NH_4^+ was obtained in the course of the amino-acid analyses: this was 0.49 mol m^{-3} in the NO_3^- -N and 0.97 mol m^{-3} in the NH_4^+ -N

Table 3.13 Summary of contribution of free amino acids and amides to the nitrogen and charge balance of leaves from plants grown on NO_3^- -N or NH_4^+ -N solution. Data are derived from Table 3.12.

	NO_3^- -N leaves	NH_4^+ -N leaves
concentration of amino acids and amides / mol m^{-3}	9.35	58.55
concentration of organic nitrogen / mol m^{-3}	11.62	147.30
approximate net charge / mol m^{-3} equivalents	-2.1	+21.3

Table 3.14 Approximate contribution of the major solutes to Ψ_s of leaf-cell sap for plants grown on NO_3^- -N or NH_4^+ -N solution. †Ions are considered as salts on basis of total +ve charge contributed by monovalent and divalent cations and amino acids.

	approximate contribution to Ψ_s / MPa	
	NO_3^- -N leaves	NH_4^+ -N leaves
non-reducing sugars	-0.04	-0.09
reducing sugars	-0.05	-0.13
ions†		
$\text{K}^+ + \text{Na}^+$	-0.74	-0.84
$\text{Mg}^{2+} + \text{Ca}^{2+}$	-0.45	-0.39
<i>amino acids</i> (+ve) + NH_4^+	~ 0	0.08
uncharged amino acids + amides	-0.03	-0.09
TOTAL	-1.31	-1.62
∴ approximate leaf-cell sap Ψ_s accounted for	89%	96%

leaf-cell sap. The values in Table 3.14 show that about 90% of the osmotically active solutes in the NO_3^- -N leaves were ionic, whereas about 80% were ionic in the NH_4^+ -N leaves. This difference was due partly to the lower concentration of sugars in the NO_3^- -N leaves, and to the lower contribution in this case from the uncharged amino acids plus amides. Positively charged amino acids plus NH_4^+ also made a significant osmotic contribution in the NH_4^+ -N leaves. For the leaves from both groups of plants, the Ψ_s accounted for by summation was around 90% relative to the values given in Table 3.4. This demonstrates that the solutes of osmotic importance in the leaves of these plants had been largely identified.

3.4 Xylem and phloem transport during growth on NO_3^- -N and NH_4^+ -N solution

From these results on the biochemical basis of solute accumulation in the leaves, it appears that the contrasting patterns of net OH^- and H^+ excretion from the roots observed in the two groups of plants were symptomatic of substantial differences in whole-plant metabolism. In NO_3^- -N plants, the cation content of the leaf-cell sap is largely balanced by malate, whereas in NH_4^+ -N plants the level of malate is very low and inorganic anions accumulate to almost an equivalent extent. The free nitrogen content of the leaves also shows significant quantitative differences in the two instances. The main purpose of this section is to determine how these differences are related to the biochemical characteristics of the long-distance transport pathways.

Xylem and phloem sap was collected for analysis by the standard methods described in section 2.3. The experiment was performed three times, but as with the leaf analyses the results are not all referable to the same experiment. The volumes of sap available for analysis were in many cases small, but comparison of the values obtained showed that variation between experiments was within acceptable limits. The NH_4^+ -N plants in particular tended to yield only small quantities of sap.

The results of the investigation of xylem and phloem water relations are given in Table 3.15. For both the xylem and the phloem, the rates of sap exudation from NH_4^+ -N plants were considerably slower than those from NO_3^- -N plants. The figures for the xylem represent net rates of exudation over a period of 10 h, the value for the NO_3^- -N plant being approximately an order of magnitude higher than that for the NH_4^+ -N plant. Rates of exudation for the phloem are given as initial rates

Table 3.15 Characteristics of xylem and phloem water relations of eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Sap Ψ_s was determined cyroscopically, sap Ψ was estimated from Table 3.4 (using assumptions described in text), and sap Ψ_p was computed from the relation, $\Psi = \Psi - \Psi_s$. Figures are means \pm S.E. for 3 plants or values from pooled samples from the same experiment.

	approximate initial rate of sap exudation / $10^{-9}\text{m}^3 \text{min}^{-1}$	sap Ψ_s / MPa OBSERVED	sap Ψ / MPa ESTIMATED	sap Ψ_p / MPa COMPUTED
<i>xylem</i>				
NO_3^- -N plants	~ 3.0	-0.17 ± 0.04	-0.41	-0.24^\dagger
NH_4^+ -N plants	~ 0.3	-0.23 ± 0.06	-0.50	-0.27^\dagger
P	-	N.S.	-	-
<i>phloem</i>				
NO_3^- -N plants	3.5 ± 0.3	-1.52 ± 0.06	-0.41^\S	$+1.11$
NH_4^+ -N plants	1.0 ± 0.5	-1.61	-0.50^\S	$+1.11$
P	< 0.01	N.S.	-	-

† these are not pressure-bomb measurements, but are computed values only

§ values assume xylem-sap $\Psi =$ phloem-sap Ψ

from incisions of equivalent size. They probably do not give a quantitatively accurate representation of the differences between the two groups of plants because exudation was always sustained for longer periods, and at rates diminishing more slowly, in the NO_3^- -N plants. The observed values of Ψ_s were determined cryoscopically: for the xylem sap they were not significantly different between the NO_3^- -N and NH_4^+ -N plants, and this was also true for the phloem-sap Ψ_s . These values for xylem Ψ_s are likely to be an over-estimate of the solute content of the xylem, since the long collection periods will have involved greater evaporative loss during exudation. On a relative level, however, the differences between the two groups of plants were not significant.

In Table 3.15, computed values are also given of the approximate sap Ψ_p . The assumptions made for comparative purposes were that xylem sap Ψ was about 0.10 MPa higher (i.e. less negative) than leaf Ψ as given in Table 3.4, and that the phloem sap Ψ was in equilibrium with that in the xylem. Gross leaf Ψ is invariably lower than xylem Ψ because of the influence of transpirational water loss from the leaf surface, and there is evidence in Ricinus that the difference is often of the order of 0.1 MPa (Dodoo, 1978; see section 5.2). Further, the rate of phloem-sap exudation is influenced by xylem Ψ in a manner suggesting that the effective L_p of the interconnecting pathway is reasonably low (Weatherley et al., 1959; Hall and Milburn, 1973). Sap Ψ_p was then computed from the relation $\Psi = \Psi_s + \Psi_p$ (although xylem Ψ_p was also determined in other experiments using the pressure bomb). The values given in Table 3.15 show that phloem Ψ_p was about 1.1 MPa in both NO_3^- -N and NH_4^+ -N plants. Although they cannot be taken as precise estimates, the values nevertheless indicate that phloem turgor was similar in the two instances.

The levels of inorganic cations in the xylem and phloem sap from these plants are shown in Table 3.16, the figures for Mg^{2+} and Ca^{2+} again being expressed in charge equivalents. In the xylem sap, K^+ was present at a significantly higher level in the NO_3^- -N plants compared with the NH_4^+ -N plants, but there were no differences in the concentrations of the other cations, nor in the total cation content. There appeared also to be no significant difference in the level of any of the four cations, or in the total content, between the NO_3^- -N and NH_4^+ -N plants. The figures for NH_4^+ -N phloem sap are given only as mean values for pooled samples from three plants because of the small volumes of sap

Table 3.16 Levels of inorganic cations in xylem and phloem sap from eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are means \pm S.E. for samples from 3 plants or values for pooled samples from the same experiment.

	K^+ / mol m ⁻³	Na^+ / mol m ⁻³	Mg^{2+} / mol m ⁻³ equivalents	Ca^{2+} / mol m ⁻³ equivalents	TOTAL / mol m ⁻³ equivalents
<i>xylem sap</i>					
NO_3^- -N plants	15.3 \pm 0.1	3.4 \pm 0.3	4.4	4.6 \pm 2.0	27.7 \pm 1.8
NH_4^+ -N plants	10.3 \pm 0.7	4.6 \pm 1.0	6.4 \pm 0.7	4.2 \pm 1.0	25.4 \pm 0.3
P	< 0.01	N.S.	N.S.	N.S.	N.S.
<i>phloem sap</i>					
NO_3^- -N plants	65.0 \pm 2.6	7.8 \pm 0.7	10.3 \pm 1.2	4.5 \pm 1.3	88.8 \pm 3.3
NH_4^+ -N plants	52.0	7.4	6.6	4.0	71.2
P	N.S.	N.S.	N.S.	N.S.	N.S.

that were obtained from bark incisions. The $K^+;Na^+$ ratio in sap from the NO_3^- -N plants was slightly higher than in that from NH_4^+ -N plants for both the xylem (4.5 versus 2.2, respectively) and phloem (8.3 versus 7.0, respectively). The ratio of monovalent to divalent cations in terms of charge equivalents was higher for the phloem sap (4.9 in the NO_3^- -N plants against 5.6 in the NH_4^+ -N plants) than the xylem sap (2.1 in the NO_3^- -N plants against 1.4 in the NH_4^+ -N plants). Phloem sap also contained about three times the concentration of inorganic-cation charge equivalents as the xylem in both types of plants.

The anionic contribution to the charge balance of the xylem and phloem sap can be considered in the same way as for the leaf tissue. Table 3.17 presents the results of the enzymatic analysis of malate levels in $mol\ m^{-3}$. Measurements were only made on pooled samples, except for the NO_3^- -N phloem sap, but there appeared to be more malate in xylem and phloem sap from the NO_3^- -N plants compared with the NH_4^+ -N plants, in which the levels were extremely low. To determine whether other organic acids made significant contributions to the charge balance in phloem sap from the NO_3^- -N plants, the organic-acid fraction was separated by ion-exchange chromatography. The fraction was reduced to dryness and washed a total of three times before titration to pH 7.00 against standard NaOH solution to obtain an estimate of total titratable acidity. Pure malic acid was used as an internal standard in duplicate samples so that values could be corrected for the efficiency of recovery. The values recorded were in the range 15.7 - 24.7 $mol\ m^{-3}$ charge equivalents. Analysis of the organic-acid fraction by TLC proved unsatisfactory because of the low concentrations of acids in the samples. However, separation of TMSi derivatives of the acids on 1.5% (w/w) QF-1 by GLC indicated that the charge equivalents in addition to malate (that is, of the order of 20 - (2 x 5) = 10 $mol\ m^{-3}$ equivalents) were largely contributed by citric and isocitric acids.

Of the inorganic anions, the levels of chloride found in the xylem and phloem sap are shown in Table 3.18. Measurements were not made on xylem samples from the NO_3^- -N plants, and the value of 1.0 $mol\ m^{-3}$ is taken from Bowling, Macklon and Spanswick (1966); these authors grew Ricinus on a NO_3^- -N nutrient solution containing a slightly higher level of Cl^- (0.13 $mol\ m^{-3}$). The results suggest that Cl^- occurs at notably higher levels in sap from the NH_4^+ -N plants than the NO_3^- -N plants. Inorganic phosphate levels were also measured in phloem sap from NO_3^- -N plants and were recorded at a concentration of $7.6 \pm 0.3\ mol\ m^{-3}$ for samples from two plants.

Table 3.17 Levels of malate in xylem and phloem sap from eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are values for pooled samples or means \pm S.E. for samples from 2 plants in the same experiment.

	malate concentration / mol m ⁻³	
	NO_3^- -N plants	NH_4^+ -N plants
xylem sap	6.0	0.2
phloem sap	5.2 \pm 0.7	1.2

Table 3.18 Levels of chloride in xylem and phloem sap from eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are values for pooled samples or means \pm S.E. for samples from 2 plants in the same experiment. The value marked † is taken from Bowling, Macklon and Spanswick (1966).

	chloride concentration / mol m ⁻³	
	NO_3^- -N plants	NH_4^+ -N plants
xylem sap	1.0 [†]	18.4
phloem sap	8.9 \pm 3.4	33.5

Previous work on Ricinus phloem sap (Hall and Baker, 1972; Mengel and Haeder, 1977) had demonstrated that the amino acids, as well as the organic acids, might contribute a net negative charge to the charge balance of the sap. Tables 3.19 and 3.20 present a complete analysis of the levels of free amino acids and their amides present in xylem and phloem sap, respectively, for both NO_3^- -N and NH_4^+ -N plants. Measurements were made on pooled samples from two or three plants in each case, the analyses being carried out in five separate experiments (NO_3^- -N xylem and phloem), two experiments (NH_4^+ -N xylem), or three experiments (NH_4^+ -N phloem). The results thus include the element of variation arising from differences between plants grown in separate batches.

The data in Table 3.19 show that quantitatively by far the most important amino acid or amide in the xylem sap of both NO_3^- -N and NH_4^+ -N plant was glutamine. This accounted for about 70% of the total amino acids and amides in both types; the glutamine concentration in the NH_4^+ -N xylem sap (15.7 mol m^{-3}) was, however, over three times the level in the NO_3^- -N xylem. Asparagine constituted over 7% of the total in the NH_4^+ -N xylem sap, and serine also was present in significantly larger amounts ($P < 0.01$) than in the NO_3^- -N sap. The total concentration of amino acids plus amides was 3.4 times greater in the xylem sap from the NH_4^+ -N plants.

Table 3.20 presents values for the levels of free amino acids and amides in the phloem sap from the two types of plants. As in the xylem, glutamine is the predominant compound in phloem sap of NO_3^- -N plants, but in the phloem it occurred at much higher levels (nearly 70 mol m^{-3}). The other compounds of quantitative importance in the NO_3^- -N phloem were glutamic acid, aspartic acid and serine. In phloem sap from NH_4^+ -N plants, glutamine was also present in substantial amounts (30 mol m^{-3}), but quantitatively the most important species was serine: this was present at about 60 mol m^{-3} and accounted for nearly half the total concentration of amino acids and proteins. Asparagine was also found at notably higher levels than in the NO_3^- -N phloem sap. Despite these changes in composition, the total level of amino acids and amides in the NO_3^- -N and NH_4^+ -N phloem differed by only 11%.

Examples of the traces obtained from the amino-acid autoanalyzers by chromatographic separation of the amino acids and amides in NO_3^- -N and NH_4^+ -N phloem sap are shown in Figs. 3.6 and 3.7. In Fig. 3.6,

Table 3.19 Levels of free amino acids and amides in xylem sap from eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are for samples from 2 to 5 experiments expressed as means \pm S.E. (mol m^{-3}) or relative amount (%).

	NO_3^- -N xylem		NH_4^+ -N xylem	
	mol m^{-3}	%	mol m^{-3}	%
Gln	4.75 \pm 0.19	71.2	15.72 \pm 0.93	68.9
Glu	0.07 \pm 0.03	1.0	0.13 \pm 0.02	0.6
Asn	0.19 \pm 0.09	2.8	1.64 \pm 0.23	7.2
Asp	0.06 \pm 0.03	0.8	0.22 \pm 0.09	1.0
Ser	0.03 \pm 0.01	0.4	1.23 \pm 0.18	5.4
Ala	0.04 \pm 0.03	0.6	0.45 \pm 0.25	2.0
Thr	0.19 \pm 0.07	0.3	0.54 \pm 0.13	2.4
Pro	0.04 \pm 0.03	0.5	0.20 \pm 0.10	0.9
Lys	0.13 \pm 0.05	2.0	0.14 \pm 0.04	0.6
Val	0.33 \pm 0.16	5.0	0.97 \pm 0.37	4.3
Leu	0.17 \pm 0.08	2.5	0.33 \pm 0.13	1.5
Phe	0.04 \pm 0.01	0.6	0.08 \pm 0.05	0.3
Ile	0.22 \pm 0.11	3.3	0.49 \pm 0.09	2.2
Arg	0.14 \pm 0.04	2.1	0.07 \pm 0.02	0.3
Gly	0.01 \pm 0.01	0.2	0.07 \pm 0.03	0.3
His	0.20 \pm 0.07	3.0	0.42 \pm 0.17	1.8
Tyr	0.04 \pm 0.02	0.5	0.08 \pm 0.03	0.3
Met	0.05 \pm 0.02	0.7	0.03 \pm 0.02	0.1
Cys	absent	-	0.02 \pm 0.01	0.1
TOTAL	6.68	100	22.82	100

Table 3.20 Levels of free amino acids and amides in phloem sap from eight-week-old plants grown on NO_3^- -N or NH_4^+ -N solution. Figures are for samples from 3 to 5 experiments expressed as means \pm S.E. (mol m^{-3}) or relative amounts (%).

	NO_3^- -N phloem		NH_4^+ -N phloem	
	mol m^{-3}	%	mol m^{-3}	%
Gln	69.47 \pm 12.53	61.7	30.09 \pm 4.61	24.0
Glu	14.58 \pm 2.29	12.9	4.87 \pm 0.75	3.9
Asn	0.35 \pm 0.25	0.3	10.01 \pm 2.88	8.0
Asp	10.38 \pm 1.94	9.2	2.81 \pm 0.84	2.2
Ser	5.36 \pm 0.39	4.8	59.03 \pm 19.36	47.1
Ala	1.90 \pm 0.29	1.7	2.49 \pm 0.77	2.0
Thr	2.83 \pm 0.14	2.5	3.05 \pm 0.54	2.4
Pro	0.40 \pm 0.25	0.4	1.52 \pm 0.45	1.2
Lys	0.86 \pm 0.35	0.8	1.05 \pm 0.15	0.8
Val	2.01 \pm 0.68	1.8	3.84 \pm 1.3	3.1
Leu	0.65 \pm 0.21	0.6	0.89 \pm 0.29	0.7
Phe	0.61 \pm 0.24	0.5	0.85 \pm 0.32	0.7
Ile	1.16 \pm 0.61	1.0	1.84 \pm 0.67	1.5
Arg	0.35 \pm 0.04	0.3	0.62 \pm 0.14	0.5
Gly	0.97 \pm 0.37	0.9	1.14 \pm 0.48	0.9
His	0.63 \pm 0.21	0.6	0.94 \pm 0.27	0.8
Tyr	0.02 \pm 0.01	0.01	0.04 \pm 0.01	0.03
Met	0.15 \pm 0.05	0.1	0.13 \pm 0.03	0.1
Cys	absent	-	0.06 \pm 0.05	0.05
TOTAL	112.68	100	125.28	100

Fig. 3.6 Traces from Jeol autoanalyzer showing separation of amino acids in $10 \times 10^{-9} \text{m}^3$ samples of phloem sap from plants grown on (a) NO_3^- -N solution, and (b) NH_4^+ -N solution. Abbreviations as in text.

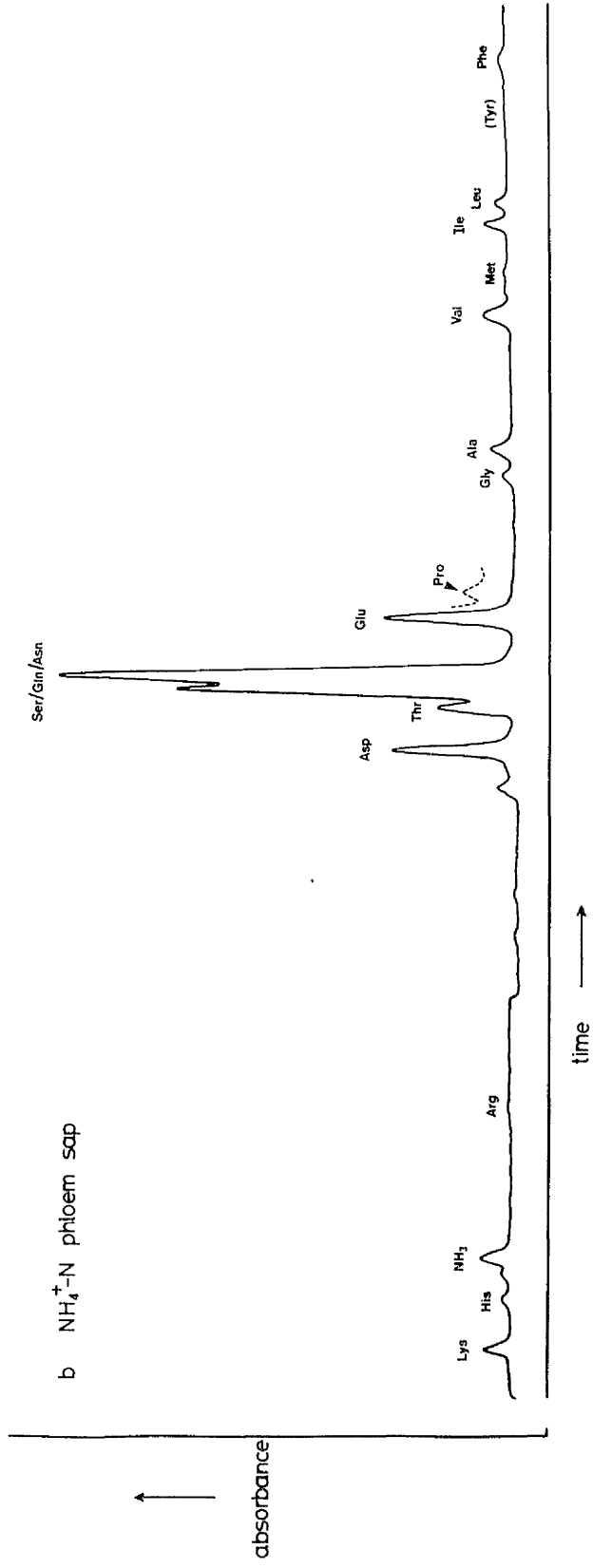
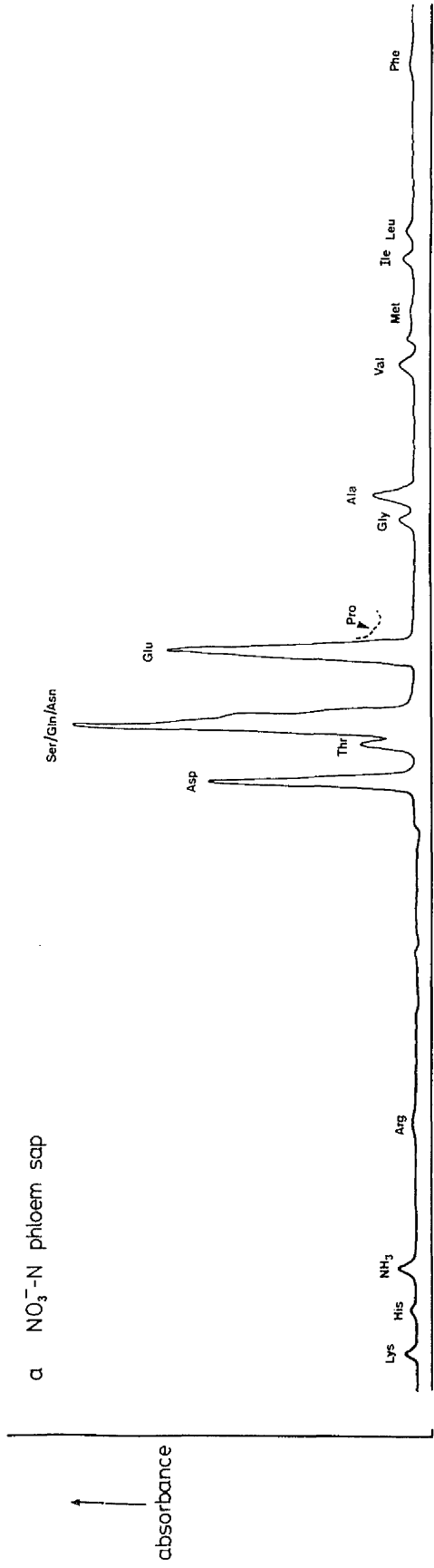
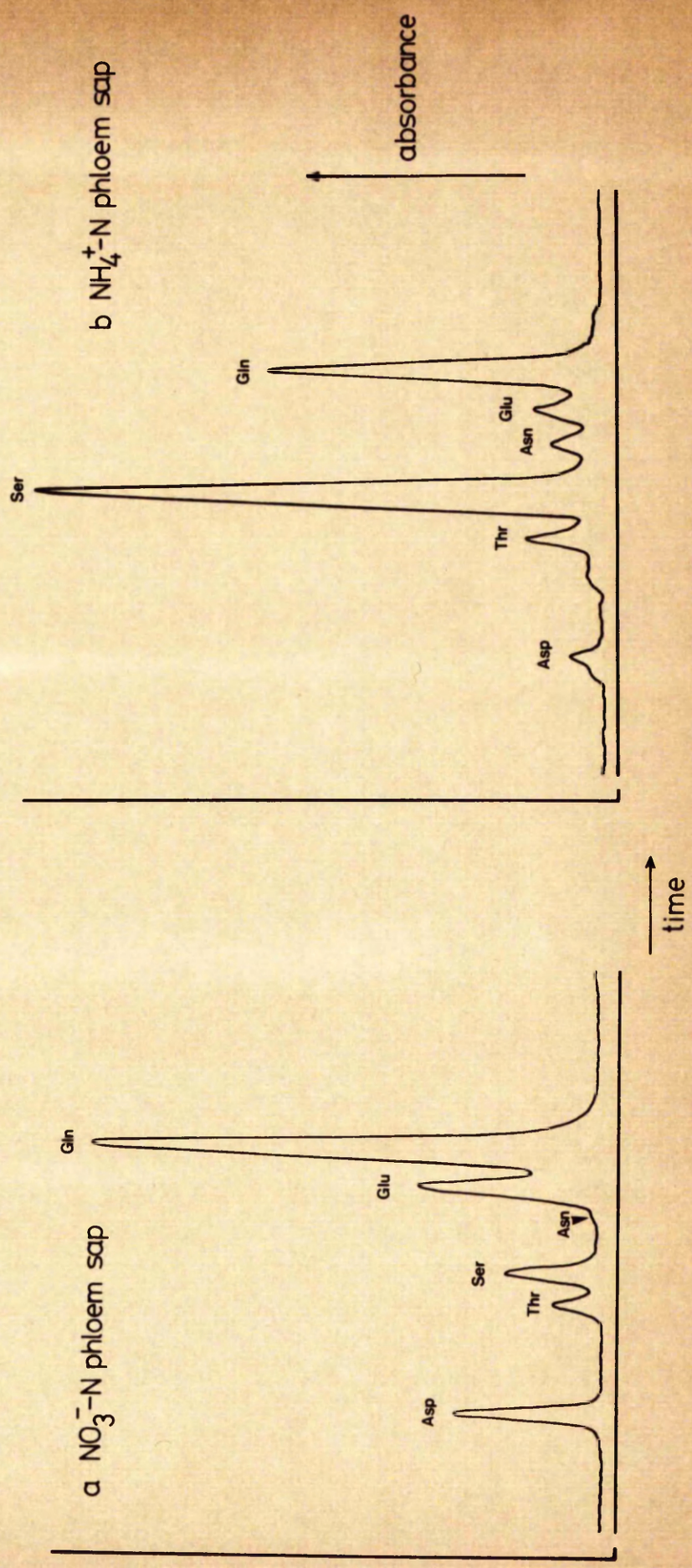


Fig. 3.7 Traces from Locarte autoanalyzer showing separation of amino acids and amides in $10 \times 10^{-9} \text{m}^3$ samples of phloem sap from plants grown on (a) NO_3^- -N solution, and (b) NH_4^+ -N solution. Abbreviations as in text.



representative traces from the Jeol autoanalyzer are given for $10 \times 10^{-9} \text{m}^3$ samples of both $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ sap samples. The instrument response was calibrated for each series of analyses with authentic standards, but since these examples are from different runs the peak areas are not directly comparable. However, the traces show that the relative proportions of the amino acids were similar in the $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ phloem sap. In this system, the peaks for serine, asparagine and glutamine tended to overlap, and Fig. 3.7 shows similar examples of the separation of these compounds achieved on the Locarte autoanalyzer using the Li citrate buffer system. The high proportion of serine in the phloem sap from $\text{NH}_4^+ \text{-N}$ plants is clearly illustrated.

The contribution made by these amino acids and amides to the total N content and the charge balance of the xylem and phloem sap is shown in Tables 3.21 and 3.22. The values have been derived in the same way as those for the leaf-cell sap, except that the positive charge contribution from histidine has been considered negligible in the phloem; this is because the pH values of the sap (given later in Table 3.27) are very close to the pI for histidine.

Table 3.21 shows that the proportionate difference in the free organic N content of the $\text{NO}_3^- \text{-N}$ versus $\text{NH}_4^+ \text{-N}$ xylem sap was similar to the difference expressed as solute concentration. The value was nearly double that of the amino acid plus amide level in both instances because of the predominance of glutamine, possessing two N per molecule. Further, this organic N fraction from the sap of both the $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ plants carried a very small net positive charge. This Table also gives values for the inorganic N fraction in the sap. The free NH_4^+ concentration was obtained from the amino acid analyses, and was not significantly different between the $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ xylem sap. Nitrate was determined by the nitrite-reduction method: it was present at relatively high levels in the $\text{NO}_3^- \text{-N}$ xylem but was absent ($< 0.1 \text{ mol m}^{-3}$) in the $\text{NH}_4^+ \text{-N}$ xylem. The total free organic and inorganic N content of the $\text{NH}_4^+ \text{-N}$ xylem sap was thus only 1.2 times higher than that of the $\text{NO}_3^- \text{-N}$ xylem, the former carrying a total small net positive charge, the latter a substantial negative charge. These figures are relevant to the metabolic relationships between the N content of the shoot and the N supply from the roots in the xylem.

The free organic and inorganic N content of the phloem is presented in the same way in Table 3.22. The figures for the organic N fraction show that the content of the phloem sap was similar in the $\text{NO}_3^- \text{-N}$ and

Table 3.21 Organic and inorganic nitrogen content, and their contribution to the charge balance, of xylem sap from plants grown on NO_3^- -N or NH_4^+ -N solution. Data for amino acids and amides are derived from Table 3.19. Figures for inorganic N are means \pm S.E. for samples from 3 plants

	NO_3^- -N xylem		NH_4^+ -N xylem	
	concentration/ mol m^{-3}	approximate net charge/ mol m^{-3} equivalents	concentration/ mol m^{-3}	approximate net charge/ mol m^{-3} equivalents
ORGANIC N				
amino acids + amides	6.68	+ 0.34	22.82	+ 0.28
equivalent organic N	12.57		41.37	
INORGANIC N				
NH_4^+	1.16 \pm 0.18	+ 1.16	1.34 \pm 0.35	+ 1.34
NO_3^-	22.3 \pm 3.0	-22.3	0	0
TOTAL N	36.0	-20.8	42.7	+ 1.6

Table 3.22 Organic and inorganic nitrogen content, and their contribution to the charge balance, of phloem sap from plants grown on NO_3^- -N or NH_4^+ -N solution. Data for amino acids and amides are derived from Table 3.20. Figures for inorganic N are means \pm S.E. for samples from 2 plants or values for pooled samples from the same experiment.

	NO_3^- -N phloem		NH_4^+ -N phloem	
	concentration/ mol m^{-3}	approximate net charge/ mol m^{-3} equivalents	concentration/ mol m^{-3}	approximate net charge/ mol m^{-3} equivalents
ORGANIC N				
amino acids + amides	112.7	- 23.8	125.3	- 6.0
equivalent organic N	185.7		170.2	
INORGANIC N				
NH_4^+	1.51 ± 1.07	+ 1.51	2.62 ± 0.23	+ 2.62
NO_3^-	3.0	- 3.0	0	0
TOTAL N	190.2	-25.3	172.8	- 3.4

NH_4^+ -N plants when expressed as concentration of organic N, although the amino acids and amides possessed an appreciably larger net negative charge in that of the NO_3^- -N plants. Inorganic N was present only at low levels, and the concentration of free NH_4^+ was not significantly different in the two instances; nitrate was present at 3.0 mol m^{-3} in the NO_3^- -N phloem but was absent from the NH_4^+ -N phloem. Thus, the total free N content of phloem sap from the two groups of plants was rather similar, being only 9% lower in that from the NH_4^+ -N plants. The total N fraction from the NO_3^- -N phloem, however, carried a considerably larger net negative charge.

For the purpose of assessing approximately to what extent the solute content of the xylem and phloem sap had been accounted for, the Ψ_s contribution of the solutes can be calculated as before. This is again determined on the basis of the levels of uncharged solutes plus those that are positively charged, assuming the latter to be balanced by negative charge equivalents. A balance sheet for solutes in the xylem sap is given in Table 3.23. Sugars were never recorded in more than trace amounts from xylem sap of NO_3^- -N or NH_4^+ -N plants, and their contribution to the total Ψ_s was assumed to be negligible. The majority of the solutes in the xylem were therefore ionic, although in the NH_4^+ -N plants nearly 30% of the calculated total Ψ_s was made up by the uncharged amino acids and amides. In terms of the Ψ_s values given in Table 3.15, over 90% of the observed xylem Ψ_s was accounted for by these solutes in both the NO_3^- -N and NH_4^+ -N plants.

Table 3.24 gives the equivalent balance sheet for the solutes quantified in the phloem sap. This includes an estimate of the Ψ_s contributions from sucrose, which was found at $287 \pm 23 \text{ mol m}^{-3}$ in the NO_3^- -N phloem sap and $276 \pm 24 \text{ mol m}^{-3}$ in the NH_4^+ -N sap, the difference between the means not being significant. Reducing sugars were detected only in trace amounts and their contribution to the total Ψ_s was taken to be negligible. In the phloem sap from the NO_3^- -N plants, 70% of the calculated total Ψ_s was made up by uncharged molecules, and in the NH_4^+ -N sap the equivalent figure was 75%. The contribution from the uncharged amino acids and amides was slightly greater in the NH_4^+ -N phloem sap, but the total Ψ_s estimated by summation of values for all the solutes was almost identical for the sap from NO_3^- -N and NH_4^+ -N plants. These solutes accounted for around 90% of the observed Ψ_s on the basis of the values given in Table 3.15.

Table 3.23 Approximate contribution of the major solutes to Ψ_s of xylem sap for plants grown on NO_3^- -N or NH_4^+ -N solution. [†]Ions are considered as salts on basis of total +ve charge contributed by monovalent and divalent cations and amino acids.

	approximate contribution to Ψ_s / MPa	
	NO_3^- -N xylem	NH_4^+ -N xylem
sugars	trace	trace
ions [†]		
$K^+ + Na^+$	-0.09	-0.07
$Mg^{2+} + Ca^{2+}$	-0.03	-0.06
<i>amino acids</i> (+ve) + NH_4^+	-0.02	-0.02
uncharged amino acids + amides	-0.02	-0.06
TOTAL	-0.16	-0.21
.*. approximate xylem sap Ψ_s accounted for	94%	91%

Table 3.24 Approximate contribution of the major solutes to Ψ_s of phloem sap for plants grown on NO_3^- -N or NH_4^+ -N solution. † Ions are considered as salts on basis of total +ve charge contributed by monovalent and divalent cations and amino acids.

	approximate contribution to Ψ_s / MPa	
	NO_3^- -N phloem	NH_4^+ -N phloem
sucrose	-0.79	-0.76
reducing sugars	trace	trace
ions †		
$\text{K}^+ + \text{Na}^+$	-0.35	-0.28
$\text{Mg}^{2+} + \text{Ca}^{2+}$	-0.05	-0.04
amino acids (+ve) + NH_4^+	-0.03	-0.04
uncharged amino acids + amides	-0.23	-0.31
TOTAL	-1.45	-1.43
∴ approximate phloem sap Ψ_s accounted for	95%	89%

3,5 Discussion

Extensive studies have been made on aspects of plant growth using NO_3^- and NH_4^+ as alternative sources of inorganic nitrogen. Under equivalent conditions, dry-matter yield in most species seems to be somewhat greater on NO_3^- -N as the N-source compared with NH_4^+ -N (e.g. Naftel, 1931; Barker, Maynard and Lachman, 1966; Kirkby, 1968; Dijkshoorn, 1969; Breteler, 1973a; Raven and Smith, 1976a). It is particularly important, however, that comparisons between species take into account genetically determined preferences in N-source, since NO_3^- -N is actually toxic to some ecotypes (see Foy, Chaney and White, 1978). Another consideration is that the responses to different nutritional regimes provided by balanced water-culture media under controlled environmental conditions may not be the same as in the field. In fact, both NO_3^- and NH_4^+ are usually present to some degree in the soil (see Hewitt, 1966; Raven and Smith, 1976a), and a number of investigations have shown that plant yield may be greater when a combined N-source is provided (Vickery, Pucher, Wakeman and Leavenworth, 1940; Weir, Paulson and Lorenz, 1972; Cox and Reisenauer, 1973; Warncke and Barber, 1973). The use of culture solutions containing solely NO_3^- -N or NH_4^+ -N is therefore likely to elicit differences in metabolism that represent two extremes of the range of types found under field conditions.

From the data presented on yield in terms of leaf area (Table 3.2), fresh weight (Fig. 3.1) and dry weight (Fig. 3.3), it is clear that growth in Ricinus was better on the NO_3^- -N compared with the NH_4^+ -N source. There are two aspects of the yields that must be considered, however, one being the significance of the absolute yields, the other the relative nature of growth on these sources. Firstly, the absolute fresh-weight and dry-weight yields must be related to the level of nutrients supplied in the culture solutions. The Long Ashton solutions used in these experiments provided the major nutrients at levels comparable with those in other commonly used media, and the concentrations of K^+ , Mg^{2+} , Ca^{2+} and PO_4^{3-} in the NO_3^- -N and NH_4^+ -N solutions were very similar. Although the NO_3^- concentration was 50% higher than that of NH_4^+ , there is evidence that NO_3^- levels in this part of the concentration range (6 to 12 mol m^{-3}) have little effect on rates of uptake, growth, yield or aspects of metabolism (Kirkby and Knight, 1977; Clement, Hopper and Jones, 1978). The metabolic implications of the differences in anion content of the NO_3^- -N and NH_4^+ -N solutions

are considered later, but the similarities in the levels of the major nutrient ions suggest that the absolute yields of these plants may be related to comparisons made for other species.

Secondly, in relative terms, the fresh-weight yield of the NH_4^+ -N plants was only 27% of that of the NO_3^- -N plants after 57 days, the difference between the two groups of plants having increased during development (Fig. 3.1). One factor that will have influenced growth of the NH_4^+ -N plants was the decrease in pH of the root medium, since low pH can depress NH_4^+ uptake (Naftel, 1931; Stahl and Shive, 1933) and reduce root growth (Sheat, Fletcher and Street, 1959). This pattern of pH changes in the root medium with an NO_3^- -N versus NH_4^+ -N source has been observed in many other species (see Coïc *et al.*, 1961; Kirkby and Mengel, 1967; Kirkby and Hughes, 1970; Breteler, 1973a; Raven and Smith, 1976a). For older plants, the pH of the NH_4^+ -N medium between changes of solution every 3 days became as low as 3.5 (Table 3.5), and after eight weeks the roots of the NH_4^+ -N plants were in visibly poorer condition than those of the NO_3^- -N plants. Such problems can largely be overcome by the use of autotitrators to maintain a constant solution pH (although this leads to increases in the levels in the solution of the elements contained in the acid or alkali), or preferably of flowing nutrient solution. For example, yield in some species on NH_4^+ -N solution can almost equal that on NO_3^- -N if the pH is maintained more or less steady (Wander and Sites, 1956; Barker, Volk and Jackson, 1966; Maynard and Barker, 1969; Breteler, 1973a; Cox and Reisenauer, 1973). There is no doubt, however, that marked differences in yield can be found even with a constant root-medium pH (Kirkby, 1969; Pill and Lambeth, 1977), and indeed the percentage reduction in yield of Lycopersicon on an NH_4^+ -N medium (Kirkby, 1969) was close to that observed here in Ricinus. Pill and Lambeth (1977) have also shown that the pH of the root medium did not affect the concentration of ions (except NH_4^+) in the shoots of Lycopersicon. Of course, there may in addition have been changes in the rate of nutrient uptake with increasing pH (i.e. in the root medium of the NO_3^- -N plants), for the solubility and uptake of some of the micronutrient cations varies with pH (Gerloff, 1963; Hewitt, 1966).

Despite these substantial differences in yield, the changes in relative growth rate of the NO_3^- -N and NH_4^+ -N plants showed similar trends during ontogeny (Fig. 3.2). Furthermore, the proportionate differences in R_W between the two groups of plants were smaller than

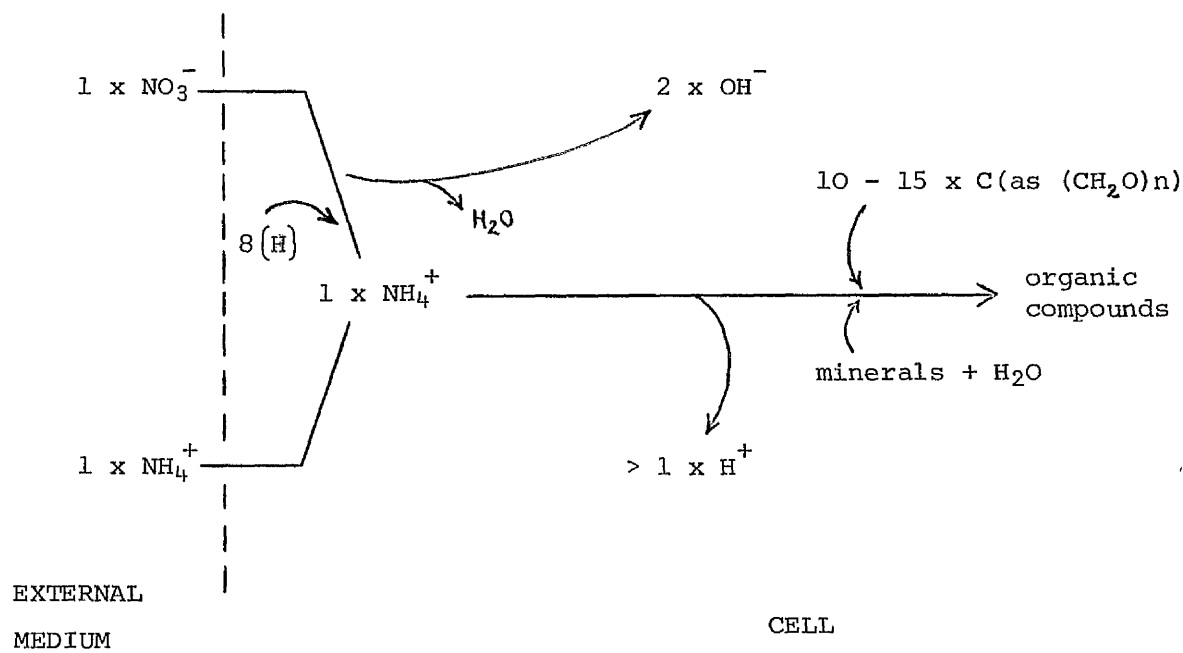
those based on fresh-weight or dry-weight yields. The similarities in the water relations of shoots (Table 3,4) also suggested that the growth characteristics of the NO_3^- -N and NH_4^+ -N plants were directly comparable. Because the trends in R_W indicated that the plants were at ontogenetically equivalent stages, the leaves used in these studies were selected as being physiologically similar. It has been shown in Ricinus, in fact, that differences between leaf Ψ for more or less 'mature' alternate leaves near to their maximum area on the same plant are relatively small (< 0.05 MPa) (Dodoo, 1978). The pressure-chamber and cryoscopic techniques used here in studying the shoot water relations are generally accepted as providing reasonable estimates of leaf Ψ and leaf Ψ_s (Barrs, 1968; Slavík, 1974; Ritchie and Hinckley, 1975). Consideration is given later on to the sources of error involved in these methods and the significance of the values obtained (section 5.4). For the present purposes, it may be noted that no difference was found in leaf Ψ_p between the NO_3^- -N and NH_4^+ -N plants. The significantly lower leaf Ψ observed with NH_4^+ -N nutrition has also been found in Lycopersicon, and may be attributable to increased root resistance to water uptake in these plants (Quebedeaux and Ozbun, 1973; Pill and Lambeth, 1977).

These results suggest that, despite the large differences in yields between the plants, physiological comparisons of the NO_3^- -N and NH_4^+ -N plants are nevertheless legitimate. As a starting point for discussing the comparative aspects of whole-plant metabolism, it is important to consider the nature of the ion uptake processes in the roots. Therefore, I shall deal firstly with the significance of the observed patterns of net H^+ and OH^- excretion from the roots, before turning to the general question of solute accumulation in the shoot.

