

THE INFLUENCE OF APHID COLONIES ON THE
MOVEMENT OF SOME PESTICIDE MOLECULES
AND INORGANIC IONS IN BEAN PLANTS.

by

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ABSTRACT

The distribution of inorganic tracers, ^{32}P - phosphate and ^{35}S - sulphate in bean plants, and the influence of Aphis fabae colonies on the movement of the isotopes was determined, either when the radio-label was added to the nutrient solution or applied to the surface of aphid-free leaves. The effect of the aphids was studied in relation to different factors such as the position of the colonies on the plants, translocation period and time of establishment of the colonies.

Following root uptake, the distribution and accumulation of ^{14}C - phorate in the leaves was determined by liquid scintillation counting and autoradiography.

The levels of uptake and excretion of phorate and metabolites by Aphis fabae and Acyrtosiphon pisum were compared and correlated with the quantities of labelled insecticide present in the infested leaves after root uptake.

The amounts of solvent and water soluble metabolites of phorate taken up by A. pisum colonies established prior to the addition of the insecticide to the nutrient solution were compared with the quantities ingested by similar colonies caged at the time of treatment.

The effect of aphid colonies on the movement of leaf applied phorate was assessed when aphid sinks were established at different positions in relation to the source of phorate.

On the basis of the data obtained, the influence of aphids on the movement of inorganic tracers and insecticide molecules is discussed in relation to the feeding behaviour of aphids and other factors affecting the movement of chemicals in plants.

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INTRODUCTION

The movement of chemicals in plants

The importance of systemically distributed chemicals in plant protection has focused a great deal of attention on the mechanisms of uptake and distribution of exogenous compounds.

According to Crafts and Crisp (1971) and Crisp (1972), the symplast-apoplast concept described by Munch (1930) provides a useful terminology and a mechanism for describing the movement of chemical compounds in plants. The symplast is defined as the total continuum of all the living protoplasm of a plant, where the sieve tubes of the phloem constitute its functional phase; while the apoplast consists of the total non-living continuum that surrounds and contains the symplast. The xylem may be considered as a specialized phase of the apoplast adapted to the rapid, long distance transport of substances absorbed by the roots. Movement through the symplast on the other hand, occurs with the assimilate stream, in a source to sink pattern; the source being the areas where photosynthesis is occurring, predominantly the leaves, while the sinks consist of the active growing parts such as roots, shoots and fruits. Movement from source to sink takes place as a flow along a gradient of hydrostatic pressure, developed

osmotically (Ford and Peel, 1966; Crafts, 1967a). It is now generally agreed that rapid movement of assimilate takes place as a mass flow through the sieve tubes of the phloem (Crafts and Crisp, 1971), though the mechanism of transport is still to be clarified. The movement of foreign compounds in plants and the critical factors which govern their systemic transport are also not completely understood, despite the large amount of research on this subject.

The uptake and distribution of herbicides and plant growth regulators however has provided some useful answers to problems in translocation. Considerable attention has been paid to the movement of these compounds (see for example Foy, 1961; Greenham, 1962; Yamaguchi, 1965; Magalhaes et al. 1968; Dekhuijzen and Bødlaender, 1973), and many results indicate that some herbicides are freely mobile in the phloem (Crafts, 1967b; Nalewaja, 1968).

Many other exogenous compounds have also been widely investigated. Wain and Carter (1967), Williams and Helton (1967), Shepard (1972), Verloop (1972), Tisdale and Lord (1973) have studied the movement of plant systemic chemotherapeutants and fungicides, and Bennett (1957), Mitchell et al. (1960), Finlayson and MacCarthy (1965) and Metcalf (1967) have reviewed the movement and mode of action of many systemic

insecticides. Peacock (1966) and Meikle (1968) have also contributed to this topic with work on nematocides.

Despite the knowledge obtained from investigations on the relationship between chemical structure and the ability of systemic compounds to be translocated, little improvement has been achieved with regard to effective symplastic transport, and the pathways of distribution of these chemicals in plants are not well understood. Some progress has been made by Crisp (1972) who presented an extensive list of endogenous compounds that are phloem mobile molecules, which represent a broad spectrum of chemical configurations, polarities, solubilities and molecular weights. From these characteristics he concluded that symplastic transport of exogenous compounds would be favoured by an acidic group attached to the molecule of these compounds. Other authors, too, have attributed the ability of chemicals to be absorbed and translocated to their physico-chemical properties (Mitchell et al. 1960; Metcalf et al. 1964), although the sizes and shapes of the molecules also appear to be contributing factors (Metcalf, 1967; Miller, 1971; Hussain et al. 1974b). However the movement and distribution of systemic compounds in plants are also functions of numerous other factors, especially

those related to environmental conditions and plant nutrition (Al-Adil et al. 1974).

The efficiency of action of systemic pesticides against a pest is closely related to its ability to translocate within the plant and its availability to the pest organism. In general, systemic insecticides are able to move only in a apoplastic manner and are thus readily carried up the plants from roots to foliage. Redistribution within the plant does not occur to any appreciable extent and the description given by de Pietri-Tonelli (1965) for dimethoate is still valid for most compounds which like dimethoate are unable to penetrate the symplast to be redistributed in the phloem.

Movement and metabolism of phorate

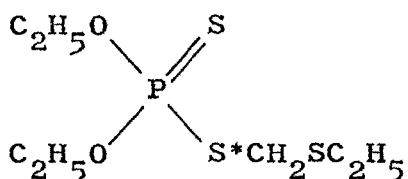
The insecticide used in this work, phorate, O,O - diethyl S (ethylthio) methyl phosphorodithioate, is a plant systemic insecticide used in a wide range of agricultural crops against the attack of soil insects and leaf feeding pests, particularly aphids and mites.

The metabolism of phorate has been thoroughly investigated both in plants (Metcalf et al. 1957; Bowman and Casida, 1957, 1958; Bowman et al. 1969) and in soils (Menzer et al. 1970; Lichtenstein et

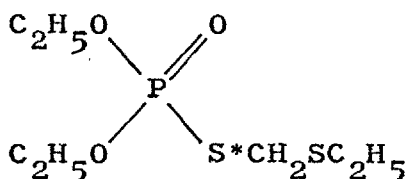
al. 1973; Schulz et al. 1973).

Five toxic metabolites are formed by oxidation of the sulphide in the side chain to the sulfoxide and sulphone; and by the formation of a P = O analogue, phoratoxon, from the P = S group of the parent compound. The chemical structure of phorate and its toxic metabolites are as follows:

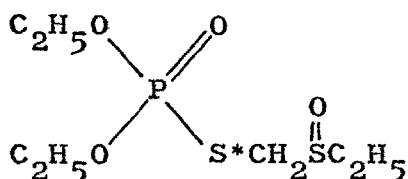
I. Phorate (P = S,S)



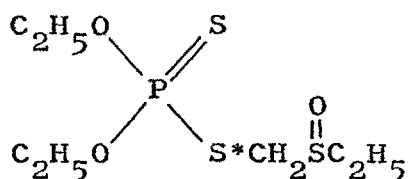
IV. Phoratoxon (P = O,S)



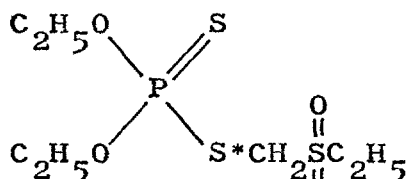
V. Phoratoxon sulfoxide (P = O,SO)



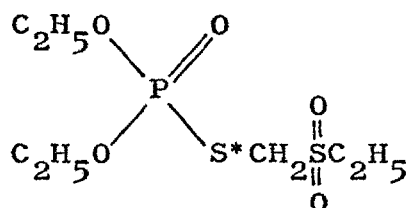
II. Phorate sulfoxide (P = S,SO)



III. Phorate sulphone (P = S,SO₂)



VI. Phoratoxon sulphone (P = OSO₂)



The asterisk indicates the labelled atom in the insecticide used during this work.

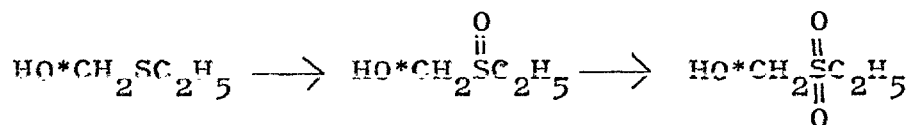
The data obtained from different studies on the metabolism of phorate in alfalfa, cotton, lemon (Metcalf et al. 1957), spinach (Menzer and Ditman, 1968) and corn (Bowman et al. 1969) appears to indicate various rates of phorate metabolism and also differing quantities and patterns of distribution of metabolite residues, especially the oxygen analogue and its metabolites. It seems clear however that phorate sulphoxide and phorate sulphone represented the major toxic metabolites found in these plant species.

Persistence of toxic residues vary considerably from crop to crop, though in the United Kingdom six weeks is the recommended interval that should elapse between spraying and harvesting for edible products (Ministry of Agriculture Fisheries and Food, 1974).

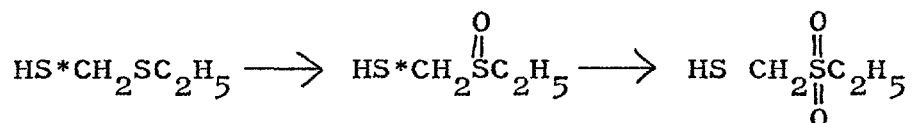
According to Mitchell et al. (1960), ready transport in plant tissues seems to be the limiting factor in the determination of systemic activity. Efficient translocation appears to be largely a function of the water solubility of the compound. The partition coefficient of phorate and metabolites in an equivolume hexane/water system range from 1.00 for phorate to 0.01 for phoratoxon sulphone (Bowman et al. (1969)). Thus, phorate owing to its lipophilic properties is readily absorbed by the roots and held in

the lipoprotein layers, where it is rapidly oxidised to its sulfoxide and to a lesser extent to its sulphone (Metcalf, 1967). The sulfoxide being approximately twice more hydrophilic than the parent compound (Bowman et al. 1969) is easily translocated to the foliage with the ascending transpiration stream, while phorate and its sulphone, which have similar partition properties, are less readily translocated. The oxygen analogue, phoratoxon, its sulfoxide and sulphone according to Bowman et al. (1969) are readily hydrolysed and therefore do not persist long enough to be of significant toxicological importance.

Phorate is concurrently metabolised to non-toxic compounds through hydrolysis. The hydrolytic products detected by radio-active analysis will depend on the position of the labelled atom in relation to the point of hydrolysis. Phorate is predominantly hydrolysed at the thiono sulphur and thus the hydrolytic products resulting from methylene labelled phorate will be probably represented by the sulphide containing side chain of the parent compound with possible oxidation of the sulphide to sulfoxide and sulphone with a sulphydril or hydroxide group at the point of cleavage, as follows:



or,



This scheme is largely conjectural as very little is known about the products of hydrolysis. Some hydrolytic cleavage may also occur at the ethoxy group though this is a less common phenomenon and the non-toxic products would be expected to be water soluble also.

The movement and metabolism of phorate have been extensively studied in relation to different factors, particularly those related to environmental conditions (Hacskaylo et al. 1961), water solubility (Reynolds and Metcalf, 1962) and soil type (Zaki and Reynolds, 1961; Getzin and Shanks, 1970).

Aphid feeding and excretion

According to Pollard (1973) the study of the feeding mechanism of aphids, apart from its biological point of view has been directed towards problems of practical importance such as those related to virus

transmission and the breeding of resistant plant varieties.

Most aphid species feed predominantly in phloem tissue, more specifically in the sieve tubes (Zimmermann, 1961, 1963; Evert et al. 1968) and since Kennedy and Mittler (1953) first described a technique for obtaining sieve-tube sap via the mouth parts of aphids, these insects have become a useful tool for the study of translocation processes in the phloem. This technique allied with the use of radio-tracers has made it possible to follow closely the movement of both endogenous and exogenous compounds within the conducting system of plants. Thus Peel (1963, 1965, 1970) and Peel and Weatherley (1962, 1963) used aphids to study the transport of water and ions in the sieve tubes and Ho and Peel (1969) have investigated bidirectional movement of radio-tracers in the sieve-tubes using the willow aphid Tuberolachnus salignus (Gmelin).

The aggregation behaviour of aphids appears to strengthen the capacity of a colony to compete with the natural sinks of the plants for food intake (Way and Cammell, 1970). Mittler (1959) discussing the feeding process of aphids pointed out that in terms of botany these insects may be regarded as perfect sinks into which all constituents of the sieve-tube

sap empty. Similar conclusions were reported by Peel and Ho (1970) after investigating the transport of ^{14}C - labelled assimilates in relation to the size of aphid colonies established in the stem of willow cuttings. The aphids in a colony may thus induce the transport of nutrients from distant parts of the plants to their feeding sites.

The excretion of honeydew is directly associated with food intake, and in toxicological studies the honeydew represents a mechanism for the elimination of toxic compounds. Quantitative and qualitative measurement of honeydew have been reported for several aphid species (Auclair, 1958, 1959, 1963; Mittler, 1958; Banks and Nixon, 1959; Maltais and Auclair, 1962). Most of the work on uptake and excretion by aphids however has investigated the performance of these insects as virus vectors, as for example Cockbain (1963), Hodges and McLean (1969), Gill (1970), Paliwal and Sinha (1970), and the relationship of aphids to susceptible and resistant varieties of host plants, as Dunn (1960, 1970), van Emden (1969), Harvey et al. (1971).

There are few toxicological studies on the uptake and excretion by aphids however, and very little is known about the fate of systemic insecticides after ingestion by aphids. Galley and Foerster

(1973) and Devonshire and Needham (1974) have demonstrated that some toxic compounds are eliminated in the honeydew and Eastop and Banks (1970) have suggested that, in the wax producing species, this too might prove to be a method by which at least some lipophilic toxic compounds might be eliminated.

It is known that feeding aphids are deterred by toxic amounts of insecticide in their food (Parry and Ford, 1967, 1969, 1971; Halimie and Ford, 1972), and though aphids may show different susceptibilities to a compound (Thomas and Bennett, 1954; Bonnemaison, 1956; Galley, 1968) this has not yet been correlated with their feeding behaviour (Lowe, 1969; Galley, 1974).

This thesis attempts to investigate the effects of sinks generated by aphids feeding on plants on the movement of labelled compounds with different transport characteristics and in particular the effect on phorate and its metabolites all of which have differing polarities. It was hoped also to obtain some information on the levels of uptake and elimination of such compounds by aphid colonies.

MATERIALS AND METHODS

1. Plants

Bean plants, Vicia faba L., variety "The Sutton", a dwarf variety, were grown in sand in a glasshouse and were used in the experiments when the third node had expanded. The sand was carefully removed from the roots under running water and the plants were placed in 100 mm x 36 mm specimen tubes containing nutrient solution (Hewitt, 1966), with ethylene diaminetetraacetic acid ferric - Na chelate (Fe EDTA) omitted.

2. Insects

The two aphid species used, Aphis fabae Scop. and Acyrtosiphon pisum (Harris) (Homoptera : Aphididae) were both reared on pot grown broad bean plants. The insects were kept in a constant temperature room at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a light regime of 16 hours light and eight hours dark, provided by four 85 W fluorescent tube lights mounted 60 cm above the bench. The relative humidity was not controlled and fluctuated from 60% during the daylight period to 80% at night. Young plants, 12 days old were transferred to pots containing vermiculite; both aphid cultures were kept in perspex cages (50 cm x 50 cm x 50 cm) with a muslin top to allow ventilation.

3. Radio-isotopes

Labelled chemicals, ^{32}P -phosphate and ^{35}S -sulphate

were obtained from the Radiochemical Centre, Amersham, the phosphate in phosphate buffer with an initial activity of 1 m Ci / mg P and the sulphate carrier free in isotonic saline, 5 m Ci / μ g S.

The insecticide used, ^{14}C - phorate, was labelled in the methylene group, and had a specific activity of 42.5 μ Ci / mg. Its purity was checked by Thin-Layer Chromatography (T.L.C.) using palladium chloride (Blinn, 1963) as visualising agent, by comparing the labelled compound against standards of unlabelled phorate, its sulphoxide and sulphone. Pure phorate was obtained using the chromatographic method described by Bowman et al. (1969). The stock solution of the insecticide was concentrated to 1.5 ml and eluted with 100 ml of benzene through a column 8 mm internal diameter, packed with 6 g of silicic acid (silic AR CC7, Mallinckrodt). All the phorate was eluted in the first 50 ml of effluent and re-checked for purity by T.L.C. The purified insecticide was made up to 10 ml in acetone and its specific activity determined by colorimetry (Getz, 1964) and radio-assay in a liquid scintillation counter.

4. Experimental design

The general procedures described below were followed in the experiments. Details regarding the position of aphid colonies on the plants, duration and timing of feeding and translocation of radio-label

are given in the description of the respective experiments.

Aphid colonies were established on different leaves of bean plants by confining the insects to the lower surface of the leaflets with perspex cages 2.5 cm in diameter, with a muslin top. The cages were lined with filter paper at the bottom and on the sides in order to collect the excreted honeydew (Galley, 1974). The cage-bearing leaflets were supported by a strip of perspex held by a clamp at a height such that the leaflets were maintained in their normal positions.

In the phosphate and sulphate experiments, 40 four day old apterous A. fabae were caged on each leaflet until the end of the experiment. For the ^{14}C - phosphate experiments, ten adult apterous A. fabae or A. pisum were caged on each leaflet to reproduce for 24 hours. At the end of this period the adults were removed and the newly born larvae were left to form the aphid sink.

The radio-labelled solutes were either added to 40 ml of nutrient solution for root uptake, or applied to aphid-free leaflets.

At the end of the experiments, the aphids and filter papers bearing honeydew were transferred to specimen tubes and stored at -20°C . The leaves for analysis were detached from the plants and also stored

at -20°C until analysed. Each pair of leaflets was analysed individually.

Leaf positions are described following the usual convention; the lowest leaf, the first to develop, is at node one; the second leaf to be formed, node two; the third, node three, and so on. All the leaves on plants at this stage of development were composed of two leaflets.

Three replicates per treatment were used in each experiment, except where otherwise stated in the experimental section.

5. Isotope extraction procedure

(a) ^{32}P and ^{35}S . In two of the experiments, the levels of ^{35}S were assessed in intact plants by positioning a Geiger - Muller tube (MX 168) against the abaxial surface in the centre of the lamina. In other experiments, ^{32}P and ^{35}S were assessed by a liquid scintillation technique. Leaves containing either isotope were homogenised with 10 ml of distilled water in an all glass hand homogeniser. The extract was filtered through a 9 cm Whatman filter paper No 1, and an aliquot of 2 ml was transferred to a counting vial containing 10 ml of scintillant solution.

The aphids were similarly extracted in a tissue grinder with 5 ml of distilled water.

Filter papers containing honeydew were transferred

to a specimen tube, 2.5 cm diameter containing 2 ml of distilled water and shaken for three minutes. The papers were removed and washed down with a 3 ml aliquot of water. Two ml were taken out and added to a counting vial with 10 ml of scintillant.

(b) ^{14}C - phorate

(b) 1. Plants. Two methods, employing three solvent systems were tried for the extraction of phorate and its metabolites from bean leaves. Recoveries were estimated by spotting known amounts of ^{14}C - phorate on a leaf immediately before extraction; identical amounts were added to counting vials, as standards.

Vacuum filter method

a. Chloroform-water. A leaf was crushed in a hand homogeniser with 10 ml of chloroform-water, 1:1 v/v and transferred to a 50 ml conical flask containing 1.5 g of finely divided activated charcoal (Norit G SX). The slurry was shaken for about five minutes and then filtered through a 4.5 cm buchner funnel. Both homogeniser and conical flask were washed with 20 ml of the same chloroform-water mixture and the washings added to the buchner funnel to facilitate the removal of any insecticide residue adsorbed on the charcoal. The filtrate was transferred to a 50 ml separating funnel and thoroughly shaken. The two phases were

allowed to separate; the chloroform fraction was collected in a 50 ml round-bottomed flask and the aqueous residue re-extracted with a further 20 ml of chloroform. The solvent extracts were pooled and evaporated to near dryness using a rotary film evaporator under reduced pressure (about 300 mm Hg). The flask containing the solvent extract was warmed in a water bath at 50 - 55°C. The water fraction was similarly concentrated to approximately 2 ml, at a temperature of 80°C. Both residues were quantitatively washed with two 5 ml aliquots of scintillant into counting vials.

b. Chloroform-acetone-water. This is a modification of the method described by Menzer and Ditman (1968). A leaf was extracted as described above except that the solvent system was acetone-water, 1:1 v/v. After the second aliquot was filtered, 20 ml of chloroform were added to the buchner funnel to remove any insecticide adsorbed on the charcoal. Separation and concentration of the two fractions followed the same procedure described above. Replicates of this procedure were carried out using 1 g of charcoal (see table below). *The charcoal was adsorbed with the chloroform*
When ~~chloroform~~ was omitted, virtually no radio-activity was recovered in the extracted solvent.

Charcoal column clean-up method

A similar procedure to that described by Watts et

al. (1969) using chloroform-methanol, 9:1 v/v was also evaluated and is shown in the table below.

Per cent recovery of ^{14}C - phorate from bean leaves^a.

Method	Solvent	g of charcoal	
		1.0	1.5
vacuum filter	chloroform- water	-	46
	chloroform- acetone-water	85	89
charcoal column	chloroform- methanol	97	93

a. Mean of five replicates.

Maximum recoveries were attained by the charcoal column method using 1 g of charcoal for decolorizing the plant sample. An increase in the amount of charcoal to 1.5 g reduced the percentage recovered from 97 to 93 per cent. The opposite happened with the vacuum filter method using chloroform-acetone-water; recoveries decreased from 89 to 85 per cent with a reduction from 1.5 to 1.0 g in the amount of charcoal.

Despite the high recoveries. the charcoal column method had two limitations; 1) the preparation of the column is time consuming, and 2) because the column is

made up under vacuum, great care has to be taken to avoid compaction of the support, which results in a slow flow of solvent through the column.

The chloroform-acetone-water procedure, though less efficient, was considerably faster and less elaborate than the charcoal column method.

