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TRANSLLOCATION IN PETIOLES AND TRANSPORT ALONG PHLOEM  
LOOPS OF *HERACLEUM LANATUM*

by



JOHN HODDINOTT

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
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IN

PLANT PHYSIOLOGY


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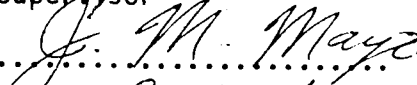
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Translocation in Petioles and Transport along Phloem Loops of *Heracleum lanatum*" submitted by John Hoddinott in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Physiology.

  
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#### 4 ABSTRACT

This study provided information on translocation in *Heracleum*. By the use of a sensitive counting method using Cab-O-Sil it was possible to demonstrate that the advancing front of the profile of radioactivity in periods following localized labeling for 2 min with  $^{14}\text{CO}_2$  was of wavy form. These pulses were apparently produced by the labeling technique in which elevated  $\text{CO}_2$  concentrations perturbed steady-state conditions and caused oscillations in labeled metabolite pool sizes and loading rates of labeled translocate into veins. Wounding the petioles caused inhibition of translocation in vascular bundles in the vicinity of the wound, with the primary effect being located above the site of the wound in the region of the junction between primary and secondary petioles. This was indicated by profiles of  $^{14}\text{C}$  in petiole sections and by autoradiography. A variety of treatments failed to overcome this inhibition. Sugar beet petioles were found to be less sensitive to wounding than *Heracleum* petioles. Isolated phloem loops of *Heracleum* transported tritiated water and ( $\text{U-}^{14}\text{C}$ ) sucrose in a manner which proved to be non-physiological. This became very evident when similar transport data were obtained for the movement of tritiated water along polyester threads. It is postulated that tritium nuclei from tritiated water moved in phloem loops and polyester threads along solid-liquid interfaces according to their own chemical potential gradients. Measurements of water potential and its component potentials for leaf blade and petiole phloem of *Heracleum* indicated that water moved passively from the leaf mesophyll to the phloem, while sucrose may have moved actively. Light quality appeared to have no effect on the nature of the translocate, or the translocation profiles in *Heracleum*.

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A mountain having labour  
With clamor rent the air.  
The neighbors who came running  
Predicted she would bear  
A city broad as Paris  
Or at least a manor house,  
But at the crucial moment  
The mountain dropped a mouse.

How like so many authors  
Who say they'll set to paper  
A vast Promethean epic  
But all that comes is vapor.

Jean de la Fontaine

1621 - 1695

## INTRODUCTION

The translocation of assimilates in the phloem of higher plants remains one of the most enigmatic subjects of plant physiology. However this does not imply that there has not been a great deal of research, both theoretical and practical, covering all aspects of the field. Despite improvements in analytical instrumentation and sample preparation techniques coupled with the use of more manipulable plant species, no clear consensus on the mechanism of translocation has emerged. The discussion still centers on the nucleus provided by the work of Münch (1930), and much of the later work is compared to this reference point.

The topic has been well reviewed in several recent books and articles. Crafts and Crisp (1971) provide the most comprehensive review but they interpret all data which they cite in terms of a mass flow mechanism. Canny (1973) provides a less comprehensive review based on the more controversial activated diffusion hypothesis. The most objective review in terms of mechanism is that of MacRobbie (1971). More specialised reviews have also been published. Eschrich (1970) surveyed the literature relating to the biochemistry and fine structure of phloem. Both of these topics have been the subject of more recent work. Great advances have been made in the understanding of the biochemistry of P-protein. Concomitant with this has been an increase in information on its fine structure and cytochemistry (Cronshaw *et al.*, 1973; Gilder and Cronshaw, 1974). The anatomy of phloem tissue has been the subject of a book by Esau (1969).

Weatherley and Johnson (1968) discuss the relationship

between form and function in the sieve tube. This has been followed up with specific papers on the nature of microfilaments in sieve tubes. Johnson (1973) gives evidence for the absence of membranous transcellular strands and the presence of microfilaments in translocating phloem. Weatherley (1972) discusses the implications of microfilament distribution as it relates to a translocation mechanism, with special emphasis on the sieve plate pores. Lee (1972) provides an additional analysis on the possible significance of microfilaments in surface flow mechanisms.

Recent literature reveals a resurgence of interest in a number of other possible mechanisms of translocation than that of Münch, and in providing models in support of these mechanisms. Thaine has revised his previous protoplasmic streaming hypothesis (Thaine, 1964) in favour of a mechanism of cytoplasmic pumping (Thaine, 1969) based on observations of transcellular strands by Thaine *et al.* (1967) and Thaine and DeMaria (1972). The cytoplasmic pumping method has been analysed quantitatively by Aikman and Anderson (1971). Protoplasmic streaming has also been revised as a model by Miller (1973).

The problem of flow in tubular semi-permeable membranes has given rise to several artificial models. Eschrich *et al.* (1972), on the basis of their model suggest replacing the concept of pressure flow in Münch type systems with that of volume flow. Weatherley (1973) has taken exception to this and has proposed the more neutral term "Münch flow" to overcome objections relating to the inability of a hydrostatic pressure gradient to cause flow through the sieve tubes. However this suggestion has not been

regarded favorably by Young *et al.* (1973). Lang (1973) in his semi-permeable membrane model retains the concept of a hydrostatic pressure gradient. The model of Christy and Ferrier (1973) also relies on such a gradient.

A recent development has been the use of *Heracleum* species to provide experimental systems for the study of translocation, because of the ease with which the vascular bundles of the petiole can be dissected out. The xylem and phloem in the bundles can also be readily separated. Ziegler (1958) first used isolated bundles of *H. mantegazzianum* to study rates of respiration in vascular tissue. He also noted the ability of an isolated phloem loop to slowly transport  $^{14}\text{C}$ -sucrose and fluorescein K, when they were applied to the upper end of a loop. Ziegler and Mittler (1959) used aphids to sample sieve tube contents of *H. mantegazzianum*. Sucrose was the only compound found in the aphid exudate at a concentration of 24%. Ziegler (1960a) carried out an electron microscope study of this species and noted the absence of mitochondria in conducting cells of the phloem, and the presence of endoplasmic reticulum which continues through the sieve plate pores. In a later paper, Ziegler (1960b) also reported on the presence of UDP-glucose in the phloem which he suggested might serve as a precursor of callose. Ziegler and Vieweg (1961) used a heat pulse method to derive a speed of solute flow in phloem loops of *H. mantegazzianum* which they found to be between 35-70 cm/hr. Ullrich (1961) used phloem loops of *H. sphondylium* and showed the presence of intensive anaerobic respiration in them which was stimulated by 0.1 M sucrose and inhibited by 0.01 M HCN. These

Observations led, in part, to his hypothesis of a peroxidase-based respiration system in phloem.

Further work has been carried out mainly by Fensom and his colleagues. Fensom *et al.* (1968) reported the existence of moving particles in living sieve tubes of *H. mantegazzianum*. These have been discussed at greater length by Lee *et al.* (1971) and have been the subject of a cine film (Lee *et al.* 1970). The ultrastructure of the moving particles in phloem loops of *H. sphondylium* was described by Robidoux *et al.* (1973). Experiments involving microinjection of tracers into sieve tubes were carried out by Fensom and Davidson (1970) using isotopic tracers, and by Barclay and Fensom (1973) using carbon black as a tracer. Microinjection into the sieve tubes has also allowed electrical measurements to be taken, but the interpretation of the data derived varies between the two published reports of these studies (Tyree and Fensom, 1970; Spanner, 1970). The research in Fensom's laboratory on *Heracleum* has culminated in a microperistaltic theory of translocation being proposed (Fensom, 1972) based on the contraction of microfibrillar material to provide a motive force for assimilate movement.

It was hoped in this study to obtain a better understanding about the nature of translocation in wounded petioles and phloem loops. Phloem has been widely regarded as a tissue which is very sensitive to manipulation, and the process of isolating phloem loops from petioles would normally be considered a rather drastic procedure. The apparent existence of an active translocation mechanism or mechanisms in phloem loops was, therefore, of great interest. However, the work of Ziegler and the work in Fensom's laboratory had only indicated the movement of isotopes applied



externally to the phloem loops or microinjected into them, while the natural introduction of isotopic tracers via  $^{14}\text{CO}_2$ -fixation and vein-loading of  $^{14}\text{C}$  assimilates had not been examined. It was, therefore, important to obtain data on the translocation of  $^{14}\text{C}$ -labeled compounds loaded normally into phloem bundles of unwounded petioles. These would serve as controls to assess the effect of the wounding of the petiole on the translocation of  $^{14}\text{C}$ -labeled compounds loaded naturally into isolated phloem loops. At the same time the degrees of slime plugging (i.e. plugging with P-protein) and callosing, which are widely regarded as indicative of phloem injury (Crafts and Crisp, 1971), were followed. Some of these results have already been published (Hoddinott and Gorham, 1974).

The *Heracleum* species chosen for the present study was *H. lanatum* Michx. as it was native to the Edmonton area and readily available. Attempts were made to germinate *H. mantegazzianum* and *H. sphondylium* from seed, but these were unsuccessful. Professor Fensom kindly provided rootstocks of *H. mantegazzianum* but these could not be multiplied rapidly enough to provide sufficient experimental material. *H. lanatum* has phloem which can be readily detached from its petiole like *H. mantegazzianum*, so it was regarded as suitable for the proposed work.

In early experiments, *Beta vulgaris* L. was used due to the initial unavailability of *Heracleum* plants. The petiolar bundles of sugar beet anastomose frequently and are more firmly attached in their surrounding parenchyma than those of *Heracleum*. These experiments were designed to test the effect of wounding

the petioles of a different species on the process of translocation through them.

The water relations of the translocation process in isolated loops were examined by determining the component potentials of the tissues involved, to see if they could be related to vein-loading and Münch flow as postulated by Weatherley (1973). The growth chambers in which the experiments were carried out were readily modified to permit a study of the effects of light quality on translocation. This was done in an attempt to find any relation between light quality and a pulsed mode of translocation of the kind first noted in intact petioles by Nelson *et al.* (1958) and in phloem loops by Fensom and Davidson (1970).

The thesis is divided into four main experimental sections relating to experiments performed on intact and wounded petioles, the effect of light quality on translocation, the transport of externally applied isotope in isolated loops, and the water relations of translocation. Each section will be self-contained with its own introduction, results and discussion. General conclusions derived from the four sections are presented at the end.

## MATERIALS AND METHODS

### A. Chemical and Instrument Suppliers

The sources of isotopes, chemicals, special materials and instruments are given in Appendix A. Other pertinent information will be given at appropriate places throughout the text.

### B. Culture Methods

The growth conditions of *Heracleum lanatum* Michx. and *Beta vulgaris* L. var. Klein Wanzleben are described in accordance with the American Society of Horticultural Science's Committee on Growth Chamber Environments "Guidelines for Reporting Studies in Controlled Environment Chambers" (1972).

Plants were grown in Environmental Growth Chambers Inc. Model M-7 chambers equipped with a Plexiglass lamp barrier. Light cycles in the chambers varied for the two species. For *Beta*, two hours with 4 x 60 W incandescent lamps giving  $5.3 \mu\text{E}/\text{m}^2/\text{sec}$ , two hours with 10 x 60 W incandescent lamps with 8 x 1500 ma Cool White fluorescent lamps giving  $70 \mu\text{E}/\text{m}^2/\text{sec}$ , eight hours with 10 x 60 W incandescent and 24 fluorescent lamps giving  $400 \mu\text{E}/\text{m}^2/\text{sec}$ , two hours at  $70 \mu\text{E}/\text{m}^2/\text{sec}$ , two hours at  $5.3 \mu\text{E}/\text{m}^2/\text{sec}$  and eight hours darkness. All light changes were abrupt. For *Heracleum* the lighting was similar except for the reduction of the  $400 \mu\text{E}/\text{m}^2/\text{sec}$  light period to two hours, with a corresponding increase to five hours in the  $70 \mu\text{E}/\text{m}^2/\text{sec}$  light periods. All light measurements were made with a Lambda LI 185 meter and LI 192S quantum sensor which measures photosynthetically active radiation from 400 to 700 nm. Light measurements were made at canopy height which was approximately 100 cm from the lamps.

The air temperature, measured with resistance thermometers, was constant at 20 C for *Heracleum*. For *Beta* during the 16-hr light period the temperature was 20 C and during the dark, 15 C. Temperature changes were achieved gradually over a one-hour period. The relative humidity, measured by dew point sensors, was maintained at 70% at all times for both species. The air speed at the canopy level was 1.609 km/hr measured with a Hastings Air Meter, RBI Series 89. Fresh make-up air was provided at the rate of 10 changes per hour.

Attempts to germinate seeds of *H. mantegazzianum* and *H. sphondylium* from various suppliers proved unsuccessful. After imbibition for various times, with and without scarification of the testas, followed by planting in soil, the seeds failed to show significant germination. Stratification or heat treatments also had no effect on the rate of germination. Imbibition in 0.5 mM indoleacetic acid or gibberellic acid A3 solutions had no effect on the germination rate. The poor germination rate was attributed to the general lack of viable embryos. Subsequently additional information on *Heracleum* seed dormancy became available (Stokes, 1952a & b). It was decided to use native *H. lanatum* plants as the phloem in their petioles was easily dissected out and they were readily available in the Edmonton area. A leaf is diagramed in Fig. 1.

*Heracleum* plants were grown in soil in six-inch pots after being collected from roadsides near Edmonton. They were watered three times a week, twice with distilled water and once with Hoagland's solution containing iron as FeEDTA. Plants were kept pruned to three leaves to keep them vegetative.

*Beta* seeds were germinated in wet Vermiculite for two weeks in the growth chamber. Seedlings with white hypocotyls were then transferred to two-liter plastic pots containing Hoagland's solution with iron as FeEDTA. The nutrient solution was aerated continually and changed weekly. Between nutrient changes the pots were kept topped up with distilled water. The plants were used after they had been in nutrient solution for six weeks.

Experiments involving  $^{14}\text{CO}_2$  fixation, net assimilation and stomatal diffusion resistance were carried out at 20 C and 70% relative humidity in a special Environmental Growth Chambers Inc. Model M-3 chamber attached to a fume hood exhaust. The light in the chamber was normally white, but by interposing coloured cellophane filters above the clear Plexiglass barrier between the lamps and the growing chamber, the spectral distribution of the white light could be altered as needed. The irradiances used with the various colours were determined on a quantum basis, and also on an energy basis using an Eppley pyranometer. The spectral qualities of the various colours were also measured with an ISCO spectroradiometer.

### C. Isotope Application Techniques for Translocation Studies

When  $^{14}\text{CO}_2$  was applied to a whole leaf or a part of a leaf of either species it was a general practice to cover the petioles with aluminum foil. This was done wherever possible to ensure that there would be no fixation of  $^{14}\text{CO}_2$  by the petiole. Three methods of application of isotope were used for various purposes:

#### 1. Whole Lamina Application

The method used to offer  $^{14}\text{CO}_2$  to the leaves of *Beta* and

*Heracleum* was based on that of Mortimer (1965). A lamina of *Beta* or the three leaflets of the compound leaf of *Heracleum* were enclosed in a polyethylene bag and sealed around the top of the petiole with the aid of a collar of Terostat VII and a wire tie. A length of thin polyethylene catheter tubing, 1.5 mm OD, passed through the Terostat. The  $^{14}\text{CO}_2$  was generated in a 50-ml syringe by the mixing of a drop of concentrated sulphuric acid and a measured volume of stock  $\text{NaH}^{14}\text{CO}_3$  solution as the plunger was being gradually withdrawn. The  $^{14}\text{CO}_2$  in the syringe was discharged through the catheter tubing into the bag surrounding the leaf blade. To ensure good mixing of the gas during the fixation period the syringe was pumped gently. After the standard two-minute fixation period the bag was removed and the assimilated  $^{14}\text{CO}_2$  was allowed to translocate for additional periods of time, as indicated, with the leaf blade in normal air. Routine experiments used 70.72  $\mu\text{Ci}$  of  $^{14}\text{C}$  and the experimental times stated include the two-minute fixation period.

When phloem loops were dissected free from the petiole (Ziegler, 1958) but left inside the hollow petiole, it was possible to cover the petiole with aluminium foil. However, if the incised petiole and loop were to be exposed to a bathing medium during the experiment this precaution could not be taken. Bathing fluids, when used, were pumped onto the wounded petiole continuously by a peristaltic pump through a Tygon tube of 1 cm OD.

## 2. Application to Localized Areas of Leaf

To enable  $^{14}\text{CO}_2$  to be offered to a more restricted area of the lamina a special cuvette was constructed. The cuvette consisted of a cylinder of glass, one centimeter high and one centimeter in diameter, with an outside flange on the bottom to allow application

of silicone grease as a sealing compound. The other end of the cylinder was enclosed by the tip of a finger from a rubber glove which formed a hemisphere approximately 1 cm high. A small side arm on the cylinder was closed with a serum cap through which projected the tip of a number 18 hypodermic needle. The needle was in turn attached to a five-centimeter length of tubing of five millimeter OD.

Generation of  $^{14}\text{CO}_2$  was carried out in a 2-ml syringe which was attached to the tubing from the side arm of the cuvette. Routinely, 70.72  $\mu\text{Ci}$  of  $^{14}\text{C}$  was used. The syringe was pumped several times during the two-minute assimilation period to ensure good mixing of the gasses in the cuvette and syringe. The rubber hemisphere on the top of the cuvette served as a pressure relief while the syringe was being pumped and prevented the cuvette from becoming detached from the lamina. The application of  $^{14}\text{CO}_2$  was always to the lower epidermis of the leaves of both species as epidermal strips showed this surface to have the greatest number of stomata per unit area. It also ensured that there was a minimum of interference, by the cuvette, to the light impinging on the leaf. At the end of the assimilation period the syringe plunger was drawn back, the cuvette was detached from the blade, closed with a sheet of plastic and rapidly removed from the chamber to a fume hood alongside.

### 3. External Application to Isolated Phloem Loops

This technique was used to apply isotope to isolated phloem loops of *Heracleum* prepared as described by Ziegler (1958). Phloem loops, still attached to the plant, were lifted clear of the petiole

and the whole plant was placed in a horizontal position. The petiole, with its attached phloem loop, was supported on a glass sheet. Filter paper flooded with distilled water was usually inserted between the loop and the glass. At the mid-point of the loop a 1.0 cm<sup>2</sup> enclosure of lanolin was built on the glass slide and the loop was laid across this. The loop was sealed in position by a second layer of lanolin. Isotopes were applied as aqueous solutions of known specific activities to the portion of the loop in the lanolin enclosure. This method is similar to that of Fensom (1972). In experiments where the time of application of an isotope was greater than several minutes the lanolin enclosure was covered with a glass cover slip to minimize evaporation.

In a few experiments, the filter paper on which the phloem loop lay was moistened with various aqueous media, or the wet filter paper was replaced by mineral oil.

#### D. Callose Fixation and Staining

To study the distribution of callose in isolated loops it was important to obtain adequate control samples of phloem from unwounded petioles. The unwounded control tissue was prepared by freezing intact petioles in liquid nitrogen and breaking sections into cold (-10 C) FAA (formalin: acetic acid: 50% ethanol, 5:5:90 by volume) fixative. When the petiole sections had thawed the phloem was dissected out under the cold fixative and left to fix for four hours. The fixative was then washed out with water and the tissue stained for callose. Phloem from isolated loops was either fixed with cold FAA while attached to the petiole and then detached for staining, or stained without fixation while still



attached.

Phloem was stained with 0.005% aniline blue in phosphate buffer at pH 8.2 (Jensen, 1962) and callose fluorescence was observed with a microscope with an ultraviolet light source. The UV light passed through a UGI transmission filter and a K430 barrier filter. The diachrome detection method for callose (Eschrich and Currier, 1964) involving a resorcinol blue stain prepared from resorcinol and ammonia was also used.

#### E. Isotope Counting Methods

Liquid scintillation counting of labeled segments of plant tissue was carried out using a scintillation fluor consisting of p-dioxane 400 ml, anisole 100 ml, 1,2 dimethoxyethane 100 ml, PPO 4.5 g and POPOP 0.5 g. When portions of isolated loops subjected to external application of isotope were being counted the samples were placed directly into the fluor. This included the section from the zone of application which was not rinsed. For sections of petiole that had been loaded with isotope by normal translocation it was necessary to improve the counting efficiency as the thick petiole caused significant self absorption. After translocating for the desired length of time the petioles were cut into one-centimeter sections with a multibladed cutter, and frozen in liquid nitrogen. The samples were then ground up in individual mortars. The ground samples were washed, with the aid of the fluor, into scintillation vials containing Cab-O-Sil M5. This preparation ensured that the ground particles of petiole remained suspended in the fluor to allow maximum counting efficiency. Quench correction curves for the Cab-O-Sil counting technique were prepared by

suspending ground one-centimeter segments of unlabeled petiole in Cab-0-Sil with a known volume of a  $^{14}\text{C}$  internal standard. Increasing quantities of water were added to the vials as a quenching agent and the efficiency of the method was determined with the aid of an external standard in a Nuclear Chicago Unilux II scintillation spectrometer.

In experiments where the efflux of externally offered isotopes from phloem loops was being determined, the loops were washed for known lengths of time in a series of 0.5 ml aliquots of water. The washings were then transferred by Pasteur pipette to vials containing fluor for counting.

When isolated loop segments were being counted, the vials were precounted with fluor for 10 min to obtain a background count rate for each individual vial. After the sample had been added, the vial was counted for 10 min to obtain a gross count rate. The significance of a count rate in a sample was determined by the following equation:

$$\text{Standard deviation of net count rate} = \sqrt{\frac{\text{Gross sample count rate}}{\text{Gross sample count time}} + \frac{\text{Background count rate}}{\text{Background count time}}}$$

For example, a sample had a background count rate of 25 cpm determined in a 10 min period and a gross count rate of 32 cpm determined in a 10 min period. This gave a net count rate of  $7 \pm 2.39$ .

However, if the ratio of the gross count rate to that of the background count rate is approximately 1.2, the background counting time needs to be 175 min and the sample counting time to be 191 min

to obtain a 10% standard error in the net count rate (Wang and Willis, 1965).

#### F. Autoradiography

In this study use was made of both freeze-drying and freeze-substitution methods. The freeze-drying method was a modification of the one described by Perkins *et al.* (1959) used by Trip and Gorham (1967). The freeze-substitution technique was based on the method of Fisher and Housley (1972).

In the freeze-drying experiments, local areas in the basipetal ends of *Beta* leaves were exposed to 42.6  $\mu\text{Ci } ^{14}\text{CO}_2$  for two minutes. Assimilated isotope was then allowed to translocate for a further 28 min. Large petiole sections from the labeled leaves were rapidly frozen in isopentane cooled to just above its freezing point with liquid nitrogen. Once frozen, five-millimeter sections of the petiole were sawn off with a jeweller's saw and placed in liquid nitrogen. Frozen sections were placed in small beakers on previously degassed Tissuemat wax. The wax was cooled with liquid nitrogen before the petiole sections were placed on it. The beakers were then placed in a cold dessicator along with an open petri dish containing phosphorous pentoxide. The dessicator was placed in a deep freeze below  $-40^\circ\text{C}$  and evacuated by a vacuum pump. The air line to the pump had a cold finger trap cooled by liquid nitrogen. The pump was allowed to run for two hours. The vacuum pump was then turned off, the dessicator closed and left for one week. On removal from the deep freeze the dessicator was allowed to warm to room temperature before the

beakers containing the wax and tissue sections were transferred to a vacuum oven. The specimens were heated to 55 C under vacuum so the wax would melt and infiltrate them. Sections were left in the vacuum oven for two days after which those that had sunk and were 95-100% infiltrated with wax were removed and mounted in blocks for sectioning.

The freeze-substitution method was used with *Heracleum*. A leaf was offered  $70.72 \mu\text{Ci } ^{14}\text{CO}_2$  for two minutes and the assimilated isotope was allowed to translocate for a further 28 min. A large petiole section was then rapidly cut off and frozen in isopentane cooled with liquid nitrogen. The frozen petiole was sawed into five-millimeter sections which were placed on frozen anhydrous propylene oxide. The vials containing frozen tissue and propylene oxide were placed in a deep freeze below -40 C where the propylene oxide thawed. The tissue was left for one week below -40 C at which time it was transferred to fresh, cold, dry propylene oxide. After a further week the vials were allowed to warm to room temperature and the propylene oxide was replaced in the tissue by anhydrous xylene through a propylene oxide:xylene series of increasing xylene concentration. The tissue was ultimately placed in a layer of xylene in a beaker containing degassed Tissuemat wax. The wax was heated in a vacuum oven and the tissue sank into the wax. The wax was allowed to infiltrate for two days. The tissue was then removed and mounted in blocks for sectioning.

Paraffin blocks containing freeze-dried or freeze-substituted tissue were treated in a similar manner. They were first prepared for sectioning by cooling in a refrigerator. The microtome knife

blade was also cooled and kept cool by a down draft of cold air from a powder funnel containing solid carbon dioxide positioned directly above the blade. Ribbons of sections, 10  $\mu\text{m}$  thick, were cut and stored in slide boxes with a dessicant. Sections were affixed to Kodak NTB nuclear track plates in the darkroom. The plates were warmed and moistened very slightly by breathing on them to facilitate the adhesion of the ribbons to the emulsion on the plates. The ribbons were placed on the plates, covered with a sheet of clean white paper, and pressed by hand onto the emulsion. The plates were then placed with a dessicant in a dark slide box in a refrigerator and exposed for one month. All work with the NTB plates was carried out under a Kodak No. 2 safelight.

At the end of the exposure period the sections were deparaffined in re-distilled xylene. All traces of xylene were then removed under vacuum. The plates were developed for five minutes in Kodak D-19 developer, followed by fixation for five minutes in Kodak X-ray fixer. The slides were then washed for 20 min in running water.

Developed slides were stained in ammoniacal basic fuchsin for 30 sec, passed through a series of ethanol: water mixtures of increasing ethanol concentration, absolute ethanol and then xylene. The sections were then mounted in Canada Balsam. The stain was prepared by mixing equal volumes of 0.5% aqueous basic fuchsin and ammonia. The precipitate was filtered off and the supernatant used as the staining solution.

Autoradiographs were photographed using a Leitz microscope with a Pentax 35-mm camera attachment. Photographs were taken

using Kodak Tri-X Pan film with a green filter in the light source.