The pattern of net H^+ efflux from roots of plants grown on the NH_4^+ -N medium and net OH^- efflux from roots in NO_3^- -N solution (Fig. 3.3) has been noted in other species for which data are available (refs. given on p. 81). There are two questions to be considered: one concerns the direction in which net H^+ / OH^- movement occurs, the other is the source of the H^+ and OH^- ions. As regards the flux across the root, it follows that there is either net H^+ efflux or OH^- influx with the NH_4^+ -N roots, and similarly net H^+ influx or OH^- efflux with the NO_3^- -N roots; in terms of pH changes in the solutions, these alternatives are operationally indistinguishable. However, the nature of the net fluxes is mechanistically of some importance because of the

consequences for the solute balance of the whole plant. In NO_3^- -N solution, for example, influx of all the NO_3^- as a neutral salt would probably give rise to a much higher level of inorganic cations in the plant than is actually observed (Cram, 1976a, b; cf. Raven and Smith, 1976a). Thus, it seems likely that part of the NO_3^- uptake is balanced by OH^- efflux in exchange (and similarly for NH_4^+ uptake/ H^+ efflux), this giving rise to the observed pH changes in the medium. Most workers have, in fact, considered ion uptake to be associated with net H^+ (H_3O^+) or OH^- (HCO_3^-) movement out of the root tissue (e.g. Hoagland and Broyer, 1936; Arnon, 1939; Jacobson and Ordin, 1954; Cořic *et al.*, 1961; Poole and Poel, 1965; Kirkby and Hughes, 1970), although the converse view has been taken by van Tuil (1965) and Dijkshoorn, Lathwell and Wit (1968).

If the H^+ and OH^- are excreted from the plant tissue, the second question to consider is where the ions come from. This may be regarded as a consequence of the assimilation of inorganic N and its incorporation into cell constituents during growth. The reactions involved may be summarized in the following way (taken from Raven, 1977c):



In other words, with CO_2 or sugar as the C source for metabolism, cell growth involves the net production of more than one excess H^+ per N when NH_4^+ is the N-source, and of almost one excess OH^- per N when NO_3^- is the N-source (Raven and Smith, 1973; 1974). The production of H^+ and OH^- during N assimilation thus constitutes a potentially enormous

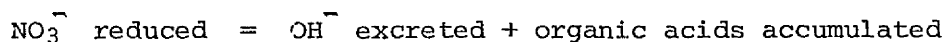
pH stress on the plant, and this excess H^+ or OH^- must largely be either physically removed from the cell or chemically neutralized (Rayen and Smith, 1974, 1976a).

It is therefore reasonable to assume that the H^+ and OH^- appearing in the root medium arises from that generated in N assimilation. The following points may be noted about the characteristics of net H^+ and OH^- excretion observed from the roots in Ricinus. Firstly, the equivalent rates of H^+ and OH^- excretion recorded were of the same order, that for the NO_3^- -N plant being only about 23% lower. The value for net H^+ excretion may, however, represent an underestimate if the rate of NH_4^+ uptake was lower than the equivalent rate of NO_3^- uptake, having been impaired by the lower pH of the root medium. Another complication is that ratio of $H^+ : OH^-$ excretion by equivalent NH_4^+ -N and NO_3^- -N plants can change during ontogeny (Breteler, 1973a). Secondly, the absolute rates of net H^+ and OH^- excretion recorded (Table 3.6) were higher than, but comparable with, the values observed in other species. In Hordeum, Pitman (1970) recorded net H^+ excretion in the range $0.05 - 0.60 \mu\text{mol (g FW root)}^{-1} \text{h}^{-1}$; Breteler (1973a) observed about $0.40 \mu\text{mol (g FW root)}^{-1} \text{h}^{-1}$ in Beta; and the equivalent rate in Ricinus (Table 3.6) averaged $1.32 \mu\text{mol (g FW root)}^{-1} \text{h}^{-1}$. And thirdly, the rates of net H^+ excretion from the roots of NH_4^+ -N plants over diurnal periods reached a maximum during the day and declined during the night. In the course of the dark period there was actually a slight net OH^- excretion, and this has been observed in grasses but was not found in Beta (Breteler, 1973a). The fall in rate of H^+ production in Beta is paralleled by a decrease in the soluble carbohydrate content of the NH_4^+ -N roots (Breteler, 1973a), and it is possible that this is a reflection of the general dependence of solute transport processes in the root on a continuing supply of photosynthetic products from the shoot (Bowling, 1968; Graham and Bowling, 1977). Although the rate of net OH^- excretion did not show a consistent correlation with photoperiod in Ricinus, there is nevertheless circumstantial evidence that NO_3^- uptake is also at least partially dependent on import of assimilates from the shoot (Pearson, 1974; Jackson, Kwik and Volk, 1976; Pearson and Steer, 1977). However, there are indications that NH_4^+ uptake may show a closer correlation with the current supply of carbon than does NO_3^- uptake (Michael, Martin and Owassia, 1970; Breteler, 1973b), and that during ontogeny net H^+ excretion may become more dependent and net OH^- excretion less dependent on the photoperiod (Breteler, 1973a). Whether this is specifically associated with the energetic requirements of NH_4^+ assimilation and

disposal of excess H^+ remains to be investigated.

To say that the H^+ or OH^- excreted to the root medium arises from the excess generated during N assimilation does not mean that all the excess H^+ and OH^- produced in metabolism is necessarily excreted. Transport of H^+ and OH^- out of the cells to the external medium certainly seems to represent the usual means by which cytoplasmic pH is controlled in aquatic algae (Eisele and Ulrich, 1975; Cram, 1976a; Raven, 1976a), but in terrestrial plants the only extracellular sink of any quantitative significance is the soil solution. One aspect of this net H^+ or OH^- excretion is that it can also be regarded as tending to maintain electroneutrality in the nutrient medium during salt uptake, since cations and anions are rarely taken up by the roots at equal rates. Short-term experiments, however, have shown that associated with this unequal uptake there is a stoichiometric accumulation of non-volatile organic acids when inorganic cation absorption exceeds anion absorption, and an equivalent decrease in organic-acid levels when anion absorption exceeds cation absorption (e.g. Ulrich, 1941; Jacobson and Ordin, 1954; Wit et al., 1963; Hiatt, 1967a). Hence, the sum of the cation equivalents (C) in the plant minus the sum of the anion equivalents (A) tends to equal the organic-acid equivalents (Pierce and Appleman, 1963; Wit et al., 1963; van Tuil, 1965).

This relationship between ion uptake and metabolism is modified during growth because, of the three major inorganic anions, NO_3^- and SO_4^{2-} are reduced and incorporated into organic molecules with the production of excess OH^- ; the oxidation state of inorganic phosphate is usually not changed during metabolism. The assimilation of SO_4^{2-} involves equivalent reactions to those involved in NO_3^- metabolism, (see above) although the quantities involved are normally only about 6% of those in N assimilation (Dijkshoorn and van Wijk, 1967) and will be ignored for the present purposes. As Dijkshoorn (1962) pointed out, assimilation effectively allows the negative charge from NO_3^- to be transferred to organic acids as an alternative to OH^- excretion. These relationships can be approximately represented by the following equations, taken from Pitman and Cram (1977):



i.e. organic N = A - C uptake + C - A accumulation

Having already considered the nature of H^+ and OH^- excretion

to the root medium, how then is organic-acid accumulation affected by the alternative N-sources in Ricinus? The data presented in Table 3.8 show that there was a sharp contrast between the malate levels in leaves of the NO_3^- -N and NH_4^+ -N plants. In the NO_3^- -N plants, malate contributed about 68% of the anion balance, but was present only in catalytic amounts in the NH_4^+ -N plants. Differences of this sort, both for malate and total organic acids, have been found in many instances (Clark, 1936; Wadleigh and Shive, 1939; Vickery and Pucher, 1940; Pucher, Leavenworth, Ginter and Vickery, 1947; Gilbert, Sheer and Gropp, 1951; Chouteau, 1960; Coïc, Lesaint and Le Roux, 1962; Kirkby, 1969; van Tuil, 1970; Houba, van Egmond and Wittich, 1971; Breteler, 1973a; Cox and Reisenauer, 1973). The contrast in levels in Ricinus, however, was more extreme than for any other species reported; Lycopersicon is the most similar, in which malate levels with NH_4^+ -N nutrition were 5% of those with NO_3^- -N nutrition (Kirkby, 1969; and see p. 81).

There are three reasons for believing that the low malate levels found in leaves of the NH_4^+ -N plants were not an artefact of the experimental methods.

- 1) Quantification by enzymic assay demonstrated the same pattern of malate levels in all the leaves analyzed in the three experiments, and variation between individual samples was within acceptable limits (Table 3.8).
- 2) Known amounts of pure malic acid added to the sap from NH_4^+ -N leaves were quantitatively recovered by enzymic assay: this demonstrated that the coupled enzyme system was not inhibited by substances in the leaf-cell sap.
- 3) The same differences were found on analysis of the organic acids by GLC (Fig. 5): this represented confirmation of the results by a reasonably sensitive and accurate physicochemical technique.

The data given on Cl^- (Table 3.10) and H_2PO_4^- (Table 3.11) levels in the NH_4^+ -N leaves indicate that inorganic anions largely substituted for malate in these leaves. Substantial increases in H_2PO_4^- levels with NH_4^+ -N nutrition have been observed frequently (e.g. Asher and Loneragan, 1967; Kirkby, 1968; Blair, Miller and Mitchell, 1970; DeKock, 1970; Cox and Reisenauer, 1973; Polizotto, Wilcox and Jones, 1975), as has accumulation of Cl^- in Beta (Breteler, 1973a).

The other group of solutes of major osmotic importance was the amino acids and their amides. Table 3.13 shows that the total concentration of soluble organic N contributed by the free amino acids and amides in the NH_4^+ -N leaves was nearly thirteen times higher than in the NO_3^- -N leaves. This difference appears to be characteristic of the response to the two N-sources (e.g. Coic et al., 1962; Barker, Volk and Jackson, 1966; Kirkby, 1968; Ferguson and Bollard, 1969). Although the total organic N content of the shoot would provide a more useful basis for relating inorganic N uptake to growth, these values are important in relation to the osmotic balance of the shoot. The levels are comparable with those found in other species, and this soluble N pool probably represents of the order of 10% of the total organic N in the leaf (Mengel and Helal, 1970; Breteler, 1973a).

Of the individual compounds (see Table 3.12), those present at the highest concentrations in the NO_3^- -N leaves were glutamic acid, serine, alanine, glutamine and aspartic acid; these are all closely related to the primary products of photosynthesis (cf. Pate et al., 1975; Miñlin and Lea, 1977). The NH_4^+ -N leaves showed a marked accumulation of asparagine and arginine, the latter having important consequences for the charge balance of the leaf-cell sap (Table 3.13) because of its net positive charge. Asparagine is generally the predominant form in legumes, where it is found in relatively high amounts in the leaves (Norton, 1970) and is the principal free organic N compound used in long-distance transport and for storage in the seeds (Pate, 1976; Atkins, Pate and Sharkey, 1975). Arginine, however, was found in these NH_4^+ -N leaves at over twice the level of asparagine. Arginine, too, is a particularly important storage form (Miñlin and Lea, 1977); it is found at high concentrations in vacuoles of the yeast, Saccharomyces cerevisiae (Wiemken and Dürr, 1974), and may be used in osmoregulation in some organisms (see Matile, 1978). The prevalence of asparagine and arginine as storage (and transport) forms of organic N has been considered by Miñlin and Lea (1977) as possibly related to their high N:C ratios. Of all the protein amino acids, asparagine (2N:4C) and arginine (4N:6C) are the most economic in their use of carbon. Furthermore, if the consumption of C in organic-acid synthesis is greatly reduced in these plants, this may be matched by the increased amounts involved in the accumulation of amino acids and amides (see Craven et al., 1972; Mott and Steward, 1972; Mueller, Feller and Erismann, 1977; Paul, Cornwell and Bassham, 1978).

From these results, the solute levels found in the leaves can be considered in relation to the effective charge balance of the sap. This analysis is presented in Table 3.25. The total inorganic cation levels were similar in the NO_3^- -N and NH_4^+ -N leaves, an observation that runs counter to the more common reports of decreased levels in the NH_4^+ -N leaves (e.g. Arnon, 1939; Cofic et al., 1961; Kirkby, 1968; Blair et al., 1970). In Nicotiana, however, K^+ levels do not seem to be affected by N-nutrition (Dekock, 1970), and in Lolium cation uptake seems actually to be enhanced by NH_4^+ -N (Dijkshoorn et al., 1968). Over 80% of the anions were accounted for in the NO_3^- -N leaves, but only about half of the negative charge equivalents were identified in the NH_4^+ -N leaves (assuming malate to be divalently charged: $\text{pK}_{a1} = 3.54$; $\text{pK}_{a2} = 5.12$). If estimates were included of the organic acids found in the sap by GLC in addition to malate, these figures would be correspondingly larger, and could account for about 60% of the anions in the NH_4^+ -N leaves. The balance is presumably made up by a certain amount of SO_4^{2-} , other unidentified acids and possibly additional negatively charged organic N compounds. The pH of the NH_4^+ -N leaf-cell sap (5.40) was slightly lower than that of the NO_3^- -N leaves (5.66), as found by Wadleigh and Shive (1939) and Kirkby and Mengel (1967).

An important aspect of these results that requires comment is that they give values only for the bulk soluble phase of the leaf tissue extracted by freeze-disruption, which represents essentially the vacuolar contents. Most of the previous studies on effects of N-source on chemical composition have referred to whole shoot (and occasionally root) content on a unit fresh weight or dry weight basis, after extraction and digestion of the tissues. Direct comparisons with these investigations can therefore only be made with caution, and the present results do not provide any information on the content of bound and insoluble substances in the leaves. The bulk-phase values may also obscure important differences due to compartmentalization within the cells and tissues. For example, although the free NH_4^+ content was not significantly different between the two groups of leaves (p.73), the small areas of necrosis found periodically on the NH_4^+ -N leaves may have been a reflection of localized NH_4^+ toxicity (see Warren, 1962; Maynard and Barker, 1969). Parenthetically, it might also be noted that the greater accumulation of soluble sugars in the NH_4^+ -N leaves (p.73) (cf. Kirkby and Hughes, 1970) could have been related to the lower growth rates of these plants, and that the induction of

Table 3,25 Effective charge balance of leaf-cell sap for plants grown on NO_3^- -N or NH_4^+ -N solution. Figures for sap pH are values for pooled samples from one experiment.

	contribution to charge balance/ mol m ⁻³ equivalents	
	NO_3^- -N leaves	NH_4^+ -N leaves
sugars	0	0
cations		
$\text{K}^+ + \text{Na}^+$	+ 159	+ 185
$\text{Mg}^{2+} + \text{Ca}^{2+}$	+ 139	+ 120
<i>amino acids</i> + NH_4^+	0	+ 21
	<hr/>	<hr/>
	+ 298	+ 326
anions		
$\text{Cl}^- + \text{H}_2\text{PO}_4^-$	- 41	- 151
<i>malate</i>	- 204	- 1
<i>amino acids</i>	- 2	0
	<hr/>	<hr/>
	- 247	- 152
<i>anions accounted for</i>	83%	47%
pH	5.66	5.40

anthocyanin synthesis may be directly associated with the levels of free sugars (Pirie and Mullins, 1976).

One area of relevance here in terms of the extraction methods concerns the rôle of oxalate. This organic acid is also found at high levels in many plants in which NO_3^- assimilation occurs in the shoots (Osmond, 1967; van Egmond and Houba, 1970; Nierhaus and Kinzel; Kirkby and Knight, 1977). Furthermore, it occurs at much lower levels in plants with NH_4^+ -N versus NO_3^- -N nutrition (Clark, 1936; Gilbert et al., 1951; Scharrer and Jung, 1954; Chouteau, 1960; Joy, 1964; Kirkby, 1969). Oxalate is found, however, in both soluble and insoluble forms, and light microscopy examination of stem petiole and leaf sections showed that there were abundant oxalate crystals (druses) in Ricinus, especially in the vascular parenchyma and bundle sheath cells. It was not possible to determine quantitatively by inspection whether the druses were less common in the NH_4^+ -N than the NO_3^- -N plants, and acid extraction of the oxalate would be required for this purpose. The significance of oxalate precipitation (usually as the Ca- or Mg-salt) remains uncertain, although it may be another aspect of the prevention of excess solute accumulation in leaves (see Raven and Smith, 1976a; Austenfeld and Leder, 1978). Calcium can also be important in balancing malate (Kirkby and DeKock, 1965; Phillips and Jennings, 1976b) and the balance sheet for the leaf-cell sap (Table 3.25) indicates that the soluble oxalate pool in Ricinus can only be of minor importance compared with malate.

There is one final aspect of the observed differences in whole-plant metabolism that must be considered, and this is the question of why there should be such large differences in organic-acid levels with the contrasting N-sources. I am going to discuss this in some detail, because it represents the most significant and widely noted feature in experiments of this sort, and because it will demonstrate the importance of understanding how long-distance transport is related to root and shoot metabolism. It is also necessary to explain how during growth on an NO_3^- -N solution, from which anion uptake usually exceeds cation uptake (Walker, 1960; Dijkshoorn, 1962; Kirkby, 1969), the plant tissues accumulate larger quantities of organic acids than during growth on an NH_4^+ -N solution, from which cation uptake is invariably greater than anion uptake (Wit et al., 1963; van Tuil, 1965; Dijkshoorn et al., 1968) (see p. 85).

The transfer of the negative charge from NO_3^- to carboxylate (as an alternative to OH^- excretion) allows organic-acid accumulation with the maintenance of electroneutrality in the tissues (Dijkshoorn, 1962). On the assumption that H^+ and OH^- production associated with NH_4^+ and NO_3^- assimilation, respectively, has effects on cytoplasmic pH, Hiatt (1967a,b) has pointed out that this could result in increased activity of the enzymes involved in carboxylation reactions (e.g. malate synthesis) as the pH increases in the range 6 - 8, the converse being true for decarboxylation. This balance has been discussed by Davies (1973a, b) as a 'biochemical pH-stat', in which formation of strong acids from neutral precursors as a result of increased pH would itself tend to lower the pH, and vice versa, thereby maintaining a relatively constant pH. Although it is hard to determine whether the enzyme characteristics in vivo are compatible with this scheme (see Osmond, 1976), relevant empirical data are certainly consistent with the scheme (see Raven and Smith, 1974; Smith and Raven, 1976; Haschke and Lüttge, 1977; Stout and Cleland, 1978; Hill and Brown, 1978).

This scheme emphasizes that the excess H^+ and OH^- generated during N assimilation can be dealt with either by transport (the biophysical pH-stat) or by chemical neutralization (the biochemical pH-stat). The control of intracellular pH in higher plants involves both of these strategies (Raven and Smith, 1974, 1976a). But in addition, the synthesis of malate is of major quantitative importance in the generation of cell turgor for growth in NO_3^- -N plants (see Table 3.8 and 3.25). As for the NH_4^+ -N plants, three types of ionic interactions might have accounted for the inhibition of malate accumulation (following Osmond, 1976). Of these, perhaps the least likely to have been operative was inhibition of phosphoenolpyruvate (PEP) carboxylase activity by malate levels in the cytoplasm (i.e. feedback control), unless malate transport across the tonoplast was specifically prevented. A balance in favour of decarboxylation rather than carboxylation as a result of continuous excess H^+ production during NH_4^+ assimilation would, however, be one mechanism consistent with the tenets of the pH-stat concept. And finally, the accumulation of inorganic anions in the cytoplasm might directly inhibit HCO_3^- (CO_2) fixation and oxaloacetate reduction, since both PEP carboxylase and malate dehydrogenase are inhibited in vitro by Cl^- (Osmond and Greenway, 1972). The high levels of Cl^- in the NH_4^+ -N leaves (Table 3.10) suggest that this last mechanism might also have been contributory, particularly as the slightly higher Cl^- levels

in the (NO_3^- -N + Cl^-) leaves compared with the NO_3^- -N leaves (Table 3.10) appeared to be matched by an equivalent decrease in malate levels in these plants (Table 3.8). In root tissue, Cl^- uptake can inhibit malate accumulation and stimulate decarboxylation (Smith, 1973; Cram, 1974), and the availability of Cl^- may also determine the quantities of malate synthesized during stomatal opening (Raschke and Schnabl, 1978). The present data do not permit a decision on the relative importance of the biochemical pH-stat and Cl^- inhibition in controlling malate accumulation in Ricinus. This will require a better understanding of the uptake processes in the root, since Cl^- and NO_3^- uptake may be competitive (Böning and Böning-Seubert, 1932; Lundegårdh, 1959; Wit et al., 1963).

The biochemical characteristics of the N-assimilation reactions may have important consequences for the location of these processes in land plants. The view of Raven and Smith (1976a) is that control of intracellular pH in the shoots must occur largely by operation of the biochemical pH-stat; this is because neither the apoplastic solution (see Pitman, Lüttge, Kramer and Ball, 1974) nor the phloem (see Raven, 1977b) appear to constitute quantitatively adequate sinks for disposal of excess H^+ and OH^- . Furthermore, the biochemical pH-stat can only cope in the long-term with excess OH^- , since the organic-acid anion needed for decarboxylation to neutralize excess H^+ must itself have previously arisen from a carboxylation reaction that generated H^+ (Raven and Smith, 1974). The implication is that NH_4^+ assimilation must be confined to the roots, and that NO_3^- reduction in the shoots is coupled with organic-acid synthesis. The model of Dijkshoorn et al. (1968) and Ben Zioni et al. (1970, 1971), on the other hand, suggests that removal of OH^- (as carboxylate) in the phloem is a quantitatively important process. These issues, however, have not previously been approached with specific reference to analytical data for xylem- and phloem-sap composition with different N-sources.

The characteristics of the water relations of the xylem and phloem in Ricinus (Table 3.15) indicate that the osmotic properties of the transport systems were not affected by the form of inorganic N supplied. The slow exudation rates observed in the NH_4^+ -N plants can be attributed to the smaller stature and lower growth rates of these plants; if expressed in terms of unit cross-sectional area of the transport paths, or unit mass of tissue, the values would be more comparable (cf. fresh-weight yields in Fig. 3.1). More than 90% of

the Ψ_s of the xylem and phloem sap was subsequently accounted for by chemical analysis in both the NO_3^- -N and NH_4^+ -N plants (Tables 3,23 and 3,24).

As with the levels in the leaves, it was found that the total inorganic cation levels in the xylem and phloem showed no significant differences between the NO_3^- -N and NH_4^+ -N plants. Although concentrations in root-pressure exudate may over-estimate those in the transpiration stream (section 1,3), there is evidence in Ricinus that at least K^+ levels in the sap are probably a close reflection of those in the xylem before excision of the shoot (Bowling and Weatherley, 1965). The present data show that K^+ was the major cation in the xylem sap, and the only one present in significantly smaller amounts in the NH_4^+ -N sap. Even with the same concentrations in such plants, the reduced cation content of NH_4^+ -N shoots found in many instances may be associated with decreased flux through the xylem (Wilcox, Mitchell and Hoff, 1977). The reasons for believing that Ricinus phloem exudate represents a true sample of the transported assimilates have been discussed elsewhere (Milburn, 1971; Hall et al., 1971; Hall and Baker, 1972; Mengel and Haeder, 1977). Cation levels in the phloem were not significantly different in the NO_3^- -N compared with the NH_4^+ -N plants either. It is the nature of the anion balance in the sap which is again more affected by the N-source.

Table 3.26 presents a balance sheet for the solutes contributing charge equivalents in the xylem that were quantified in these experiments. NO_3^- was quantitatively the most important anion in the xylem, and together with malate (here taken as about 75% malate⁻, 25% malate²⁻ at the xylem pH of 5.0) effectively accounted for all the required negative charge equivalents. The data of Bowling et al. (1966) indicate that the sum $\text{Cl}^- + \text{H}_2\text{PO}_4^- / \text{HPO}_4^{2-} + \text{SO}_4^{2-}$ contributes only about 4 mol m⁻³ negative charge equivalents in the xylem. Malate may therefore contribute significantly to the anion balance (cf. Frost, Blevins and Barnett, 1978). For the NH_4^+ -N xylem, the 30% or so of the anions not accounted for may have been made up by inorganic ions (especially SO_4^{2-} in view of the levels in the nutrient solution: Table 3.1) and other carboxylates besides malate. In the phloem (Table 3.27), amino acids made a substantial contribution to the anion balance, but more so in the NO_3^- -N plants. It is possible that the negative charges unaccounted for in the NO_3^- -N phloem sap was made up by oxalic acid, which was lost during extraction but is thought to be phloem mobile (Peel and Weatherley, 1959) as well

Table 3.26 Effective charge balance of xylem sap for plants grown on NO_3^- -N or NH_4^+ -N solutions. Figures for sap pH are means \pm S.E. for samples from 2 plants or values for pooled samples from the same experiment.

	contribution to charge balance/ mol m ⁻³ equivalents	
	NO_3^- -N xylem	NH_4^+ -N xylem
sugars	0	0
cations		
$\text{K}^+ + \text{Na}^+$	+ 18.7	+ 14.8
$\text{Mg}^{2+} + \text{Ca}^{2+}$	+ 9.0	+ 10.6
amino acids + NH_4^+	+ 1.5	+ 1.6
	<hr/>	<hr/>
	+ 29.2	+ 27.0
anions		
$\text{Cl}^- + \text{NO}_3^-$	- 23.8	- 18.4
malate	- 7.5	- 0.3
	<hr/>	<hr/>
	- 31.3	- 18.7
anions accounted for	107%	69%
pH	5.00 \pm 0.20	5.00

Table 3.27 Effective charge balance of phloem sap for plants grown on NO_3^- -N or NH_4^+ -N solutions. Figures for sap pH are means \pm S.E. for samples from 3 plants or values for pooled samples from the same experiment.
 *denotes figure for chloride + nitrate only;
 §denotes figure for malate only.

	contribution to charge balance/ mol m ⁻³ equivalents	
	NO_3^- -N phloem	NH_4^+ -N phloem
sugars	0	0
cations		
$\text{K}^+ + \text{Na}^+$	+ 74.0	+ 60.6
$\text{Mg}^{2+} + \text{Ca}^{2+}$	+ 14.8	+ 10.6
NH_4^+	+ 1.5	+ 2.6
	-----	-----
	+ 90.3	+ 73.8
anions		
$\text{Cl}^- + \text{HPO}_4^{2-} + \text{NO}_3^-$	- 25.2	- 33.5*
organic acids	- 20.2	- 2.4 [§]
amino acids	- 23.8	- 6.0
	-----	-----
	- 69.2	- 41.9
anions accounted for	77%	57%
pH	7.43 \pm 0.04	7.40

as by trace levels of SO_4^{2-} and HCO_3^- (Hall and Baker, 1972). About 30 mol m^{-3} negative charge equivalents were unidentified in the NH_4^+ -N phloem. In neither the xylem nor the phloem were the sap pH values significantly different with the two forms of N supplied.

Given the significance of the sites of N assimilation in land plants (Raven and Smith, 1976a) and of the quantities of organic N transported in the phloem (section 1.3), the N balance of the xylem and phloem is of particular importance with respect to solute accumulation in the whole plant. The total N content of the xylem sap in the NO_3^- -N and NH_4^+ -N plants was in fact very similar (Table 3.21), although in the former about twice as much N was transported as NO_3^- than in reduced form. The amino-acid and amide composition of the xylem saps was also similar by proportion (Table 3.19), glutamine being by far the most important N form, as in many other species (see section 1.3; Mifflin and Lea, 1977; and Weissmann, 1964; Waldron, 1976). It is worth noting also that the NH_4^+ -N xylem sap did not contain more free NH_4^+ than the NO_3^- -N sap, but that in some species the $\text{NH}_4^+:\text{NO}_3^-$ ratio can be as high as five under field conditions (Waldron, 1976).

Despite marked differences in the proportions of amino acids present in the phloem sap, Table 3.22 shows that the level of total N in the NH_4^+ -N phloem was within 10% of that in the NO_3^- -N phloem. As in the xylem, glutamine was the predominant reduced N compound in the NO_3^- -N phloem (Table 3.20; see section 1.3), and asparagine was of increased importance in the NH_4^+ -N phloem. However, both the level and proportion of glutamine in the NH_4^+ -N phloem was lower than that in the NO_3^- -N phloem, and instead the compound occurring in the largest amounts was serine. Although serine is generally of some quantitative importance in the phloem (see Ziegler, 1975; Pate, 1976), why it should appear in the NH_4^+ -N phloem sap at levels of around 60 mol m^{-3} is not clear. There appear to be three possible pathways of serine biosynthesis (Mifflin and Lea, 1977), although there is evidence that by far the most important in leaves of C_3 plants is that via glycollate in photorespiration (Waidyanatha, Keys and Whittingham, 1975; see Schnarrenberger and Fock, 1976). It has been argued that the flux of photosynthetically derived carbon through the glycollate pathway may represent the major route for sucrose synthesis in C_3 plants, rather than 3-phosphoglycerate being formed directly from the photosynthetic carbon reduction cycle (Kumarasinghe, Keys and Whittingham, 1977; Bird, Cornelius, Keys and Whittingham, 1978). Thus, the higher levels of sugars in the NH_4^+ -N

leaves might cause accumulation of C in compounds earlier in the biosynthetic pathway, but on the other hand there is no evidence for this happening in soil-grown plants (see Hall and Baker, 1972), which can also possess higher soluble carbohydrate levels in the leaves. Another possibility is that there may be considerably reduced formation of oxalate (from glycollate and glyoxylate) in the NH_4^+ -N plants because of the biochemical pH-stat (see Raven and Smith, 1976a), causing accumulation of the photorespiratory C in a compound (i.e. serine) that can be readily loaded and transported at high concentrations in the phloem (see Clauss, Mortimer and Gorham, 1964; Leckstein and Llewellyn, 1975; Housley, Peterson and Shrader, 1977; Peterson, Housley and Shrader, 1977). Measurements of the levels of soluble and insoluble oxalate in the NO_3^- -N and NH_4^+ -N plants would help to resolve this issue.

These data on xylem- and phloem-sap composition can be used to consider the possible significance of K^+ recirculation, as envisaged by Dijkshoorn *et al.* (1968) and Ben Zioni *et al.* (1970, 1971), for control of the solute balance between root and shoot in Ricinus. This model assumes that all the organic N in the shoot (and possibly in the whole plant) is produced by NO_3^- assimilation in the shoot. Further, it is suggested that all the OH^- appearing in the root medium arises from K carboxylate which is transported in the phloem and subsequently decarboxylated in the roots. The evidence supporting this hypothesis comes from grasses in which the carboxylate content of the shoot is lower than would be expected if all the N was transported to the shoot as NO_3^- and then stoichiometrically converted to organic N and organic acids (implying that some carboxylate is exported); in addition, net rates of K^+ uptake can be lower than those required to give the postulated 1:1 ratio of $\text{K}^+:\text{NO}_3^-$ in the xylem (see Wit *et al.*, 1963; Dijkshoorn *et al.*, 1968).

The first point of note is that the xylem sap in Ricinus contains substantial quantities of reduced N. It could be argued that this simply reflects recirculation of organic N derived from the shoot, but studies on the activity of nitrate reductase in root systems (Pate, 1973; Radin, 1977) and work on incorporation of radiotracers (Ingversen and Ivanko, 1971) suggest that this organic N is likely to represent that reduced in the root. The data can also be used to consider what proportion of NO_3^- reduction occurs in the root versus the shoot. It is unsatisfactory to try to estimate this ratio with any precision, since part of the organic N produced in the root is used in root growth

and consequently never appears in the xylem exudate (see Radin, 1977). However, the ratio of organic N;total N in the sap (see Table 3.21) indicates that at least 38% of the NO_3^- assimilation occurs in the roots, and this figure would probably be higher with lower NO_3^- levels in the nutrient solution (see Kirkby, 1974).

In Ricinus, therefore, substantial amounts of inorganic N appear to be assimilated in the roots, and this differs from one premise on which the K^+ -recirculation model was originally based. One important consequence of this is that it may explain why in many species the carboxylate content of the shoot is lower than its organic N content, for only a part of the total N supplied to the shoot by the xylem is in the form of NO_3^- (Raven and Smith, 1976a). Furthermore, Pitman and Cram (1977) have also shown by collation of published data from a number of species that the proportion of carboxylate equivalents to organic N in the shoot does tend to equal the proportion of NO_3^- to total N supplied in the xylem. On these grounds, then, there may be no need to invoke K carboxylate export in the phloem as a quantitatively important means of OH^- disposal from the shoots. The question of whether any of the OH^- excreted by the roots is derived from carboxylate transported in the phloem (Pitman and Cram, 1977) can only be answered by quantitative comparison of rates of transport in the phloem with rates of NO_3^- reduction in the root and shoot during growth.

The levels of malate found in the NO_3^- -N phloem in the present study (about 10 mol m^{-3} equivalents) were low compared with those recorded by Hall and Baker (1972) ($30 - 47 \text{ mol m}^{-3}$ equivalents). However, Mengel and Haeder (1977) detected only around 1 mol m^{-3} equivalent of malate in phloem sap from water-cultured plants; these were grown on a combined $\text{NH}_4^+ + \text{NO}_3^-$ source of N, and the presence of even small amounts of NH_4^+ can decrease carboxylate production (Breteler and Smit, 1974). If both carboxyl groups of malate can be metabolized to give OH^- in the roots, and the sucrose level in the phloem is taken as 300 mol m^{-3} (see p. 79), this effectively allows the transport of about 0.03 OH^- per sucrose down in the phloem; if the total carboxylates at about 30 mol m^{-3} equivalents were taken instead, this would give a ratio of 0.1 OH^- per sucrose. With assumptions about the pH at the ends of the xylem and phloem, it is also theoretically possible that OH^- could be directly transported down the phloem as a result of the $\text{H}_2\text{PO}_4^- \rightarrow \text{HPO}_4^{2-} + \text{H}^+$ reaction (Raven, 1977b); with an H_2PO_4^- concentration in the sap of 7.6 mol m^{-3} (see p. 76), this could

transport about 0.025 OH⁻ per sucrose. Since the free OH⁻ present at the phloem pH of 7.4 (Table 3.27) is quantitatively insignificant, this gives the phloem a total capacity for OH⁻ transport of about 0.13 OH⁻ per sucrose at a maximum. This line of reasoning has been considered in detail by Raven (1977b), who has calculated from the data of Kirkby and Mengel (1967) that if the phloem was to remove the excess OH⁻ generated during NO₃⁻ assimilation in the shoot, the required ratio would be about 1.4 OH⁻ per sucrose. Thus, to a first approximation it seems that the theoretical capacity of the phloem to transport OH⁻ is an order of magnitude too low to account for disposal of excess OH⁻ from the shoots.

Although these considerations suggest that the phloem does not make a major contribution to disposal of excess OH⁻, the composition of the sap is nevertheless of great significance for the maintenance of the charge balance. The end-products of C and N assimilation (proteins, cell-wall material and so on) carry a net negative charge, and some organs, such as developing fruits, receive most of their nutrients via the phloem (e.g. Pate, Sharkey and Atkins, 1977; Pate and Hocking, 1978). Consequently, the organic solutes in the phloem **must** also carry a net negative charge if excess H⁺ production is to be avoided during metabolism in the sinks (Raven and Smith, 1976a). This is another facet of the control of phloem-sap composition in the NO₃⁻-N and NH₄⁺-N plants (Table 3.22), for not only is the total organic C and N content of the sap almost the same, but in addition both carry a net negative charge albeit to a less significant extent in the NH₄⁺-N phloem. The importance of phloem transport in redistribution of organic C and N within the shoot is also emphasized by the fact that the ratio of carboxylate to organic N tends to change during leaf development, older leaves possessing relatively less N (Chouteau, 1960; Coic et al., 1962; Martin, 1970; van Egmond, 1971).

As with the phloem there is a similar requirement for the xylem that the net charge on the organic N compounds in the sap matches that of the assimilation products in the shoots. The total N content of the xylem was also very similar in the NO₃⁻-N and NH₄⁺-N plants (Table 3.21), and in the former the charge balance was dominated by NO₃⁻. It is likely that the small net positive charge carried by the NH₄⁺-N xylem would contribute to the pH stress in the shoot, and xylem sap with a predominance of basic amino acids or free NH₄⁺ has been noted in other species (Weissmann, 1964; Ivanko and Ingversen, 1971b). The slightly

lower pH of the leaf-cell sap in the NH_4^+ -N plants (Table 3.25), however, is unlikely to represent increased H^+ storage in the vacuoles, because this has to be set against the low levels of carboxylates present, these compounds being the most significant buffers in this part of the pH range.

The presence of high NO_3^- levels in the NO_3^- -N xylem was associated with a NO_3^- :K ratio in the sap of greater than one. This argues against a requirement for stoichiometric transport of the two (postulated by Ben-Zioni et al., 1971) and ^{suggests} that other cations are also involved as counter-ions for NO_3^- transport (see Kirkby, 1974; Breteler and Hänisch Ten Cate, 1978; Frost et al., 1978). The marginally lower concentration of K^+ in the NH_4^+ -N xylem (Table 3.16) may have been partly related to competitive interaction between K^+ and NH_4^+ uptake in the roots (Tromp, 1962; Breteler, 1977), although this must also be considered in terms of the $\text{K}^+:\text{NH}_4^+$ ratio in the medium (see Cox and Reisenauer, 1973). The malate levels in the NO_3^- -N xylem (Table 3.17) may in addition have made a significant contribution to the carboxylate content of the shoot in these plants (see Tiffin, 1970; Bradfield, 1976; Cox and Reisenauer, 1977). Under the present conditions, the malate may have been produced by the biochemical pH-stat as a response to the high NO_3^- levels in the medium.

These arguments suggest that, in Ricinus, K^+ recirculation in the form envisaged by Dijkshoorn and Ben-Zioni et al. is of limited significance. This may be characteristic of most dicotyledons in which cation:anion uptake ratios tend to be just below one when NO_3^- supply is adequate (Walker, 1960; Kirkby, 1969). In these plants, therefore, the amount of NO_3^- taken-up by $\text{NO}_3^-/\text{OH}^-$ exchange is relatively small, whether or not the OH^- excreted originates from the shoot; the current view, though, is that OH^- is derived from NO_3^- assimilation occurring in the roots that is not balanced by carboxylate synthesis (Blevins, Hiatt and Low, 1974; Cram, 1976b; Raven and Smith, 1976a). It follows that K^+ recirculation may be principally important in plants where the fraction of NO_3^- taken up from the root medium by $\text{NO}_3^-/\text{OH}^-$ exchange is greater than the fraction of NO_3^- reduced in the root (Cram, 1976b). In cereals and grasses, anion uptake tends to be much greater than cation uptake, there is little accumulation of carboxylates in the shoot, and K^+ constitutes a high proportion of the total cations in the plant (see van Tuil, 1970; Kirkby, 1974). Most charge transfer from NO_3^- is thus directed towards OH^- excretion, and transport of carboxylate to the roots in the phloem may then be a controlling element in NO_3^- uptake.

4. PHLOEM TRANSPORT, SAP EXUDATION AND SOLUTE FLUX

4. PHLOEM TRANSPORT, SAP EXUDATION AND SOLUTE FLUX

4.1 Introduction

Phloem-sap composition in higher plants appears to be determined largely by the biochemical and physiological properties of the loading process. Transport out of the phloem and metabolism within the sieve elements undoubtedly modify the solute content to varying degrees, but these can be regarded as secondary effects, since they are both ultimately related to the continued net influx of solutes into the tissue. The results presented in the previous chapter suggest that sap composition can be controlled in several important respects despite significant changes in whole-plant metabolism. With the alternative sources of inorganic nitrogen, it was found that the total organic C and N content, as well as the cation levels, were hardly affected by the different patterns of solute accumulation in the leaves. This was reflected in similar values of phloem-sap Ψ_s and Ψ_p in the plants, implying that the osmotic characteristics of the phloem were not altered by these changes in metabolism.

The purpose of the remaining chapters is to examine how the solute content of the phloem is influenced by plant water relations. This is another fundamental aspect of the control of phloem transport, and one that is directly connected with the issue of longitudinal flux through the system. If the nature of the source-sink relationships observed during ontogeny (section 1.4) is to be better understood, it is important to clarify the mechanism by which the transport systems as a whole can respond to changes in demand. At the same time, the phloem will almost inevitably be affected by alterations in water availability (section 1.5). Consequently, the processes that determine the driving force for phloem transport must be carefully distinguished from more general osmotic characteristics of the system.

Analysis of sap composition during prolonged exudation from bark incisions in Ricinus provides one direct means of investigating these aspects of phloem transport. Evidence has been presented elsewhere that exudate from Ricinus represents, in most qualitative respects, a true sample of the substances normally transported in the phloem (Hall *et al.*, 1971; Hall and Baker, 1972). Quantitative aspects of sap exudation in Ricinus have also been considered in some detail (see Milburn, 1974, 1975). One further point about phloem-sap exudation, however, is that under controlled conditions it may provide a valuable model for the study of source-sink relations in the whole plant. In effect, the

incision represents a sink whose capacity is limited only by the sealing reactions that occur after wounding (see Eschrich, 1975), and it is thus possible to examine the response of the physiological sources to artificially induced changes in demand. Nonetheless, the potential usefulness of sap exudation as a means of studying the transport system rests upon a detailed appraisal of its characteristics.

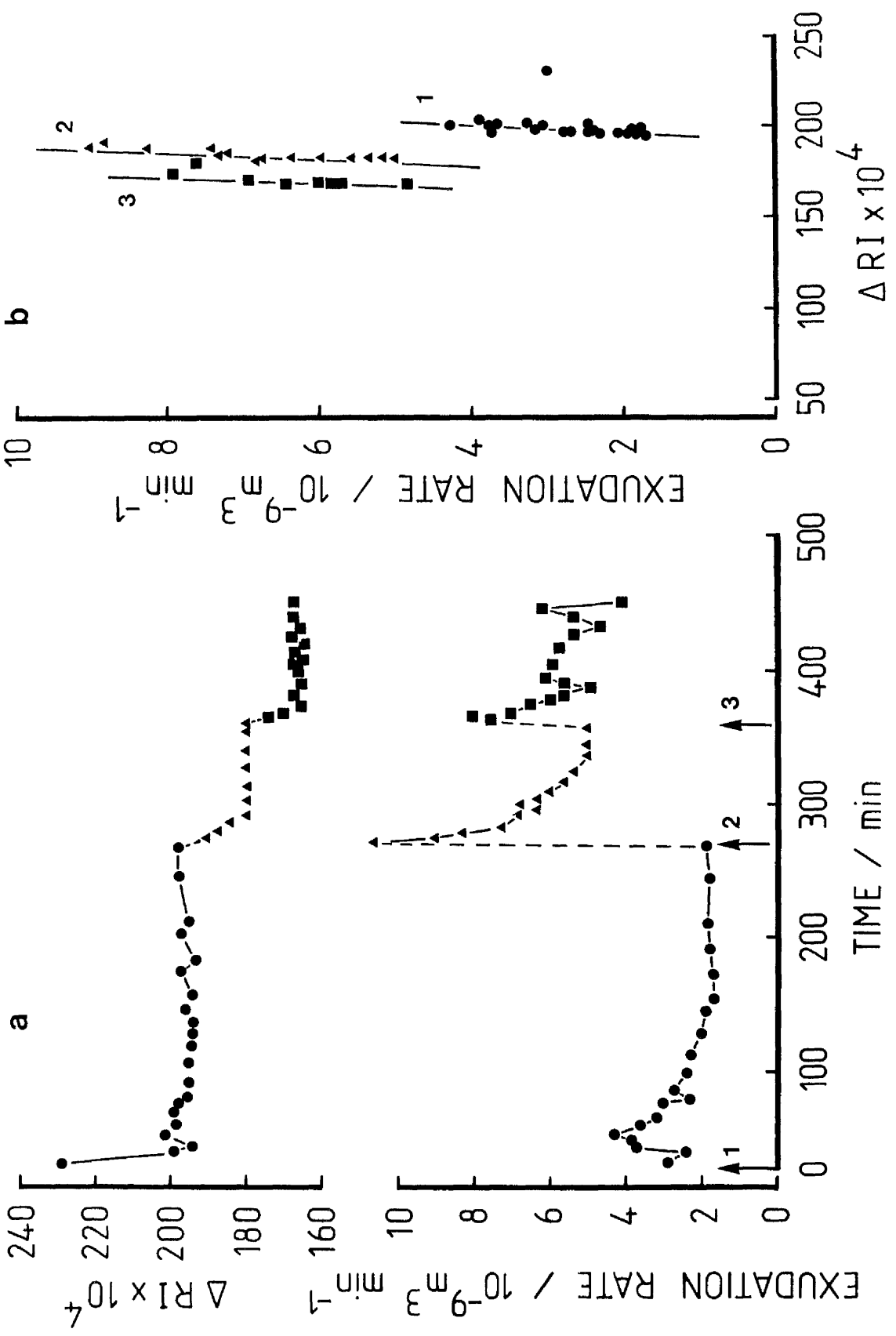
In this chapter, a number of quantitative aspects of sap exudation have been investigated, with specific reference to water-cultured plants under controlled environmental conditions. Firstly, the rates of sap exudation as measured by solution flow and solute flux were examined in terms of the bulk solute content of the sap. This approach was then extended to an analysis of the relationships between sap exudation and solute loading in the leaves using foliar-applied radioisotopes as tracers. Lastly, the kinetics of transport were considered by studying the responses of sap exudation to experimental manipulation of the source-sink relations. The results are discussed in terms of the properties expected of an osmotically controlled transport system.