The chloroform-water system has been widely used, with modifications in the extraction methods for phorate and other organophosphorus insecticides, see for example Metcalf et al. (1957), Metcalf et al. (1964) Coleby et al. (1972). A major inconvenience with this procedure, however, lay in the formation of an emulsion between the chloroform and water fractions after filtration of the extract. A complete separation of the two components was not obtained, even after the samples had been allowed to stand for two hours. This fact, coupled with the comparatively low recoveries, precluded the adoption of this procedure for the present work.

The chloroform-acetone-water system had the advantages of consistency of results, speed, and relatively good recovery rates. For these reasons this procedure was adopted for this study.

(b) 2. Insects. Extraction and clean-up of phorate from aphid colonies consisting of 100 - 110 individuals were carried out as described for plants. using the chloroform-acetone-water system. The volumes

of tissue were small compared with the leaf extracts, and thus the amount of charcoal was reduced to 0.2 g and the chloroform aliquots reduced to 15 ml and 10 ml in the filtering and separating funnel steps.

(b) 3. Honeydew. The disks and strips of filter paper bearing the excreted honeydew were transferred to a 50 ml separating funnel with 10 ml of chloroform-water; 1:1 v/v and thoroughly shaken for five minutes. The filter papers were withdrawn from the funnel and washed down with 5 ml of chloroform and then with 5 ml of water. The two phases were separated and concentrated as previously described for the treatment of plant and insect extractions. Recovery of radio-label was satisfactory, since no more radio-activity could be extracted from or detected in the filter papers.

Honeydew extractions were also tried with toluene-water and toluene-methanol-water. Complete ^{14}C - recovery was obtained in both cases; however, the advantage of the chloroform-~~acetone~~-water system is that these solvent solutions were concentrated considerably faster than toluene and methanol, thus reducing the risk of loss in the rotary film evaporator.

6. Separation of metabolites

Analysis of phorate, its sulfoxide and sulphone in plants, aphids and honeydew followed a modification

of the method described by Bowman et al. (1969).

The solvent extract from the homogenate was concentrated to 2 ml and transferred to the top of a chromatographic column containing 6 g of silicic acid.

Phorate, its sulphone and sulfoxide were eluted with 40 ml aliquots of benzene, benzene-acetone, 95:5 v/v and acetone respectively. Each effluent was collected in a 50 ml round-bottomed flask and concentrated to near dryness as described before. The concentrated residues were quantitatively washed into counting vials with two 5 ml aliquots of scintillant cocktail.

7. Isotope counting procedure

(a) ^{32}P and ^{35}S . Radio-activity in intact leaves was assessed with a thin end-window G-M tube, Mullard MX 168, connected to an Isotope Developments Ltd 1700 Scaler. Counting efficiencies for ^{35}S were about 0.5 per cent over the area of the G-M tube window, which was taken as 3.1 cm^2 . Each sample was counted five times for two minutes, and an average value was taken. Results were corrected for background, radio-active decay and counting efficiency.

Liquid scintillation samples were counted in a PANAX Equipment Ltd scintillation head and associated PX equipment. The scintillant cocktail used was N.E. 240 (Nuclear Enterprises, Edinburgh). Counting efficiencies with this method were 70 per cent for ^{35}S

and 82 per cent for ^{32}P .

(b) ^{14}C - Phorate. ^{14}C was counted in a Beckman LS 250 scintillation counter. The activity in each sample was counted either for 100 minutes or until the 2 σ counting error was $\pm$ 1 per cent, whichever occurred first.

Counting efficiency was assessed by the external standard method, and the results were corrected for background and efficiency.

Scintillation media Two scintillant solutions were used for ^{14}C counting;

I - Solvent extracts: ^{14}C compounds in solvent solution were evaluated in a butyl PBD cocktail. Eight grams of butyl PBD, (2 - (4' - t - butylphenyl) - 5 - (4'' - biphenyl) - 1,3,5 - oxadiazole) were dissolved in one litre of toluene. Counting efficiencies with this medium ranged from 91 to 94 per cent.

II - Aqueous extracts: a slightly modified formulation of Bray's scintillant solution (Bray, 1960) was used for the water soluble fraction of the extracted insecticide. Naphthalene (60 g); PPO, 2,5 diphenyloxazole (4 g) and POPOP, 1,4 - Di (2 - (5 - phenyloxazolyl) - benzene (0.2 g) were dissolved in 100 ml of Analar grade methanol, and the solution was made up to one litre with Analar grade dioxan. Counting efficiencies for Bray's cocktail were between 84 and 86 per cent.

8. Thin-Layer Chromatography

Bean leaves were homogenised and cleaned-up as described before. The solvent fraction was concentrated to 2 ml and transferred to a 10 ml centrifuge tube to be further concentrated to approximately 100 μ l under a nitrogen stream. A 30 μ l sample was spotted on a glass plate, 20 cm x 20 cm coated with silica gel G, 0.250 mm in thickness and previously activated at 100°C for two hours. Each spot was fortified with 5 μ g of ^{or each!}unlabelled insecticide containing phorate and its five metabolites, in order to facilitate the detection of the spots. The plates were developed in a benzene-methanol, 92:8 v/v solvent system for a distance of 15 cm from the point of application. The spots were visualised by spraying with 0.5 per cent solution of palladium chloride in 2 ml of concentrated hydrochloric acid. The silica gel in the area of each spot was carefully scraped from the plate and transferred to a counting vial with 10 ml of scintillant (butyl PBD) for radio-assay. pl.?

9. Autoradiography

Leaf samples selected for autoradiography were detached from the plants and immediately arranged between two sheets of polythene in a deep freezer at -20°C. to prevent further movement of label within the plant tissue. A piece of cardboard, 16 cm x 14 cm

was placed underneath the lower sheet of polythene to support the leaves and the polythene sheet. The samples thus arranged were enclosed in a film holder 16.2 cm x 14.2 cm with a Kodak "Kodirex" X-ray film above the upper polythene sheet. This operation was carried out in a dark room using a Kodak safe light with a 6B filter. The holder was wrapped in a black polythene bag to exclude light and placed at -20°C under a lead brick to ensure maximum contact between the sample and the film. After a suitable period of time, depending on the isotope and its approximate concentration in the plant tissue, the film was taken from the holder and developed following conventional methods in Ilford ID - 19. Residues of the developer were removed under tap water for 30 seconds and the film was fixed in a solution of Amfix for 8 - 10 minutes before the final washing and drying.

EXPERIMENTS AND RESULTS.

PART ONE:

INORGANIC TRACERS.

1. The effect of aphid sinks on the translocation of ^{35}S .

The uptake of ^{35}S by three successive colonies of aphids feeding on the same leaf and the levels of the isotope present in the caged area of the leaf were measured. The distribution of ^{35}S in plants bearing aphid colonies and aphid-free plants was also compared.

Experimental procedure. Aphid colonies were caged on both leaflets of the second node and at the same time 5 μCi of ^{35}S - sulphate were added to the nutrient solution. The aphids were changed at two and four days and on the sixth day the experiment was terminated. The radio-activity in the leaflets of the first three nodes of the plants was assessed by the Geiger-Muller technique between the removal of each colony and the caging of the next batch. Control plants without aphids were assessed at similar time intervals.

Results. The radio-activity in the leaves is presented as nCi/cm^2 of plant tissue; aphid and honeydew counts are related to the caged area. Figure 1.1 shows the distribution of ^{35}S in the leaves and the respective proportions removed by the aphids in relation to time. Clearly, the colonies had a significant effect on the movement of label to the second

leaf in comparison to aphid-free plants. Accumulation of ^{35}S in the leaves and intake by the colonies increased with time. The amounts of isotope removed by the aphids accounted for approximately 80 per cent of the total ^{35}S translocated to the second leaf at each feeding interval. Of the quantity ingested, about 70 per cent was excreted in the honeydew. The concentration of ^{35}S in the leaves of both infested and control plants showed an apically dominant pattern.

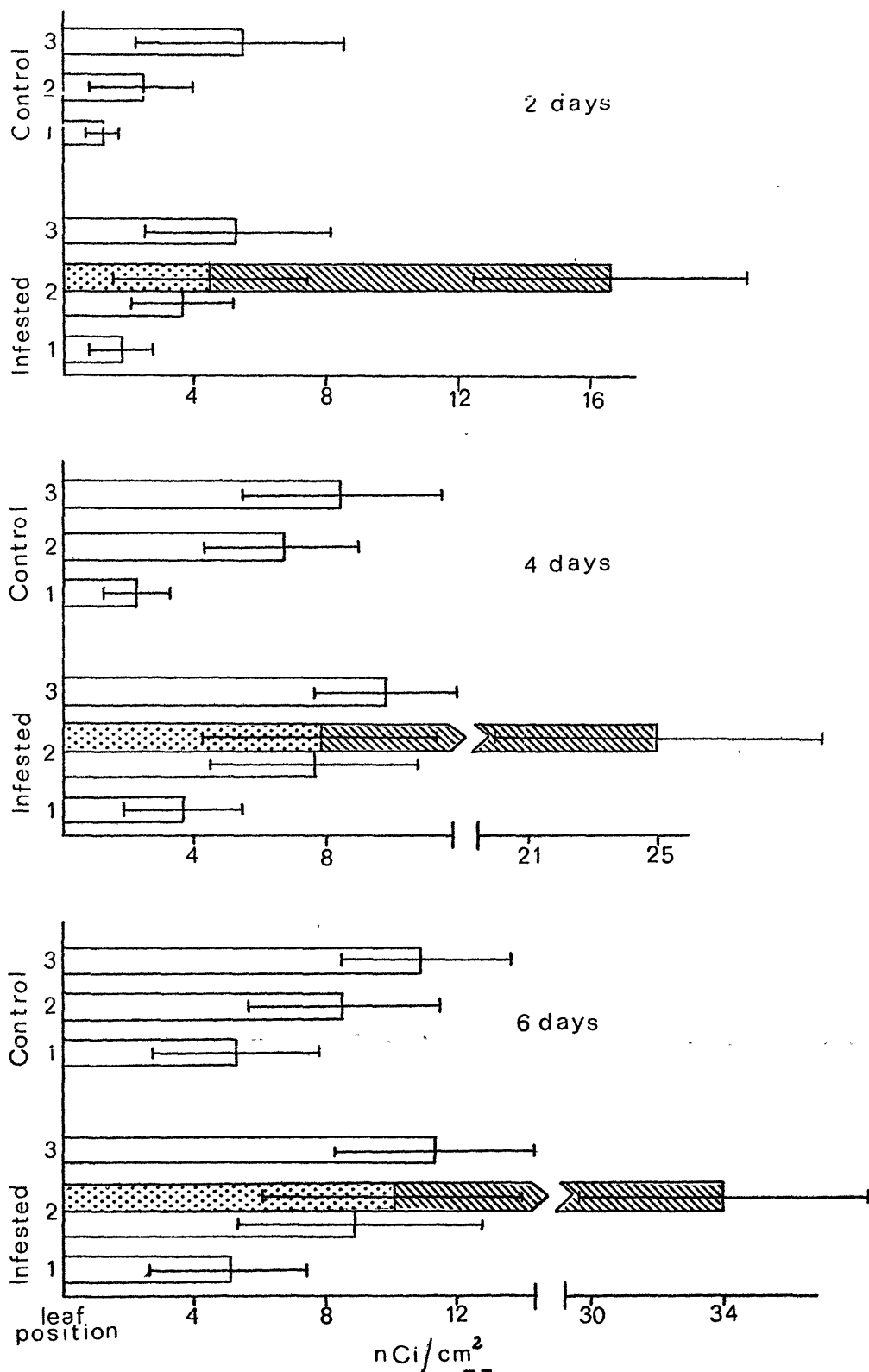


Fig. 1.1 Distribution of ^{35}S in the leaves and quantities removed by aphid colonies in relation to time, with 95% confidence limits. (Appendix 1)

2. The influence of aphid colonies caged after the absorption of ^{35}S - sulphate by the plants.

The accumulation of ^{35}S in aphid-free plants was followed for six days; the aphids were then caged and allowed to feed continuously for four days, after which the leaves, aphids and honeydew were assessed for radio-activity.

Experimental procedure. Four μCi of ^{35}S sulphate were added to the nutrient solution of three plants, and the radio-activity was measured in the first three leaves on every other day, for six days. On the sixth day, aphid colonies were caged on both leaflets of the first node of two of the plants and were left to feed for four days. The remaining plant was kept without aphids as a control. The radio-label was left in the nutrient solution throughout the experimental period.

Results. The assessment of radio-activity in the leaves was carried out with the Geiger-Muller detector, and the results were expressed as nCi/cm^2 of leaf; aphid and honeydew counts were related to the caged area. Transport of label to the leaves increased throughout the experiment, as shown in figure 1.2. When aphid sinks were established on the leaflets of the first node, further amounts of ^{35}S were translocated to this leaf and taken up by the colonies. However, despite the considerable quantities of label removed

by the aphids, accumulation of label in the upper leaves of the infested plants was not affected in comparison to the distribution in the aphid-free plant. After feeding on labelled tissue for four days, the aphids had excreted in the honeydew three quarters of the total amount imbibed.

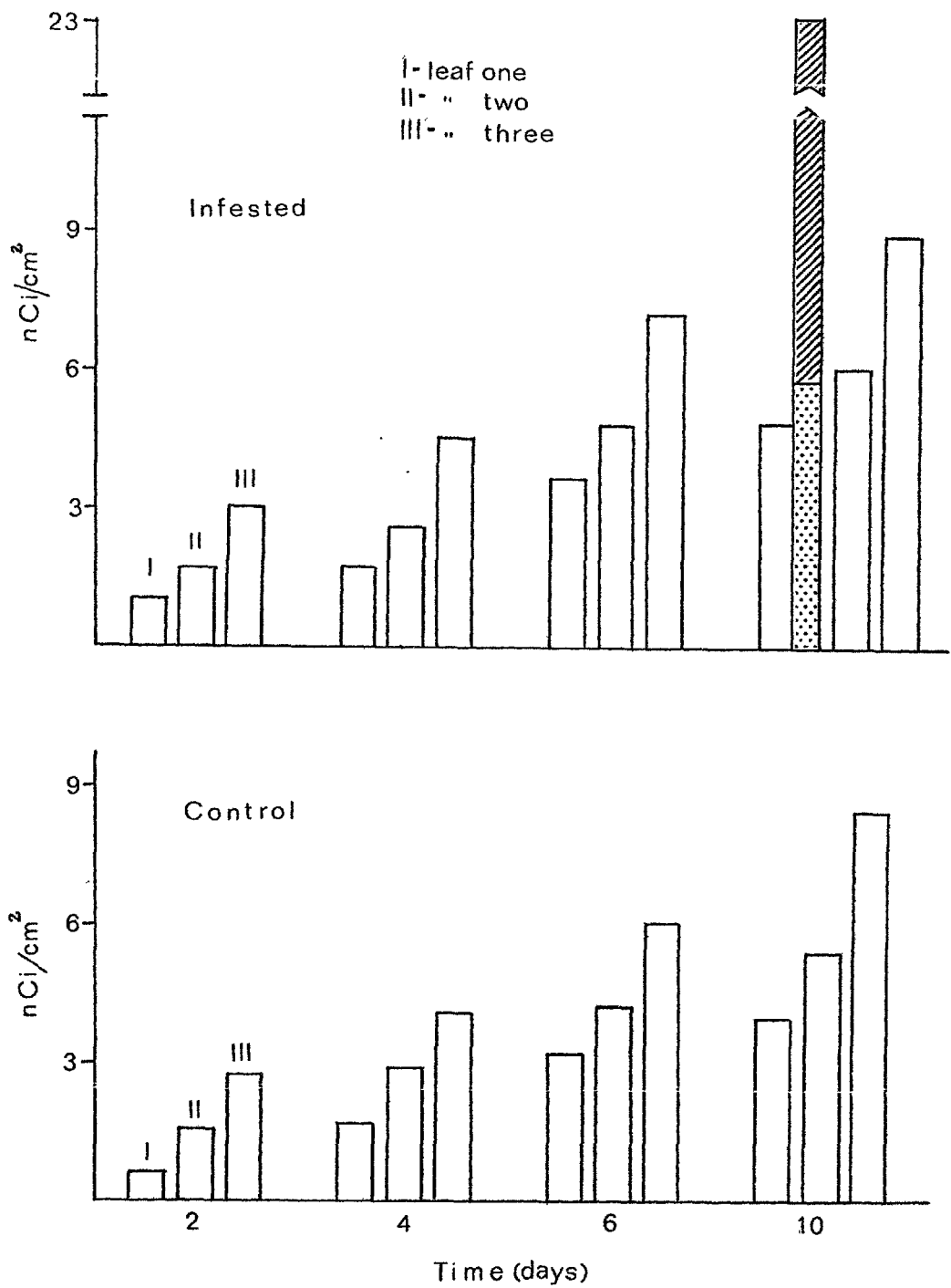


Fig. 1.2 Distribution of ^{35}S in the leaves and quantities removed by aphid colonies feeding on the lower leaf following root uptake. (Appendix 2)

Leaves
 Aphids
 Honeydew

3. Export of ^{35}S to aphid sinks situated above or below the treated leaf.

The distribution of ^{35}S applied to the leaf was studied when aphid colonies were established either above or below the source of label.

Experimental procedure. Aphid sinks were formed on both leaflets of the first node in a set of plants; at the same time 0.5 μCi of ^{35}S were spotted on the second leaf. Another group of plants was similarly treated, except that the aphids were caged on the leaflets of the third node. A third set of plants received the same dose of ^{35}S and was kept without aphids as a control. After three days of translocation, radio-active analyses were carried out on the first and third leaves, roots, stem, aphids and honeydew. Extraction of ^{35}S from the roots and stems followed a similar procedure to that previously described for the leaves, using 20 ml of distilled water.

Results. Figure 1.3 shows the distribution of ^{35}S in the samples analysed and the proportions removed by aphid colonies established above or below the treated leaf. Similar amounts of label were ingested by the aphids in both colonies, representing approximately 50 per cent of the total quantities translocated to the leaves supporting the aphid colonies.

The highest amounts of ^{35}S were found in the roots however, where in all treatments more than half the exported isotope was found. From the data in figure 1.3 it can be seen that neither colony had any significant effect on the distribution of ^{35}S through the plant, compared with the control treatment.

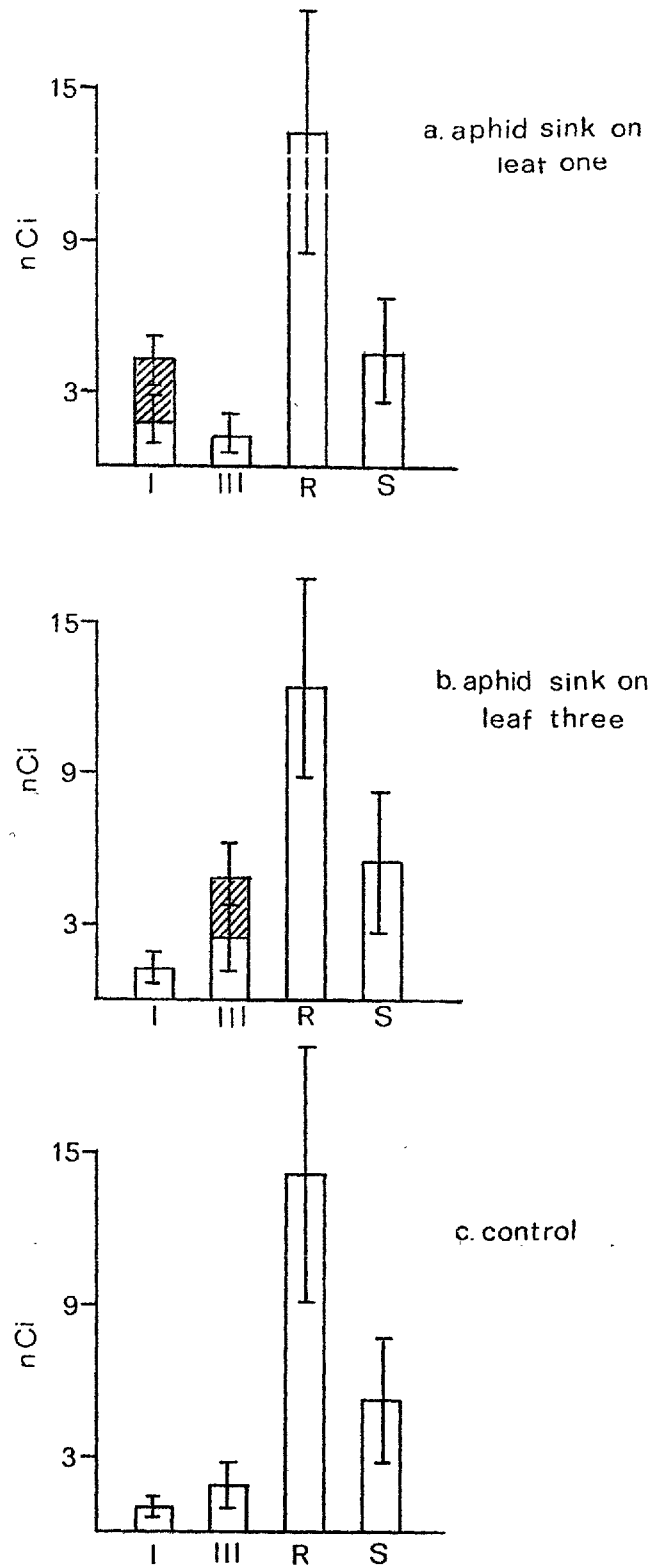


Fig. 1.3 Export of ^{35}S from the second leaf and proportions removed by aphid colonies above or below the treated leaf with 95% confidence limits. (Appendix 3)

I-Leaf one; III-Leaf three; R-Roots; S-Stem

 Aphids + honeydew

4. The effect of aphid sinks on the movement of ^{32}P - phosphate applied to the leaf.

The quantities of isotope absorbed by aphid colonies established two days before the treatment were compared with the proportions removed by similar colonies established at the time of application of ^{32}P - phosphate. The aphids were allowed to feed for three days after application of the labelled phosphate. The distribution of label was assessed in plants supporting aphids and in one aphid-free plant.

