The susceptibility of the aphids *Myzus persicae* and *Brevicoryne brassicae* to systemic pesticides

by

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ABSTRACT

M.persicae was more tolerant to pesticide incorporated in its diet than was B.brassicae, and up to a fifteenfold difference in EC_{50} was found between the species. The rate of feeding of B.brassicae was found to be twice that of M.persicae, feeding on ^{35}S-labelled artificial diet. The feeding rate of B.brassicae was substantially reduced by sublethal doses of pesticide in the diet, M.persicae was much less affected. When sublethal doses of ^{14}C-labelled phorate were fed in the diet, analysis of the residues in the aphids and their honeydew, showed that a larger proportion of radioactive material was retained by B.brassicae than M.persicae.

Horizontal starch gel electrophoresis of whole aphid esterases showed differences in activity and mobility of enzymes from the two species. In vitro analysis of cholinesterase and carboxylesterase activity showed the enzymes in M.persicae to be more active than those in B.brassicae, except when α-naphthyl acetate was the substrate and the B.brassicae esterases were the more active. I_{50} values, using three inhibitors, indicated that the esterases of B.brassicae were more easily inhibited than those of M.persicae, although again a conflicting result was obtained when α-naphthyl acetate was used as the substrate.

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1. INTRODUCTION

Aphids are one of this country's most economically important crop pests, not normally by causing physical damage, but by their involvement in the spread of viruses. Alate forms are principal virus transmitters due to their behaviour of flying to many plants before settling, their exploratory probing on each plant quickly spreading the virus throughout a crop (Watson and Plumb, 1972).

The polyphagous aphid Myzus persicae has long been regarded as one of the principal spreaders of viruses. Another aphid of significant pest status, particularly of Brussels-sprouts (Strickland, 1957), is Brevicoryne brassicae. This insect is less important as a transmitter of virus, being a specific pest of Cruciferae and dependant upon the presence of a secondary plant substance sinigrin which acts as a feeding stimulant. These two species were the subject of this study which extends earlier observations by Galley (1974) that there is a considerable difference in the susceptibility of the two species to insecticides acting systemically, yet topical treatments were similar in their effects.

Insecticides are still the main control agent of aphids, but problems have always existed. Contact insecticides are not very successful due to the difficulty of reaching aphids feeding on the underside of leaves, although this situation is likely to change with the advent of new techniques such as U.L.V., C.D.A. and electrostatic spraying. Systemic insecticides give better control of aphids, but are relatively less successful in preventing virus transmission, the aphids having to feed before coming into contact with the insecticide, thus introducing the virus before it dies. Infection in this way may even be accentuated by an insecticide since an initial symptom of the presence of toxic

residues is the increased probing of aphids (McEwen, 1953) searching perhaps for a fresh feed supply. Most viruses spread by aphids are of the 'non-persistant' type such as cucumber mosaic and potato virus are normally carried on the cuticle of the aphid, probably in stylet grooves (Forbes, 1969) but are often lost when the aphid moults. There are over 38 'persistant' or circulative viruses (Gibbs, 1969), for example potato leaf roll, which are taken in by the aphid and circulate in the body before being passed back into the plant via saliva. This second type is very difficult to control with insecticides which are only successful if aphid probing, and therefore distribution of virus, is reduced to a minimum.

1.1. Systemic insecticides

Systemic insecticides are absorbed into plants and translocated, primarily in the xylem, in biologically active quantities (Bennett, 1949; 1957). Systemic pesticides can be subdivided according to their path of translocation. Symplastic compounds are those which are able to pass through the plasmalemma into the living continuum, or symplats, and move with the assimilate stream in the phloem tissue to other parts of the plant (Crisp, 1972). Conversely an apoplastic compound is one that is transported predominantly by the transpiration stream in the xylem or apoplast, which makes up the non-living cell wall continuum. These compounds partition poorly into other plant tissues and tend to accumulate in the regions of water loss. Insecticides belong to this latter group of compounds.

The effectiveness of systemic insecticides is governed by their ability to reach the areas of the plant where the insects are feeding. Penetration of leaves, fruits and stems is influenced by the lipophilic properties of insecticides. However translocation in the xylem depends upon exhibition of hydrophilic properties (Mitchell et al, 1960).

Crisp (1972) reported that all the organophosphorus insecticides he tested were apoplastic (xylem transported) and concluded that a weak acid functional group was necessary for effective uptake and mobility in the phloem. Organophosphates are readily soluble in organic solvents and are therefore lipophilic in nature. They are also, to a lesser extent, soluble in aqueous solutions but are then unstable and rapidly hydrolysed to sulphoxides and sulphones. These hydrolysis products retain their toxicity but are hydrophilic and therefore mobile within the phloem giving them their systemic properties. A similar situation occurs with carbamates, such as aldicarb and pirimicarb, which are readily converted to their hydrophilic and toxic sulphoxides (Weiden, 1968) which behave systemically in plants.

According to Finlayson and MacCarthy (1965), transport of systemic insecticides is generally upward with limited downward movement, a theory born out by de Pietri-Tonelli (1965) investigating the translocation of dimethoate in plants. This insecticide moved mainly in the transpirational stream, but some downward translocation movement in the phloem also occurred at a slower rate and was attributed to radial transfer from the xylem. Work by Galley and Foerster (1973) and Foerster and Galley (1976) using phorate has showed that in broad bean plants aphids feeding on lower leaves contained toxic compounds when ^{14}C-phorate was added to distal foliage, again suggesting downward movement of this apoplastic sytemic chemical.

Thus only limited phloem mobility occurs, usually in insufficient quantities for effective pest control on a crop. The compounds distribute predominantly in the transpirational stream with enough reaching areas local to the site of accumulation to affect control of many insect pests.