#### G. The Chemical Nature of the Translocate

To assess the chemical nature of the translocate in *Heracleum* under white or coloured light conditions, a local two-minute application of  $^{14}\text{CO}_2$  (70.72  $\mu\text{Ci } ^{14}\text{C}$ ) was carried out and the isotope was allowed to translocate down the petiole for a total of 5 or 40 min. The leaf was then divided into three samples; labeled leaflet, secondary petiole subtending the labeled leaflet plus the upper part of the primary petiole, and the lower part of the primary petiole. Each of the three samples was frozen with liquid nitrogen, ground and extracted for one hour in hot 80% ethanol. The residue was filtered off, washed with hot 80% ethanol and transferred to scintillation vials containing Cab-0-Sil and fluor for counting. The filtered extract was evaporated to dryness in a stream of air and taken up in a known volume of 80% ethanol. Aliquots of this solution were taken for counting.

Sugars in the aqueous ethanolic extracts were examined by thin-layer chromatography on 0.25 mm layers of silica gel N buffered with 0.1 N boric acid. Two-dimensional chromatograms were prepared. The solvent used in the first direction was n-butanol:acetone:water (4:5:1); that used in the second direction was methyl ethyl ketone:acetic acid:water (3:1:1). Sugars were located on the plate with an anisaldehyde and sulphuric acid spray (Randerath, 1966) and compared to standards run on other plates for identification.

Amino acids in the extracts were examined by two-dimensional thin-layer chromatography using unbuffered silica gel N layers. The first direction was run with a chloroform:methanol:17% ammonia

(2:2:1) solvent, the second with a phenol:water (3:1) solvent. The amino acids were located by spraying with 0.3 g ninhydrin in 100 ml acetone, and identified by comparing their R<sub>f</sub> values to known amino acids run on separate plates. Ninhydrin positive areas from the plates were also scraped off, eluted in citrate-phosphate buffer, and these eluates were examined with a Beckman amino acid analyser.

Organic acids were examined by thin-layer chromatography on silica gel N layers by separation with an ethanol:water:ammonia (8:1:1) solvent. Acids were detected by spraying the plates with a solution of bromocresol green, pH 7 (Hall and Baker, 1972).

To ascertain the distribution of radioactivity in relation to the compounds detected on the thin-layer plates, areas of chromatograms were scraped off into scintillation vials containing Cab-0-Sil and fluor and counted.

#### H. Water Relations

The water relations of the leaf and phloem tissues of *Heracleum* were determined by vapour phase psychrometry. The sampling was carried out over a 24-hr period to provide data on the diurnal changes in the water status of the plants.

Leaf discs were taken from each of the three leaflets and their water potentials were determined in Spanner-type psychrometers suspended in a constant-temperature water bath (Spanner, 1951). A phloem loop was rapidly dissected out of the primary petiole subtending the sampled leaflets to minimize drying, and its water potential was determined in a Wescor C51 sample chamber attached to a Wescor HR33 Dew Point Microvoltmeter. The principle of operation of the latter equipment was similar to the Spanner-type psychrometers.

Following determinations of water potentials, the combined osmotic and matric potentials were determined for the samples by freezing them in liquid nitrogen and replacing them in the psychrometers. The turgor of the tissue was determined as the difference between the water potential and the combined osmotic and matric potentials.

Attempts were made to examine gradients in any of the component potentials within a phloem loop by cutting the loops into portions five centimeters long and determining the potentials with the Wescor psychrometer.

All psychrometers used in this study were first calibrated with solutions of known osmotic concentration.

The diffusion resistances of the leaves were also determined over a 24-hr period using a Lambda Instruments LI 60 diffusion resistance meter with a Kannemasu type sensor. This was carried out in the growth chamber where the plants were normally cultivated. The sensor had previously been calibrated at the chamber temperature using the calibration plate supplied with the instrument. Leaf resistances were also measured under various spectral irradiances at the chamber temperature.

#### I. Net Assimilation and Dark Respiration

The net assimilation capacity of *Heracleum* plants in the growth chamber was determined by infra-red gas analysis. Leaflets were enclosed in Plexiglass cuvettes through which a flow of air could be passed. The leaflets were placed in the cuvettes in a manner which would ensure even illumination from above and maximum air flow over both leaf surfaces. The leaflet temperature was monitored with a leaf thermocouple. The leaflets were illuminated by



exposing them to a range of intensities of white or coloured light from incandescent and fluorescent sources in the ceiling of the growth chamber.

Net assimilation was measured with a Unor 2 infra-red gas analyser. Compressed air from the building supply was passed through the cuvette containing a leaflet. A gas flow rate of 3400 ml/min was maintained through both the reference and sample cells of the analyser which was calibrated to span 100 ppm full scale. Net assimilation and leaf temperature were recorded directly on a dual range Honeywell Electronik 16 multipoint recorder with a center zero reading so that both net assimilation and dark respiration could be measured. Light measurements were made with the quantum sensor alongside the cuvette under a layer of Plexiglass of the same thickness as that used in the cuvette. One leaflet on each of three plants was examined under a range of light conditions as indicated later.

## EXPERIMENTS; RESULTS AND DISCUSSION

### A. Translocation in Petioles Following Foliar Application of $^{14}\text{C}\text{O}_2$

#### 1. Introduction

Before attempting to follow the translocation of  $^{14}\text{C}$ -labeled compounds in isolated phloem loops of *Heracleum* or petiole wedges of sugar beet it was important to obtain normal translocation profiles for reference purposes. With these it would then be possible to test if isolated loops or wedges would translocate  $^{14}\text{C}$ -labelled compounds, and to assess the effects of wounding the petioles on translocation. This was determined by isotope counting techniques and by autoradiography of the translocating petioles.

Two approaches to offering  $^{14}\text{C}\text{O}_2$  to the plant were used. Either a general application to the total lamina, or a more restricted application to a portion of the lamina. The wounded *Heracleum* petiole was very amenable to the application of bathing fluids which might affect translocation in isolated loops. This was especially true when the bathing fluid contained reagents that could affect callosing and slime plugging.

#### 2. Results

##### a. Translocation from whole lamina with intact petioles

###### Sugar beet

The graph of  $^{14}\text{C}$  level against distance down the petiole gave a linear profile when the data were transformed to a semi-logarithmic plot (Fig. 1). On one occasion a profile was not linear on a semi-logarithmic plot (Fig. 2). This profile had the appearance of the redrawing of Mortimer's (1965) data by Canny (1971). On analysing the data in Fig. 2 by the error function method of

Canny (1973) a line with a translocation coefficient  $K=9.96 \times 10^{-3} \text{ cm}^2/\text{sec}$  gave a close fit to the data.

#### Discussion

Intact petioles showed semi-logarithmic translocation profiles of  $^{14}\text{C}$  label which became less steep with time (Fig. 3). On the basis of this data 30- or 40- min translocation times were used for further work on the effects of wounding.

#### b. Translocation from whole lamina with wounded petioles

##### Sugar Beet

Experiments to obtain translocation profiles in wounded petioles following  $^{14}\text{CO}_2$  assimilation by whole lamina were not carried out. The sugar beet system was very suitable for the study of the effects of wounding in conjunction with localized  $^{14}\text{CO}_2$  application which provided more critical data.

##### Heracleum

When a phloem loop was dissected from a petiole just before tagging the lamina with  $^{14}\text{CO}_2$ , a profile typified by Fig. 4 was produced in the petiole. A peak of activity was common in the wounded petiole at either the acropetal or basipetal end of the wound, indicating the build-up of isotope in those regions. No activity was found in the isolated loop, even when further lengths of a loop were isolated from intact regions of the petiole above the wound just prior to counting.

Flooding the wound with water during the incision, assimilation and translocation period did not cause  $^{14}\text{C}$ -translocate to move into the loop. This was also the case with 0.1 M phosphate, borate or Tris buffers with or without the addition of 0.1 M sucrose. The

use of a salt solution consisting of enough  $\text{Na}_2\text{CO}_3$  crystals to shift a 0.1 M sucrose solution to pH 8.5, followed by the addition of enough  $\text{KH}_2\text{PO}_4$  to lower the pH to 8.0 (prepared according to G. Mitton, personal communication), and the addition of 20 mM  $\text{KCl}$ ,  $\text{CaCl}_2$ , 10 mM EDTA or 0.05 M 2-mercaptoethanol to water or 0.1 M sucrose bathing solutions also had no effect on promoting translocation of  $^{14}\text{C}$ -labeled assimilates into the loops. However, if 1.0 M sucrose was used as the bathing medium, translocation was severely inhibited in the remainder of the wounded petiole. Isolating the loop up to 96 hr before tagging the lamina with or without bathing the loop with water or 0.1 M sucrose failed to restore translocation in the loop.

Several petioles were dissected down to single phloem loops. This procedure was carried out under water prior to the tagging of the leaf and the strand was kept under water throughout the experiment. Isotope was found in the water near the cut end of the petiole and in the first few centimeters of the phloem loop. No isotope was found further down the petiole. The activity recorded in the upper part of the loop is regarded as contamination by exuding labeled translocate from the cut petiole.

To attempt to evaluate how damaging the incisions into the petiole were to the translocation pattern, pairs of incisions were made in the petiole to isolate increasingly larger sectors or wedges of tissue with increasing numbers of bundles in the wedge. A typical hollow petiole in cross section may contain 16 outer and 10 inner bundles. When a wedge of petiole about 10 cm long containing one outer bundle was isolated by two parallel incisions and the

lamina tagged with  $^{14}\text{CO}_2$ , no isotope was recovered from the wedge, although it was recovered from the remaining sector. This was also the case with a wedge containing two outer bundles. When the wedge was large enough to contain three outer bundles a small amount of  $^{14}\text{C}$  was detected in it (Fig. 5).

- c. Translocation from a localized area of  $^{14}\text{CO}_2$  application down intact petioles

#### Sugar Beet

Profiles of  $^{14}\text{C}$  in the petiole were linear when plotted semi-logarithmically following application of  $^{14}\text{CO}_2$  to an area between two veins in a lower quadrant of the lamina. With increasing translocation times, the slopes of the profiles decreased and the presence of peaks and hollows near the front became less evident (Fig. 6), as though a transient mixing phase of  $^{14}\text{C}$ -loading and transport had been followed by a more steady state phase of  $^{14}\text{C}$ -loading and transport.

#### Heracleum

Profiles were linear in 40 min translocation experiments when plotted semi-logarithmically. Where the profile was obtained in both the primary and secondary petioles subtending the labeled leaflet the profile was generally continuous through the junction region (Fig. 7).

The fact that labeled translocate was redistributed in the region of the commissural ring of vascular tissue at the junction of the primary and secondary petioles is shown by sampling the two secondary petioles not subtending the labeled leaflet. In both of these secondary petioles isotope was present. Figure 7 also

shows a profile obtained in a five-minute translocation experiment which shows pronounced peaks and hollows.

- d. Translocation from a localized area of  $^{14}\text{CO}_2$  application down wounded petioles

#### Sugar Beet

Sugar beet plants were wounded by incisions being made parallel to the bundles coming from the tagged area of the leaf. That  $^{14}\text{C}$  translocate was passing primarily through these bundles was shown by making two-centimeter incisions, immediately prior to labeling, on either side of the two bundles in the petiole which served the tagged area of the lamina. This isolated a wedge of tissue containing these bundles (Fig. 8). After a 15-min translocation time the petiole was cut into one-centimeter segments. The wedge of tissue isolated by the wound incisions was counted separately from the remainder of the petiole (Fig. 8). This showed that the bulk of the isotope was passing through the wedge, so it was possible to predict the path of the isotope in further experiments.

Wounds of various lengths were made in the petiole adjacent to the  $^{14}\text{C}$ -loaded bundles. The wounds were made just prior to, or 90 min before offering  $^{14}\text{CO}_2$  to the lamina. With longer wounds, e.g. six centimeters, it was impossible to avoid cutting some of the anastomosing bundles which are common in sugar beet petioles. With two-centimeter wounds this was avoided. In all cases where a wound was made there was a deviation on the normal semi-log profile in the vicinity of the wound, often with accumulation of isotope in the wounded area above the level predicted by the profile (Fig. 9).

#### Hexacolum

Experiments in this category were not carried out for two reasons. Firstly, the presence of the commissural ring of vascular

tissue at the petiolar junction made it impossible to trace the vascular tissue from the tagged area of one leaflet to the primary petiole. Secondly, on the basis of whole lamina labelings it was known that small wedges of tissue or isolated bundles did not carry labeled translocate.

Some experiments with localised  $^{14}\text{CO}_2$  application were carried out on leaves which had been detached from the plant while the plant was held under water. The leaves were therefore deprived of their normal sink by a drastic form of wounding. The detached leaves were kept with their cut petioles under water and were placed in a chamber in the dark for two hours to prevent wilting. The leaf blade was then illuminated with  $70 \mu\text{E}/\text{m}^2/\text{sec}$  and the leaf was given a localised tag of  $^{14}\text{CO}_2$  in a 40-min experiment. If the leaf was detached under water and transferred to 20 mM EDTA, pH 7, for two hours prior to and during a 40-min experiment, there was a decrease in isotope in the petiole compared to the water control (Fig. 10). In both cases the translocation profile had the appearance of a series of peaks of activity with no semi-log component along the profile.

#### e. Callosing

Callose was present on the sieve plates in both control and isolated *Heracleum* phloem tissue stained with aniline blue and examined in ultra-violet light. No distinction could clearly be made between heavily and lightly callosed sieve plates, such as reported by McNairn and Currier (1968) in *Gossypium*. Using the diachrome stain the sieve plates in the isolated loops showed a greater amount of staining than the control sieve plates. When unfixed tissue still attached to the plant was stained with

resorcinol blue before severing the loop, from the plant, there was no rapid increase of callosing such as observed by Knight (In Fenson, 1972).

#### f. Autoradiography

The localization of isotope in the petiolar bundles basipetal to the region of the sugar beet lamina where the isotope was applied can be seen in the diagram in Fig. 11. The localization of the isotope within the bundle can be seen in Fig. 13 and 14. An unlabeled bundle is seen for comparison in Fig. 15.

The localization of isotope in a *Heracleum* petiole reflects the fact that the leaflets were offered isotope since the majority of the bundles contained isotope, as indicated in the diagram in Fig. 12. Upon wounding, however, the isotope showed a more restricted distribution (Fig. 16). The isotope was found in bundles at a distance from the site of the incision into the petiole. If a wedge of tissue was isolated from the petiole the label was found only in the bundle furthest from the site of wounding (Fig. 17).

Both Fig. 13 for sugar beet and Fig. 18 for *Heracleum* showed that the isotope was contained primarily in the phloem. The localization of the isotope was always more sharply defined in freeze-dried as compared to freeze-substituted preparations.

### 3. Discussion

#### a. Sugar Beet

Sugar beet, on the basis of pulse-labeling experiments, has been reported to produce a linear profile with an arithmetic plot of radioactivity versus distance down the petiole (Mortimer, 1965). In the present study the more typical semi-log plot of activity



*versus* distance produces a linear profile (Fig. 1). This profile is in accordance with the first of the models of Horowitz (1958) where the isotope in the translocating system is reduced in its level of activity due to irreversible loss from the transport pathway. The plants used in the present study were of a similar variety to those used by Mortimer, and their growth conditions were also comparable. Even if sample preparation techniques for isotope detection similar to those of Mortimer were used the profile plots remained semi-logarithmic. No explanation of this difference was obvious.

Canny (1971) redrew the data of Mortimer (1965) showing linear profiles on arithmetic plots of count rate *versus* distance. Canny on semi-log plots found a curve which was fitted by an error function complement curve giving a translocation coefficient of  $K=3.30 \times 10^{-2} \text{ cm}^2/\text{sec}$ . The K value for the data in Fig. 2 was lower than that of Mortimer's data, but it was well within the range of translocation coefficients given by Canny (1971, Table 1). In his book, Canny (1973) gave a range of K values for the data of Mortimer. He suggests that the profiles were of a transition type where the error function curve was developing at the top of the petiole while lower down the petiole was a steeper loading profile. In the present study this loading profile was not evident in the single experiment that produced this class of data.

Canny (1973) emphasized a need for intense local applications of isotope to laminae to overcome problems produced when whole leaves were treated. If the application of  $^{14}\text{CO}_2$  covered a large area, a large number of veins at different distances from the petiole

would be loaded, and the profile in the petiole would be a composite profile. He also emphasised the need for petioles to be kept darkened to prevent re-fixation of  $^{14}\text{CO}_2$  released by respiration in the petiole. When this approach was adopted as far as possible, i.e. application of isotope to a  $3.14 \text{ cm}^2$  area of lamina between two veins followed by translocation down a darkened petiole, the resulting profiles were typical of whole lamina applications.

The decreasing slope of the translocation profiles with increasing times (Fig. 6) was typical of experiments of this type, (Mortimer, 1965; Qureshi and Spanner, 1971; Whittle, 1971). The fronts of the profiles in this study were very different from the fronts in the typical translocation profiles outlined by MacRobbie (1971, Fig. 1). MacRobbie diagrams profiles where the front was marked by a steep region (1) followed by a less steep region (2). It is region (2) which decreased in slope with increasing time and which corresponded to the slopes reported in this thesis. In the present study no region (1) was evident, and it was replaced by a series of peaks of activity. The nature of these peaks will be discussed in conjunction with the data from *Heracleum*.

Bundles of sugar beet which were translocating labeled compounds from a zone of localized application showed some effect of petiolar wounding (Fig. 9). A build-up of activity in the vicinity of a wound was usually observed. This build-up may have been initiated by the creation of a local sink as a result of wounding the petiole. However, translocate continued to move through the bundles contained in wedges of tissue between parallel wounds (Fig. 8).

That the isotope applied to a restricted area of lamina remains in a particular vascular bundle from that area was shown by autoradiography. A bundle from the basal area of the fed leaf was shown to contain isotope (Fig. 13). This agreed with the results of Suzuki and Mortimer (1973) which showed that bundles drain particular areas of the sugar beet leaf. The isotope was clearly seen to be primarily in the younger phloem as was the case with the autoradiographic study of Mortimer (1965). The absence of isotope from other bundles of the same section was very evident (Fig. 15).

b. *Heracleum*

The translocation profiles of *Heracleum* showed the typical linear profiles on semi-log plots of activity versus distance (Fig. 5). The decrease in the slope of the profile with increasing time was also typical of pulse-labeling experiments. With increasing translocation times it could be predicted that the slope would become negative as the pulse of label passed down the petiole. Compared to the number of counts in the intact petiole there was a depression of activity in wounded petioles (Fig. 3). The peak of activity in the region of a wound may indicate increased lateral movement of label as a result of artificially inducing a sink by wounding. The phenomenon of wound respiration, i.e. the increase in the respiratory rate of a tissue following its wound (Lipetz, 1970) would result in a lowering of the osmotic concentration in the cells in the wounded area as substrates were used in respiration, and this would provide a secondary sink along the translocation pathway. This sink would accentuate the lateral movement of labeled assimilates from the transport pathway in the vicinity of

the wound and produce the elevated count rate.

Assimilated  $^{14}\text{C}$  was never detected in the isolated loops lifted free of overlying parenchyma following the opening of the petioles by incisions. The failure of the various bathing treatments to restore the ability of isolated loops to transport  $^{14}\text{C}$  assimilates was significant. The fact that all the bathing media were aqueous rules out the possibility of desiccation as the cause of the loss of function of the loops, except in the case where the bathing medium was 1.0 M sucrose. This latter solution had the effect of inhibiting translocation in the unwounded petiole, presumably by lowering the water potential of the tissue by plasmolysis. The use of buffers to guard against any drastic pH changes implies that the inhibition of translocation was not due to this cause. It was possible, however, that an intracellular pH change was occurring which was not checked by the extracellular application of buffers. The use of 0.1 M sucrose as a bathing medium was intended to provide less of an osmotic shock to the tissue than that caused by water.

The presence of P-protein in phloem is quite common (Evert *et al.*, 1973), although not ubiquitous (Evert *et al.*, 1971). The nature of P-protein has been widely studied, especially in *Cucurbita* species (Kleinig *et al.*, 1971a; Kleinig *et al.*, 1971b; Kollman *et al.* 1970; Walker, 1972; Walker and Thaine, 1971; Weber and Kleinig, 1971; and Eschrich *et al.*, 1971). It has been reported to occur in *Heracléum sphondylium* (Robidoux *et al.* 1973). Sabnis and Hart (1973) and Hart and Sabnis (1973) worked with P-protein isolated from phloem loops of *H. mantegazzianum* while

Yapa and Španner (1972) studied P-protein obtained from phloem exudate of this species.

The possibility that in excising the phloem loops of *Heracleum* the P-protein structure was altered on a molecular and hence cellular level was examined by treating the loops with reagents that would alter or stabilize the P-protein (assuming that the reagents penetrated the tissue). Kleinig *et al.*, (1971) noted that KCl, CaCl<sub>2</sub> or Vinblastine precipitated microfibrillar or P-protein material in phloem exudate of *Cucurbita* while Sabnis and Hart (1973) reported Vinblastine to have no effect on the P-protein tubules of *Heracleum*. Injection of 20 mM KCl or CaCl<sub>2</sub> into the hollow petiole and isolation of the loops under a flow of 20 mM KCl or CaCl<sub>2</sub> was carried out to precipitate any P-protein material in its normal state and prevent it from plugging sieve plate pores. If any plugging of this nature had occurred by disulphide bond formation between protein molecules during the isolation of loops, it was hoped that injection and bathing with 0.05 M 2-mercaptoethanol would prevent the gelling reaction. A gelling reaction of this type had been reported by Walker (1972) in *Cucurbita* P-protein.

Neither the KCl, CaCl<sub>2</sub> or 2-mercaptoethanol treatments had any effect on the translocation capacity of the isolated loops. The inconclusive nature of this type of experiment is typified by the fact that Williamson (1972) reported an absence of an effect of Cytochalasin B on translocation in *Lepidium sativum* and on the structure of P-protein from *Ricinus communis*, while Thompson and Thompson (1973) reported a reversible inhibition of translocation of externally applied label in *Heracleum* loops caused by Cytochalasin B.

Yapa and Spanner (1972) reported that the isoelectric point of P-protein from *H. mantegazzianum* was 4.9 so the buffers used in this study should have maintained the P-protein on the correct side of its isoelectric point. The constituents of the sieve tube are usually regarded as being slightly alkaline (Crafts and Crisp, 1971; Hall and Baker, 1972).

Eschrich *et al.* (1965) recorded an acceleration of assimilate movement in *Cucurbita* when borate was injected into its hollow petiole, even though borate induced increased callose deposition. In *Heracleum* the borate bathing media had no effect on maintaining assimilate movement in phloem loops.

In addition to the reported effect of  $\text{CaCl}_2$  on P-protein,  $\text{Ca}^{2+}$  ions are implicated in the mechanism of callose formation (Eschrich, 1965), and the addition of calcium ions may not have been overcoming a translocation inhibition because while fixing the P-protein they may have been inducing the formation of callose. This should not have been the case where calcium was replaced by potassium. Addition of EDTA to the bathing medium should chelate  $\text{Ca}^{2+}$  ions resulting in the inhibition of callose formation, and this was postulated to be the mode of action of Mitton's solution (G. Mitton, personal communication). King and Zeevart (1974) report that EDTA maintained phloem exudation from *Perilla* petioles by inhibiting callose formation, while  $\text{Ca}^{2+}$  ions inhibited the activity of EDTA. Similar experiments with *Heracleum* showed no increase in translocation of  $^{14}\text{C}$  assimilates in cut petioles standing in 20 mM EDTA compared to controls standing in water (Fig. 10).

In isolated loops of *H. sphondylium* it has been reported by

Knight (Fensom, 1972) that callosing did not occur until the ends of the loops were severed from the petiole. This callosing was monitored by aniline blue vital staining and fluorescence microscopy. This experiment, when repeated with *H. lanatum*, was performed by dissecting out two phloem loops from one petiole and mounting them side by side in aniline blue; one loop was then cut from the petiole. When the two strands were compared no difference in callose fluorescence was observed even though the staining times were identical. Care was taken in viewing both control and cut loops on similar faces, preferably the cambial one. While the callose was being studied observations were also made on slime plugging. During the severing of the loop there was no obvious rush of material towards the sieve plates to create slime plugs.

On the basis of these results several conclusions on the site(s) of the inhibition of the translocation of  $^{14}\text{C}$ -labeled compounds may be made. The phloem loop itself was unlikely to be the site of inhibition if the inhibition was due to dessication, pH change, slime plugging (i.e. P-protein plugging the sieve plate pores) or callosing as attempts were made to control these effects to no avail. The possibility then exists that the inhibition occurred at the site of vein loading, or at the commissural ring of vascular tissue at the petiolar junction. The role of the commissural ring in the distribution of isotope could be seen where only a local area of leaflet was offered  $^{14}\text{CO}_2$ . This should have localised the  $^{14}\text{C}$  assimilated between two major leaf veins and as there was no anastomosing visible in the secondary petiole the label should have remained in those two veins alone. When the secondary

petioles subtending the two unlabeled leaflets were cut off and counted there was some isotope present in them. This isotope was assumed to have moved into these regions following redistribution in the commissural ring. It was, of course, possible that isotope was present in the unlabeled leaflets due to leakage of  $^{14}\text{CO}_2$  when a leaflet was tagged, or to  $^{14}\text{CO}_2$  produced by respiration in the labeled leaflet being fixed by supposedly untagged leaflets. It would have been possible to prevent this by shading the unlabeled leaflets with aluminium foil. However this would have had the effect of converting the shaded leaflets into sinks and would have enhanced the movement of  $^{14}\text{C}$  assimilates from the leaflet through the commissural vascular tissue into the unlabeled leaflets. Experiments showing the ease with which a translocate source can become a sink were performed by Qureshi and Spanner (1971).

The fact that vein-loading continued following wounding was indicated by two facts. Firstly, following incisions isolating increasingly wider wedges of petiole a critical distance was reached following which  $^{14}\text{C}$  translocate moved through the wedge (Fig. 5). Even when the wedge was not translocating the rest of the petiole was. This implied a relatively restricted effect of wounding not transmitted to the area of vein loading. Secondly, when a petiole was dissected down to a single vascular bundle no isotope was found in the bundle. It was, however, found in the intact petiolar tissue above the wound. The petiolar bundles had continued to be loaded but there was some exudation of isotope from the cut petiole indicating some passage of assimilates through the commissural tissue.



Further evidence that incisions into the petiole caused cessation of function was obtained by autoradiography. Bundles in the region of a wound did not show the presence of isotope in them (Fig. 16). This agrees with the data from the labeling experiments. If large wedges of tissue were isolated then activity was shown to be in a bundle at some distance from the sites of the wounds (Fig. 17).

It was concluded that the most probable site of regulation of movement of  $^{14}\text{C}$ -translocate in isolated phloem loops of *H. lanatum* was in the junction region between the primary and secondary petioles. It was possible, however, that the site of inhibition was along the length of the isolated loops, and that none of the treatments used in this study overcame this inhibition.