Experimental procedure. Colonies of A. fabae were caged on both leaflets of the first node of two plants while three other plants were kept without aphids. After three days, 0.5 μCi of ^{32}P were spotted on the second leaf of all the plants and at the same time similar groups of aphids were caged on the lower leaf of two of the remaining plants. The fifth plant was left without aphids as a control.

Results. The distribution of ^{32}P in the plants and the proportions removed by the aphids is shown in figure 1.4. Most of the tracer present in the samples analysed was concentrated in the roots and stem, and only a small proportion had accumulated in the leaves above and below the source. The amount of tracer diverted to the leaves supporting the aphids however

was greatly affected by the presence of aphids, particularly when the aphid sinks had been established before the ^{32}P - phosphate treatment. Nearly half of the total label exported from the treated leaf was ingested by the colonies caged prior to the treatment, a large proportion of it being excreted in the honeydew. When the colonies were caged at the time of treatment their influence was markedly reduced. Accumulation of label in the control plant was mainly restricted to the roots and stem, in similar proportions to those found in the roots and stem of the infested plants. Insignificant amounts of radio-activity were detected in the lower leaflets.

The results in figure 1.4 indicate some accumulation of tracer in the infested leaflets in relation to the control treatment. An autoradiographic study was carried out to assess any further accumulation of label in the leaflets after the removal of the colonies. An aphid sink was formed on one leaflet of the second node for six days. After this period, 5 μCi of ^{32}P were added to the nutrient solution; three hours later the insects were removed and the isotope was allowed to translocate for a total period of 12 hours. The autoradiograph shown in figure 1.5 failed to demonstrate any significant difference between the infested and control leaflets, the latter bearing an empty cage during the period in which the aphids were confined on the adjacent leaflet.

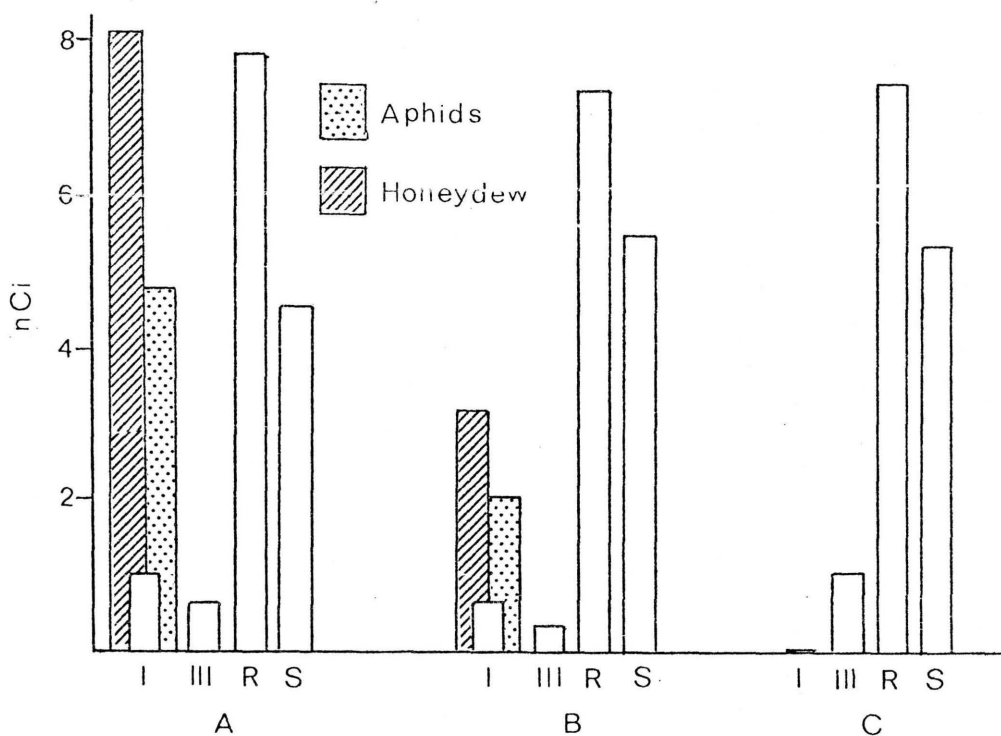


Fig. 1.4 Distribution of ^{32}P in plants and proportions removed by (A) previously established aphids, (B) aphids caged at the time of treatment and (C) in an aphid free plant. (Appendix 4)

I-Leaf one; III-Leaf three; R-Roots; S-Stem.



Fig. 1.5 Distribution of ^{32}P - phosphate in infested (I) and aphid-free (F) leaflets 12 hours after the removal of the aphid colony, following root uptake.

PART TWO:
¹⁴C-PHORATE.

1. Estimation of ^{14}C - phorate in the leaf by Thin-layer chromatography.

The relative proportions of phorate and its oxidation products were evaluated after this compound had been absorbed and translocated for one, four and seven days in bean leaves, following root uptake.

Results. Phorate was rapidly oxidised to its sulphoxide and sulphone as shown in table 2.1

Table 2.1 Relative distribution of phorate and its oxidation products in bean leaves.

Metabolite	Per cent occurrence after		
	1 day	4 days	7 days
P = S,S	19.0	18.0	16.0
P = S,SO	57.0	52.7	47.0
P = S,SO ₂	14.0	16.0	17.9
P = O,S	0.8	0.3	0.1
P = O,SO	8.0	10.0	15.0
P = O,SO ₂	1.2	3.0	4.0

Phorate, its sulphoxide and sulphone represented percentages varying from 90 per cent after one day to 80 per cent after seven days, of the total toxic metabolites present in the leaf. The oxygen analogue, phoratoxon, and its sulphone occurred in relatively small amounts, the former decreasing with time whereas the latter increased during the experimental period.

Phoratoxon sulphoxide appeared to be more stable during these translocation periods, representing eight and 15 per cent of the oxidation products after one and seven days respectively.

In view of the comparatively small amounts of phoratoxon and its metabolites, particularly after one and four days translocation, the qualitative analysis of phorate was restricted to phorate its sulphoxide and sulphone.

2. Distribution of ^{14}C - phorate in the leaves of bean plants.

The levels of phorate and its metabolites were estimated in the first three leaves during a 24 hour translocation period from the nutrient solution.

Results. After 24 hours translocating, 11.4 per cent of the dose applied (20 μg) had accumulated in the first three leaves. The distribution of the insecticide followed an apically dominant pattern, as indicated in figure 2.1. Approximately 61 per cent of the label present in the leaves was concentrated in the leaflets of the third node in contrast with 23 and 16 per cent present in the second and first leaves respectively. These results indicate a rapid oxidation of phorate to its sulphoxide and sulphone. Phorate sulphoxide was the major metabolite found in all the leaves, accounting for 71 per cent of the total label present in the leaves. Unchanged phorate represented 13 per cent and phorate sulphone, nine per cent of the total radio-activity translocated to the leaves. Hydrolysis products after 24 hours translocation accounted for seven per cent of the tracer accumulated in the leaves.

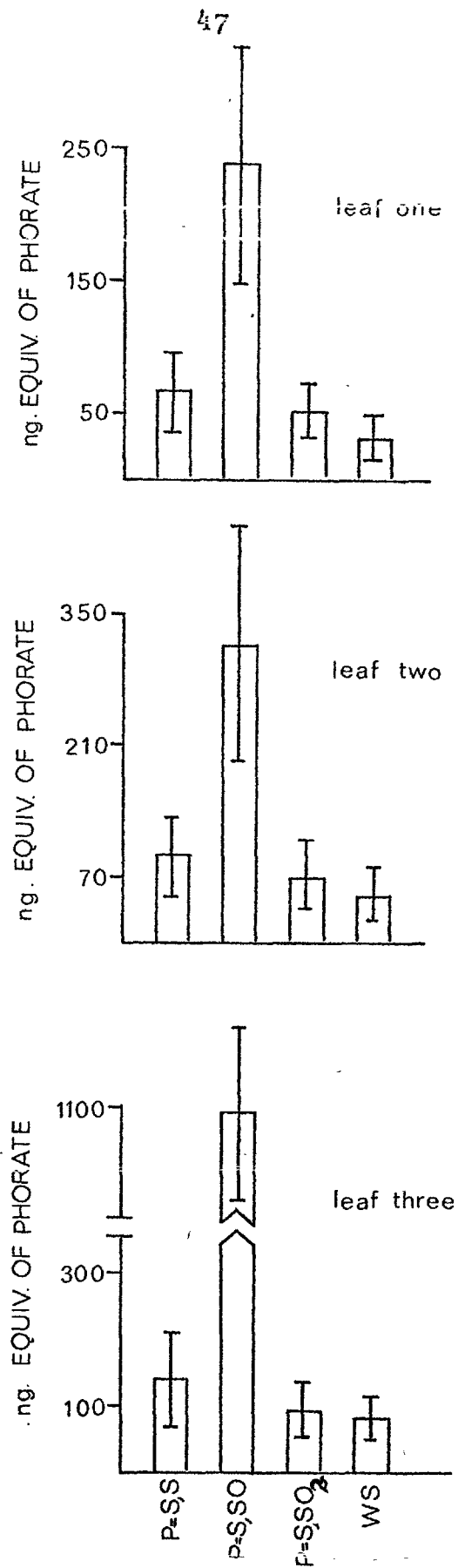


Fig 2.1 Distribution of phorate and its metabolites in the first three leaves, after root uptake, with 95% confidence limits. (Appendix 6)

3. Gross distribution of phorate and its metabolites within the leaf.

Radio-active analysis and autoradiography were used to study the distribution of phorate and its metabolites within the leaf at different time intervals.

Experimental procedure. 20 µg doses of labelled phorate were added to the nutrient solution of two sets of plants and were left to translocate for $\frac{1}{2}$, one, two, four and seven days. At the end of each time interval, the leaflets of the second node were detached from the plant and divided into two parts; a peripheral area cut approximately 5 mm around the edge, and the remaining central portion. Both fractions were weighed and quantitatively analysed. The distribution of radio-label in the second group of plants was followed by autoradiography after similar translocation periods.

Results. The levels of accumulation in both areas are expressed in figure 2.2 as a percentage of radio-activity per 100 mg fresh weight of plant material. It is evident from figure 2.2 that accumulation of the insecticide occurs rapidly around the periphery of the leaf. After 12 hours the total label concentrated in the marginal area accounted for 59 per cent of the total translocated to the leaf; by the

seventh day, 87 per cent of the radio-activity found in the leaf was present in the marginal area.

Phorate sulphoxide was the most abundant metabolite at all periods, representing 50 per cent or more of the metabolites analysed, although about a third of this fraction was probably oxygen analogue metabolites which when present would be eluted from the column with phorate sulphoxide. In the central area, the percentages of phorate and its sulphone slowly decreased with time, whereas the opposite occurred with the hydrolysed metabolites. After seven days, 50 per cent of the metabolites present in the centre of the leaf consisted of water soluble metabolites. In the marginal area however, only 15 per cent of the extracted radio-label was present as water soluble metabolites.

The sequence of autoradiographs shown in figure 2.3 confirms the steady increase in concentration of label at the periphery of the leaves, and by the seventh day the amount of tracer present in the centre of the leaf was small in relation to that at the margin.

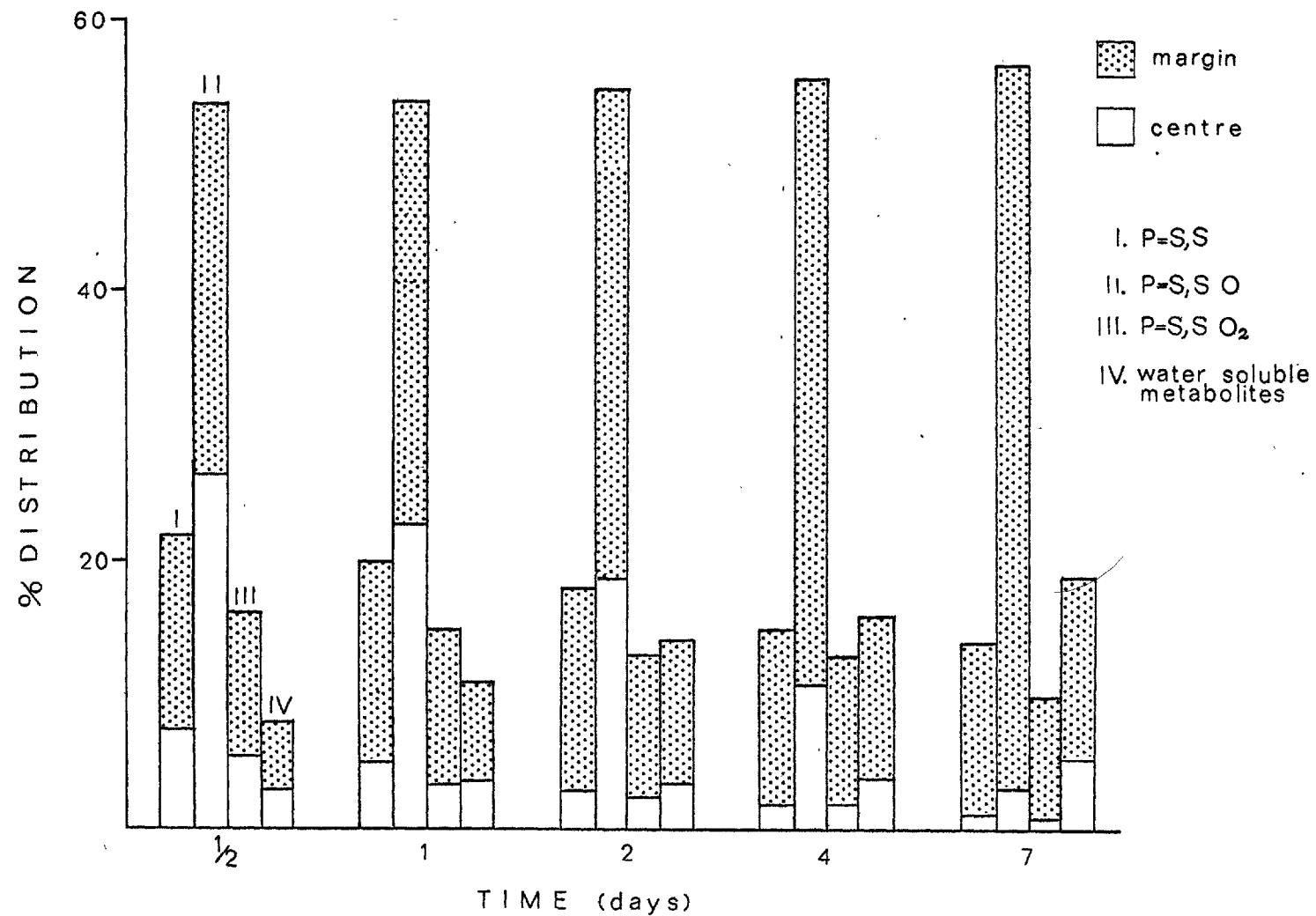


Fig. 2.2 Distribution of phorate and its metabolites within the leaf.
(Appendix 7)

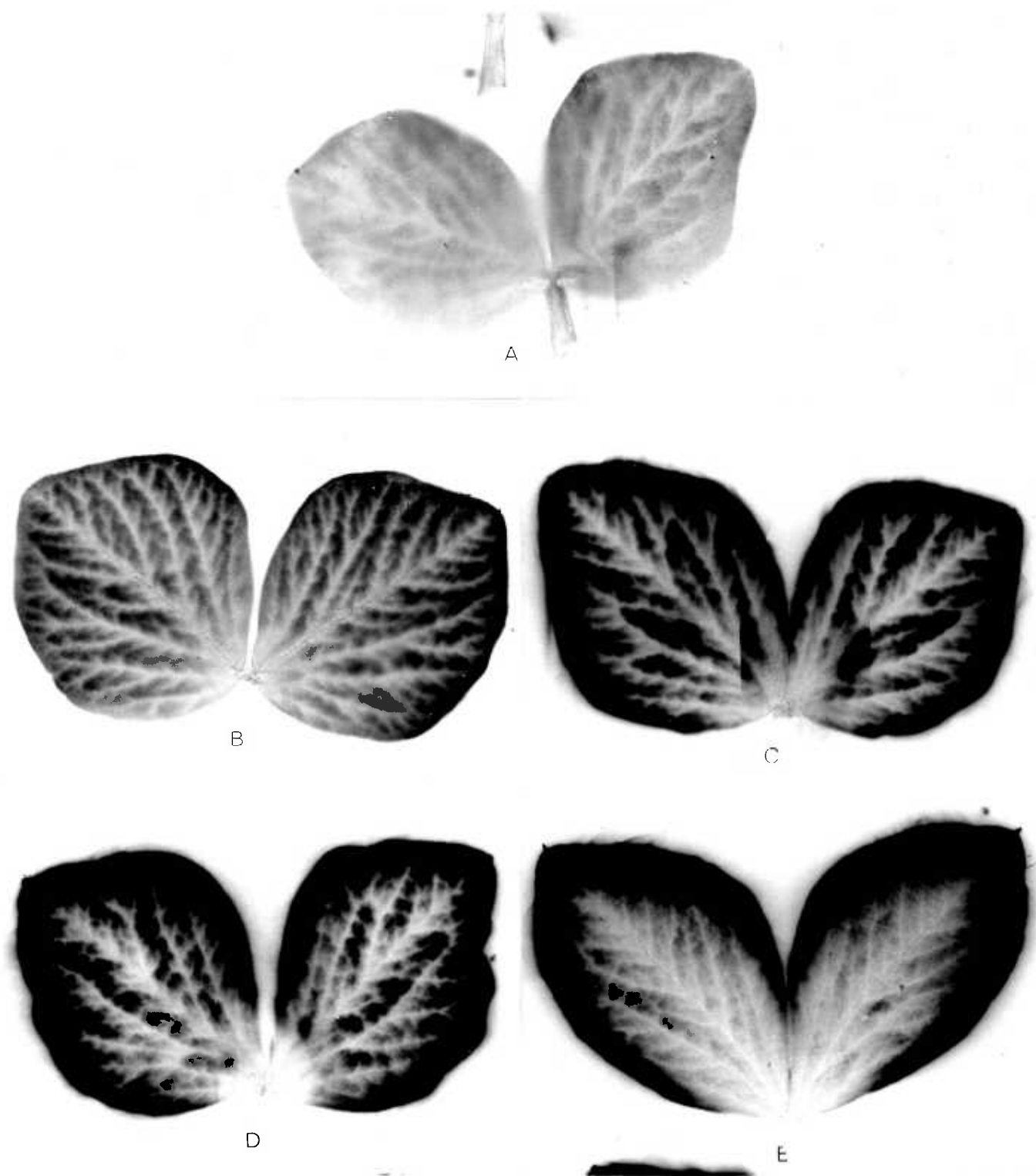


Fig. 2.3 Accumulation of ^{14}C by leaves at different times following the addition of ^{14}C - phosphate to the root nutrient.

A. $\frac{1}{2}$ day B. 1 day C. 2 days D. 4 days E. 7 days

4. Uptake and excretion of the insecticide by A. fabae and A. pisum.

The amounts of phorate and its metabolites ingested and excreted by colonies of A. fabae and A. pisum were assessed and compared to the quantities of the insecticide present in the leaves supporting the colonies.

Experimental procedure. Colonies of A. fabae were reared on both leaflets of the second node of three plants; similarly, colonies of A. pisum were grown in other three plants. After six days, 22 µg of labelled phorate were added to the nutrient solution and left to translocate for 18 hours, when analyses were performed on the foliage and the aphid sinks.

Results. A. fabae produced more larvae than A. pisum during the 24 hour reproduction period; the mean number of larvae produced by the former species was 131, whereas A. pisum adults produced 96 individuals.

The quantities of toxic metabolites ingested and excreted by both species are shown in figure 2.4b and 2.4c. Despite the difference in the number of aphids produced, both colonies ingested similar quantities of phorate and its toxic metabolites. The total amount of solvent soluble metabolites found in the

colonies of A. fabae represented 0.4 per cent of these metabolites present in the leaves supporting the aphids. Of the amount absorbed, 28 per cent had been excreted in the honeydew. A. pisum colonies removed 0.35 per cent of the solvent soluble present in the leaves, of which 30 per cent was eliminated through the honeydew. The pattern of distribution in leaves, aphids and honeydew was similar, in the following order: phorate sulphoxide > phorate > phorate sulphone, although the concentration of phorate sulphoxide was proportionally higher in the leaves.

Water soluble metabolites however were found in considerable quantities in the aphid sinks, particularly with A. pisum, as shown in figure 2.5. A. fabae absorbed nearly half of these metabolites present in the leaf, whereas A. pisum ingested 77 per cent. The distribution of the hydrolysis products imbibed by the aphids was different in the two species; A. fabae excreted in the honeydew 44 per cent of the total label ingested, while A. pisum eliminated a smaller proportion (26.5 per cent) in relation to the amount retained in their bodies.

Table 2.2 gives the mean quantities of solvent soluble and water soluble metabolites found in both aphids and their honeydew.

Table 2.2 Quantities of solvent and water soluble metabolites per aphid, detected after 18 hours translocation in the plants.

species	mean picogram equivs. per aphid	
	solvent soluble	water soluble
<u>A. fabae</u>	16.6	190
<u>A. pisum</u>	19.2	420

The data shows that individual A. pisum absorbed about 15 per cent more solvent soluble metabolites than individual A. fabae, and 2.2 times more water soluble metabolites.