1.2. Artificial aphid diets

Many attempts have been made at feeding aphids artificially. Early experiments by Hamilton (1930; 1935) were unsuccessful but Lindemann (1948); Day and Irzykiewicz (1953); and Mittler (1954) improved the diet, though these and many more, were only able to maintain the survival of aphids for a few days. Not until 1962 did Mittler and Dadd have success in rearing M.persicae for many generations on a chemically defined diet. The diet containing a mixture of 18% sucrose, vitamins, cholestrol, salts, amino acids and amides was presented to the aphids in a sachet made of stretched Parafilm 'M' membrane through which they fed.

Development continued and has led to improvements in diet composition and techniques for making up sterile sachets. Sachets made of Parafilm 'M' first used by Mittler and Dadd (1963a) were gradually improved to reduce chemical and microbial contamination (Mittler and Dadd, 1964a). The diet could also now be stored for long periods of time at -20°C with no loss of vitamins, especially ascorbic acid (Dadd et al, 1967).

Survival of aphids is dependent on sucrose but amino acids are essential for larval growth (Dadd and Mittler, 1965). Sucrose alone was found to be completely unacceptable (Mittler and Dadd, 1965) though it is necessary as a phagostimulant (Mittler and Dadd, 1963a; Parry and Ford, 1967; Srivasta and Auclair, 1971) and is optimal at concentrations ranging from 10-20% for M.persicae (Mittler and Dadd, 1962; 1965a; Dadd and Mittler, 1965). Complex diets gave greatest success (Mittler and Dadd, 1963b) but B.brassicae requires the presence of sinigrin as this is a specific phagostimulant to this species (Nault and Styer, 1972).

Development has resulted in a totally synthetic diet with trace metals added (Dadd and Mittler, 1966). This diet can maintain aphids

over many generations but aphids never attain the same physical size feeding on artificial diet as they do feeding on plants.

1.3. Aphid feeding

Aphids are normally considered to be phloem feeders although other feeding sites have been recorded (Auclair, 1963; Lowe, 1967; Pollard, 1973). The constitution of the sap taken up by aphids has been investigated by using a cut-stylet technique devised by Kennedy and Mittler (1953) and used by Mittler (1953; 1957; 1958a; 1958b). Estimations of feeding rate have been obtained by the measurement of honeydew excretion rate (Auclair, 1958; Mittler, 1958a; 1958c). Radiotracers incorporated in the aphid's diet can be used to quantify the results (Watson and Nixon, 1953; Day and Irzykiewicz, 1953; Barks and Nixon, 1959; Galley, 1974; Ho, 1978) and by analysing the amount of radioactivity in both aphids and honeydew the the total uptake of diet can be determined.

Mclean and Kinsey (1965) have developed an electron recording device which automatically produces a trace record of probing, salivation and ingestion. Later modified by Mclean and Weight (1968), the aphid acts as a variable resistor which has reduced resistance between the liquid in the plant or diet and the aphid body when the stylet canals are filled with saliva or plant sap; both are electrically conductive. However this technique will only record the incidence of feeding and is of little use in quantitative studies.

1.3.1. Honeydew excretion

Several techniques have been used to collect honeydew and measure excretion rates. Filter papers on revolving turntables were employed by some workers (Smith, 1937; Broadbent, 1951; Auclair, 1958; 1959; Mittler and Sylvester, 1961). Other devices such as pulling long filter papers

at a constant speed under feeding aphids has been used (Banks and MacAulay, 1965), as well as mineral oil (Mittler and Sylvester, 1961; Banks and MacAuley, 1964) and waxed card (Auclair, 1958, 1959; Auclair and Maltias, 1961).

Honeydew excretion rates are affected by environmental factors such as: Temperature (Smith, 1937; Mittler, 1958c); Wind (Broadbent, 1951; Mittler, 1959c); Light intensity (Maxwell and Painter, 1959) and diurnal changes in total soluble carbohydrates in the food (Cull and van Emden, 1977). Cessation of honeydew excretion has been noted in post-reproductive adults of Aphis fabae (Banks and MacAuley, 1964). Various periods of non-excretion during ecolysis have also been shown to occur in Myzus persicae (Broadbent, 1951), Acyrthossiphon pisum and Tuberalachnus salignus (Mittler, 1958c; Auclair, 1959).

Excretion of unchanged insecticide has been suggested to be a method of resistance (Busvine and Feroc, 1972) and aphid honeydew has been recorded to be a mechanism by which toxic compounds can be eliminated from the insect (Eastop and Banks, 1970; Galley and Foerster, 1973; Devonshire and Needham, 1974).

1.3.2. Feeding rate on artificial diets

Various methods have been used to determine the feeding rates of aphids on artificial diet sachets, such as sachet weight loss and the addition of dyes to the diet (Mittler and Dadd, 1964b; Parry and Ford, 1967; 1969) but the most accurate method is by the use of radioisotopes. ^{32}P-phosphate is the most commonly used (Parry and Ford, 1967; 1969; 1971; Danneel, 1969a; 1969b), exact concentrations can be added to the diet and feeding rate calculated following analysis of the isotope in aphids and honeydew.

Exact concentrations of insecticide can also be added to the diet in order to investigate any effect upon feeding rate as well as on

mortality. Numerous authors have found a reduction of feeding rate in the presence of pesticide in the diet (Mittler and Pennell, 1964; Parry and Ford, 1967; 1969; Javadi, 1971; Patsakos, 1972; Holtgrawe and Kloft, 1974; and Ho, 1978).