Spot feeding of  $3.14 \text{ cm}^2$  of the central leaflet of *H. lanatum* produced a linear exponential profile normally running through the secondary petiole subtending the tagged leaflet and the primary petiole. The fronts of the profile were similar to those found in the sugar beet. Beyond the exponential portion of the profile the actual front consisted of a series of peaks and hollows of activity. These were very apparent in the five-minute experiment in Fig. 7.

It was initially thought that these peaks were induced by the pumping of the syringe in the tagging process. If experiments were carried out where the syringe was discharged into the cuvette with no further pumping the peaks were still present in the petiolar profile. It was concluded that the peaks were not artifacts created by the tagging procedure. This conclusion was also reached by comparing the periodicity of the peaks with

that of the pumping of the syringe. The syringe was pumped at least eight times a minute for the two minute tagging time, while the peaks were much less frequent.

The peaks may also have been due to local accumulations of isotope along the petiole. Serial sections of *Heracleum* petiole were made to try to find areas of anatomical specialization which might account for the local accumulation. No such specialization was readily apparent at the light microscope level and the anatomy of the petiole appeared to be uniform throughout its length.

Localized application of two-minute pulses of  $^{14}\text{CO}_2$  resulted in a temporary increase in the partial pressure of carbon dioxide in the region of application. The carbon dioxide level was increased 19-fold. Prior to the introduction of the increased  $\text{CO}_2$  level the plant was equilibrated to a steady state condition in the growth chamber. The pulse of  $^{14}\text{CO}_2$  would thus serve to introduce a perturbation into this steady state condition. This perturbation would be expected to cause oscillations in the levels of photosynthetic intermediates and this would ultimately be reflected in sucrose pool sizes and in the rates of vein loading. These oscillations would then be carried along the translocation pathway.

Induced oscillations are common in photosynthetic systems particularly following a dark to light transition with elevated  $\text{CO}_2$  levels. This subject was well reviewed by Rabinowitch (1956). Oscillations in metabolic intermediates caused by changes in  $\text{CO}_2$  levels are known in photosynthetic systems. Wilson and Calvin (1955) showed great variations in levels of ribulose-1,6-diphosphate,

phosphoglycerate and other intermediates of the Calvin cycle when the level of  $\text{CO}_2$  was changed from a steady 1% state to a 0.03% state. These oscillations were damped down after several minutes to a new steady state. The cycle of oscillations was approximately 50 sec. Lewenstein and Bachofen (1972) reported transient induced oscillations in the levels of ATP in *Chorella fusca*. When  $\text{CO}_2$  was suddenly withdrawn, less ATP-consuming reactions were carried out, but ATP-consuming reactions continued for several minutes and the ATP levels oscillated with a cycle of approximately 50 sec.

Several workers have postulated mechanisms of phloem transport based on some form of pulsing and Fensom and Davidson (1970) have provided data purporting to be evidence for a pulse-flow mechanism. The frequencies and wavelengths of these models and results are compared with the parameters of the oscillations in the present study in Table 1. If the data recorded in this study represented a pulsed mode of translocation operating at a greater speed than a mass flow mode they must be compared with other models. The models of Hejnowicz (1970) and Aikman and Anderson (1971) postulated pulses of quite different orders of magnitude to the ones in the present study. This was to be expected as their models were based on mechanisms operating at a subcellular level. Miller's (1973) model and his calculations based on Fensom and Davidson's (1970) data were of a similar order of magnitude to the ones recorded in the present study.

It is concluded that the pulses in the present study represent an artifact induced in the system by the pulse-labeling method.

It is possible that Fensom and Davidson's data also represent artifacts induced by the perturbation of a steady-state condition upon microinjection of  $^{14}\text{C}$ -sucrose. The model of Miller deals with a protoplasmic streaming mechanism. If such a system were in operation in *H. lanatum* the changes in the flow of cytoplasm postulated by Miller would have been visible due to the presence of the marker particles in the sieve tubes. Streaming on a sub-microscopic level would not have been observed.

Previous reports of pulsed modes or wavy profiles of labeled translocates in advance of more typical profiles have appeared. The pulsed mode was first observed by Nelson *et al.*, (1958) and was considered to be an aspect of the phenomenon termed "rapid translocation". This was more fully discussed by Nelson *et al.*, (1959) and Nelson (1962). The latter reported that it was capable of moving  $^{14}\text{C}$  rapidly from the labeled leaves to the roots in the stem of soybean at speeds of 7200 cm/hr, with voids of detectable tracer in the profile. He discussed the possibility of the total amount of carbon moved in translocation being the result of a low-speed movement of a low concentration of translocate as in mass flow, or a high-speed movement of a low concentration as he observed. Lateral accumulation along the pathway was proposed to account for the observed semi-logarithmic distribution in the petiole behind the rapid translocate. The possibility was suggested that the "slow" translocation of sucrose in the phloem was due to the spread of the accumulation front down the stem. The fact that the front of the  $^{14}\text{C}$  localized in the petiole was discontinuous was taken as being indicative of movement mediated by metabolism. This

discontinuous pattern may have indicated a true "wave-like" translocation, or a continuous stream of material below the limits of detection which accumulated at certain loci to a level high enough to be detected. Nelson (1963) also reported a similar wavy mode of  $^{14}\text{C}$  translocate in sunflower. Fensom (1972) reinterpreted these data in terms of his trimodal flow model. The possibility that the pulses represented an artifact induced by the labeling technique was not considered.

By using a more sensitive counting method than that used by Nelson *et al.*, and by cutting the petioles into smaller segments greater resolution of the "wave-like" translocation profile was possible.

The pulses in this study were more pronounced and easier to observe than any reported by Nelson because the shift in the steady state kinetics due to elevated  $\text{CO}_2$  levels in the area of application was much greater, and the area of application much smaller. It is likely that the pulses observed by Nelson *et al.* were caused by perturbing a steady-state condition as in the experiments reported here, and were not associated, as assumed, with "fast translocation". These facts still do not entirely rule out Nelson's suggestion of a continuous stream of small amounts of labeled translocate at low levels, but they make it unlikely.

While the waves may have been artifacts, their speed of movement is not. It is possible that previous estimates on the speed of transport of material in the phloem were serious underestimates of the true speed of translocation. The inadequacy of deriving estimates of speed of translocation from profiles has been thoroughly discussed by Crafts and Crisp (1971), MacRobbie (1971) and Canny (1973). In this study, sugar beet shows speeds of movement of label at the upper

end of the range quote by Mortimer (1965) of 50-135 cm/hr and possibly much faster. In Fig. 6 the pulse had moved at least 15 cm in two minutes giving a minimum speed of 450 cm/hr. In *Heracleum* the pulses in Fig. 7 had moved at least 21 cm in five minutes giving a minimum speed of 252 cm/hr, a much greater value than the 35-70 cm/hr of Ziegler and Vieweg (1961) from their heat pulse experiments. This study did not establish the existence of several modes of translocation occurring simultaneously (Nelson *et al.* 1959; Fensom, 1972), but it did not definitely rule out the possibility.

## B. Effects of Light Quality

### 1. Introduction

The studies on the effects of light quality were initially undertaken to test whether peaks of radioactivity observed in short-term translocation experiments were simply artifacts due to the pulse-labeling process, or whether they were indicative of a physiological process. It has been suggested (O. Biddulph, personal communication to P.R. Gorham) that light quality may have been responsible for inducing the pulses. Therefore short-term experiments were carried out under different light qualities to test this possibility, and also to evaluate the effect of light quality on several other physiological processes.

### 2. Results

To compare the effects of light quality on translocation, green, red, blue and white light of approximately  $80 \mu\text{E}/\text{m}^2/\text{sec}$ . used to irradiate the plants. The transmission spectra for the various coloured lights in the growing area of the growth chamber were measured by an ISCO spectroradiometer at the level of the

plant canopy and are given in Fig. 19. The energy of the light was also measured by an Eppley pyranometer and these values are given with Fig. 19.

a. Net assimilation

The net assimilation rate of a *Heracleum* leaflet per unit area *versus* light intensity is plotted in Fig. 20. The same information plotted on a leaf dry weight basis is shown in Fig. 21. These graphs are typical of the three pairs of results obtained. On both graphs are included the net assimilation rates for a variety of light qualities. No difference was observed in net assimilation rate with differences in light quality expressed on an equivalent quantum basis.

b. Diffusion resistance

Figure 36 shows the changes in leaf diffusion resistance plotted against light intensity. Over the range of light intensities used the leaves show decreased resistance with increasing light intensity. Also plotted on this graph are the diffusion resistance values of leaves irradiated with light of different qualities.

c. Translocation from a localized area of application of  $^{14}\text{CO}_2$

Translocation experiments of 5- and 40-min total duration were carried out in each light colour and the profiles obtained at both times are shown in Figs. 22, 23 and 24. These profiles were compared with the white light controls in Fig. 7. At the 40-min translocation time profiles were generally continuous through the junction region, the site of the commissural ring of vascular tissue. However in the junction region the count rate was frequently higher than that predicted by the profile through the

primary and secondary petioles.

There was no effect of light quality on the nature of the major labeled compounds in the leaf of *Heracleum*. At both 5- and 40-min from the start of an experiment the major labeled compounds in the leaf were sucrose and glutamic acid. The only labeled compound extracted from the petiole was sucrose.

The average fixation of available  $^{14}\text{CO}_2$  in these experiments was 7.63%. Of the total fixed, less than 1% was translocated in 40 min from the area of localized isotope application to the petiole.

After five minutes the average ratio of hot-80%-ethanol-extractable  $^{14}\text{C}$  to non-extractable  $^{14}\text{C}$  was 7.46:1. This rose to 12.77:1 after 40 min. In the petiole, after five minutes the ratio was 12.04:1 rising to 12.26:1 after 40 min.

### 3. Discussion

The only significant study on the effect of light quality on translocation was carried out by Hartt (1966). This study was with sugar cane, a so-called "C4" plant, and involved the use of coloured fluorescent lamps whose output was measured on an energy basis. Hartt gave results that indicated that red or blue light induced more translocation, measured on a percentage basis, than equivalent energies of green or white light. This present study utilized *Heracleum*, a "C3" plant, and coloured cellophane filters with a combination of fluorescent and incandescent lamps to provide coloured light of equal quanta. *Heracleum* was determined to be a "C3" plant on the basis of the absence of a pronounced bundle sheath in the leaf (Black, 1973). The measurement of



irradiance between 400-700 nm was regarded as a better measure of light usable by the plant than measurement on a wider energy basis (McCree, 1972).

The effects of light quality on several plant processes which might have a direct or indirect effect on translocation, e.g., stomatal diffusion resistance and net assimilation rates, were studied in addition to the effects on the translocate and the profiles of the translocate.

Light qualities produced by the three coloured filters used in this experiment did not cause leaf diffusion resistance greatly different from those caused by equivalent quanta of white light. The net assimilation rates of *Heracleum* for the various light qualities also did not vary from rates for equivalent quanta of white light. Red light is known to cause stomatal opening and efficient photosynthesis, however blue light is regarded as being more efficient in causing stomatal opening than would be expected on the basis of its efficiency in photosynthesis, and green light on an energy basis is usually less effective in photosynthesis than red or blue light (Salisbury and Ross, 1969).

Under all light qualities assimilated  $^{14}\text{C}$  was found primarily as sucrose and glutamate in the leaf after 5 or 40 min. The increase in the ratio of hot-80%-ethanol-extractable  $^{14}\text{C}$  to non-extractable  $^{14}\text{C}$  is presumably due to the loss of starch synthesized in the leaf during pulse labeling. It is obvious that *Heracleum* is not a rapid exporter of  $^{14}\text{C}$  assimilates as less than 1% was exported in 40 min.

It has been reported that blue light favors the incorporation

of  $^{14}\text{C}$  into glutamate at the expense of sucrose in reactions independent of photosynthesis (Voskresenskaya, 1972). Under the light conditions tested the proportions of activity in sucrose and glutamate did not vary widely. The presence of high levels of glutamate in the leaves may have been the result of the culture conditions used for *Heracleum*. Joy (1969) reported that an NADH-dependent glutamic dehydrogenase is very active in the leaves of *Lemna* when the plant is grown on nitrate or ammonia media. As *Heracleum* plants were watered once a week with Hoagland's solution they would have a high nitrate status which could have stimulated glutamate synthesis. Despite the high specific activity of glutamate in the leaves, only sucrose was labeled in the petiole. This agrees with the study of Ziegler and Mittler (1959) who found sucrose to be the only translocated compound in the phloem of *H. mantegazzianum*.

At short translocation times the profiles produced by all light qualities were very pulsed, with no obvious differences between the colours. This pulsing was probably due to the shifting of the leaf sucrose loading pools from a steady state due to the pulse-labeling process. With longer translocation times a typical profile on a semi-log plot was seen as the pools returned to a new steady state and as more  $^{14}\text{C}$  assimilates became loaded.

The count rate in the junction region of the petiole was generally higher than expected on the basis of the translocation profile through the primary and secondary petiole. The junction region is the place where the commissural ring of vascular tissue occurs. As a result the path length of phloem carrying isotope in

the junction region is greater than the normal one centimeter in the other segments with bundles running parallel through them. It is also where some isotope was diverted into the untagged leaflets. This greater path length probably accounts for the increased count rate above the expected one.

### C. Transport of Labeled Compounds Applied to Isolated Phloem Loops

#### 1. Introduction

This property was initially examined in an attempt to reproduce some of the data from Fensom's laboratory. This was intended to show whether or not *H. lanatum* would transport labeled compounds when they were applied externally to the phloem loops. If they had this ability then it would be legitimate to compare data obtained with this species to that obtained in Fensom's laboratory with *H. mantegazzianum* and *H. spondylium*. It was also hoped to provide greater insight into the nature of the transport of labeled compounds applied externally to phloem loops.

#### 2. Results

In initial experiments, isolated loops with (6,6'-<sup>3</sup>H) sucrose (20  $\mu$ l of aqueous solution containing 10  $\mu$ Ci) applied to their surface at their mid-point transported the label acropetally and basipetally. With increasing transport times from 15 to 45 min, more isotope was transported basipetally from the point of application than acropetally (Fig. 25). These experiments were carried out with the loops laid directly on the supporting glass plate, and the humidity kept high in the region of the loops by a series of water droplets alongside. Times shorter than 15 min gave little significant movement from the region of isotope application.

Subsequent experiments with labeled sucrose and glucose, in which the loops were laid on moist filter paper, gave different results. (U-<sup>14</sup>C) sucrose (6,6'-<sup>3</sup>H) sucrose and (6-<sup>3</sup>H) glucose all failed to move more than a few centimeters along the loops supported on moist filter paper. Tritiated water, however, was transported very rapidly along loops supported on wet filter paper. Figure 26 shows the distribution of tritium both acropetally and basipetally following a two-minute treatment with 20mCi of tritiated water in 25  $\mu$ l of water. Bathing of the loops with water or Mitton's solution produced no significant difference in the movement of tritium. The replacement of water on the apical or basal side of the feeding chamber with 0.5 M sucrose did not produce a flow of tritiated water predominantly in one direction (Fig. 27) following a 15-min application of 10  $\mu$ l of water containing one millicurie of tritium. When this experiment was repeated using (U-<sup>14</sup>C) sucrose there was a somewhat greater movement of sucrose towards the portion of the loop with the highest water potential. This movement was confined to the first one or two centimeters and never over long distances (Fig. 28).

If tritiated water (10  $\mu$ l containing one millicurie) was placed in the treatment enclosure for 15 min, then removed, the tissue thoroughly rinsed several times, and the enclosure refilled with unlabeled water for an additional 15 min, there was no pulse chasing of tritium out of the loop, and isotope was still present in the tissue exposed to the tritiated water in the feeding chamber (Fig. 29).

When the filter paper supporting the loops treated with tritiated water was placed in scintillation vials and counted it

was found to contain a considerable amount of label (Fig. 30). To ensure that this movement into the paper was not from a leak in the feeding chamber a barrier of light mineral oil (3 in 1 brand) was interposed between the feeding chamber and the support paper. The oil covered the loop. Isotope still moved through the oil covered segments and into the paper beyond. If the whole loop was covered with oil without the use of filter paper, tritium still moved along the loop.

When the apical or basal portions of phloem loops labeled with tritiated water ( $10 \mu\text{l}$  containing one millicurie) for 15 min were cut off and washed in a series of aliquots of water which were then counted, the efflux of label from the loop could be followed. The nature of these efflux data suggested the presence of two compartments in the system which both contained label. One compartment emptied much more rapidly than the other during washing in unlabeled water (Fig. 31).

A 25 cm length of polyester thread was soaked in 0.1 M sucrose or water in a vacuum oven and then laid through a treatment enclosure onto wet filter paper or under oil, to provide a model system to follow isotope movement. When 1.0 m Ci tritiated water was applied to a thread soaked in water and laid on wet filter paper the isotope moved rapidly through the thread in both directions from the treatment enclosure (Fig. 32). Results with thread soaked in 0.1 M sucrose and/or laid under oil were similar. The profile compares favorably with the one obtained from a phloem loop (Fig. 26) but it took longer to achieve using one-tenth the specific activity of that employed for Fig. 26. Figure 32 compares well

with Fig. 27 which involved the same amount of tritium applied to the loop. Efflux data from threads and loops were similar.

### 3. Discussion

The shapes of the tritiated water profiles (Fig. 26) are similar to those reported by Fensom and Davidson (1970). The fact that similar profiles are obtained from polyester threads and loops is very significant. The similarities between the phloem loops and polyester threads are few. Both are made up of longitudinally oriented fibers, cellulose fibers in the phloem tissue and polyester fibers in the thread. However, the cellulose fibers are embedded in a hemicellulose matrix. Both are also saturated with water, the threads artificially, the phloem naturally. The movement of tritiated water must then be related to these properties if the nature of the tritiated water movement in the two systems is similar as the profiles suggest. Further evidence on the similarity of movement is gained from efflux experiments where the efflux of tritiated water from a sample indicates the presence of two compartments of tritium in both systems. In the phloem loop (Fig. 31) the two compartments could represent the apoplast and symplast, or bulk tritiated water in the apoplast and exchanged tritium in the apoplast or symplast. For the polyester thread there is obviously no symplast component so the compartments are probably bulk tritiated water in the interfibrillar spaces of the thread and exchanged tritium in or on the fibers themselves. As a result of the similarity in the efflux data from phloem loops and threads it is suggested that the symplast is not behaving as a significant, discrete compartment in the phloem loops during the

experiments.

If the movement of tritiated water in the two systems is similar then the driving force for the movement could be similar. A minimum estimate of the speed of isotope movement in Fig. 26 is 240 cm/hr. This is greater than the 160 cm/hr bidirectional movement of tritiated water in maize roots reported by Anderson and Long (1968) which they ascribed to streaming transcellular strands in the sieve tubes. For the polyester threads where experiments were conducted over 15-min time periods the minimum speed is 40 cm/hr which is a serious underestimate of the true speed. These speeds of movement are clearly greater than those possible by diffusion. The labeled molecules of water would move down their own chemical potential gradient as reported by Nims (1962), so there would be a tendency for the labeled water to diffuse out of the isotope application chamber in both directions along a loop or a thread. This depends on the relation between the mole fraction of the labeled substance in a mixture with the unlabeled substance. If the mole fraction of the labeled substance approaches zero the observed flow of tracer bears little relation to the flow of unlabeled substance. If the mole fraction of labeled substance is appreciable the flow of the substance has a measurable effect on the flow of the labeled substance. The mole fraction of tritiated water is low and it will be moving independently. However, during the period of the experiment it will not diffuse more than two centimeters from the zone of application. This would explain the initial abrupt decline in the count rate in the first few centimeters from the zone of application. This rapid decline in the count rate with increasing distance from

the feeding chamber ~~may~~ also be a result of the slow filling of the slowly emptying compartment seen in the efflux data. Labeled sucrose would diffuse slower than labeled water and it too would not diffuse far from the zone of application (Fig. 28). The presence of isotope more than a few centimeters from the zone of application requires some means of moving the labeled compound other than diffusion. As labeled glucose or sucrose did not show non-diffusional movement the rapid transport may be a property of the tritium or tritiated water itself, linked to the common properties of the loops and threads.

The tritium nucleus in the tritiated water molecule may be capable of proton transfer as described by Eigen (1964). Protons in aqueous media are able to move with a speed that differs from that of the conduction band electrons in metals by two orders of magnitude. This occurs as the proton ( $H^+$ ) becomes hydrated ( $H_3O^+$ ) followed by the alignment of water molecules around the hydrated proton by hydrogen bonding ( $H_9O_4^+$ ). By forming additional hydrogen bonds this complex forms a tertiary hydration complex. By structural diffusion the proton can move within the complex and the structure changes accordingly. The lifetime of the  $H_3O^+$  ion is  $0.8 \times 10^{-13}$  to  $1.0 \times 10^{-13}$  sec. Deuterated water,  $^2H_3O^+$ , has a lifetime larger by a factor of six to eight, and it is assumed that for  $^3H_3O^+$  it is larger still. The water continuum in the phloem loop or polyester thread would provide a path for the movement of tritium nuclei at speeds that could conceivably be three to four orders of magnitude greater than those observed.

Assuming that the hydrated proton moves one structural unit



at a time, that each hydrogen in a water molecule is  $2.76 \text{ \AA}$  from the closest hydrogen on another water molecule in the  $\text{H}_9\text{O}_4^+$  complex and that the lifetime of the  ${}^3\text{H}_3\text{O}^+$  is larger by a factor of 100 than the  $\text{H}_3\text{O}^+$  ion, then a tritium nucleus could move 27.6 cm in 0.01 sec. It would only do this if the proton transport were ordered in one direction, while in a bulk water phase it would be a random process capable of moving in all directions, There will however be a tendency to move away from the zone of labeling down a chemical potential gradient.

The problem with the proton transport hypothesis is that it does not fit the data of other workers. Choi and Aronoff (1966) applied tritiated water vapour to darkened petioles. The tritiated water showed little tendency to translocate from the zone of application until the light was turned on and then it moved toward the transpiring lamina. If proton transport was occurring it should not be light dependent. Gage and Aronoff (1960) had earlier reported the inability of tritiated water to move in translocating systems. In the study of Choi and Aronoff (1966) it is not clear that the tritiated water is getting into the plant in significant amounts. This may only be possible after the light is turned on and the stomata open. Any tritium penetrating into the plant during the dark might have spread throughout the tissue beyond the zone of application, and become diluted below the level of detection in the large bulk of the water phase of the petiole. However, Biddulph and Cory (1957), Plaut and Reinhold (1967), Trip and Gorham (1968), Thompson and Nelson (1971), Cataldo *et al.*, (1972) all have reported data which indicated that tritiated water had been translocated.

In the experimental systems in this study it is unlikely that the movement of tritiated water is due to translocation in a physiological sense as the polyester thread is clearly incapable of this, and the phloem loops behave like the polyester threads.

An alternative explanation may be postulated due to an interfacial flow mechanism such as that described by van den Honert (1932). The only interface common to both loops and threads would be a water:fibril one. In the loops the fibrils would be of cellulose; in the threads, of polyester. If movement of this type occurred in translocating systems then it should have been revealed in the experiments of Choi and Aronoff (1966).

It is possible that the flow of tritiated water results from proton transport along a surface. Ordered water molecules along the surface of cell wall or polyester fibers may be the path of movement.

The observed rates of tritium movement in this study are obviously greater than can be explained on the basis of diffusion. It has not been possible to provide an answer as to what is the driving force for this movement. The fact that the tritiated water cannot be pulse-chased from a phloem loop indicates that there is no normal flow of water going on along the loop. It is possible, however, that due to tritium exchange reactions with hydrogen atoms in the phloem, the pulse chase of tritiated water is masked.

The apparent ability of  $6,6\text{-}^3\text{H}$  sucrose to move rapidly along the loops in the initial experiments when the loops were not supported by moist filter paper may have been due to a failure to keep the loops from desiccating, and this in turn caused the tritiated

sucrose to be drawn along the loops. It was also possible that the sample of tritiated sucrose, which was several years old when used, contained a large proportion of tritiated water due to breakdown or exchange with the tritiated sucrose. This tritiated water could then have moved rapidly along the loops giving the observed profiles. A freshly acquired sample of (6,6'-<sup>3</sup>H)sucrose did not display any movement of tritium rapidly along the loops supported on wet filter paper due to a lack of an evaporative suction and/or the lack of tritiated water in the sucrose.

By setting up an osmotic gradient through the zone of isotope application it was possible to induce some movement of (U-<sup>14</sup>C) sucrose down a chemical potential gradient (Fig. 28). The fact that the label moved only a few centimeters indicated that this was not a mass flow, as the water would be flowing in the opposite direction to sucrose down its own gradient. It did emphasize that the movement of labeled sucrose was more diffusional than representative of the physiological ability of the loops. Ziegler (1958) in his pioneer work with phloem loops of *Heraclium* reported that there were no differences in the rates of movement of <sup>14</sup>C sucrose and fluorescein K in living or freeze-killed phloem loops. He also noted that the tracers moved only a few centimeters in six hours. This is more like the rates of movement observed in the present study than those reported by Fensom (1972).

Tritiated water could not be induced to flow predominantly in one direction down a loop from a feeding chamber. This is probably due to the low mole fraction of tritiated water compared to unlabeled water with the result that the tritiated water r

down its own chemical potential gradient. It still moves faster than would be possible by diffusion.

As a result of the similarities in the efflux curves from phloem loops and polyester threads it was concluded that the two compartments revealed by graphing the data were due to a rapid efflux from the bulk water in the fiber matrices of the loops and threads, and a slow efflux due to tritium exchange with hydrogen in the molecules of the loops and threads. Jarvis and House (1967) discuss the role of exchangeable tritium in the identification of slowly emptying compartments from maize roots. However, they make the assumption that tritium atoms remained attached to water molecules and did not exchange with hydrogen ions of organic molecules. This assumption does not appear to be valid.

It must be concluded that the movement of tritiated water in phloem loops is an artifact and that no natural translocation is occurring. No evidence was seen in this study of a pulsed sucrose transport as described by Fensom (1972).

#### D. Water Relations

##### 1. Introduction

The Münch pressure-flow theory requires appropriate gradients in component potentials along the conducting tissues to drive the solute from source to sink. In addition, the loading of the veins in the leaf is generally regarded as being a metabolically active process. It was hoped that information could be obtained on the water relations of vein loading and translocation by determining the component potentials of leaf and phloem tissues.