Despite the different amounts of label removed by both aphid species, the quantities of phorate and metabolites accumulated in the leaves supporting the colonies were not significantly different (figures 2.4a and 2.5)

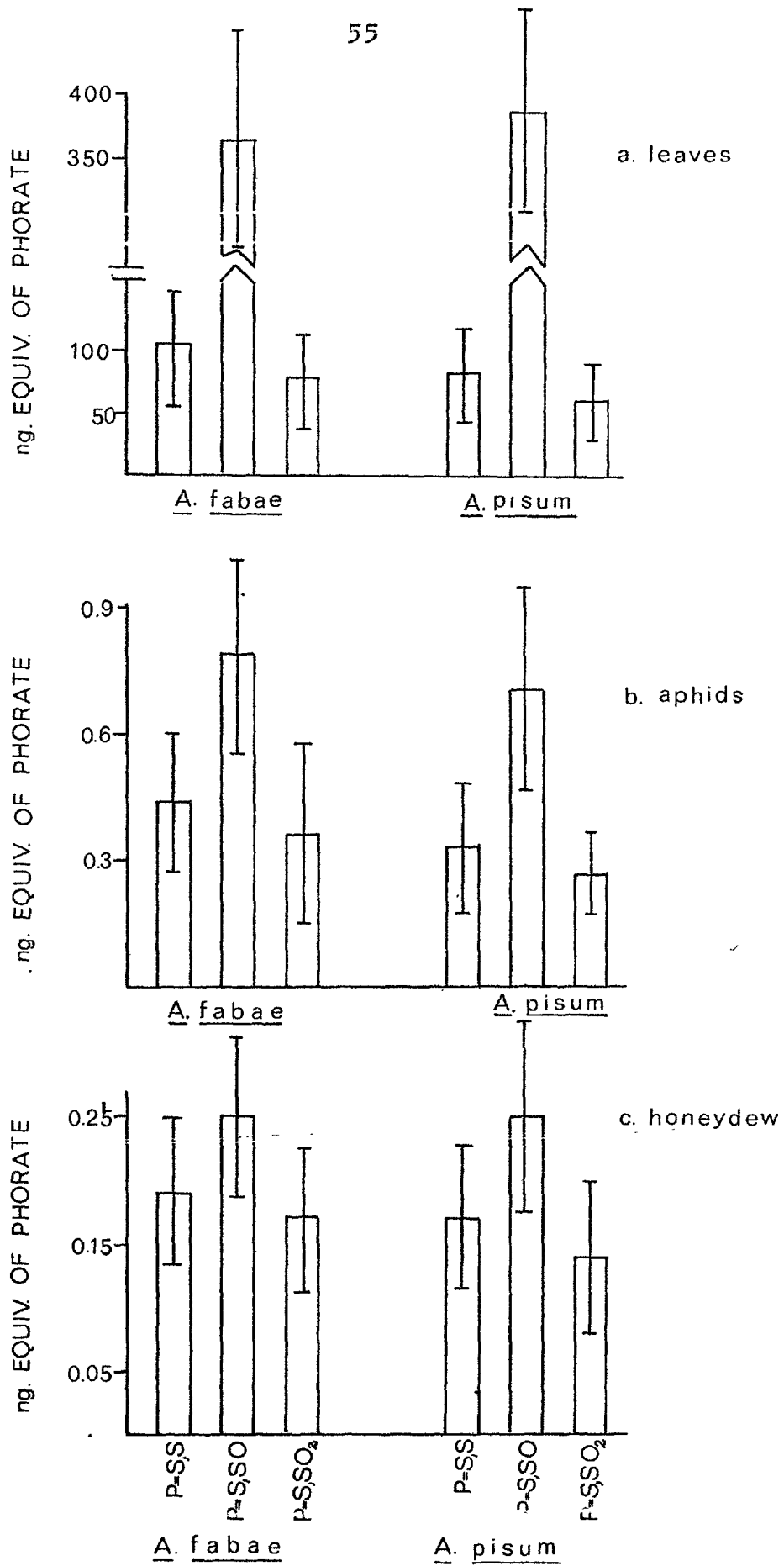


Fig. 2.4 Distribution of solvent soluble metabolites in leaves, aphids and honeydew, with 95% confidence limits. (Appendix 8)

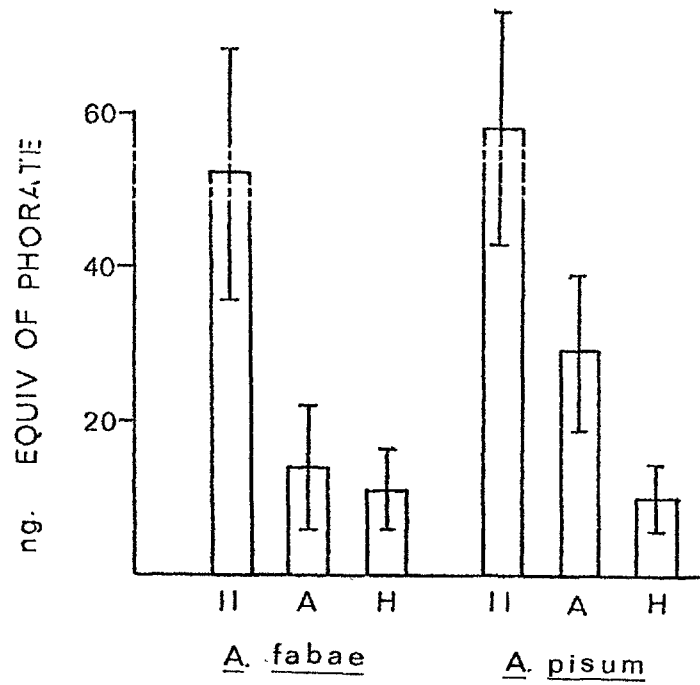


Fig. 2.5 Accumulation of water soluble metabolites in the leaves supporting colonies of *A. fabae* and *A. pisum* and its distribution in aphids and honeydew, with 95% confidence limits. (Appendix 8)

II-Leaf two; A-Aphids; H-Honeydew.

5. The influence of aphid colonies on the movement of phorate within the caged area.

The quantities of phorate and its metabolites taken up by colonies of A. fabae and A. pisum were compared with the amounts accumulated in the leaf area where the colonies had been feeding, and the marginal portion of the leaf.

Experimental procedure. Colonies of A. fabae or A. pisum were established on both leaflets of the second node of two plants. After five days, 20 µg of phorate were added to the nutrient solution for 24 hours. At the end of this period the unabsorbed nutrient solution was discarded and 40 ml of fresh insecticide-free nutrient solution were given to each plant. The absorbed insecticide was left to translocate for a further 24 hour period, after which aphids, honeydew and the leaves supporting the colonies were analysed. The leaflets were divided into two parts; the central area occupied by the cage, and the remaining marginal portion. Equivalent areas were taken from control plants without aphids.

Results. As shown in figure 2.6a, the largest proportion of toxic metabolites was present in the marginal portion of the leaf, outside the caged area. The quantities of these metabolites within the caged area did not seem to be affected by the presence of

aphid colonies when compared to the control treatment.

By the end of the experimental period, the colonies of A. fabae absorbed 1.6 per cent of the toxic products present in the caged area of the leaf and A. pisum 0.9 per cent. Of the amounts ingested, A. fabae eliminated 29 per cent compared with 34 per cent excreted by A. pisum.

Water soluble metabolites however were strongly influenced by the aphid sinks, as shown in figure 2.7. By the end of the translocation period, A. fabae had removed twice the amount of hydrolysed products accumulated in the caged area whereas A. pisum had an even greater influence and removed 4.8 times the quantity present in that part of the leaf. A. fabae eliminated half and A. pisum 42 per cent of the absorbed water soluble metabolites.

The distribution of hydrolysed material in the leaves of the control treatment was similar to that in the infested leaves, indicating that these metabolites appeared in the area at the same rate at which they were removed by the aphid colonies.

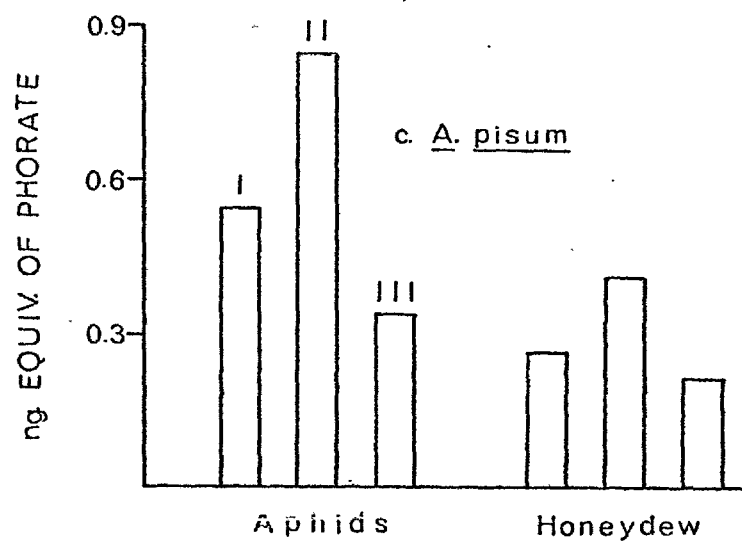
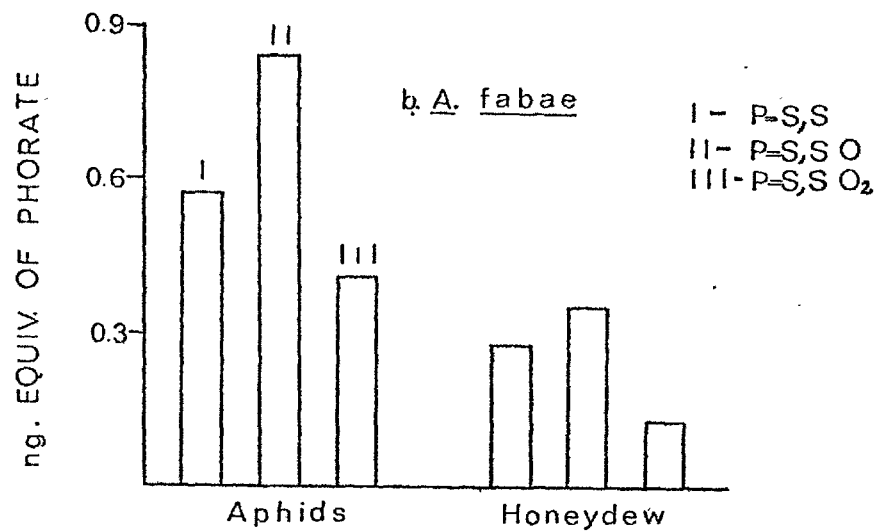
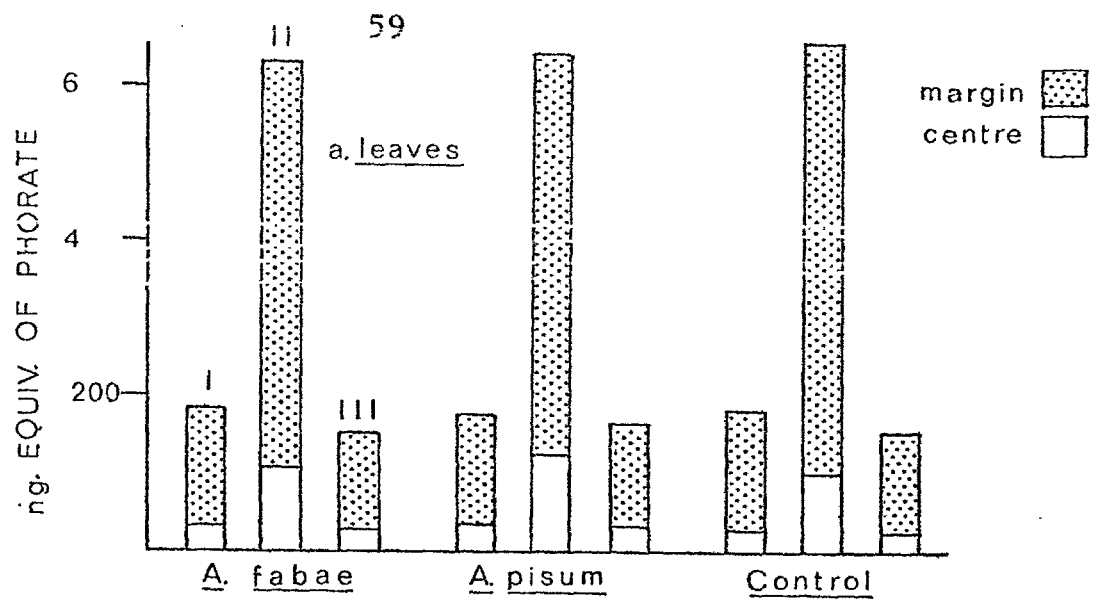


Fig. 2.6 Distribution of the solvent soluble fraction in the leaf, aphids and honeydew after root uptake. (Appendix 9)

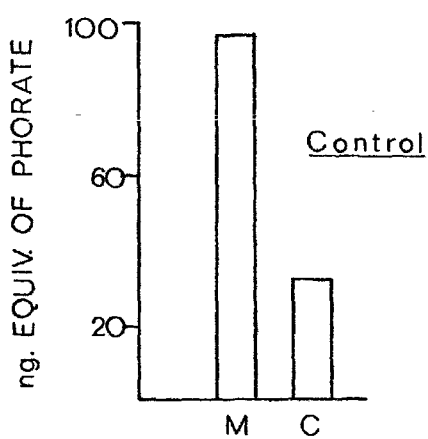
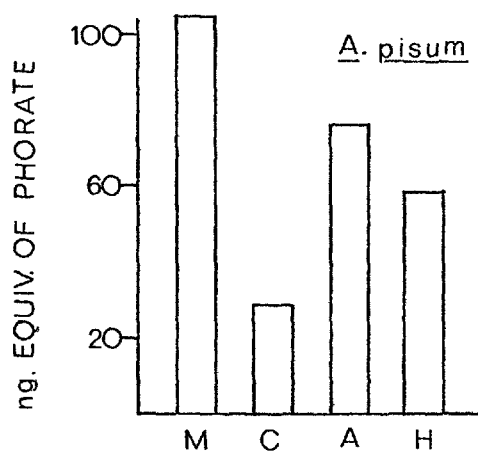
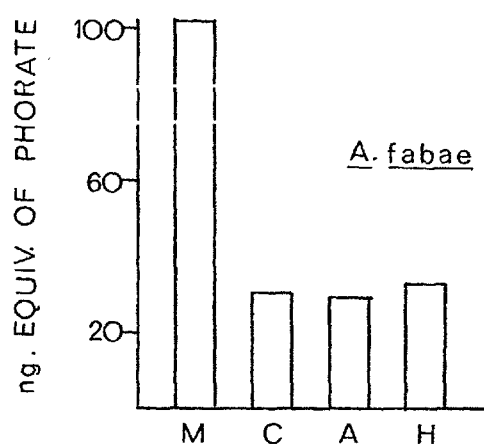


Fig 2.7 Distribution of hydrolysed metabolites in leaves supporting colonies of A. fabae and A. pisum, following root uptake. (Appendix 9)

M-Leaf margin C-Leaf centre

A-Aphids H-Honeydew

6. The effect of aphid sinks in relation to the translocation period.

The influence of aphid colonies on the movement of phorate was studied in relation to three different concentrations of insecticide acting for distinct translocation periods, after the addition of the insecticide to the nutrient solution.

Experimental procedure. Colonies of A. pisum were reared on both leaflets of the second node for seven days; at three, five and six days after the colonies were established, the insecticide was added to the nutrient solution in doses of 4 µg, 8 µg and 16 µg respectively. On the seventh day, the levels of radio-activity were assessed in the first three leaves, as well as in aphids and honeydew. Control plants were established for the shortest and longest translocation periods.

Results. Table 2.3 gives the concentration of the solvent soluble fraction of phorate during the three translocation periods and the respective proportions found in aphids and honeydew. The data suggest that there was a stronger initial uptake of these metabolites by the plants in the first 24 hours, when 13 per cent of the dose applied was found in the leaves. After two days the percentage of solvent soluble metabolites translocated corresponded to 7.5

per cent per day, and after four days the amounts present in the leaves corresponded to a rate of translocation of 4.6 per cent per day.

Table 2.3 Distribution of solvent soluble metabolites in the leaves, aphids and honeydew.

Time	Plant	ng. equivs. of phorate $\pm$ s.e				
		Leaf 1	Leaf 2	Leaf 3	Aphids	Honeydew
1 day	Infested	382 $\pm$ 177	644 $\pm$ 229	1072 $\pm$ 467	1.6 $\pm$ 0.6	0.45 $\pm$ 0.29
	Control	408 $\pm$ 237	639 $\pm$ 187	1061 $\pm$ 270		
2 days	Infested	106 $\pm$ 78	393 $\pm$ 220	662 $\pm$ 193	3.0 $\pm$ 0.8	0.8 $\pm$ 0.3
4 days	Infested	115 $\pm$ 62	250 $\pm$ 138	366 $\pm$ 160	3.5 $\pm$ 0.6	0.7 $\pm$ 0.2
	Control	107 $\pm$ 77	256 $\pm$ 145	322 $\pm$ 187		

The proportion of toxic metabolites found in the aphids increased with time; after one day the aphids and honeydew contained 0.3 per cent of the amount present in the infested leaf, of which 22 per cent was detected in the honeydew. By the second day the proportion in the colonies accounted for 0.9 per cent, with one fifth being eliminated and on the fourth day 1.6 per cent of the amount found in the leaf was present in the aphid sink, of which 17 per cent was excreted in the honeydew.

The distribution of water soluble metabolites along the leaves and the quantities removed by the aphid colonies during the three periods is shown in figure 2.8. The amounts of hydrolysed products in the leaves increased with time and their translocation was markedly influenced by the aphids. After one day, 61 per cent of the label entering the second leaf was absorbed by the aphids; and when this proportion is added to the amount present in the second leaf, it can be seen that 67 per cent of the total quantity of hydrolysed products found in the infested plants were translocated to the second leaf, in comparison with 26 present in the same leaf of the control plant.

Similar proportions of hydrolysed metabolites were found to have been translocated to the second leaf after two and four days. In the control plants the water soluble fraction had increased to 33 per cent of the quantity present in the plant but this still is only about half of the amount found in the second leaf of the infested plants.

The proportion of hydrolysed metabolites in the honeydew increased with time; after one day, 28 per cent of the quantity found in the sink was eliminated, while on the fourth day the proportion excreted was more than twice the amount present in the insects' bodies.

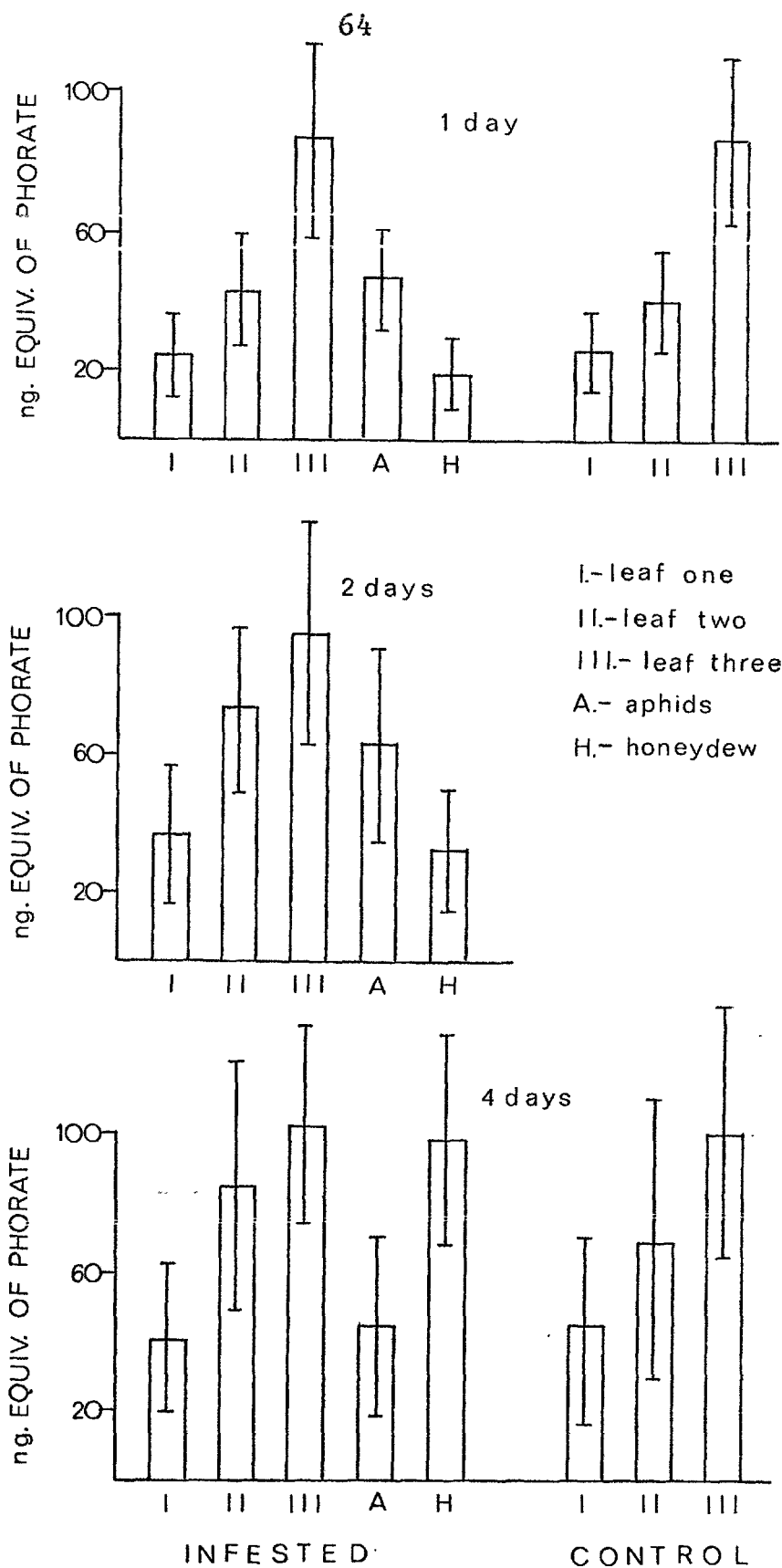


Fig. 2.8 Accumulation of hydrolysed metabolites in the leaves and quantities ingested and eliminated by A. pisum in relation to the translocation period, with 95% confidence limits. (Appendix 10)

7. Uptake and excretion of ^{14}C - phorate metabolites by A. pisum in relation to the time of establishment of the aphid sink.

The amount of solvent soluble and water soluble metabolites removed by aphid colonies established before the addition of the insecticide was compared with the quantities ingested by colonies of the same size and age which were transferred to the leaves at the time of treatment.