1.4. Selectivity of pesticides

Differences in response to insecticides between species is a measure of the chemical's selectivity to these species, ideally a pesticide should be toxic to the pest but safe to other life forms (Winteringham, 1969). Bonnemaison (1956) was the first to report that B.brassicae was more susceptible than M.persicae to a number of insecticides acting systemically in rape crops. A field trial by Way et al (1969), using a soil treatment of menazon on Brussels-sprouts, yielded similar results especially apparent a few weeks after treatment. Laboratory experiments by Galley (1974) confirmed a six fold difference in the susceptibility of M.persicae and B.brassicae to dimethoate feeding on excised foliage. This work was extended to artificial diets by Handique (1977) who showed a fifteen fold higher concentration was needed to kill M.persicae than B.brassicae. It is this difference that initiated further studies to systemic pesticides.

Original techniques for testing the toxicity of systemic pesticides to aphids involved caging them on either the underside of a leaf and recording toxicity after root treatment (Way et al, 1969) or on a leaf disc to which pesticide is directly applied (Galley, 1968). These techniques each have their own advantages, but an unknown factor is common to them both, in that the amounts of toxic material absorbed by the aphids is uncertain. This uncertainty arises from the characteristic unequal distribution of pesticide in plant tissue and the possibility

of different feeding sites and rates of uptake of the two species. The total insecticide residues in the leaves in relation to that taken up by the aphids may vary enormously (Galley and Foerster, 1976) and so in this study artificial diet sachets were used (Mittler and Pennel, 1964; Parry and Ford, 1967; 1969; Patsakos, 1972; and Ho, 1978) in an attempt to standardise feeding conditions.

1.4.1. The selective toxic action of Organophosphorus Carbamate insecticides

Corbett (1974) lists six factors which might affect susceptibility of insects to pesticides:-

1. Susceptible insects may possess behaviour patterns that bring them into contact with insecticides that others avoid, for example, a systemic pesticide will affect only phytophagous insects.
2. Tolerant insects may come into contact with an insecticide but not take it up, or else take it up at a reduced rate.
3. The insect may take up the pesticide but not transport it to the site of action, for example, by storing it in fat bodies.
4. The more tolerant insect may alter the chemical metabolically so that it is detoxified at a greater rate.
5. The insect may possess a site of action which is not attacked effectively by the insecticide, for example, a less sensitive cholinesterase.
6. The insecticide may be excreted in an unchanged form (Busvine and Feroz, 1972).

Many of these factors are examined in this study in relation to the susceptibility differences of M.persicae and B.brassicae to systemic insecticides. The fifth factor is studied in depth by investigations into activity and inhibition of B-esterases of the two species.

1.5. B-esterases

Carbamate and organophosphate insecticides work by interfering with the passage of impulses in the nervous system. Although by no means conclusive, the general view is that these compounds kill by the inhibition of acetylcholinesterase (O'Brien, 1960). These insecticides block cholinesterases leading to accumulation of acetylcholine in the synaptic tissues followed by failure of stimulus transmission (Smallman, 1956; Colhoun, 1959; Metcalf, 1959). However,many workers have suggested that other esterases may also be involved in poisoning, even if not necessarily causing death (Lord and Potter, 1951; 1954; Van Asperen and Oppenoorth, 1959; Bigley and Plapp, 1960). The most frequently studied enzymes in this second group being those classified as carboxylesterases or carboxylic ester hydrolases (Dixon and Webb, 1964). Alteration of these target enzymes is thought to be one of the methods by which resistance to insecticides is achieved.

1.5.1. Carboxylesterases

Most of the initial work on carboxylesterase activity was carried out in the early sixties by Dutch workers who found that treatment of insects with organophosphate insecticide resulted in higher inhibition of carboxylesterases. Asperen and Oppenoorth (1959) showed the activity of carboxylesterases in resistant houseflies was less than in susceptible varieties. This was later followed by work done by Asperen and Oppenoorth (1960) suggesting that the parathion-resistant strains of houseflies contained modified carboxylesterases which broke down paraoxon and diazoxon, the oxygen analogues of parathion and diazinon, more rapidly than normal strains. They concluded that organophosphate resistant strains possessed a converted carboxylesterase which acted as a breakdown enzyme, a phosphatase, with a very high affinity for its substrates paraoxon and

diazoxon. Thus it was difficult to correlate low carboxylesterase activity with resistance in houseflies, although mechanisms vary in other strains (Oppenoorth and Asperen, 1961). The view held at this time was that carboxylesterase-like degrading enzymes were able to prevent the toxicant from reaching a fatal concentration at the site of action, whereas total elimination was brought about by other mechanisms (Oppenoorth and Asperen, 1961). Other workers also showed differences in carboxylesterase activity between susceptible and resistant species, although the cholinesterase activities were similar. Bigley (1966) found lower activity of carboxylesterase in parathion-and malathion-resistant strains of houseflies, and Townsend and Busvine (1969) demonstrated a similar situation with malathion resistant blowflies Chrysomya putoria (Wild), and stated that reduced activity of aliesterases and malathion resistance were genetically inseparable.

However, since these early experiments, workers have found reversed relative activities in other species. Ozaki et al (1966) have ascribed malathion-resistance to high carboxylesterase activity in the leaf hopper Nephottetix cincticeps. Ozaki (1969) extended the work to Laodelphax striatellus (Fallen) and again found increased activity in resistant strains. Ogita (1961) likewise found a higher activity in resistant strains of Drosophila melanogenter than in susceptible ones. Aphids, in particular M.persicae seem to show a relationship between high caboxylesterase activity and resistance (Needham and Sawicki, 1971; Sudderuddin, 1972), and a loss of resistance reflected by a corresponding loss of carboxylesterase activity (Needham and Sawicki, 1971). Later work on M.persicae again showed this relationship (Devonshire and Needham, 1974; Needham and Devonshire, 1975). So consistant were these results that Needham and Sawicki (1971) proposed that the measuring of carboxylesterase activity

was a promising alternative to bioassay in testing for field resistance, because individual insects could be assayed, an important factor when few test insects are available and where field populations of aphids are composed of mixed strains.