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## 2. Results

### a. Diurnal changes

The water potential ( $\psi$ ) and combined osmotic and matric potentials ( $\psi_{\pi}\psi_{\tau}$ ) showed a diurnal fluctuation in both phloem and leaf. The difference between the two values represents the turgor potential of the tissue ( $\psi_p$ ). Figures 33 and 34 show the changes in the potentials of leaf and phloem, respectively, plotted over a 24-hr period with the light intensities indicated on the time axis. The potentials in both tissues were more negative under the highest light intensities than in the dark. Figure 35 shows the differences throughout the day in the water potential and osmotic and matric potentials between the leaf and phloem tissues. At all times there were differences between the leaf and phloem potentials. The leaf values were always less negative than the phloem values giving positive gradients between leaf and phloem tissues. The magnitude of this difference varies between -8 and -1 atm.

### b. Gradients within the phloem

Attempts to find gradients in water potential, or combined osmotic and matric potentials within the phloem produced erratic results. Leaf potentials measured at the time of phloem sampling were consistent with results obtained in the study of diurnal changes, but the phloem values were consistently more negative and unduly low. In the range of phloem material sampled there were gradients in component potentials running apically and basally, but there was always a water potential gradient (from a less negative to a more negative tissue) from the leaf to the phloem

of the petiole.

### 3. Discussion

Diurnal changes in the water potential of leaves and other organs are well known (Slatyer, 1967) and changes of the magnitude recorded for *Heracleum lanatum* in Fig. 33 were not unexpected. As the light intensity increased the rate of evapotranspiration, as measured by a diffusion resistance porometer, increased (Fig. 36) resulting in a greater water deficit in the leaves. The parallel trace of the combined osmotic and matric potentials to that of the water potential indicated that the lowering of the water potential was paralleled by the increase in the concentration of osmotically active solutes in the leaf. Whether this occurred as a result of higher light intensities giving greater net assimilation rates, or as a result of the greater water loss due to increased transpiration, or both, was not clear. Under the conditions in the growth chamber the plants showed no sign of wilting and the leaves always gave positive turgor values.

The situation was very similar in the phloem tissue of the petiole (Fig. 34). The osmotic potentials in the phloem could have become more negative because of increased loading of assimilates into the phloem in the leaves as a result of increased net assimilation associated with increasing light intensities. The fact that the turgor in the phloem was negative at light intensities near the compensation point may have been due to the insensitivity of the method used to determine the component potentials, or as a result of the sample preparation. In the latter case cutting the phloem loops free of the plant could be expected to cause a

loss of turgor. At times of day with high light intensities the turgor may have been so high as to rapidly block the sieve plates by P-protein plugging when the loops were cut, thus retaining a measure of the functional turgor of the tissue in the psychrometer sample chamber. At periods of low light intensity the turgor may have been reduced and on cutting the phloem loops free, it was lost altogether. The presence of negative turgor did not, however, preclude the ability of the phloem to remain functional. In the model of Christy and Ferrier (1973) the system could work with a negative turgor as long as there were a gradient of turgor along the phloem.

When the differences in the component potentials between the leaf and the phloem were studied (Fig. 35), it was found that there was always a positive water potential difference between the leaf and the phloem. This indicates that there could be a passive flux of water occurring throughout the day. There was also a positive osmotic gradient with a lower osmotic potential i.e. higher sugar concentration in the phloem than the leaf. Thus for sucrose to move it would have to move against this concentration difference. This would be additional evidence to support the concept of the active vein loading of sucrose (see review in Crafts and Crisp, 1971).

The values reported for osmotic and matrix potentials could be compared to those reported by Geiger *et al.* (1973) for sugar beet leaves. Their plasmolytic method gave values for the osmotic potentials of individual cells in a tissue, while the present study gave a combined osmotic and matrix potential for the whole tissue or organ. The leaf mesophyll value of sugar beet was 13 bars



(1 bar = 0.987 atm) while the leaf osmotic and matric potentials of *Heracleum* which would be dominated by the mesophyll component were between -9 and -19 atm depending on the light intensity. These compared well with the values for sugar beet. In sugar beet the leaf sieve elements and companion cells had an osmotic potential of 30-bars, while the phloem of *Heracleum* had an osmotic and matric potential of between -14 and -24 atm, a somewhat higher value than sugar beet had. The *Heracleum* phloem value may have been an underestimate of the sieve element and companion cell value due to dilution by the component potentials of the phloem parenchyma and cambium which would have much higher water potentials.

Attempts to find gradients of component potentials in the loops of phloem were regarded as optimistic. Hammel (1968), in his study on turgor pressure in oak, found turgor pressures to be zero to three atmospheres larger in his upper sensing zone than his lower one with two zones about five meters apart. This gave a pressure gradient of between 0.2 to 0.4 atm/m. To resolve pressure gradients of this magnitude over a distance of up to 30 cm was beyond the capabilities of the technique used. Concentration gradients have been reported in *Fraxinus* by Zimmerman (1957) to 0.01 mole/m and this too was unlikely to be resolved in a small herbaceous plant.

## CONCLUSIONS

This study was primarily undertaken to provide a better understanding of the physiology of translocation in *Heracleum*, both in intact plants and also plants wounded to isolate parts of their phloem as individual loops. The behaviour of labeled compounds applied externally to these loops was also examined.

By the use of a sensitive counting method it was possible to analyze the front regions in translocation profiles. These were found not to be steep profiles marking a rapid increase in isotope level, but to be composed of a series of pulses. These pulses were regarded as artifacts of the pulse labeling method rather than as evidence for rapid translocation, or translocation by several mechanisms. The pulses indicated that previous estimates of the minimal speed of translocation derived from pulse-labeling data were serious underestimates of the true speed.

Wounding the petiole of *Heracleum* caused inhibition of translocation in the vicinity of the wound. This was demonstrated in experiments involving the study of profiles and also by autoradiography. Sugar beet is not so sensitive to wounding as was demonstrated in similar experiments to those carried out on *Heracleum*. The nature of the vasculature in the petiole in *Heracleum* helps to explain why such different responses to wounding were shown between the plant species used. *Heracleum* may simply redirect translocate from wounded regions via a commissural ring of vascular tissue, an anatomical feature not found in sugar beet where petiolar vascular bundles come more directly from specific areas of lamina.

Isolated phloem loops do not seem to offer a good system in which to study translocation. It was never possible to induce  $^{14}\text{C}$ -labeled assimilates in the leaves to translocate through isolated loops even though attempts were made to overcome possible reactions such as callosing or slime-plugging. If translocation was occurring in the loops then the labeled sugars utilized were not loaded, while the labeled water was loaded. It is more likely, however, that data derived from studies on phloem loops reflect the behaviour of the isotope in the system rather than a physiological process. This is especially true because it was found that polyester threads transport tritiated water in a similar manner to the phloem loops. Tritiated water appeared to move as tritium nuclei along interfaces rather than as a result of translocation in the sense of a bulk flow. The efflux of tritiated water from phloem loops indicated the presence of two compartments in which the isotope was found, and this was also reflected in the efflux from polyester threads.

Additional studies were carried out on *Heracleum* to examine the water relations of phloem transport and the effect of light quality on translocation. The data from the water relations study indicates that water may move passively from leaf tissue to the petiolar phloem, while sucrose is moving against a gradient over the same distance. This would tend to support the concept of the active vein loading of translocate. Light quality appeared to have no effect on the profiles of translocate in the petiole or on the nature of the translocate itself, sucrose being the only labeled compound found in the petiole.

Phloem translocation remains an enigmatic area of plant physiology despite repeated attempts to fully understand it. The prospects of the process being fully resolved in the near future are remote and the continued application of Occam's razor is essential to prevent the subject being swamped in a surfeit of experimental and theoretical data obtained for its own sake.

TABLE 1  
Parameters of postulated waves in phloem transport

<u>Reference</u>	<u>Wavelength (cm)</u>	<u>Frequency (min)</u>	<u>Velocity (cm/min)</u>
Aikman and Anderson, 1971	0.1	300	0.00033
Hejnowicz, 1970	0.0001	600	0.0000000166
Miller, 1973	1	1	1
Fensom and Davidson, 1970 (In Miller, 1973)	7.5	1.5	5
Hoddinott, 1974	3	1.33	2.25

FIGURE 1. Diagram of the trifoliate leaf of *Heracleum* (1/2 life size). C = commissural ring of vascular tissue, arrows indicate region of wounds.

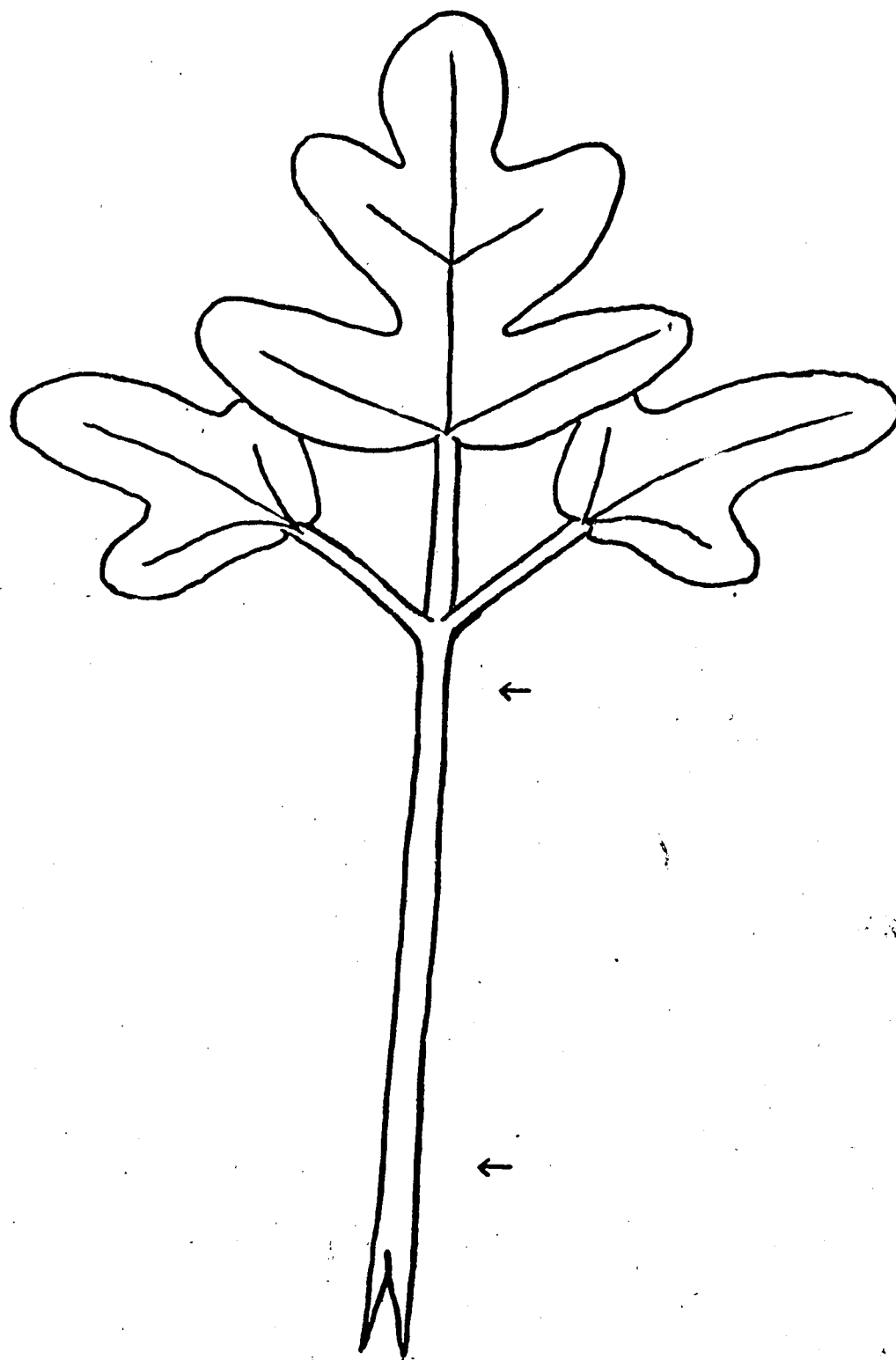


FIGURE 2. Profile of  $^{14}\text{C}$ -assimilates in unwounded sugar beet petiole 40 minutes after a two-minute exposure of the whole lamina to  $70.72 \mu\text{Ci}$  of  $^{14}\text{CO}_2$ .



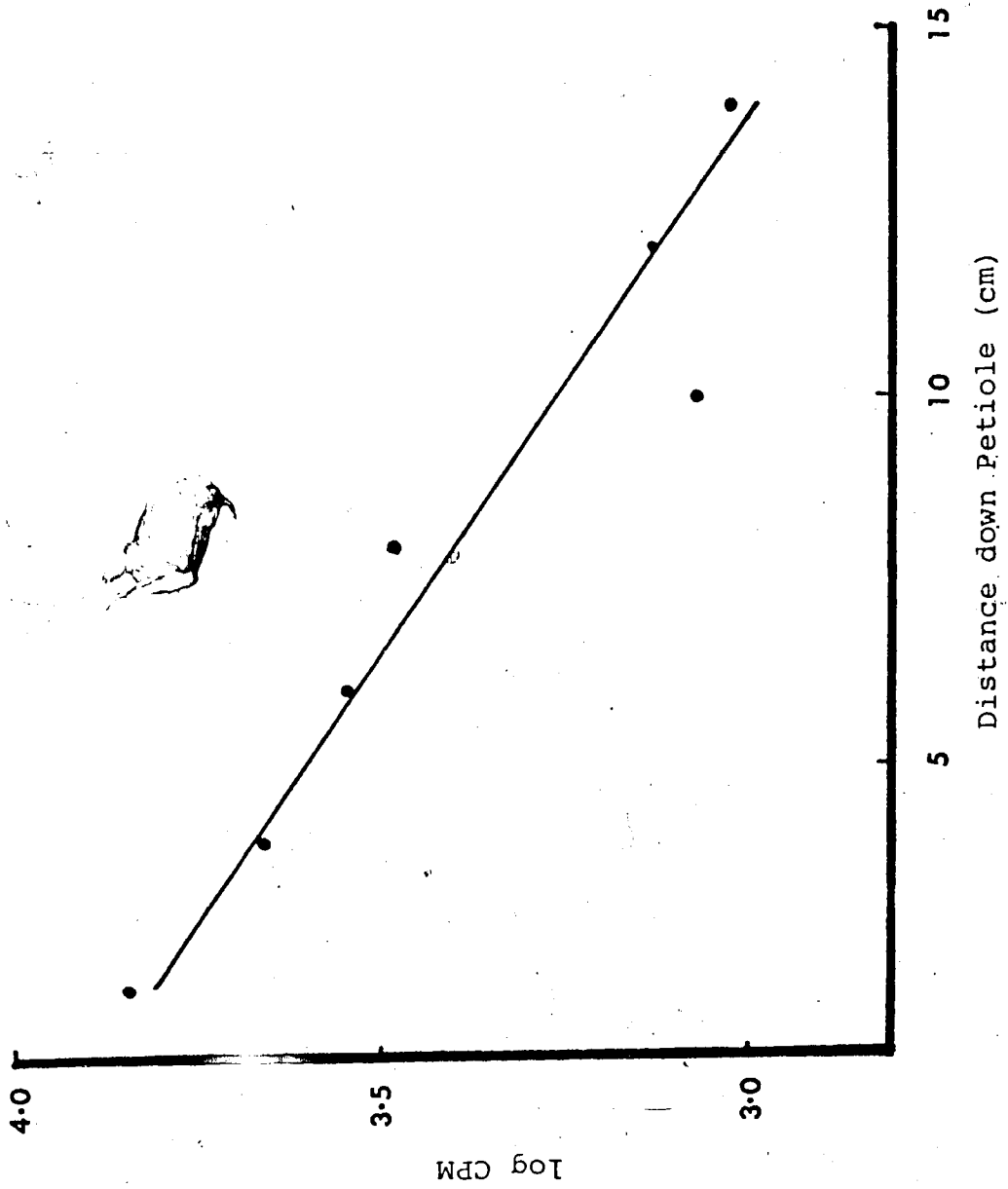


FIGURE 3. Continuous Line: profile of  $^{14}\text{C}$ -assimilates in an unwounded sugar beet petiole 40 minutes after a two minute exposure of the whole lamina to  $70.72 \mu\text{Ci}$  of  $^{14}\text{C}$ . Dashed line: error curve fit of the data using the method described by Canny.

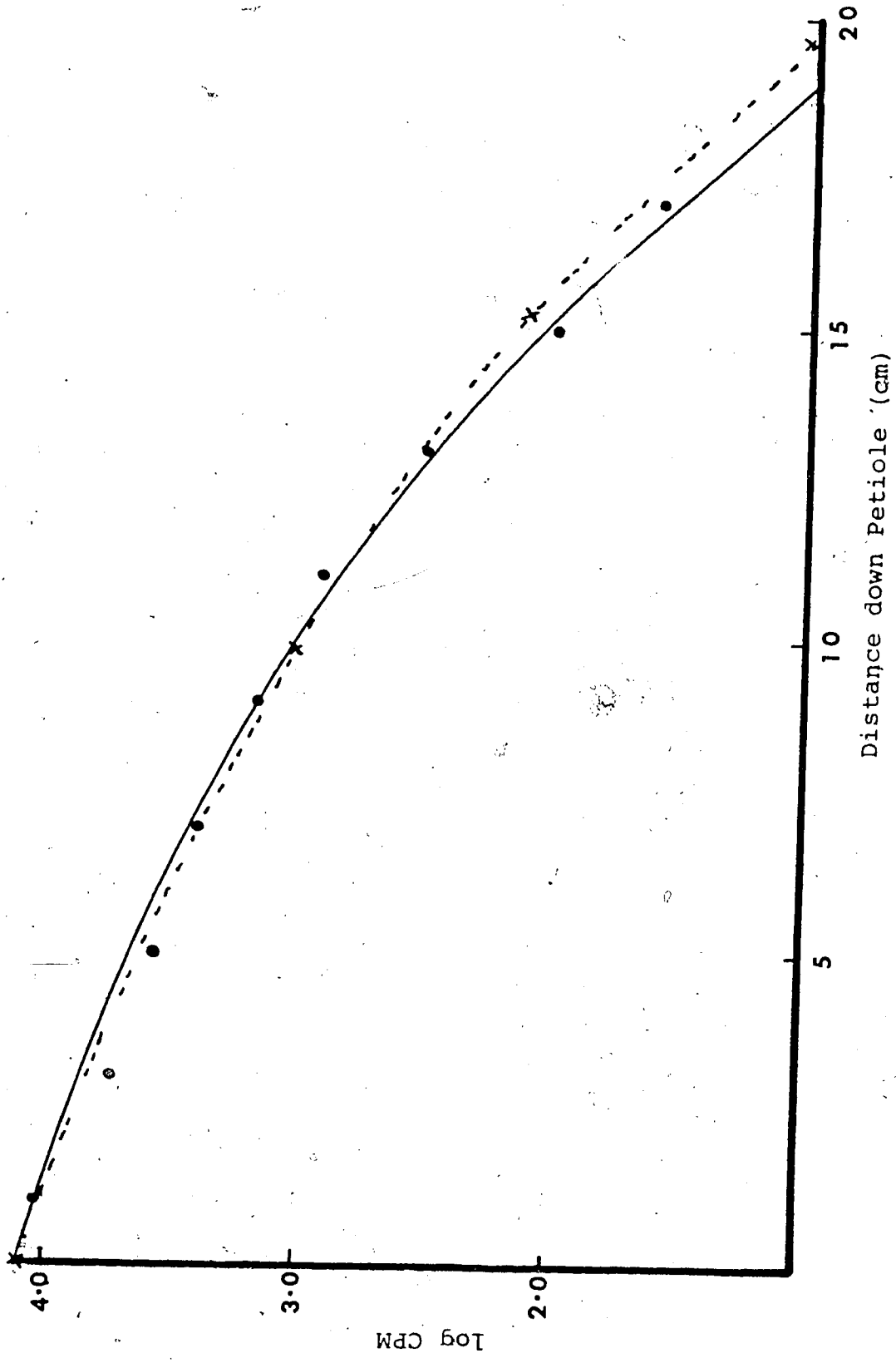


FIGURE 4: Profiles of  $^{14}\text{C}$ -assimilates in intact primary petioles of *Heracleum* with increasing translocation times, from 30 to 100 min. The total time includes the two-minute tagging time with  $70.72 \text{ Ci } ^{14}\text{CO}_2$  (some intermediate points have been omitted for clarity).

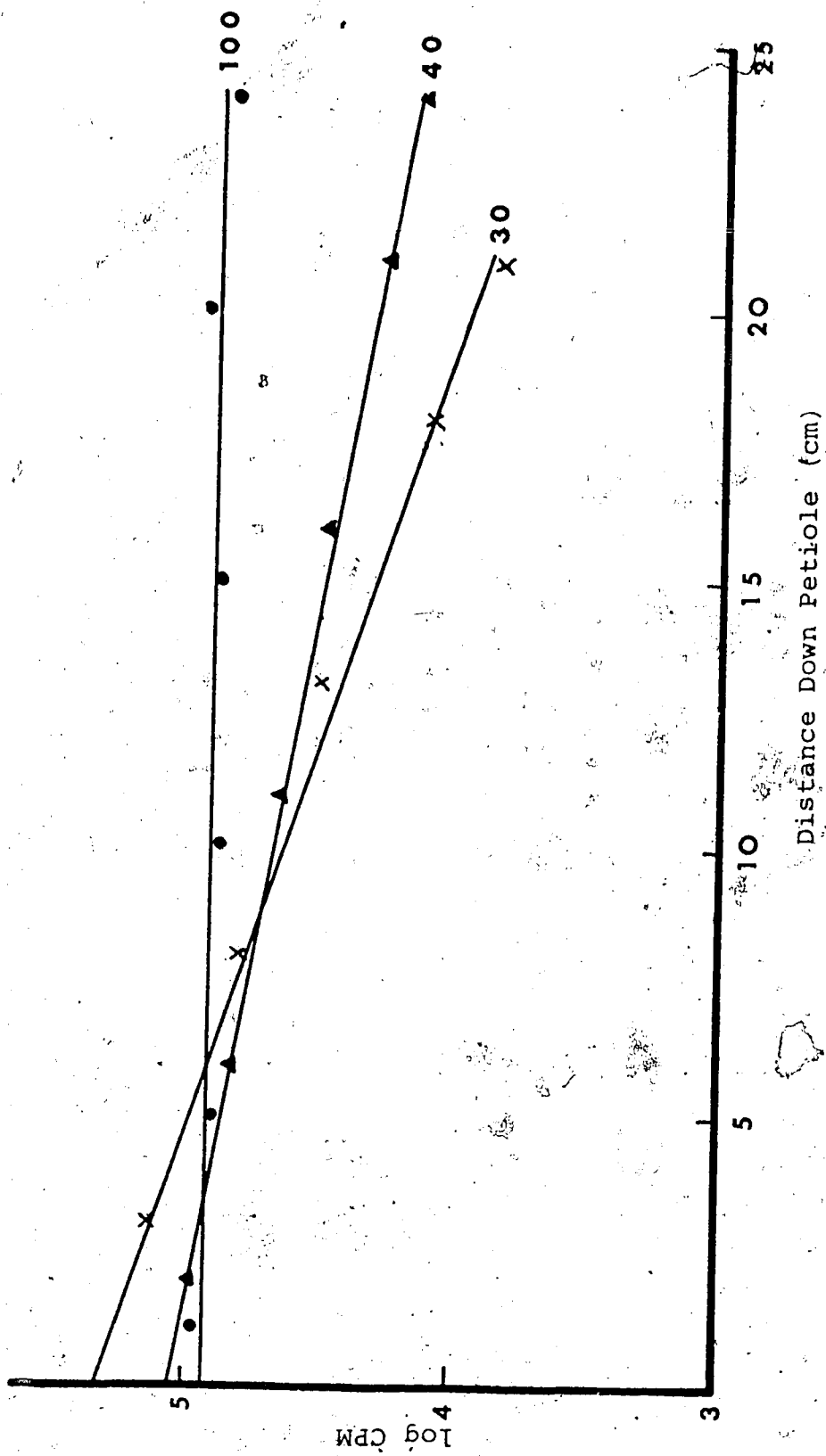


FIGURE 5. Profile of  $^{14}\text{C}$ -assimilates in a wounded primary petiole of *Heracleum* exclusive of the isolated loop which is not labeled. Translocation time 30 min. Arrows indicate the length of the wound incision and the phloem loop.