Experimental procedure. Two sets of plants were placed in nutrient solution and colonies of A. pisum were established on the second leaf of one group of plants for five days. At this time, colonies of five day old aphids were caged in the same position on the second set of plants, and eight μg of phorate were added to the nutrient solution of all plants. The insecticide was allowed to translocate for two days and at the end of this period the levels of radioactivity were estimated in the leaves supporting the colonies, aphids and honeydew.

Results. The most striking feature of this experiment was the relatively large proportion of mortality observed in the colonies caged at the time of application of phorate, which is shown in table 2.4 with the respective amounts of solvent soluble metabolites in the leaf, aphids and honeydew.

Table 2.4 Distribution of solvent soluble metabolites translocated to the leaves supporting aphid colonies.

Treatment	No. of aphids per plant	per cent mortality	ng. equivs of phorate $\pm$ s.e		
			Leaf	Aphids	Honeydew
with previous sink effect	104	0	393 $\pm$ 220	3.0 $\pm$ 0.8	0.8 $\pm$ 0.3
without previous sink effect	100	16	417 $\pm$ 237	1.3 $\pm$ 0.6	0.6 $\pm$ 0.4

The quantities of label concentrated in the leaves of both treatments were approximately the same; however the aphids established prior to the addition of phorate removed a larger amount of solvent soluble metabolites, partly reflecting the higher number of active individuals at the end of the experiment. The quantity ingested by the aphids born on the plants was twice the proportion removed by the other aphids, although the amounts retained in the insects and excreted in the honeydew were not proportional in the two cases.

The effect of both colonies on the movement of water soluble metabolites is shown in figure 2.9. Clearly, the colonies longer established had a greater

influence than those caged at the time of treatment.

The quantity of label removed by the former was twice the amount ingested by the latter.

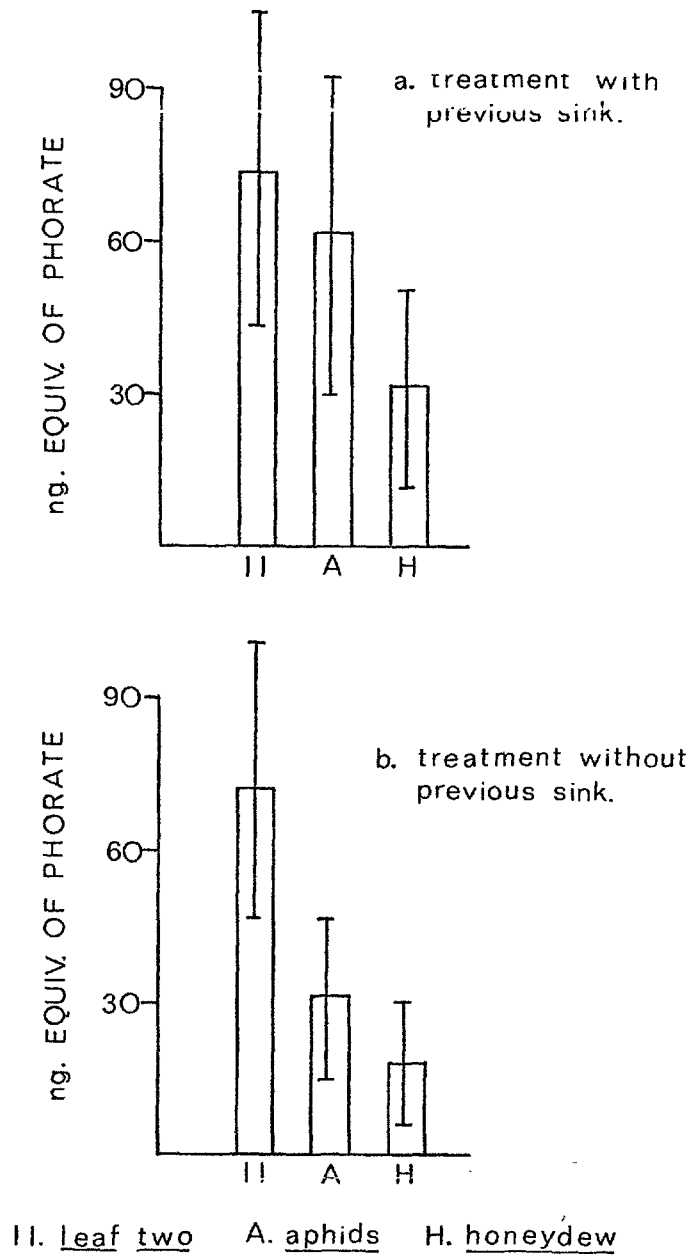


Fig. 2.9 Absorption and excretion of water soluble metabolites by colonies of *A. pisum* caged before and at the time of application of ^{14}C - phorate, with 95% confidence limits. (Appendix 11)

8. Export of phorate from a treated leaflet to an aphid sink on the adjacent leaflet.

The distribution of solvent soluble and water soluble metabolites was followed when an aphid sink was established on a leaflet adjacent to one treated with the insecticide. The amounts of label exported to the leaves above and below the source of phorate were also investigated.

Experimental procedure. Newly born larvae of A. pisum were reared for four days on one leaflet of the second node. At this time 3 μ g of phorate were spotted on the surface of the aphid-free leaflet of the same node. A set of plants without aphids followed the same treatment, as controls. The insecticide was left to translocate for three days. After this time, both leaflets of the third and first nodes were analysed and also the aphid-bearing leaflet of the second node. The aphids and honeydew were also analysed.

Results. Preliminary control experiments having aphids caged on other plants close to phorate treated leaflets showed no visible harmful effects arising from vapour action of the insecticide.

Accumulation of phorate in the leaves above and below the treated leaflet was extremely low in comparison to the concentration of the insecticide after

root uptake. However the influence of aphid sinks on the movement of phorate is more evident after leaf application as shown in figure 2.10. During the three day translocation period the aphid colony removed twice the amount of solvent soluble metabolites present in the leaflet supporting the colony. The honeydew contained 30 per cent of the quantity ingested. Figure 2.10a indicates a different pattern of distribution of these metabolites in the leaves of infested and control plants. It can be seen that more label accumulated in the leaflet supporting the colony, whereas more solvent soluble metabolites concentrated on the leaf above the source in the control treatment. The movement of water soluble metabolites was greatly influenced by the aphid colonies as shown in figure 2.10b. The quantity of hydrolysed products ingested by the colonies represented 24 times the amount found in the leaflet supporting the colony. The distribution of these metabolites in aphids and honeydew was quite distinctive from that presented by the solvent soluble fraction; in this case, 58 per cent of the hydrolysed metabolites ingested were eliminated in the honeydew. The pattern of distribution of these metabolites in the leaves was basically the same in infested and control plants.

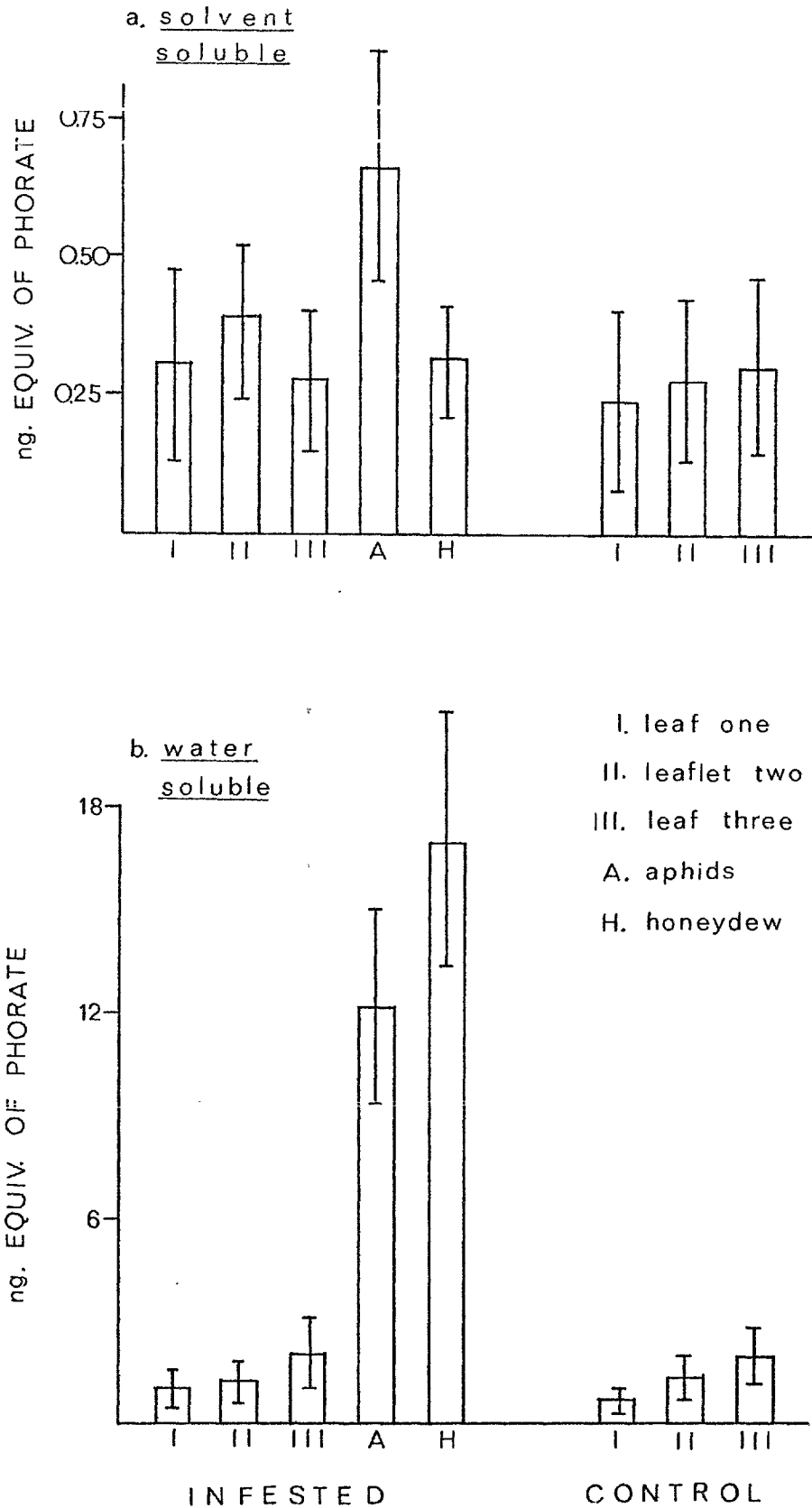


Fig. 2.10 Distribution of solvent and water soluble metabolites after application of phorate to a leaflet of the second node, with 95% confidence limits. (Appendix 12)

9. Movement of ^{14}C - phorate to aphid sinks established below the treated leaf.

Experimental procedure. Colonies of A. pisum were caged on both leaflets of the second node and after four days, 6 μg of labelled phorate were added to the third leaf. The insecticide was allowed to translocate for three days, and after this period the leaves supporting the colonies, roots, aphids and honeydew were analysed. Aphid-free plants were similarly treated and analysed.

The extraction of phorate and its metabolites from the roots followed a similar procedure to that described for the leaves using 20 ml of acetone-water 1:1 v/v and 40 ml of chloroform.

Results. Only a very small proportion of the applied insecticide was found in the second leaf and roots. Figure 2.11a shows the amounts of solvent soluble metabolites present in the samples analysed; the influence of the aphid colonies on the movement of these metabolites, despite their reduced mobility was noticeable when compared to the amounts exported in the control plants. The movement of solvent soluble metabolites was 4 times greater in the infested plants, most of it being removed by the colonies. The data indicate a slight accumulation of label in the leaves supporting the colonies in relation to the control

treatment. It was also noticeable that a proportion of these products accumulated in the roots of both infested and control plants, in similar quantities.

Of the toxic metabolites ingested by the colonies during the translocation period, 36 per cent had been excreted in the honeydew.

Water soluble metabolites were more mobile and therefore exported in larger quantities, as shown in figure 2.11b. The proportion of label in the leaf was insignificant in comparison with the quantities removed by the colonies; thus, the amount absorbed by the aphids corresponded to 28 times the quantity in the leaf. Unlike the solvent soluble metabolites, more hydrolysis products were excreted in the honeydew than retained in the insects' bodies; of the amount imbibed, 56 per cent had been eliminated by the colonies. Accumulation of water soluble metabolites in the roots showed different patterns between infested and control plants; the proportion of these metabolites in the roots of the control treatment was about 2.5 times greater than the quantity present in the roots of the infested plants, perhaps reflecting the influence of the aphids in reducing the movement of these metabolites to the roots.

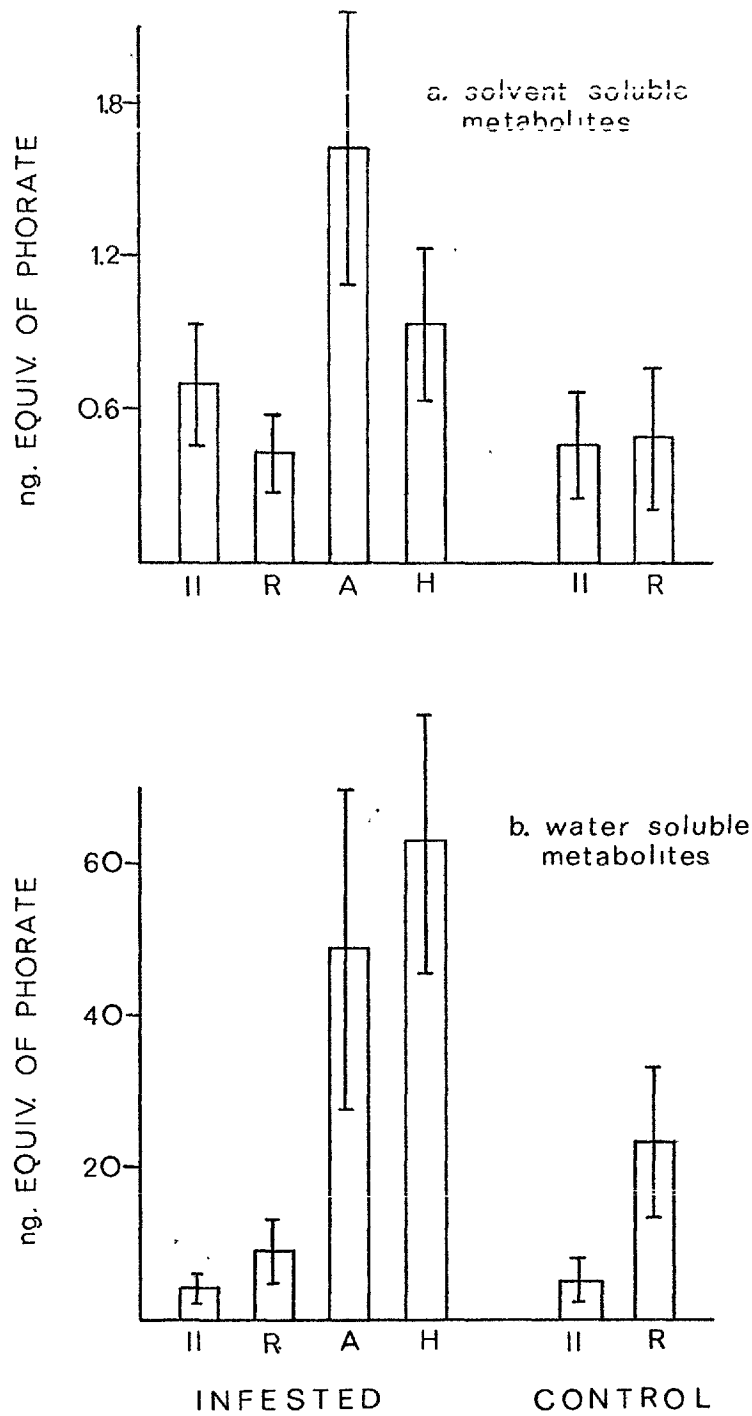


Fig. 2.11 Distribution of solvent soluble and water soluble metabolites of phorate in aphid sinks established below a treated leaf, with 95% confidence limits. (Appendix 13)

II-Leaf two; R-Roots;
A-Aphids; H-Honeydew.

10. Export of ^{14}C - phorate to aphid sinks formed above the treated leaf.

Experimental procedure. Colonies of A. pisum were established on the third leaf and when four days old, 6 μg of phorate were spotted on the second leaf. Analyses of the radio-active fractions of the infested leaf, aphids and honeydew were carried out after a three day translocation period. Export of label to the third leaf of aphid-free plants was also assessed.

Results. The quantity of solvent soluble metabolites accumulated in the third leaf of both treatments was negligible in relation to the dose applied, as indicated in figure 2.12a. The data shows that despite the low levels of these metabolites in the leaf, aphid colonies attracted almost three times the quantity of label accumulated in the leaf. Most of the solvent soluble products were retained in the insects' bodies; however a proportion of non-hydrolysed metabolites was excreted in the honeydew.

The effect of aphids on the movement of water soluble metabolites was considerable in comparison to the export of these products in the control treatment. Similar quantities were concentrated in the third leaf of both infested and control plants, as shown in figure 2.12b. The proportion removed by the aphids

however, corresponded to ten times the amount concentrated in the leaves. Excretion of water soluble metabolites accounted for 54 per cent of the quantity ingested by the colonies.

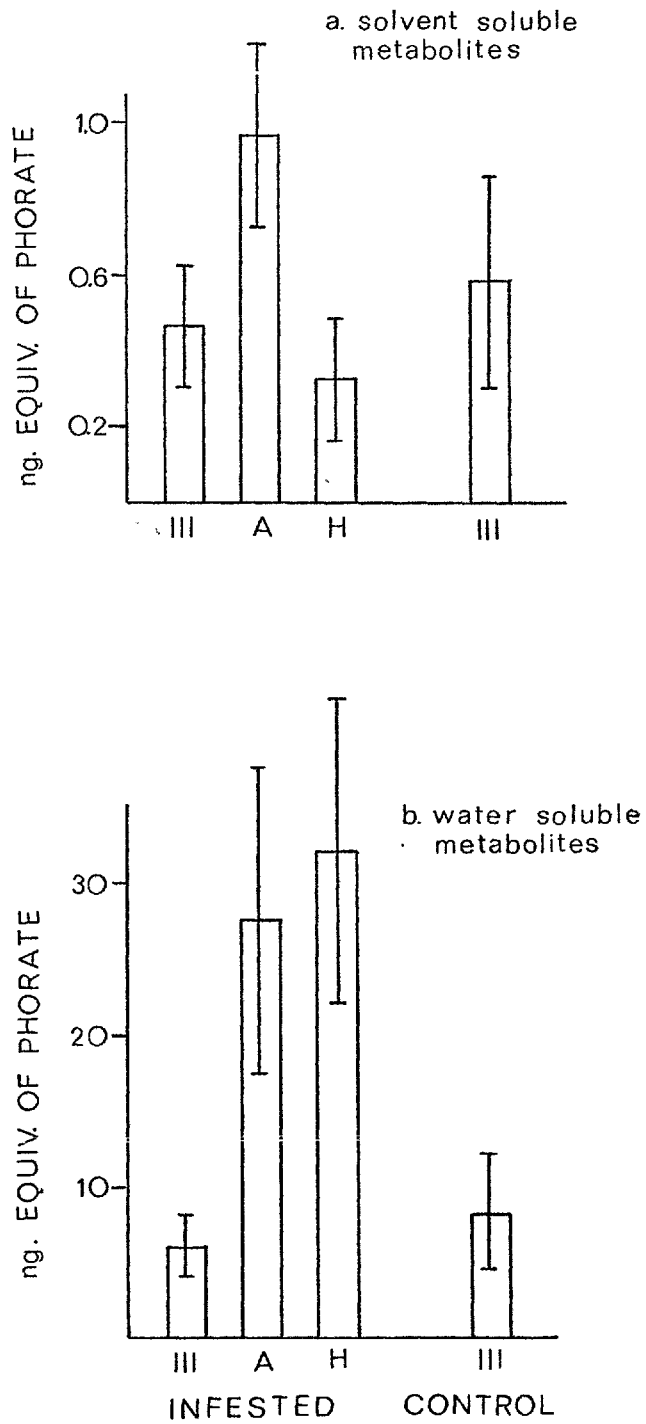


Fig. 2.12 Distribution of solvent and water soluble metabolites of phorate in aphid sinks established above the treated leaf, with 95% confidence limits. (Appendix 14)

III- Leaf three; A-Aphids; H-Honeydew.

11. Translocation of ^{14}C - phorate to two simultaneous aphid sinks.

The movement of phorate and its metabolites to aphid sinks established above and below the treated leaf was assessed, as well as the proportions concentrated in the leaves.

Experimental procedure. Colonies of A. pisum were established on both leaflets of the second and fourth nodes of four bean plants. After four days, 6 μg of ^{14}C - phorate were spotted on the third leaf and allowed to translocate for a further four day period.

Results. Although the number of adults caged initially for reproduction was the same on both leaves, the production of larvae in the 24 hour period was considerably smaller on the lower leaf. The average number of aphids born on the second leaf was 66, whereas the number on the fourth leaf was 93. This difference was not further investigated but a possible explanation may lie in the quality of food available.

Table 2.5 shows the distribution of total radioactivity translocated to the leaves above and below the source of ^{14}C - phorate. The amount of label exported to the second leaf was 3.3 times greater than to the fourth leaf. Most of the tracer exported to

the leaves was removed by the aphids, representing about 95 per cent of the total translocated in each case.

Table 2.5 Distribution of total radio-activity exported to the leaves supporting aphid colonies caged above and below the treated leaf.

Leaf position	ng. equivs. of phorate		
	Leaflets	Aphids	Honeydew
Leaf 2	3.4	21.2	66.0
Leaf 4	1.4	8.6	17.3

Export of solvent soluble metabolites to the infested leaves was small, as shown in figure 2.13a. Both aphid sinks however were able to remove a noticeable amount of these products in comparison with the proportions present in the leaves. Thus, the colonies feeding on the second leaf removed the equivalent of 34 picograms of phorate per aphid, whereas the aphids on the fourth leaf ingested 10.7 picogram equivalents of phorate per insect. Of the quantities ingested, 44 per cent of solvent soluble metabolites was excreted in the honeydew by the colonies caged on the second leaf, and those on the upper leaf excreted 39 per cent.