Electrophoretic separation of carboxylesterases can often show differences between insect strains of the enzymes themselves, for example Velthuis and van Asperen (1963) and van Asperen et al (1965) showed that one esterase band in houseflies was linked to resistance and Kassai and Ogita (1965) demonstrated one esterase band (hydrolysing β-naphthyl acetate) was more intense in a resistant strain of green rice hopper. Other workers (Menzel et al, 1963; Collins and Forgash, 1968; Ahmed, 1968) have shown only eight hydrolying α-nephthyl acetate after electrophoresis in resistant houseflies compared to ten in susceptible flies. In M.persicae increased activity of the enzymes is caused predominantly by changes in a single esterase (Beranek, 1974; Devonshire, 1975a).

Devonshire (1977) proved that purified enzymes from resistant and susceptible strains of M.persicae had the same catalytic centre of activity, and concluded that any difference must result from different amounts of the same enzyme following mutation of the regulator gene(s), rather than of the structural gene.

Electrophoresis can now be used successfully to detect organophosphate and carbamate resistant M.persicae by the comparative activity of their carboxylesterases (Sawicki et al, 1978).

1.5.2. Cholinesterases

Early work on cholinesterases showed no difference between resistant and susceptible strains (van Asperen and Oppenoorth, 1959; Bigley and Plapp, 1960; Forgash et al, 1962). van Asperen (1964) summarises a "mutant aliesterase theory" but this has been criticised by O'Brien (1967)

who quotes work by Smissaert (1964) which showed that although resistant strains of the two-spotted spider mite had lower aliesterase activity, the resistance was in fact due to insensitive cholinesterase in the resistant strain. Further work by Smissaert et al (1970) substantiated these findings although much earlier work by Stegwee (1960) had shown that considerable carboxylesterase inhibition is houseflies treated with tri-o-totyl phosphate (a selective carboxylesterase inhibitor) did not cause death. Devonshire and Sawicki (1974) and Tripathi and O'Brien (1973) using houseflies, confirmed the presence of a modified cholinesterase, and that this was responsible for resistance. Devonshire (1975b) concluded that modified cholinesterase would confer slight resistance in houseflies when isolated genetically, but can however interact with other resistance mechanisms, resulting in high levels of resistance (Devonshire and Sawicki, 1974).

Electrophoretic studies on houseflies have shown four acetylcholine isoenzymes in the head and three different ones in the thorax (Tripathi et al, 1973; Tripathis and O'Brien, 1973). These isoenzymes varied considerably in their sensitivity to inhibitions. However reports have appeared suggesting that these isoenzymes appear during in vitro manipulation and that their in vivo existance is uncertain. Their toxicological significance has also been questioned by Steele and Maneckjee (1979).

1.6. Techniques used to study esterases

1.6.1. Electrophoresis

Initially developed for human esterases by Smithies (1955) this provides the best qualitative method of separating esterases. The principal of the techniques is that a charged particle will move in an electric field according to the type of charge it possesses. The

rate of migration of the particle will be influenced by: the molecular weight, shape and charge of the molecule; the type of supporting medium; the potential gradient applied and the concentration of the buffering system.

There are two types of gel medium in use: starch/agar and polyacrylamide. Most of the early work in insect esterases involved starch gel electrophoresis (Laufer, 1960; Menzel et al, 1963; Velthius and van Asperen, 1963; Collins and Forgash, 1968) and in fact most of the comparative carboxylesterase work between resistant and susceptible strains mentioned earlier used this technique. However starch gels do suffer from several disadvantages, such as poor resolution due to the gel reacting chemically with substrates and the dye solutions used in the detention of enzyme bands; the completed gels cannot be stored for more than a few hours, and finally if quantitative yields of the separated components are required for subsequent kinetic studies, starch gel is of little value due to its low carrying capacity and difficulties encountered in recovery of the enzymes. Certain advantages are to be gained by using starch gels: they are easy and cheap to run; are far less time consuming and depending on the size of gel, can enable many replicates to be performed in one run.

Polyacrylamine gels developed by Raymond and Weintraube (1959) are more satisfactory for quantitative work on the separated components. These gels can be stored almost indefinitely in acetic acid and the pore size is inversely proportional to the square root of the gel concentration and thus may be altered as desired.

Electrophoresis has become increasingly important in esterase studies on field populations in comparing resistant strains of pest species, for example resistant strains of M.persicae show a good correlation with high esterase activity (Sawicki et al, 1978). The

technique is also of value in taxonomy where it can provide a method of speciation. This study has employed only starch gel electrophoresis, but as Sudderuddin (1972) showed, the results obtained are qualitatively comparable with separations on acrylamide gels.

Quantitative determinations of esterase activity has been achieved using a variety of colourimetric techniques:-

1.6.2. Hestrin's method

Developed by Hestrin in 1949 this method enables short chain carboxylic acid esters to be estimated by their ability to react with hydroxylamine in an aqueous alkaline solution. Modifications of the technique (Robbins et al, 1958; Bigley and Plapp, 1960) allows cholinesterase and carboxylesterase activity to be studied. The ester (substrate) reacts with the hydroxylamine to produce a hydroxamic acid which gives a dark brown colour when ferric ions are added. The amount of unhydrolysed substrate is determined colourimetrically and when subtracted from the total amount of substrate in the control, provides an estimation of the ester hydrolysed by the enzyme under investigation. In this work two substrates were used: Acetylcholine chloride to investigate cholinesterase activity, and ethyl butyrate, a specific substrate for carboxylesterases.