4.2 Characteristics of phloem-sap exudation

The essential characteristics of sap exudation from bast incisions in Ricinus have been described by Milburn (1971, 1974). By way of summary, data representative of experiments performed on soil-grown plants are shown in Fig. 4.1; this figure is redrawn from unpublished results of J.A. Milburn, used with permission. The principal features of exudation profiles of this sort were as follows:

- 1) the rate of sap exudation declined after each incision and increased immediately in response to successive incisions in the series (Fig. 4.1a);
- 2) during the course of exudation from each incision, the solute content of the sap (measured by refractometry) declined for 10 to 20 min and subsequently remained constant (Fig. 4.1a);
- 3) an incision as little as 1 to 2 mm above the preceding one in the series produced a sharp increase in exudation rate; and
- 4) a plot of exudation rate against solute content (Fig. 4.1b) shows that the concentration of solutes in the sap was virtually independent of exudation rate.

Fig. 4.1 Time course of changes in solute content of phloem sap and exudation rate in response to successive incisions (denoted thus ↑) in the stem internode of a soil-grown plant. a Associated changes in solute content and exudation rate with time. b Same data plotted as exudation rate versus solute content. Units of solute concentration, $\Delta RI \times 10^4$, represent difference between RI of sap and that of distilled water.



Neither histological nor biochemical tests have been made of the specific responses of the phloem tissue in Ricinus to wounding. Point 2 (above), however, suggests that the decline in exudation rate was a result of localized blockage of the sieve elements around the site of the incision (cf. Crafts, 1936). The protein fraction of the phloem sap does not appear to coagulate on exposure to air, as happens in other species (see Crafts, 1961; Milburn, 1971), and the reduction in exudation rate may be caused primarily by deposition of callose in the sieve-plate pores. Callose formation can occur within seconds of injury to the sieve tubes (Currier, 1957; Eschrich, 1965), but the kinetics of this process have yet to be studied in terms of changes in the rate of longitudinal transport.

Transverse sections of stem tissue examined by light microscopy showed that razor-blade incisions penetrated no further than the phloem; in addition, sap did not exude unless the incision reached the inner layers of the bark. The absence of any lactiferous canals or ducts in these regions of the stem suggested that the sap originated from the phloem tissue (see Hall et al., 1971). Direct observation of exudation from cut ends of small stems or petioles also showed that sap came from the phloem region of the tissues. Similar demonstrations have been made with sap exudation from curcubit and Yucca inflorescences (Crafts, 1936; Die and Tammes, 1966). Since there is no evidence that phloem fibres, phloem parenchyma or companion cells are able to conduct large volumes of sap, it is probable that the exudate arose from severed sieve elements.

These results suggest that phloem sap is released from the sieve elements when the turgor is released on incision. An important point, however, is that the rates of exudation are frequently maintained from one incision over prolonged periods at significantly higher levels than from previous incisions in the series into the same cross-section of tissue. Given that the solute content of the sap can remain relatively constant over such periods, this implies that the transport system is able to respond to changes in the effective strength of the artificial sink created by these incisions. Another aspect of this occurrence is that sap concentration tends to be relatively independent of the rate of exudation (Fig. 4.1b). The significance of this feature of the exudation profiles will be considered later in relation to analogous studies on sap exudation from severed aphid stylets.

Phloem-sap exudation from stem incisions in water-culture plants showed the same characteristics as noted above for soil-grown material. However, it was generally found that exudation from a single incision was more prolonged than from soil-grown plants, that exudation rates expressed per unit cross-sectional area of tissue were higher, and that the solute content of the sap (measured by refractometry) fluctuated only slightly ($\pm 5\%$) over prolonged periods (4-6 h). These features may have been associated with the fact that availability of water in the root medium was effectively constant and non-limiting.

In all the present studies on exudation from water-cultured plants, the transport pathway was modified at least 24 h before experimentation by the method described in section 2.3 (and see Plate 2.4). The isolation of a single longitudinal band of bark meant that exudation rates from successive incisions (made in an acropetal direction, each across the whole width of the band) could be directly related to approximately the same cross-sectional area of tissue. Furthermore, after the first incision in the series, the stem was effectively ring-girdled, and exuding sap must have originated solely from the upper part of the shoot. This strategy was adopted because incisions into the phloem seem to constitute sinks of a strength sufficient to induce sap flow even from organs such as the roots, which must have been net importers of photosynthetic assimilates in the intact plant (Milburn, 1974).

Measurements of refractive index (RI) of the sap gave estimates of the bulk solute content of the solution, but the time course of changes in levels of the principal solutes was also followed in a number of experiments. In Table 4.1, a summary is presented of the concentrations of the major solutes occurring in the phloem sap from water-cultured plants grown on NO_3^- -N solution. The sap samples were obtained from a group of nine-week-old plants in one experiment, and the proportion of the measured Ψ_s accounted for was determined as described in section 3.4. The plants had been kept under growth-room conditions for 3 d and were morphologically similar to those used in all the exudation studies; further, the values from this experiment were representative of those obtained in other experiments. The figures all refer to means \pm S.E. for values from 6 plants except for those for malate, which were derived from 16 plants used in different experiments. The metabolic significance of the solute content has been discussed already (section 3.5), and there are two points concerning the bulk solute content that are of relevance. Firstly,

Table 4.1 Summary of solute content of phloem sap from nine-week-old plants grown on NO_3^- -N Long Ashton solution. Figures are means \pm S.E. for samples from 6 plants or values for pooled samples from section 3.4.

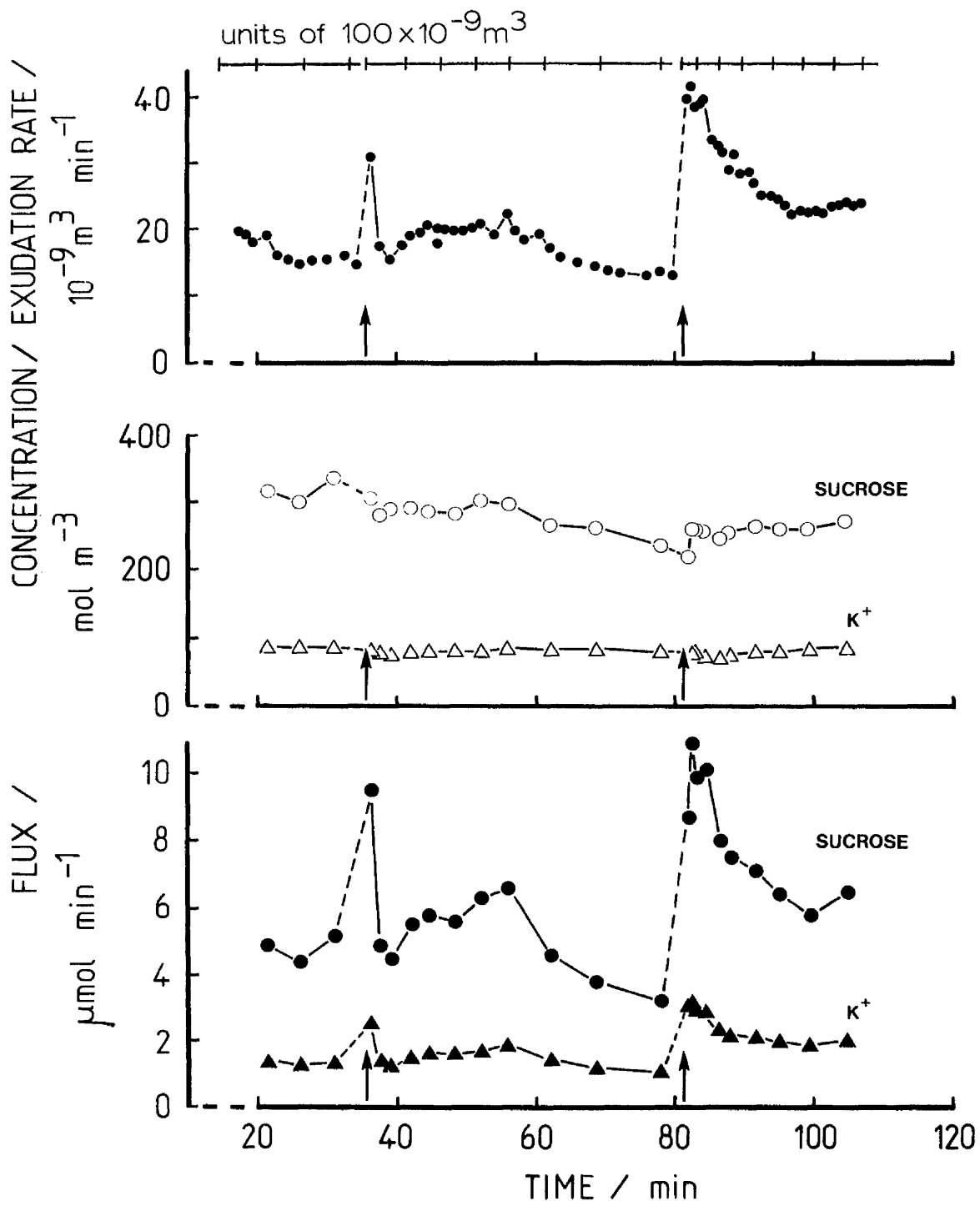
	concentration/ mol m^{-3}	theoretical contribution to sap Ψ_s / MPa
carbohydrates		
<i>sucrose</i>	259 \pm 21	-0.71
<i>reducing sugars</i>	trace	0
cations		
K^+	68.1 \pm 4.1	-0.36
Na^+	6.7 \pm 1.9	
<i>amino acids</i> (+ve) + NH_4^+	2.7	
Mg^{2+}	3.9 \pm 0.3	-0.04
Ca^{2+}	1.3 \pm 0.4	
anions		
Cl^-	8.9 \pm 3.4	-
H_2PO_4^- / HPO_4^{2-}	7.6 \pm 0.3	
NO_3^-	3.0	
<i>organic acids</i>	20.7	
(<i>malate</i>)	3.6 \pm 0.6)	
<i>amino acids</i> (-ve)	23.8	
neutral compounds		
<i>uncharged amino acids</i> + <i>amides</i>	86.5	-0.22
TOTAL		-1.33
observed sap Ψ_s =	-1.36 \pm 0.10	
.*. sap Ψ_s accounted for \approx 98%.		

chemical analysis accounted for 98% of the observed sap Ψ_s , demonstrating that these methods provide a quantitatively adequate basis for considering the significance of the solute content in terms of the osmotic relations of the phloem. And secondly, the two solutes occurring at the highest levels (besides glutamine: see Table 3.20) were sucrose and K^+ , which together accounted for 75% of the observed sap Ψ_s .

Sucrose and K^+ were used in a series of experiments as specific indicators of the solute content of the phloem sap to investigate whether the constancy in RI over prolonged periods was reflected in a constancy in the levels of these solutes. Fig. 4.2 shows the results from one experiment, in which the time course of changes in sucrose and K^+ concentrations is plotted against changes in exudation rate. This experiment was performed a total of seven times, and the results in Fig. 4.2 are representative of those recorded on each occasion. Incisions were made at 0.5 min (not shown), 35.6 min and 81.2 min, and sap samples were collected as aliquots of $25 \times 10^{-9} m^3$; for sucrose and K^+ analyses, these samples were diluted to $10 \times 10^{-6} m^3$ and the assay carried out in duplicate. As regards exudation rate, the graph shows that the incisions brought about an immediate response in sap flow (top graph). At $t = 35.6$ min the change was only transitory, suggesting that the fresh incision had only partly removed the obstruction to flow around the site of the previous incision; at $t = 81.2$ min, a substantial increase resulted, and the rate was maintained subsequently at higher values than found with the previous incisions. In addition, it may be noted that the exudation rates were high compared with values from soil-grown plants of comparable size (cf. Fig. 4.1), and also that the volumes of sap exuded were relatively large (top scale marked in units of $100 \times 10^{-9} m^3$; shoot fresh weight ≈ 100 g).

The sucrose and K^+ concentrations recorded in Fig. 4.2 showed only small fluctuations despite the large changes in exudation rate (middle graph). Over the course of 80 min the sucrose concentration decreased by less than 15% of the initial value, and this is comparable with the trends observed in sap RI during exudation. A slight transient fall in sucrose levels was observed immediately after each incision, but over most of the time course the levels were relatively constant. The K^+ concentrations exhibited a similar constancy and varied over this time period by less than $\pm 8\%$. The results thus indicate that the independence of sap RI from exudation

Fig. 4.2 Time course of changes in exudation rate together with sucrose and K^+ levels in phloem sap in response to successive incisions (denoted thus \uparrow) in the stem internode of a water-cultured plant. *Top graph:* changes in exudation rate. *Middle graph:* changes in sucrose and K^+ concentrations. *Bottom graph:* changes in sucrose and K^+ flux. Scale at top of figure indicates collection of successive units of $100 \times 10^{-9} m^3$ of sap.



rate of these conditions was reflected in an equivalent constancy of the levels of two solutes accounting for about 75% of the sap Ψ_s .

An important quantity that can be derived from the data on exudation rate and solute concentration is the magnitude of the solute flux through the phloem. For comparative purposes, the cross-sectional area of tissue through which the sap is collected can be taken as constant from one incision to the next, given the experimental arrangement shown in Plate 2.4. The figure for solute flux is then, in effect, related to the units of specific mass transfer (SMT), with the cross-sectional area term in the denominator implicitly assumed to be equal for successive incisions in the same series. Thus, the product of exudation rate and sap concentration gives the values for sucrose flux shown in the bottom graph of Fig. 4.2. The point emphasized by presentation of the data in this form is that the marked changes observed in exudation rate corresponded directly to equivalent changes in sucrose and K^+ flux through the phloem. Because of the constancy of sucrose concentration, this implies that there was a response of solution flow through the system to the new incisions, and consequently of the processes by which solutes and water were transferred through the tissue. What must be established is whether the solutes appearing in the exudate during the course of such experiments represented substances that had been loaded into the phloem since making the first incision in the series. If this can be demonstrated, then the method offers a potentially valuable means of examining the responses of the transport system as a whole to changes in the source-sink balance.

Quantification of the cross-sectional area of the transport pathway was carried out using a light microscope to examine transverse sections of stem tissue stained with lacmoid. Callose deposits associated with the sieve elements constituted one criterion by which these cells were identified. In addition, the sieve elements possessed clear lumina and were situated adjacent to companion cells, which were stained dark brown; transverse sieve plates without callose deposits were also observed in a small proportion of the sieve elements. Bicollateral bundles of phloem tissue were not found in stem sections. The parenchyma cells, although highly vacuolate, were distinguished from the sieve elements by the presence of a conspicuous nucleus and a layer of cytoplasm close to the cell wall. Detailed tissue plans were made on cartridge paper of the cell types found between the

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cambium and phloem fibres, a camera lucida arrangement being used for the transposition. The cross sectional area of the cells identified as sieve elements was determined by cutting the cell types out from the drawing and weighing: the weights were converted to areas by calibration against standard sizes of paper, and then by proportion to account for the magnification factor involved in the microscopy.

From these anatomical studies it was calculated that the sieve elements occupied 7-9% of the total cross-sectioned area of the phloem excluding phloem fibres. This is at the low end of the values found in a range of species (see Canny, 1973). The figures for Ricinus are likely to represent something of an underestimate because the values were not based on a comparison of serial sections from the same segment of stem. To be set against this, however, is the possibility that not all of the sieve elements were actually conducting sap. Milburn (1972) has previously estimated that in Ricinus the sieve-element cross-sectional area is unlikely to exceed 8% of the bark, and direct # identification of cell types in sections of Ricinus peduncles showed that sieve elements occupied 5-8% of the phloem area including fibres (Colquhoun, 1976).

If a figure of 10% is taken for this proportion as an approximation, it can be calculated that the sieve elements occupy about 0.8% of the cross-sectional area of the stem including pith. With the assumption that this relationship is roughly the same in all parts of the shoot, the phloem-transport pathway can then be regarded as making up of the order of 0.8% of the total volume of the shoot. To take the example shown in Fig. 4.2, in which the volume of the shoot above the site of the incision was about $75 \times 10^{-6} \text{ m}^3$, the total sieve element sieve-tube volume will have been around $600 \times 10^{-9} \text{ m}^3$. With the observed rates of sap exudation, the equivalent of this volume was therefore displaced roughly once every 25 to 30 min. Since the solute content of the sap remained relatively constant throughout exudation, this demonstrates that the observed rates of solute flux could only have been sustained by loading of new material into the phloem.

These considerations indicate that if there is bulk (mass) flow of solution through the phloem, then the large changes in solute flux found on making fresh incisions may be associated with equivalent changes in the rate of solute loading. The experiments discussed in the next two sections attempt to investigate this relationship more directly,

but there is one further aspect of the sucrose and K^+ content of the sap that requires comment beforehand. This concerns the fact that under certain conditions the sucrose and K^+ levels in aphid honeydew or sap exuding from severed stylets have been found to vary reciprocally. Hoad and Peel (1965a) observed that when the K^+ concentration in the phloem sap was raised by perfusion of isolated stem segments with K^+ , the sucrose concentration in the sap declined with the same time course. Conversely, when the sucrose levels in the sap decreased over a period of days as a result of respiratory loss from the segment, there was a tendency for the K^+ level to increase. This reciprocity was also observed during exudation from several stylets over periods of a few hours, although the magnitudes of the changes were different in the case of these short-term variations.

In the experiments with Ricinus of which Fig. 4.2 is representative, relatively high exudation rates (i.e. $> 5 \times 10^{-9} \text{ m}^3 \text{ min}^{-1}$ from incisions into 15-20% of the circumference of stems of eight-week-old plants) from water-cultured plants were always accompanied by a constancy of sucrose and K^+ levels in the sap. In three additional experiments, however, in which exudation rates were markedly lower, substantial fluctuations in sucrose concentrations were observed. The results from one of these experiments are shown in Fig. 4.3. The exudation rate over this time period declined slowly (top graph), but there were large changes in sucrose concentration in the sap, of the same magnitude as found in short-term experiments by Hoad and Peel (1965a). As the sucrose levels decreased there was a slight rise in K^+ level (middle graph). With the same data, though, plotted as solute flux (bottom graph), it becomes apparent that because of the declining exudation rate there was also in fact a decrease in the rate of K^+ transport.

The significance of these trends was tested by plotting the values for sucrose concentration against those for K^+ concentration found in this experiment, and then performing a regression analysis. These results are given in Fig. 4.4. In this graph the scale for K^+ concentration has been doubled relative to that for sucrose, because the K^+ will have been balanced by anions and its contribution to sap Ψ_s would thus be nearly double that of sucrose on a mol/mol basis (about 1.75 times in this part of the concentration range). The slope of the regression line (-0.057) indicates that large changes in sucrose levels were associated with only small changes in K^+ levels,

Fig. 4.3 : Time course of change in exudation rate together with fluctuations in sucrose and K^+ levels in phloem sap in response to an incision (at $t = 47.6$ min) in stem internode of a water-cultured plant. *Top graph*: change in exudation rate. *Middle graph*: changes in sucrose and K^+ concentrations. *Bottom graph*: changes in sucrose and K^+ flux.

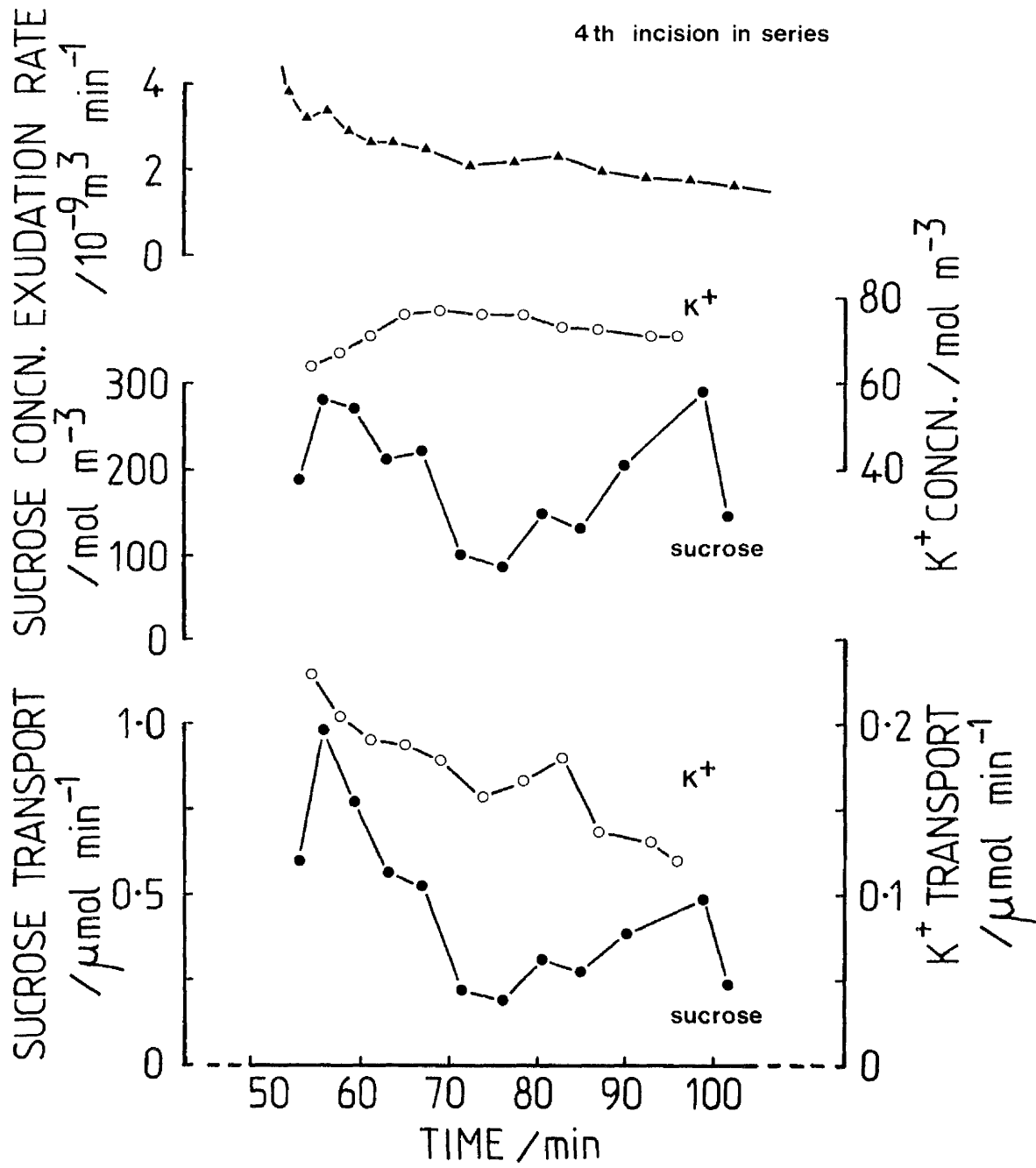
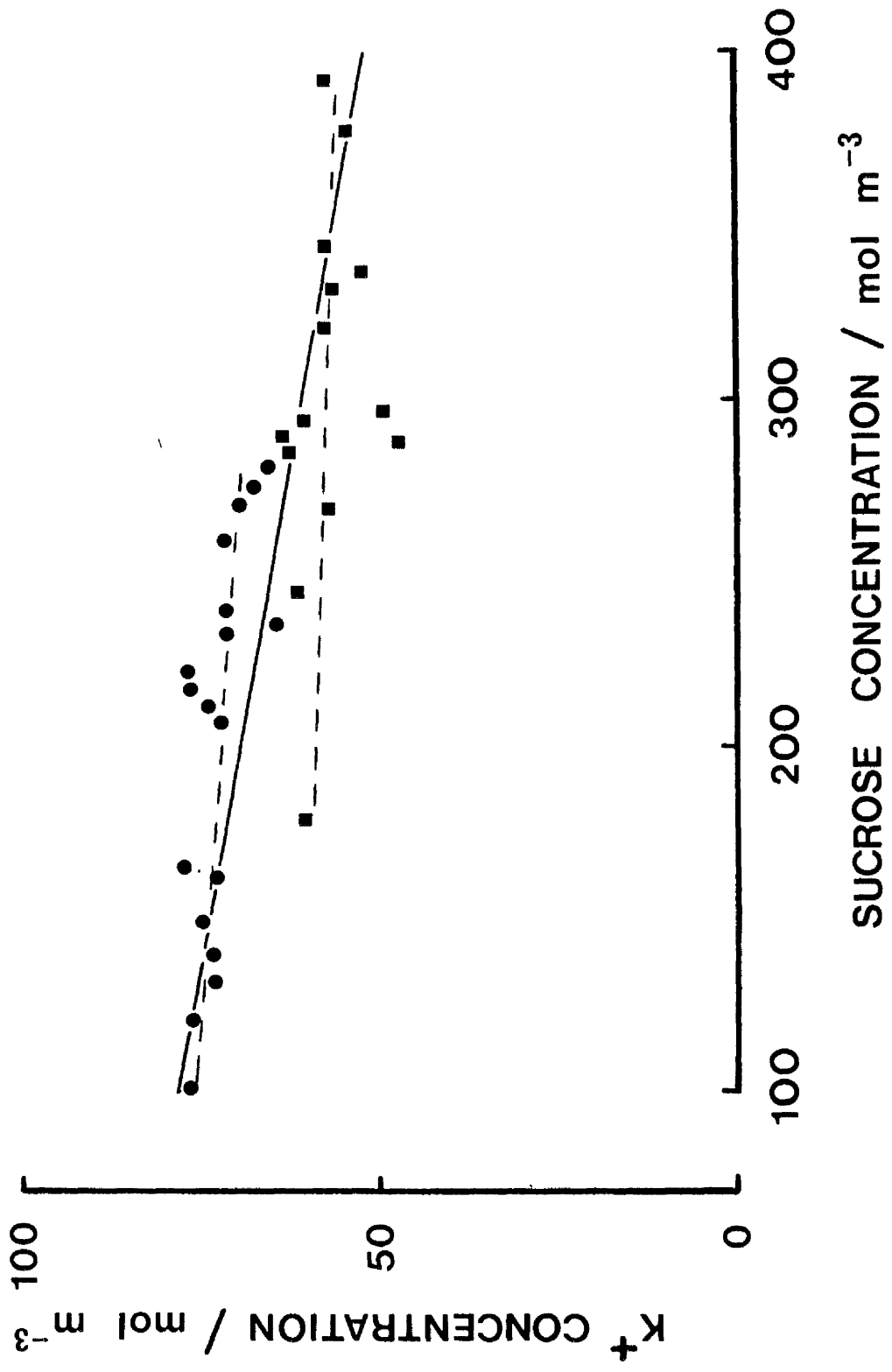


Fig. 4.4 Plot of sucrose concentration against K^+ concentration for data from two successive incisions in experiment from which results in Fig. 4.3 were taken. Note different scales on two axes. Regression line fitted by least squares' analysis to equation $y = 86.286 - 0.057x$ ($r = 0.791, P < 0.001$).

Circles represent values from 2nd incision in series, squares those from 4th incision. Broken lines are related to these two separate populations.



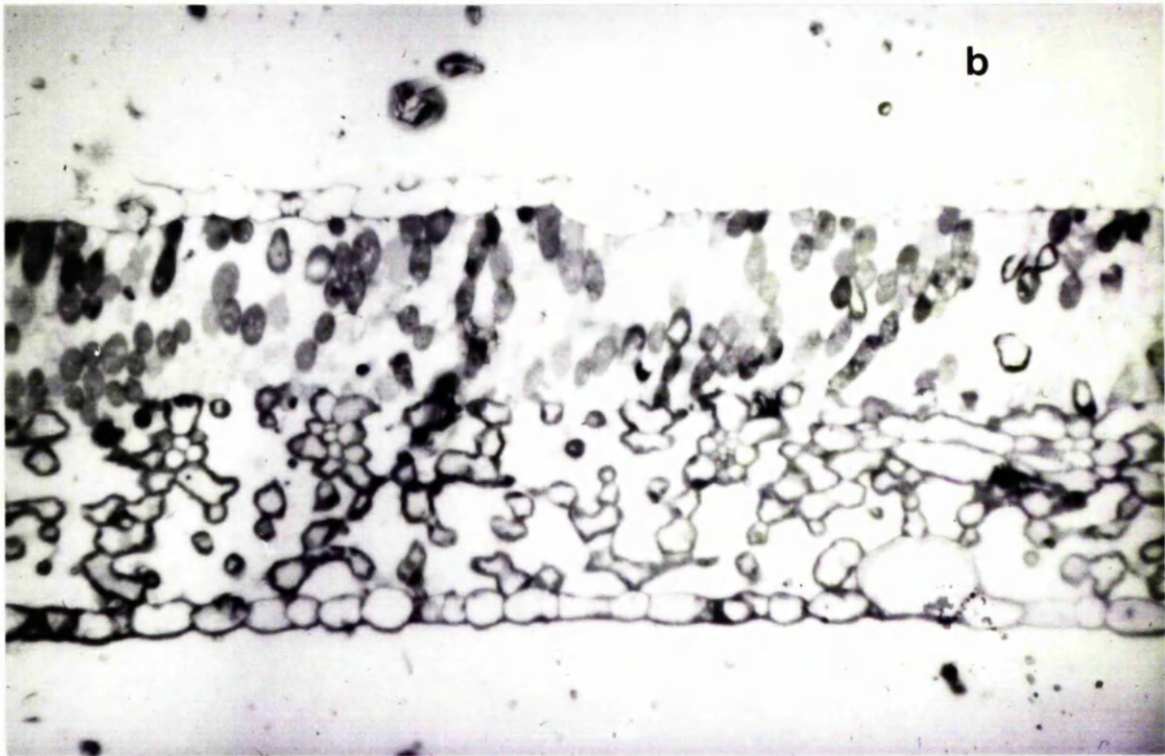
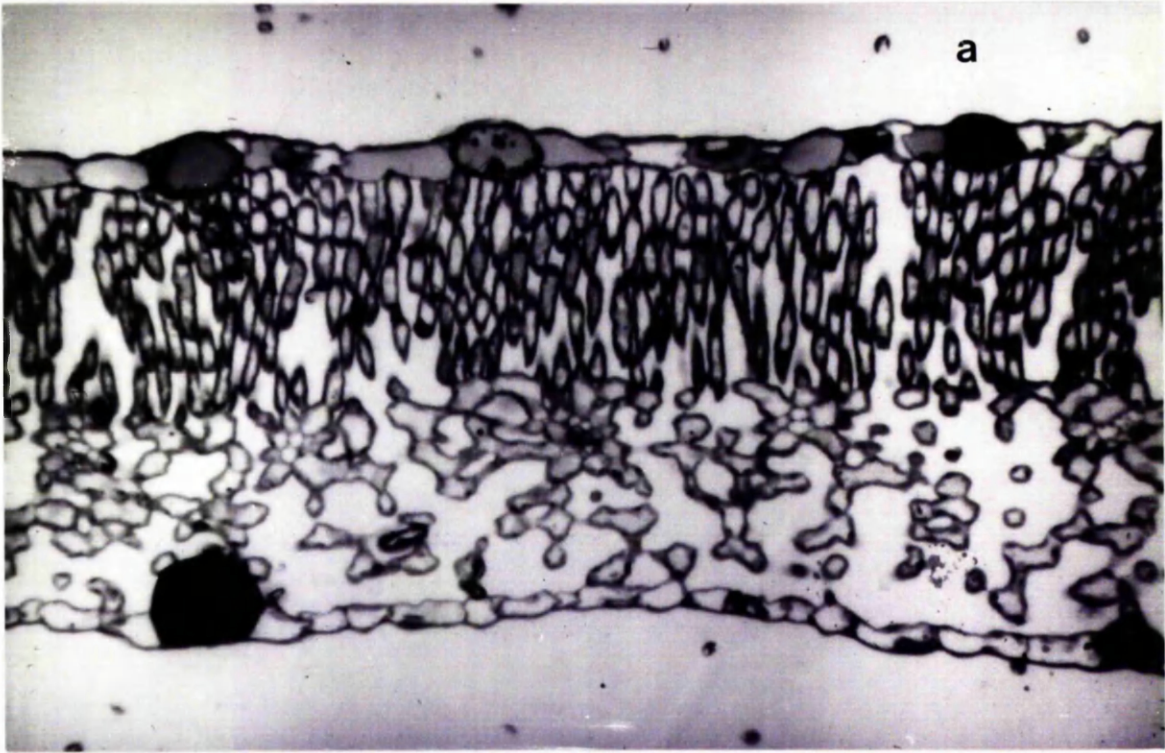
but the trend, with $r = 0.791$, was significant at the level of $P < 0.001$. Thus, although this inverse relationship between sucrose and K^+ levels needs to be explained, in none of the experiments performed did it contribute markedly towards the maintenance of sap Ψ_s .

4.3 Relationships between solute loading and longitudinal flux

A direct consequence of the large changes in exudation rate caused by successive incisions in a series and the constancy of solute levels in the sap over the same period is that substantial fluctuations occur in longitudinal flux through the sieve tubes. Since the volumes of sap exuded are large compared with the calculated total volume of the sieve tubes in the shoot, flux must be maintained by continued loading of solutes. These considerations, however, say nothing about the source of the solutes, and the following experiments were carried out to investigate the origins of the transported substances. In particular, it was important to establish whether the solutes were transported from the leaves, which from their proportion of whole-plant fresh weight alone (see Milburn, 1972) would be expected to constitute the major sites of phloem loading.

As a means of following the kinetics of solute transport from the leaves, experiments were performed in which a $50 \times 10^{-9} \text{ m}^3$ aliquot of solution containing radioactively labelled sucrose in MES buffer (pH 5.5) was applied to a small abraded area of an adaxial leaf surface. The appearance of radioactivity in the exuding phloem sap was then monitored over a time period of 2 to 7 h. The isotopes were applied to a fully expanded alternate leaf, which was assumed to be exporting assimilates. Examination of leaf sections by light microscopy demonstrated that the vascular tissue was not damaged by the abrasion procedure. Plate 4.1a shows a section of control leaf tissue and Plate 4.1b that of an abraded leaf, in which it can be seen that disruption of the cells was restricted to the epidermis and mesophyll. The abrasion permitted a faster and more direct penetration of the labelled solution to the vascular bundles than would otherwise have occurred. Further, labelled sucrose was used in preference to $^{14}\text{CO}_2$ feeding, since a portion of the labelled sucrose formed during assimilation of $^{14}\text{CO}_2$ seems to become sequestered into a storage pool, which can further complicate interpretation of the tracer profiles (see Qureshi and Spanner, 1973; Outlaw *et al.*, 1975; Wardlaw, 1976; Troughton *et al.*, 1977).

Plate 4.1 Transverse sections of leaf tissue. a Control leaf.
b Leaf with adaxial surface abraded with Aloxite
powder: vascular tissue is not disrupted. Sections
stained in toluidene blue. Magnification X900.



The technique was first used with a solution containing both ^3H -sucrose and ^{14}C -sucrose to test for possible isotopic discrimination at any stage during uptake and transport of the tracers. The highest concentration of sucrose supplied in labelled form was 0.4 mol m^{-3} . Fig. 4.5 shows the results from one experiment of this type, using a soil-grown plant, the experiment having been performed twice with similar results on both occasions. Sap exudation was maintained continuously during the experiment. The fastest component of the front of radioactivity appeared in the sap after about 10 min, the levels of radioactivity themselves only increasing very gradually after 30 min. The results show, however, that both the ^3H and ^{14}C label appeared at the same time in the sap, and that the ratio of $^3\text{H}:^{14}\text{C}$ radioactivity remained relatively constant over the time course. An important point is that the mean $^3\text{H}:^{14}\text{C}$ ratio in the sap (around 1.8) was significantly lower than that in the solution applied to the leaf surface (2.4). It seems unlikely that the tritium would have exchanged with hydrogen atoms to any great extent at these pH values (close to neutrality), but it is possible that the discrepancy represented molecular discrimination at the sites of loading. At any rate, this did not affect the usefulness of the ^3H compared with the ^{14}C tracer for the study of transport kinetics, because the isotope ratio manifestly remained more or less constant during the course of exudation.

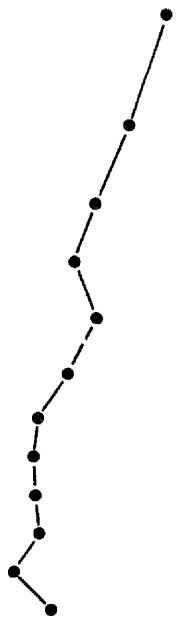
The form of such tracer profiles is determined partly (and perhaps mainly) by the kinetics of loading in the leaves. An element of temporal variation is introduced by the differential rates of transfer into the phloem, but this is compounded by a degree of spatial variation as the tracer diffuses away from the site of application. The magnitude of this latter effect was investigated by removing a series of leaf discs at the end of one experiment from the abraded leaf, radiating outwards as a transect from the site of application to the edge of the lamina. The radioactivity was removed from the leaf discs by methanolic extraction. The results presented in Fig. 4.6 show the radial distribution of radioactivity about 5 h after application of the tracers, and indicate that over longer time periods this source of variation might modify considerably the shape of the profiles.

One feature of the transport system that can be investigated by this means is the speed of transport between the site of tracer application and the stem incision. Similar experiments were therefore conducted to investigate the kinetics of transport more closely. As

Fig. 4.5 Appearance of ^3H and ^{14}C label in phloem sap exuding from stem incision following foliar application of a solution containing ^3H -sucrose and ^{14}C -sucrose. Experiment carried out with soil-grown plant. Graph also shows ratio of $^3\text{H}:^{14}\text{C}$ label in phloem sap.

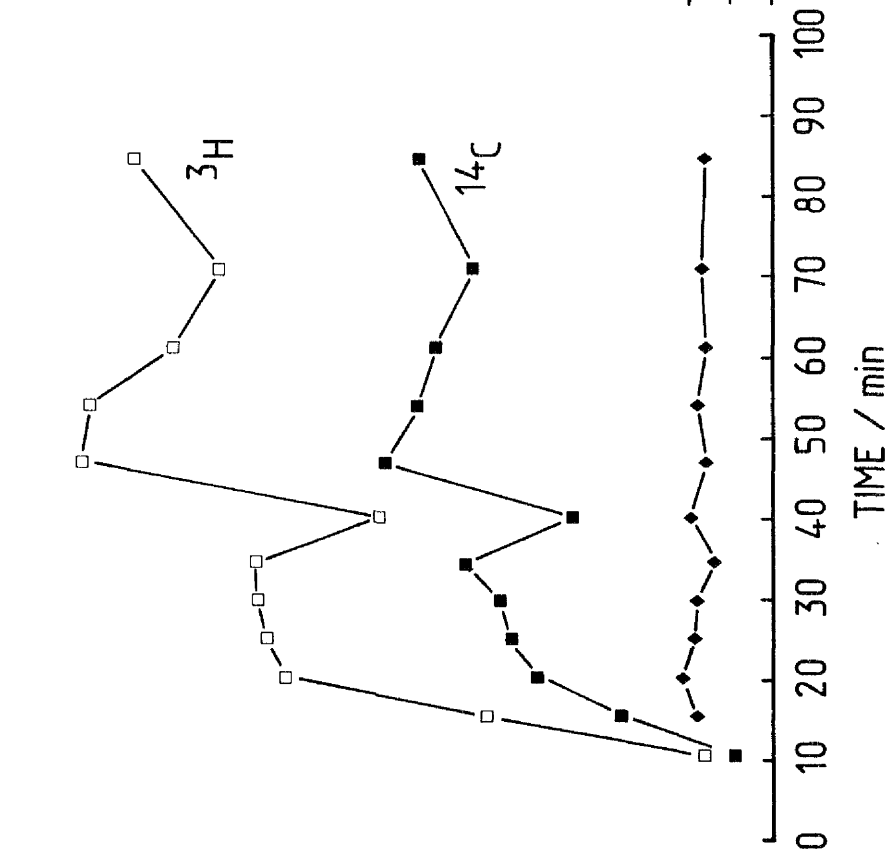
EXUDATION
RATE /
 $10^{-9} \text{ m}^3 \text{ min}^{-1}$

3.0
2.0
1.0
0



TRACER CONCENTRATION / Bq sample⁻¹

15
10
5
0



³H:¹⁴C RATIO

2.0
1.0

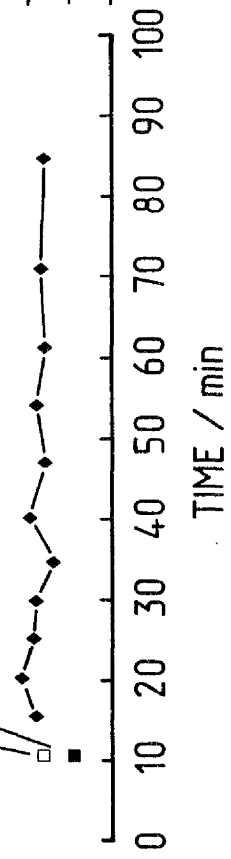
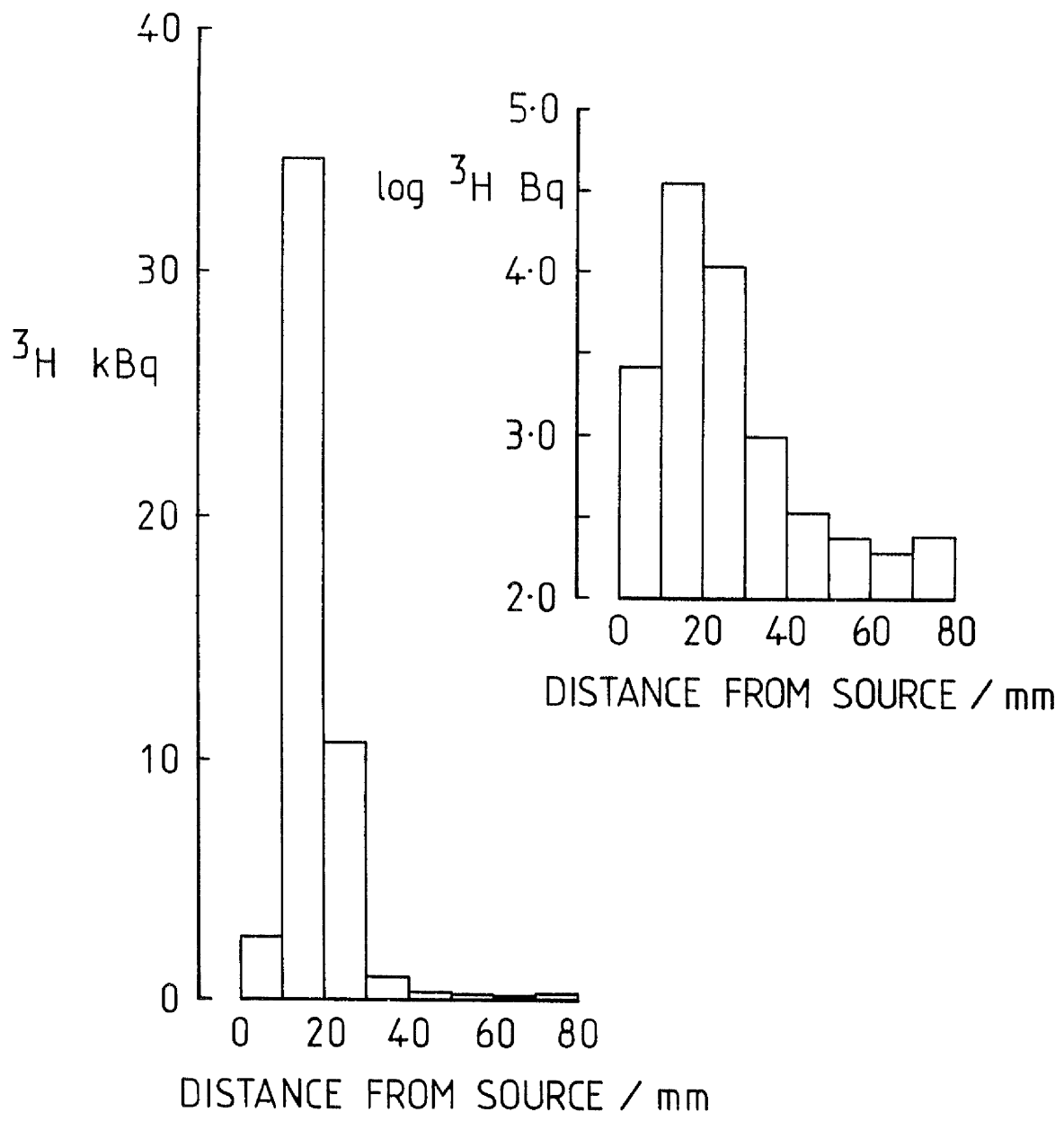


Fig. 4.6 Radial distribution of ^3H label in leaf lamina 5 h after application of ^3H -sucrose to abraded area of adaxial epidermis at source (= 0 mm).