The distribution of water soluble metabolites in the leaves, aphids and honeydew is shown in figure 2.13b. Like the solvent soluble products, the proportion of hydrolysed metabolites translocated to the second leaf was about three times higher than the quantity present in the fourth leaf. Aphids feeding on the second leaf excreted 76 per cent of the water soluble products ingested, in contrast to 68 per cent eliminated in the honeydew produced by the aphids feeding on the fourth leaf.

The mean amounts of hydrolysed metabolites ingested by the aphids represented 1.3 ng equivalents of phorate per individual for the colonies on the lower leaf, and 0.3 ng equivalents per aphid for the colonies established on the upper leaf.

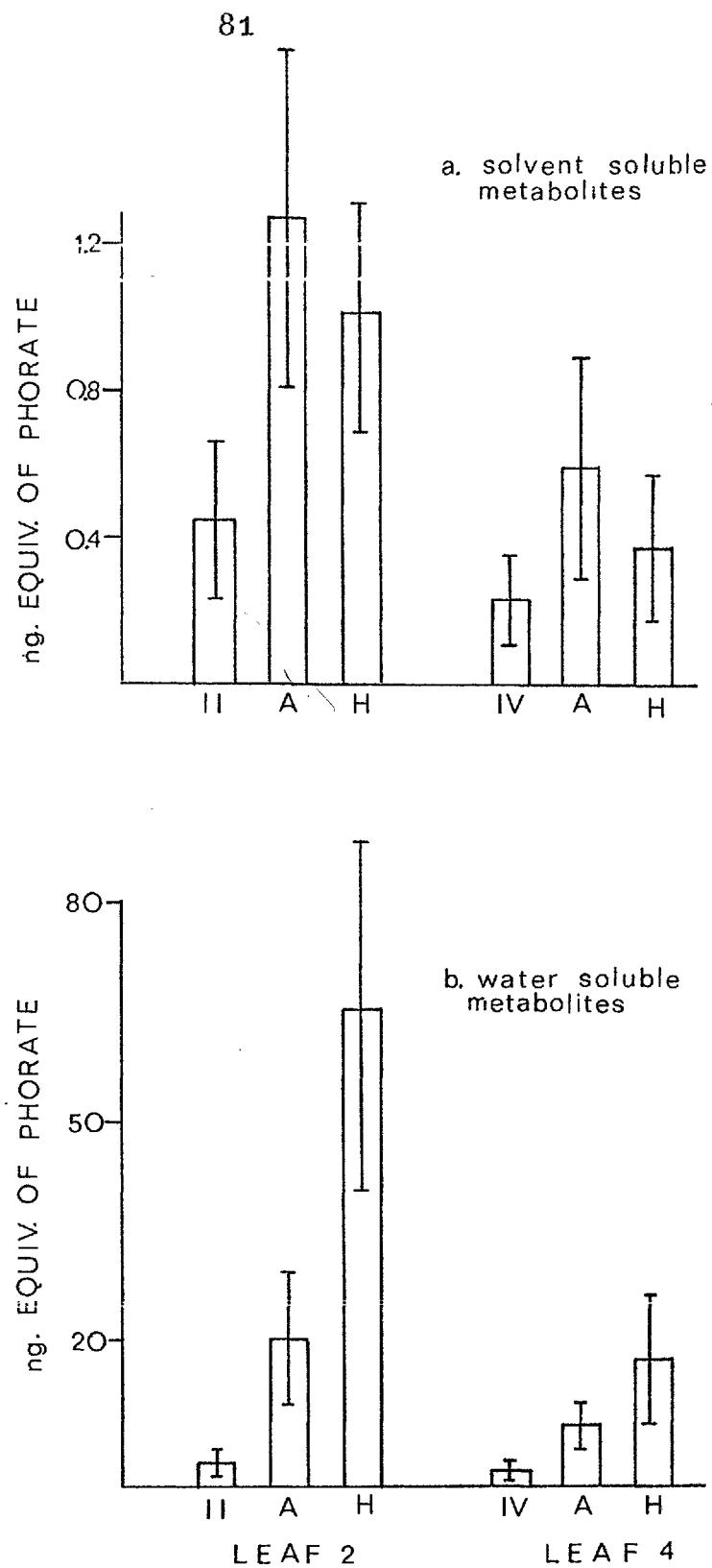


Fig. 2.13 Distribution of solvent and water soluble metabolites in aphid sinks established above and below a treated leaf, with 95% confidence limits. (Appendix 15)

II - Leaf two IV - Leaf four
 A - Aphids H - Honeydew

DISCUSSION AND CONCLUSIONS

Previous work on the aggregation behaviour and feeding mechanism of aphid species has shown that these insects can act as adventitious sinks by altering the movement of nutrients within the plant (Kennedy and Stroyan, 1959; Mittler, 1959; Way and Cammell, 1970). The results reported here assessed the extent to which aphid colonies influenced the uptake and movement of inorganic ions and insecticide molecules in bean plants.

Inorganic tracers

Both ^{32}P - phosphate and ^{35}S - sulphate were strongly attracted to the feeding sites established on the leaves, either when the radio-isotope was added to the nutrient solution or spotted on the surface of the leaf. Preliminary results by Galley and Foerster (1973) indicated that the effect of aphid sinks increased when the colonies had been feeding prior to the addition of radio-label to the nutrient solution, the greatest effect occurring with the longest established colonies. When aphid sinks were formed on a lower leaf, the proportion of label translocated to the young upper leaves was reduced in comparison to aphid-free plants.

Further experiments on root uptake and leaf

absorption of both isotopes are described here. The amounts of ^{35}S removed by successive aphid colonies feeding on the second leaf increased with time, as the accumulation of radio-label in the leaf increased. The distribution of ^{35}S in the other leaves however did not seem to be significantly affected, despite the considerable quantities of tracer diverted to the infested leaf. A possible explanation for this fact would lie in the availability of ^{35}S in the nutrient solution throughout the experimental period to supply the leaves above and below the aphid sink.

The levels of ^{35}S absorbed from the nutrient solution and translocated to aphid-free leaves was followed for six days, after which aphid colonies were allowed to feed on the labelled leaflets of the first node for a further four days. Similarly, even though the aphids absorbed significant amounts of tracer in relation to the control treatment, there was no noticeable difference in the levels of ^{35}S accumulating in the leaves above the aphid sink before or during the feeding period.

The export of leaf applied ^{35}S was studied when aphid colonies were established above or below the treated leaf; the amount of tracer attracted to the leaf supporting the colonies was markedly higher than

to aphid-free leaves, irrespective of their position in relation to the source of label. Distribution of ^{35}S within the plant after leaf treatment was mainly toward the roots where approximately 40 per cent of the amount recovered had accumulated.

From the data obtained it seems that ^{35}S moves freely both in the xylem and phloem. When applied to the nutrient solution ^{35}S was readily absorbed by the roots and transported to the leaves with the transpiration stream, while leaf absorption resulted in movement out of the treated leaf and into the stem and roots with the assimilate stream, via the phloem.

Aphid sinks had a similar effect on the movement of ^{32}P , though its distribution within the plant was notably different from that found for ^{35}S . Compared with other ions, phosphorus is particularly mobile being retranslocated extensively (Fisher, 1967). This fact may explain its more even distribution between the different leaves following root uptake (Galley and Foerster, 1973) in comparison to the apically dominant distribution of ^{35}S .

As was previously shown for root uptake (Galley and Foerster, 1973) the presence of aphid colonies established before a foliar treatment had a greater effect on the movement of ^{32}P than colonies caged at

the time of treatment. This difference could be attributed to some extent to an initial slow feeding rate of the aphids, since it takes between 12 - 16 hours for A. fabae to reach their normal feeding rate (Banks and Nixon, 1959). However aphid colonies caged one day before the isotope application also removed less label than those caged three days beforehand, indicating that the full sink effect was not established until sometime later, even though the aphids were probably feeding at their normal rate by the time of treatment.

It is likely that the enhanced movement of radio-label to aphid sinks is due to alterations in the physiological processes of plants. Kloft et al. (1968) have suggested that aphid feeding directly affects the mechanisms of transpiration and respiration of the host plant. Way and Cammell (1970) referring to the sink capacity of old leaves stated that in normal conditions the potential supply of photosynthetic products may often exceed the sink capacity, with the latter factor regulating the production of assimilates. The increase in the drain of nutrients by an aphid sink however causes the assimilation rate to increase, with a consequent enhancement in the transpiration rate. This transpiration flow will also bring with it a proportion of isotope from the

labelled root system which would be used by the plant to contribute to the osmotic potential in the sink system.

Despite the considerable amounts of radio-tracer attracted to infested leaves there is little evidence that either ^{32}P or ^{35}S accumulated in the leaf area occupied by the cage; an autoradiograph of a leaflet prepared 12 hours after the removal of an aphid colony did not show any significant difference in the concentration of ^{32}P in the caged area in relation to the concentration in an aphid-free leaflet. Similarly, no clear evidence of accumulation of ^{35}S could be found 30 minutes after the aphids were removed (Galley and Foerster, 1973), indicating that both isotopes were transported to the infested areas of the leaves at the same rate at which they were taken up by the colonies.

Large proportions of label were excreted in the honeydew. Between 68 and 76 per cent of the ^{35}S imbibed by the aphids were found to have been excreted after two and four days feeding on labelled tissue. The proportions of ^{32}P eliminated were reduced in relation to ^{35}S . After three days, previously established aphid colonies excreted 63 per cent of the quantity ingested, whereas colonies caged at the time of treatment eliminated 57 per cent during the same

feeding period. Differences in the rates of excretion of the two isotopes may well reflect the interactions of a given element within the insects, particularly phosphorus which participates in many processes involving energy transfer reactions. Absolute comparisons between the rates of uptake and excretion cannot be made since in these experiments different feeding times and methods of application were used. However the data obtained provides useful information on the levels of uptake and excretion by aphids, since in toxicological terms it gives a rough idea not only of the ability of these insects to absorb a toxicant present in the sap, but also because the honeydew represents a route of elimination for these substances.

Phorate and metabolites

The performance of a systemic insecticide is directly dependent upon interactions between the chemical, plant and insect complexes. Factors such as rates of uptake and translocation, accumulation, activation and detoxification will dictate the efficiency of a toxicant on the insect pest. Despite the recent advances in methodology and instrumentation, a great deal of clarification is needed on the exact processes involved in the movement of exogenous substances within the conducting system of plants. As Peel (1972) pointed out, much of the basic information

concerning transport in plants was obtained during the first three decades of the present century and since then remarkably few advances have been made.

A preliminary assessment of the behaviour of phorate within the plant clearly confirmed its apoplastic characteristics. Phorate was rapidly absorbed from the nutrient solution and distributed in an apically dominant pattern. After 24 hours of root uptake, 84 per cent of the insecticide present in the leaves had oxidised and nine per cent remained in the form of the parent compound. Hydrolysis was also rapid; after the same period, seven per cent of the radio-activity was present in the water soluble fraction of the extract. It is likely that some of the hydrolysis products were formed in the nutrient solution and were subsequently absorbed by the plants; further experiments with leaf application however demonstrated that hydrolysis occurred readily in the plants. Hacskeylo et al. (1961) found that after two days in nutrient solution, 32 per cent of the phorate present had been hydrolysed.

Estimation by T.L.C. of the relative proportions of phorate and its toxic metabolites present in treated plants indicated that most of the products found in the leaves consisted of phorate, its sulphoxide and sulphone. Phoratoxon metabolites,

predominantly the sulphoxide did occur however and formed a small, though not insignificant proportion of the total solvent soluble fraction. In the circumstances it was felt justified in restricting the analysis of solvent soluble fractions to the parent thio analogue and its sulphoxide and sulphone.

This step both simplified and shortened the analytical procedure and enabled a greater range of experiments to be attempted. The oxygen analogue and its side chain oxidised forms taken together probably formed about one third of the phorate sulphoxide fraction over the shorter translocation times employed here and when present would have shown up in the analysis in this fraction.

The distribution of labelled phorate and its metabolites within the leaf was followed by gross autoradiography after root uptake for different periods up to seven days. The results indicated that accumulation of the tracer occurred rapidly around the periphery of the leaf as has been demonstrated by de Pietri-Tonelli (1965) with dimethoate. The effect of a toxicant with such a pattern of distribution on insects feeding some distance away from the source of photosynthate will thus be greatly reduced due to its inability to move in the assimilate stream. Moreover, according to Crisp (1972), the large volume

of xylem tissue represents large diluent volumes for apoplastic insecticides. The volume of phloem tissue however is comparatively small and thus the dilution effect would be much reduced and the total amount of toxicant needed to reach a lethal concentration would be considerably reduced. This aspect is of particular importance in the control of aphids, since the majority of the species feed from phloem tissues (Auclair, 1963; Lowe, 1967; Pollard, 1973).

The detection of phorate and its toxic metabolites in aphids and honeydew however indicates that some leakage of the insecticide must have occurred. According to Peel and Weatherley (1962), the loss of sap from an aphid sink in a sieve element is replaced by two fluxes of solutes; a lateral movement from surrounding elements and a longitudinal movement down the pierced element. It seems therefore that this leak may be induced or accentuated by the nutrient sink developed by the feeding aphids. The movement of solutes from xylem to phloem may occur over a considerable distance from a large aphid colony, since the contributory length within the phloem described by Weatherley et al. (1959) for an individual aphid may extend for many centimetres along the pierced element on either side of the aphid stylets. Peel (1972) discussing the control of solute movement in the phloem stated that any

solute which is able to penetrate into the sieve tubes is capable of being subsequently transported. After investigating the relative mobility of sugars, aminoacids, ions and growth regulators into sieve elements, this author concluded that if synthetic substances such as pesticides can be induced to enter the sieve tubes then they too will be transported. A similar conclusion was reached by Crisp (1972).

The effect of aphids on the distribution of phorate after root uptake

The apoplastic tendency of phorate and the large amounts of solvent soluble toxic metabolites present in the leaves obscured any slight influence the colonies may have had on the ascending insecticide. Thus, after an 18 hour translocation period, colonies of A. fabae ingested 0.4 per cent and A. pisum 0.34 per cent of the solvent soluble metabolites accumulated in the infested leaves. However there were more A. fabae than A. pisum, and assuming that the uptake of label was uniform between the individuals of each species then the mean quantity of solvent soluble metabolites ingested per individual of A. pisum was 15 per cent higher than in A. fabae. Differences in the uptake of isotopes by aphids feeding on radio-labelled plants have been found by other workers

(Watson and Nixon, 1953; Banks and Nixon, 1959), even when the insects fed on the same radio-active surface for the same time (Watson and Nixon, 1953).

The levels of excretion of solvent soluble metabolites were similar between the two species. It is notable that after 18 hours about 30 per cent of the toxic metabolites detected in the aphid sinks had been eliminated in the form of non-hydrolysed metabolites. Separation and identification of the toxic metabolites indicated that they were present in proportions which followed similar patterns in plants, aphids and honeydew. Phorate sulphoxide was the major toxic metabolite followed by phorate, which was more abundant than the sulphone; but it must be borne in mind here that a proportion of the sulphoxide was made up of the oxygen analogue metabolites. The relative proportion of the sulphoxide in the plants however was markedly higher in comparison with the proportions present in the aphids and honeydew of both species. This relative difference in the sulphoxide might represent some degree of detoxification by the insects, since according to numerous workers (Mitchell et al. 1960; Bull, 1965; Bowman et al. 1969) the sulphoxide is more readily hydrolysed and therefore could be more readily degraded by the aphid to water soluble products. This

would be particularly so if the aphid gut is a more favourable environment for hydrolytic reactions. Little is known however about the physiological conditions in the digestive system of aphids, but acid or alkaline conditions favourable for hydrolysis might occur. Recent work with Myzus persicae has indicated the presence of free ammonium ion in the honeydew (Hussain et al. 1974a).

The influence of aphid colonies on the distribution of toxic products within the caged area of the leaf was also small; nevertheless it demonstrated that after two days translocation, less than 20 per cent of the solvent soluble metabolites present in the infested leaves was distributed within the area occupied by the cage.

The transport of water soluble metabolites into the infested leaves was however greatly enhanced by the aphid colonies in comparison with the appearance of these compounds in aphid-free plants. Comparative estimations on the uptake of A. fabae and A. pisum showed that the latter species imbibed significantly more water soluble metabolites than did A. fabae, even though the amounts of these products eliminated in the honeydew were markedly lower in A. pisum. Thus, after 18 hours translocation, A. fabae eliminated 44 per cent of the hydrolysis products

ingested in comparison with 26 per cent excreted by A. pisum. It is not known what proportions of these quantities had been hydrolysed by the insects, though it was probably very little. The abundance of hydrolysis products in the leaves and their higher mobility in the phloem suggest that most of the water soluble metabolites found in the aphids and their honeydew were removed from the plant in this form.

The differences in the uptake and excretion of water soluble metabolites between A. fabae and A. pisum must play an important role in toxicological studies, since a greater ingestion of sap is correlated with an increased uptake of toxicant. Very little is known however about the fate of systemic insecticides after ingestion by aphids. The different patterns of retention and excretion between these two species may well represent different rates of detoxification.

The effect of aphids on the distribution of phorate after leaf application.

The influence of aphid colonies on the movement of phorate and its metabolites was more evident when the labelled insecticide was applied to the surface of the leaves. In aphid-free plants, the export of phorate following leaf application was

negligible in relation to the dose applied.

Colonies of A. pisum established on different leaves however were able to attract significant quantities of both solvent and water soluble metabolites, irrespective of their position in relation to the source of ^{14}C - phorate. The amounts of hydrolysed metabolites exported to the aphid sinks and their accumulation in the roots suggest that these products were translocated in a similar manner to ^{32}P and ^{35}S . Pesticide molecules on the other hand are larger and less readily transported; the results obtained however do indicate that aphid sinks were able to enhance the movement of these compounds out of the treated leaf and into the sinks.

When two aphid colonies were established simultaneously above and below a treated leaf, movement of solvent soluble and water soluble metabolites occurred to both aphid sinks, although downward movement was noticeably higher. Bidirectional movement of substances has been found to occur simultaneously in different bundles of the phloem (Biddulph and Cory, 1965; Eschrich, 1967; Petterson and Currier, 1969). Biddulph and Cory (1965) were able to demonstrate the mechanism of bidirectional transport in relation to the anatomy of the phloem. They concluded that radio-label applied to a mature

leaf of intermediate position moved downwards until anastomosing of the bundles allowed a division into upward- and downward- moving components. The pathway of movement however varies in relation to the position of the treated leaf; old leaves export primarily to the roots, while young upper leaves export to the stem apex (Crafts, 1967a). The greater effect of the aphid colonies caged below the source of phorate may therefore be due to the physiological status of the source leaf in relation to the aphid and natural plant sinks.

The comparatively small amounts of toxic metabolites exported from treated leaves suggest that the hydrolysed products were formed in the treated leaves and subsequently exported rather than being hydrolysed in the sink. Some hydrolysis should be expected in the infested leaves and aphids, but the small amounts of solvent soluble metabolites present seem to indicate that most of the hydrolysed products entered the aphid sink in this form.

Aphid feeding in relation to the presence of the insecticide.

A critical factor in this study was the interference of the insecticide with the feeding behaviour of the aphids. Despite the large amount of work on the feeding mechanism of aphids, comparatively little

is known about the influence of systemic insecticides on the feeding behaviour of these insects. Most of the work so far has been done with one aphid species (Myzus persicae) using artificial feeding techniques (Mittler and Pennell, 1964; Parry and Ford, 1967, 1969, 1971) and one insecticide, phosphamidon. Even less is known about similar effects on living plants. It is likely however that different aphid species will show different degrees of susceptibility in relation to other systemic insecticides.

It is not known to what extent the presence of phorate affected the feeding process of the aphids during this work. Sub-lethal doses were used, but in a few of the experiments some mortality could have been expected, had the aphids been allowed to feed for longer periods. The increase in uptake of solvent and water soluble metabolites by A. pisum seems to suggest that the presence of phorate exerted little influence on the feeding process of the colonies. It was noticeable however that colonies caged at the time of treatment exhibited a different response to the insecticide than those previously established on the plants. The colonies born and reared on the treated plants removed more solvent soluble and water soluble metabolites and were better able to survive than the colonies established later, in which there

was 15 per cent mortality. The reduced uptake by the latter colonies is easily understood, since the amount of radio-activity could not increase so rapidly once part of the population had died. The reason for this higher susceptibility may well be associated with a repellent effect during the initial probing process before settlement, whereas the colonies previously established were feeding at their normal rate by the time of treatment. Halimie and Ford (1972) studying the uptake of phosphamidon by Myzus persicae on radish plants suggested that either the ingested insecticide reduces feeding as a result of malfunctioning or the insecticide acts as a repellent after being detected by chemosensory receptors during probing. It could also be postulated that the established sink system which is present when aphids have been caged prior to the treatment, affects a greater part of the plant symplast. In this system mainly water soluble metabolites which have been shown to be drawn readily by the sink are present. A freshly established colony once feeding will take time to exert such an extensive influence on the plant and the local sink conditions will draw more heavily on the xylem to replace lost turgor. Thus higher concentrations of solvent soluble and therefore toxic compounds could appear in the phloem by solvent drag

across the xylem / phloem boundary to be withdrawn by the aphids.

The results obtained here with phorate are not conclusive and further work fell rather beyond the scope of this project; more research is evidently required to clarify the interactions between systemic insecticides and the feeding behaviour of aphids.

Hydrolysis of phorate in plants and insects

Little is known of the ability of insects to hydrolyse insecticide molecules; Bowman and Casida (1958) studying the metabolism of ^{32}P - phorate in bean plants and armyworms (Prodenia eridania) concluded that the insects were more efficient than the plants in the hydrolysis of this compound.

Their results indicated that the major hydrolytic products found in plants and insects were O,O- diethyl phosphorothioic acid and O,O- diethyl phosphoric acid. The hydrolytic products of Di-Syston, a close relative of phorate in cotton plants and boll weevils (Anthonomus grandis Boheman) were also similar both in plants and insects (Bull, 1965). The water soluble metabolites of phorate were not further identified during this work, but since the labelled carbon was in the methylene group it is likely that the hydrolytic metabolites consisted of

the sulphide containing side chain, together with sulphoxides and sulphones all having a sulphydril or hydroxide group at the point of cleavage from the parent molecule. The chemical structures of the hydrolysed products were given before in the introduction.