1.6.3. Gomori's method

Based on a method devised by Gomori (1953) to study human esterases, this technique depends on the enzymic breakdown of phenyl-or naphthyl-esters to phenol or naphthol which can be coupled with diazonium salts to produce stable dyes whose optical density can be measured spectrascopically. This test has the advantage over Hestrin's method in that it is very sensitive, responding to tiny quantities of enzyme, normally one aphid provides enough material. It must be noted however that substrates are non-specific, being affected by carboxyl- and cholin- esterases, unlike

the specific substrates used with Hestrin's method.

1.7. Experimental insecticides

Three systemic insecticides were used in this study, two organophosphates (Dimethoate and phorate) and one carbamate (pirimicarb). The organophosphorus chemicals in common with other compounds are very apopolastic insecticides but are susceptible to oxidative desulphuration, thioether oxidation and hydrolysis. Early work by Metcalf et al (1954; 1955; 1957) on phorate elucidated the oxidative reactions and the metabolic pathways in plants, and found that phoratoxon and its sulphoxide and sulphone were more effective anticholinesterase agents than phorate derivatives. Later work by Bowman (1973) and le Patourel and Wright (1976) showed these anticholinesterase agents to be more water soluble, extraction procedures can therefore be designed to remove toxic and non-toxic pesticide residues from samples. Organophosphates are also open to detoxification by hydrolysis, the rate developing upon such factors as pH, temperature and the presence of catalysts e.g. free metal ions (Eto, 1974; Fest and Schmidt, 1973).

Pirimicarb is a fast acting aphicide with good contact, fumigant and translaminar effects (Baranyvits, 1970). It is systemic when taken up by the roots of herbaceous plants and is readily translocated in the xylem. In common with other carbamate compounds it is a powerful cholinesterase inhibiter (Matsumura, 1975) but is not as readily metabolised as many organophosphate pesticides. Metabolism is dominated by mixed function oxidase systems, but the products, for example, sulphoxides normally show high toxicities and are rarely without any effect (Oonnithan and Casida, 1968). Confugation and hydrolytic reactions both detoxify carbamates, but oxidation is the primary detoxifier and is normally of two types: ring hydroxylation, often with further oxidation to ketones, and oxidation of side chains.

1.8. Aim of the study

This study uses dimethoate, phorate and pirimicarb as examples of commercially used systemic aphicides. The object of the investigation was to examine the physiological, biochemical and behavioural factors affecting susceptibility of M.persicae and B.brassicae to these insecticides added to artificial diet.

2. MATERIALS AND METHODS

2.1. Plants

Brussels-sprout plants (Evesham Special) were grown individually in 10 cm diameter pots under greenhouse conditions and away from any pesticide treatments. At about the ten-leaf stage the plants were transferred to aphid culture cages after first being cleared of any unwanted insects.

2.2. Insects

Two cultures, one of Myzus persicae and the other of Brevicoryne brassicae (Homoptera: Aphididae) were maintained on Brussels-sprout plants in a constant temperature room maintained at 20$\pm$ 2°C on a 16:8 day/night cycle and uncontrolled humidity.

Adult apterous aphids from the stock culture were transferred to artificial diet sachets in the same environment conditions. After 48 hours the adults were removed, and the nymphs left to feed for a further 72 hours, before being used in experiments. Aphids were therefore always of the same age for experimental work.

2.3. Artificial diet

An artificial aphid diet medium as described by Dadd and Krieger (1967) was used to rear experimental animals. The constituents of the diet were individually weighed on a Mettler H20 five-figure balance and dissolved in water which was first glass-distilled, then deionised and redistilled with potassium permanganate, and finally filtered through charcoal. The diet was prepared by dissolving the chemicals in the order, amino acids: sugar: salts: vitamins; to prevent precipitation (van Emden, 1972) and the pH adjusted to 7.0 using a saturated KOH solution. Sinigrin

(Koch-Light Laboratories) was also added (200 mg/100 ml diet) to provide the necessary phago-stimulus for B.brassicae. The diet was stored in 10 ml vials at -20°C.

Diet sachets were prepared from Parafilm 'M' Laboratory film (American Can. Co.) as devised by Mittler and Dadd (1963a) and modified by Fosbrooke (Pers. Comm.). A 0.5 ml aliquot of diet was delivered from a syringe through a disposable sterile Acrodisc filter (Gelman - pore size 0.20 µm), onto a piece of parafilm stretched across a brass curtain ring 1" in diameter. Suction was applied under the membrane using a simple mouth line, and a second membrane stretched over the drop of diet. A piece of thin fuse wire was trapped between the membranes to allow the air to escape as the suction was released, and the wire removed before finally sealing the sachet. In this way contamination from trapped air is reduced. In order to reduce the risk of contamination still further, the whole process was carried out in a Lamina Flow sterile air cabinet.

Diet sachets normally last for about two days before needing replacement. In order to keep disturbance down to a minimum, aphids were placed under a single layer of parafilm stretched over a second curtain ring(Fosbrooke, Pers. Comm.), the diet sachet can then be placed on top and changed whenever necessary without seriously disrupting the aphid's feeding.

A further refinement of positioning a green filter above the sachets was also employed; this appeared to encourage the adult aphids to settle more readily on the sachets after transfer from the main culture feeding on plants.

2.4. Radioassay techniques

2.4.1. Liquid scintillation counting

Quantitative determination of radioactivity was obtained by liquid scintillation methods. Two scintillation solutions were employed:-

1. Bray's scintillant solution (Bray, 1960) was used for all samples containing water; it consisted of: 60 g naphthalene, 4.0 g P.P.O. (2,5 -Diphenyl oxazole), 0.2 g POPOP (1,4 -Di (2- (5 -phenyloxazolyl)) -benzene, and 100 ml methanol made up to one litre with dioxan,
2. Butyl -PBD (Turner, 1971) was used for non-aqueous samples. It was made up by dissolving 4 g 2- (4^1-t-Butyl phenyl) -5- (4'' -biphenyl) -1, 3, 4-Oxadiazole in one litre of toluene.