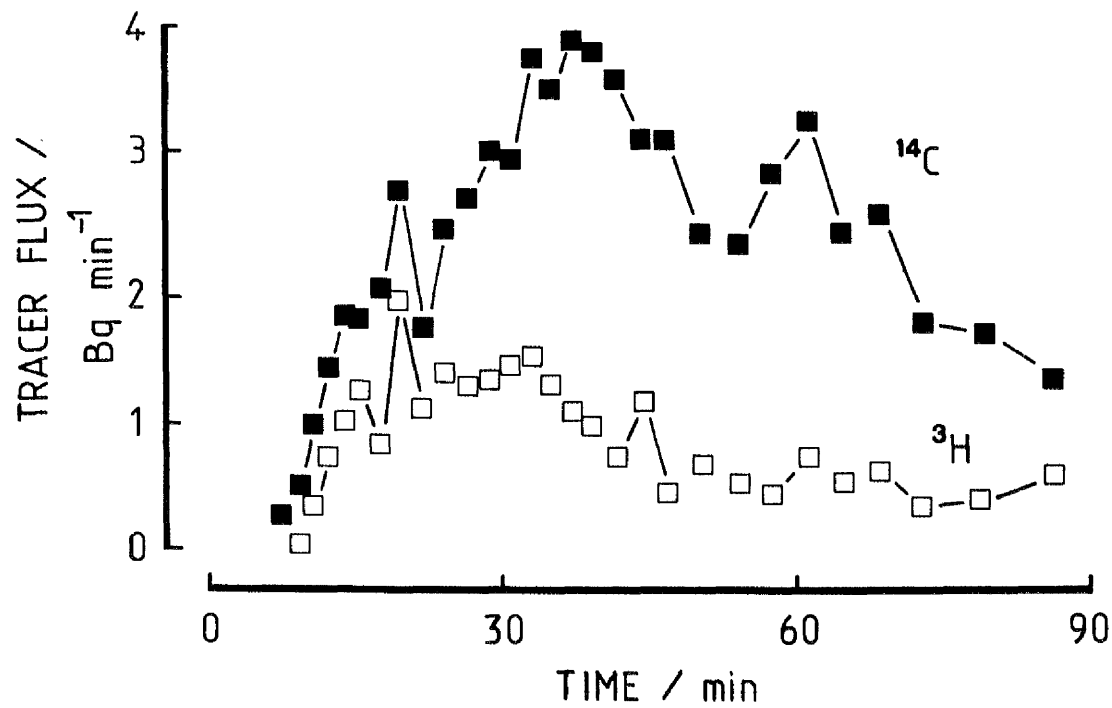
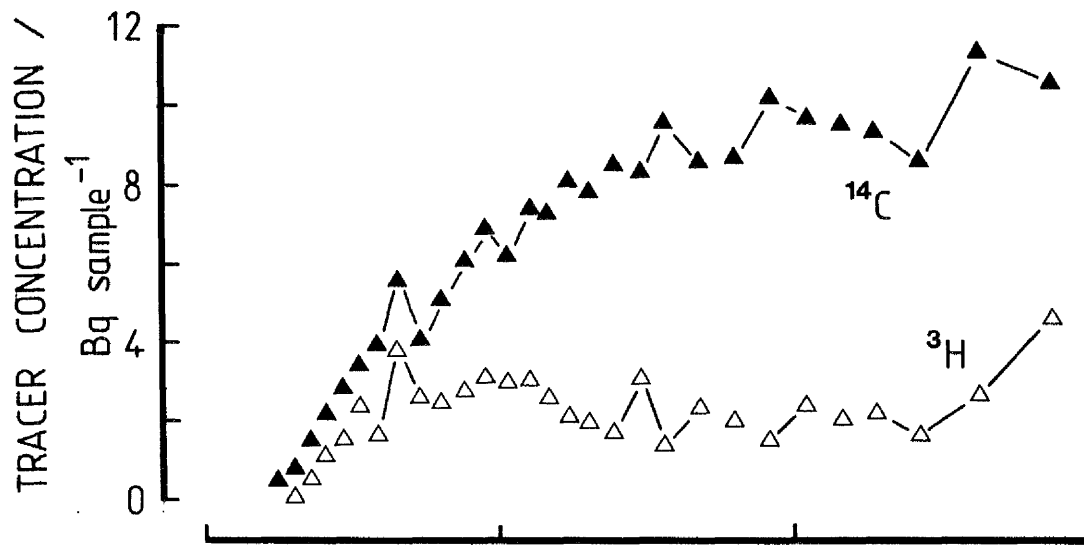
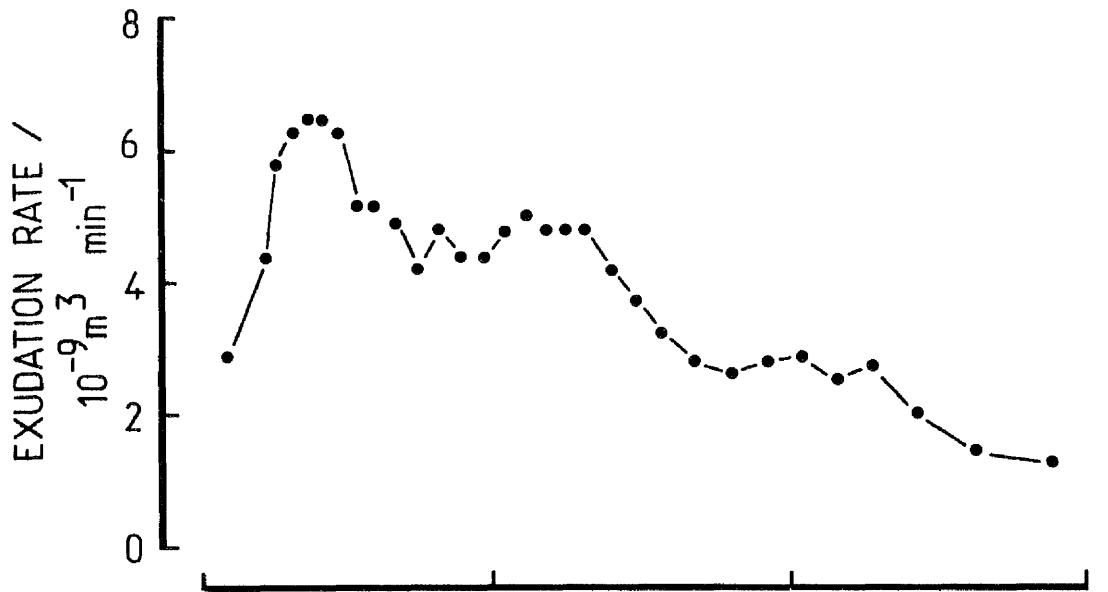


with the analyses described in the previous section, the results can be considered both in terms of transport of label per unit volume of sap and transport per unit time (i.e. flux). In order to compare the kinetics of transport from labelled sucrose with those of other compounds known to be transported in the phloem in Ricinus, double-label experiments were carried out using either ^3H -ABA or ^3H -IAA in combination with the ^{14}C -sucrose. These compounds were used because they occur endogenously in the sap in Ricinus at concentrations that have no significant effect on the osmotic relations of the phloem (see Hoad, 1973; Hall and Medlow, 1974), yet their loading and transport are presumably influenced by the mass flow of assimilates and rates of longitudinal transport. The characteristics of these processes specifically relevant to aspects of hormone transport in the phloem have been discussed elsewhere (Smith, Weyers and Hillman, 1978).

Fig. 4.7 shows the results from one experiment in which tracer profiles were determined following application of ^{14}C -sucrose and ^3H -ABA to a leaf surface in the normal manner. These results are characteristic of those obtained from five experiments of this sort performed with soil-grown plants. The exudation profile (top graph) represents the time course of exudation from a single incision made at $t = 0$ min. In terms of tracer per unit volume (middle graph), the fronts of ^{14}C and ^3H label appeared at similar times, although the amount of ^{14}C tracer recovered per sample continued to increase after $t = 25$ min. Plotted as the flux of tracer (bottom graph), the transport of ^{14}C tracer itself started to decline after about 40 min. It is possible that the different profiles for ^{14}C and ^3H label were a reflection of continued accumulation of ^{14}C -sucrose in the phloem against a concentration gradient, whilst ^3H -ABA was loaded only passively in response to the prevailing diffusion gradient. Judgement on this could only be made by characterization of the endogenous pools of sucrose and ABA in the leaf, so that the values for flux might then be comparable on the basis of specific activities. Similar tracer profiles were also observed for the transport of label following foliar application of ^3H -IAA.

In some experiments it was found that the rate of transport of label did not remain steady for long periods, but instead decreased relatively quickly after the first front of label had appeared in the sap. Profiles of this sort are shown in Fig. 4.8, which again follows label derived from ^{14}C -sucrose and ^3H -ABA, but during exudation from a

Fig. 4.7 Time course of exudation rate and appearance of label in phloem sap from stem incision (at $t = 0$ min) following foliar-application of ^{14}C -sucrose and ^3H -ABA. *Top graph*: exudation profile. *Middle graph*: label per unit volume ($10 \times 10^{-9}\text{m}^3$) of sap. *Bottom graph*: label per unit time. Soil-grown plant.

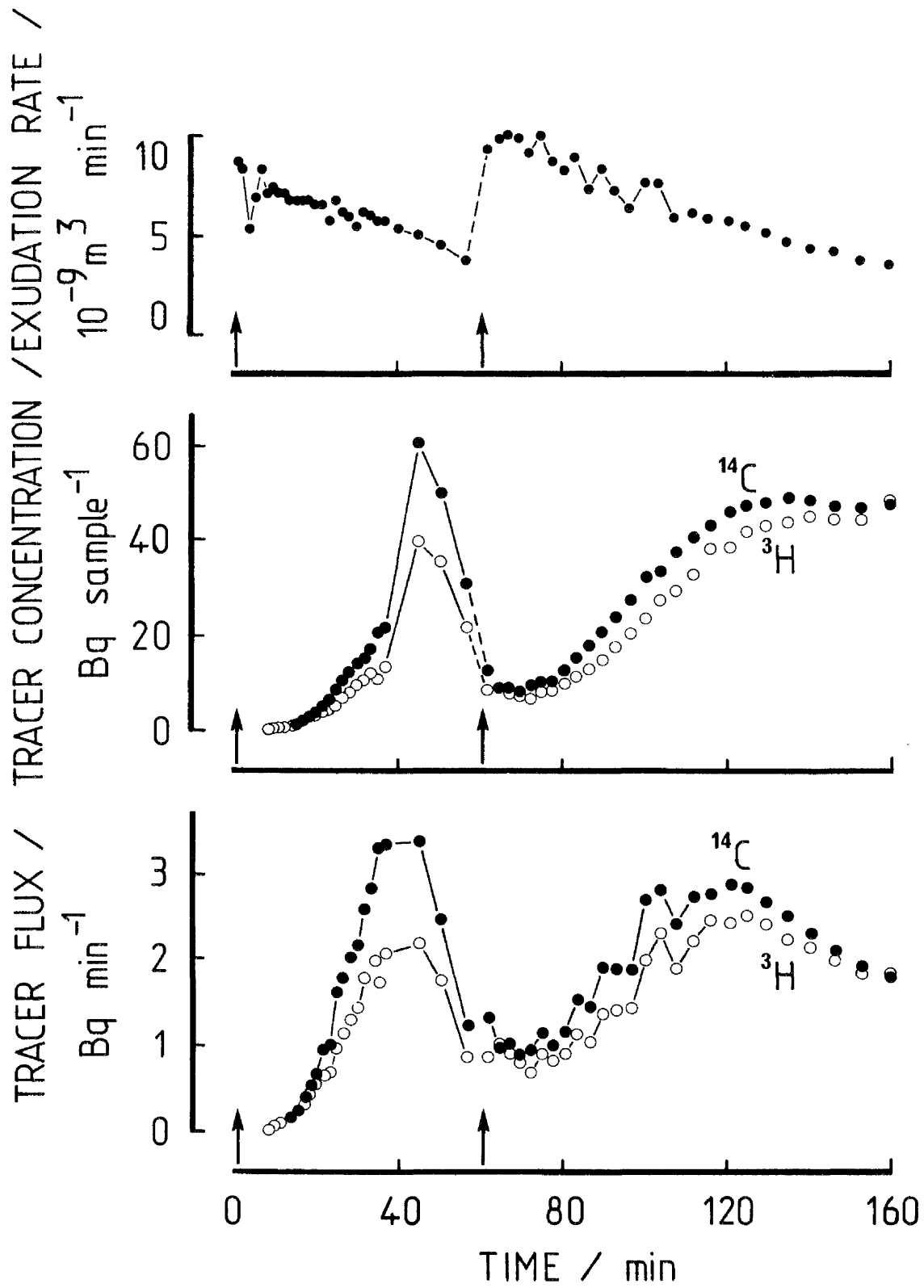


plant in which the transport system had been modified 2 d before experimentation by excising all the leaves except one, to which the tracers were then supplied. The graphs are similar in form to those of Fig. 4.7, except that a second incision was made during this time course at $t = 60.5$ min. The notable feature in Fig. 4.8 is that both the transport and flux of label started to decline soon after 40 min, before the second incision was made. The fresh incision, however, brought about an immediate increase in exudation rate, after which the transport and flux level also rose gradually (and to almost the same levels as during exudation from the previous incision). This response was observed in three experiments in which the transport of tracer had started to decline before making the second incision. It is probably reasonable to assume that over these relatively short time periods the large part of the label was still in the source leaf, rather than removed into tissues bordering the phloem in the stem (cf. Fig. 6.4). Thus, the effect of the second incision in this series seems to have been to cause a response in the source leaf that resulted in increased loading and transport of the applied tracers.

From these data it is possible to obtain some information on the speed of phloem transport in this system. For these calculations, the quantity is taken as 'an apparent mean speed of translocation measured from the time delay in arrival of the midpoint at the front of the pulse over a known path length' (Troughton *et al.*, 1977). Because there are variations in the speed of loading and of transport along the pathway, such estimates represent only mean speeds under these particular conditions. Nevertheless, they can be extremely useful, given sufficient standardization, as a means of examining transport kinetics on a comparative basis.

The appearance of a shallow S-shaped front of tracer in the phloem sap may be taken as representing the appearance of the first-loaded portion of labelled solute. Thus, the midpoint of the front occurred in Fig. 4.7 at $t = 15$ min, and in Fig. 4.8 at $t = 26$ min. The measured distance between the site of application and the incision (in most experiments around 200 to 250 mm) was then divided by this time delay to give the mean speed of translocation. For five experiments, the figure derived on this basis was 627 ± 34 mm h⁻¹. The relatively low standard error for this value indicates that there were close similarities in the speeds recorded in different experiments under equivalent conditions. However, this value probably represents a

Fig. 4.8 Time course of exudation rate and appearance of label in phloem sap from stem incision (at $t = 1.0$ min) following foliar-application of ^{14}C -sucrose and ^3H -ABA, and response of transport to a second incision at $t = 60.5$ min. *Top graph*: exudation profile. *Middle graph*: label per unit volume ($10 \times 10^{-9}\text{m}^3$) of sap. *Bottom graph*: label per unit time. Soil-grown plant with all leaves excised except source leaf.



minimum estimate of the mean speed, for two reasons. Firstly, the calculation is based on the time from application of the tracer to the leaf surface, and does not allow for the lag before the tracer was actually loaded into the phloem. And secondly, no direct estimate is available of the extent to which there was lateral leakage of the tracer from the phloem to surrounding tissues during longitudinal transport. Both these processes would tend to lower the apparent mean speed of transport.

Estimates of the SMT values given by the relationship (Canny, 1960):

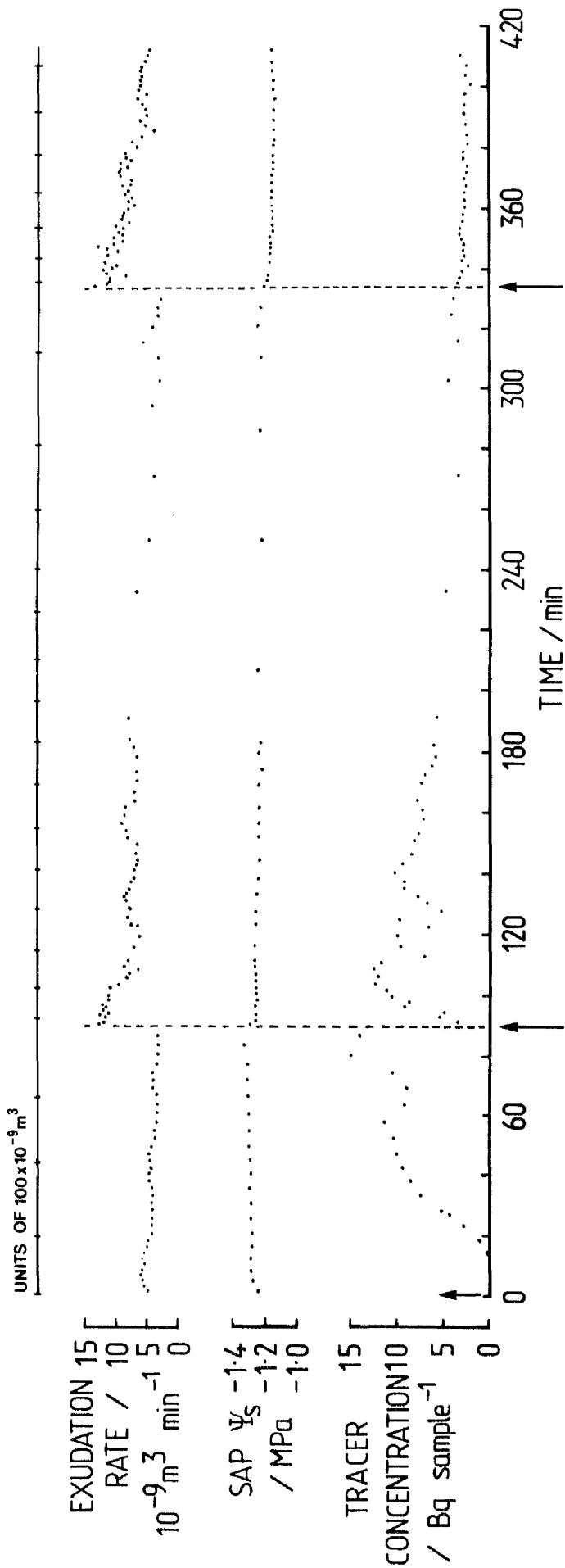
$$\begin{array}{rclcl} \text{SMT} & = & \text{speed} & \times & \text{concentration} \\ \text{g m}^{-2} \text{ s}^{-1} & & \text{m s}^{-1} & & \text{g m}^{-3} \end{array}$$

can also be derived from these results. Thus, taking the sucrose concentration as 259 mol m^{-3} (Table 4.1), i.e. 88.6 kg m^{-3} , and the speed as 0.174 mm s^{-1} , the SMT value will be $15.4 \text{ g m}^{-2} \text{ s}^{-1}$ for sucrose. For the reasons given above, a figure such as this is likely to represent the lower limit of the possible range of values.

As regards the source of the solutes in the phloem sap, these results also demonstrate in a qualitative way that there was a relatively rapid loading of solutes in the leaves and transfer to the site of the incision. Furthermore, the calculations in the previous section indicated that, even with rather higher rates of exudation than observed here, the equivalent of the total volume of the sieve tubes in the shoot would take 25-30 min to be displaced. The fact that the midpoint of the tracer front always arrived at the incision within this time (accepting that the mean speeds were minimum estimates) suggests that there was preferential transport of sap from the source leaf compared with the rest of the plant. A corollary of this is that the comparison sets an upper limit on the extent to which solute loading from cells surrounding the sieve tubes could have contributed to the solute flux. It was also shown in these experiments that excision of the source leaf resulted in a rapid decline in flux of label per unit time, to levels marginally above background within 60 min.

One further experiment of this type was carried out with a water-cultured plant to examine how the kinetics of changes in tracer transport were related to those of the bulk solute content of the sap over a prolonged period. The results presented in Fig. 4.9 illustrate the time course of these changes over 7 h, with the tracer (^{14}C -sucrose

Fig. 4.9 Time course of responses of exudation rate, bulk solute content and amount of label in phloem sap to successive stem incisions at $t = 0.5, 90.0$ and 334.3 min following foliar application of ^{14}C -sucrose. *Top graph*: exudation profile. *Middle graph*: changes in bulk solute concentration (expressed as approximate sap Ψ_s). *Bottom graph*: label per unit volume ($10 \times 10^{-9}\text{m}^3$) of sap. Experiment carried out with water-cultured plant. Scale at top of figure indicates collection of successive units of $100 \times 10^{-9}\text{m}^3$ of sap.



only) applied to the source leaf at t_0 , and three incisions at $t = 0, 5, 90, 0$ and $334, 3$ min. Sap samples were collected in aliquots of $10 \times 10^{-9} \text{ m}^3$ to give sufficient resolution to the time course, but this also resulted in a greater scatter in estimates of exudation rate at the high end of the range (top graph). The sap Ψ_s was estimated from values of refractive index from a calibration curve relating the two variables, made up for samples analyzed during this experiment (see section 2.4). The bulk solute concentration changed only fractionally during the entire time course, and showed the dilution characteristically associated with fresh incisions into the stem (middle graph). In comparison, relatively large fluctuations were found in the transport per unit volume of tracer (bottom graph). The label appeared in a front with a shallow slope, which had at least partially levelled off by 90 min. With the second incision, however, there was a substantial dilution of the tracer, which then within 15 min was largely restored to its previous level. This almost instantaneous dilution of the tracer was much greater than that of the bulk solute content of the sap. Over the next 3 h the level of tracer in the sap declined gradually, and the 3rd incision in the series did not bring about a markedly greater dilution than found for the bulk solute levels. During the complete experimental period of 7 h, 40% of the foliar-applied tracer was recovered in the exuding phloem sap.

4.4. Transport kinetics

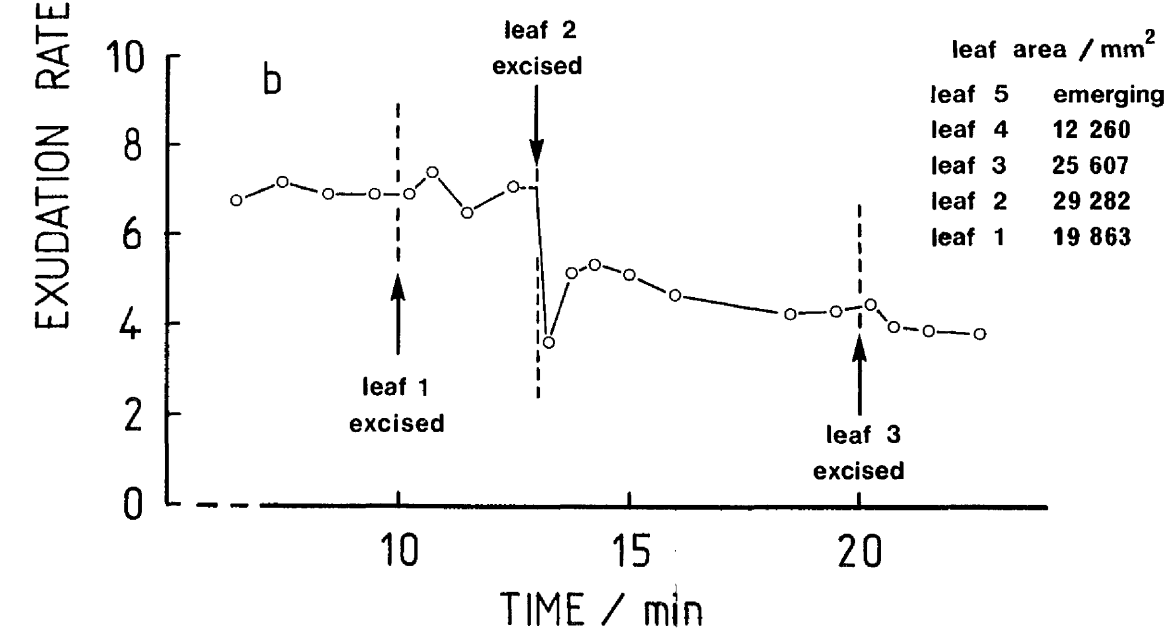
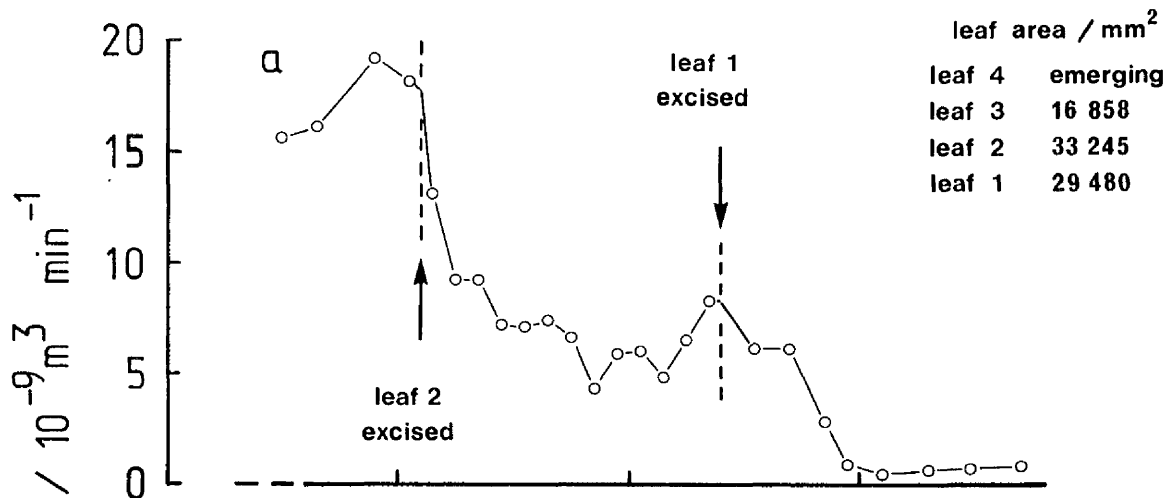
The experiments following the appearance of foliar-derived tracer in exuding phloem sap have established that the large changes in solute flux caused by stem incisions seem to be associated with transport of solutes from the source leaves. One aspect of the transport kinetics has already been considered, viz. the speed of longitudinal transport. Another consideration, however, is how fast the changes in transport (or flux) occur in response to the incisions. The increase in exudation rate resulting from a fresh incision into the stem is brought about more or less instantaneously (see Figs. 4.1, 4.2, 4.7, 4.8 and 4.9). Since columns of water are virtually inextensible and incompressible (the compressibility coefficient of water corresponding to a volume change of only $0.1\% \text{ MPa}^{-1}$; see Hüsken, Steudle and Zimmermann, 1978), the rate at which more solvent must be transferred into the sieve tubes in response to exudation is dependent on the physical characteristics of the tissue. The kinetics of these processes are considered briefly in this section.

The dependence of sap exudation on the presence of source leaves can be demonstrated by excision of the leaf laminae, which results in a very rapid decline in exudation rate (Hall and Milburn, 1973). Experiments of this sort were extended to examine the specific contributions to exudation made by individual leaves and the kinetics of changes in exudation rate. Results from two experiments are given in Fig. 4.10, in which the alternate leaves were excised at the point of inception of the petioles. Fig. 4.10a shows that excision of the two mature alternate leaves brought about an almost complete cessation of exudation, the decline in exudation rate being detected very quickly after excision of each leaf. The inference is that the two leaves were contributing most of the solutes to exudation, and that the younger leaves and stem tissue were not able rapidly to provide equivalent sources. Fig. 10b shows similar results from an experiment with a slightly older plant, in which it is notable that excision of the oldest leaf on the plant had no significant effect on exudation rate. This last observation is important, because it demonstrates that the decreases in exudation rate observed were not simply the result of exposing the ends of the vascular system to air and disturbing xylem Ψ . Separate experiments in which phloem transport was interrupted without excision by ring-massaging of the petioles (cf. Milburn, 1974) also gave quantitatively similar responses in exudation, and thus are consistent with this view.

The speeds with which these decreases in exudation rate occurred were difficult to quantify, partly because they were brought about almost instantaneously by leaf excision, and partly because the exudation rates were rarely completely steady before or after treatment. However, it is possible to obtain estimates from such graphs of the time delay between excision of the leaf lamina and the observed response in exudation rates. Combined with the known distance between the sites of excision and exudation, this also allows calculation of the speed with which the change in rate of transport must have been propagated down the transport pathway (Watson, 1976). Derived in this way, these data indicate that the change in rate of transport (i.e. exudation rate) must have been effectively transmitted down the phloem at a rate of 15 m h^{-1} or greater. This is about an order of magnitude greater than the normal speed of longitudinal transport itself.

One of the general characteristics of sap exudation seems to be

Fig. 4.10 Effect of leaf excision on exudation rate (a) Excision of alternate leaves from four-week-old water-cultured plant. (b) Excision of alternate leaves from eight-week-old water-cultured plant. Areas of all alternate leaves on the plants are given in mm^2 .



its sensitivity to any form of environmental perturbation (see Hall and Milburn, 1973). An apparent correlation observed consistently in growth-room experiments, and not previously noted, was one between small changes in exudation rate and fluctuations in ambient temperature for both soil-grown and water cultured plants. The thermostatically controlled heating and ventilation unit in the growth-room in fact gave rise to cyclical variations in temperature, and an example of the correlation with fluctuations in exudation rate is shown in Fig. 4.11. This correlation was found in the six experiments in which temperature was recorded throughout. Although this phenomenon was not investigated rigorously with controlled variations in temperature, the periodicity of the temperature fluctuations was different in each experiment (ranging from 8 to 29 min), yet was always synchronously correlated with the observed changes in exudation rate. In addition, the temperature changes gave rise to large variations in humidity, which fluctuated between about 60% RH (at the temperature maximum) and 85% RH (at the temperature minimum). Further experimentation is required to determine whether the fluctuations in exudation rate are directly associated with humidity or temperature.

4.5 Discussion

Before sap exudation can be used as a model of phloem transport in the intact plant, it is important to determine the extent to which wounding induces a change in the transport properties of the system. If the characteristics of exudation can be defined with sufficient accuracy, these responses may turn out to have a direct bearing on the way in which transport is controlled during the course of natural alterations in the source-sink balance.

Table 4.2 summarizes some of the observations described in the previous sections concerning exudation from stem incisions. These points merely outline the facets of sap exudation that must be related to phloem transport if the associated techniques are to be physiologically useful. The direct observation of sap exudation discussed in section 4.2 shows that exuding sap does come from the phloem. There are also broad similarities between the chemical composition of exudate from incisions (Table 4.1; Hall and Baker, 1972; Mengel and Haeder, 1977) and sap obtained from feeding aphids in other species (Mittler, 1958; Peel and Weatherley, 1959; Weatherley et al., 1959). Since the aphid stylets have been shown

Fig. 4.11 Example of apparent correlation between sap exudation rate and fluctuations in ambient temperature (six-week-old soil-grown plant).

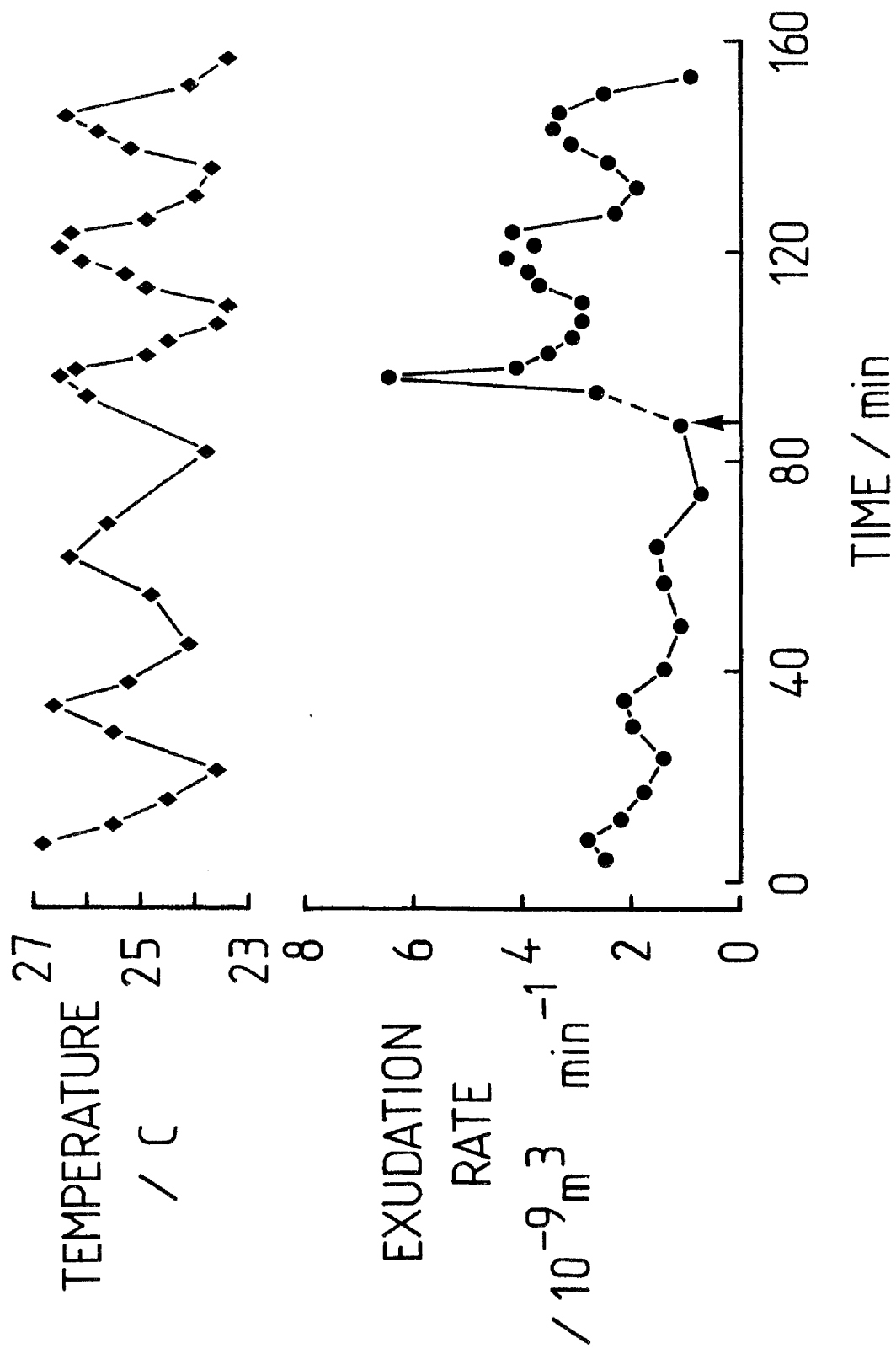


Table 4.2 Summary of results indicating that exudate from stem incisions in Ricinus is a representative sample of normal phloem sap.

observation	comments	other examples
1. Direct observation	exudate originates from phloem	<u>Cucurbita</u> (Crafts, 1936) <u>Yucca</u> (Die and Tammes, 1966)
2. Composition	similar to aphid-stylet exudate constant over prolonged periods	<u>Salix</u> (Peel and Weatherley, 1959) <u>Salix</u> (Kennedy and Mittler, 1953) <u>Yucca</u> and other monocots (Die and Tammes, 1975)
3. Source	large proportion of solutes derived from leaves	<u>Salix</u> (Ho and Peel, 1969) <u>Yucca</u> (Die, Vonk and Tammes, 1973)
4. Speed of transport	within accepted range of values, but may vary widely	Canny (1973) Troughton et al. (1977)
5. Specific Mass Transfer	within usual range, although relatively high — related to speed, not solute content	Canny (1975)

specifically to penetrate sieve elements (Zimmermann, 1961; Evert, Eschrich, Medler and Alfieri, 1968), this further supports the inference that exudate from incisions represents the contents of the transport stream (given reservations about the ways in which solute composition may be modified by the aphids themselves: see Dixon, 1975).

These characteristics suggest that in certain qualitative respects the exuding sap constitutes a sample of the solutes transported in the phloem. This issue has been discussed in greater depth by Zimmermann (1960), Crafts and Crisp (1971) and Die and Tammes (1975). There is, however, much less certainty about whether such exudate quantitatively represents a true sample of the solutes transported in the phloem. It is this question that needs to be examined in some detail. In particular, it is important to establish how the incisions affect the levels of solutes in the phloem, the transport kinetics, and also the sources of the solutes and water.

Perhaps the most striking feature observed in the experiments with water-cultured plants was the relative constancy of the bulk solute content of the sap over periods of hours (e.g. Fig. 4.9). The slight dilution associated with each incision generally lasted for less than 15 min, and over a time course of 7 h the bulk solute levels decreased by less than 10%. A relative constancy in sap concentration has also been noted in aphid-stylet exudate from Salix (Kennedy and Mittler, 1953; Mittler, 1958; Weatherley et al., 1959), from Yucca (Die and Tammes, 1966; Tammes, Die and Ie, 1971) and other monocotyledons (see Die and Tammes, 1975), and also previously from soil-grown Ricinus (Milburn, 1971, Hall et al., 1971). However, the present results obtained by analysis of successive small volumes of sap (rather than of bulk samples) indicate a much greater consistency of solute levels than recorded in these other reports. In fact, the exudation rates found in Ricinus permit a higher degree of resolution of the temporal variations in solute concentrations than is possible with the small volumes of sap obtained by the aphid techniques.

Another important feature of these results was that bulk solute concentration was largely independent of sap exudation rate (e.g. Figs. 4.1, 4.9). As a direct consequence of this, the large changes in exudation rate caused by fresh incisions corresponded to substantial changes in solute flux. The fact that both sucrose and K^+ levels remained more or less constant over the same period

(see Fig. 4.2) further suggests that these changes in flux can be regarded in practice as equivalent to changes in the rate of transport of solution (cf. Peel and Weatherley, 1963). This does not necessarily mean that there is an obligatory coupling of solute and solvent transfer into the sieve tubes, but may at least serve to describe longitudinal transport in part of the system.

The inverse relationship between sucrose and K^+ levels found under certain conditions by Hoad and Peel (1965a) appeared to be absent in Ricinus when exudation was sustained at relatively high rates. Although a reciprocity was found in three experiments in which exudation rates were low, the changes in K^+ concentration were too small to have been significant in osmotic terms. Nevertheless, the fact that sucrose and K^+ (with its accompanying anions) account for 75% of the sap Ψ_s (Table 4.1) means that the absolute K^+ level will be of considerable quantitative importance. Indeed, it has been found that there seems to be a proportional relationship between the averaged exudation rate and mean K^+ levels in sap from soil-grown Ricinus plants (F. Malek and D.A. Baker, unpublished results). The significance of the association between sucrose and K^+ transport will be returned to in the next chapter.

One aspect of these results that must be explained is why in the present experiments the bulk solute concentration of the sap remained nearly constant over prolonged periods. This is closely associated with the question of whether sap composition is 'controlled' in an osmotic (as opposed to a metabolic) sense. That is, the solute levels must be considered not simply as a consequence of solute flux into and out of the tissue, but also of net solvent movement. This point is forcefully demonstrated by results from experiments with Salix and Ricinus in which changes in water availability were imposed, and were found to have marked effects on sap exudation. In studies with isolated stem segments of Salix, Weatherley *et al.* (1959) showed that when xylem Ψ was lowered by perfusion of the stem with osmoticum, the concentration of the sap exuded by aphid stylets increased (i.e. sap Ψ_s decreased). Moreover, when xylem Ψ was raised again with water, the sap Ψ_s increased back to its earlier level. With de-rooted Ricinus shoots, removal of the water supply brought about an immediate decrease in exudation rate, and restoration of the water supply produced a rapid increase in rate (Hall and Milburn, 1973).

These observations indicate that phloem-sap exudation is markedly

influenced by xylem Ψ , and the speed of the responses in Ricinus suggests that the L_p of the membranes separating the xylem and the phloem is relatively high, or the resistance correspondingly low. (However, the half-time for water exchange between the tissues will also be influenced by the value of the elastic modulus of the cells.) In other words, lowering xylem Ψ will tend to cause an osmotic withdrawal of water from the phloem down the modified water-potential gradient, and vice versa. Thus, one interpretation of the significance of the constant sap Ψ_s of exudate from the water-cultured plants is that it is simply a reflection of a constant xylem Ψ . This would be consistent with the fact that bulk leaf Ψ in water-cultured Ricinus plants does not change over a wide range of transpiration rates (Tinklin and Weatherley, 1966). Consequently, if the xylem presented a relatively low resistance to water flow through the plant, xylem Ψ might remain correspondingly steady even with changes in water flux. In some species, though, the stem xylem can constitute a significant resistance to flow (see Kaufmann, 1976), but this is perhaps less likely to be the case in young plants grown in water culture, which have probably not suffered extensive cavitation in the xylem vessels.

Consideration of the effects of xylem Ψ therefore represents one way of examining the significance of changes in sap Ψ_s . The importance of this approach, as stressed earlier, lies in the fact that the phloem will almost inevitably show osmotic responses to water availability. And these responses, which are conventionally regarded as passive, must be distinguished from those caused primarily by changes in solute transport processes. Peel and Weatherley (1962) have suggested, for instance, that the changes in sap Ψ_s found in aphid stylet exudate during alternating periods of light and darkness arose principally from associated changes in xylem Ψ (see also Peel, 1965; and cf. Milburn and Zimmermann, 1977a, b). To what extent, then, can studies such as these provide information on the characteristics of solute transport?

The most useful way in which to refer to movement of solute is in terms of flux i.e. the amount transported per unit time (per unit cross-sectional area). Thus, in the experiments of Weatherley et al. (1959) and Hall and Milburn (1973), the changes in water availability affected not only sap Ψ_s but solute flux as well. With regards to the origins of the solutes, however, the solutes collected in the first volumes of sap are likely to have been those

already within the sieve tubes prior to incision. Although in this sense the solute flux will be the result of longitudinal movement through the sieve tubes, it is also important to determine whether the flux is related to movement into the tissue. The calculations presented in section 4.2 based on anatomical investigations indicated that the sieve tubes occupied about 0.8% of the volume of the shoot. Consequently, with the usual exudation rates, the equivalent of the total volume of the sieve tubes was displaced roughly once every 25 to 30 min. This shows that the large changes in solute flux resulting from successive incisions were maintained by continued solute loading into the phloem.

If longitudinal transport along the sieve tubes took place in the same manner as flow through impermeable pipes, then there would be a direct correspondence between the rates of transfer out of the pipes unloading, (i.e. exudation) and transfer into them at the other end (loading). In essence, the rest of this discussion is concerned with the ways in which transfer through the phloem differs from this hypothetical condition. One of the important features of the sieve tubes is that there appears to be influx and efflux of both solutes and solvent along the pathway. However, the extent to which there is net flux into and out of the phloem down the stem seems to vary considerably between species. Thus, the question of where the bulk of the solutes are being loaded is of considerable importance.

The results presented in section 4.3 on transport of foliar-applied radioactivity provide some evidence that solute loading is associated with events in the leaf. This has been shown in other studies on sap exudation with radioisotopes (Peel and Weatherley, 1962; Ho and Peel, 1969; Die, Vonk and Tammes, 1973), and in the intact plant the mature leaves certainly constitute the most important sources of assimilates. Without information on the specific activity of the tracer along the pathway, it is not possible to estimate the extent of lateral leakage of tracer from the sieve tubes versus influx of unlabelled solutes. Furthermore, interpretation of the kinetics of transport of label is complicated by the interaction of factors affecting both loading and longitudinal transport. In the present experiments, loading will have been influenced by the time course of feeding of the tracers (which were not applied as a pulse), by spatial variation in the sites of loading (cf. Fig. 4.6), by the mixing of radiochemicals with endogenous pools, by the kinetics of

transfer to the sites of loading, and by the mechanism of uptake into the sieve elements themselves. In turn, the kinetics of longitudinal flow will have been the result of net efflux of tracer along the pathway, the speed distribution between different sieve tubes, and the speed distribution within each sieve tube (Fisher, 1975; Tyree and Dainty, 1975; Troughton *et al.*, 1977; Christy and Fisher, 1978). Thus, quantitative estimates of the kinetics of transport, as for the mean speed of longitudinal flow, represent average values determined by all these variables.