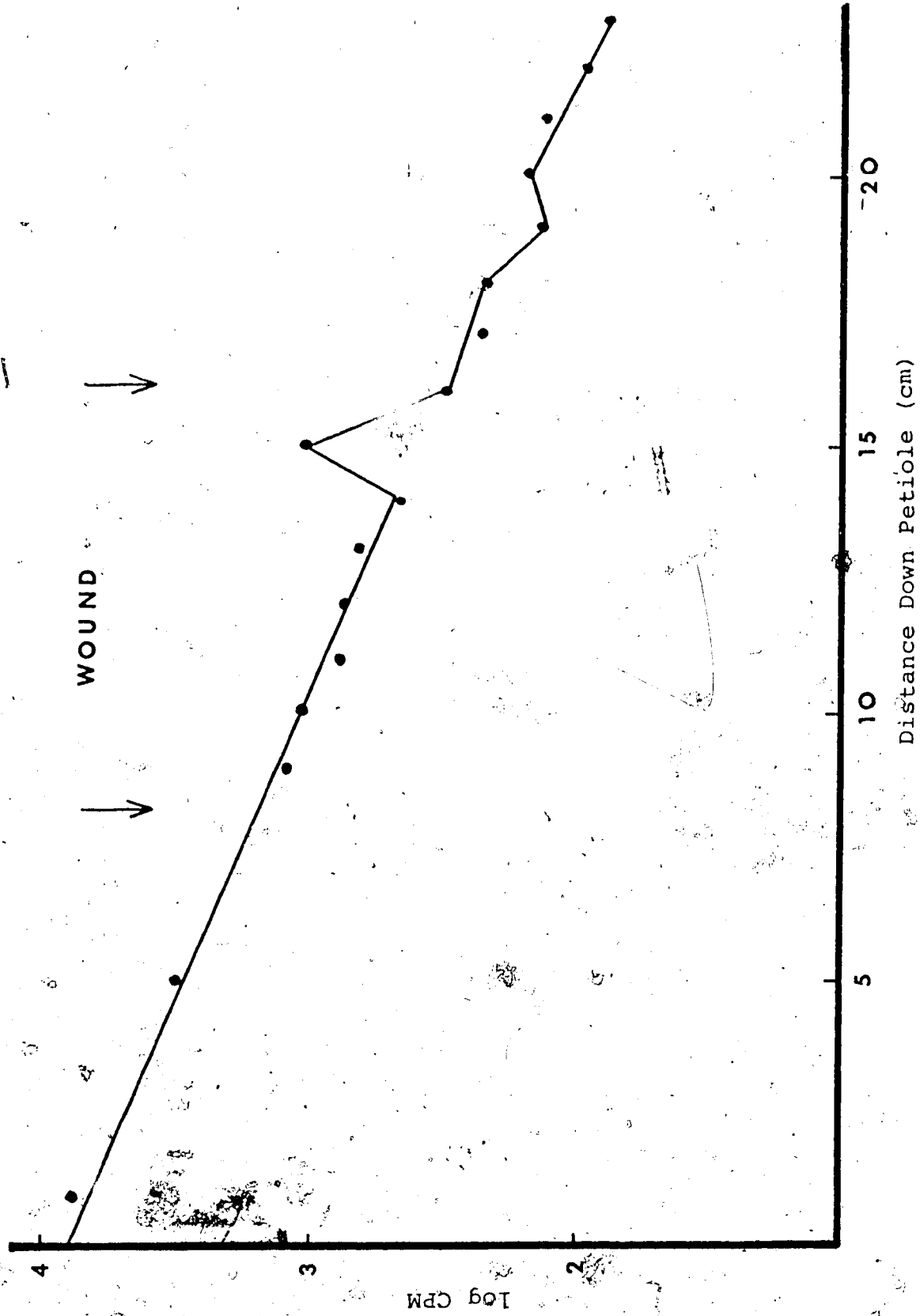
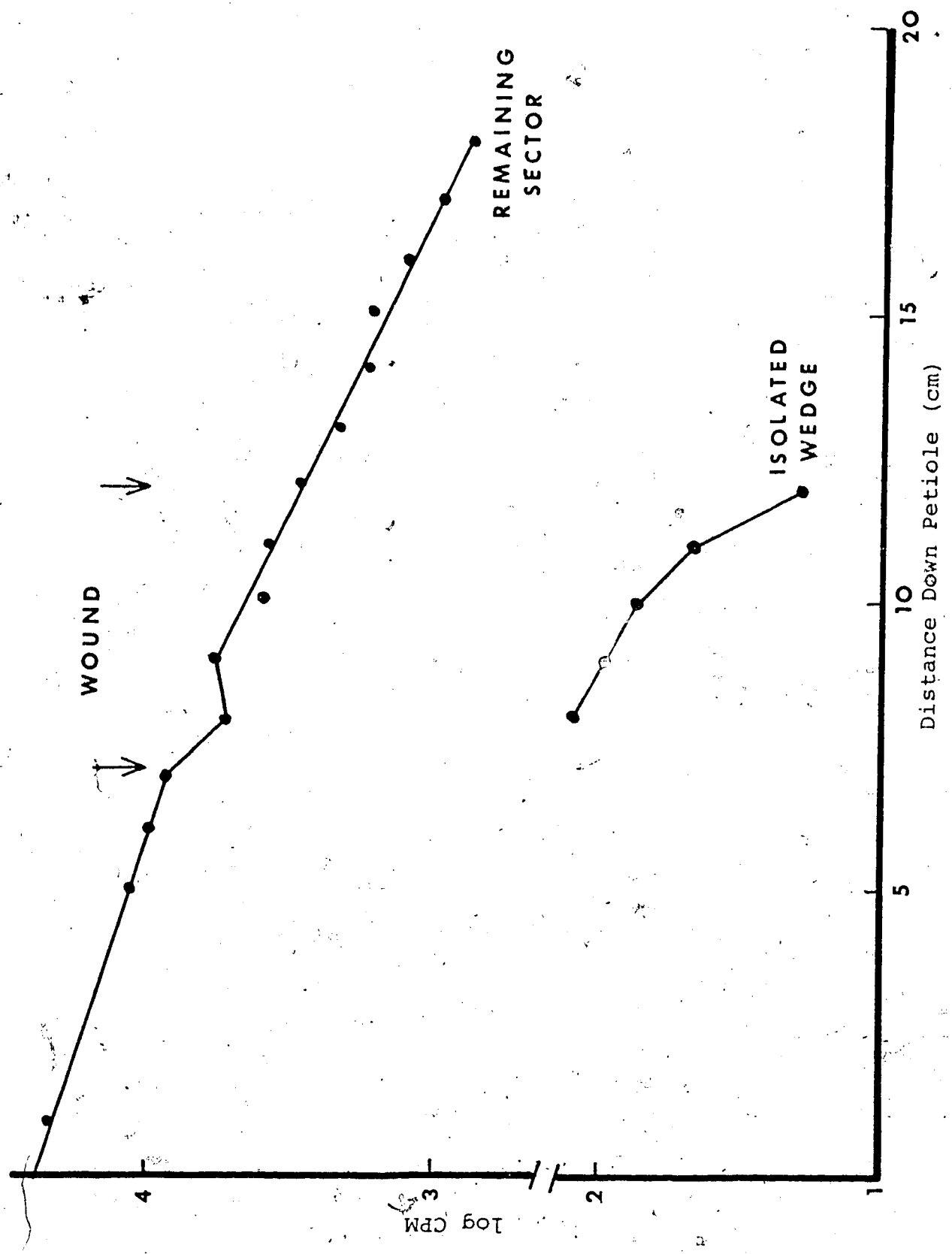


FIGURE 6. Profile of  $^{14}\text{C}$ -assimilates in an isolated wedge containing three outer bundles and in the remaining sector of a wounded primary petiole of *Heracleum*. Translocation time 30 min.





WOUND

REMAINING SECTOR

ISOLATED WEDGE

log CPM

Distance Down Petiole (cm)

FIGURE 7. Profiles of  $^{14}\text{C}$ -assimilates in petioles of sugar beet with increasing translocation times from 2 to 15 min following a localized application of  $70.72 \text{ Ci } ^{14}\text{CO}_2$ .

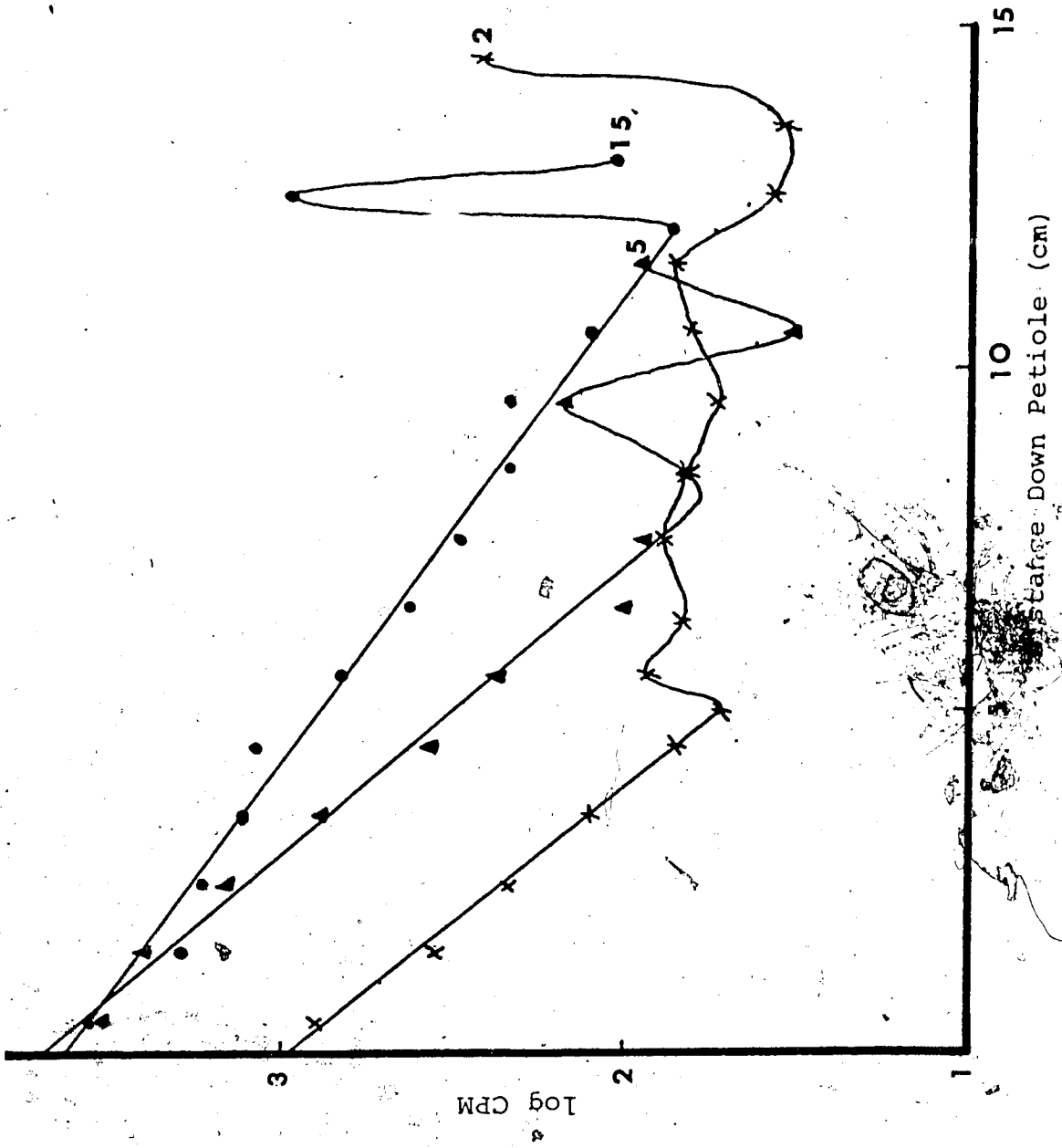


FIGURE 8. Profiles of  $^{14}\text{C}$ -assimilates in intact petioles of *Heracleum* 5 and 40 minutes after a two-minute localized application of  $70.72 \text{ Ci } ^{14}\text{CO}_2$ . Experiments performed in white light. J = Junction between primary and secondary petioles.

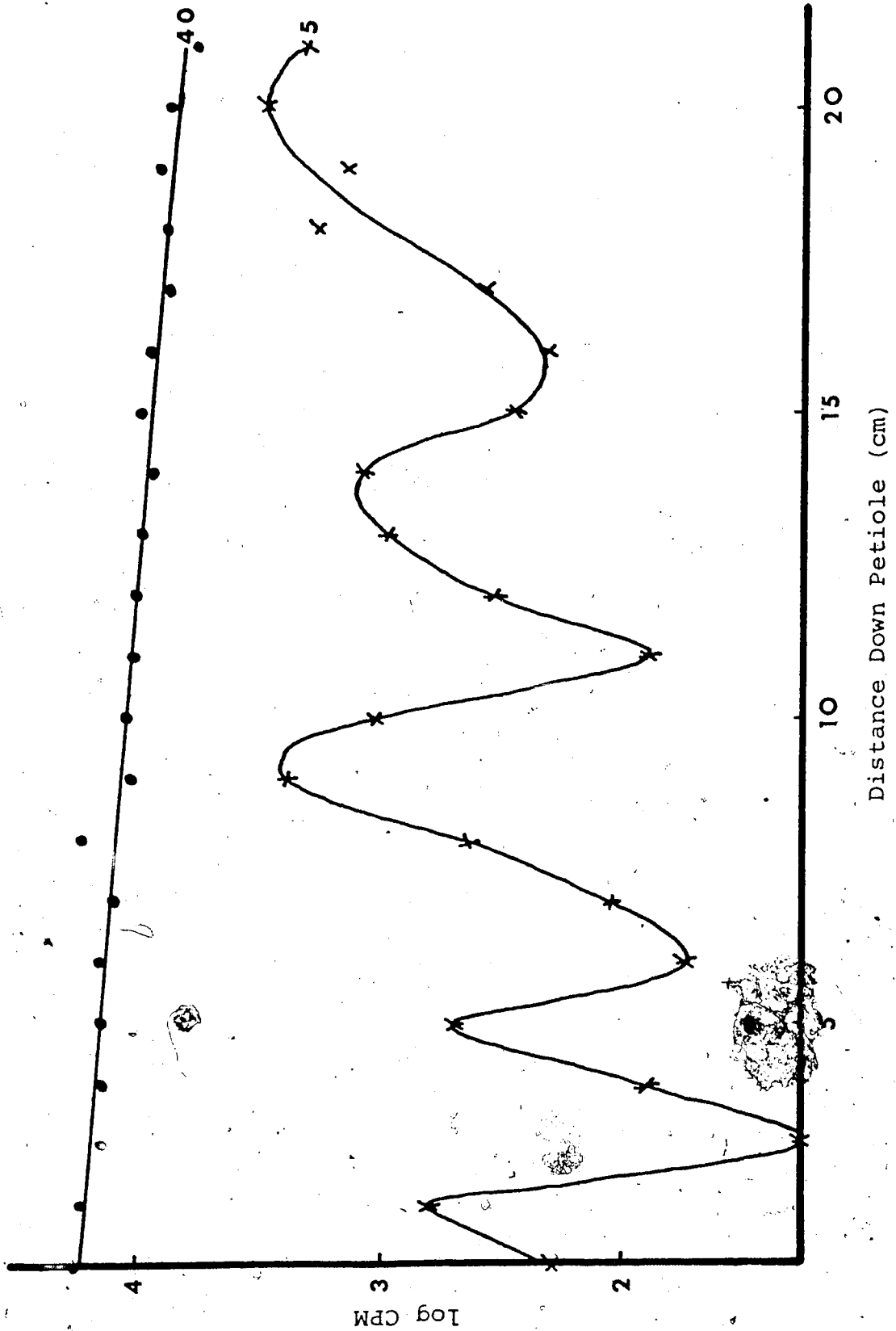
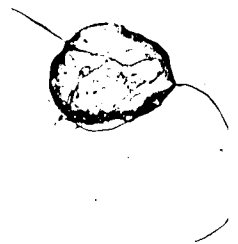
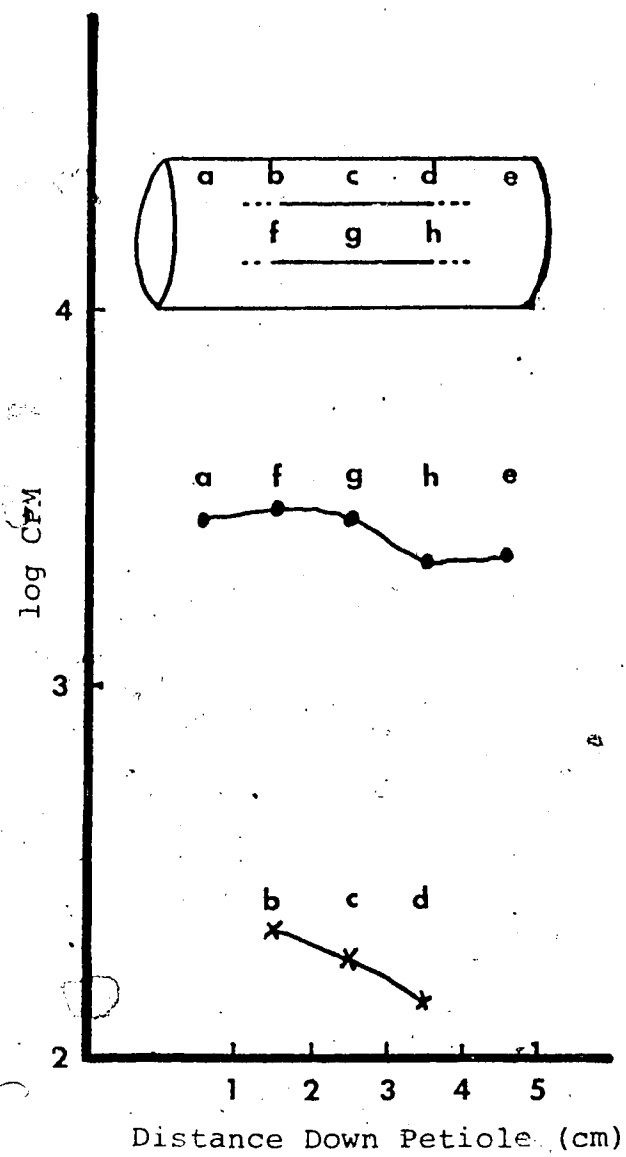


FIGURE 9. Profile of  $^{14}\text{C}$ -assimilates in a wounded petiole of sugar beet 40 min after a localized application of  $70.72 \text{ Ci } ^{14}\text{CO}_2$  to a region of lamina drained by vascular bundles running through an isolated wedge of petiole. *a* and *e* are unwounded regions of petiole, *b*, *c*, *d* are the remaining sectors of the wounded area of petiole, *f*, *g*, *h* are parts of the isolated wedge enclosing the bundles serving the tagged area of the lamina.





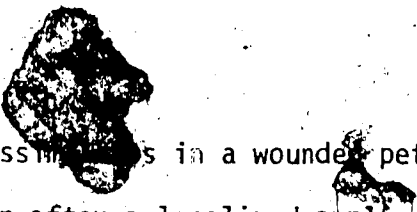


FIGURE 10. Profile of  $^{14}\text{C}$ -assimilates in a wounded petiole of sugar beet 40 min after a localized application of  $70.72 \mu\text{Ci } ^{14}\text{CO}_2$ .



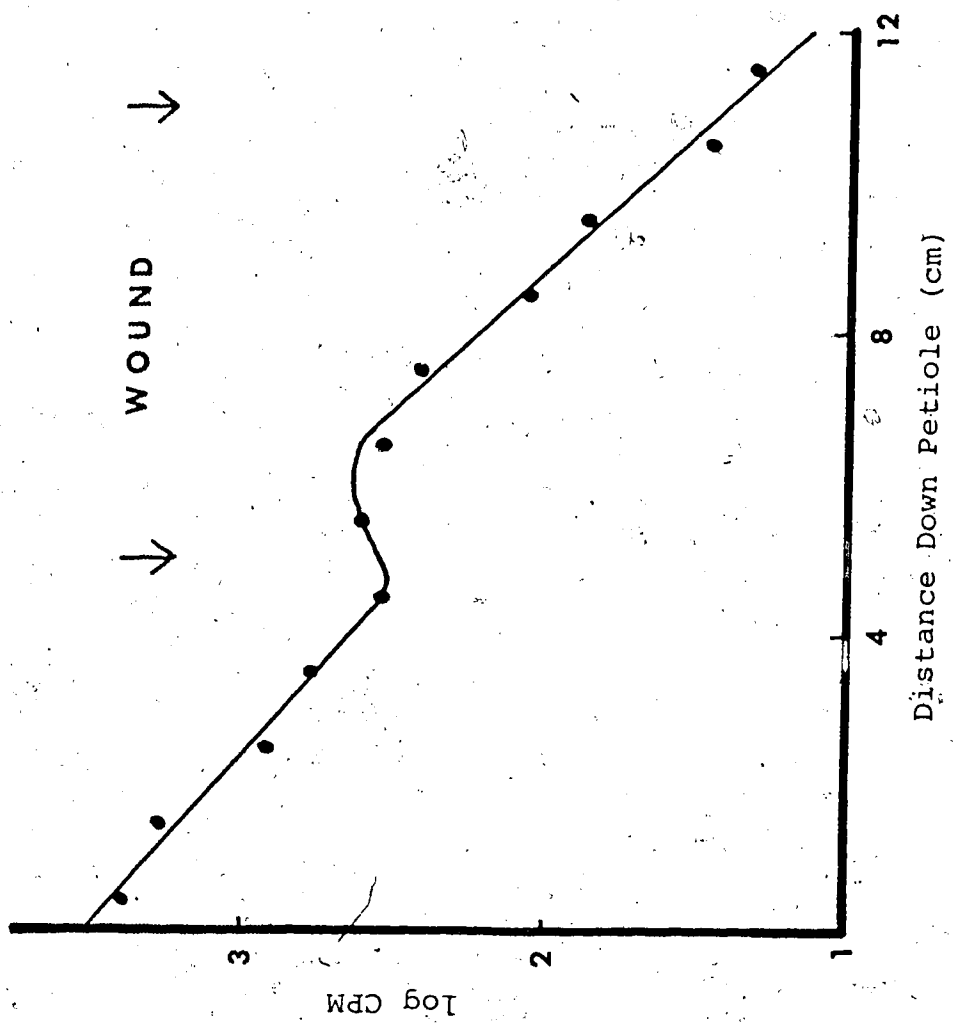


FIGURE 11. Forty-minute profiles of  $^{14}\text{C}$ -assimilates in detached leaves of *Heraclium* following whole leaf tagging with  $70.72 \text{ Ci } ^{14}\text{CO}_2$ . Points represent the profile of a control plant with its cut petiole in water. Crosses represent the profile of a test plant with its cut petiole in 20 mM EDTA.

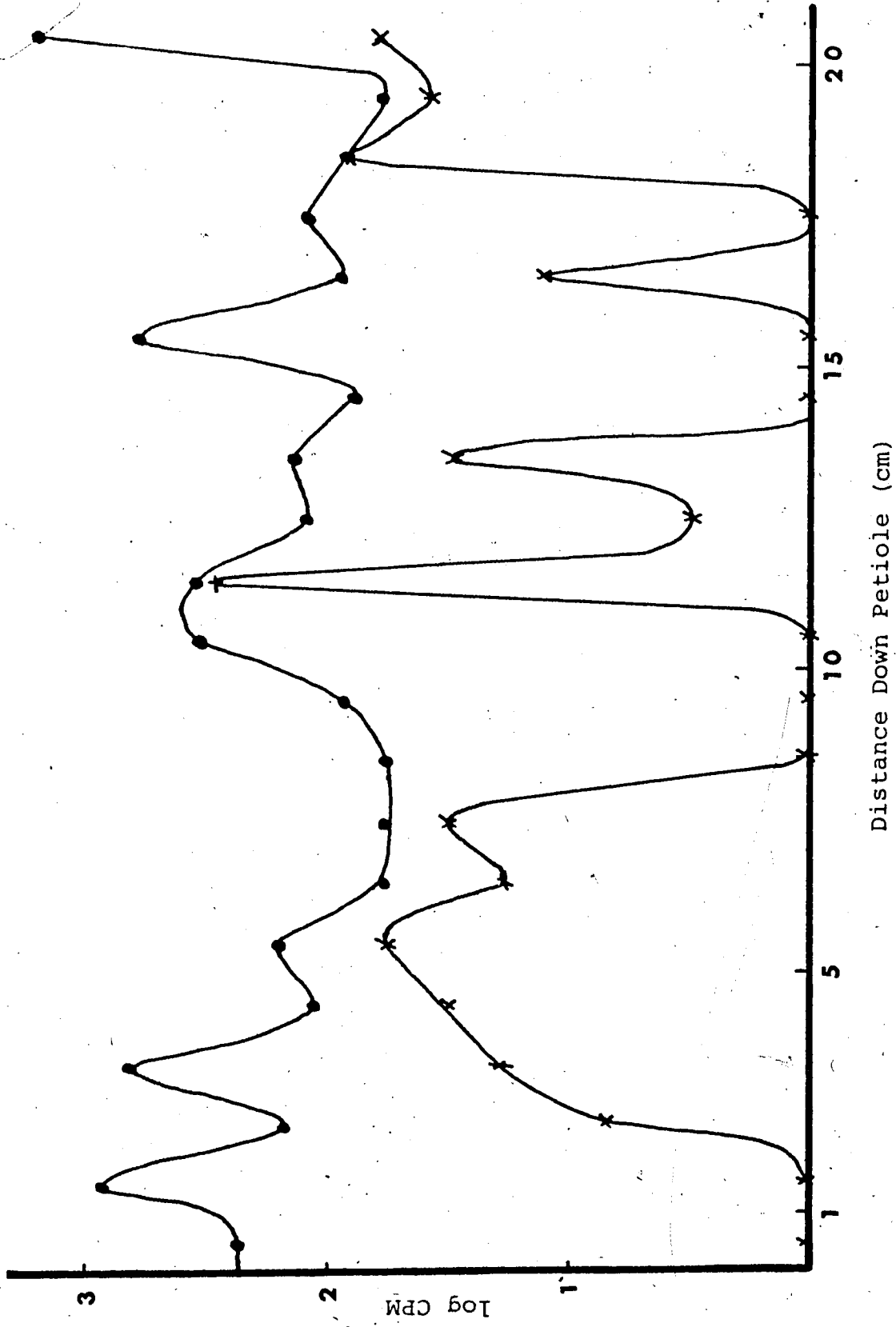


FIGURE 12. Localization of  $^{14}\text{C}$ -assimilates in the mid-point of a petiole of sugar beet following a localized application of  $^{14}\text{C}$  to the lamina and a 30-min translocation time. L indicates phloem containing labeled assimilates.

FIGURE 13. Localization of  $^{14}\text{C}$  assimilates in the mid-point of a petiole of *Helianthemum* following the application of  $^{14}\text{C}$  to the whole lamina and a 30-min translocation time. L indicates phloem containing labeled assimilates.

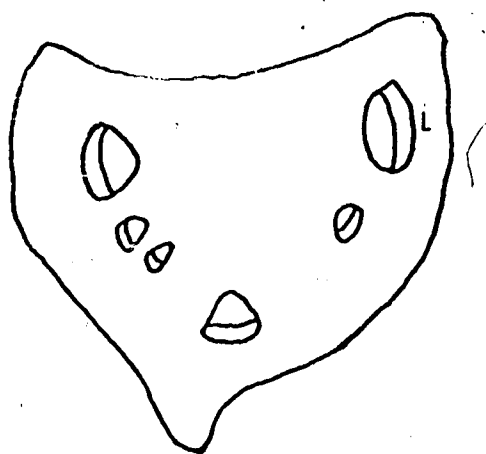


FIGURE 12

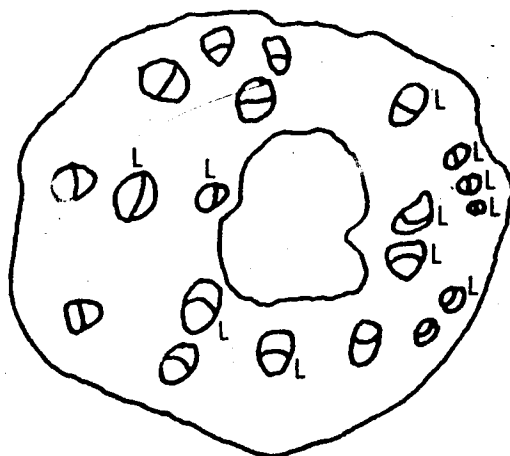


FIGURE 13

FIGURE 14. Autoradiograph of freeze-dried tissue showing the localization of  $^{14}\text{C}$  assimilates in a bundle of sugar beet following a localized application of  $^{14}\text{CO}_2$  to the lamina, and a 30-min translocation time.