10 ml of scintillant was used per sample in low potassium glass scintillation vials. The activity in the samples was counted with a Beckman L.S.250 liquid scintillation counter for 50 minutes or until a 20 error of 0.5% of the observed radioactivity in the sample was reached. All results were corrected for background and counting efficiency assessed and corrected for by the external standard channels ratio method.

2.4.2. Radiochemicals

For feeding rate, evaporation and absorption experiments two radiolabelled salts were used:

1. Sodium ^{35}S-sulphate
2. Sodium ^{32}P-phosphate

Both were obtained from the Radiochemical Centre at Amersham, in sterile saline solution. In experiments where these tracers were used, an allowance was made for decay, the half life for ^{32}P being 14.3 days and that of ^{35}S, 87.2 days.

A labelled organophosphorus insecticide, methyl-labelled ^{14}C-phorate was also used. This was obtained from American Cyanamid Co. and has a specific activity of 42.5 µci/mg.

2.4.3. Extraction and sampling techniques for ^{32}P and ^{35}S

A known volume of radiolabelled salt solution delivered from a Drummond microcap was added with the diet to sachets when these were made up. Aphids were transferred to this radioactive diet for the course of an experiment. Analysis procedures were as follows:-

2.4.3. (i) Diet

At the end of each experiment the sachet was removed and punctured. A 20µl sample was taken using a Drummond microcap and transferred to a scintillation vial containing 10 ml of Bray's scintillant. The amount of radioactivity present was then determined using the scintillation counter.

2.4.3. (ii) Honeydew

Honeydew was collected on large glass coverslips (35 x 64 mm), which when cut in half were just the right size to accommodate the curtain rings supporting the diet sachets. Each coverslip with its honeydew sample was broken directly into a scintillation vial using a glass rod and covered funnel, to ensure that all the sample entered the vial. 0.5 ml of a 1:1 methanol-water mixture was added to the vial to dissolve the honeydew, which because of its high water content, would not dissolve completely in Brays scintillant alone. Once the scintillant was added, the sample was assessed as described above.

2.4.3. (iii) Aphids

Aphids were counted, transferred to scintillation vials (using a paintbrush), and six drops of tissue digestant (methyl bezethonium hydroxide) added. The aphids were then crushed using a glass rod and

left in the dark to digest for five days. After this period 0.5 ml of distilled water and one drop of glacial acetic acid was added to each vial to neutralise the alkaline digestant. 10 ml of Bray's scintillant was then added and the activity assessed in the scintillation counter.

2.5. Feeding rate determination

Determination of feeding rate was achieved be feeding aphids on radiolabelled diets and collecting their honeydew over regular time intervals. Subsequent analysis of radioactivity in the honeydew gave an estimation of the quantity of honeydew produced and this added to the quantity of radioactivity in the aphids gave the amount of radioactivity imbibed, and thus an estimation of the feeding rate.

The honeydew was collected on glass coverslips arranged on an "Aphid clock" (Mittler, 1958c), consisting of a turntable which rotated a fixed distance every pre-set time interval. Aphids were allowed to feed on a sachet suspended above the turntable and the honeydew collected on a coverslip underneath them. At the end of each time interval the turntable would rotate and a new coverslip would be positioned below the aphids.

2.6. Extraction procedures for ^{14}C-phorate

2.6.1. Diet

A 200 µl sample was taken from the sachet containing the ^{14}C-labelled pesticide and added directly to a separating funnel containing 20 ml of chloroform : water : acetone 2: 1: 1 and the radiolabelled components separating into toxic and non-toxic fractions (Galley and Foerster, 1976c) by a procedure based on that of Menzer and Ditman (1968). The two phases were shaken and then allowed to settle before the lower chloroform layer was removed into a round bottom flask. The aqueous layer was extracted

twice more using 10 ml aliquots of chloroform, the aqueous layer was run off into a second flask. The chloroform extracts were pooled, 1 ml of Risella M oil was added and most of the chloroform removed using a rotary film evaporator at 55°C under reduced pressure. The chloroform content must be reduced as much as possible because it is a powerful quenching agent reducing the efficiency of scintillation counting. The oily residue was transferred to a scintillation vial and 10 ml of butyl-PBD (4g/l) solution added before assessment with the scintillation counter. The volume of the aqueous fraction was reduced to about 1 ml in a similar way but at a higher temperature of 65°C (10 ml of Bray's scintillant will only take about 2 ml of water before precipitation occurs). The aqueous extract was then transferred to a scintillation vial and 10 ml of Bray's scintillant added.

2.6.2. Honeydew

Glass coverslips on which honeydew had been collected were broken into a conical flask and 10 ml of water : acetone (1: v/v) mixture was added and shaken to dissolve the honeydew. The solution was then transferred with a pasteur pipette to a separating funnel containing 10 ml of chloroform. The conical flask was washed with a further two 10 ml aliquots of water and one 10 ml of chloroform in order to remove all the radioactivity, and all the washings added to the separating funnel. The partitioning into chloroform and water soluble components was then carried out as before.

2.6.3. Aphids

The method described by Menzer and Ditman (1968) and modified by Galley and Foerster (1976b) was employed. Aphids were homogenised in 1 ml of the water : acetone mixture in an all-glass hand homogeniser. The homogenate was then transferred to a 25 ml conical flask. The

homogeniser was washed several times with the water mixture, and the washings also added to the flask. 0.2 g of powdered charcoal ("NORIT GSX") was added to decolourise the extract and the flask and contents shaken for 15 minutes. The solution was then filtered with reduced pressure, using a buchner funnel, directly into a separating funnel containing 10 ml of chloroform. The homogeniser, flask and charcoal were washed through twice more with 19 ml of aqueous acetone, and once with 10 ml of chloroform. These washings were all added to a separating funnel and the partitioning, concentration and radioassay carried out as before.