For these reasons, the speed of transport of 0.63 m h^{-1} computed from the tracer profiles is both a mean value and probably also a minimum estimate. Loading of tracer into the phloem after $^{14}\text{CO}_2$ feeding can take 3-5 min (Moorby, 1977), and although ^{14}C -sucrose may reach the site of loading more quickly when supplied directly to the leaf, there will still have been a significant lag before tracer appeared in the sieve tubes. A number of tracer studies have also shown that there may not be very much lateral leakage or exchange from the pathway, one line of evidence being that the shape of the tracer profile in the stem seems to be a close reflection of source-pool kinetics in the leaves (see Evans *et al.*, 1963; Qureshi and Spanner, 1973; Troughton *et al.*, 1974; Fisher, 1975; Geiger, 1976*b*; Troughton *et al.*, 1977; Fisher, Housley and Christy, 1978). Lateral transfer of solutes between the phloem and surrounding tissues is manifestly important in some instances (e.g. Peel and Weatherley, 1962; Hoad and Peel, 1965*a, b*; Grange and Peel, 1978), but it is not clear exactly what determines the extent of lateral exchange. This may turn out to be a function of the size of the solute pools in the vascular parenchyma in relation to the relative capacities of source and sink.

Calculation of SMT values for Ricinus on the basis of these estimates of mean speed gives values of around $15 \text{ g m}^{-2} \text{ s}^{-1}$ for sucrose. This compares with a value of $12.5 \text{ g m}^{-2} \text{ s}^{-1}$ ($= 4.5 \text{ g cm}^{-2} \text{ h}^{-1}$) for the whole phloem cross-sectional area quoted by Canny (1975) as common for relatively fast transport systems. However, the more relevant figure is for flux through the sieve tubes themselves, which corresponds, if the sieve elements are said to occupy 20% of phloem cross-sectional area (Canny, 1973), to a value of $62.5 \text{ g m}^{-2} \text{ s}^{-1}$. On this basis, therefore, the apparent levels of mass transfer during exudation are not exceptionally high. However, it is also possible to derive estimates using the direct measurements of sieve-element

cross-sectional area from the equation:

$$\begin{array}{ccccccc} \text{SMT} & = & \text{exudation rate} & \times & \text{concentration} & \times & \text{area} \\ \text{g m}^{-2} \text{ s}^{-1} & & \text{m}^3 \text{ s}^{-1} & & \text{g m}^{-3} & & \text{m}^2 \end{array}$$

To take as an example the values from Fig. 4.9, the observed exudation rates computed on this basis corresponded to a range of values between 47 and 225 g m⁻² s⁻¹. The fact that this is an order of magnitude higher than the values calculated from the estimated mean speeds probably results from two sources of error. Firstly, the mean speeds represent minimum estimates, as discussed previously. Secondly, the value taken for the total cross-sectional area of sieve elements may well be an underestimate, and adopting a figure of 20% for their proportion of the whole phloem (see Canny, 1973) would approximately halve the SMT values. There can be no doubt, however, that high rates of transport do occur during exudation, for SMT values of over 700 g m⁻² s⁻¹ would correspond to the exudation rates of 40 x 10⁻⁹ m³ min⁻¹ observed on some occasions (e.g. Fig. 4.2). Furthermore, values of this magnitude have been found in a number of other species (Passioura and Ashford, 1974; Die and Tammes, 1975; Milburn and Zimmermann, 1977b).

In view of what has been said about the influence of xylem Ψ , it is likely that the solute concentration in the exuding sap corresponds closely to that in the sieve tubes prior to wounding. This is strengthened by the observation that successive incisions have little effect on sap Ψ_s (Fig. 4.9). The implication is that the large changes in solute flux correspond to equivalent changes in the speed of longitudinal transport, assuming that bulk flow is occurring through the sieve tubes (see Grange and Peel, 1978). However, in none of the experiments in which mean speed was estimated by tracer movement was the exudation rate much higher than 10 x 10⁻⁹ m³ min⁻¹. Since, on the other hand, sucrose concentrations did not vary greatly between plants, this suggests that speeds of over 2.0 m h⁻¹ may be found when exudation rates are high. There have, in fact, been very few studies on the explicit rôle of the speed component in determining SMT values, but if water-cultured plant material in general shows a similar constancy in phloem-sap concentration this might make the variables easier to study. The importance of the speed of longitudinal transport is demonstrated clearly in the work of Troughton *et al.* (1977) with *Zea*, in which it was found that different levels of irradiance alone gave rise to speeds

anywhere within the range 0.15 to 6.60 m h^{-1} , i.e. almost as wide a range as found in all the different species that have been studied (see Crafts and Crisp, 1971; Canny, 1973). Thus, the speed of longitudinal transport (and the values of SMT) might depend primarily on the strengths of the sources and sinks, and particular values may be of little significance except under carefully defined conditions.

To turn to the question of the sites of loading, there are two respects in which the experiments with tracers suggest that there was preferential transport of solutes from the leaves. One is that tracer from the ^{14}C -sucrose applied to the presumed source leaf arrived at the incision in a considerably shorter time than it would have taken to displace the equivalent of the total sieve-element volume of the shoot. The other is the more specific observation that a second incision following application of tracer apparently resulted in an increased flux of tracer from the source leaf (Fig. 4.8). Within these relatively short time periods it seems unlikely that the second profile could have been generated by loading of tracer from sites of accumulation in the stem tissue. The fact that the front of the second profile was generally shallower than that of the first (Fig. 4.8) was probably caused by loading of tracer over a larger area of the leaf following its diffusion away from the site of application (cf. Fig. 4.6). The similarity of the kinetics of hormone transport to those of the label from ^{14}C -sucrose further suggested that transport was occurring in the form of mass flow following loading. The effects of ABA and IAA on the loading process itself have yet to be examined, but the transport kinetics were similar in these experiments to those in which only labelled sucrose was applied to the leaf.

In the absence of quantitative information on the specific activity of the tracers along the pathway, the most direct demonstration of the importance of the source leaves in solute transport came from the leaf-excision experiments (Fig. 4.10). Removal of the leaf laminae presumed to be exporting assimilates brought about very large reductions in exudation rate (Fig. 4.10a), whereas excision of the leaves that were not sources had little effect (Fig. 4.10b). Exudation in these plants after leaf excision was sustained at very low rates and eventually stopped completely, indicating that the stem tissues (and younger leaves) were unable to maintain equivalent rates of solute loading (see also Hall and Milburn, 1973). In corresponding experiments with foliar-applied tracers, it was also found that

excision of the source leaf resulted in a rapid decrease in the levels of tracer found in the exuding sap (cf. experiments with Yucca quoted by Die and Tammes, 1975).

These experiments demonstrate that prolonged exudation in Ricinus is maintained by solute loading, and that the solutes involved are largely derived from the leaves. However, if the changes in solute flux caused by successive incisions also correspond in some way to alterations in the rate of loading, it is important to determine how these responses are brought about. This comes down to the question of what direct effects the incisions may have on the transport system.

The instantaneous effect of an incision into the sieve tubes will be to cause a fall in Ψ_p at the site of the incision. If the phloem sap has a Ψ_s of about -1.4 MPa (Table 4.1) and is assumed to be in equilibrium with the xylem Ψ of about -0.4 MPa (Table 3.15), this means that phloem Ψ_p will be of the order of +1.0 MPa (from equation 1.5, p.32), assuming Ψ_m to be numerically small or implicit in the Ψ_s term. There are indications from direct pressure measurements that sieve tubes are normally at hydrostatic pressures of this magnitude (see Weatherley et al., 1959; Hammel, 1968; Milburn and Zimmermann, 1977b; Wright and Fisher, 1978; J.A. Milburn, unpublished data). Thus, a fall in Ψ_p to around atmospheric pressure at the incision will represent a profound change in the water relations of the phloem in this region.

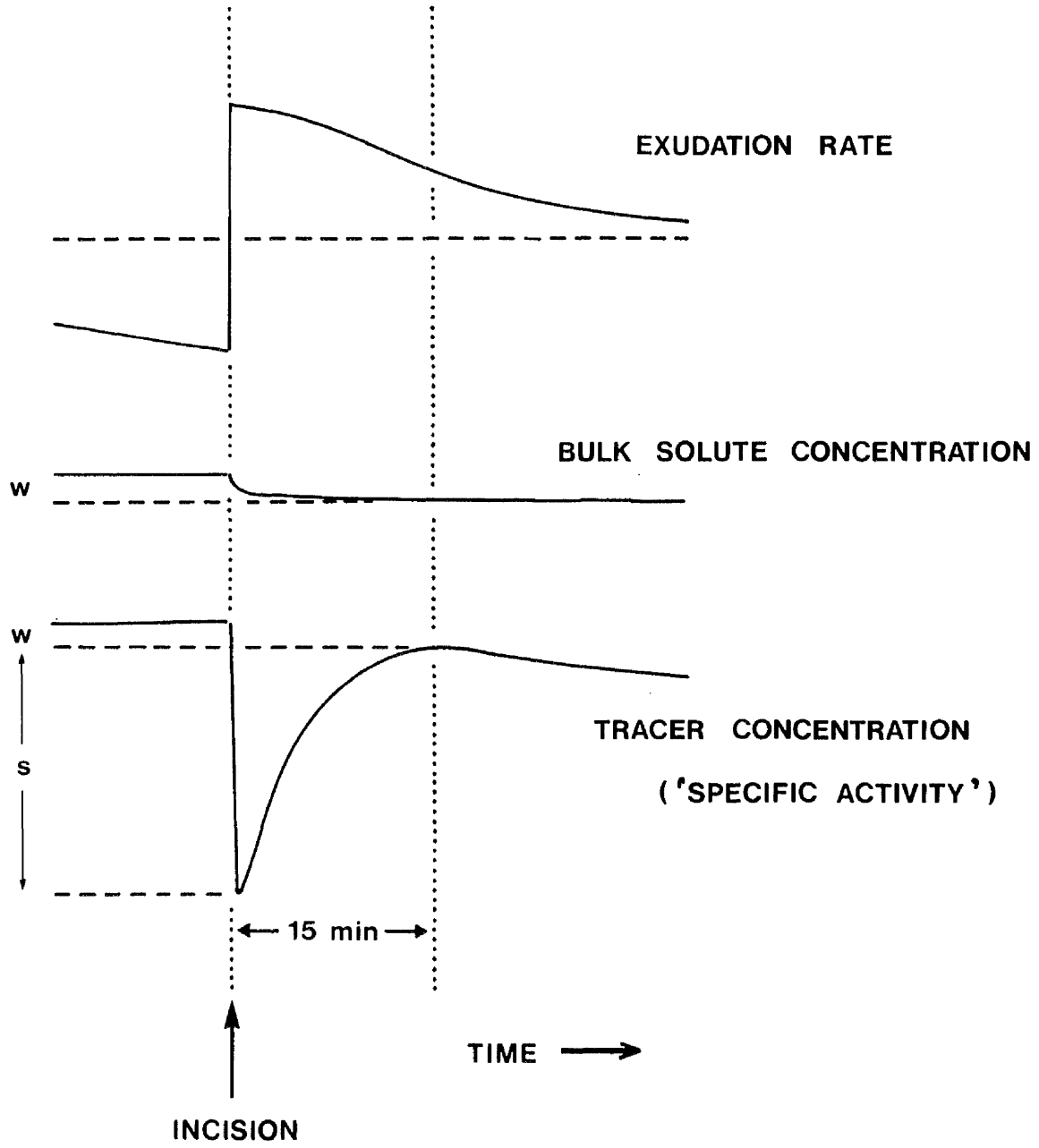
Two effects secondarily associated with the incision will be important in determining exudation rate. One is that an instantaneous fall in phloem Ψ_p will be accompanied by a decrease in phloem Ψ : this will increase the water-potential gradient between the phloem and the surrounding tissues, thereby tending to cause an osmotic influx of water into the sieve tubes. The other is that the drop in Ψ_p will not be confined to the severed sieve elements, but will be transmitted along the pathway the instant the solution within the sieve tubes moves (i.e. when exudation occurs) (Peel and Weatherley, 1962). However, the magnitude and speed of this decrease in Ψ_p along the pathway will be determined both by the resistance to flow through the pathway and the elasticity of the cell walls. If there is a gradual and localized deposition of callose on the sieve plates close to the incision (in response to wounding), this will then tend progressively to increase the resistance to flow at this end of the pathway, leading to an increase in Ψ_p in the sieve tubes.

The extent to which these responses can qualitatively account for

the kinetics of exudation can be considered in relation to the observed changes in exudation rate, bulk solute concentration and solute flux. Fig. 4.12 presents a schematic interpretation of the results obtained in the experiments of the sort depicted in Fig. 4.9, with emphasis on the changes occurring during the first 20 to 30 min following a fresh incision. Exudation rate increases immediately in response to an incision and thereafter declines slowly; it remains for prolonged periods at a higher level than found from the previous incision, and may take more than an hour to stabilize at a new value. In contrast, the net changes in the solute content (RI) and concentration of tracer in the sap are generally completed within 10 to 15 min. The bulk solute concentration decreases by about 5%, and this can be attributed to the osmotic influx of water occurring with the release of turgor. The tracer concentration also stabilizes within the same time to a level about 5% lower than during exudation from the previous incision, but in this case there is a much larger transient dilution of tracer: the concentration falls rapidly to as low as 25% of the initial level and then rises to within 5% of the original concentration by about 15 min. This effect is superimposed on the small change in bulk solute concentration. In other words, whereas the 5% dilution seems to be caused by osmotic influx of water, the further dilution of tracer must be the result of influx of phloem sap not containing significant quantities of tracer. Microscopic investigations have shown that a large proportion of the sieve elements in Ricinus possess lateral sieve areas, and thus flow could occur in response to the modified gradients of Ψ_p not just longitudinally through the sieve tubes but also laterally. This would not result in any change in sap concentration, but would dilute the tracer contained within the sieve tubes previously contributing to exudation. That the effect is only transitory may be explained by a greater reduction in the driving force ($\Delta\Psi_p$) for lateral transport compared with longitudinal flow as the sieve-plate pores near the incision become occluded by callose.

The interpretation of the effects of incisions on exudation in terms of alterations in the phloem water relations is thus qualitatively consistent with the observed characteristics of changes in sap composition. It is also important to consider whether the transport kinetics can be accounted for quantitatively on this basis. The values of the volumetric elastic modulus (ϵ) of the sieve elements and the resistance of the longitudinal pathway are critical in this

Fig. 4.12 Schematic interpretation summarizing changes observed in tracer experiments in exudation rate, bulk solute concentration and concentration of tracer in phloem sap following one incision in a series. Horizontal broken lines represent values (on a relative scale) at which the three variables tend to stabilize under steady-state environmental conditions. Net changes in concentration are completed within 15 min, w denoting dilution by water of contents of sieve tubes previously contributing to exudation, s denoting dilution by phloem sap from other sieve tubes.



respect. To take again the example of a rigid impermeable pipe, this would have an extremely high ϵ and a very low (frictional) resistance to longitudinal flow. In this case, a drop in Ψ_p at one end of the system would, as soon as flow occurs in this direction, be almost instantaneously transmitted to the other end, because water is virtually inextensible (see p.111). The sieve tubes will have a much lower value of ϵ and a much larger resistance to flow through them, but the quantitative importance of these components can be inferred from the kinetics of the response of sap exudation to change in Ψ_p .

Fig. 4.10 presented results characteristic of experiments on the effects of leaf excision on exudation rate. In practice, these were opposite in their consequences to the responses of exudation to incisions in the stem: the instantaneous reduction of phloem Ψ_p in the cut petiole to somewhere near atmospheric pressure brought about an almost immediate decrease in exudation rate from the stem incision. It was estimated that this change in rate must have been transported down the pathway at a speed of at least 15 m h^{-1} . This agrees closely with a value of $> 13 \text{ m h}^{-1}$ derived in a similar way by Watson (1976) from observations on the speed with which heat ringing caused a reduction in the rate of longitudinal transport in Helianthus. Disturbance to the transport pathway has also been found to have very rapid effects on transport in Cucurbita (Webb, 1971), Salix (Walding and Weatherley, 1972) and Ipomoea (Pickard and Hill, 1975; Pickard, Minchin and Troughton, 1978b, c). These results provide one of the strongest indications possible that a bulk flow of solution (i.e. mass flow) is occurring in the phloem through a relatively low-resistance pathway. Furthermore, the same principle may serve to explain the measured speeds of movement of 'concentration waves' down the transport pathway (e.g. Huber, Schmidt and Jahnel, 1937), which are more likely to represent a phase velocity than the true solution velocity (Ferrier, Tyree and Christy, 1975).

The speed with which these changes in rate are transmitted must set an upper limit on the resistance to flow within the phloem, this being determined largely by the sieve plates (Cataldo, Christy, Coulson and Ferrier, 1972; Tyree and Dainty, 1975). However, the speed of transmission is also dependent on the value of ϵ for the sieve tubes, since the decrease in cell volume occurring when the turgor is released on incision will dampen the speed at which the change in Ψ_p is propagated. Thus, the pressure change will be

transmitted more slowly the lower the value of ϵ (i.e. the higher the cell elasticity). It is possible to derive a value for ϵ from the data of Milburn (1972) by applying equation 1.7 (p.33). Using isolated portions of bark tissue, Milburn (1972) estimated the turgor displacement volume (TDV) of the sieve elements to be around 12%, this representing the proportion by which the cell volume decreases as Ψ_p falls from the value at full turgor to zero on incision. If this is assumed to correspond to a decrease in Ψ_p of about 0.8 to 1.0 MPa, substitution of the figures into equation 1.7 now gives an approximate value of ϵ between 6.0 and 7.5 MPa. This value is of no practical use because ϵ is itself pressure-(and volume-) dependent, increasing hyperbolically with Ψ_p ; values are consequently only relevant for small changes in Ψ_p over a defined range, of which few accurate estimates are so far available (see Dainty, 1972, 1976; Steudle, Zimmermann and Lüttge, 1977). However, it is at least similar to the values used in theoretical studies on the osmotic behaviour of the transport system (Ferrier, 1978).

The magnitude of this term is of considerable importance, because it will partly determine the speed with which phloem Ψ (and Ψ_p) responds to change in xylem Ψ . The appropriate expression for the half-time of water exchange ($t_{\frac{1}{2}}$) is given by the equation (Philip, 1958; Dainty, 1972)

$$t_{\frac{1}{2}} = \frac{V \ln 2}{A L_p (\epsilon + \Psi_s)} \quad (4.1)$$

where V and A are the cell volume and surface area, respectively, and L_p , ϵ and Ψ_s have their usual meaning. Thus, the higher the value of ϵ , the quicker will be the response to changes in xylem Ψ . Since the phloem ϵ will be higher than 7.5 MPa at full turgor, this may be an order of magnitude larger than the Ψ_s term (~ 1.4 MPa), demonstrating that ϵ will largely control the way in which phloem Ψ changes with cell volume.

Of more immediate interest is the fact that the same data allow calculation of the speed with which sap was expelled from the sieve elements in the bark tissue, taking the driving force to be represented by the release of turgor (~ 1.0 MPa). Milburn (1972) showed that this speed was 14.4 m h^{-1} , which represents the maximum speed with which flow can occur given this driving force and the fixed resistance of the sieve plates in the sieve tubes (plus frictional resistance

in the lumina). The agreement with the value of 15 m h^{-1} derived from the leaf-excision experiments demonstrates that this may constitute a reasonable estimate of the speed with which changes in pressure can be transmitted through the sieve tubes.

The almost synchronous changes in ambient temperature and exudation rate recorded in growth-room experiments may constitute further evidence that changes in the driving force for flow can have extremely rapid effects on the speed of transport. Whether these changes were directly associated with fluctuations in ambient temperature or humidity remains problematic. If temperature fluctuations were responsible, the effects must have taken place in the leaves, since the thermal conductivity of the tissues would not have allowed such changes to be perceived quickly enough in the stems or petioles to elicit the response in phase. Stimulation of solute loading would certainly increase the driving force for transport, and it is conceivable that the activity of the enzyme(s) involved in membrane transport of the major solutes is modulated by temperature, although this response would have to be extremely rapid. The alternative is that there is some association between increase in transpiration rate and enhancement of transport. There is clear evidence that stomatal aperture in some species is extremely sensitive to changes in humidity (Schulze, Lange, Buschborn, Kappen and Evenari, 1972; Hall and Kaufmann, 1975; Ackerson and Krieg, 1977), and large changes in transpiration rate may occur with little effect on bulk leaf Ψ (Camacho-B, Hall and Kaufmann, 1974; cf. Tinklin and Weatherley, 1966). The extremes of the temperature and humidity cycling in the growth-room corresponded to a change in the effective water potential of the vapour phase between about -22 MPa (at 23 C, 85% RH) and -71 MPa (at 27 C, 60% RH). This represents a large change in the driving force for evaporation at the leaf surface, but how an increase in transpiration water flux might be associated with increased osmotic influx into the phloem is not clear. Whether the primary effect is related to humidity or temperature remains to be investigated, but short-term effects correlated with temperature changes have previously been interpreted in terms of effects on solution viscosity or the biochemical sealing mechanisms in the phloem (see Tammes, Vonk and Die, 1969; Lang, 1974; Pickard, Minchin and Troughton, 1978a).

These considerations taken together indicate that the responses of the transport system to successive incisions are associated with changes

in the osmotic relations of the tissue. The high values of SMT found during exudation almost certainly overestimate the rates of transport in the intact system. This has been demonstrated in Lupinus (Sharkey and Pate, 1976) and Cocos (Milburn and Zimmermann, 1977b), in which mass transfer during exudation was compared with that occurring during fruit-filling in similar plants: in both species, exudation represented SMT values up to ten times higher than those calculated on the basis of fruit growth. The evidence in Ricinus suggests that the large SMT values are the result of high speeds of longitudinal transport rather than increases in sap concentration. An incision may therefore bring about a change in the source-sink balance in the plant by providing an artificial sink of potentially unlimited capacity. Although localized deposition of callose eventually slows down the rate of exudation, the fact that exudation can continue from fresh incisions for periods of hours with only small changes in sap composition suggests that the loading process is able to respond to the alterations in demand.

This in itself does not answer the question of how solute loading responds to changes in sink activity, yet this mechanism may be fundamental to the control of source-sink relations in the whole plant (see section 1.4). The constancy of sap concentration during exudation superficially suggests that Ψ_s may be 'controlled'. It can be argued, however, that this is not achieved by regulation of the solute transport processes, but that it is simply a reflection of a constancy in xylem Ψ , such that passive (osmotic) movement of water occurs in response to any perturbation in phloem Ψ_s (see p.115). Because successive incisions cause large changes in solute flux, it is possible instead that the effects are directly associated with the alterations in phloem Ψ_p (or conceivably phloem Ψ). As discussed above, the changes in pressure seem to be propagated through the phloem at high speeds, and thus alterations in the rate of solute loading could occur almost immediately in response to the incision. The significant point is that it is not possible to decide from the characteristics of exudation under these conditions whether phloem Ψ_s or Ψ_p is the more important component in the control of solute flux into the sieve tubes.

5. SOLUTE ACCUMULATION AND OSMOREGULATION IN THE PHLOEM

5. SOLUTE ACCUMULATION AND OSMOREGULATION IN THE PHLOEM

5.1 Introduction

The results presented thus far have shown that solute flux through the phloem can respond to artificially induced changes in sink demand. This suggests by implication that the supply of photosynthetic assimilates at the source is not effectively limiting the rates of mass transfer through the phloem under these conditions. Consequently, it is reasonable to assume that the major short-term variable in the source-sink balance was the effective sink strength. Over longer periods, however, alterations in sink demand may have effects on assimilate metabolism in the leaves (section 1.4). In addition, although the rates of mass transfer during sap exudation were high, evidence was presented consistent with the view that this corresponded to the way in which transport in the intact plant responds to changes in source-sink relationships.

These aspects of phloem-sap exudation represent the fundamental characteristics of the transport process under relatively controlled environmental conditions. In themselves, they do not help unequivocally to distinguish the mechanism by which loading responds to changes in sink demand. This chapter is therefore devoted to a consideration of how the solute content of the sap, and the loading process specifically, is affected by two forms of environmental stress. The aim was to see whether the responses of the transport system to these treatments revealed properties of the loading process that were not apparent under 'steady-state' environmental conditions. This is an important issue in relation to the biochemical characteristics of phloem-sap composition. For if the similarities that exist between the solute content of phloem sap and cell cytoplasm (section 1.3) are not purely circumstantial, it should be possible to demonstrate that there are similar constraints determining the ways in which sap composition is controlled in the two cases. One aspect of this has already been discussed, namely that the markedly different patterns of whole-plant metabolism on different inorganic N sources seem not to affect the total organic C and N content of the phloem sap. In the present chapter, the alterations in sap composition induced by the experimental conditions are considered primarily in terms of the osmotic balance of the phloem.

Firstly, changes in the bulk solute content of the sap were

examined in relation to the effects of water deficits on transport. A reduction in xylem Ψ constitutes one form of osmotic stress on the phloem, and yet there are indications that in certain species solute transport can be maintained during periods of decreased water availability (see section 1.5). Interpretation of the results from whole-plant studies is complicated by the effects of water deficits on source and sink metabolism. With Ricinus, it is possible that successive incisions into the phloem provide a way of standardizing sink demand. This would then permit a more direct assessment of how water deficits affect the driving force for longitudinal transport and the loading process itself.

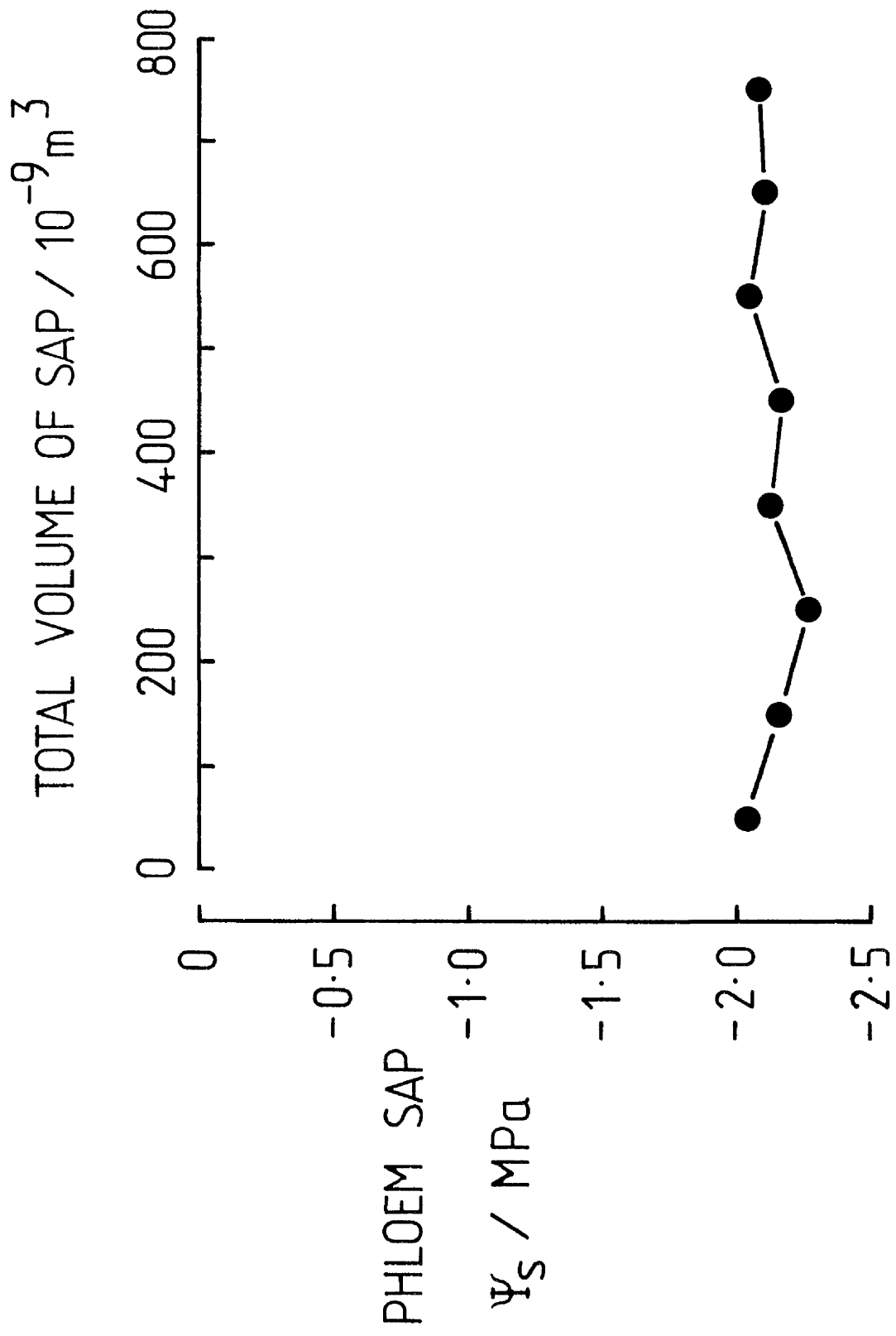
Secondly, phloem-sap composition was studied during the course of a stress on shoot metabolism imposed by continuous darkness. The intention was to examine how the solute content of the sap was influenced by a shortage of assimilates derived from photosynthetic carbon metabolism (i.e. source limitation). In particular, this constituted a means of investigating further the nature of the relationship between sucrose and K^+ levels in the phloem discussed briefly in the previous chapter. Thus, whereas the effects of osmotic stress were considered in terms of the bulk solute content of the sap, the response to continuous darkness was examined to determine the importance of individual solutes in the water relations of the phloem.

5.2 Effects of water deficits on phloem transport

The characteristics of phloem-sap exudation described for water-cultured plants in the previous chapter refer to conditions in which water availability was constant and non-limiting. However, it has also been shown that sap exudation will occur from severely wilted plants (Milburn, 1975). In this section, quantitative aspects of sap exudation are discussed in terms of the water relations of the shoot over a wide range of water deficits.

Water deficits were imposed in these experiments by excision of the root systems of the plants. This allowed low values of leaf Ψ to develop quickly, and also ensured that the shoot was the only source of solutes for exudation. Control plants with an unrestricted water supply were ring-girdled at the start of the experimental period. Fig. 5.1 shows an example of the changes in sap Ψ_s in successive $100 \times 10^{-9} m^3$ volumes of sap exuding from a water-stressed plant. There are two characteristics to be noted from this graph.

Fig. 5.1 Phloem-sap Ψ_s determined by osmometry on successive $100 \times 10^{-9} \text{m}^3$ volumes of sap exuding from an incision in stem internode of eight-week-old water-stressed plant.



One is that the bulk solute concentration of the sap remained relatively constant during exudation; in this example, the solute content per unit volume was about 50% higher than in control plants. The other is that the volumes of sap were large in terms of the calculated total volume of the sieve tubes in the shoot (in this plant $\sim 400 \times 10^{-9} \text{m}^3$). As in the experiments described in the previous chapter, this again means that exudation must have been maintained by solute loading.

The fact that exudation occurs from plants subject to water stress implies that phloem Ψ_p remains positive even under these conditions. The exact relationship between leaf Ψ and the water relations of the xylem and phloem was investigated in plants stressed for different times after excision of the roots. Fig. 5.2 presents the results obtained from individual plants over a range of values of leaf Ψ . In Fig. 5.2a the relationship is shown between leaf Ψ and both xylem Ψ and phloem Ψ_s . Pressure-bomb measurements were corrected for a value of xylem-sap Ψ_s of 0.1 MPa to give the estimates of leaf Ψ and xylem Ψ . This represented an approximate mean value for samples analyzed during the experiment; there are also indications that xylem-sap Ψ_s does not vary significantly with changing xylem Ψ (Dodoo, 1978). Xylem Ψ decreased linearly with decreasing leaf Ψ , the slope of the line calculated by regression analysis indicating that there was an almost 1:1 relationship between these variables. Phloem-sap Ψ_s was determined by osmometry on $100 \times 10^{-9} \text{m}^3$ volumes of sap and also decreased with increasing water stress, although to a lesser extent. Measurements were all made within 20 h of excising the root system, and sap flow was periodically maintained in these plants by making successive incisions into the stem. This was to eliminate the possibility that the increasing solute concentration in the sap (i.e. decreasing Ψ_s) could have been caused by continued solute loading into the phloem in the absence of transport. The constancy of solute levels during prolonged exudation (Fig. 5.1) also provided evidence that solute loading was maintained during periods of water stress.

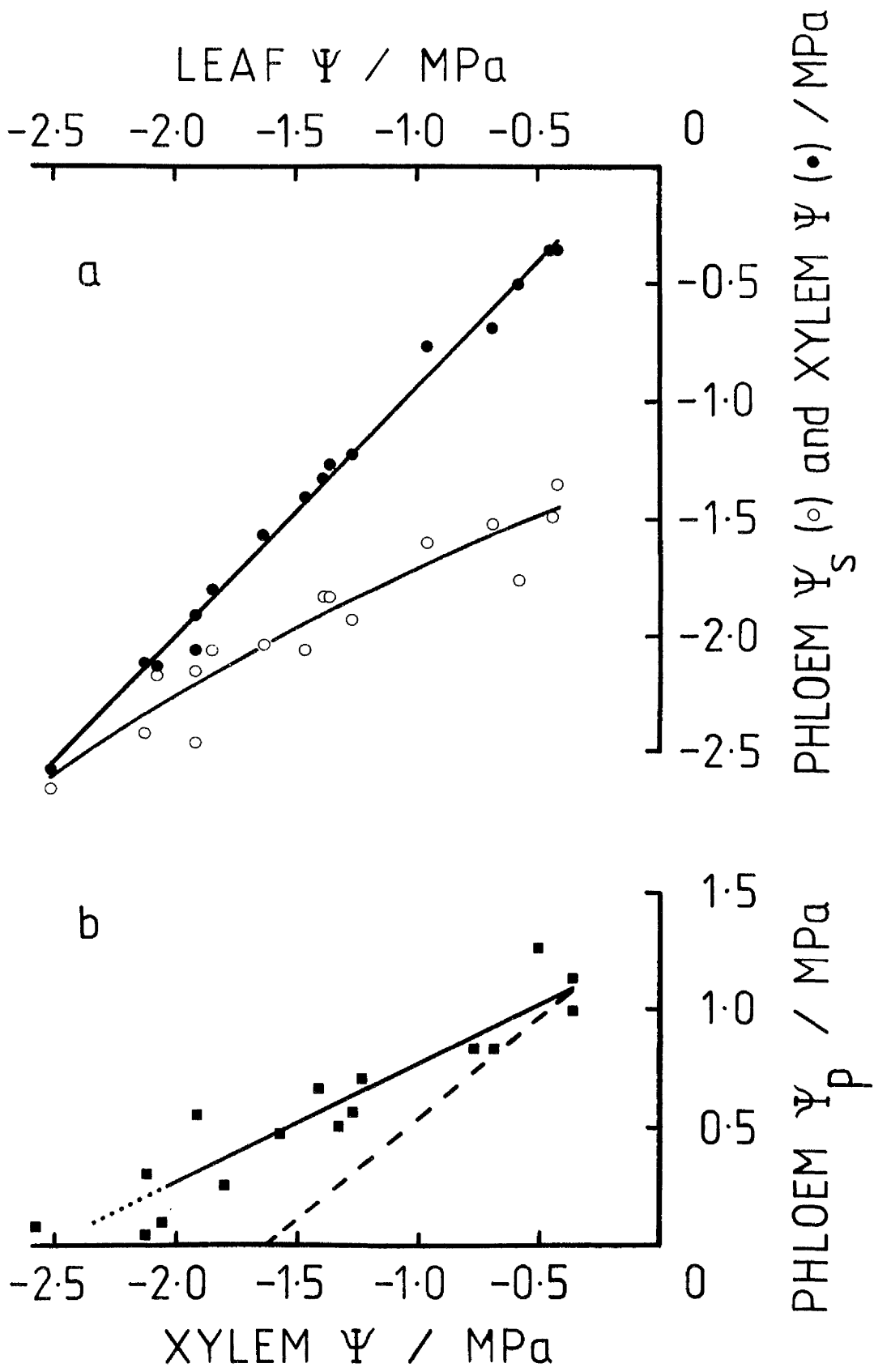
From these data it is possible to derive estimates of the values of phloem Ψ_p in the different plants. The values can be obtained from the equation

$$\Psi_p = \Psi - \Psi_s \tag{5.1}$$

(see equation 1.5, p.32), providing the usual assumptions are made about

Fig. 5.2 Changes in osmotic characteristics of phloem sap during water stress. (a) Relationships between leaf Ψ and xylem Ψ (\bullet), and between leaf Ψ and phloem-sap Ψ_s (\circ), with curves fitted by least squares' analysis to equations $y = 0.133 + 1.074x$ ($r = 0.996$, $P < 0.001$) and $y = -1.297^{0.279x}$ ($r = 0.941$, $P < 0.001$), respectively.

(b) Relationship between xylem Ψ and estimated phloem Ψ_p (\blacksquare), with line of equation $y = 1.270 + 0.504x$ ($r = 0.937$, $P < 0.001$) calculated from the relation $\Psi_p = \Psi - \Psi_s$, and assuming phloem to be in equilibrium with xylem Ψ ; dotted portion of line represents region where pressure-bomb estimates of leaf and xylem Ψ likely to have been less reliable. Broken line shows values of phloem Ψ_p anticipated if the rate of solute loading and unloading remained constant with changing xylem Ψ ; it is extrapolate to the point at which $\Psi_p = 0$, and $\Psi = \Psi_s$, and allows for a TDV of 12.0%. The slope of the experimental line is 0.504 ± 0.051 which is significantly different from the predicted slope of 0.868 at $P < 0.001$ (Student's t -test).



the status of the Ψ_m component and equilibration of Ψ between the xylem and phloem. The values of phloem Ψ_p calculated in this way are plotted in Fig. 5.2b against the corresponding values of xylem Ψ , from which it can be seen that estimated phloem Ψ_p declined with increasing water stress. The dotted portion of the line represents the region in which pressure-bomb estimates of leaf and xylem Ψ are likely to have been less reliable because of cavitation in the xylem vessels. In the same graph, the broken line represents the values of phloem Ψ_p that would be anticipated for the given xylem Ψ if the rates of solute loading into and unloading from the phloem were unaffected by water stress. That is, this would correspond to a condition under which solute influx and efflux are not altered by the stress treatment, with the assumption that the instantaneous solute content of the phloem is determined by the flux equilibrium between loading (influx) and unloading (efflux). Because these processes are spatially separated, with loading occurring largely in the leaves and unloading at the incision, longitudinal flux through the phloem simply represents the direct consequence of the two. The slope of the line for the predicted relationship between xylem Ψ and phloem Ψ_p under these conditions was determined by fixing two co-ordinates thus:

- 1) *xylem Ψ at which phloem Ψ_p maximum:* derived from the lines fitted by regression analysis to the experimental points shown in Fig. 5.2a, i.e. xylem Ψ (-0.36 MPa) - phloem Ψ_s (-1.45 MPa) = phloem Ψ_p (1.09 MPa); and
- 2) *xylem Ψ at which phloem Ψ_p zero:* taken as point at which xylem Ψ = phloem Ψ_s , which for constant rates of loading and unloading is -1.45 MPa; this also has to be corrected for a turgor displacement volume of 12.0% of sieve-element volume at full turgor (Milburn, 1972), because the decrease in cell volume with the fall in Ψ_p will itself increase the solute concentration (i.e. decrease Ψ_s); hence, the Ψ_s when $\Psi_p = 0$ would be $-1.45 \times 11.4\%$ (sic) = -1.62 MPa, which equals the xylem Ψ at which phloem turgor would be zero.

The slope of the unbroken line in Fig. 5.2b fitted by regression analysis to the inferred values from the experimental data is 0.504 ± 0.051 ; this is significantly different from the predicted slope of 0.868 at $P < 0.001$. The difference would still be significant

at $P < 0.01$ allowing for a TDV of 50%.

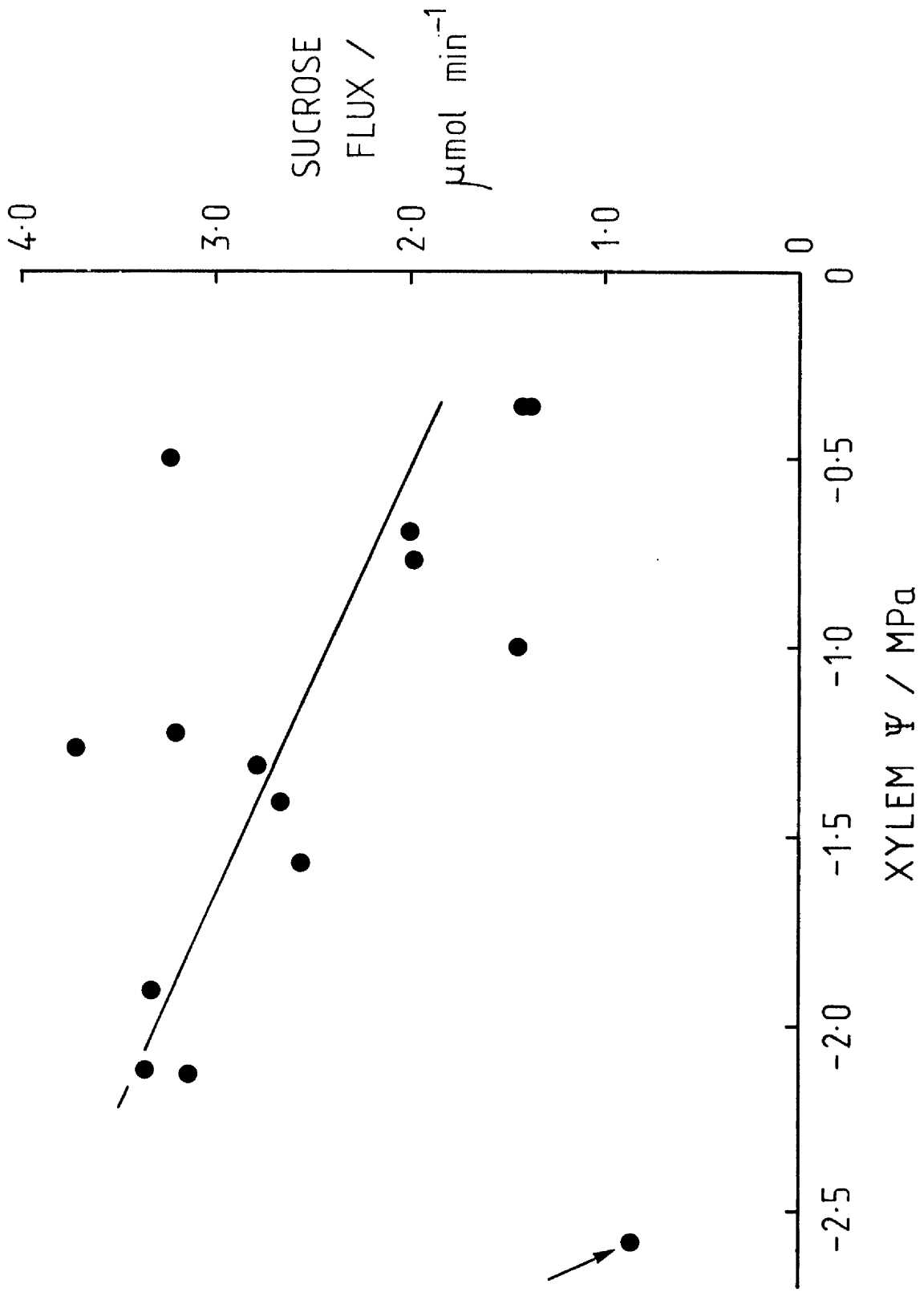
These results suggest that phloem Ψ_p remained positive at lower values of xylem (and leaf) Ψ than could be accounted for on the assumption that solute flux was not affected by water stress. Xylem and phloem Ψ are likely to have been in equilibrium, because of the relatively long time course of the changes involved, and therefore the maintenance of phloem Ψ_p can be attributed to the accumulation of solutes in the phloem. This could have been the result of either an increase in the rate of solute loading in the absence of an equivalent increase in the rate of unloading, or a decrease in the rate of solute unloading without an equivalent decrease in the rate of loading (or a combination of the two).

These possibilities can be distinguished by considering how solute flux was affected by decreasing water availability. An estimate of net flux can be obtained from the product of exudation rate and solute concentration, as discussed in section 4.2. The results are expressed in this way in Fig. 5.3. Solute flux is given as the amount of sucrose transported per unit time: this was calculated from the corresponding value of sap Ψ_s , since there was shown to be a linear relationship between sucrose concentration and Ψ_s . The results are also approximate in so far as they are based on values of exudation rate averaged over the time taken to collect $100 \times 10^{-9} \text{m}^3$ volumes of sap.. Fig. 5.3 nevertheless shows that the trend observed was for solute flux to increase with decreasing xylem Ψ , and the slope of the line fitted by regression analysis was significantly different from zero at the level of $P < 0.01$. One measurement was not included in this analysis, namely that at the xylem Ψ of -2.58 MPa . Exudation occurred only slowly in this plant, and tended to stop relatively quickly, so that several successive incisions were required in order to collect the full aliquot of sap. This may be a reflection of a certain value of leaf or xylem Ψ below which transport cannot be maintained. The results indicate that the decline in phloem Ψ_p during water stress was associated with an increase in the rate of solute loading.