FIGURE 15. Autoradiograph showing localization of  $^{14}\text{C}$ -assimilates in a bundle of sugar beet following a localized application of  $^{14}\text{CO}_2$  to the lamina.



FIGURE 14 .



FIGURE 15 .

FIGURE 16. Autoradiograph of unlabeled bundle from the same section shown in Fig. 14.

FIGURE 17. Autoradiograph of freeze substituted tissue showing the distribution of  $^{14}\text{C}$  assimilates in the remaining sector of a wounded petiole of *Heracleum* following a whole leaf application of  $^{14}\text{CO}_2$  and a 30-min translocation time.



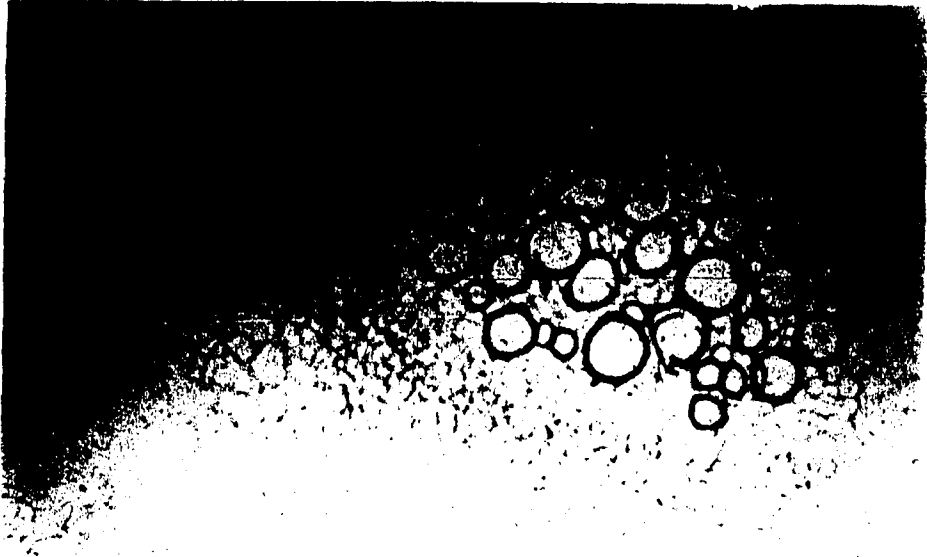


FIGURE 16.



FIGURE 17.

FIGURE 18. Autoradiograph showing distribution of  $^{14}\text{C}$  assimilates in the isolated wedge containing 6 bundles of a wounded petiole of *Heracleum* following a whole leaf application of  $^{14}\text{CO}_2$ .

FIGURE 19. Autoradiograph showing the distribution of  $^{14}\text{C}$  assimilates in a bundle in an isolated wedge of a wounded petiole of *Heracleum* following a whole leaf application of  $^{14}\text{CO}_2$ . (An enlargement of Fig. 18)

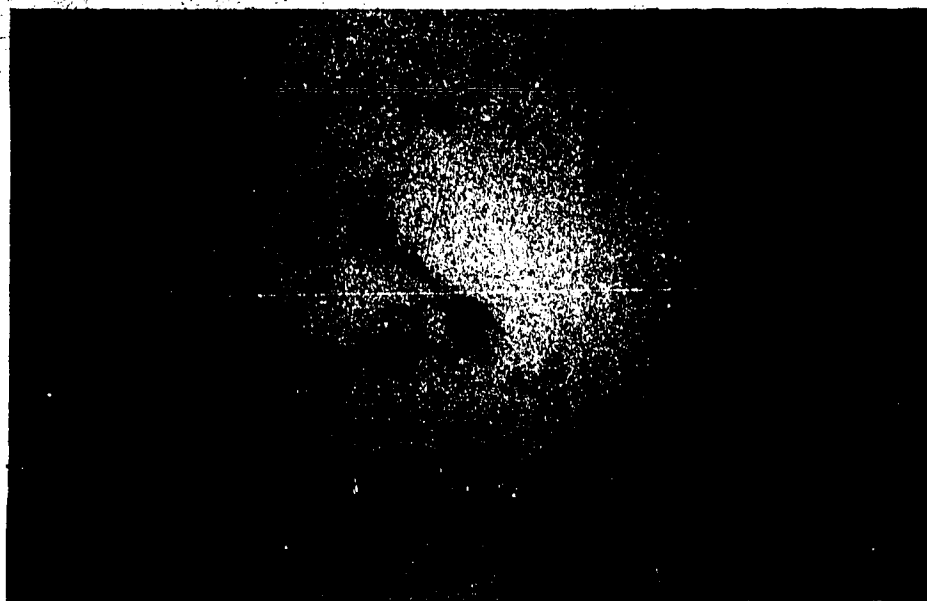


FIGURE 18.

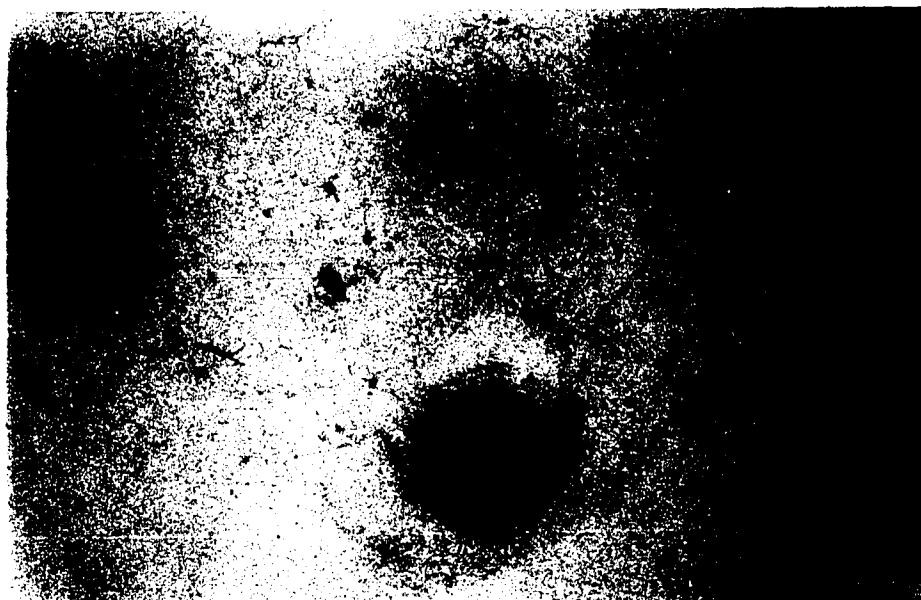


FIGURE 19.

FIGURE 20. Spectroradiometer data for the various light qualities used in the growth chamber. Energy values for the various colours as measured by an Eppley pyranometer were

White	$\cdot 1621 \times 10^{-2}$	cal cm <sup>-2</sup> min <sup>-1</sup>
Blue	$\cdot 040 \times 10^{-2}$	"
Red	$\cdot 160 \times 10^{-2}$	"
Green	$\cdot 038 \times 10^{-2}$	"

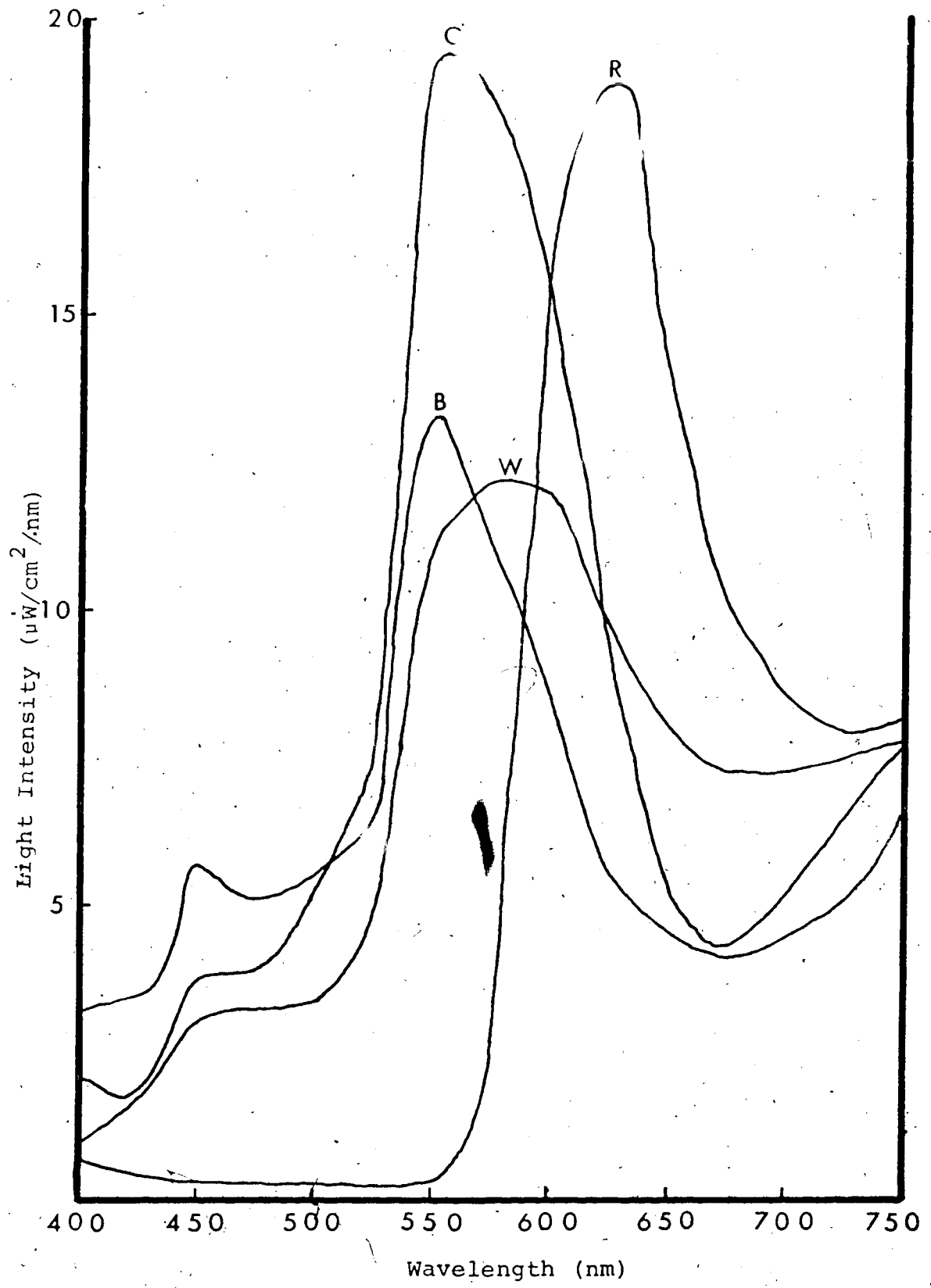


FIGURE 21. Net assimilation rate of an *Heracleum* leaflet measured on a leaf area basis. Points indicate fixation rates with increasing white light intensity. Crosses indicate spot readings with various light qualities. O = Orange, G = Green, R = Red, B = Blue, W = White

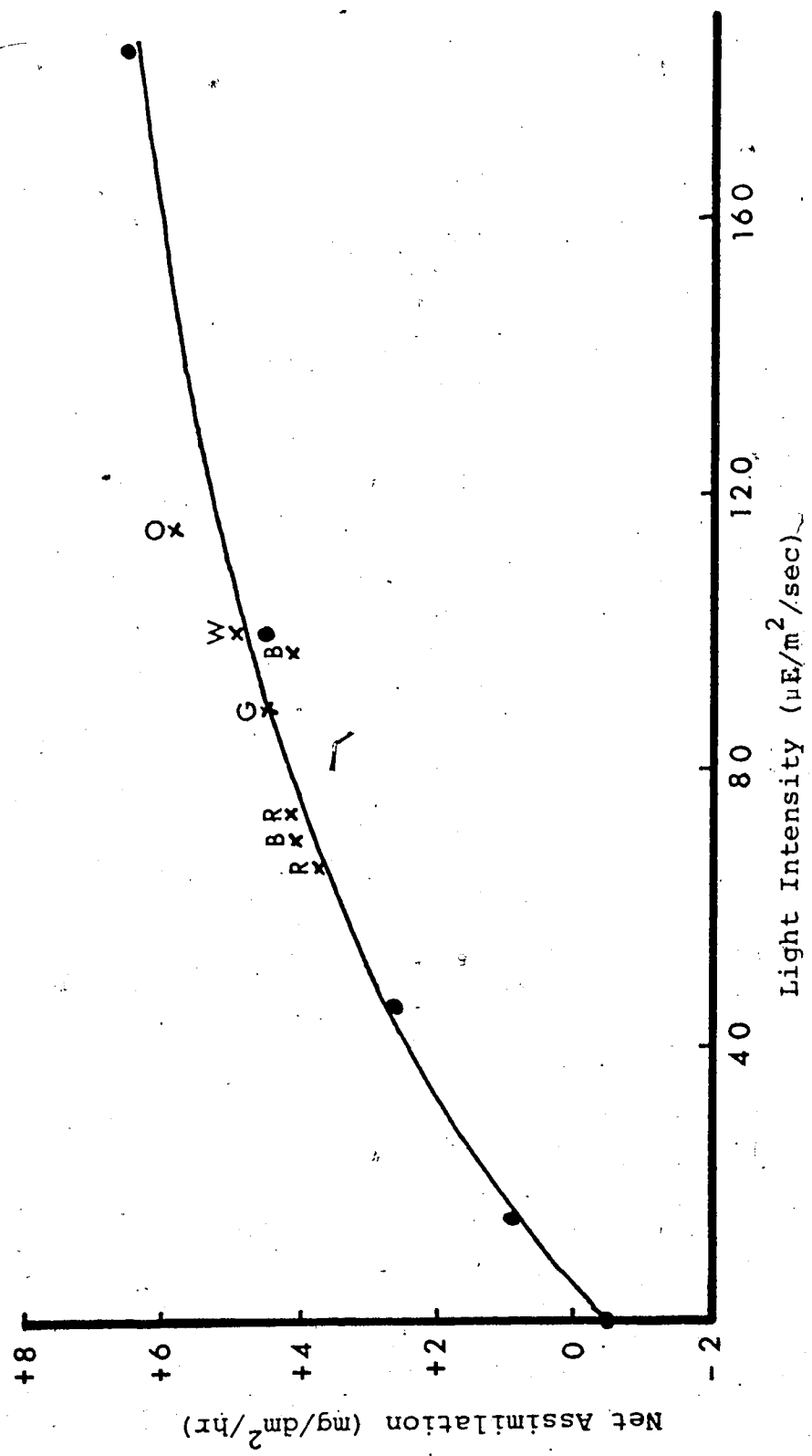


FIGURE 22. Similar data to Fig. 20 but expressed on a dry weight basis.



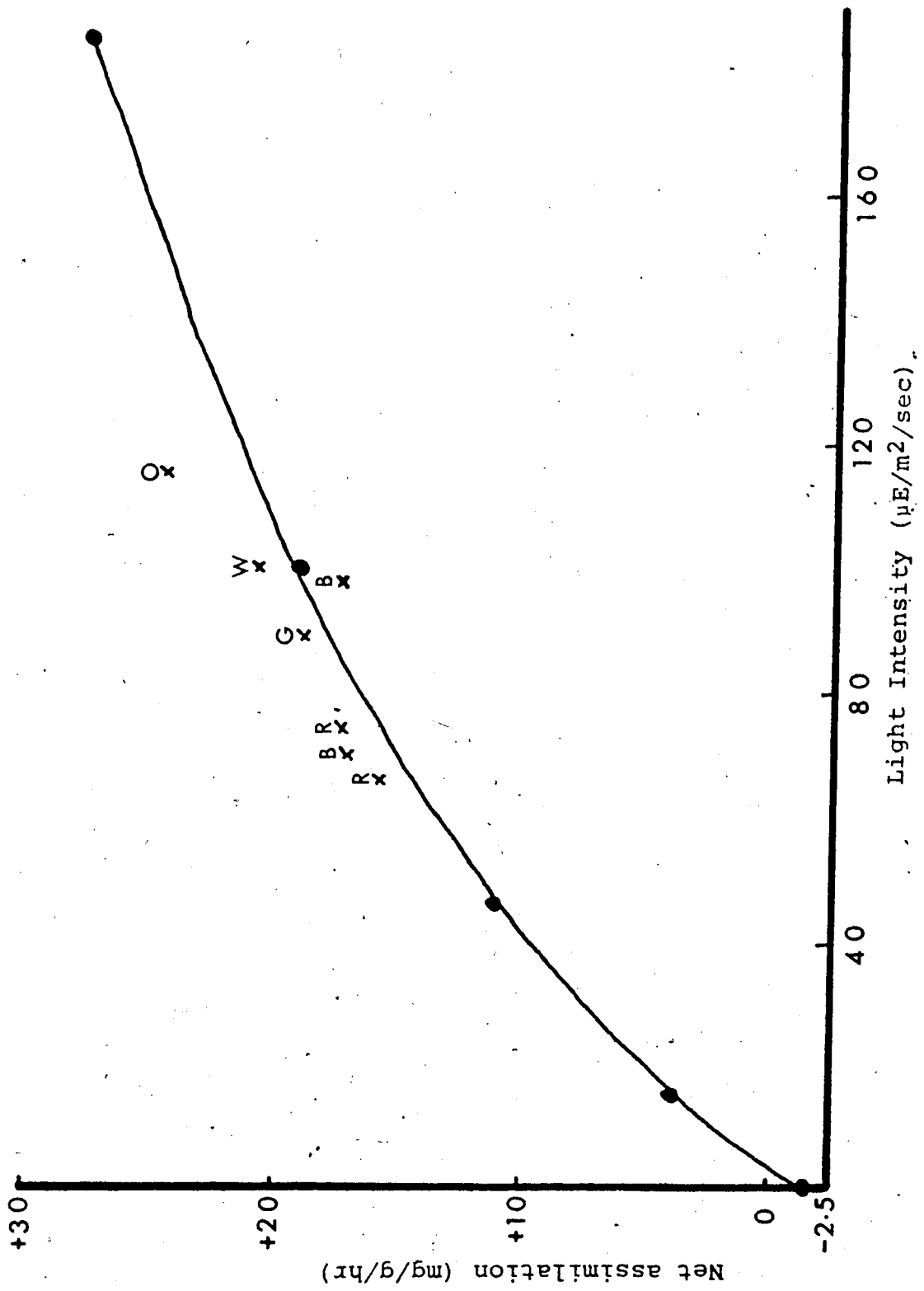


FIGURE 23. Profiles of  $^{14}\text{C}$ -assimilates in intact petioles of *Heracleum* 5 and 40 minutes after a localized application of 70.72 Ci  $^{14}\text{CO}_2$ . Experiments performed in Blue Light.

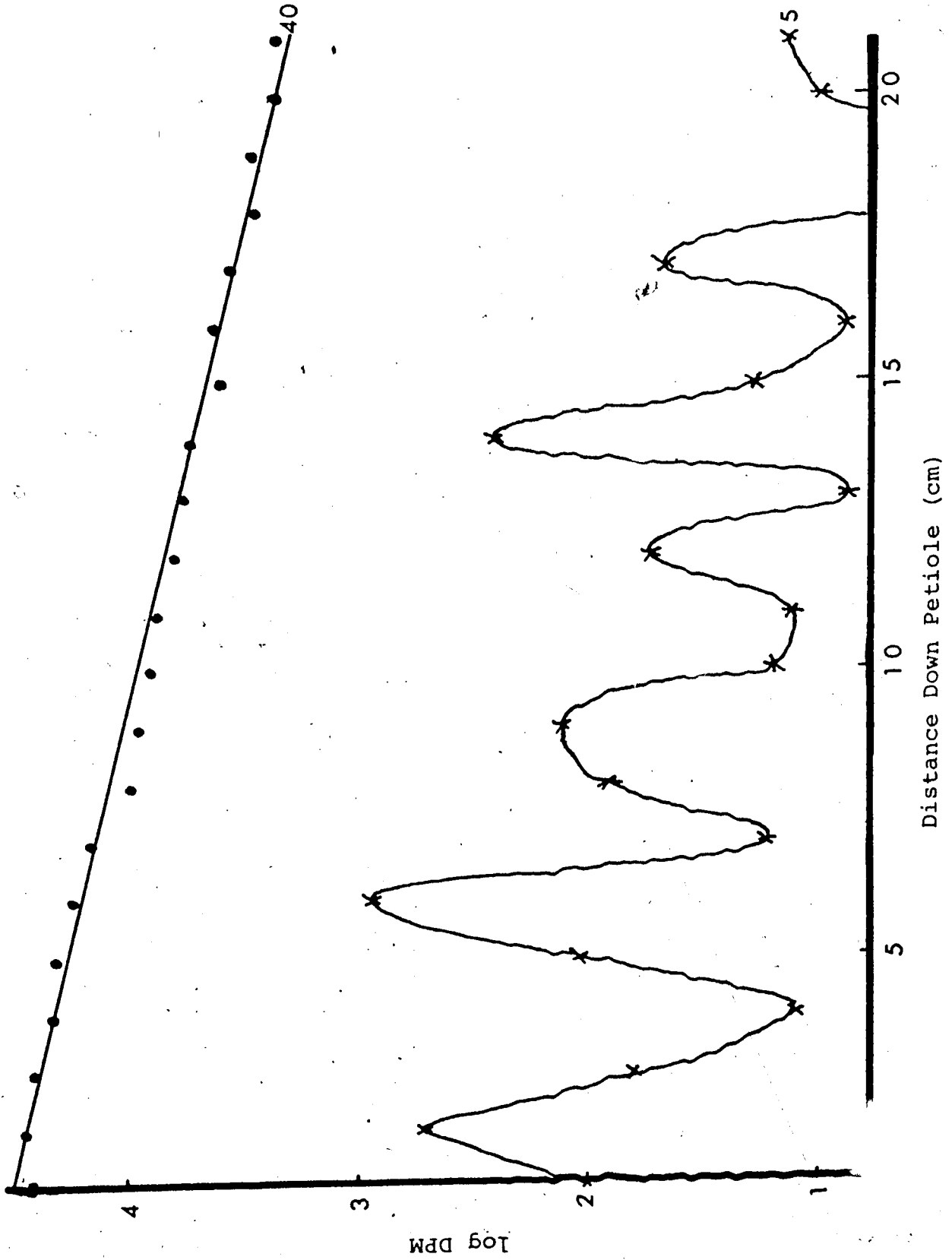
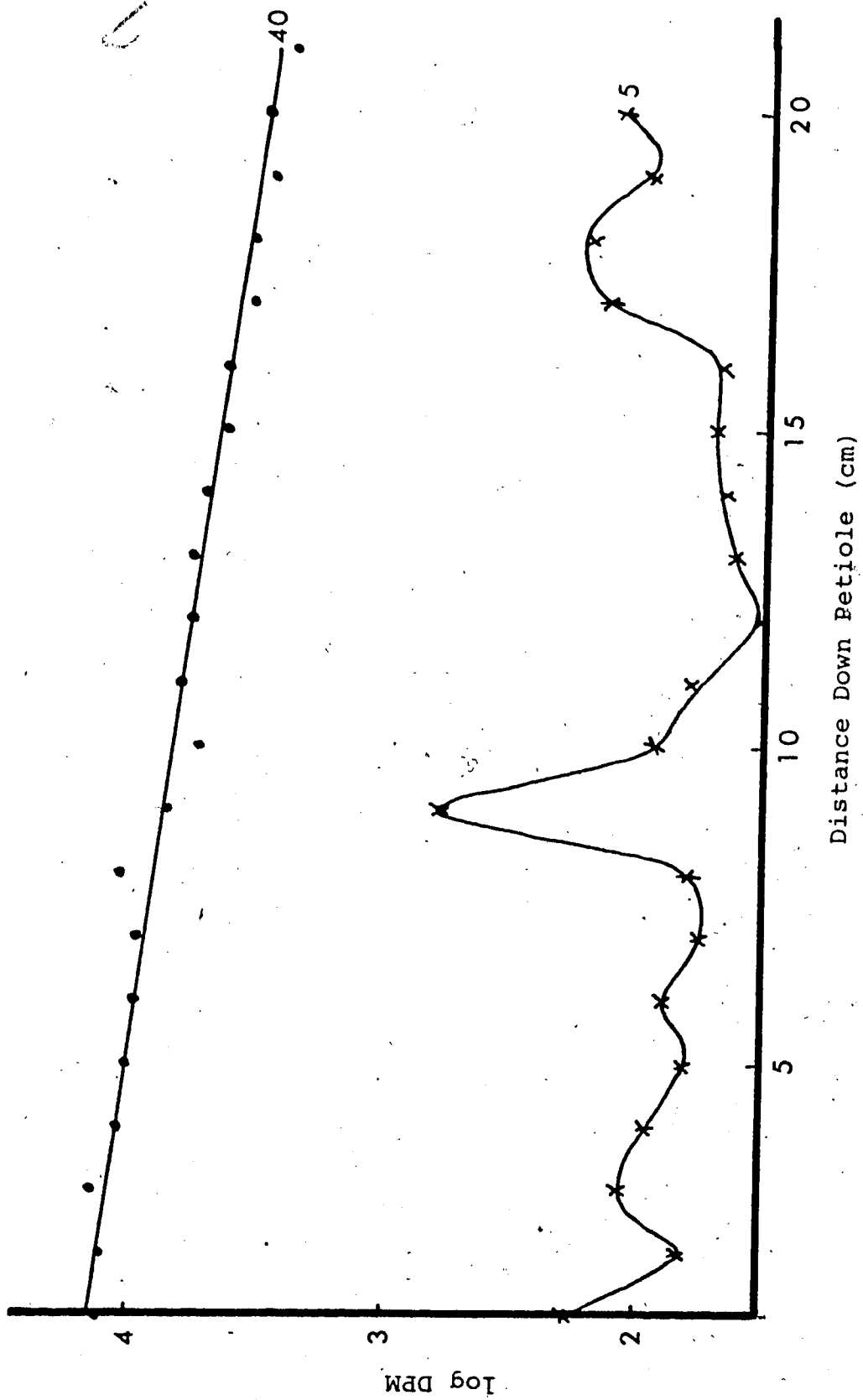


FIGURE 24. Data similar to that in Fig. 22 but obtained in Red light.

1



0

40

5

10

15

20

Log DPM

Distance Down Petiole (cm)

FIGURE 25. Data similar to that in Fig. 22 but obtained in green light.