2.7. B-Enzyme studies

2.7.1. Starch gel electrophoretic separation of esterases

Esterases were separated by horizontal starch gel electrophoresis as described by Smith (1968).

2.7.1. (i) Starch gel mould

The construction of the mould is shown in Fig.1. The mould was designed to split into two halves to facilitate horizontal slicing of the gel before treatment with substrate and dye. The sides were sealed with silicone grease.

2.7.1. (ii) Preparation and running of gel

The gel was made from 22 g of hydrolysed starch dissolved in 200 ml of 0.03M borate buffer, pH 8.5 (1.8 g boric acid + 12 ml of 1M NaOH made up to 1 litre with distilled water and adjusted to pH 8.5 using 0.1 M HCl). The starch and buffer were heated gently in a conical flask and stirred constantly until nearly boiling. A vacuum line was attached to the conical flask in order to remove dissolved gases. This procedure prevented bubbles from forming when the gel set in the mould.

25 mg of aphid material was homogenised in 1 ml of distilled water using an all-glass hand homogeniser, and applied to grooves (0.5 cm wide)

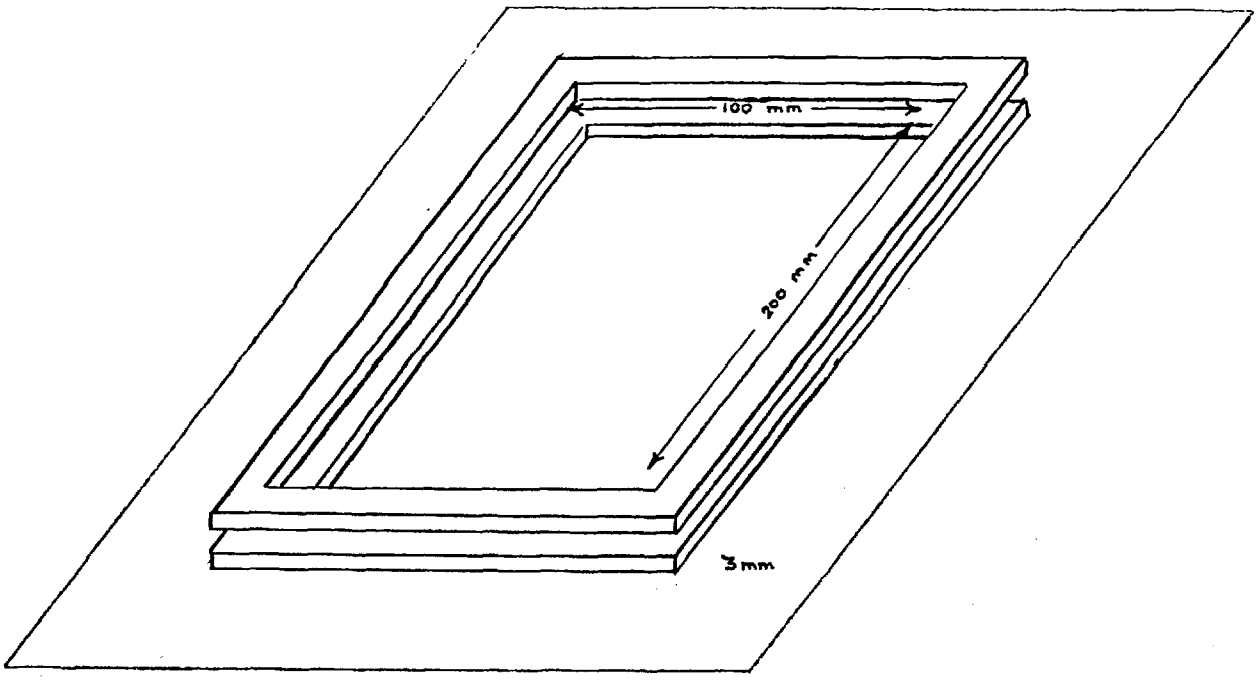


Fig.1 Costruction of Perspex gel mould used for electrophoresis.

at the cathode end of the gel. The top of the gel was covered by a layer of "Clingfilm" plastic sheet to prevent the gel from drying out. The electrode buffer used was 0.3M borate at pH 8.0 (18.6 g boric acid + 50 ml 1M Na OH made up to 1 litre with distilled water). The system was connected to a constant current/voltage D.C. power supply and run at a voltage applied at 5 volts per cm. of gel for 16 hours at 4°C.

2.7.1. (iii) Treatment of gels

After separation of the enzymes the gel was cut in half horizontally by drawing a fine nylon fishing line between the halves of the perspex mould and the enzymes located using the method described by Smith (1968). The gels were transferred to a bath containing 1% α-naphthyl acetate substrate (1 g α - naphthyl acetate + 50 ml Analar acetone + 50 ml deionised distilled water). After 20 minutes at room temperature the gel was washed in deionised distilled water to remove excess substrate, and placed in dye solution (100 mg Fast blue RR salt + 10 ml Tris Ci buffer pH 7.0 + 3 ml 1% α - naphthyl acetate + 87 ml deionised distilled water). After about 30-45 minutes, blue bands appeared on the gel representing the different esterases. The gel was finally washed in deionised distilled water, and remained usable in this state for several hours. Both the substrate and dye solutions were freshly prepared for each gel and kept away from the bright light.

Splitting the gel has two functions: Firstly the esterases tend to move in the middle of the gel, slicing therefore being necessary in order to stain them correctly. Secondly, one half of the gel can be treated with inhibitors and then compared to the other which acts as a control.