5.3 Responses of phloem-sap composition to darkness

The effects of continual darkness were examined in a series of experiments primarily to determine how restrictions on the photosynthetic activity of the source leaves affected phloem-sap composition and the

Fig. 5.3 Relationship between solute flux through the phloem and xylem Ψ for data given in Fig. 5.2. Solute flux was calculated from sap exudation rate \times solute concentration (Ψ_s), and was converted to sucrose flux from the relationship between sap Ψ_s and sucrose concentration determined separately. Regression line fitted by least squares' analysis to equation $y = 1.54 - 0.88x$ ($r = 0.663, P < 0.01$).



water relations of the phloem, Sucrose and K^+ were used as indicators of the osmotic status of the phloem sap to assess how large changes in sucrose levels affected K^+ concentrations, with particular reference to the effects noted by Hoad and Peel (1965a). The connection between solute levels in the leaves and shoot water relations was also investigated in similar experiments.

Fig. 5.4 shows the changes found in sucrose and K^+ levels in phloem sap from plants transferred at the start of the experimental period to continuous darkness compared with those in control plants maintained under a photoperiod of 8 h. The experiment was carried out three times, and the results presented here are representative of those found on all three occasions. In the control plants (Fig. 5.4a), sucrose levels increased during the course of the experiment, whilst K^+ levels did not alter significantly from the t_0 values. (This change in sucrose concentration was not observed in the two other experiments of this kind.) In the plants kept under continuous darkness (Fig. 5.4b), on the other hand, there was a marked decrease in sucrose levels within 12 h, and this was associated with an increase in K^+ levels. From 24 h onwards, however, no significant changes occurred in the levels of these two solutes. Calculated in terms of their relative effects on the sap Ψ_s , the increase in K^+ levels during the first 24 h of darkness compensated to a considerable extent for the corresponding fall in sucrose levels.

In Fig. 5.5, the data from this experiment are plotted in the form of a scatter diagram of K^+ versus sucrose concentration. Regression analysis showed that the power curve fitted to the experimental points was significant at the level of $P < 0.001$, this curve giving a significantly higher correlation coefficient than that for a linear regression. The graph indicates that with decreasing levels of sucrose there was a relatively large increase in K^+ concentration, but that at high sucrose levels there was relatively little change in K^+ concentration. This latter part of the curve is a reflection of the fact that K^+ levels remained constant in the control plants while the concentration of sucrose in the sap increased.

The consequences of these changes in solute content for the osmotic relations of the phloem were examined by making additional measurements of phloem-sap Ψ_s and xylem Ψ . Table 5.1 presents results from an experiment in which the values found for plants kept in continuous darkness for 24 h were compared with those from control

Fig. 5.4 Changes in concentrations of sucrose and K^+ in phloem sap from plants (a) under a normal photoperiod of 16 h, and (b) transferred to continual darkness at t_0 . Points are means of values obtained from 4 to 6 plants, expressed relative to the values at t_0 ; the vertical bars represent 2 x S.E.

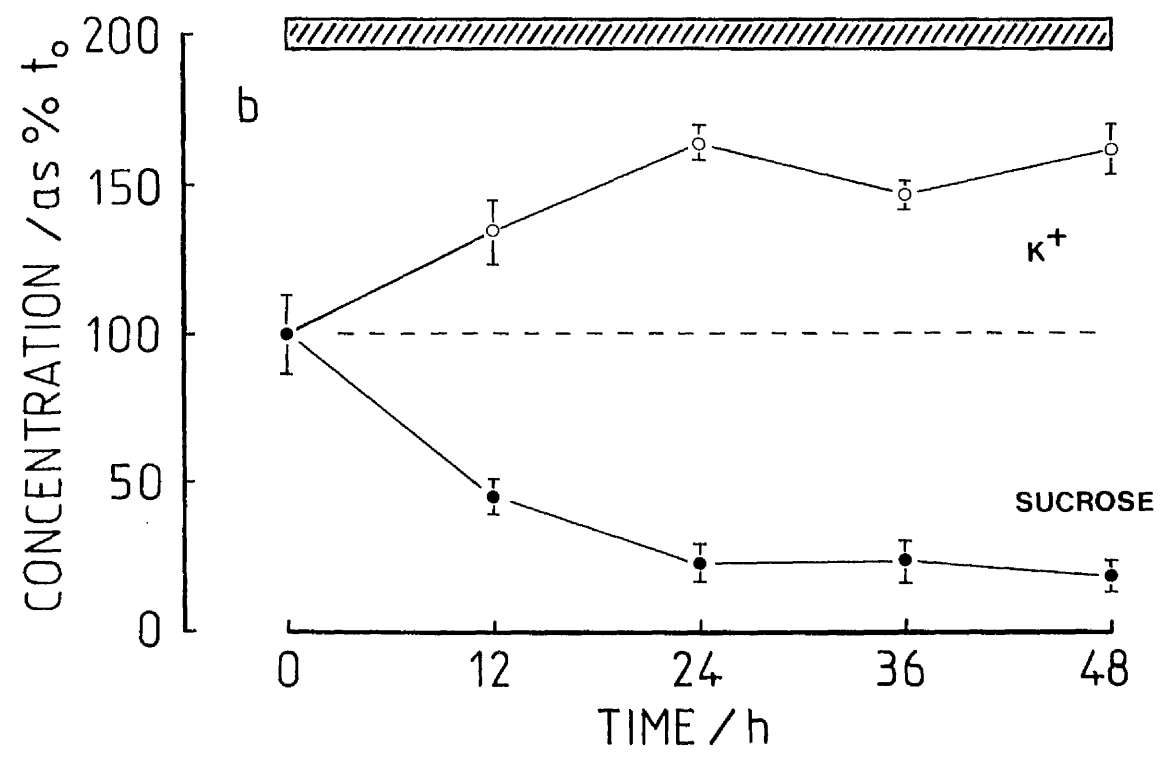
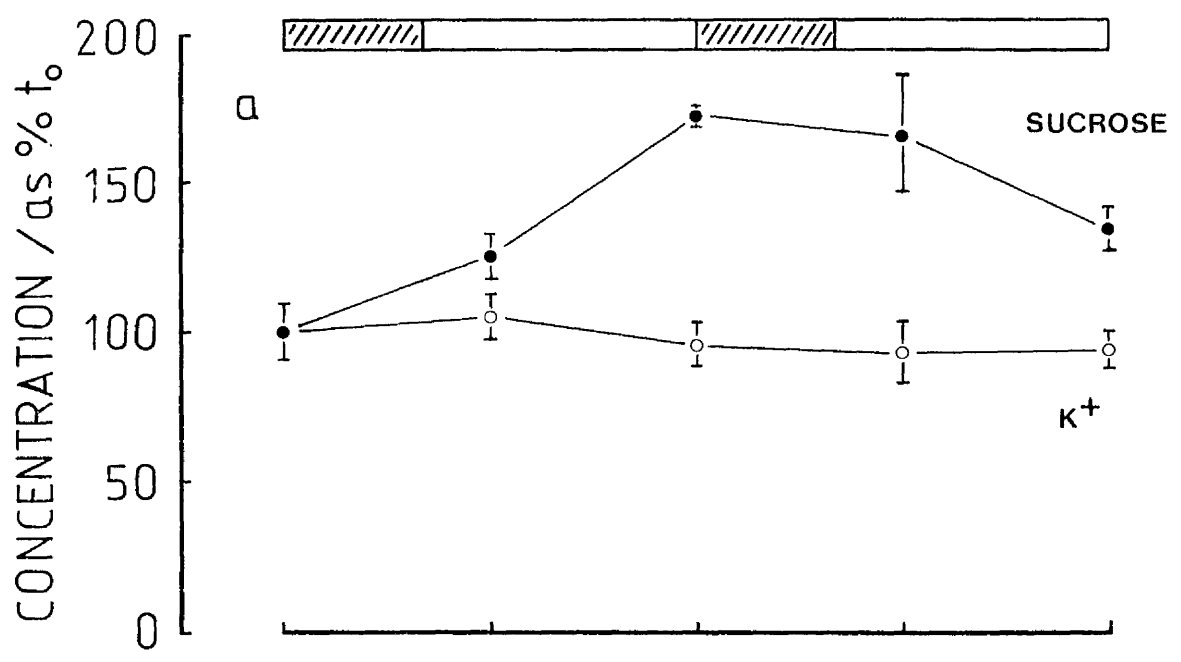


Fig. 5.5 Scatter diagram of mean values of sucrose and K^+ concentrations from experiment shown in Fig. 5.4. Values are expressed as absolute concentrations, but note different scales. Points shown thus (●) are values from control plants, (■) are values from dark-treated plants.

Broken lines are fitted to these two separate populations.

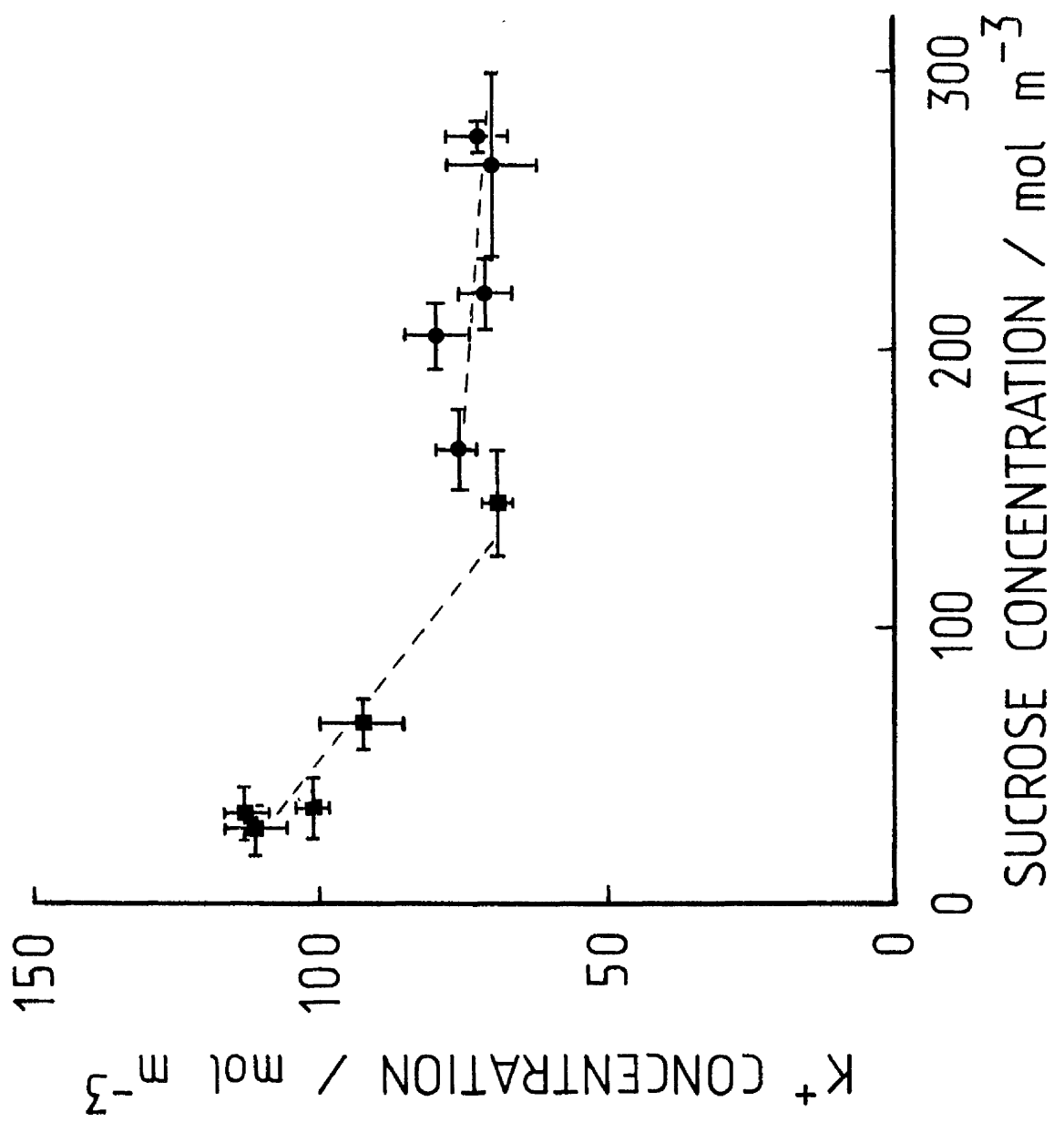


Table 5.1 Effect of 24 h darkness on phloem-sap sucrose and K^+ concentrations, phloem-sap ψ_s , xylem ψ and estimated phloem ψ_p (calculated assuming phloem in equilibrium with xylem ψ) for dark treated compared with control plants. Figures are means \pm for 4 plants, together with levels of significance.

	sucrose / mol m ⁻³	K^+ / mol m ⁻³	phloem ψ_s / MPa	xylem ψ / MPa	estimated phloem ψ_p / MPa
CONTROL	265 \pm 33	70 \pm 8	-1.42 \pm 0.07	-0.36 \pm 0.01	+1.06
DARK TREATED	33 \pm 9	113 \pm 4	-0.97 \pm 0.07	-0.33 \pm 0.01	+0.64
P	< 0.001	< 0.01	< 0.01	N.S.	-

plants maintained under the normal photoperiod. The results show that there was a characteristically large fall in the sucrose levels in the sap after 24 h darkness (to 12% of the control value), and that this was accompanied by a 61% increase in the K^+ concentration. The Ψ_s values as determined by osmometry indicated that the bulk solute concentration in the sap had decreased in the dark-treated plants to 68% of that in the control plants. It can be computed from the recorded sucrose and K^+ levels that the changes in the concentration of these two solutes alone would have given rise to an increase in Ψ_s of about 0.43 MPa. The agreement with the observed increase of 0.45 MPa suggests that these solutes may have been largely responsible for the change in sap Ψ_s . Xylem Ψ , however, was not significantly affected by continuous darkness over 24 h, and thus the difference in estimated phloem Ψ_p between the two groups of plants, calculated in the normal way, was a close reflection of the differences in sap Ψ_s . These results show that despite a fall in sucrose concentration to 12% of the control value, the increase in K^+ level was associated with a maintenance of phloem Ψ_p in the dark-treated plants at about 60% of the value found in the control plants.

An important aspect of these alterations in the solute content of the phloem sap, as emphasized in Chapter 3, is the question of how they may be related to changes in leaf metabolism. This is of relevance to the control of both sucrose and K^+ supply to the phloem. On the one hand, although the sucrose levels in the sap declined rapidly during the first 24 h in darkness, thereafter they remained relatively steady: in similar experiments they were found not to have decreased any further even after 72 h in darkness. Since the starch reserves are likely to have been largely depleted within 12 h (N.M. Stitt, personal communication), one question concerns the origin of the organic C for sucrose mobilization. On the other hand, it is important to establish whether the increase in K^+ concentration in the phloem sap has consequences for the levels of this ion in the bulk leaf tissue.

The results presented in Table 5.2 show that over a period of 24 h in darkness there was a significant decrease in the total solute levels in the bulk leaf tissue.

Table 5.2 . Effect of 24 h darkness on leaf-sap Ψ_s . Figures are means \pm S.E. for samples from 6 leaves,

Time in darkness / h	leaf-sap Ψ_s / MPa	
0	-1.25 \pm 0.03	
24	-1.09 \pm 0.01	P < 0.001

This represented a decrease in the solute content of about 13% compared with the t_0 value. This increase in leaf sap Ψ_s was investigated in terms of the levels of malate and K^+ in the bulk tissue, these solutes contributing a large proportion of the total Ψ_s (see section 3.3). The results of these analyses are given in Tables 5.3 and 5.4. Malate levels were found not to have decreased significantly after 12 h darkness, but after 24 h had fallen to about half the level in leaves from the control plants (Table 5.3). In contrast, no significant differences were observed in the K^+ concentrations in the leaves from the two groups of plants at either 12 h or 24 h (Table 5.4). Calculation of the predicted effect of the fall in malate levels after 24 h on leaf-sap Ψ_s shows that this quantitatively accounts for the observed change noted in Table 5.2.

5.4 Discussion

The observed responses of phloem-sap composition and solute flux to these two forms of environmental stress contrast with the constancy in bulk solute concentration found under the conditions described in the previous Chapter. In these earlier experiments, the characteristics of sap exudation suggested that rates of flux were largely determined by effective sink strength. Under conditions of restricted availability of water or photosynthetic assimilates, limitations are imposed on the activity of the transport system in a different way. It is thus important to determine whether the nature of the responses to these forms of stress help to clarify the mechanism by which solute loading can be affected by the osmotic relations of the transport system.

One of the issues underlying these considerations is the extent to which the solute content of the phloem is 'regulated' or 'controlled'.

Table 5.3 Effect of continuous darkness on malate levels in leaf-cell sap from dark-treated plants compared with controls under normal photoperiod. Figures are means \pm S.E. for samples from 4 plants.

time / h	malate concentration / mol m ⁻³		P
	control	dark-treated	
12	83 \pm 18	70 \pm 12	N.S.
24	85 \pm 7	38 \pm 6	< 0.01

Table 5.4 Effect of continuous darkness on K⁺ levels in leaf-cell sap from dark-treated plants compared with controls under normal photoperiod. Figures are means \pm S.E. for samples from 4 plants.

time / h	K ⁺ concentration / mol m ⁻³		P
	control	dark-treated	
12	143 \pm 12	148 \pm 13	N.S.
24	155 \pm 14	143 \pm 7	N.S.

Because these terms have important conceptual implications for the characteristics of solute transport processes, it is worth distinguishing the way in which they can be defined. For this purpose I shall follow the distinction made by Cram (1976a) in using 'control' in the sense of 'exercise of restraint or direction upon', and reserving 'regulation' for the more specific sense of adjustment 'with regard to some law, standard or reference'. Consequently, the use of 'regulation' is restricted to the immediate effect of an inflow of information (or energy) on a process (such as solute transport), and is not used to describe the indirect effect on an output (such as solute concentration).

Having made this distinction, Cram (1976a) proceeds to inveigh against the use of the term 'osmoregulation'. On semantic grounds, however, the criticism involves an inconsistency in logic, because it presupposes that the term refers to an adjustment of 'osmotic pressure'. In fact, if the distinction between 'control' and 'regulation' is tenable, the term must by definition refer to the regulation of osmosis (i.e. a process, not an output). This usage also avoids the other objection to the term raised by Cram (1976a), namely that it implies that 'osmotic pressure' is the output of the control system involved in adjustment of the rate of solute accumulation (whereas it may often prove to be turgor or cell volume, for example). The point here is that 'osmoregulation' sensu stricto refers to the adjustment of the rate of solvent movement, which occurs irrespective of the nature of the output from the accumulatory control system. In this context, 'osmoregulation' in fact represents an extremely useful, general term by which to refer to the observed phenomenon of adjustment of solute accumulation, since in only a few species is the mechanism of feedback control understood anyway. Further aspects of the terminology have been considered by Zimmermann (1977).

If the effects of water deficits on phloem transport are to be properly assessed, it is essential to examine not only the water relations of the system in what is taken to represent flux equilibrium, but also the magnitude of solute flux through the sieve tubes. The fact that the bulk solute concentration of the sap remained constant over prolonged periods of exudation (Fig. 5.1) indicated that even during water stress the processes of solute loading and longitudinal transport were maintained. This demonstrated that sap Ψ_s did not decrease simply because the rate of longitudinal flux was inhibited by water stress more severely than the rate of solute loading.

The measurement of xylem Ψ as well as phloem-sap Ψ_s at given levels of leaf Ψ allowed estimates to be made of phloem Ψ_p . Although the curves to which the points were fitted were associated with high correlation coefficients, the possibility of systematic errors in the measurements has to be carefully considered. The pressure-bomb technique itself has

been widely used in the investigation of water potentials (see Boyer, 1969; Ritchie and Hinckley, 1975; Tyree, Cheung, MacGregor and Talbot, 1978), and it has been argued that it is impossible to determine whether this or thermo-couple psychrometry represents the more accurate method for measuring leaf Ψ (Talbot, Tyree and Dainty, 1975). It is likely, however, that the technique is less valid at lower values of leaf (and xylem) Ψ when cavitation of the water columns in the xylem vessels become extensive (Milburn, 1975): the consequence of this is that the degree of water stress is overestimated, because a greater balance pressure is required to force the xylem sap to the cut end of the petiole (West and Gaff, 1976). In the present experiments, nevertheless, the relationship between Ψ and xylem Ψ was linear across the whole range of observed values (cf. West and Gaff, 1971). The quantitative importance of this source of error at these levels of water stress remains to be accurately determined.

The values of leaf Ψ estimated by this method in addition have little meaning in a rigorous thermodynamic sense. Because of the structural heterogeneity of the organ, there is unlikely to be equilibration of Ψ between the different phases within the leaf; thus, the values obtained represent some sort of average for the bulk tissue. The problems of assigning absolute values to Ψ and its components in leaves have been extensively discussed (Warren Wilson, 1967a, b, c; Noy-Meir and Ginzburg, 1967, 1969; Weatherley, 1970). There are also difficulties in deciding on how the different components of Ψ are affected by decreasing leaf Ψ (e.g. Warren Wilson, 1967c; Noy-Meir and Ginzburg, 1969; Al-Saadi and Wiebe, 1973). Nevertheless, the concept is of considerable usefulness for comparative purposes as a means of assessing the water status of the bulk tissue.

Given that sap Ψ_s seems to decrease with increasing water stress, (Fig. 5.2a), further assumptions are still involved in the estimation of phloem Ψ_p . One is that the phloem is taken to be in flux equilibrium with xylem Ψ . This appears to be reasonable in view of the speed of the responses to incision noted in the previous Chapter (and see Hall and Milburn, 1973). In the present experiments, moreover, xylem Ψ decreased gradually over a period of hours. Thus, although the half-time for water equilibration may have been greater at lower values of phloem Ψ_p because of a decrease in the elastic modulus of the cells (see equation 4.1, p.124), conditions of equilibration may nevertheless have existed to a first approximation. Another major assumption is

that the value of the Ψ_m component in the phloem only changes with increasing water stress in exact proportion to the total Ψ . There have been no direct studies on the importance of Ψ_m in phloem water relations, but the low dry-matter content of the phloem sap (see section 1.3) may restrict the quantitative effect of this component. It is also possible that part of the Ψ_m value is included in the value of Ψ_s determined cryoscopically (Gardner and Ehlig, 1965; Warren Wilson, 1967a), and Ψ_m seems to be numerically small in the tissues for which data are available (Wiebe, 1966; Boyer, 1967b). Nevertheless, the potential importance of the Ψ_m component at low values of phloem Ψ should not be neglected (see Geiger, 1976b).

Calculation of phloem Ψ_p as the algebraic difference between xylem Ψ and phloem-sap Ψ_s shows that Ψ_p decreased with increasing water stress (Fig. 5.2b). This response would be anticipated in a qualitative way as a consequence of progressive osmotic efflux of water from the sieve tubes with decreasing xylem Ψ . The first question to ask, therefore, is whether the observed change in phloem Ψ_p with increasing water stress is significantly different from that expected if the phloem responded entirely passively to changes in xylem Ψ . This predicted response is shown in Fig. 5.2b as the broken line extrapolated to the abscissa, and is derived from the computation of the point at which incipient plasmolysis would have been reached without any change in the amount of solute in the sieve tubes. With regards to the absolute values of Ψ_p , allowance also has to be made for the fact that an increase in cell-wall elasticity (i.e. a decrease in ϵ) would result in the maintenance of a positive Ψ_p to lower values of xylem Ψ (Weatherley, 1965; Noy-Meir and Ginzburg, 1969). The magnitude of this effect can be accounted for by correction of the predicted values of Ψ_p for the turgor displacement volume, since this term implicitly includes an estimate of both the pressure and volume changes taking place between full turgor and zero turgor. Fig. 5.2b shows that phloem Ψ_p was maintained at significantly higher levels than expected if the tissue responded passively to changes in xylem Ψ .

This analysis suggests that with increasing water stress there was also an increase in the amount of solute in the sieve tubes. Because sap exudation was maintained during the experimental period, it can be inferred that this did not result from continued solute loading in the absence of longitudinal transport. In fact, exudation of the relatively large volume of sap ($\approx 2 \times 10^{-6} \text{m}^3$) collected during the experimental period is itself likely to have contributed

to the water stress on the shoot. Within 20 h, leaf Ψ had decreased to about -2.5 MPa, whereas in soil-grown Ricinus plants it was found that even after two months without watering, leaf Ψ was no lower than -1.7 MPa (Dodoo, 1978). Furthermore, exudation occurred in the present experiment whatever the degree of water stress, and there is no justification for assuming by extrapolation of these data that it might not have been maintained at values of leaf Ψ considerably lower than -2.5 MPa.

In order to determine how this change in solute content of the sap is related to the rate of loading, it is necessary to derive estimates of flux through phloem. The results presented in Fig. 5.3 indicate that there was a significant increase in the rate of solute flux with the decrease in xylem Ψ (and phloem Ψ_p). It is important to recognize, however, that the treatment of the data in this way involves introducing a number of sources of variation. Discussion of the significance of solute flux in the previous Chapter was based on a comparison of exudation from successive incisions into the same cross-sectional area of tissue for a single plant under steady-state environmental conditions. In the present experiment, the cross-sectional area of the transport pathway was not defined with the same precision, although the plants were all of similar stature and the length of the incision virtually identical in each measurement. The estimate of exudation rate was also based on a mean value integrated over the time taken to collect a sample of $100 \times 10^{-9} \text{m}^3$: in this case the source of variation is compounded, because marked differences are found between the exudation profiles even of different plants at a given leaf or xylem Ψ , let alone between plants with disparate water relations. Nonetheless, these effects may have introduced largely random variation into the analysis, in spite of which the alteration of solute flux during water stress was significant at the level of $P < 0.01$.

The most serious limitation imposed on interpretation of these estimates of flux is likely to be that introduced by the changing source-sink relations with increasing water stress. The results in Chapter 4 were interpretable in terms of the interaction between controlled modifications to sink demand and an effectively non-limiting supply of solutes in the source leaves. Irrespective of xylem Ψ , the incisions represent a means of standardizing sink strength with increasing water stress, but it is likely that source metabolism was

also affected by decreasing leaf Ψ , Photosynthesis in mesophytic species is inhibited by water deficits to various degrees in different species (Maximov, 1929; Iljin, 1957; Slatyer, 1967; Crafts, 1968; Hsiao, 1973). A large part of the reduction in assimilation rate can be attributed to stomatal closure (e.g. Troughton, 1969; Boyer, 1970; Raschke, 1975), but changes in intracellular processes may also be important (e.g. Boyer, 1971; Jones, 1973; Bunce, 1977a). The effect of sink demand on solute loading may thus be restricted by the availability of photosynthetic assimilates for transport. In spite of this, however, an increase in solute flux and loading was observed at lower values of leaf Ψ , which implies that the response to low Ψ might be even greater in the absence of inhibitory effects on photosynthesis.

The relative effects of water stress on source and sink metabolism must influence the patterns of assimilate movement in intact plants. This makes it extremely hard to interpret the changes in phloem transport occurring at low leaf or xylem Ψ in terms of specific effects on a particular component of the transport system, although inhibitory effects of water deficits on transport have been widely noted (Plaut and Reinhold, 1965; Hartt, 1967; Crafts, 1968; Munns and Pearson, 1974; Reinhold, 1975; Allaway, 1976). However, experiments in which flux has been related to the changes in photosynthesis and growth rate of the sinks have indicated that water stress per se may not have much effect on the transport system (Wardlaw, 1969; Munns and Pearson, 1974; Moorby et al., 1975). The present results for Ricinus further suggest that there may be different effects on the various parts of the transport system. Thus, the speed of longitudinal transport (i.e. exudation rate) is lower with increasing stress, and this may be the result of a reduced Ψ_p difference between the source and sink; but on the other hand, solute flux is higher, which in turn corresponds to an increase in the rate of loading.

Consequently, it is important that the effects of environmental stress on transport are considered not just in terms of how the system may respond passively to changes in water relations, but also in relation to how the transport process may adapt to different conditions. It is in this sense that alteration of the rate of solute loading may be important in 'osmoregulation', for the manifestation of this adjustment is that phloem Ψ_p remains positive even at very low values of xylem Ψ . The question of the mechanism by which loading responds

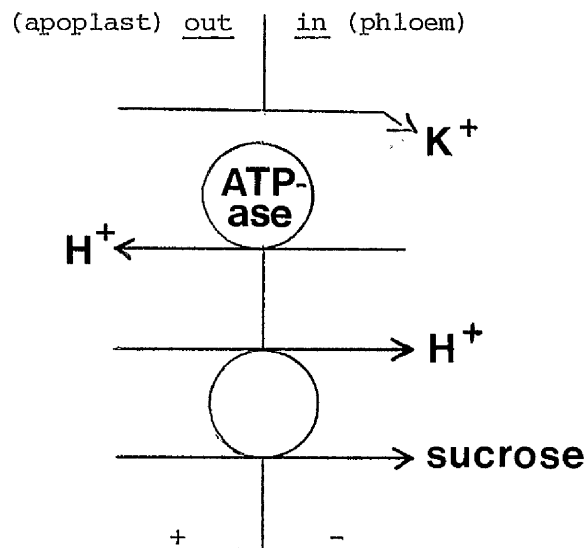
to changes in the water relations of the phloem was raised in section 4.5. The results presented here argue against the modulation of influx Ψ_s , because a decrease in sap Ψ_s was associated with an increase in the rate of loading, whereas under 'steady-state' conditions (Chapter 4) the increases in loading resulting from incisions were associated with (if anything) increases in sap Ψ_s . These are not the characteristics that would be expected of a feedback mechanism in which solute concentration was the output of the control system regulating the accumulatory process (see Cram, 1976a). The results suggest instead that loading responded to changes in phloem Ψ_p or some function of it, such as phloem Ψ or cell volume.

Certain aspects of the changes in sap composition found in the plants kept under continuous darkness are similar to these observed responses to water stress. Despite a severe source limitation in the dark on the supply of photosynthetic products, phloem Ψ_p was maintained at higher levels than would have been expected if the rate of loading of all the solutes had declined in proportion with that of sucrose. The increase in K^+ concentration found as sucrose levels decreased is similar to the changes observed in sap composition in isolated stem segments of Salix as sugar levels declined because of respiration (Hoad and Peel, 1965a). How these changes are related to alterations in the rate of K^+ influx and efflux in the phloem is less clear, since there may have been either a stimulation of loading or a decrease in lateral loss from the sieve tubes, or a combination of the two. The fact that the small increases in K^+ concentration noted earlier were nevertheless associated with a net decrease in loading rate (Fig. 4.3) suggests that both possibilities must be considered.

The relationship between sucrose and K^+ flux is of particular importance because of its bearing on the mechanism of sucrose loading. There is now substantial evidence that sucrose uptake into the phloem in Ricinus occurs by sucrose- H^+ cotransport (Komor et al., 1977; Komor, 1977; Malek and Baker, 1977, 1978; Hutchings, 1978a, b). It is postulated that the driving force for sucrose uptake is provided by an electrochemical potential gradient generated by active, electrogenic H^+ efflux in the manner proposed by Mitchell (1967). This H^+ efflux is presumed to be driven by a vectorial membrane-bound ATPase, and seems to be associated with K^+ influx (Hutchings, 1978b; Malek and Baker, 1978). Phloem loading also seems to occur by a

similar mechanism in Beta (Glaquinta, 1977b), and experiments with Samanea pulvini (Racusen and Galston, 1977) and Zea scutella (Humphreys, 1978) have suggested that there may be sucrose- H^+ cotransport in these tissues. In addition, hexose- H^+ cotransport has been described in a wide range of species, and this may represent the principal mechanism for sugar uptake in plant cells generally (Komor, 1973; Jones, Novacky and Dropkin, 1975; Raven, 1976b; Novacky, Ullrich-Eberius and Lüttge, 1978; see also Slayman, 1974; Raven, 1976a).

Sucrose loading will thus tend to dissipate both the ΔpH and $\Delta\psi$ components of the electrochemical potential gradient, $\Delta\bar{\mu}_H^+$, whereas K^+ influx will only discharge the $\Delta\psi$ component. The postulated loading mechanism can be summarized thus



K^+ influx is represented here in the form of carrier mediated K^+/H^+ exchange, but it is not possible as yet to distinguish between this possibility and that of passive diffusion of K^+ in response to the $\Delta\psi$ generated by H^+ extrusion. The significant point about this mechanism is that it can explain at the level of solute loading how a reciprocity is found between sucrose and K^+ levels in the phloem sap. It has been shown in Ricinus cotyledons that sucrose uptake is about three times greater than K^+ uptake from a medium containing equal concentrations of the two solutes (Hutchings, 1978b). The larger turnover of sucrose may occur because its uptake tends to discharge both ΔpH and $\Delta\psi$ (Hutchings, 1978b), but the influx ratios also correspond to the relative concentrations of these two solutes in the phloem sap of normal plants (see Fig. 5.5). Consequently, any decrease in the amount of sucrose available for loading may allow

increased uptake of K^+ . Although K^+ supply to the shoot may also have been partially inhibited in the dark-treated plants because of a decrease in transpiration rate, it seems probable that the reduction in sucrose availability was considerably greater in proportion. This apparent 'antagonism' between sucrose and K^+ loading at the membrane level may also account for another observation made by Hoad and Peel (1965a), namely that increasing the K^+ level in the phloem in Salix by perfusing the xylem with high K^+ concentrations actually caused a decrease in sucrose levels in the sap.

These considerations show that the mechanism of solute loading may have important consequences for the maintenance of phloem Ψ_p under conditions of changing solute availability. It is possible that a similar explanation may account for the increase in amino-acid levels noted by Hill (1955) (cited in Hoad and Peel, 1965a) in sap from Salix cuttings transferred to continuous darkness (cf. Sharkey and Pate, 1976). This increase was correlated with a fall in the sucrose content of the sap, and there is evidence now that amino-acid uptake in plant cells may also be mediated by cotransport with H^+ (van Bel and van Erven, 1976; Novacky, Fischer, Ullrich-Eberius, Lüttge and Ullrich, 1978; Etherton and Rubinstein, 1978).

In addition to the control of solute loading, it is possible that K^+ levels in the phloem are also determined by the rates of lateral leakage along the pathway. This may be an important component determining net flux, because K^+ seems to be rather freely exchangeable between the sieve tubes and the surrounding tissues (see Qureshi and Spanner, 1971; Grange and Peel, 1978). The rôle of K^+ transport in osmoregulation has been most intensively studied in the alga Valonia, in which it has been found that net K^+ influx increases in response to reduced turgor, and vice versa (Gutknecht, 1968; Hastings and Gutknecht, 1974). There is also evidence, however, that in V. utricularis the decreased net influx is brought about by a stimulation of K^+ efflux, as well as by inhibition (and possibly reversal) of K^+ influx (Zimmermann, Steudle and Lelkes, 1976). This may occur because of changes in the permeability characteristics of the membrane as cell Ψ_p varies (Zimmermann et al., 1976; Coster, Steudle and Zimmermann, 1976). Since there may be similarities between the physical characteristics of all plant cell walls and plasma membranes (see Steudle et al., 1975; Zimmermann, 1978), there is a possibility that K^+ efflux from the phloem is directly affected by phloem Ψ_p .

During the dark treatment there was a significant decrease in the bulk solute concentration of the leaf-cell sap (Table 5.2). As absolute values of Ψ_s , these may underestimate the solute content of the leaves, because the sap tends to become diluted by apoplastic water during extraction (Gaff and Carr, 1961). There are indications that this may not introduce serious errors for Ricinus leaves (Weatherley, 1965). Furthermore, it has been shown by Dodoo (1978) that the values of leaf-sap Ψ_s obtained from pressure-volume curves (see Tyree and Hammel, 1972) agree closely with those estimated cryoscopically after extraction of the sap by freeze-disruption of the tissue.

Although there was no significant decrease in K^+ levels over this period (Table 5.4), the malate concentration in the bulk leaf tissue decreased markedly during 24 h in darkness (Table 5.3). This may be indicative of another aspect of the relation between leaf metabolism and phloem loading, because the sucrose levels in the phloem sap, after declining relatively quickly, remained steady for prolonged periods. This maintenance of sugar levels was also found in similar studies by Sharkey and Pate (1976), and suggests that in the absence of significant starch reserves there may be a mobilization of organic C from malate in the leaves to provide sucrose for transport. A pathway serving this purpose has been indicated by experiments with Kalanchoë tubiflora (Kluge, 1977), and may exist in Lycopersicon (Farineau and Laval-Martin, 1977) and Spinacea (Böcher and Kluge, 1978). How large a proportion of this sugar may be synthesized via gluconeogenesis rather than by re-fixation of CO_2 generated by metabolism of malate in the tricarboxylic acid cycle remains to be established. It should be noted, however, that gluconeogenesis seems to be of principal importance in lipid metabolism (e.g. in germinating seeds) as opposed to metabolism of organic acids (see ap Rees, 1974). Nevertheless, evidence has been presented for its occurrence in CAM plants (Dittrich, Campbell and Black, 1973) and stomata (Dittrich and Raschke, 1977). The biochemical nature of the relationships between leaf metabolism generally and phloem transport warrants further investigation.

6. REGULATION OF PHLOEM LOADING

6. REGULATION OF PHLOEM LOADING

6.1 Introduction

Evidence has been presented in the last two chapters that phloem transport is able to respond to changes in the osmotic relations of the vascular system. In part, these adjustments are associated with water movement into and out of the tissue, which are conventionally regarded as passive responses. However, the results suggest that in addition the rate of solute loading can be affected by the osmotic balance of the phloem. The experiments in Chapter 4 indicate that the responses of sap exudation to successive incisions may correspond to the way in which transport in the intact plant is geared to changes in source-sink relationships. One consequence of this, as shown in Chapter 5, is that an increase in the rate of loading maintains phloem turgor during periods of environmental stress at higher levels than would be possible if the loading rate remained constant.

Since for experimental purposes such incisions constitute the sites of unloading from the phloem, an attempt to characterize the exudation system in more detail relies heavily on a better understanding of the mechanism of solute loading. As discussed previously, it is difficult to quantify the contributions made by different organs to bulk solute flow, and under conditions of sufficiently strong sink demand even natural sinks (such as roots and developing fruits) can be induced to export assimilates (e.g. Die and Tammes, 1966; Milburn, 1974). Nonetheless, the studies on transport kinetics and the effects of leaf excision (sections 4.3 and 4.4) suggested that the mature alternate leaves are the principal sites of solute loading in Ricinus. One aspect of the loading process considered briefly in this Chapter is therefore how the ultrastructure of the sieve element-companion cell complex is adapted to solute transfer into the phloem. The fine structure of the minor veins in Ricinus leaves has not previously been examined by transmission electron microscopy. Furthermore, it is important to consider how the high rates of mass transfer found during exudation are accommodated at the sites of entry into the transport system.

A second aspect of solute loading examined here is the nature of sucrose uptake by leaf discs. The studies on phloem-sap exudation

strictly allow inferences to be made only about the consequences of solute loading, i.e. the characteristics of longitudinal flux through the sieve tubes. These may correspond closely to the kinetics of loading in the leaves if there is little lateral exchange along the pathway (see p. 118). However, it is important to determine whether the putative osmotic characteristics of loading can be demonstrated more directly. One way in which this can be approached is to compare the characteristics of sucrose and glucose uptake by leaf discs, because there is evidence that, at least in some species, the uptake process into the phloem is specific for sucrose. The limitations of this system as a means of examining the characteristics of loading are nevertheless considerable, and have an important bearing on the relevance of this approach to the properties of transport in the intact plant.

6.2 Ultrastructural aspects of phloem loading

The minor veins of the leaf laminae appear to constitute the principal sites of phloem loading in leaves carrying out a net export of assimilates (Geiger, 1975a). Towards the ends of the minor veins, the sieve elements may actually lie adjacent to the xylem vessels, or are separated from them by only a few cell layers. There may thus be a reasonably direct pathway for osmotic movement of water from the transpiration stream to the phloem in response to solute loading. The present study concentrates on the ultrastructure of this part of the vascular system in Ricinus, with emphasis on the characteristics of the sieve element-companion cell complex.

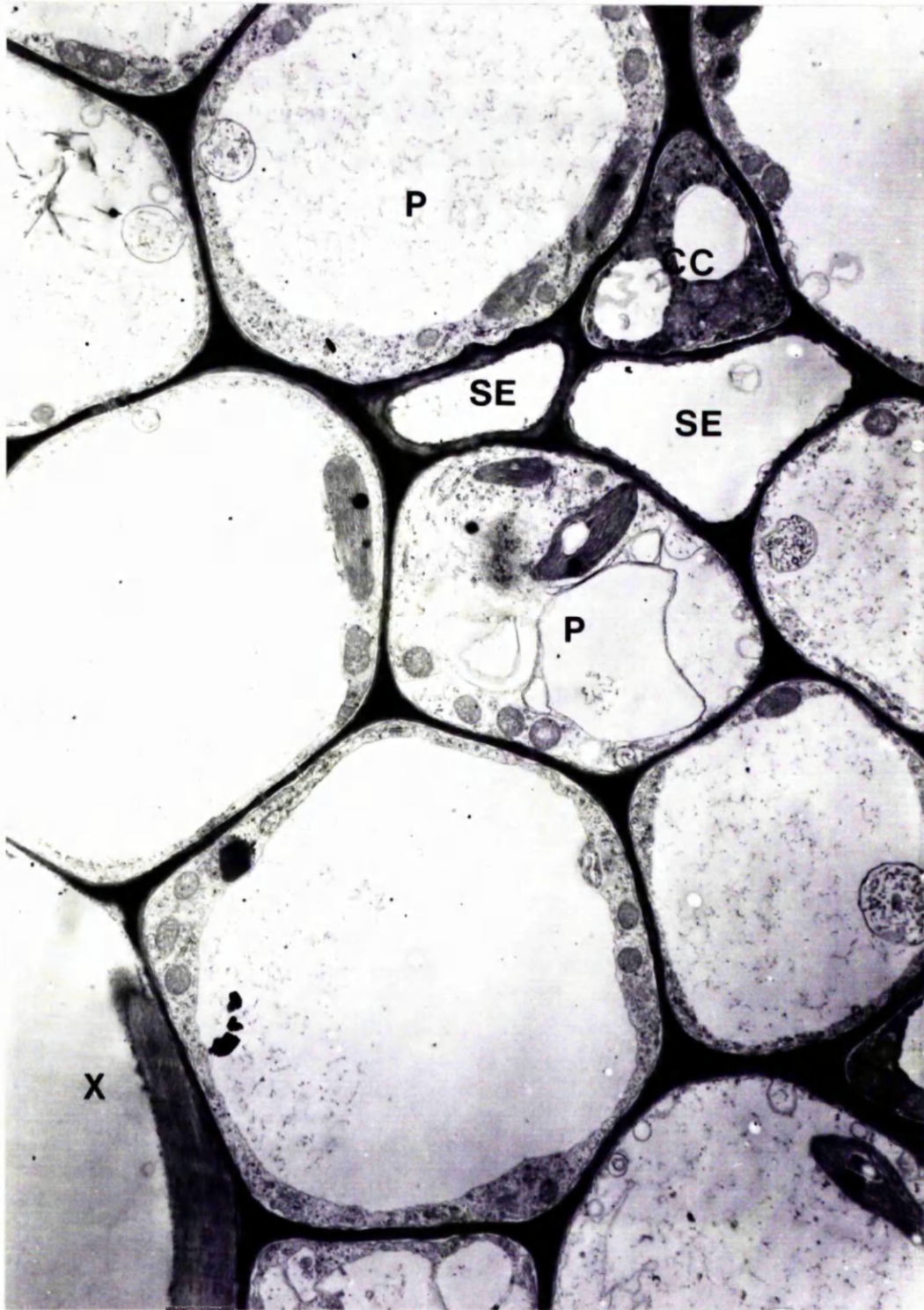
The material prepared for electron microscopy was taken from peripheral regions of laminae of fully expanded alternate leaves. Plate 6.1 shows the typical arrangement of the cell types near the vein endings. Sieve elements were characterized by relatively clear lumina and the presence of only parietal organelles. These were abutted by parenchyma cells, of which Esau (1973) has suggested it is possible to distinguish two different types. One can be described functionally as a companion cell, containing a densely staining cytoplasm with abundant mitochondria, endoplasmic reticulum and ribosomes. The other is more vacuolate, has a lighter staining cytoplasm with fewer organelles, and is not necessarily adjacent to a sieve element (whereas the first type invariably is). Of the cells adjacent to the sieve elements in Plate 6.1, one is recognizably a

Plate 6.1 Low-power micrograph showing arrangement of cell types in minor vein in source leaf of Ricinus. Phloem parenchyma cells lie adjacent to sieve elements together with one companion cell. Note also lignified xylem vessel. Fixation was in glutaraldehyde followed by osmium tetroxide. Magnification X7060.