The increased research on the identification of such compounds seems justified since it could provide useful information on the structural design of new pesticide molecules with symplastic characteristics.

In conclusion, it can be said that aphid sinks had little effect on the movement of large toxic molecules, especially on the xylem transported compounds from the roots, though leaf applied phorate was withdrawn from the source leaf by the aphid sink. The solvent soluble products, despite their different polarities were not differentially attracted to the sink. Only the water soluble metabolites and similarly ^{32}P and ^{35}S most probably present as phosphate and sulphate, appeared sufficiently polar to penetrate the symplast and be drawn with the phloem contents to the aphid sink.

In practical terms, root applications give more uniform distribution of toxic material within a plant and therefore, provided the dose is adequate,

are more effective in pest control procedures. Foliar applications may be less effective since larger doses of chemical are required in order to ensure that any infested leaves which may have escaped treatment receive an adequate dose. This work has shown that in these circumstances established aphid populations are more effective in attracting the pesticide than aphid populations established subsequently.

SUMMARY

Inorganic tracers

Studies on the effect of A. fabae on the movement of ^{32}P - phosphate and ^{35}S - sulphate showed that aphid colonies were able to attract considerable amounts of tracer to the infested leaves in comparison to the quantities translocated to aphid-free leaves. After a two day translocation period the proportion of ^{35}S which entered an infested leaf corresponded to approximately five times the quantity found in aphid-free leaves, of which 80 per cent was removed by the aphids. There was no noticeable difference in the distribution of ^{35}S in the leaves of infested and control plants.

The gross distribution of ^{35}S within the plant after root uptake showed an apically dominant pattern; aphid colonies caged subsequently for four days on the lower leaf however attracted a markedly higher quantity of label to this leaf, even though accumulation of label in the upper leaves was not affected.

The movement of ^{35}S was similarly enhanced when aphid sinks were established above or below a treated leaf, irrespective of the position of the colonies in relation to the source of ^{35}S . Accumulation of label in this case was mainly in the roots and stem.

Previously established aphid colonies had a

greater influence on the translocation of ^{32}P from a treated leaf than did colonies caged at the time of treatment. Auto-radiographic studies failed to show any noticeable difference in accumulation of ^{32}P after a colony was removed from the leaf.

^{14}C - phorate

Analysis of the toxic fraction of phorate by T.L.C. indicated that phorate sulphoxide was the major metabolite present in the leaves after one, four and seven days. Phoratoxon sulphoxide and phoratoxon sulphone increased with time and by the seventh day they represented about 20 per cent of the total toxic metabolites found in the leaves. The distribution of phorate and its metabolites in the leaves after root uptake followed an apically dominant pattern. Accumulation of label within leaves occurred rapidly around the marginal area, where after seven days translocation, 87 per cent of the ^{14}C present was found. One tenth of this amount was composed of water soluble metabolites. These water soluble metabolites were more uniformly distributed, and in the central area formed about a half of the radio-activity present.

The small effect of aphid colonies on the movement of solvent soluble metabolites was

obscured by the large amounts of insecticide accumulated in the infested leaves after root uptake. The quantities of toxic metabolites found in A. fabae and A. pisum represented less than 0.5 per cent of the quantity present in the infested leaves after an 18 hour translocation period. The amounts of toxic metabolites ingested by A. pisum colonies increased with the translocation period.

Water soluble metabolites appeared to be more mobile and were withdrawn by aphid sinks in large amounts. After feeding for 18 and 48 hours on labelled tissue, A. pisum imbibed considerably more water soluble metabolites than did A. fabae, even though the latter species eliminated a proportionally higher amount of these products in the honeydew.

Colonies of A. pisum established before the addition of phorate to the nutrient solution removed more toxic and water soluble metabolites than similar colonies caged at the time of treatment. These latter colonies seemed to be more affected by the presence of the insecticide than those previously established.

Little phorate was exported from a treated leaf when compared to the dose applied; colonies of A. pisum established at different leaves in relation to the treated leaf however enhanced the movement of both solvent soluble and water soluble metabolites

to the feeding sites. When aphid sinks were simultaneously formed on the leaves above and below a treated leaf, considerably more label was attracted to the colonies feeding on the lower leaf.

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Appendix 1. (Fig 1.1)

Distribution of ^{35}S in the leaves and quantities removed by aphid colonies in relation to time.

DPM/cm² of leaf tissue

Replicate	Time (days)	Leaf 1	Leaf 2	Leaf 3	Aphids	Honeydew
Plant 1	2	5889	8181	15561	13943	23190
	4	10852	18305	22839	23075	36221
	6	14275	24599	24230	29038	58343
Plant 2	2	2934	5097	8867	8321	32470
	4	6595	12983	16987	13770	46132
	6	8891	15291	22818	19940	55379
Plant 3	2	3431	9044	10133	7239	25366
	4	7643	20131	26124	15489	35976
	6	11288	19763	30126	22145	48874
Control 1	2	1951	3986	7916		
	4	3978	12884	17442		
	6	11237	15391	23108		
Control 2	2	3364	8042	15013		
	4	6521	18214	23081		
	6	15036	22176	27102		
Control 3	2	2986	4268	12358		
	4	4376	13621	15972		
	6	8879	19317	21931		

^{35}S distribution in the leaves. The efficiency of the Geiger-Muller detector for whole leaf tissue was derived by homogenising the counted area and recounting the homogenate in liquid scintillant. The mean efficiency of the Geiger-Muller detector by this method was 0.5%.

Appendix 2. (Fig. 1.2)

Distribution of ^{35}S in the leaves and quantities removed by aphid colonies feeding on the lower leaf.

DPM/cm² of leaf tissue

Replicate	Sample	Time (days)			
		2	4	6	10
Plant 1	Leaf 1	1749	3061	7420	9433
	Leaf 2	3478	4979	9582	12144
	Leaf 3	5891	10585	17920	19214
	Aphids				10514
	Honeydew				34740
Plant 2	Leaf 1	2675	3952	8504	12274
	Leaf 2	3634	6194	11953	15088
	Leaf 3	7443	9987	14442	22688
	Aphids				15007
	Honeydew				45108
Control	Leaf 1	1310	3847	6917	9519
	Leaf 2	3109	5724	9377	12214
	Leaf 3	5868	8808	13713	19086

^{35}S distribution in leaves. As in appendix 1

Appendix 3. (Fig. 1.3)

Export of ^{35}S from the second leaf and quantities removed by aphid colonies caged above or below the leaf source.

Aphid sinks on the first leaf			
	D P M		
	Plant 1	Plant 2	Plant 3
Leaf 1	3216	4618	3824
Leaf 3	2128	2071	1394
Roots	30118	19684	37226
Stem	9235	13268	8347
Aphids + Honeydew	5226	6628	3894
Aphid sinks on the third leaf			
Leaf 1	3318	2297	1479
Leaf 3	6127	6642	3965
Roots	19691	47215	36285
Stem	8226	15274	9897
Aphids + Honeydew	7268	3793	4826
Control Plants			
Leaf 1	1942	2032	3110
Leaf 3	5216	2796	4064
Roots	30566	22148	42070
Stem	8142	11256	15948

Appendix 4. (Fig. 1.4)

Distribution of ^{32}P in plants and proportions removed by aphid colonies established before and during treatment.

	D P M				
Sample	Plants with previous sink		Plants without previous sink		Control
	Plant 1	Plant 2	Plant 1	Plant 2	
Leaf 1	1843	2519	1721	932	ND
Leaf 3	1277	1553	866	588	2246
Roots	19931	14976	14433	19234	16495
Stem	11144	8830	9080	14818	11845
Aphids	9479	11610	3773	6594	
Honeydew	16804	19226	5038	8948	

Appendix 5. (Table 2.1)

Distribution of phorate and its oxidation products
in bean leaves.

Metabolite	D P M		
	Time (Days)		
	1	4	7
P = SS	2542	2377	2121
P = SSO	7467	7161	6317
P = SSO ₂	1786	2108	2324
P = QS	105	42	21
P = QSO	1025	1348	1985
P = QSO ₂	165	369	526

Appendix 6. (Fig. 2.1)

Distribution of phorate and metabolites in bean leaves after 24 hours translocation.

Leaf position	Metabolite	D P M			
		Plant 1	Plant 2	Plant 3	Plant 4
Leaf 1	P = SS	7125	3978	6104	7221
	P = SSO	17380	22348	30146	18947
	P = SSO ₂	7832	3288	4467	4126
	WS	4137	2813	3195	2364
Leaf 2	P = SS	11243	9021	11243	7309
	P = SSO	32281	23987	37126	26303
	P = SSO ₂	7104	3989	6336	7200
	WS	4220	2813	3335	6631
Leaf 3	P = SS	18048	10677	13825	11230
	P = SSO	82568	69977	84137	92108
	P = SSO ₂	11147	6712	7661	8250
	WS	10141	6328	5986	9200

Appendix 7. (Fig. 2.2)

Distribution of phorate and its metabolites within the leaf.

Time (Days)	Leaf part	METABOLITE			
		D P M			
		P = SS	P = SSO	P = SSO ₂	W S
$\frac{1}{2}$	centre	1117	4791	986	522
	margin	2871	4998	1914	928
1	centre	2343	10773	1642	1640
	margin	7026	14522	5384	3513
2	centre	1752	13554	1751	2802
	margin	8756	25675	7356	8406
4	centre	1749	9796	1748	3498
	margin	11370	39180	9620	10495
7	centre	1113	3808	1123	6135
	margin	14475	59568	10021	15020

Appendix 8. (Figs. 2.4 and 2.5)

Distribution of phorate and its metabolites in leaves, aphids and honeydew.

Sample	Metabolite	D P M					
		<u>A. fabae</u>			<u>A. pisum</u>		
		Plant 1	Plant 2	Plant 3	Plant 1	Plant 2	Plant 3
Leaf 2	P = S,S	8430	7974	12106	7341	5932	10028
	P = S,SO	40078	28534	33871	33919	35105	41088
	P = S,SO ₂	7072	5937	9206	5876	4672	8293
	WS	6119	4038	4628	4576	5574	6032
Aphids	P = S,S	30	43	51	31	22	37
	P = S,SO	77	83	59	68	56	74
	P = S,SO ₂	28	50	32	24	20	33
	WS	1745	918	1206	2241	3217	2876
	No. of aphids	132	126	135	100	96	93
Honeydew	P = S,S	14	24	18	22	16	14
	P = S,SO	26	31	23	26	20	31
	P = S,SO ₂	20	16	12	19	10	13
	WS	1338	803	1023	904	880	1228

WS = water soluble metabolites

Appendix 9. (Figs. 2.6 and 2.7)

Distribution of phorate and metabolites in the caged area and margin of the leaves and quantities removed by aphid colonies.

Replicate	Metabolite	D P M									
		<u>A. fabae</u>				<u>A. pisum</u>				Control	
		Margin	Centre	Aphids	Honeydew	Margin	Centre	Aphids	Honeydew	Margin	Centre
Plant 1	P = SS	17612	2725	51	25	10388	4155	47	24	16415	3754
	P = SS ₀	48332	11655	73	28	43367	13333	79	42	44488	6871
	P = SS ₀ ₂	15395	1950	38	11	9528	2560	28	18	14625	2110
	WS	10178	3301	3257	3876	7735	2435	7740	5038	7151	2001
Plant 2	P = SS	10349	3592	59	18	16480	1894	54	26	10959	2358
	P = SS ₀	59824	6137	85	38	58184	6911	77	33	62077	10616
	P = SS ₀ ₂	17903	3652	37	16	15387	3346	35	23	18681	3107
	WS	9180	2439	2320	2987	12443	2973	6838	5742	10981	3897

Appendix 10. (Fig. 2.8 and table 2.3)

Distribution of phorate and metabolites in plants, aphids and honeydew in relation to the time of translocation.

Sample		D P M					
		Plant 1	Plant 2	Plant 3	Control 1	Control 2	Control 3
<u>1 DAY</u>							
Leaf 1	SS	27031	43512	37626	29296	51030	35268
	WS	1887	3013	2020	3022	1936	2273
Leaf 2	SS	48819	63182	70100	70272	53622	56818
	WS	2913	4570	4088	4331	2926	3787
Leaf 3	SS	85740	91305	126376	113612	88370	98263
	WS	7412	8084	10100	9206	6888	8840
Aphids	SS	133	187	146			
	WS	4232	3836	5030			
Honeydew	SS	29	41	57			
	WS	1328	2172	1669			
<u>2 DAYS</u>							
Leaf 1	SS	14208	6975	8930			
	WS	4628	2530	2972			
Leaf 2	SS	39088	25881	46377			
	WS	8013	5966	6724			
Leaf 3	SS	72513	54725	60183			
	WS	10184	9381	7268			
Aphids	SS	287	319	248			
	WS	3987	7308	5825			
Honeydew	SS	64	90	69			
	WS	4172	2604	2384			
<u>4 DAYS</u>							
Leaf 1	SS	13820	10495	7921	14022	6807	9321
	WS	4638	2521	3956	3937	2918	5703
Leaf 2	SS	22624	17347	30425	30309	25531	16834
	WS	10037	5989	7626	5509	4809	8799
Leaf 3	SS	34502	26962	42105	40107	28224	22831
	WS	8021	9428	11659	7797	8843	12133
Aphids	SS	328	359	301			
	WS	5394	2866	4231			
Honeydew	SS	67	70	64			
	WS	8337	7895	11979			

Appendix 11. (Fig. 2.9 and table 2.4)

Distribution of phorate in the leaf and quantities removed by aphid colonies caged before and at the time of treatment.

Sample	Metabolite	D P M					
		Treatment with previous sink			Treatment without previous sink		
		Plant 1	Plant 2	Plant 3	Plant 1	Plant 2	Plant 3
Leaf 2	SS	39088	25881	46377	40211	50220	27864
	WS	8013	5966	6724	4946	8132	7520
Aphids	SS	287	319	248	107	110	156
	WS	3987	7308	5825	3612	3076	1888
Honeydew	SS	64	90	69	46	40	75
	WS	4172	2604	2384	2126	1831	970

SS=solvent soluble metabolites; WS=water soluble metabolites

Appendix 12. (Fig. 2.10)

Export of phorate from a treated leaflet to an aphid sink on the adjacent leaflet.

Sample	Metabolite	D P M					
		Infested			Control		
		Plant 1	Plant 2	Plant 3	Plant 1	Plant 2	Plant 3
Leaf 1	SS	30	37	19	32	20	17
	WS	44	67	80	41	77	48
Leaflet 2	SS	44	37	30	18	32	26
	WS	130	141	90	89	147	162
Leaf 3	SS	19	26	31	22	40	27
	WS	182	151	204	188	227	149
Aphids	SS	50	74	68			
	WS	988	1144	1317			
Honeydew	SS	17	13	22			
	WS	1389	1835	1596			

Appendix 13. (Fig. 2.11)

Distribution of phorate in aphid sinks established below the treated leaf.

Sample	Fraction	D P M					
		Infested			Control		
		Plant 1	Plant 2	Plant 3	Plant 1	Plant 2	Plant 3
Leaf 2	SS	81	64	57	29	52	46
	WS	488	395	286	563	277	474
Roots	SS	36	51	45	38	36	60
	WS	1074	695	737	1681	2646	2275
Aphids	SS	137	200	143			
	WS	5458	5091	3322			
Honeydew	SS	72	110	83			
	WS	7003	5726	4975			

Appendix 14. (Fig. 2.12)

Distribution of phorate in aphid sinks established above the treated leaf.

Replicate	Fraction	D P M		
		Leaf 3	Aphids	Honeydew
Plant 1	SS	42	79	42
	WS	526	1987	2801
Plant 2	SS	37	91	25
	WS	663	3071	4026
Plant 3	SS	51	102	31
	WS	496	2632	2772
Control 1	SS	65		
	WS	916		
Control 2	SS	38		
	WS	498		
Control 3	SS	59		
	WS	886		

Appendix 15. (Fig. 2.13 and table 2.5)

Distribution of phorate in aphid sinks established simultaneously above and below the treated leaf.

Replicate	Fraction	D P M					
		Leaves		Aphids		Honeydew	
		Leaf 2	Leaf 4	Leaf 2	Leaf 4	Leaf 2	Leaf 4
Plant 1	SS	44	31	145	58	98	50
	WS	161	142	2083	568	4981	1522
Plant 2	SS	26	16	120	76	120	26
	WS	336	260	2420	926	5308	2103
Plant 3	SS	51	22	161	39	89	37
	WS	265	108	1396	714	8105	990
Plant 4	SS	41	19	96	44	94	28
	WS	292	179	1745	759	6423	1420

EFFECTS OF APHIDS ON THE DISTRIBUTION OF PESTICIDE
MOLECULES AND INORGANIC IONS IN BEAN PLANTS

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Summary Aphids were caged on foliage at one node on young broad bean plants, and radio-labelled solutes were added to the nutrient solution or applied to non-infested leaves.

When ^{32}P -phosphate was added to the nutrient solution, aphid colonies caged 1-3 days before this treatment removed more radio-label than aphids caged 1 day after. ^{35}S -sulphate was affected similarly, both in cut and rooted plants. The amounts of labelled phosphate or sulphate removed from the plants by an established aphid sink were greater than those present in the leaf at the feeding site.

Solvent-soluble radio-label derived from ^{14}C -phorate and ^{14}C -ethirimol added to the nutrient solution was less strongly attracted to established aphid sinks, and the aphids did not significantly increase movement of phorate applied to an adjacent leaflet. Water soluble-phorate metabolites, however were strongly attracted to and removed from the feeding sites by the aphids.

Résumé Les pucerons ont été encagés sur le feuillage de jeunes plantes de fève au niveau d'un noeud et on a, ou ajouté des isotopes radioactifs marqués aux solutes du milieu nutritif, ou on les a appliqués aux feuilles non infectées.

Si on ajoutait du phosphate ^{32}P , au milieu nutritif, les colonies de pucerons encagés un jour plus tard. Le sulfate ^{35}S a été affecté de la même manière dans les plantes coupées comme dans les plantes enracinées. Les quantités de phosphate ou de sulfate retirées des plantes par le système d'écoulement d'un puceron établi étaient plus élevées que celles présentes dans la feuille à l'endroit où il se nourrissait.

Un isotope marqué soluble dans le solvant, dérivé du phorate ^{14}C et de l'ethirimol - ^{14}C ajouté au milieu nutritif était moins fortement attiré par le système d'écoulement du puceron, et les pucerons n'augmentaient pas de façon significative le mouvement du phorate appliqué au foliole adjacent. Cependant les métabolites aquasolubles du phorate, étaient fortement attirés et évacués des endroits où les pucerons se nourrissaient.

INTRODUCTION

All plant systemic insecticides in general use move predominantly in the xylem and tend to accumulate in areas of the plant where evaporative water loss occurs. Crisp (1972) applying the Munch concept of plant transport mechanism, has concluded that the pattern of movement of insecticides is predominantly apoplastic and that, unless an acidic group is attached to the molecule these compounds are normally unable to penetrate and to be transported by the symplast. Although the symplast/apoplast boundary appears to be a considerable barrier to these compounds, aphids feeding predominantly in the phloem pick up significant amounts of toxicant. Thus a degree of leakage must occur between apoplast and symplast. This leak may be induced or accentuated by the nutrient sink developed by feeding aphids; in the transpiration process only water loss occurs, whereas at an aphid sink both water and solutes are taken from the plant. This loss of sap is replaced in an individual sieve element by longitudinal movement down the pierced element and by lateral movement from surrounding elements. The length of the pierced element affected in this manner has been termed the contributory length and may extend for a considerable distance on either side of the point of stylet penetration (Weatherley *et al.*, 1958; Peel and Weatherley, 1962; Ford and Peel, 1966). Larger aphid colonies increase the contributory length accordingly (Peel and Ho, 1970).

Sap lost from the aphid-infested area must be replaced from neighbouring regions. The water may be obtained by an increased flow in the ascending apoplast, but a large proportion of the osmotica must be synthesised by the plant, though storage material may be used temporarily. In this context an aphid infestation on a brussels sprout plant leaf has been shown to increase assimilation rate in adjacent leaves (Way and Cammell, 1970).

Thus the sink may affect both symplastic and apoplastic compounds to a variable extent depending on the magnitude of the sink in relation to the size and physiological state of the plant.

This hypothesis, coupled with an observation that aphids already present on the plant appeared to be more rapidly affected by a root application of a systemic insecticide than aphids caged subsequently, prompted the investigations described in this report.

METHODS AND MATERIALS

Plants

Broad bean plants (*Vicia faba* L., var Sutton) were pot-grown in a 3: 1 peat: sand mix in a glasshouse. Plants with at least three fully expanded leaves were used in the experiments and were either cut just above the cotyledons or taken complete from the pots. The compost was carefully removed from the roots under running water. Both cut shoots and rooted plants were maintained in a nutrient solution (Long Ashton solution, trace elements omitted) in 100 mm x 36 mm specimen tubes. The experiments were carried out in a constant temperature room at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a 16 h daylength provided by four 80 W fluorescent lamps mounted 60 cm above the bench. The relative humidity was not controlled and fluctuated from about 60% RH during the daylight period to about 85% RH at night.

Cut plants were treated with ^{32}P -phosphate and ^{35}S -sulphate by standing the shoot in about 0.5 ml of nutrient solution to which the radio-isotope had been added. This volume was absorbed in 1-3h after which a further 0.5 ml of 'cold' nutrient solution was added. When this too was absorbed the nutrient solution

was restored to its original volume (50 ml) and maintained at that level for the remainder of the experiment.

This technique could not be followed with the rooted plants and in these experiments the radiolabelled compounds were added directly to the nutrient solution surrounding the roots.

Aphids

Black bean aphids (*Aphis fabae* L.) were used to produce the aphid sinks. The aphids were cultured on bean plants brought into the constant temperature room at the two-leaf stage.