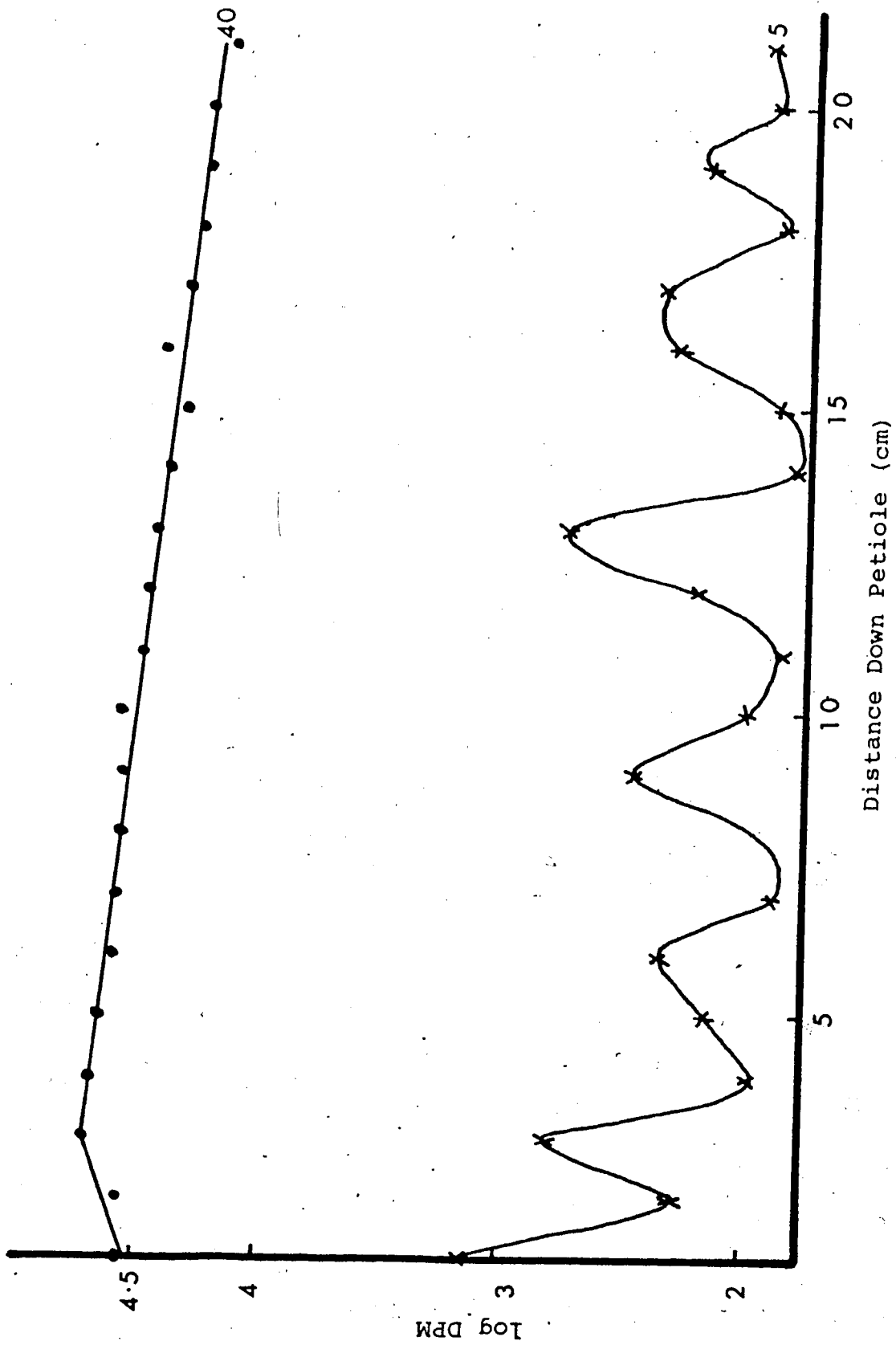


FIGURE 26. Transport profiles of externally applied 6,6'-<sup>3</sup>H-sucrose along isolated loops with increased times of 15 to 45 min. The loops were laid directly on a glass plate with a series of water drops alongside to keep humidity high. F = width of application of labeled compound.



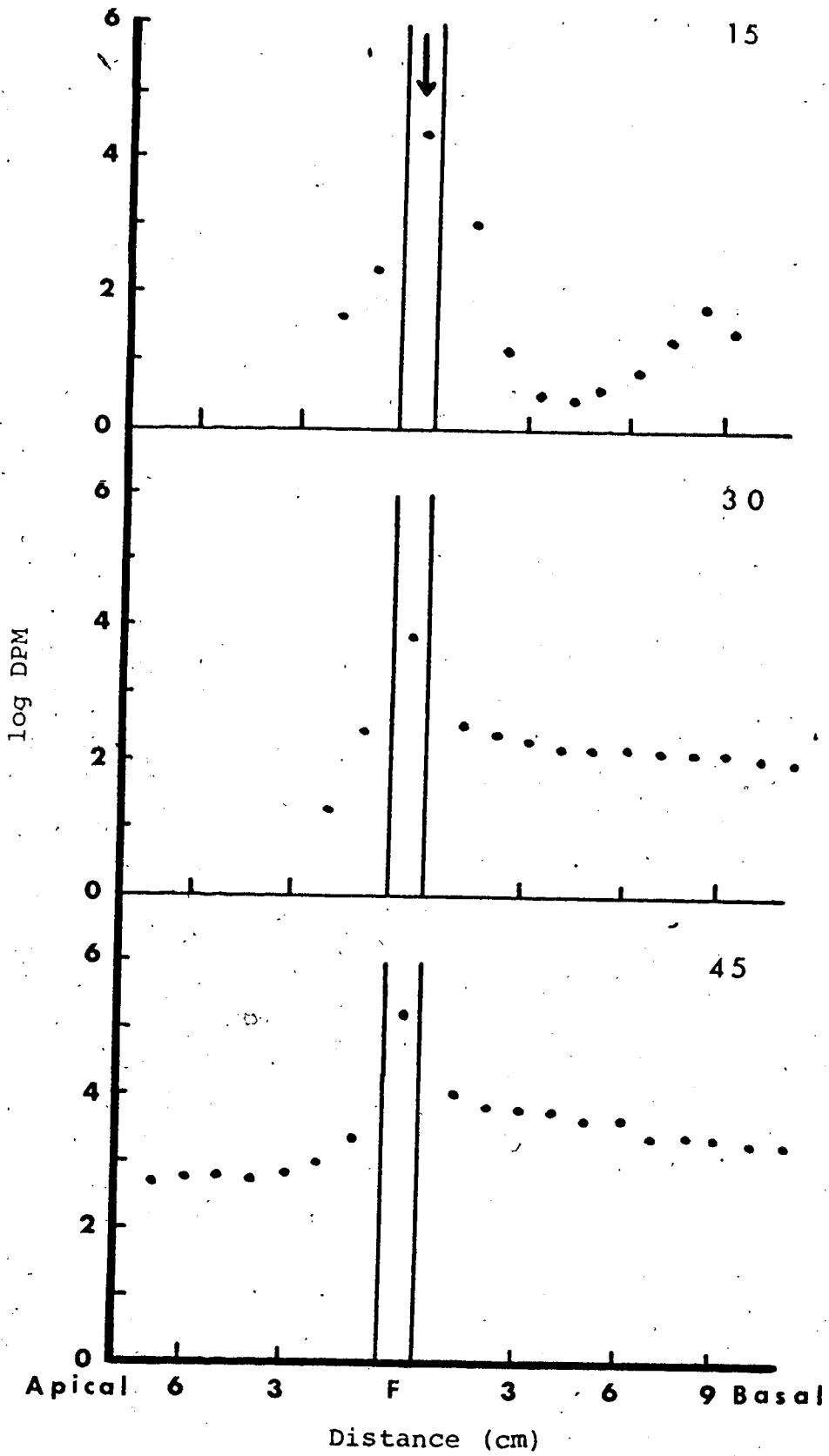


FIGURE 27. Distribution of tritium in a phloem loop after a two-minute exposure to 20 mCi tritiated water at the center of the loop. The loop is supported on wet filter paper.

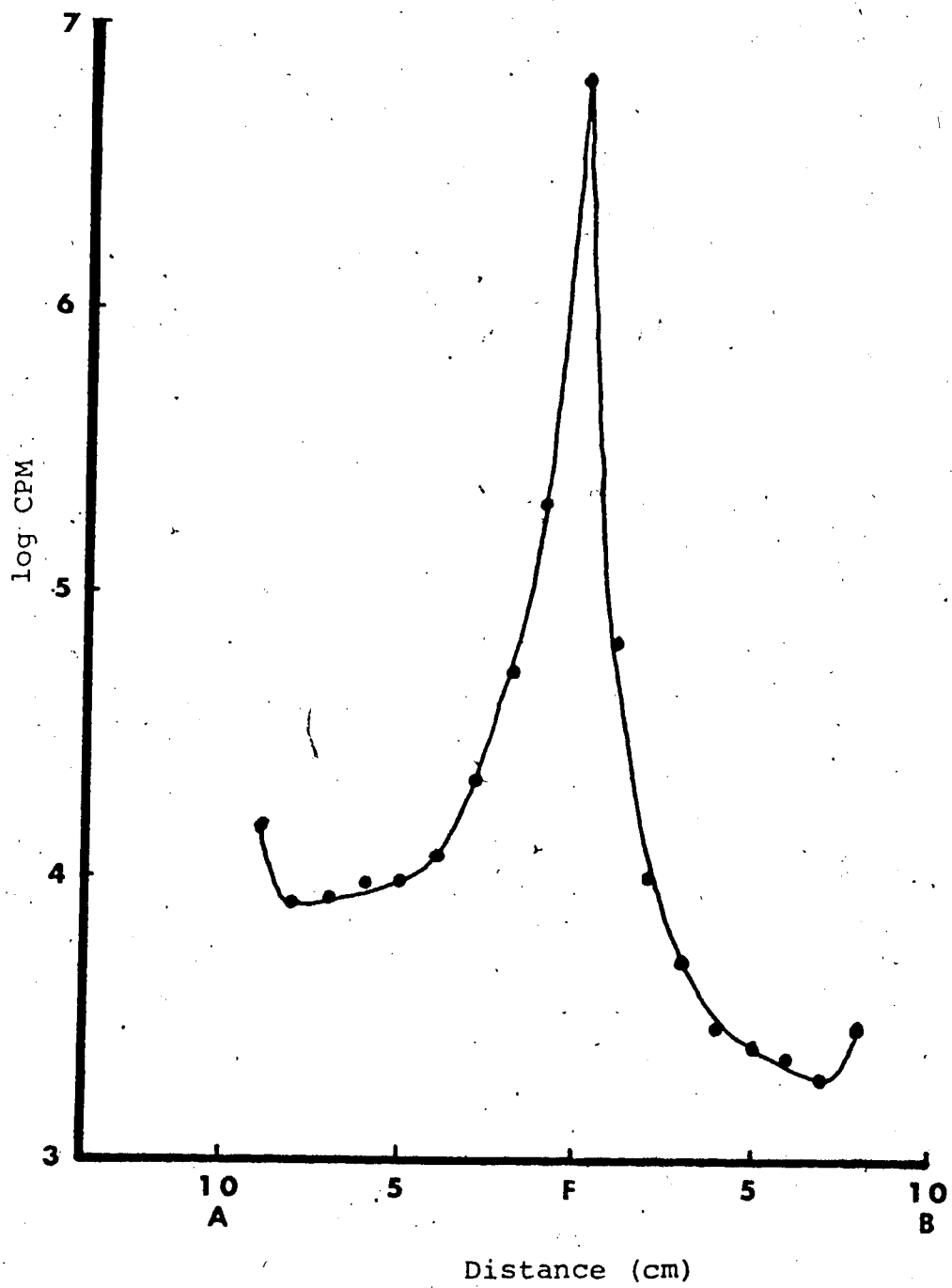


FIGURE 28. Distribution of tritium in a phloem loop after a 15-min exposure to 1.0 mCi of tritiated water at the center of the loop. The apical portion (A) of the loop is supported on wet filter paper, the basal portion (B) is supported on filter paper soaked with 0.5 M sucrose.

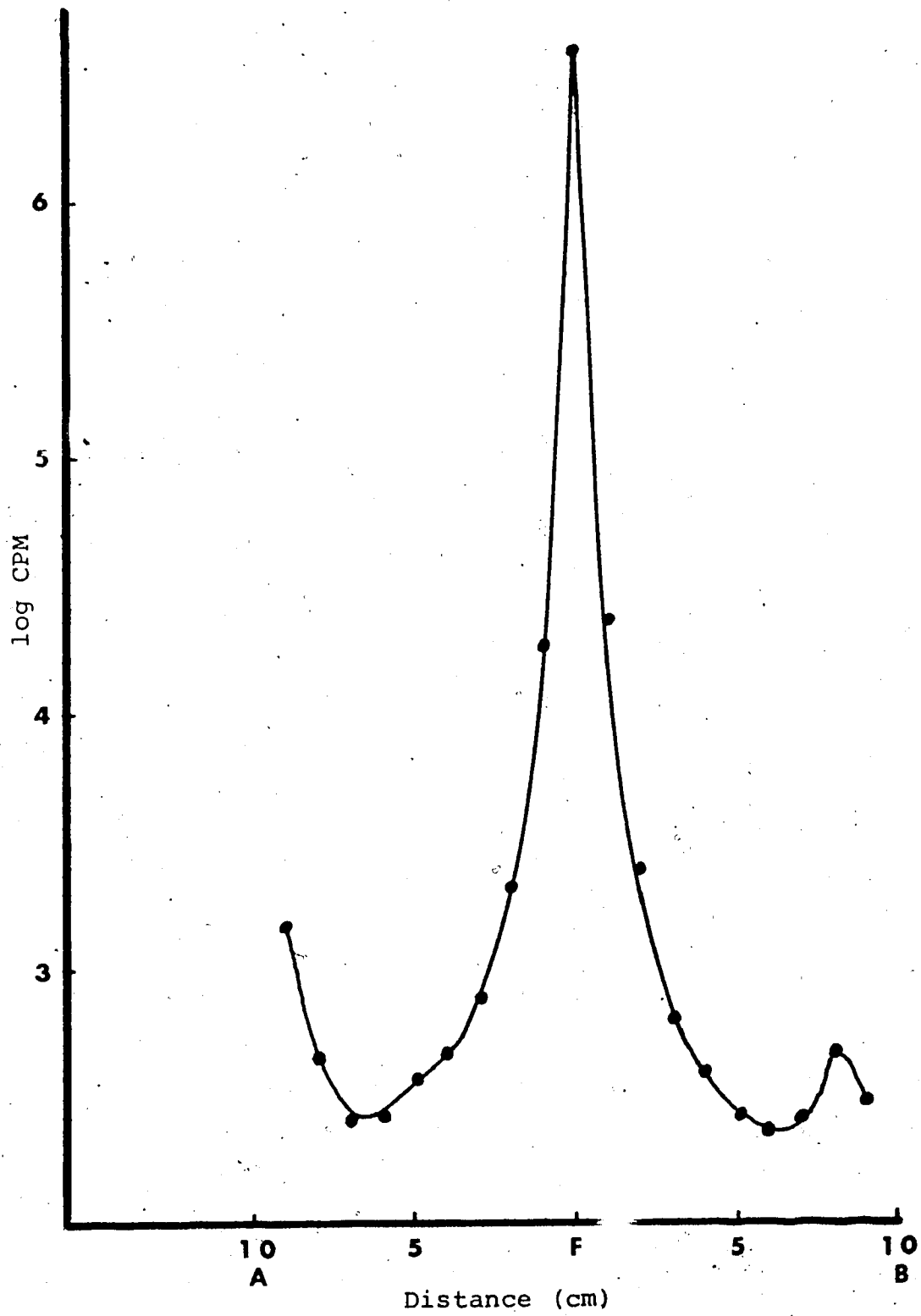


FIGURE 29. Distribution of  $^{14}\text{C}$  in a phloem loop after a 15 min exposure of (U- $^{14}\text{C}$ ) sucrose at the center of the loop. The apical portion (A) of the loop is supported on filter paper soaked with 0.5 M sucrose, the basal portion (B) on wet filter paper.

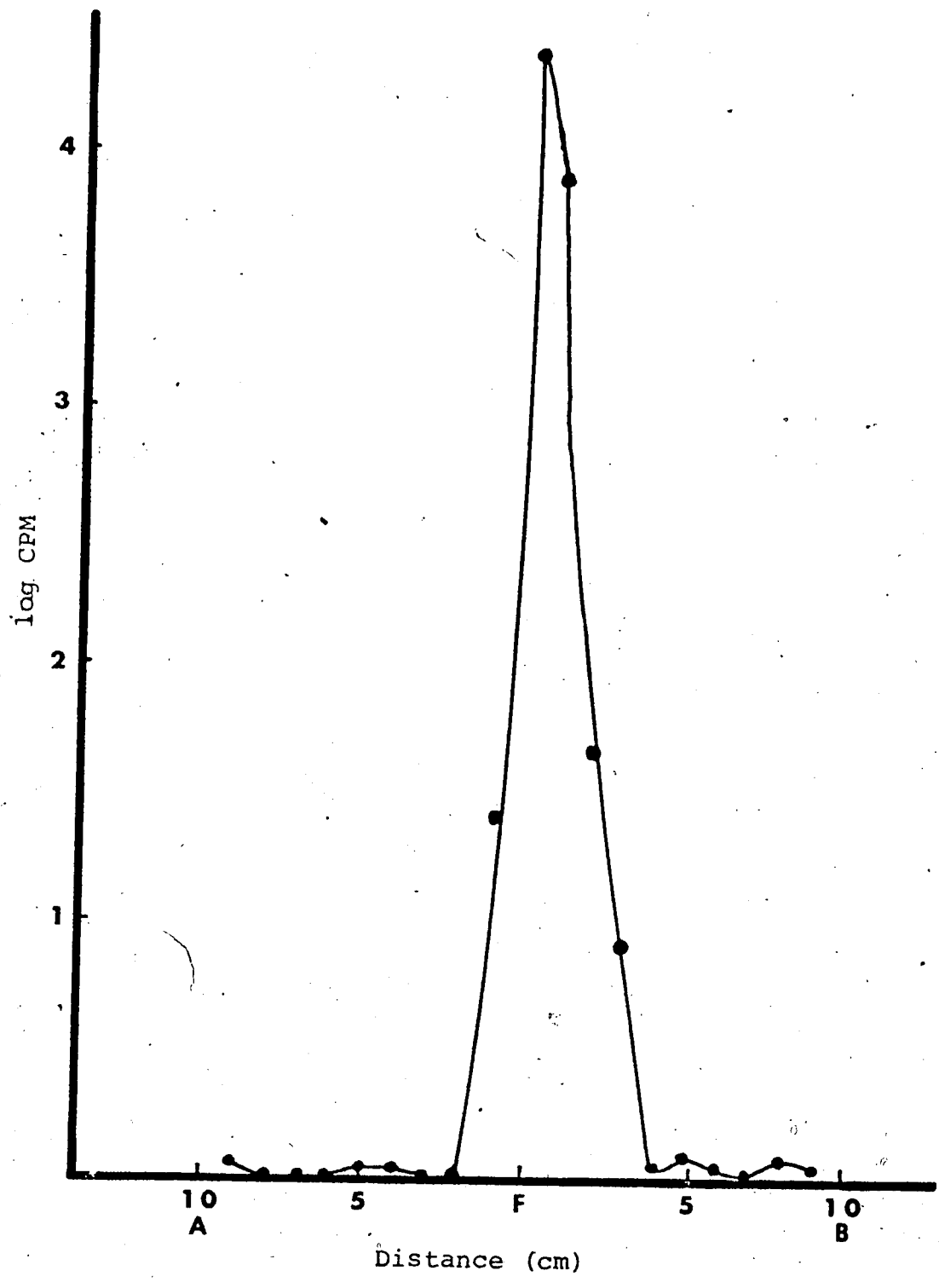


FIGURE 30. Distribution of tritium in a phloem loop after a 15-min exposure to 1.0 mCi tritiated water followed by a 15-min pulse chase with unlabeled water.



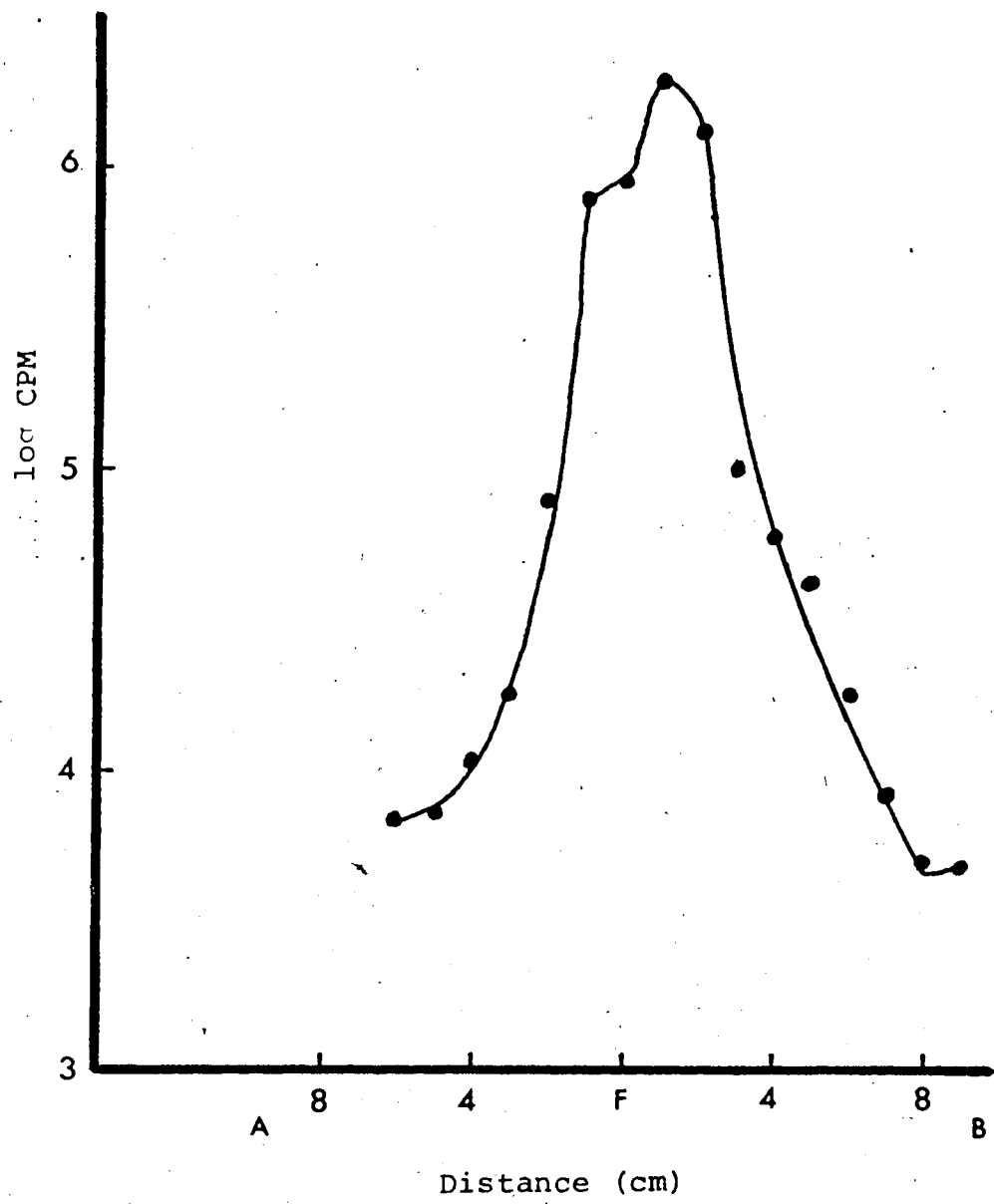


FIGURE 31. Distribution of tritium in phloem tissue and wet filter paper following a 15 min exposure of the center of the loop to 1.0 Ci of tritiated water.

1. Fed phloem
2. Apical phloem
3. Basal phloem
4. Proximal apical support paper
5. Distal apical support paper
6. Proximal basal support paper
7. Distal basal support paper

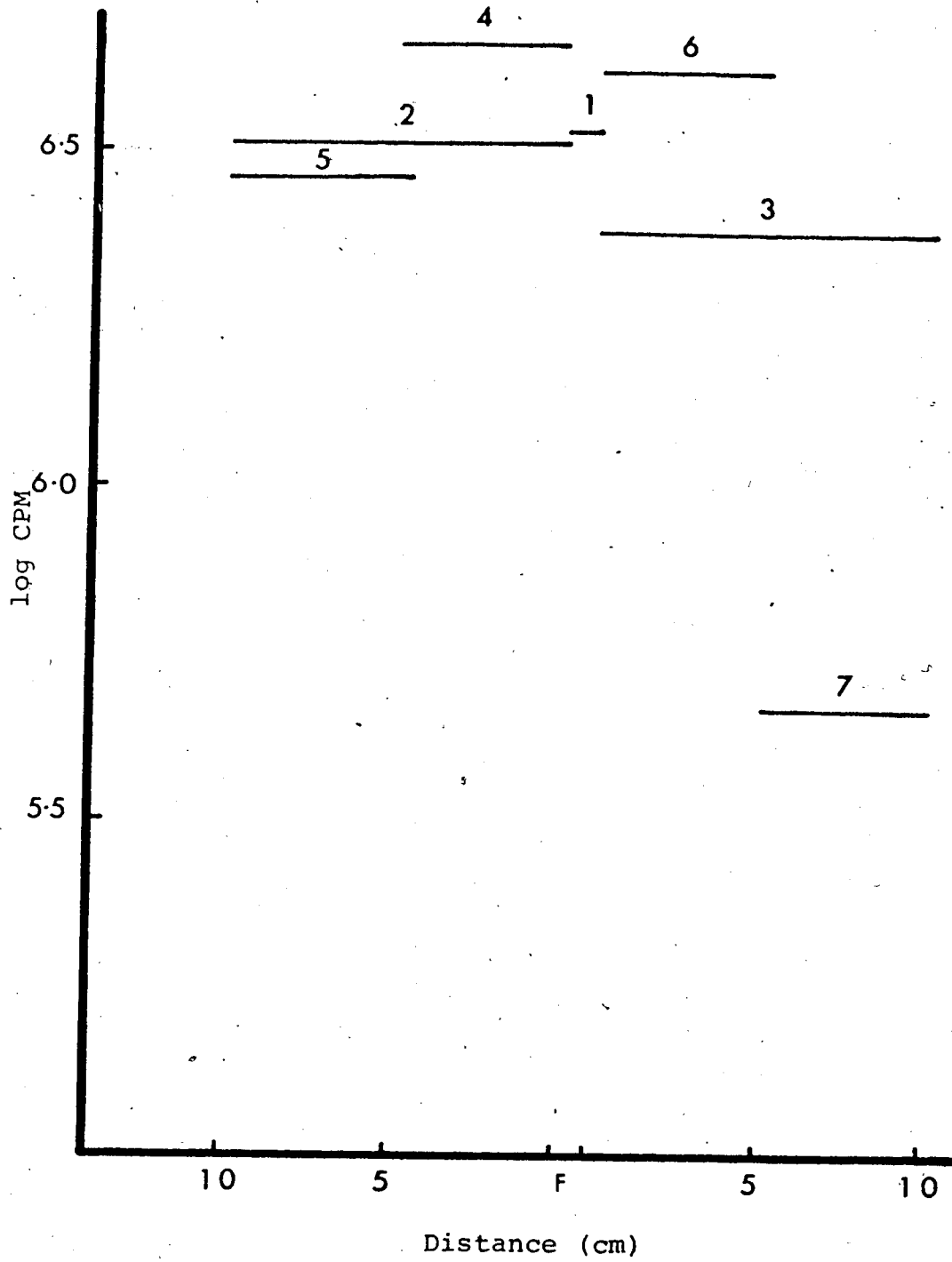


FIGURE 32. Efflux of tritium into unlabeled water from an apical piece of phloem loop following exposure of the center of the loop to 1.0 mCi of tritiated water for 15 min. Points represent initial efflux rate. Crosses represent efflux from an initial compartment following correction of the data for the efflux from the second compartment.