The following abbreviations are used in Plates 6.1 to 6.6, capitals denoting cell types, lower case denoting subcellular structures:

SE	sieve element
CC	companion cell
P	phloem parenchyma
X	xylem vessel
pm	plasmodesma
d	desmotubule
mc	median cavity
ca	callose
m	mitochondrion
p	plastid
r	ribosomes
rer	rough endoplasmic reticulum
v	vacuole
gv	golgi vesicles

The results described in this section (6.2) were obtained in collaboration with Mr R. A. Hustwaite and Mrs D. L. Leake. I gratefully acknowledge their essential contribution to this work.



companion cell in the strict sense, possessing small vacuoles, numerous mitochondria and an electron-dense 'ground cytoplasm'. The others can be regarded as phloem parenchyma, although there is a gradation of types from those that are highly vacuolate through to those that contain smaller vacuoles and more numerous organelles. The distinction between companion cells and phloem parenchyma can, in fact, only be made properly in ontogenetic terms. Plate 6.1 also shows a portion of a large metaxylem element with spiral lignification of the wall, this being separated from the sieve elements by only one cell layer.

In cross-section, these sieve elements in the minor veins were small relative to the neighbouring parenchyma and companion cells. This contrasts with the dimensions of the cells in stem tissue, in which the sieve elements are usually of much larger cross-section than the companion cells. The small sieve elements in Ricinus leaves were characterized by a number of subcellular features. Plate 6.2 shows an example of this type, the sieve element being surrounded by four phloem parenchyma cells. The sieve element possessed a clear lumen, with organelles confined to the peripheral cytoplasm. In addition, the cell wall was thick relative to the walls of the neighbouring cells, and also possessed at least one multiple branched plasmodesma. These 'compound' plasmodesmata were always found associated with wall swellings on the companion-cell side, converging on only one or two canals on the sieve-element side of the wall. Another example is shown in Plate 6.3, but in this instance the sieve-element lumen contained a number of vesicles and a considerable amount of apparently fibrillar P-protein. This may represent a less mature condition in the development of the sieve elements, but the walls appeared nevertheless to be heavily thickened, and contained branched plasmodesmata. The surrounding parenchyma/companion cells possessed relatively large mitochondria and numerous dark-staining ribosomes.

Because of their presumed rôle in symplastic transfer of solutes from the companion cells to the sieve elements, it is worth considering the ultrastructure of these plasmodesmatal junctions in greater detail. A similar example is shown in Plate 6.4, where a thick-walled sieve element again lay adjacent to a relatively large companion cell. The wall between the cell contained two swellings on the companion-cell side, and the desmotubules within the

Plate 6.2 Electron micrograph of relatively thick-walled sieve element surrounded by phloem parenchyma cells. Cell wall contains a single plasmodesma which branches into several canals on parenchyma side of the wall. Fixation in glutaraldehyde followed by osmium tetroxide. Magnification X17670.

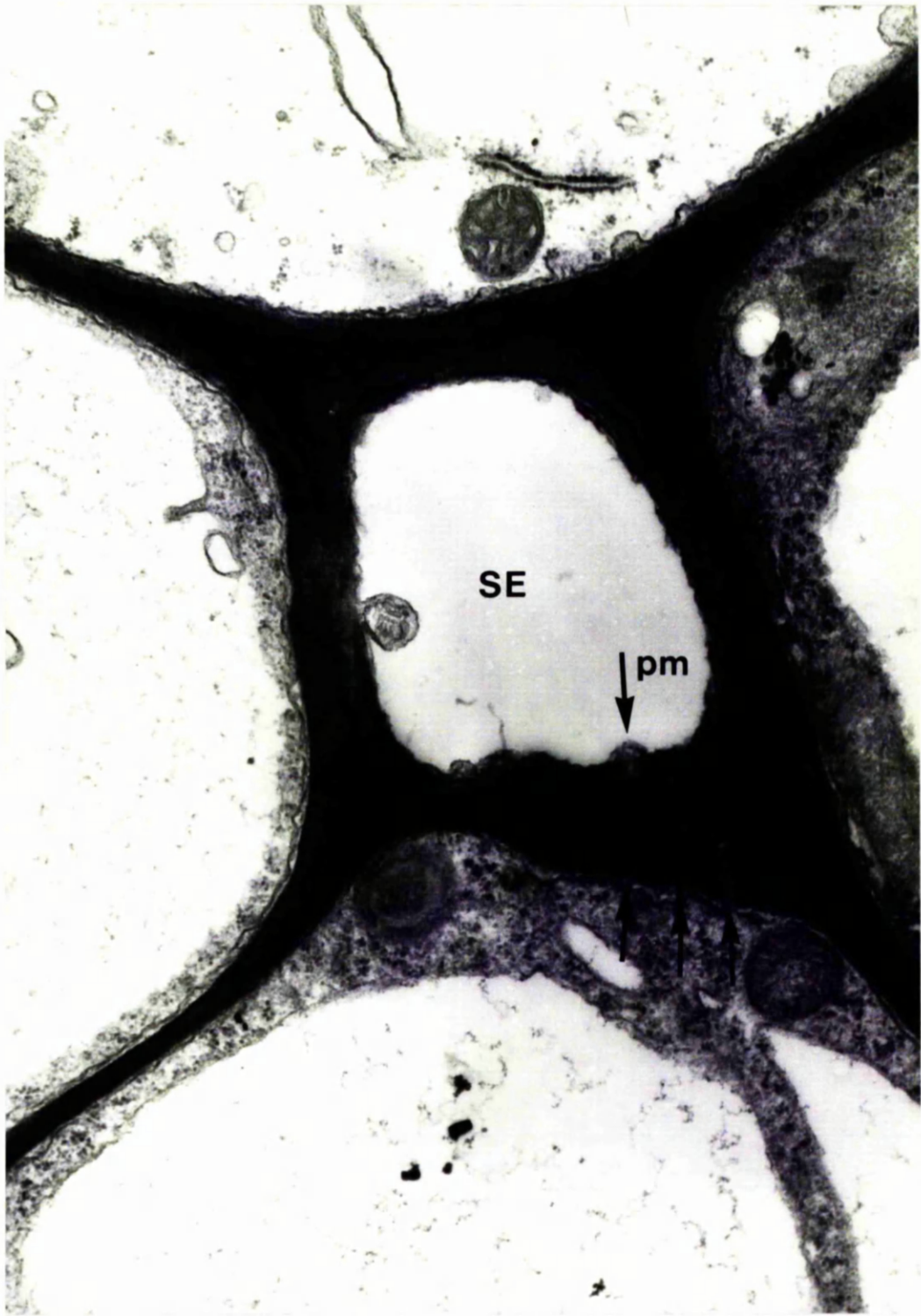


Plate 6.3 Electron micrograph of relatively thick-walled sieve element with lumen containing strands of fibrillar P-protein. Cell wall has branched plasmodesmata, and neighbouring companion cells/phloem parenchyma possess large mitochondria and numerous ribosomes. Fixation in glutaraldehyde followed by osmium tetroxide. Magnification X23560.

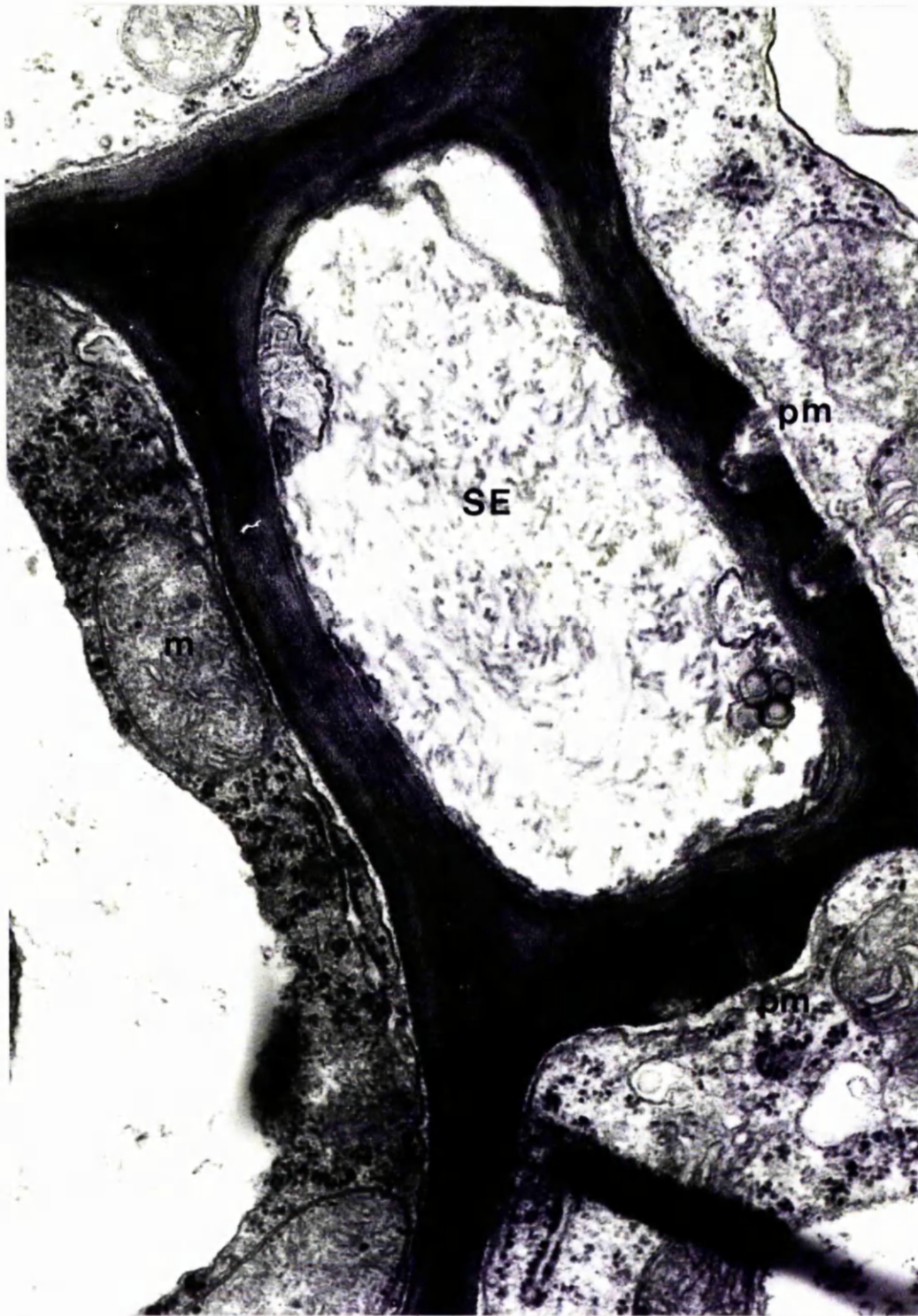


Plate 6.4 Electron micrograph showing ultrastructure of companion cell and sieve element wall. The wall possesses marked swellings on the companion-cell side, which contain multiple branched plasmodesmata. Companion cell has numerous mitochondria and ribosomes, small vacuoles and a poorly developed plastid. Fixation in glutaraldehyde/formaldehyde followed by osmium tetroxide. Magnification X 16940.



plasmodesmatal cavities were quite distinct. The cytoplasmic contents of the companion cell are indicative of a high level of metabolic activity, notably the large number of mitochondria and abundant ribosomes. The companion cell also possessed rough endoplasmic reticulum, a plastid with rather poorly defined lamellae (which may represent a photosynthetically inactive leucoplast), and several small vacuoles. An enlargement of one of the wall swellings in Plate 6.4 is presented in Plate 6.5. This shows the plasmodesmatal cavities converging on the median cavity in the region of the middle lamella; the suggestion from Plate 6.4 is that there are at least two plasmodesmatal orifices on the sieve-element side of this junction. Each of the branches meeting at the median cavity contains a desmotubule, which may be continuous with endoplasmic reticulum at the companion-cell side. There are indications that the plasmalemma of the companion cell is contiguous with the lining of the plasmodesmatal cavities. This Plate also suggests that the wall swelling on the companion-cell side consists of fairly uniform layers of cellulose microfibrils orientated transversely to the length of the sieve element. This structure is also apparent in Plate 6.6, which shows membranous stacks of the Golgi apparatus close to the wall swelling; the vesicles associated with the Golgi body may be involved in transfer of substances to the cell wall for biosynthesis of new wall material. There is also electron-lucent callose deposited around the openings of the plasmodesmatal cavities on the sieve-element side, a feature that seems to be characteristic of phloem plasmodesmata generally (Esau, 1969; Gunning, 1976; Jones, 1976).

These results indicate that there is good symplastic continuity between the companion cells and sieve elements in Ricinus. It seems unlikely that transfer of solutes between these cell types would show a net requirement for metabolic energy, in which case the considerable metabolic capacity of the companion cells is likely to be directed towards uptake of solutes from the bulk leaf tissue. The plasmodesmatal ultrastructure further suggests that there may be various forms of structural as well as physiological continuity between these cell types.

6.3 Sugar uptake by leaf discs

The characteristics of sugar uptake by leaf discs have been investigated in a few species, but the system has only recently been

Plate 6.5 Enlarged portion of Plate 6.4 showing ultrastructure of branched plasmodesmata and swelling of secondary wall of companion cell. The desmotubules within the plasmodesmatal cavities appear to be continuous with plasmalemma of companion cell (*arrows*), and fuse at the median cavity in the region of the middle lamella. Microfibrils in secondary wall are orientated transversely with respect to the longitudinal axis of sieve element. Magnification X 90320.



Plate 6.6 Electron micrograph of multiple branched plasmodesmata in wall between sieve element and companion cell. There appear to be at least two plasmodesmatal orifices on sieve-element side (*arrows*); these are surrounded by electron-lucent deposits of callose. Note on companion-cell side close association of golgi vesicles with wall swelling. Fixation in glutaraldehyde/formaldehyde followed by osmium tetroxide. Magnification X90320.



applied specifically to the study of phloem loading (Giaquinta, 1977b). Although the use of leaf discs in such studies presents certain advantages compared with other forms of excised tissue (Vickery and Mercer, 1964), it must be recognized at the outset that the direct relevance of the system to sugar transport and metabolism in intact plants is strictly limited. As regards uptake by the phloem, the major restriction is that sugar taken up by the sieve tubes is not transported away from the sites of loading. Nevertheless, some of the properties of the uptake process may be similar to those of loading in the whole plant, and the principal aim of this work with Ricinus leaf discs was to examine whether solute uptake was influenced by the osmotic relations of the tissue. The basis of these experiments was to adjust the turgor of the leaf tissue by incubating the discs on solutions containing different concentrations of the relatively impermeant solute sorbitol. This approach has been used in analogous studies on the relation between malate transport and cell water relations in CAM plants (see Lüttge, Kluge and Ball, 1975).

The following points of experimental design were included in all the studies described in this section. Firstly, discs were taken from inter-veinal regions of fully expanded alternate leaves to ensure that the material possessed true source characteristics. Secondly, the adaxial epidermis was gently abraded with moist Aloxite powder to facilitate entry of solution into the leaf tissue: this method has been used in studies on Beta (Geiger, Sovonick, Shock and Fellows, 1974; Giaquinta, 1977b), and evidence was presented earlier (Plate 4.1) that the treatment does not disrupt the vascular tissue in Ricinus. Thirdly, the discs were floated, after rinsing, on distilled water at ambient temperature containing $1.0 \text{ mol m}^{-3} \text{ CaCl}_2$ for 0.75 h. This allowed the water deficits incurred on incision to be at least partially alleviated. The discs were then transferred to a pre-incubation medium containing osmoticum (but not tracer) to allow for a standardized degree of equilibration (for 0.5 h) of the tissue water relations with the water potential of the incubation medium. Finally, after incubation on the media containing labelled sugars, the discs were given three washes of 10 min each to remove free-space label: analysis of the wash-out kinetics showed that more than 98% of the freely diffusible label was removed by the end of the second wash.

A suitable incubation time for examining sugar uptake was determined by following the time course of absorption of tracer by

the leaf discs. Fig. 6.1 shows this time course expressed both as cumulative amounts of tracer taken up (lower graph) and rate of uptake (upper graph) from a medium containing ^{14}C -sucrose and 250 mol m^{-3} sorbitol. The rate of uptake in fact declined over the first hour of incubation, then rose to a maximum after about 4 h, and subsequently decreased slowly. This initial decline may correspond to the period when the water balance of the tissue was still being restored following incision. Another possibility is that there may have been some leakage of absorbed solutes from the cut ends of the sieve elements in the minor veins; it would be expected, however, that occlusion of the sieve-plate pores by callose and membrane synthesis might seal the ends of these elements fairly quickly. This period of uptake may also represent what Vickery and Mercer (1964) referred to as 'osmotic uptake', in Vicia leaf discs i.e. uptake into the free space. However, with the Ricinus discs the length of the diffusion paths will be much reduced by abrasion of the epidermis, and thus equilibration with the apoplastic solution should have been more rapid.

To make certain that the observed characteristics of sugar uptake in these studies were a reflection of transfer into the cells, an incubation time of 8.0 h was adopted as standard in all the subsequent experiments. Longer incubation times would have introduced more error if the accumulation of sugars in the tissue had an effect on rates of uptake, as has been shown for Ricinus cotyledons (Komor, 1977; Waldhauser and Komor, 1978). In addition, growth of the leaf discs over extended incubation periods presents further problems in interpretation of uptake kinetics. Moreover, uptake of sorbitol itself will also tend increasingly to disturb the osmotic balance between the cells and incubation medium: although mannitol and sorbitol are relatively impermeant solutes, their uptake nevertheless proceeds at a significant rate (Trip, Krotkov and Nelson, 1964; Jones, 1973; Fondy and Geiger, 1977).

The distribution of label taken up from solutions containing either ^{14}C -sucrose or ^{14}C -glucose was compared by soluble-compound autoradiography. Examples of these autoradiographs are shown in Plate 6.7; controls for positive and negative chemography proved negative. One general problem encountered with this method was that adhesion of the leaf discs to the subbed slides gave rise to cracking of the tissue on freeze-drying. This problem was never completely overcome, but was found to be less serious if the discs were not actually attached

Fig. 6.1 Time course of ^{14}C uptake by Ricinus leaf discs. Lower graph: cumulative amount of ^{14}C -tracer absorbed, calculated assuming ^{14}C -sucrose not metabolized prior to uptake; points are means for 6 discs, with bar representing \pm S.E. where larger than symbol. Upper graph: Data plotted as rate of ^{14}C uptake.

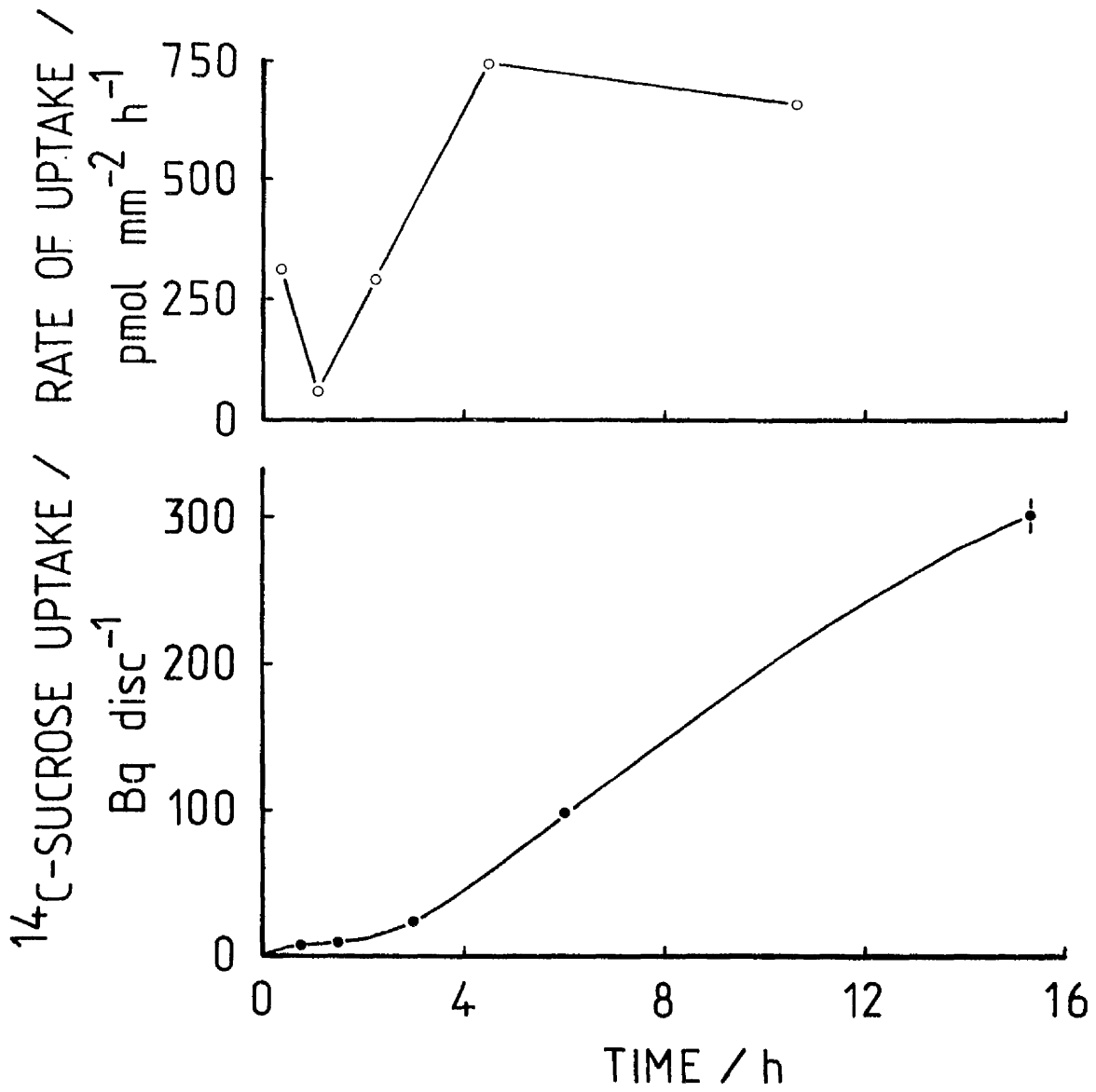
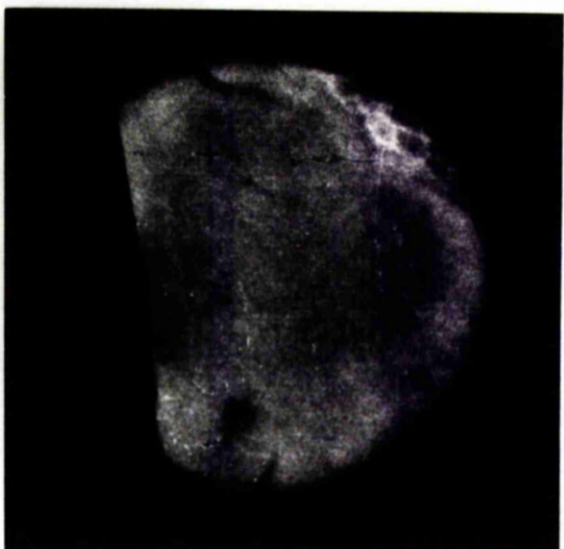
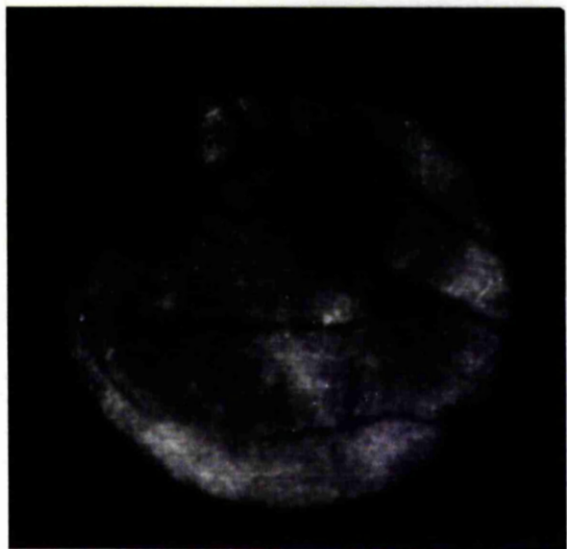
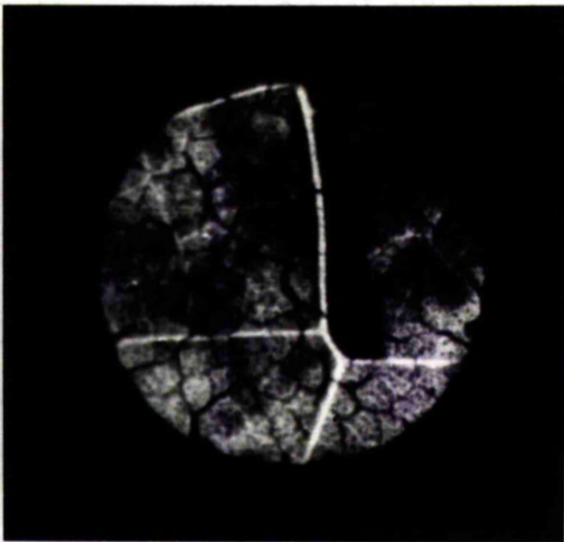
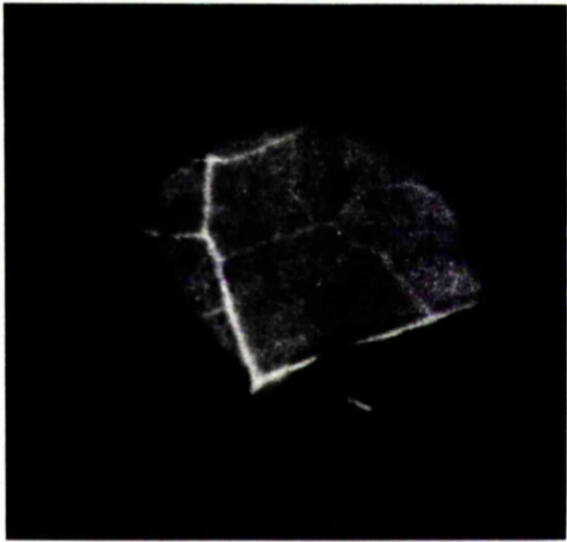


Plate 6.7 Autoradiographs showing distribution of radioactivity (white areas) in leaf discs following 8.0 h incubation on media at pH 6.0 ($\Psi_s = -1.1$ MPa) containing either ^{14}C -sucrose (*top row*) or ^{14}C -glucose (*bottom row*). Magnification X8.0.



to the slides during this stage of the processing. The autoradiographs nevertheless indicate that there seemed to be preferential accumulation of label from ^{14}C -sucrose in the minor veins of the tissue, whereas that derived from ^{14}C -glucose was more evenly distributed. Following the ^{14}C -sucrose treatment, there was usually a relatively high level of background radioactivity in the bulk leaf tissue (mesophyll), and this may have been caused by metabolism and redistribution of the solute following uptake. Shorter incubation times with higher specific activity ^{14}C -sucrose might also have made the labelling pattern of the lowest order veins in the tissue somewhat clearer.

The osmotic characteristics of solute uptake were examined by comparing rates of absorption from incubation media containing different concentrations of osmoticum. One other variable introduced was the use of either sorbitol (as a slowly permeating solute) or ethylene glycol (as a rapidly permeating solute) for the osmoticum (Greenway and Leahy, 1970). This permitted an investigation of which particular components of the total water potential might influence solute uptake, for the following reasons. When the leaf disc is in equilibrium with pure water, the equation describing the water relations of the cells is

$$\psi^i = \psi_s^i + \psi_p^i = 0 \quad (6.1)$$

where the symbols have their usual meanings, and the superscript i denotes the values inside the cell. Now, if the water potential of the medium is lowered to $\psi^o (= \psi_s^o)$ by addition of a rapidly permeating non-electrolyte, a new equilibrium will soon be reached, where

$$\psi^i = (\psi_s^i + \psi_s^o) + \psi_p^i = \psi^o \quad (6.2)$$

In this case, therefore, both the cell ψ and ψ_s are changed, but the cell regains the original ψ_p (and cell volume) indicated by equation 6.1. The alternative case may be taken as that in which ψ^o is lowered by addition of a non-electrolyte to which the cells are impermeable (i.e. with a reflection coefficient, σ , of unity): water is exchanged between the cell and the medium until a new equilibrium is reached thus

$$\psi^i = (\psi_s^i + \Delta\psi_s) + (\psi_p^i + \Delta\psi_p) = \psi^o \quad (6.3)$$

The terms $\Delta\psi_s$ and $\Delta\psi_p$ represent the adjustments resulting from the change in cell volume caused by efflux of water from the cell to the

medium, although the magnitude and time course of the changes depends on the values of ϵ and L_p (see equation 1.7, p.33, and equation 4.3, p. 124). For slowly permeating solutes, equation 6.3 represents a form of quasi-equilibrium, since true equilibrium (equation 6.2) will only be reached over a much longer period. The reflection coefficient of mannitol and sorbitol is sufficiently close to unity to permit a comparison with the effects of ethylene glycol in distinguishing between the importance of Ψ_p and the other components of Ψ in such studies (see Greenway and Leahy, 1970; Jones, 1973).

An example of the characteristics of uptake of label from media containing ^{14}C -sucrose and adjusted to different Ψ_s values with sorbitol is shown in Fig. 6.2. Five experiments of this sort were carried out, and similar trends were observed in each of the experiments. The Ψ_s values of the incubation media were determined cryoscopically, and the total sucrose concentration (or glucose concentration, as appropriate) in the solution was 30 mol m^{-3} . Fig. 6.2 shows that there was little change in the mean values of tracer uptake down to a Ψ_s of about -1.55 MPa in the medium, but that at lower values of Ψ_s the rate of uptake declined markedly. The estimates of standard error are relatively large in this graph, since only 6 leaf discs were used per treatment; in subsequent experiments at least 12 discs were routinely used for each treatment.

In view of the Ψ_s values of leaf-cell sap and phloem sap, it seems likely that these decreases in uptake rate below -1.55 MPa were associated with cell plasmolysis, i.e. the point at which cell $\Psi_s = \Psi_s$ of the incubation medium. However, both the sieve tubes and the cells of the bulk leaf tissue probably plasmolyzed in this range, since the sap Ψ_s values of the tissues are similar (see Chapter 3). Separate light-microscope studies indicated that the mesophyll and vascular parenchyma generally plasmolyzed in the range -1.2 to 1.5 MPa , but if phloem Ψ_s was about -1.4 MPa at the source, the sieve tubes themselves would be expected to plasmolyze at around this stage (allowing for a TDV of 12%). Another complication is that in some of the experiments a sharp decline in uptake was observed at values higher than -1.55 MPa . This was presumably associated with variability in the osmotic relations of the leaf tissue, although plants were grown under growth-room conditions for several days prior to experimentation and were used at similar times of day (see note in Evert, Eschrich and Heyser, 1978).

Fig. 6.2 Relationship between ^{14}C uptake by Ricinus leaf discs from solutions containing ^{14}C -sucrose and adjusted to different Ψ_s values with sorbitol. Values for sucrose uptake calculated assuming tracer not metabolized prior to uptake. Points represent means, with bars denoting 2 x S.E. for samples of 6 discs per treatment.

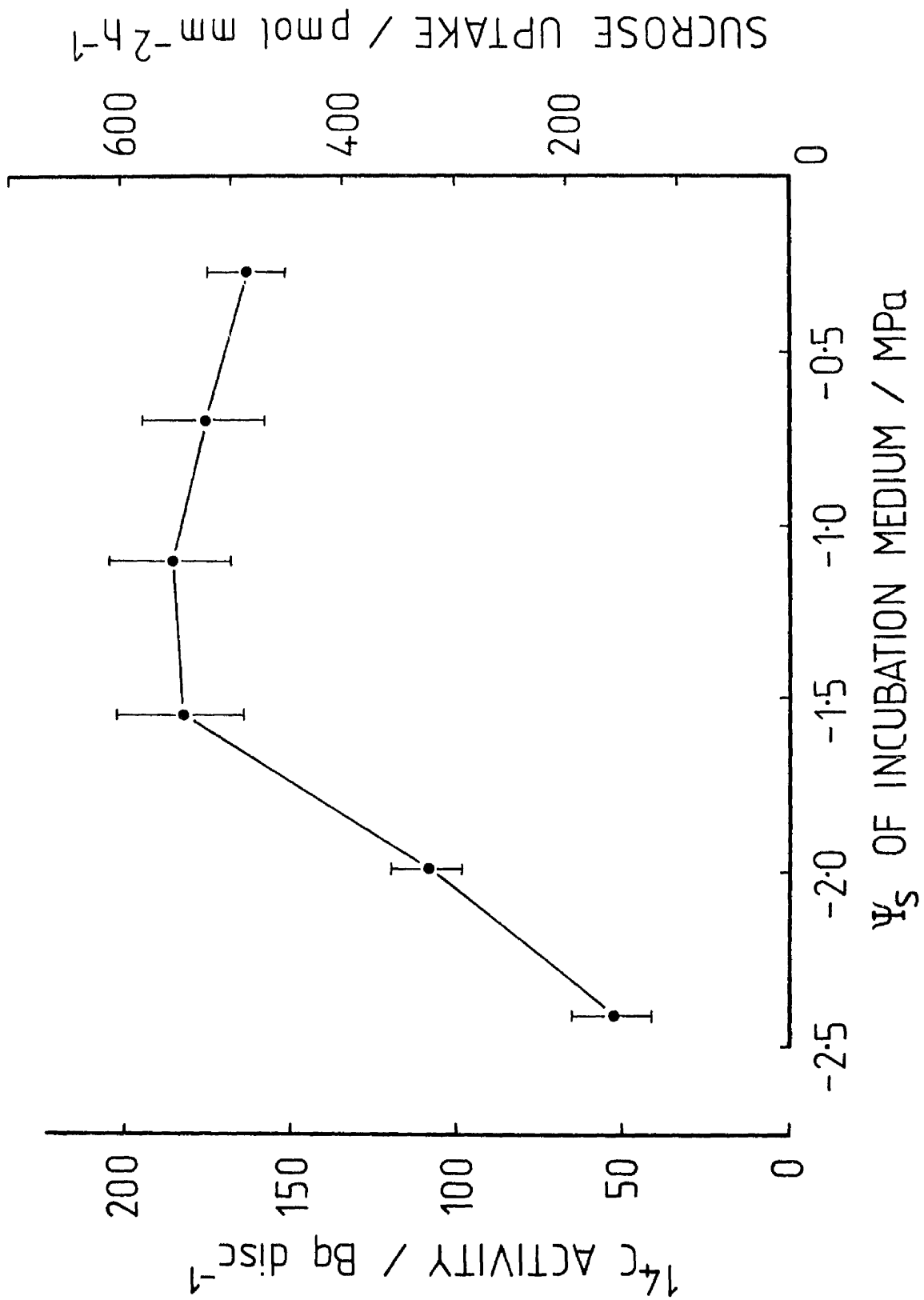


Fig. 6.3 shows an example of an experiment in which sucrose uptake decreased markedly below about -1.2 MPa. In this experiment, uptake of tracer from ^{14}C -glucose was studied in addition to that from ^{14}C -sucrose. If the assumption is made that there was no metabolism prior to uptake, the results indicate that the rates of glucose uptake were nearly three times higher in absolute terms than sucrose uptake. Glucose uptake has been found to be at least as great as sucrose uptake in leaf discs from other species (e.g. Weatherley, 1954), but the autoradiographic evidence for Ricinus suggests that the uptake patterns (or subsequent redistribution of the radioactivity) were different for the two sugars (Plate 6.7). There is, in fact, a qualitative difference apparent in Fig. 6.3 between the change in rate of uptake for sucrose and glucose with decreasing Ψ_s of the medium. Whereas sucrose uptake was stimulated by the decrease in Ψ_s to about -1.2 MPa, glucose uptake was inhibited over this same part of the range; both these trends were significant at the level of $P < 0.001$. Thereafter, the uptake of both sugars decreased with a further decline in Ψ_s of the medium. If sucrose uptake occurs preferentially into the phloem in comparison with glucose, this suggests that there may be a distinct response of absorption into the sieve tubes as the Ψ_s difference between the cells and the medium decreases.

As discussed earlier, the changes in cell water relations induced by using a relatively impermeant solute affect all the components of the water potential. To help distinguish between the various components, a similar experiment was therefore carried out with ethylene glycol as the osmoticum in the incubation medium. The results of this experiment are shown in Fig. 6.4, from which it can be seen that there was no marked change in the rate of uptake of either sucrose or glucose down to a Ψ_s of less than -2.0 MPa in the incubation medium. Between -0.24 and -0.91 MPa there was a slight increase in sucrose uptake (about 8%, significant at $P < 0.05$), and a decrease in glucose uptake of a similar order (about 15%, significant at $P < 0.01$). These results suggest that the major part of the stimulation of sucrose uptake observed with decreasing Ψ_s of the sorbitol media in the non-plasmolytic range was associated with a decrease in Ψ_p (or some function of a decrease in cell volume). On the other hand, the decrease in glucose uptake over this range in the ethylene glycol media accounted for over half of the decrease observed in the sorbitol media, so that cell Ψ (or Ψ_s) may be more important in this instance. The results also indicate

Fig. 6.3 Relationship between sucrose and glucose uptake by Ricinus leaf discs from solutions containing ^{14}C -sucrose and ^{14}C -glucose, respectively, and adjusted to different Ψ_s values with sorbitol. Values for sugar uptake, calculated assuming tracer not metabolized prior to uptake. Points are means, with bars denoting 2 x S.E. for samples of 12 discs per treatment.

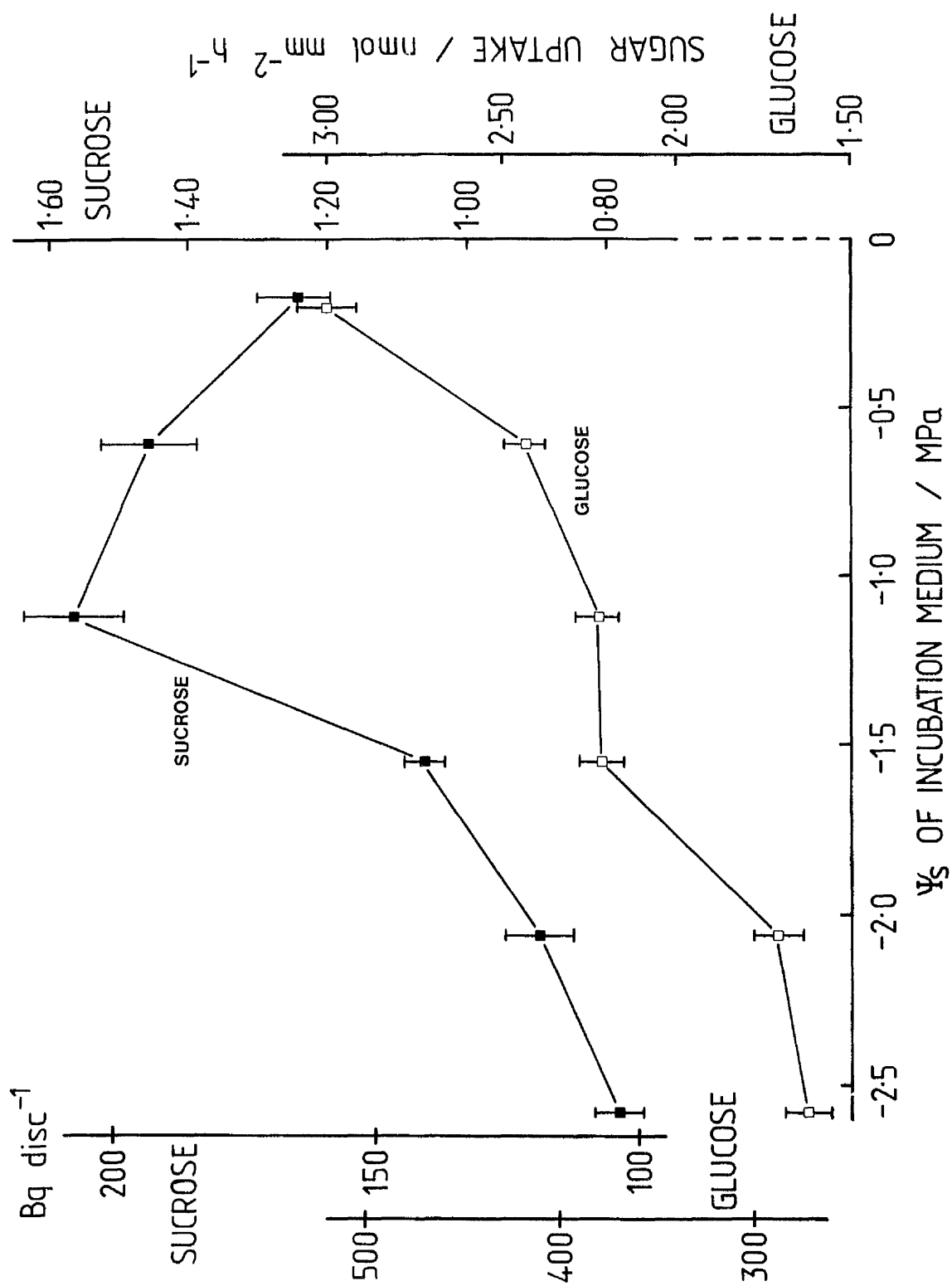
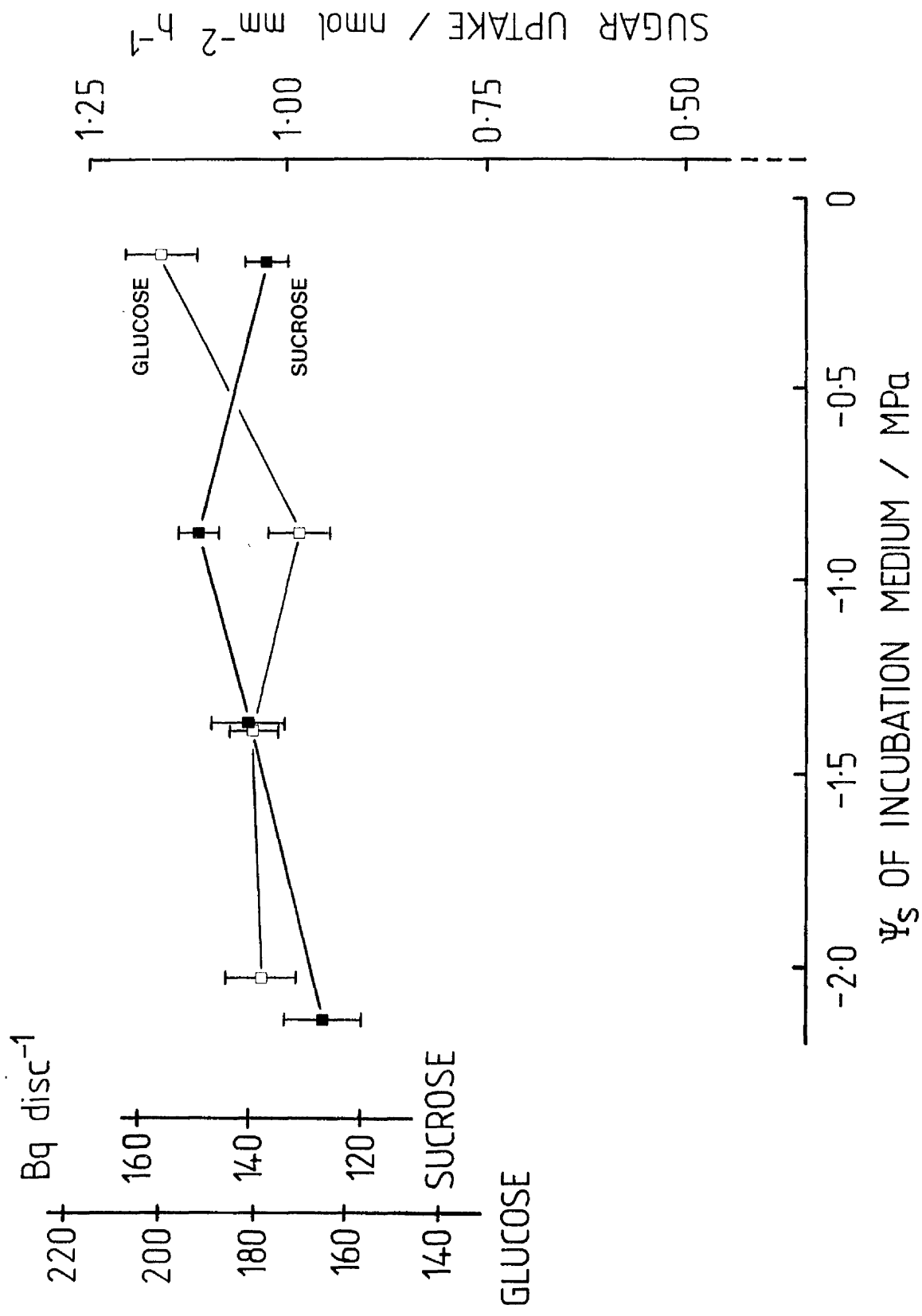


Fig. 6.4 Relationship between sucrose and glucose uptake by Ricinus leaf discs from solutions containing ^{14}C -sucrose and ^{14}C -glucose, respectively, and adjusted to different Ψ_s values with ethylene glycol. Values for sugar uptake calculated assuming tracer not metabolized prior to uptake. Points are means, with bars denoting $2 \times \text{S.E.}$ for samples of 12 discs per treatment.



that plasmolysis of the leaf tissue had not occurred even at -2.1 MPa in ethylene glycol, whereas in sorbitol plasmolysis seemed to have been caused at values as high as -1.2 MPa. This shows that σ for ethylene glycol was less than 0.6, because the effective osmotic potential of a solution divided by its true water potential gives a measure of σ (Dainty, 1963).