For the sulphate and phosphate experiments 50-60 fourth instar and adult aphids were selected and when caging times exceeded two days, young aphids were removed periodically to prevent a greater number of aphids producing a correspondingly greater sink.

In the pesticide experiments about 20 alatae were caged on one leaf and allowed to produce young for 24 h after which time the adults were removed and the larvae reduced to about 200. The cage was retained for the phorate metabolite experiments but not for the other experiments. Aphids seldom leave a leaf except when overcrowded. The pesticides were applied five days later, before adults had begun to appear in the colonies, and the experiments terminated for analysis after a further four days.

Honeydew collection

In the phosphate, sulphate and phorate metabolite experiments, honeydew was collected on untreated filter-paper linings to the aphid cages (Galley, 1974). In the other two experiments, with labelled phorate or ethirimol, the honeydew was collected on pieces of aluminium foil 70 mm x 50 mm supported under infested leaves.

Radio-isotopes

^{35}S -sulphate and ^{32}P -phosphate were obtained from the Radiochemical Centre, Amersham, the phosphate in phosphate buffer (initially 1mCi/mg P) and the sulphate carrier free in isotonic saline (5mCi/ μgS).

The ^{14}C -phorate was labelled in the ethoxy-group with a specific activity of 42.5 $\mu\text{Ci/mg}$ and the ^{14}C -ethirimol was ring-labelled with a specific activity of 22 $\mu\text{Ci/mg}$.

Isotope extraction and counting

Autoradiographs of the leaves at the end of these experiments showed that the distribution of radio-activity in the leaf following the ^{32}P -phosphate and ^{35}S -sulphate treatments was relatively uniform. The ^{14}C -pesticide distribution showed a pattern more typical of apoplastic movement with accumulation of label beginning to occur round the leaf margins.

The concentrations of label in the phosphate and sulphate experiments were determined by punching 10 mm diameter disks from the centre of each leaflet coincident also with the caged area in infested leaves, and counting these for 10,000 counts with a Geiger-Müller detector and Timer/Scaler. Disks were stored at 0°C in polythene film when long counting times caused delay between samples.

In order to correct the counting efficiencies of the disks to that of the aphid and honeydew counts some disks were also ground in a glass pestle and mortar with 0.5 g sand and 5 ml water containing 0.05% w/v laboratory detergent and mM of both sulphate and phosphate ions (sodium salts). The homogenate was filtered and washed with a further 2 x 2.5 ml of the water solution and a 1 ml aliquot of the filtrate was counted in 10 ml Bray's scintillant.

Aphids were homogenized in an all glass hand-homogenizer with a little of the sulphate, phosphate, detergent solution. The homogenate was made up to a known volume and an aliquot counted in Bray's scintillant.

The honeydew was extracted from the filter papers by shaking them in vials with 2 x 2 ml of the same aqueous solution followed by washing down with a further 1 ml. A 2 ml aliquot was counted in Bray's scintillant. These procedures gave better than 95% recoveries from samples to which known amounts of the isotopes had been added.

For ^{14}C extractions, whole leaves were ground as described above but the solvent used was chloroform: methanol (9:1). Leaves were ground and washed with 3 x 10 ml aliquots of solvent into a conical flask containing 1.5 g finely divided activated charcoal and 2 g anhydrous sodium sulphate. After swirling for two to three minutes, the solvent was filtered through a 5 cm buchner funnel and evaporated almost to dryness in a rotary evaporator. The residue was transferred to a counting vial with three aliquots of toluene totalling 10 ml and containing 8 g/l butyl-PBD. In the phorate metabolite experiments this residue was taken up in benzene and transferred to the top of a silicic acid column 8 mm internal diameter and containing 6 g silicic acid ('Silicar', Malinkrodt CG7). Phorate, its sulphone and sulphoxide were eluted with 40 ml benzene with 5% v/v acetone and 40 ml acetone respectively. This procedure was a modification of the method developed by Bowman et al. (1969) for phorate and metabolites. The column effluents were concentrated nearly to dryness and transferred to counting vials as before. The water soluble fraction was extracted with distilled water from the charcoal/homogenate residues and an aliquot from a known volume counted in Bray's scintillant.

Aphids were extracted similarly by using the hand homogenizer with the chloroform/methanol solvent system.

Aluminium foils with honeydew samples were carefully rolled into a cylinder and inserted into 10 cm B24 stoppered test tubes. About 5 ml of methanol: water (3:1) were added and the tubes rolled for thirty min. The foils were carefully withdrawn and washed down with 1 ml methanol into the tube. This extract was shaken in the tube with 3 x 5 ml of the chloroform/methanol solvent and the chloroform fractions concentrated in the rotary evaporator and transferred to counting vials with the toluene-based scintillant.

Recovery from spiked samples with these procedures were good with the exception of ^{14}C -phorate in honeydew. Counts were lost from 'honeydew' samples of ^{14}C -phorate dissolved in a 10% sucrose/50% aqueous acetone 'honeydew' applied to foils and filter papers, when the samples were dried. This loss probably arose from evaporation of the pesticide during the drying process and may extend also to the toxic metabolites. Though up to 50% of the phorate and possibly less of the sulphoxide and sulphone may have been lost from the honeydew samples, the water soluble fraction would not be so affected. Similarly, ethirimol has a low vapour pressure, and breakdown is less rapid in the plant; thus the organic solvent fraction would have removed the greater proportion of radio-label at the end of these relatively short experiments.

RESULTS

Phosphate and sulphate in cut plants

The amounts of radio-activity present in the leaves varied considerably between individual plants though the same trends were found among the replicates for each set of treatment conditions. The results are expressed as means of the proportions found in each of 3 plants. Fig. 1 shows the distribution of ^{32}P at each leaf with aphids caged at the third leaf for various times before and after the application of $2\mu\text{Ci}$ of ^{32}P -phosphate to the cut shoot. The levels of activity are expressed as a percentage of the total counts per min per cm^2 with aphid and honeydew counts related to the caged area (1.95 cm^2). It can be seen from Fig. 1 that the proportions of isotope removed by the aphids were greatest when the aphid colony was established before isotope application, the greater effect occurring with the longer established colony.

A similar effect was found when $5\mu\text{Ci}$ ^{35}S -sulphate was added to the nutrient, though in this case the distribution pattern in the plant was markedly different with more sulphate accumulating in the younger leaves. (Fig. 2a) When an established aphid colony was present on the third leaf the proportion of label entering the younger leaves was reduced.

Sulphate in rooted plants

$5\mu\text{Ci}$ ^{35}S -sulphate added to rooted plants showed a distribution pattern similar to that in the cut plants and aphids caged four days beforehand again attracted more label than those caged on the plant after the isotope application (Fig. 2b)

Autoradiographs were prepared of the leaves on which the aphid colonies were sited from both cut and rooted plants. The position of the cage site was never clearly marked with either isotope, indicating that the radio-label was being transported into the region at the same rate at which it was removed by the aphids. Experiments were also conducted to detect if there was any overshoot of labelled material into the caged area once the aphids were removed. Leaves autoradiographed thirty min after aphid removal occasionally showed slight evidence of the site of the aphid cage but with the general variability of the plant material the results were inconclusive.

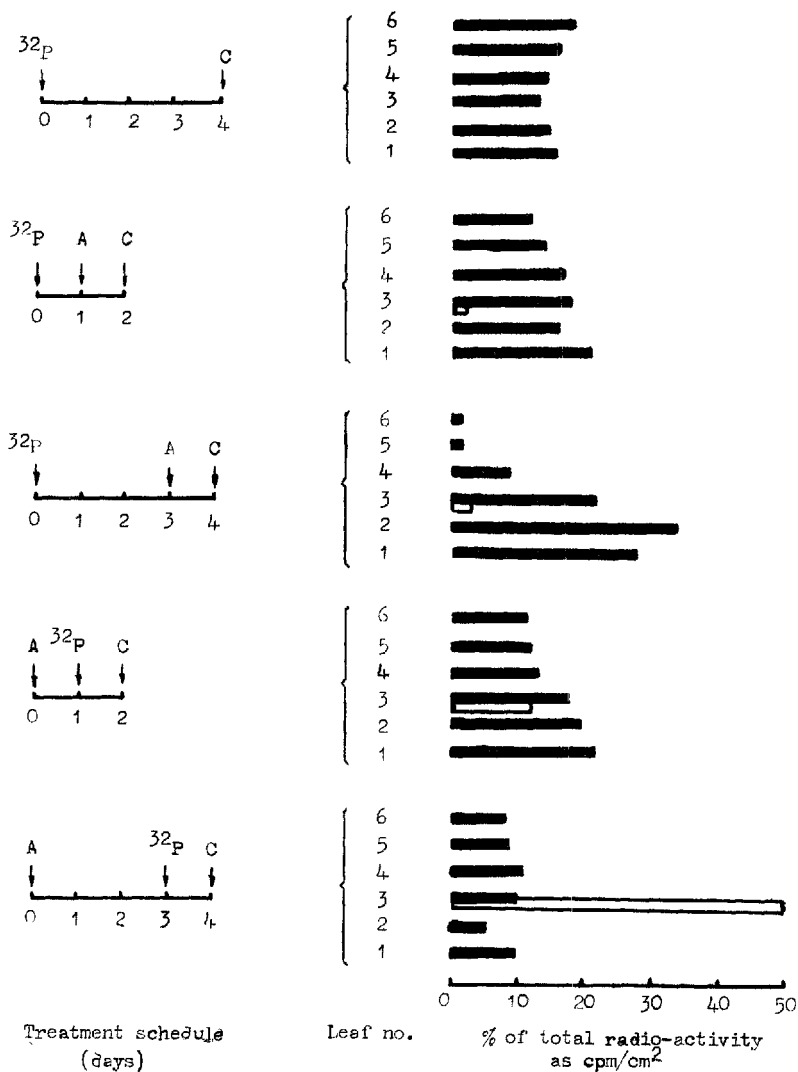
Ethirimol in rooted plants

The radio-activity found in the leaves, aphids and honeydew four days after adding $64\mu\text{g}$ ^{14}C -ethirimol to the nutrient of each of two plants are given in Table 1. The control plants with no aphid colonies are included for comparison. Distribution in the plants tended to be of the sulphate pattern with the younger leaves receiving more label and, as in the phosphate and sulphate experiments, the aphids removed a proportion of the radio-activity from the leaflet.

Phorate in rooted plants

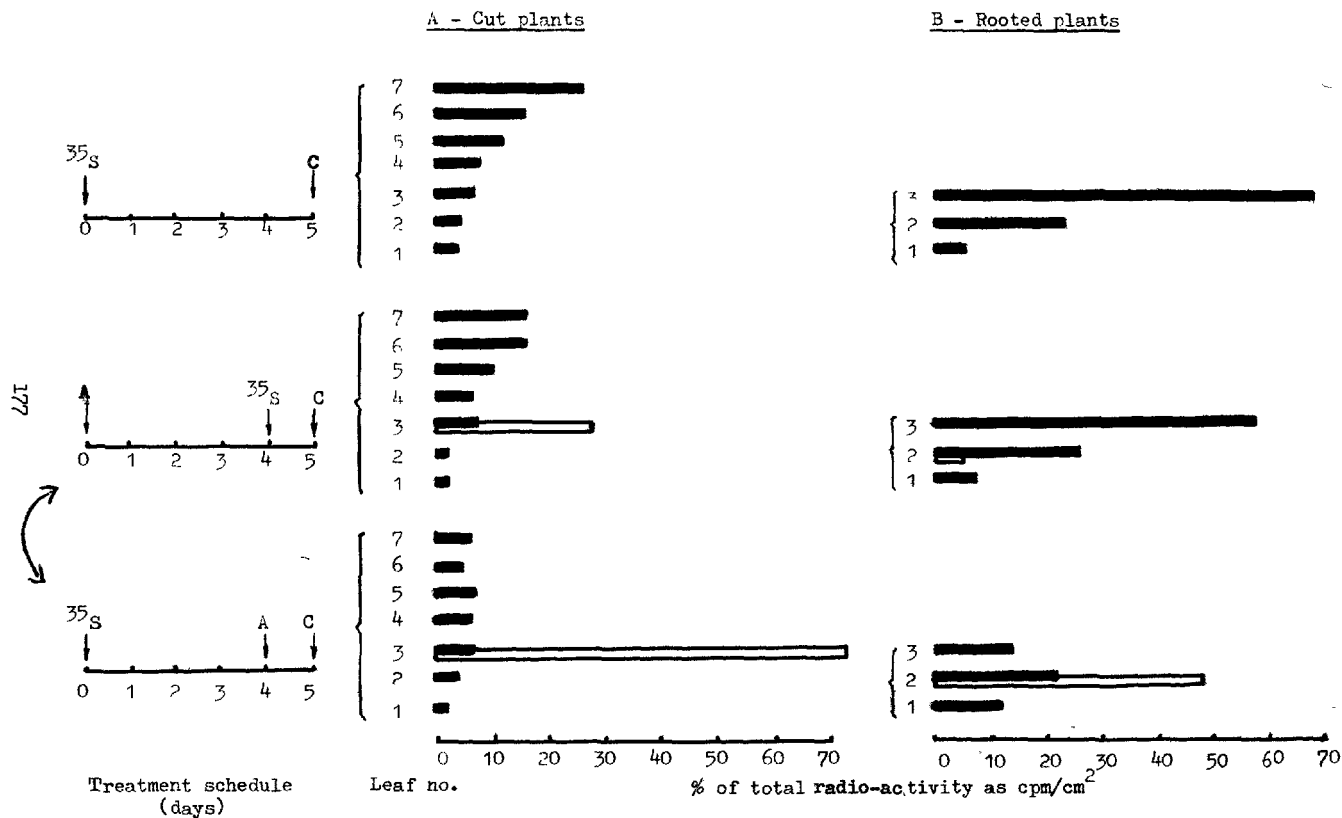
The distribution of label following the application of a sublethal quantity ($8.8\mu\text{g}$ per plant) of ^{14}C -phorate to the nutrient solution, 5 days after infestation, is shown in Table 2. A similar trend was found in this experiment but the aphids, again on the second leaf took up a little less from the infested leaf ($1/20$ and $1/10$ of the amount of phorate present) compared with $1/2$ and $1/6$ for ethirimol. The reduction is probably attributable to the response of the aphids to the insecticide, even though the dose was sub-lethal for the duration of the experiment. Two days

Fig 1. Distribution of ^{32}P in cut plants and in an aphid sink on the third leaf



^{32}P = addition to nutrient solution; A = aphids caged on plant, C = radio-activity counts; 1 = oldest, 6 = youngest leaves: open areas on histograms are combined aphid and honeydew counts.

Fig 2. Distribution of ^{35}S in the plants and the aphid sink



Explanations given under Fig 1.

after application, the feeding rate fell and many individuals left the leaf in search of more suitable feeding sites. These aphids were taken from the plant and kept at -20°C until homogenized with the remaining insects. In both root uptake experiments the amounts of phorate and ethirimol taken up varied considerably from plant to plant. The quantities found in the aphids and honeydew did not contribute significantly to the amounts present in the infested leaves nor was there any discernible effect on the general pattern of distribution.

Table 1
Radio-label distribution following the addition of
 ^{14}C -ethirimol to the nutrient solution

	Leaf no.	Leaves of control plant	Leaves of infested plant	Aphids	Honeydew
Replicate A	3	430	686	-	-
	2	310	376	43.2	130
	1	208	251	-	-
Replicate B	3	464	170	-	-
	2	379	128	5.7+3%	14.3+2%
	1	395	85	-	-

Values given are ng equivalents of ethirimol on both leaflets. Counting errors were better than $\pm 1\%$ except where indicated.

Table 2
Solvent-soluble radio-label distribution following the
application of ^{14}C -phorate to the nutrient solution

	Leaf no.	Leaves of uninfested plant	Leaves of infested plant	Aphids	Honeydew
Replicate A	4	110	26.0	-	-
	3	94	115	-	-
	2	82	67.6	1.3+4%	2.5+3%
	1	95	97.7	-	-
Replicate B	4	108	43.4	-	-
	3	150	51.6	-	-
	2	121	46.6	1.8+4%	3.1+3%
	1	100	64.8	-	-

Values are ng equivalents of phorate. Counting errors as in Table 1.

Phorate applied to an adjacent leaflet

A sub-lethal dose (3.5 μg) of ^{14}C -phorate was applied in 5x5 μl droplets of acetone to the axial surface of one leaflet of the second leaf. Table 3 gives the relative quantities of label as phorate, phorate sulphoxide and phorate sulphone found six days after application. Though toxic compounds did move from leaflet to leaflet, what movement there was seems to have been depressed by an aphid sink established on an adjacent leaflet 5 days before application of phorate.

Table 3
Distribution of radioactive phorate and toxic
metabolites in treated and adjacent leaflets

Plant	Leaflet	Phorate	Phorate sulphoxide	Phorate sulphone
Control A	treated	129	144	200
	adjacent	3.1 $\pm$ 3%	4.2 $\pm$ 3%	2.4 $\pm$ 3%
	aphid-free			
Control B	treated	176	140	504
	adjacent	2.8 $\pm$ 3%	1.7 $\pm$ 4%	1.6 $\pm$ 4%
	aphid-free			
Infested A	treated	131	146	235
	adjacent	1.2 $\pm$ 4%	0.45 $\pm$ 7%	0.34 $\pm$ 8%
	aphid-infested			
	aphids	0.17 $\pm$ 11%	0.15 $\pm$ 12%	0.14 $\pm$ 12%
	honeydew	0.32 $\pm$ 8%	2.2 $\pm$ 3%	0.99 $\pm$ 5%
Infested B	treated	217	117	123
	aphid-infested	0.52 $\pm$ 7%	0.55 $\pm$ 6%	0.46 $\pm$ 7%
	aphids	0.50 $\pm$ 7%	0.36 $\pm$ 8%	0.31 $\pm$ 9%
	honeydew	0.20 $\pm$ 10%	0.40 $\pm$ 8%	1.1 $\pm$ 5%

Units and counting errors as in Table 2.

Table 4
Distribution of radioactive phorate and metabolites in the third
leaf following application of 7 μ g 14 C-phorate to the first leaf

	Phorate	Phorate sulphoxide	Phorate sulphone	Water soluble fraction
Leaf	1.1 $\pm$ 0.9	1.1 $\pm$ 0.7	0.78 $\pm$ 0.6	2.8 $\pm$ 1.1
Aphids	1.5 $\pm$ 0.4	1.2 $\pm$ 1.1	1.2 $\pm$ 1.1	10.4 $\pm$ 3.2
Honeydew	0.15 $\pm$ 0.1	1.2 $\pm$ 0.9	0.72 $\pm$ 0.7	4.1 $\pm$ 2.1

Values given are mean ng equivalents of phorate with standard deviations.

Phorate applied to a lower leaf

In this experiment 200 aphids were reared on each leaflet of the third leaf and 3.5 μ g 14 C-phorate applied in acetone to each leaflet of the first (oldest) leaf. Table 4 gives the activity appearing in the different metabolite fractions present in the third leaf and in the aphid sink after 4 days. The figures are the means from three plants, all of which showed the same general pattern but differed considerably in the levels of activity present. The most striking aspect of this experiment is the amount of radioactivity found in the water-soluble fraction from the honeydew and to a lesser extent in the aphids, very much more than in the leaf from which it originated. The water soluble phorate metabolites were not further identified but since the compound was labelled in the ethoxy-group the fraction probably contained a mixture of diethyl phosphoric acid and analogues.

DISCUSSION

The phosphate and sulphate experiments showed that the aphid sink had a greater effect on these isotopes ascending the plant than when they were distributed in the leaves. Some of this effect could perhaps be attributed to a slower feeding rate at the beginning of the caging period. It takes 12-16 h

for *A.fabae* to achieve a normal feeding rate (Banks and Nixon, 1959). The aphids caged 1 day before the isotope application also removed less from the plant than those caged 3 days beforehand, indicating that even when the aphids were feeding at their optimum rate at the time of the isotope application the full sink effect was not established until sometime later.

A factor which may apparently enhance movement to the sink from the ascending stream is the additional transpiration caused by the increased assimilation rate that would be expected from the observations of Way and Cammell (1971). This transpiration flow will bring with it a proportion of label which, combined with other solutes, would be used by the plant to contribute to the osmotic potential in the sink system.

The distribution of radio-label in the leaves of the two plants given the root application of phorate was atypical. Generally a pattern more like that found in the ethirimol treatment might be expected with higher concentrations of insecticide in the younger leaves. However this experiment did demonstrate the appearance of compounds which were solvent-soluble and therefore probably toxic in the aphid honeydew, and this was confirmed in the subsequent experiments by the separation of phorate and its sulphoxide and sulphone. The separation technique used did not distinguish the phorate metabolites from phoratoxon and its sulphoxide and sulphone, but preliminary studies using TLC techniques failed to show any appreciable quantities of these compounds in the plants. Any traces that were present would also be eluted from the column with the phorate metabolites.

The movement from xylem to phloem may occur over a considerable distance from a large aphid colony since the contributory length for an individual aphid extends for many centimetres along the pierced element on either side of the aphid stylets (Weatherley et al, 1959). When very many of the sieve elements are affected it is likely that this effect is extended further both longitudinally and laterally. Sulphate and phosphate readily move laterally in phloem tissue, more readily even than water (Peel, 1970) though in general lateral movement in vascular tissue is poor (Canny, 1971). Pesticide molecules are larger and less readily transported, except in a passive manner, and appeared from these experiments to be less affected by the sink than the mobile ions. Water soluble phorate metabolites however were drawn by the sink and the amounts present there suggest that it may have entered the leaf in that form, perhaps in the transpiration stream, rather than have been produced there. Some hydrolysis would be expected in the sink leaf and in the aphids and honeydew while water was present, but the export of toxic compounds from treated leaves was low and the levels of these compounds in untreated leaves were also low. Thus it seems likely that much of the water soluble fraction in the sink may have been formed in the treated leaf and then exported to appear in the sink as did the ^{32}P -orthophosphate in the earlier experiment.

It may be concluded that an established aphid colony had a negligible influence on the movement of pesticide molecules in the bean plants but had a much greater effect on smaller anionic species. The attraction to the feeding site was greater when the anions were ascending the plant than once they were dispersed in the leaf.

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