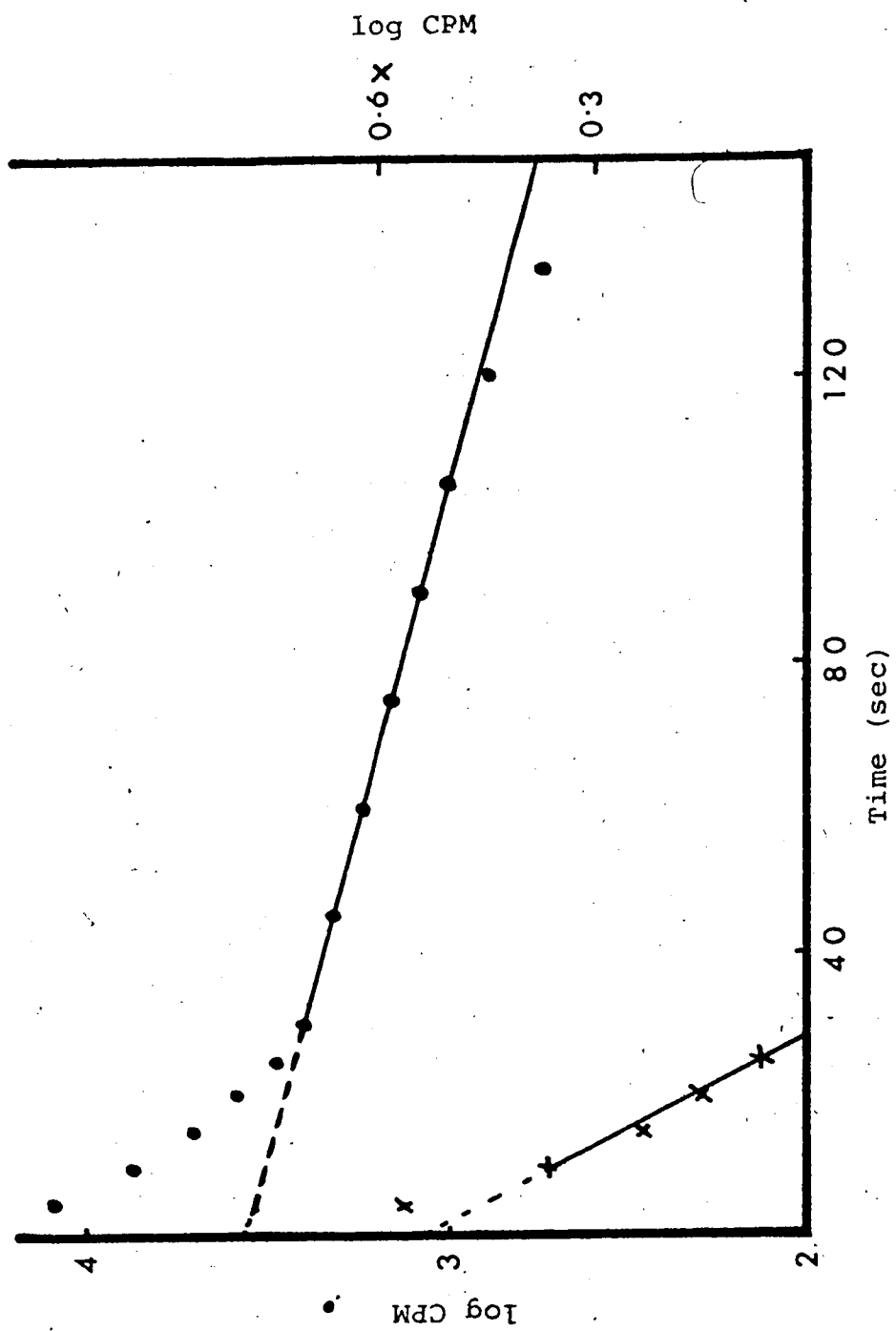


FIGURE 33. Profile of tritium in a polyester thread soaked in water and laid on wet filter paper following a 15-minute exposure of the center of the loop to 1.0 mCi tritiated water.

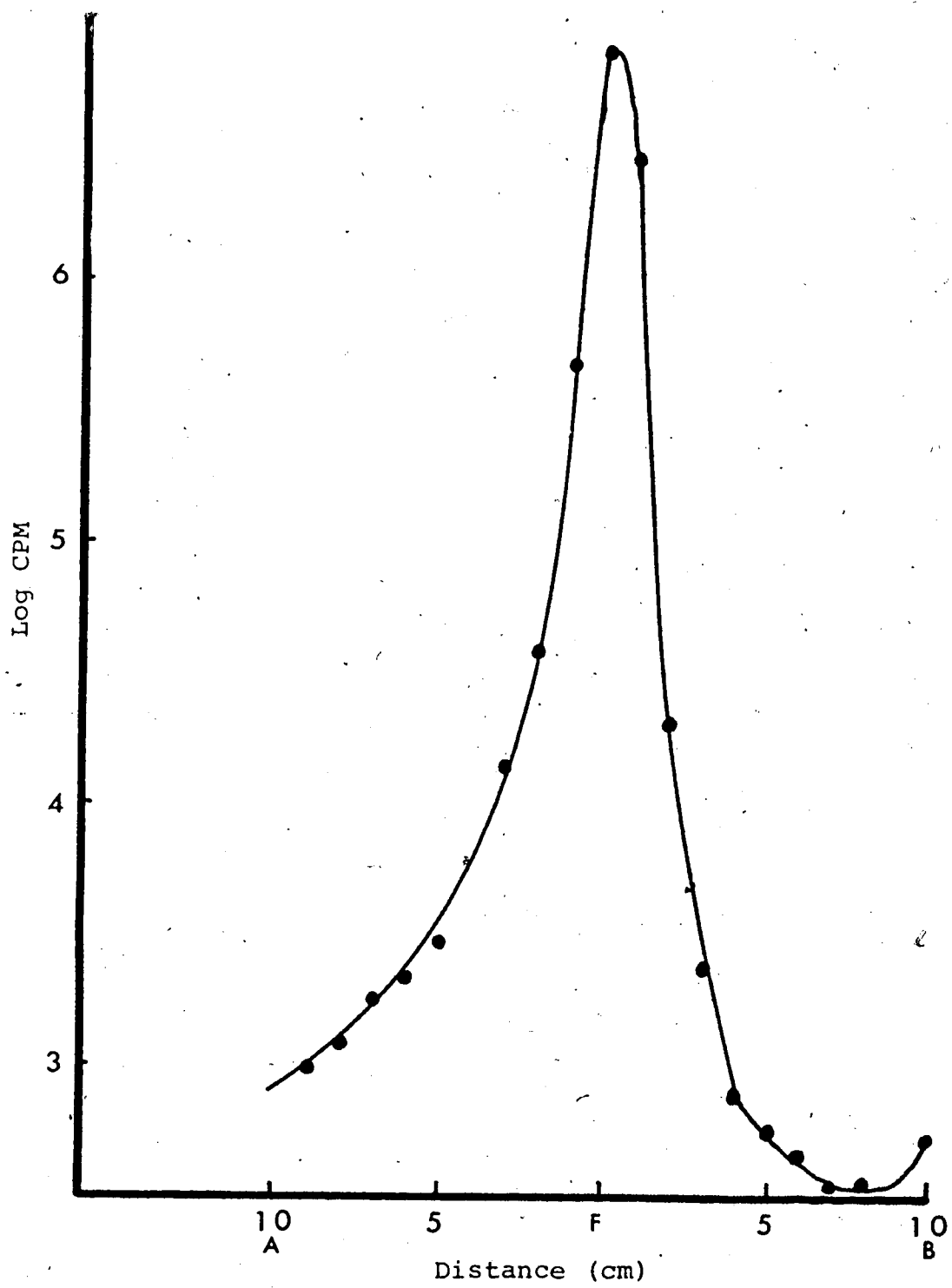


FIGURE 34. Water potential ( $\Psi$ ) and combined osmotic and matric potentials ( $\Psi_{\pi}\Psi_{\tau}$ ) of a *Heracleum* leaf measured over a 24 hr period.



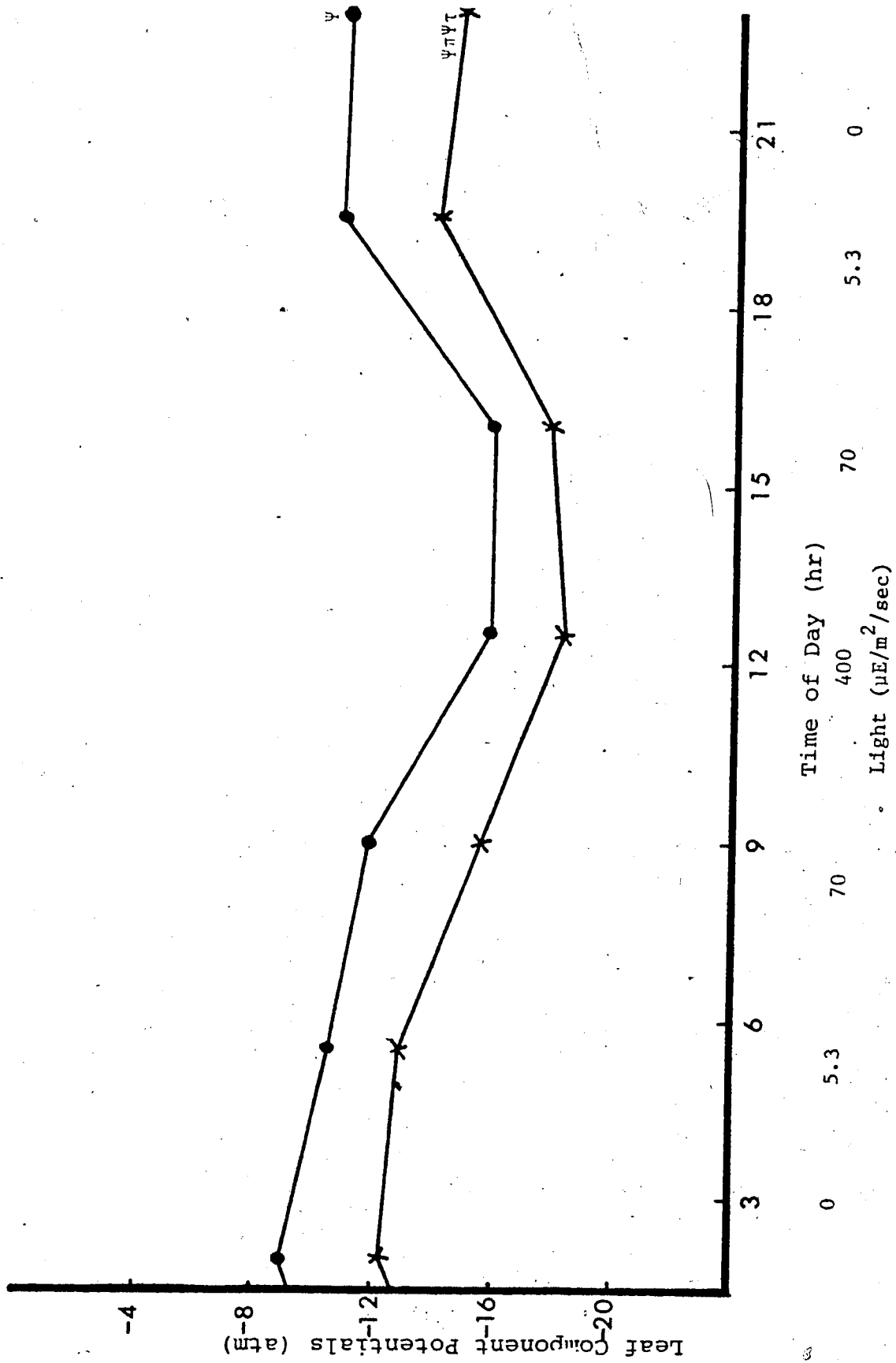
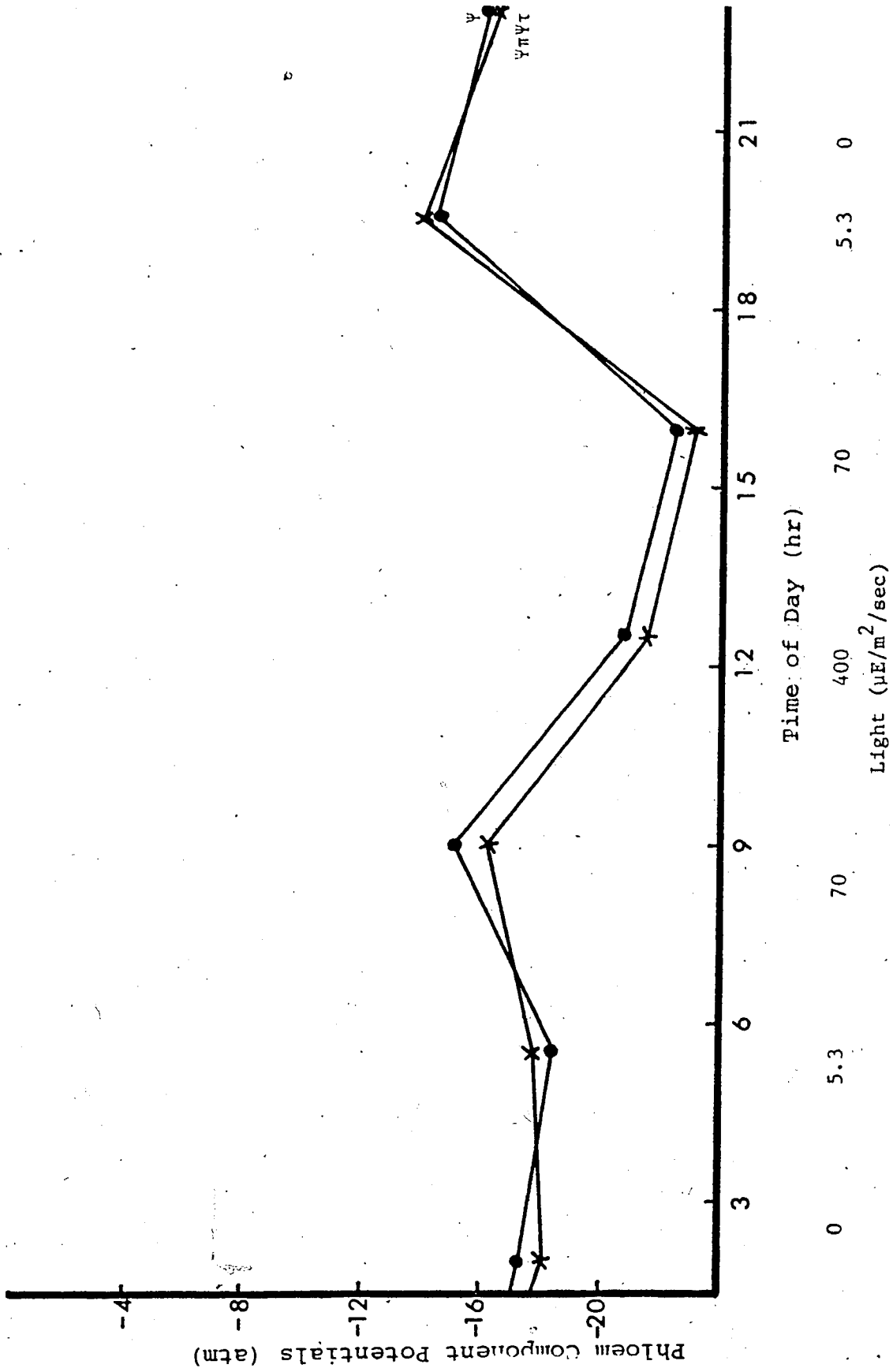


FIGURE 35. Water potentials and combined osmotic and matric potentials of an *Heracleum* phloem loop measured over a 24 hr period.



6

$\psi$   
 $\psi_{\pi\psi\tau}$

FIGURE 36. Difference in water potential and combined osmotic and matric potentials between *Heracleum* leaves and phloem loops measured over a 24 hr period.

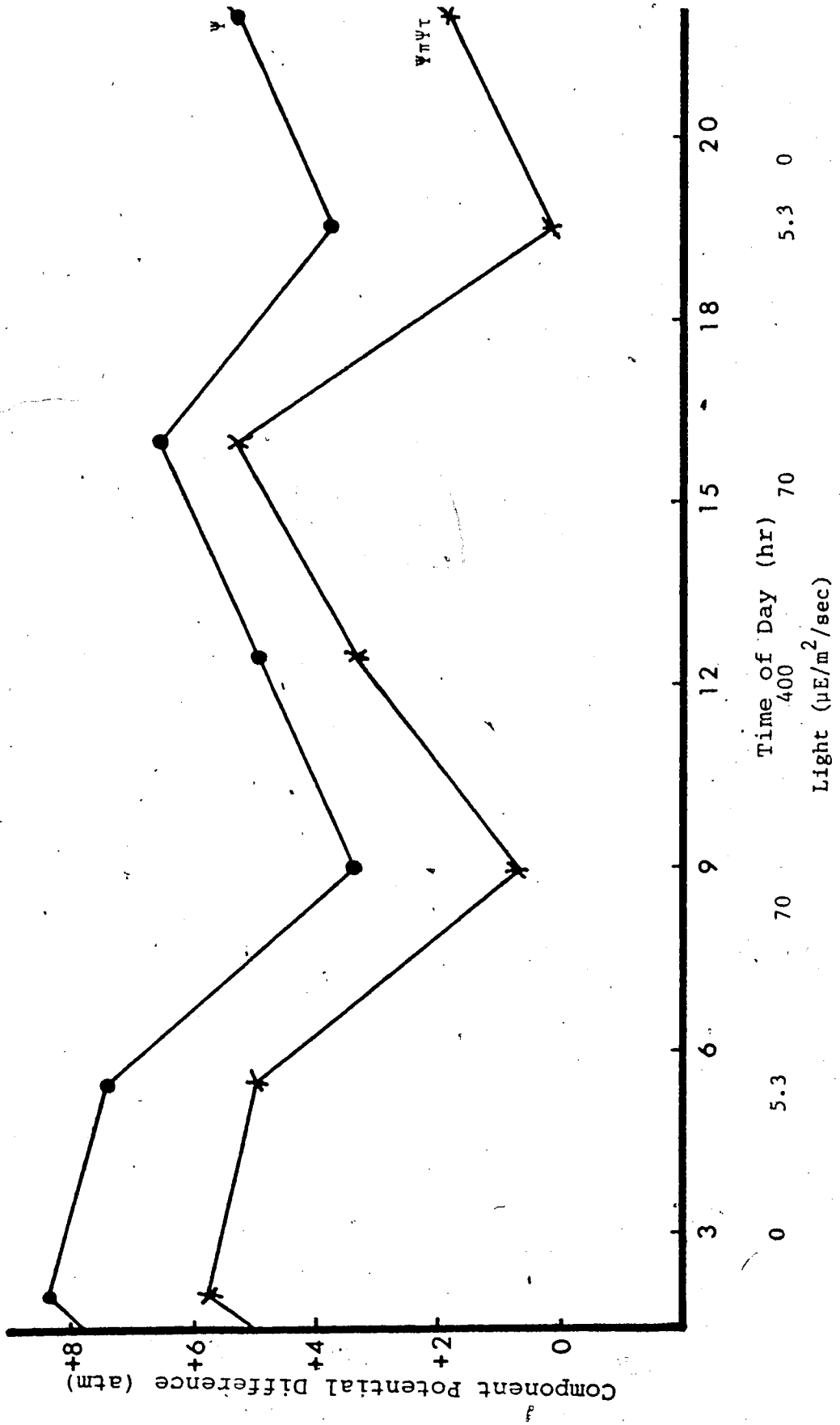
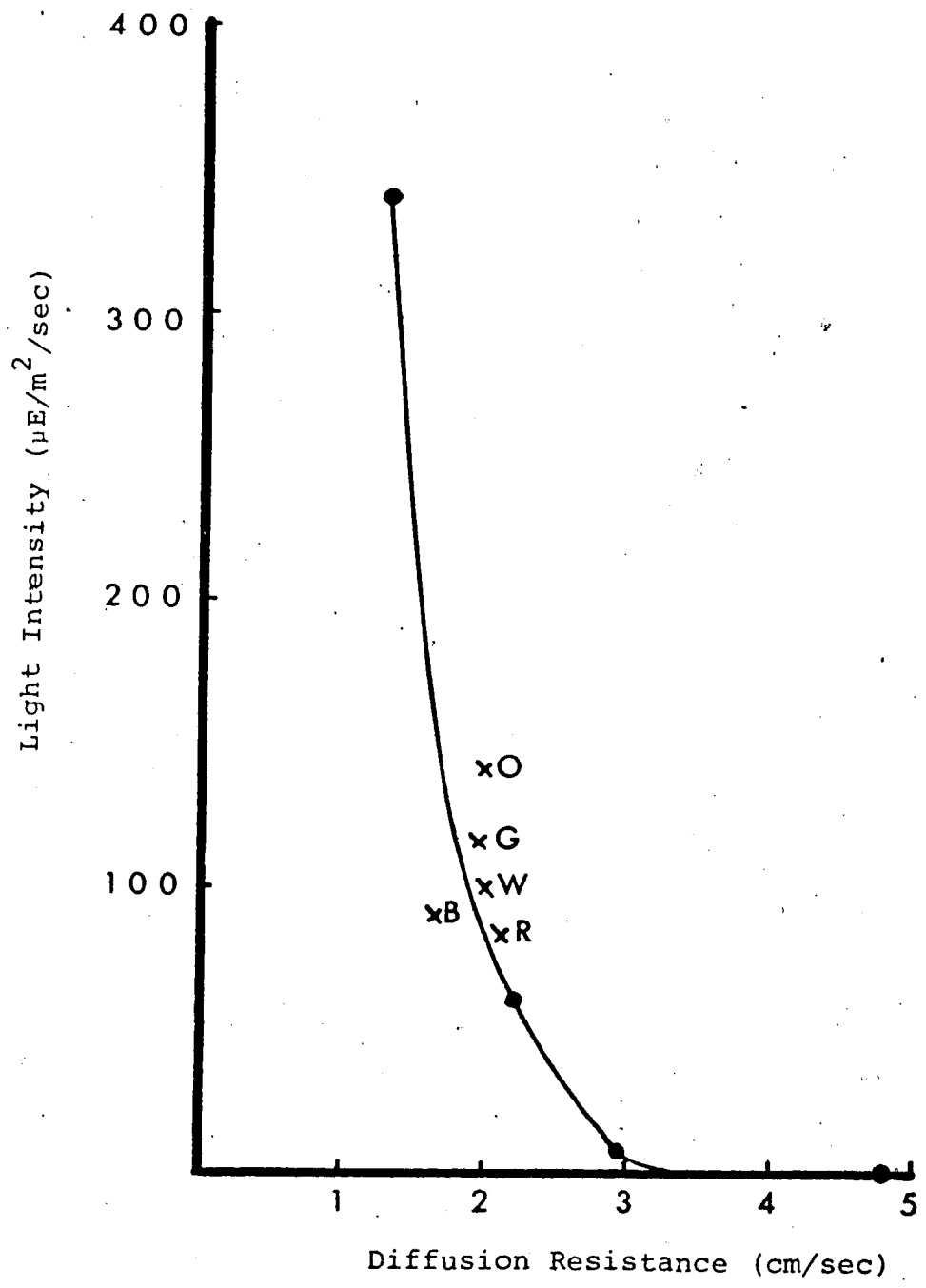


FIGURE 37. Diffusion resistance of the lower surface of *Heracleum* leaves. Points represent resistance with various intensities of white light. Crosses represent spot readings under various coloured lights.



## APPENDIX A

## Chemical and Instrument Suppliers

$\text{NaH}^{14}\text{CO}_3$ : Atomic Energy of Canada, Commercial Products, P.O.  
Box 93, Ottawa.

Radioisotope Labelled Compounds: New England Nuclear (Canada)  
11475 Cote de Liesse, Dorval.

Amino Acid Analyser: Beckman Instruments, 10538 - 124 St.,  
Edmonton.

Cab-0-Sil M5: Van Walters and Rorers (BC) Ltd., 2625 Skeena St.  
Vancouver.

Growth Chamber: Environmental Growth Chambers, P.O. Box 407,  
Chagrin Falls, Ohio.

Eppley Pyranometer: Eppley Laboratories Inc., Newport, R.I.

Hastings Air Meter: Hastings-Raydirt, P.O. Box 1275 Hampton,  
Virginia 23361, U.S.A.

Honeywell Recorder: Honeywell Ltd. 14830 - 119 Ave., Edmonton.

Lambda Meters and Sensors: Lambda Instruments Co. Inc., 2933  
North 36 St., Lincoln, Nebraska, 68504.

Leitz Microscope: Walter A. Carveth Ltd., 1367 Richards St.,  
Vancouver 2.

Maihak Unor 2 Infra-red Gas Analyser: Bendix Process Instru-  
ments Div., Drawer 477, Ronceverte, West Virginia 34990.

Unilux II Scintillation Spectrometer: Nuclear Chicago Corp.,  
7511 - 104 St., Edmonton.

Spectroradiometer: Instrumentation Specialities Co., Lincoln,  
Nebraska.



Terostat VII: Terason Werk, GmbH6900, P.O. Box 1720, Heidelberg 1, West Germany.

Tissuemat: Canadian Laboratory Supplies Ltd., 10989 - 124 St., Edmonton.

Wescor Psychrometer: Wescor Inc., 459 South Main St., Logan, Utah 84321.

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