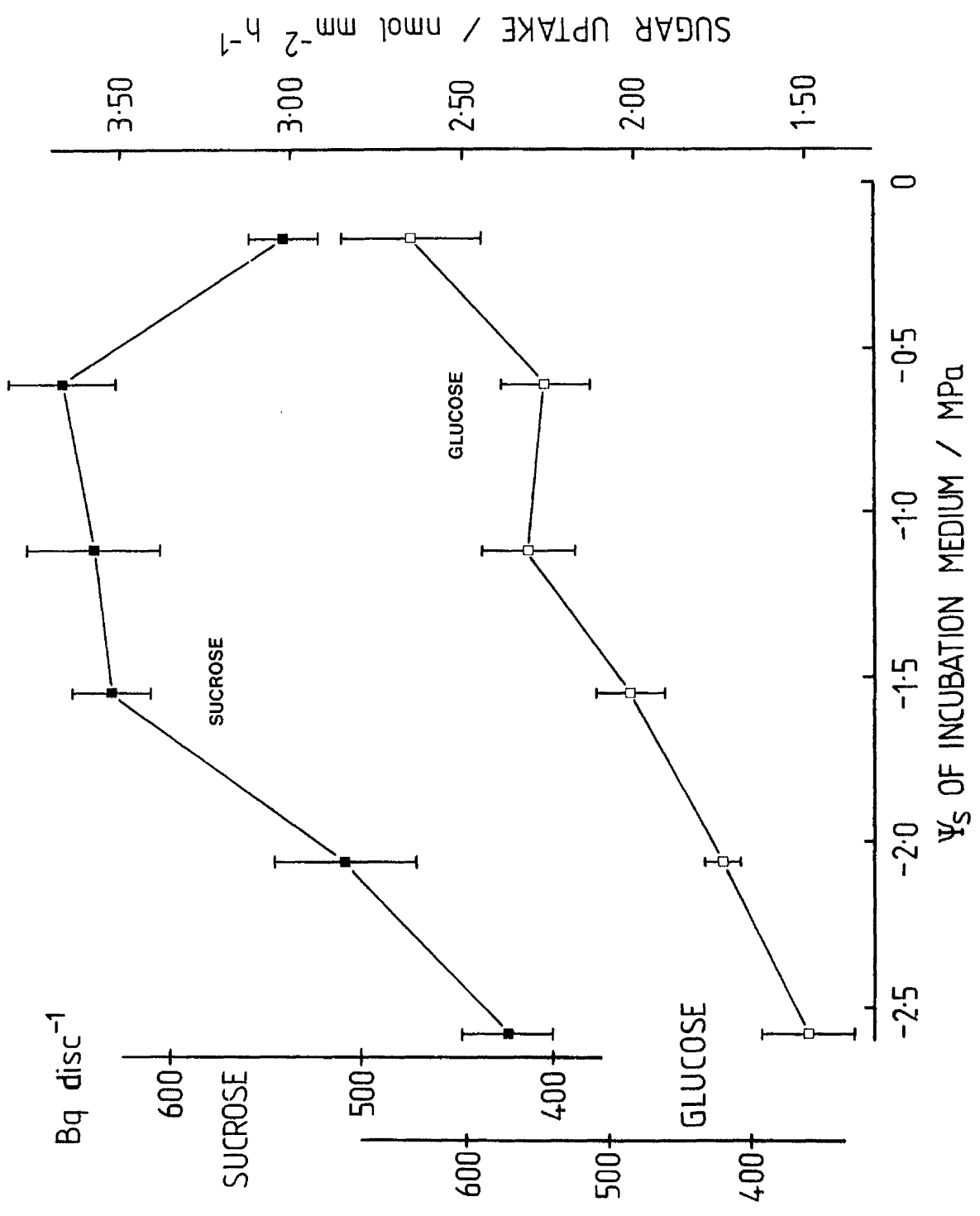
One further experiment of this sort was performed with the sorbitol media, but using Beta vulgaris leaf discs instead of Ricinus tissue. This was prompted by observations made by Giaquinta (1977b) to the effect that sucrose uptake by Beta leaf discs was greater from media containing 0.95 kmol m^{-3} sorbitol ($\sim -2.6 \text{ MPa}$) than from those containing only 0.30 kmol m^{-3} sorbitol ($\sim -0.8 \text{ MPa}$). Since the mesophyll cells plasmolyze in Beta leaves at about -1.3 MPa, but the phloem is still at positive Ψ_p at -2.6 MPa (Geiger, Giaquinta, Sovonick and Fellows, 1973), this would suggest that sucrose uptake into the sieve tubes is stimulated by the decrease in Ψ_s of the medium. The results from the present experiments with Beta discs are shown in Fig. 6.5. Although the rate of sucrose uptake increased with decreasing Ψ_s at the highest end of the range, a significant fall in the rate of uptake was observed below a Ψ_s of about -1.55 MPa in the medium. Glucose uptake declined gradually with decreasing Ψ_s , the absolute values being slightly lower than those for sucrose uptake. These values, however, were two or three times higher than the rates of sucrose uptake by Ricinus discs.

Thus, Beta leaf discs showed a similar pattern of increased sucrose uptake under these conditions with decreasing Ψ_s of the medium at the high end of the range, as found in Ricinus. The decline in sucrose uptake below -1.55 MPa, however, suggests that either the mesophyll contributed to sucrose uptake by the Beta discs to a quite significant extent, or possibly that the phloem is plasmolyzing at higher Ψ_s values than anticipated. It is difficult to make a direct comparison with the observations made by Giaquinta (1977b) without more detailed information on the water relations of the leaf tissues, and the way in which these may be influenced by growth conditions.

6.4 Discussion

One of the notable features of the companion cells in minor veins of Ricinus leaves is that they do not possess transfer cells. The conspicuous cell-wall ingrowths of this cell type may sometimes

Fig. 6.5 Relationship between sucrose and glucose uptake by Beta leaf discs from solutions containing ^{14}C -sucrose and ^{14}C -glucose, respectively, and adjusted to different Ψ_s values with sorbitol. Values for sugar uptake calculated assuming tracer not metabolized prior to uptake. Points are means, with bars denoting 2 x S.E. for samples of 12 discs per treatment.



be visible at the level of the light microscope (see Gunning^{and} Pate and Briarty, 1968; Gunning and Pate, 1969), although none of the companion cells or phloem parenchyma examined in Ricinus showed this modification. In general, transfer cells tend not to be found in woody dicotyledons, although there is a dearth of information on vein structure in other members of the Euphorbiaceae (Pate and Gunning, 1969).

The ultrastructure of the sieve element-companion cell complex in the minor veins is in most respects highly typical of that found in other dicotyledonous species (see Esau, 1969; Gunning, 1976). Towards the vein endings themselves, the sieve elements may lie only one cell removed from (and possibly adjacent to) the xylem elements. The small sieve elements in these regions possessed relatively thick cell walls compared with the larger sieve elements and those of neighbouring cells. In Zea (a C₄ plant), thick-walled sieve elements commonly lie next to xylem elements, and it has been suggested that these sieve elements may have a rôle in retrieving solutes entering the apoplast from the transpiration stream (Evert *et al.*, 1978). These sieve elements in Zea have abundant plasmodesmatal connections with neighbouring parenchyma cells, but do not seem to be associated with companion cells. In Ricinus, on the other hand, these small sieve elements had particularly good symplastic contact with companion cells and phloem parenchyma.

The companion-cell ultrastructure in Ricinus was indicative of a high level of metabolic activity, the cells containing numerous mitochondria, significant quantities of rough endoplasmic reticulum, and abundant ribosomes. Plasmodesmatal connections were found between companion cells and adjacent parenchyma, but those between the companion cells and sieve elements were the best developed. It appears to be characteristic of this junction in a large number of species that the plasmodesmata are branched on the companion-cell side of a median cavity (Esau, 1969; Gunning, 1976). These branches do not seem to arise during cell-plate deposition, and may thus be regarded as secondary modifications of a simple plasmodesma (Jones, 1976). Callose formation around the plasmodesmatal orifice on the sieve-element side of the wall is also characteristic of these plasmodesmata in many species (see Jones, 1976).

Some details of the ultrastructure of sieve element-companion cell plasmodesmata differ considerably between species, such as the

degree of branching of the cavities and the extent of the median cavity. The wall swellings on the companion-cell side are found commonly in other species (e.g. Esau, 1969; Gunning, 1976; Bentwood and Cronshaw, 1978), and may even occur to a slight extent when the plasmodesmata are unbranched (Evert, Eschrich and Heyser, 1977). In Ricinus, these wall swellings are unusually large, and may contain over ten separate plasmodesmatal cavities. It is clear, however, that these may be derived from more than just a single plasmodesma (see Plates 6.4 and 6.6), and it would be worth investigating the equivalent structure in young sieve elements for information on their ontogeny.

The frequency of these branched plasmodesmata suggest that there is a good symplastic pathway for vectorial transfer of solutes from the companion cells to the sieve elements. In fact, it seems likely that these structures must be able to accommodate far higher rates of symplastic transport than could be achieved through single plasmodesmata without any wall amplification on the companion-cell side of the wall. The diameter of the pores is of critical importance in determining rates of flow (see Gunning and Robards, 1976; Blake, 1978), and the size of the plasmodesmatal cavity on the sieve-element side may thus constitute something of a 'bottle-neck'. However, if flow through the plasmodesmata can be described by Poiseuille's Law (where volume flow would be proportional to the fourth power of the radius of the canal), then even a small increase in the size of the pore on the sieve-element side would permit a large increase in flow rate. On the companion-cell side, increased wall synthesis may be a precondition for the proliferation of plasmodesmatal cavities. In this sense, the structure may be functionally analogous to the wall amplification found in transfer cells, for the branched plasmodesmata may permit high rates of flux through the symplasm in the same way that transfer cells are presumed to allow high flux rates across the apoplast-symplast boundary. It would be especially useful to know if these wall swellings and branched plasmodesmata tend to be found in species that show high rates of phloem loading.

How much sucrose can be transported from the sites of synthesis to the companion cells entirely in the symplasm remains uncertain. In some species (e.g. Beta) the phloem Ψ_s is considerably lower than that of the mesophyll, which suggests that membrane transport is needed at some stage to transfer sucrose against the concentration gradient (see Geiger, 1975a). In Ricinus, however, the Ψ_s of the

sieve tubes and bulk leaf tissue is more similar. This is of direct relevance to the leaf-disc studies, because it means that plasmolysis of all the cell types will occur at about the same stage. The differences between the characteristics of sucrose and glucose uptake nevertheless allow some inferences to be made about the uptake systems.

The autoradiographic evidence presented in Plate 6.7 suggests that sucrose accumulation occurs preferentially in the veins in these leaf discs, whereas glucose uptake (or redistribution) occurs more evenly. The long incubation periods used for these experiments complicate interpretation of the autoradiographs, because transfer and metabolism of the solutes will obscure the patterns of uptake. The background level of radioactivity in the bulk leaf tissue is considerably higher in these Ricinus discs than in those of Beta (see Giaquinta, 1977b). This is probably a function of the prolonged incubation times used for Ricinus, but it is conceivable that there may also be more transfer of sucrose from the mesophyll to the phloem (rather than direct loading into the phloem from the apoplast) in Ricinus compared with Beta. These incubation times are also likely to have introduced more errors due to the gradual uptake of sorbitol, although estimates of the Ψ_s of the tissues based on plasmolysis after 8 h should have been too negative if this was a significant effect. If anything, the cells seemed to plasmolyze at slightly higher Ψ_s values than anticipated. Results from experiments in which mannitol has been used in incubations of up to 13.5 h indicate that errors of this sort may be within acceptable limits (Lüttge et al., 1975). MES buffer is also an effectively non-permeating solute (Good, Winget, Winter, Connolly, Izawa and Singh, 1966). At any rate, these sources of error in experiments of this sort have long been appreciated (Bennet-Clarke, Greenwood and Barker, 1936).

The results in Fig. 6.3 show that these differences in uptake patterns may also be significant in terms of the responses of sucrose versus glucose absorption to decreasing Ψ_s of the medium. Because it is likely that phloem uptake is accounting for a large part of the ^{14}C uptake from ^{14}C -sucrose media (even if there is subsequent redistribution of solutes), the decline in uptake rate below about -1.1 MPa implies that this is where the phloem may be reaching incipient plasmolysis (cf. Vickery and Mercer, 1967). In fact, it is possible that uptake is inhibited even before incipient plasmolysis

is reached, if (for example, membrane deformation altered the activity of the carrier enzymes. The most important part of the effect of Ψ_s of the incubation medium on sugar uptake is thus the nature of the response in the non-plasmolytic range. Fig. 6.3 shows that there was a significant increase (+26%) in sucrose uptake over this range and an equivalent decrease (-26%) in glucose uptake.

There is evidence from studies on sucrose uptake from incubation media for Ricinus cotyledons (Kriedemann and Beevers, 1967a, b; Komor, 1977; Hutchings, 1978a) and for other leaf disc systems (Weatherley, 1955; Vickery and Mercer, 1967; Giaquinta, 1977b) that absorption from the apoplast is an active process. There is a marked pH optimum between about 5.5 and 6.0 in Beta discs, though Komor (1977) has reported that the pH dependence of sucrose uptake into Ricinus cotyledons (which autoradiographic evidence suggests is into the phloem: Kriedemann, 1967) is not especially pronounced; a pH of 6.0 was used for the incubation medium in the present studies. The kinetics of sucrose uptake by the leaf discs, however, are greatly influenced by the way in which the tissues are prepared. Without abrasion, uptake proceeds mainly through the cut edges of the discs rather than over the whole surface (Pennell and Weatherley, 1958), and this suggests that the passive ('osmotic') uptake during the first 4 h with such discs largely corresponds to diffusion of solute into the free space (Pennell and Weatherley, 1958; Vickery and Mercer, 1967). With the epidermis abraded, this complication is to some extent avoided. Giaquinta (1977b) has shown that over the first 0.5 h of uptake with Beta discs the absorption exhibits Michaelis-Menten characteristics with respect to the sucrose concentration in the medium. In these Beta discs, the K_m value for sucrose accumulation at pH 5.0 was 25 mol m^{-3} , which is the same as that found by Komor (1977) for Ricinus cotyledons. Preliminary experiments in the present system with Ricinus discs that were incubated for only 0.5 h gave K_m values for sucrose uptake of $15\text{-}20 \text{ mol m}^{-3}$, as calculated from a Lineweaver-Burk plot. Considerable variability seems to be found within most systems, however, and the corresponding values for uptake by Vicia discs ranged between 5.2 and 15.3 mol m^{-3} .

One important aspect of these K_m values is that they are similar to the K_m values estimated for sucrose loading in intact plants (see Cataldo, 1974; Sovonick, Geiger and Fellows, 1974). Beta is by far the most extensively studied species in this respect,

and loading in source leaves does seem to involve active sucrose uptake from the apoplast (Sovonick *et al.*, 1974; Geiger *et al.*, 1974; Fondy and Geiger, 1977). It has also been shown using intact Ricinus seedlings that the Michaelis-Menten characteristics of sucrose uptake from the incubation medium coincide with those for sucrose export from the cotyledons, implying that uptake and longitudinal transport may be part of the same mechanism (Waldhauser and Komor, 1978). The leaf-disc system may therefore provide information on the osmotic characteristics of sucrose uptake that has some bearing on the loading process in whole plants. Beta leaf discs used in the present experiments also showed the same increase in sucrose uptake (and decrease in glucose uptake) with decreasing Ψ_s of the medium at the high end of the range studied.

The equivalent experiment carried out with Ricinus leaf discs using ethylene glycol as the osmoticum showed that decreasing Ψ_s of the medium had only a slight effect on sucrose uptake, although the effect on glucose uptake was somewhat greater. A summary of the relative effects of the sorbitol and ethylene glycol media is presented in Table 6.1. The values are expressed relative to those found for uptake from the media of highest Ψ_s used in these experiments; this is reasonably close to the Ψ_s of the xylem sap, and is arbitrarily referred to as 'full turgor'. The values for uptake from the ethylene glycol media indicate how much of the change in uptake observed with decreasing Ψ_s in the sorbitol media can be attributed to alteration of cell Ψ or Ψ_s . Thus, the difference between these two sets of values represents the change in uptake associated with alteration of cell Ψ_p (or cell volume). It may be inferred that about two-thirds of the increase in sucrose uptake caused by decrease in Ψ_s of the medium was associated with a fall in cell Ψ_p , whereas more than half of the decrease in glucose uptake over a similar range was associated with alterations to cell Ψ or Ψ_s .

Despite the limitations of this leaf-disc system, the results taken together suggest that sucrose accumulation in the phloem is in some way affected by cell turgor (or volume). Because of the experimental conditions involved in studies with isolated tissues, such results do not constitute a reasonable basis on which to make predictions about the characteristics of phloem loading in the intact plant. The data are, however, relevant to the observations made in section 5.2, in which a decrease in phloem Ψ_p during water stress was

Table 6.1 Summary of effect of decrease in ψ_s of incubation medium on sucrose and glucose uptake by Ricinus leaf discs. Values are expressed as percentages of those at 'full turgor'. P values were determined for original data.

ψ_s of incubation medium/MPa	sorbitol medium			ethylene glycol medium		
	approximate ψ_p of tissue	sucrose uptake	glucose uptake	approximate ψ_p of tissue	sucrose uptake	glucose uptake
-0.15	100	100 ± 4	100 ± 3	100	100 ± 3	100 ± 4
-1.08 / -0.88	30	126 ± 6	74 ± 2	~100	108 ± 3	85 ± 3
		(P < 0.01)	(P < 0.001)		(P < 0.05)	(P < 0.01)

associated with an increase in the rate of loading. Moreover, under some conditions (as with the short-term responses considered in Chapter 4) a decrease in phloem Ψ_p may be accompanied by an at least transient fall in phloem Ψ . Insofar as they refer to the same part of the transport process, these results with leaf discs are at least consistent with the responses expected of a negative feedback system in which sucrose loading is modulated by phloem turgor.

7. GENERAL DISCUSSTION AND CONCLUSIONS

7. GENERAL DISCUSSION AND CONCLUSIONS

Control of phloem transport in higher plants must be exercised at two levels. On the one hand, developing organs are largely dependent on the phloem for their supply of nutrients, and thus the chemical composition of the sap must be controlled within the limits set by whole-plant metabolism. On the other, the rate of mass transfer of these solutes through the phloem is directly determined by the water relations of the plant and the rate of phloem loading and unloading. An understanding of both these aspects of control is required to characterize the transport system, and the way in which they impinge specifically on the loading process has been the main concern of the preceding chapters.

The principal solutes in the phloem sap of Ricinus are organic C and N. Whereas sucrose is derived directly from photosynthetic assimilation, the organic N content of the sap is dependent on the nitrogenous compounds supplied to the shoot in the xylem and on subsequent metabolism in the leaves. The extent to which the sap composition of the long-distance transport pathways is buffered against changes in nutrient supply from the external medium was examined in Chapter 3. By comparing the effects of NO_3^- -N versus NH_4^+ -N nutrition, it is possible to determine how the solute content of the xylem and phloem is functionally related to root and shoot metabolism. Although the growth characteristics of plants on these alternative N-sources have been extensively studied in a large number of species, the consequences of the differences in metabolism have not been directly investigated in terms of long-distance transport processes.

The most pronounced difference in shoot metabolism associated with the two inorganic N forms is in the organic-acid content of the leaf tissues. The greatly reduced level of carboxylates when NH_4^+ is the N-source has been widely noted in other species, and is consistent with the notion that bulk accumulation of organic acids is dependent on charge transfer from NO_3^- . Mechanistically, this difference may be related to the net OH^- or H^+ produced during N assimilation (see p.83). Thus, whereas the excess OH^- generated during NO_3^- assimilation can be neutralized by synthesis of organic acids, excess H^+ must be disposed of by excretion from the roots. Essentially all the N assimilation in the NH_4^+ -N plants is therefore

carried out in the roots, and the shoot receives largely organic N in the form of amides and amino acids. With the NO_3^- -N plants, the OH^- excreted to the root medium seems to be a reflection of the amount of NO_3^- reduction occurring in the roots without associated synthesis of organic acids. The amount of NO_3^- uptake occurring by $\text{NO}_3^-/\text{OH}^-$ exchange may also be significant in terms of the control of the bulk solute content of the whole plant (Cram, 1976a).

One point emphasized by the data in Chapter 3 is that the biochemical pH-stat can be regarded not just as a process by which excess OH^- can be chemically neutralized but as one that is fundamental to the mechanism of solute accumulation in NO_3^- -N plants as well. In the absence of NO_3^- , the shoot accumulates mainly inorganic anions (section 3.3), so that under these circumstances the growth rate may be limited by the rate of ion uptake from the nutrient medium. This is certainly one aspect of the proportionality that exists between R_W and the combined process of uptake and transport to the shoot (Pitman, 1972), but under some conditions the rate of NO_3^- uptake by the roots may also be a limiting factor (Radin, 1978). It is possible that the availability of Cl^- in the NH_4^+ -N medium in the present experiments (and subsequent accumulation in the shoot) also accounts for the extremely low levels of malate found in the NH_4^+ -N leaves. There is considerable evidence that high Cl^- levels can directly inhibit malate synthesis, and in other species grown on NH_4^+ -N media containing predominantly SO_4^{2-} there is still found to be substantial accumulation of carboxylates. It would be worth investigating systematically the relative importance of these two effects, i.e. the absence of NO_3^- versus the presence of Cl^- , in the control of malate accumulation in the shoot.

The inorganic cation content of the leaves (as well as the xylem and phloem) in Ricinus is not significantly affected by the N-source, and in the NH_4^+ -N plants Cl^- substituted for NO_3^- as the predominant anion. The net charge carried by the N compounds transported to the shoot in the xylem is also important if net pH changes are to be avoided during assimilation. In fact, although the total N compounds in the NH_4^+ -N xylem do not bear a net negative charge, because of the absence of NO_3^- , the reduced N compounds themselves carry only a very small net positive charge. Although the concentration of these compounds is higher, the net charge is similar to that on the amino acids in the NO_3^- -N xylem. With a small amount of carboxylate in the

NH_4^+ -N xylem in addition, it is thus possible that the shoot can cope with a certain level of excess H^+ production by the biochemical pH-stat. The small amounts of malate in the NH_4^+ -N phloem, however, argue against a spatial separation of the biochemical pH-stat in the shoots and roots of the type discussed by Raven and Smith (1976a).

The two forms of N nutrition have more radical effects on organic N accumulation in the leaf. Storage of large quantities of arginine in the NH_4^+ -N leaves (Table 3.12) presumably imposes a further stress on the requirement for accumulation of negative charge equivalents. However, this consideration must not obscure the osmotic significance of amino-acid accumulation in the NH_4^+ -N plants, where organic N levels were about six times higher on a molecular basis than in the NO_3^- -N plants (Table 3.13). Thus, although the restriction on carboxylate accumulation in NH_4^+ -N plants requires that inorganic anions are stored instead, the amino acids also contribute to the more general process of 'solute' accumulation for the maintenance of turgor. A shift in the balance between carboxylate and amino-acid metabolism (whatever the mechanism) may turn out to be characteristic of the response to different forms of environmental stress, since similar effects have been observed in plants exposed to saline conditions (see Joshi, Dolan, Gee and Saltman, 1962). The significance of the amino acids used in solute accumulation has been more extensively studied in microorganisms and animals (e.g. Hochachka and Somero, 1973; Gilles, 1978). In plants, current interest is inclined towards the nature of reduced N compounds accumulated as 'compatible solutes' in the cell cytoplasm.

Phloem-sap composition in the NO_3^- -N and NH_4^+ -N plants is very similar, the total organic C and N concentrations being almost identical in the two instances. This is consistent with the view that phloem sap can be regarded as a symplastic phase, for the solute content must be controlled in this way to preserve the metabolic activity of the sap. In this respect, the high concentrations of serine found in the NH_4^+ -N phloem may indicate that it possesses some of the characteristics of other 'compatible solutes', although there is no evidence in this case that it is synthesized for osmoregulatory purposes. As with arginine, enhanced accumulation of serine may also contribute to the metabolic stress on the leaves, since formation of serine from glycine involves the release of NH_4^+ . In fact, if carbon flow through the glycolate pathway constitutes the principal route

for sucrose biosynthesis in Ricinus, this flux of NH_4^+ may be an order of magnitude greater than that resulting from primary assimilation of inorganic N (Keys, Bird, Cornelius, Lea, Wallsgrave and Mifflin, 1978).

Although the inorganic cation content of the phloem sap is not affected by the N-source, it is notable that the phloem is able to carry a considerably higher level of Cl^- in the NH_4^+ -N compared with the NO_3^- -N plants. In saline environments, the low NaCl content of the shoot seems to be determined by prevention of its entry to the transpiration stream or its elimination from aerial salt glands (see Clarkson, 1974; Raven, 1977a). Furthermore, in some species it is found that there is very little retranslocation of Cl^- out of leaves (Greenway and Thomas, 1965). There are a few indications that retranslocation of Cl^- in the phloem may be significant in halophytes (e.g. von Willert, 1968), but this may simply be a passive response to the high levels of Cl^- transported to the shoot in the xylem (Lessani and Marschner, 1978). This is likely to be the explanation for the high Cl^- levels in the NH_4^+ -N phloem in Ricinus (see Table 3.18), and in a similar way the presence of NO_3^- in the phloem of the NO_3^- -N plants may be the result of carry-over from the transpiration stream, in view of the high concentrations in the xylem.

As well as the agronomic implications of the growth responses to these contrasting N-sources, this issue is also relevant to the ecological distribution of various species on different soil types (see Nightingale, 1937; Street and Sheat, 1958). In acidic soils, NH_4^+ tends to be the predominant N-form available to plants, whereas in neutral or alkaline soils it tends to be NO_3^- ; the activity of nitrifying bacteria is reduced at lower pH values, and consequently in some acidic mineral soils little or no nitrification occurs (Gigon and Rorison, 1972). In general terms, therefore, it is possible that acidophilous plants are characterized (other things being equal) by a better ability to use NH_4^+ -N than calcicolous species. The soil-plant nitrogen relations are also profoundly influenced by environmental variables such as the water regime (e.g. Havill, Lee and De-Felice, 1977), but in terms of metabolism such a correlation is qualitatively consistent with the soil acidification found under certain species on chalk heaths (Grubb and Suter, 1971). Further studies are also needed to determine how the consequences of di-nitrogen fixation in nodulated plants compare with utilization of NO_3^- or NH_4^+ as the N-source (Raven and Smith, 1976a; Ryle, Powell and Gordon, 1978)

before the ecological implications of N-utilization can be properly related to aspects of whole-plant metabolism.

As regards the specific relationship between phloem transport and whole-plant metabolism, these data demonstrate that phloem-sap composition is to a considerable extent buffered against changes in patterns of organic C and N accumulation in the leaves. Although this is one respect in which phloem loading seems to be 'controlled', it was argued previously (section 1.2) that consideration of the transport process as a whole involves quantification of the flux through the pathway, together with identification of the driving force for flow. This requires that investigation of the solute transport includes an adequate description of the water relations of the system, and this issue was approached in Chapters 4 and 5.

Evidence was presented in Chapter 4 that sap exudation in Ricinus is likely to possess the same characteristics as phloem transport in the intact plant in all qualitative respects. There is no doubt that the rates of solute flux during exudation are higher than those occurring under 'natural' conditions, and yet the constancy of solute concentration over prolonged periods implies that the source leaves are able to meet this artificially high sink demand. Furthermore, if the constancy of sap Ψ_s is essentially a reflection of constant xylem Ψ , the primary component in determining rates of solution flow must be the longitudinal speed of transport. In terms of solute loading, this would correspond to proportional alterations in the rate of influx to the sieve tubes, this being coupled to osmotic influx of water. The cumulative amount of solute transported over periods of hours is so high in relation to the total content of the phloem that continued loading must occur to sustain transport. In the long term, only the leaves are able to generate flux rates of this magnitude.

If loading is able to respond to changes in demand, and sap exudation represents a reasonable model of phloem transport in the intact plant, the question arises as to how these changes in solute flux are brought about. The kinetic experiments described in section 4.4 suggest that the longitudinal resistance of the sieve-tube pathway is low enough to allow rapid changes in pressure to pass through the cells. Thus, fresh incisions into the phloem are likely to cause an almost immediate change in the size of the Ψ_p gradient along the pathway: at the incision, Ψ_p will drop to approximately atmospheric

pressure, and although Ψ_p will decrease at the source, the absolute change here will be smaller because of the elasticity of the cell walls and the finite resistance of the pathway. The pressure gradient will then be modified as the sieve-plate pores become blocked near the site of the incision.

The changes observed in exudation rate following an incision are compatible with the view that the Ψ_p difference between the source and sink provides the driving force for longitudinal transport. If metabolic energy is supplied along the pathway (as well as at the source) to drive transport, the results suggest that this is a quantitatively minor part of the total driving force, since a response to increased demand dependent on chemical intermediates is unlikely to be mediated this quickly. The rigorous characterization of pressure flow in an intact plant nevertheless depends on the demonstration that a change in the Ψ_p gradient is correlated with a change in the speed of transport. There are indications in the few studies that have been made that this condition may be met (see Sheikholeslam and Currier, 1977a, b; Fisher, 1978), and the work on manipulation of transport in isolated Zea leaves also supports this view (Heyser, Heyser, Eschrich and Fritz, 1977). This has an important bearing on the nature of changes in the source-sink balance during development, which it is important to characterize at the mechanistic level (section 1.4). For example, if the effects of 'hormone-directed transport' can be accounted for solely in terms of a stimulation of sink metabolism (da Cruz and Audus, 1978), then this may simply be the result of an enhancement of the Ψ_p difference between source and sink, and there is no need to invoke the possibility that the hormone is acting directly on the transport system (cf. Patrick and Wareing, 1978).

Although a change in sink demand may thus be the primary cause of an alteration in the Ψ_p difference between source and sink, the absolute value of Ψ_p at the source will also change to a certain extent if the resistance of the pathway is sufficiently low. A response of phloem loading to a change in turgor would provide one means by which flux could be geared to changes in demand. In the steady-state, however, there is not only a gradient of Ψ_p along the sieve-tube pathway, but also a gradient of Ψ_s . In fact, almost all previous discussions of the mechanism by which solute loading can be controlled have centred on a postulated response to changes in phloem Ψ_s (e.g. Weatherley et al., 1959; Tammes et al., 1967; Zimmermann, 1971; Ziegler, 1974;

Moorby, 1977). Even though the characteristics of sap exudation exhibited under constant environmental conditions help to describe the osmotic relations of the system, they do not permit a distinction to be made between the absolute importance of Ψ_s versus Ψ_p in controlling loading.

The effects of water deficits on transport provide one means of separating these components, because with increasing stress the values effectively move in opposite directions, i.e. the sap becomes more concentrated and phloem turgor decreases. If loading was occurring in response to changes in Ψ_s , it would be expected that the flux would decrease with increasing stress, whereas it is found that flux increases. Furthermore, this stimulation of loading occurs at a stage when the amount of assimilate available for transport would if anything be decreasing. It seems likely, therefore, that - of the two - loading is modulated by changes in Ψ_p rather than Ψ_s .

These results contrast with those obtained in studies on aphid-stylet exudate from Salix stem segments. In the latter experiments, Weatherley et al. (1959) found that irrigating the inner surface of the bark with mannitol solutions to decrease xylem Ψ brought about a decrease in phloem-sap Ψ_s ; in this case, however, there was actually a decrease in the rate of solute flow (see data redrawn by Peel, 1975). Exactly how the loading rate was related to sap Ψ_s or Ψ_p is not clear, but it is possible that the difference between this response and that in Ricinus is connected with dissimilarities in the source characteristics of the two tissues. Although the vascular parenchyma and ray cells of the Salix stem may contain substantial carbohydrate reserves, the associated tissues may not possess the same capacity to load sucrose against a concentration gradient as expected of the companion cell-sieve element complex of source leaves. Further, the rates of solute flux in the aphid-stylet system represent a smaller throughput in terms of the sieve-tube contents themselves than do the rates of mass transfer in Ricinus. Thus, the exudation rates may not be such a clear reflection of events at the sites of loading. Effects may also be found in isolated systems of this sort that are more exaggerated than those observed under normal circumstances. For example, the fact that application of hydrostatic pressure to the xylem in the region of the aphid stylet actually produced a decrease in exudation rate (Peel and Weatherley, 1963) may be caused by a decrease in membrane L_p

with increasing Ψ_p (see Zimmermann and Steudle, 1975; Powell, 1978; but note Steudle, Lüttge and Zimmermann, 1975; Palta and Stadelmann, 1977).

One important consequence of the decline in phloem-sap Ψ_s during water stress in Ricinus is that it keeps phloem Ψ_p positive to lower values of xylem Ψ than would be expected if the net solute flux through the sieve tubes remained constant. In many respects, this response is similar to the solute accumulation observed in leaves of many species during imposition of water stress (e.g. Hsiao, Acevedo, Fereres and Henderson, 1976; Morgan, 1977; Cutler, Rains and Loomis, 1977; Osonubi and Davies, 1978; Jones and Turner, 1978; Fereres, Acevedo, Henderson and Hsiao, 1978). This response is also found in Ricinus during prolonged drought (Dodoo, 1978). This form of osmoregulation can be regarded as a means of maintaining Ψ_p required for cell growth (see Ray, Green and Cleland, 1972). Part of the response, however, can result from lowering of cell ϵ without accumulation of solutes, and this is at least a contributory factor to the adaptation found in some species (e.g. Lawlor, 1969; Acevedo, Hsiao and Henderson, 1971; Bunce, 1977b). This effect is of only limited significance in the phloem, representing the changes accounted for by the turgor displacement volume. The relative importance of the two effects may vary, however, because plant responses to water deficits can be different in controlled environments to those found in the field (Begg and Turner, 1976; Bunce, 1977b).

Osmoregulatory responses in higher-plant tissues are also found over relatively short periods (Greacen and Oh, 1972; Meyer and Boyer, 1972; Hsiao, 1973), and the present experiments indicate that alterations in phloem loading may be brought about quite rapidly. The mechanism of these osmoregulatory responses has been far more extensively investigated in algae, in many species of which membrane transport of the principal osmotica is sensitive to changes in cell Ψ_p (see Cram, 1976a; Hellebust, 1976a; Kauss, 1977; Gutknecht and Bisson, 1977; Zimmermann, 1978; Kauss, 1978). In many instances, it seems legitimate to describe these responses in terms of negative feedback regulation of the solute flux by cell Ψ_p or Ψ_s (Cram, 1976a). Most algae, however, accumulate inorganic ions, and only a few cases have been described of non-electrolyte accumulation being controlled in this way (see Kauss, 1978; Müller and Wegmann, 1978). Although there is much less information on the control of transport of osmotically important solutes in higher-plant cells, certain experiments suggest

that sugar accumulation may be feedback regulated by similar mechanisms (see Gayler and Glasziou, 1972; Humphreys, 1973; McNeil, 1976). In Kalanchoë, malate transport across the tonoplast appears to be sensitive to Ψ_p specifically (Lüttge, Ball and Greenway, 1977).

These examples show that there is some sort of precedent for considering that phloem loading may be turgor-pressure dependent. In the same way that Ψ_p is essential for cell expansion, maintenance of phloem turgor may be fundamental to the preservation of a Ψ_p gradient as the driving force for transport. This can be regarded as another aspect of the fact that phloem Ψ_s is maintained in the same way as Ψ_s in other cells, but that in the phloem this is associated with a large throughput of solutes as opposed to cell growth. Furthermore, the cytoplasmic nature of the phloem sap implies that osmoregulation may be an underlying characteristic of the loading process.

The changes in phloem-sap content occurring during continuous darkness (section 5.3) can also be interpreted as an osmoregulatory response occurring as a result of deficiency of the principal osmoticum. The efficiency of K^+ accumulation as a means of maintaining turgor may depend on the nature of the accompanying anions and rate of K^+ efflux (see Gradmann, 1977; Hastings and Gutknecht, 1978). However, the fact that the reciprocity between sucrose and K^+ levels can be explained in terms of the cotransport of loading shows that the scheme can account for the general process of solute accumulation. This meets some of the criticisms levelled against the chemiosmotic hypothesis on the grounds that it cannot adequately explain how transport at the membrane level is associated with the net solute uptake required for plant-cell growth (Steward and Mott, 1970; Mott and Steward, 1972b). In certain circumstances, therefore, the question of whether non-electrolytes or ions are accumulated may be secondary to the requirement for non-specific solutes to maintain cell Ψ_p (see Pitman, Mowat and Nair, 1971; Mott and Steward, 1972a). The electroosmotic theory, on the other hand, would hold that there is an absolute requirement for K^+ at the level of sieve-tube metabolism (see Spanner, 1975; Willenbrink and Schuster, 1978).

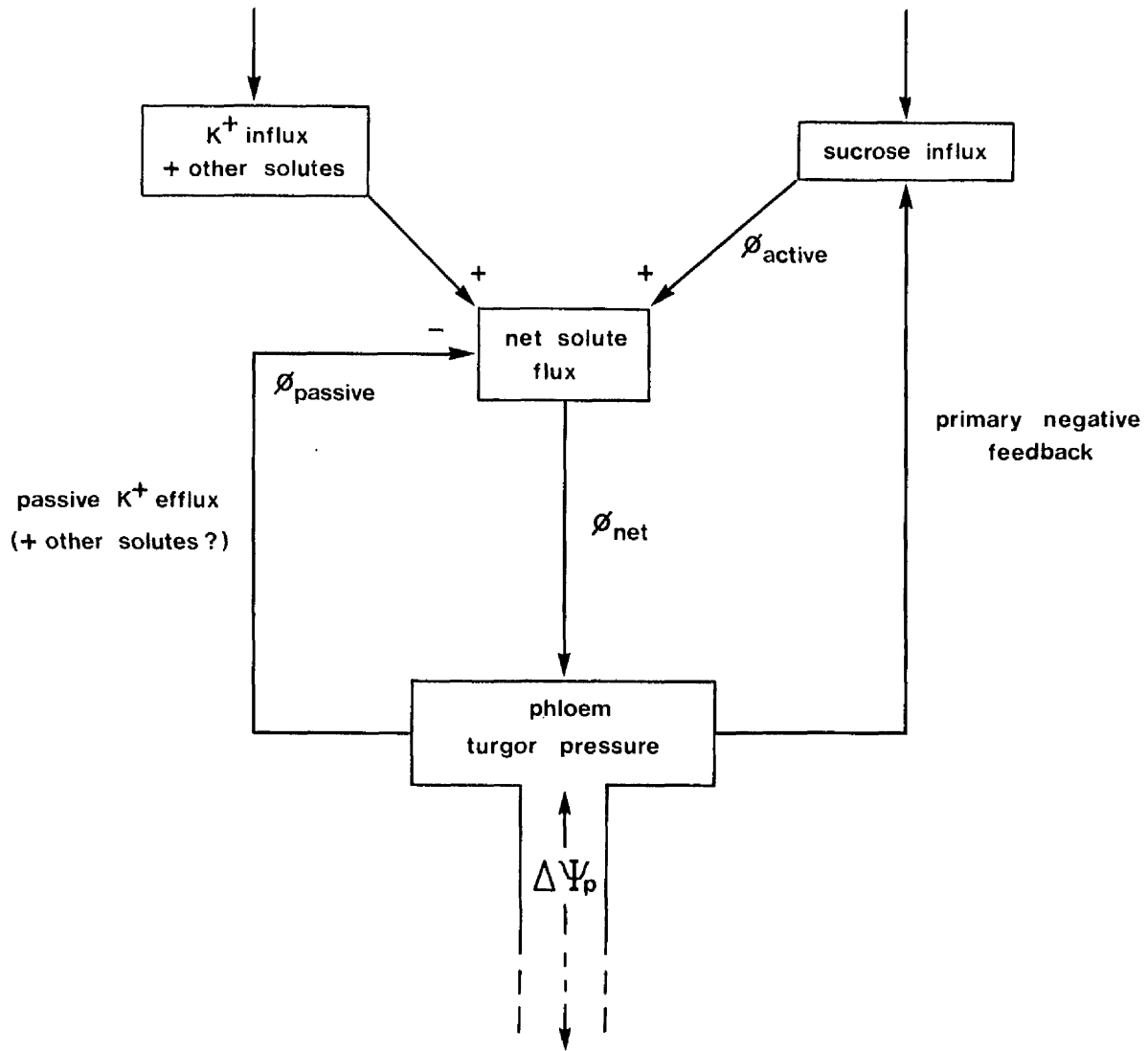
It must be stressed that modulation of sucrose loading by turgor does not pre-empt an influence of Ψ_s in addition. Indeed, there is considerable evidence that the rate of sucrose export is determined by sucrose levels in the bulk leaf tissues (see section 1.4). In

terms of sucrose loading itself, this is different to feedback regulation by the osmotic relations of the sieve tubes, because export shows a direct proportionality with source concentration. The sensitivity of the latter response may still be considerable, especially if the sucrose concentration at the 'loading membrane' is markedly below the K_m for loading (see Fondy and Geiger, 1977). Biochemical control of loading may also be related to the energetic requirements for sugar influx, although the importance of this aspect remains uncertain (see Morgan and Whitfield, 1973; Geiger, 1975a; Ho and Thornley, 1978).

The results from the studies with leaf discs suggest that changes in Ψ_p are more important in affecting sucrose loading than any associated changes in Ψ (section 6.3). The magnitude of the responses observed with decreasing Ψ_p in this system cannot be directly related to the whole-plant experiments, in which sucrose loading is coupled to export. Furthermore, alterations in Ψ_p will be accompanied by changes in cell volume. Whereas the consequences of changes in cell volume for solute transport have been partly characterized in animals (see Hoffmann, 1977), assessment of their importance in plants requires investigation of the ϵ component as well. This presents formidable technical difficulties in the case of the small cells of higher-plant tissues.

The way in which phloem turgor may affect phloem loading of sucrose and K^+ in Ricinus is summarized in the form of a block diagram in Fig. 7.1. This diagram does not attempt to represent the forms of thermodynamic control that operate on the transport processes. The evidence for a turgor-pressure dependent sucrose flux must be regarded as circumstantial, and direct manipulation of phloem turgor would constitute the most direct means of studying the system further. Direct pressure measurements have been made in higher-plant cells as yet only in stomata (Meidner and Edwards, 1975) and epidermal bladder cells of Mesembryanthemum (Steudle *et al.*, 1975), but the inaccessibility of the sieve tubes may pose special problems in experiments of this sort. The mechanism by which the effect of turgor is transduced into an effect on membrane transport is also not yet clearly understood. The electromechanical model (Coster *et al.*, 1976; Zimmermann, 1978) highlights several important aspects of this issue (cf. Kleinzeller, 1972), but the water activity of the membrane matrix may also influence solute transport (Cereijo-Santaló, 1972; Gould and Measures, 1977).

Fig. 7.1 Block diagram depicting the way in which net solute flux into the phloem (i.e. phloem loading) may be affected by phloem turgor. The scheme considers only sucrose and K^+ transport, on the basis of results described in Chapters 4, 5 and 6.



Although these discussions have been devoted to the nature of solute loading in the phloem, the same issues serve to emphasize the importance of solute unloading in the control of transport. The response of sucrose loading to changes in phloem Ψ_p may set the limits within which longitudinal transport can take place; this also applies to the influence of source metabolism on uptake into the phloem. However, as shown by the characteristics of sap exudation, it is sink strength that determines the rate of flux through the phloem under conditions in which source supply is non-limiting. In this sense, the response of phloem loading can be regarded as 'passive and correlative' rather than 'specific and regulatory'. A clearer understanding of the relationship between sink metabolism and phloem unloading is thus also required before the transport process as a whole can be adequately described.

8. BIBLIOGRAPHY

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