2.7.2. Quantitative determination of esterases using Hestrin's colourimetric method

2.7.2. (i) Reagents

The reagents used for the quantitative determinations were similar to those described by Robbins's et al (1958), with appropriate modifications to the concentrations and volumes described by Bigley and Plapp (1960). The reagents used were:-

1. Phosphate buffer - A 0.04M buffer, pH 7, was prepared by dilution of a stock solution of 0.4M made with equimolar concentrations of K_2HPO_4 and KH_2PO_4 in deionised distilled water.
2. Substrates - (a) Acetylcholine chloride - a3 x 10^{-2}M stock solution was prepared in deionised distilled water and diluted as required.
   (b) Ethyl butyrate - a3 x 10^{-2}M stock solution in deionised distilled water was prepared and diluted when required. The stock solution had one drop of Triton - X100 added per 10 ml of stock solution in order to dispense the substrates and give a homogenous mixture. The stock solutions were made every fourth day and stored at $4^{\circ}C$.
3. Hydroxylamine hydrochloride - A 2M stock solution in distilled water was prepared each week.
4. Sodium hydroxide solution - 3.5M concentration
5. Alkaline hydroxylamine - Solutions were prepared daily from equal volumes of 3 and 4.
6. Hydrochloric acid (concentrated, specific gravity 1.18), diluted with two parts by volume of distilled water.
7. Ferric chloride - a 0.37m solution in 0.1M HCL.

2.7.2. (ii) Determination of esterase activity

The procedure for esterase determination ran as follows:-

Blank - 1ml(2) + 1ml(1) + 1ml(6) + 2ml(5) + 1ml(7)

Control - 1ml(2) + 1ml(1) + 2ml(5) + 1ml(6) + 1ml(7)

Enzyme - 1ml(2) + 1ml enzyme + 2ml(5) + 1ml(6) + 1ml(7)

Enzyme + inhibitor - 1ml(2 + inhibitor) + 1ml enzyme + 2ml(5) + 1ml(6) + 1ml(7)

The substrate with enzyme, buffer or inhibitor and enzyme were always incubated at 37°C for 30 minutes in a water bath before the other solutions were added. The control sample provided a correction factor for any non-enzymic hydrolysis of the substrate. The final solutions were filtered using a Millipore filter (pore size 0.22μm) and the colour intensities recorded on a Beckman D.B.spectrophotometer at 540 μm for acetylcholine, and 620 μm for ethyl butyrate. By comparing the optical densities with a standard curve, the μg of substrate present per assay could be determined. Subtracting the value obtained when the enzyme was present from the control gave the quantity of substrate hydrolysed by the enzyme. Likewise subtracting the value with inhibitor present showed the effect of that inhibitor on substrate hydrolysis.

2.7.3. Quantitative studies of B-esterases using Gomori's colourimetric method

The reagents used were based on those listed by van Asperen (1962):-

1. Enzyme preparation
2. Phosphate buffer - A stock solution of 0.4M phosphate buffer, pH 7.0, stored at 4°C and diluted to 0.04M when required.
3. Substrate solutions - Prepared from stock solutions of α or β-naphthyl acetate in acetone and stored at -20°C and freshly prepared every week. The stock solution was diluted with 0.04M phosphate buffer to give the required concentration. It must be noted here that dilutions always contained the same percentage of acetone, in

this case 1%, for example, 0.5 ml acetone was added to 0.5 ml stock solution before dilution with 99 ml buffer.

4. Inhibitors - made from stock solutions in phosphate buffer stored at $4^{o}C$, and made freshly each day.

5. Diazo blue - sodium laurylsulphate solution (DBLS) -

   (a) 1% w/v solution of Diazo blue B (tetrazotised di-o-anisidine; also known as Fast Blue

   (b) and a 5% w/v solution of sodium laurylsulphate were made up as stock solutions and stored at $4^{o}C$. When required solutions were mixed in the ratio 1 : 5 to give the working solution.

Blanks, controls and inhibitors were made up from these reagents as follows:-

Blank - 4ml(3) + 1ml(2) + 1ml(4)

Control - 4ml(3) + 1ml(2) + 1ml(1)

Enzyme + Inhibitor - 4ml(3) + 1ml(4) + 1ml(1)

The total volume of reagent mixtures was constant at 6ml, and these were incubated at $28^{o}C$ for 30 minutes with constant shaking, before 1ml of DBLS solution (5) was added to stop the reaction 10 minutes later a stable blue (α - naphthol) or red (β- naphthol) colour developed and its optical density measured using a Beckman D.B. spectrophotometer at 550 or 600 μm for β and α-naphthol respectively.

3. EXPERIMENTS AND RESULTS

3.1. Standardisation experiments

Various preliminary experiments were carried out to determine the margins of error arising from the many techniques used.

3.1.1. Aphid weights

Individual aphids of both species reared under experimental conditions on artificial diet were weighed on a Beckman LM500 microbalance (Table 1). The results show that at the same age B.brassicae was significantly heavier than M.persicae (T-test sig. $p < 0.001$, $T = 11.7$) and weighed approximately 57% more.

3.1.2. Radiotracer sampling techniques

Different extraction techniques were used in radiotracer experiments, and the effect of these on background count was investigated in order to eliminate any error arising from phenomena such as chemiluminescence.

3.1.3. Coverslip

A single glass coverslip on which aphid honeydew had been collected was broken into a scintillation vial of known background count containing 0.5 ml water : acetone mixture. When the honeydew was dissolved, 10 ml of Bray's scintillant was added and the vial re-counted in the scintillant counter and the change in background count calculated (Appendix Table 1).

The results show that the glass and honeydew do cause a slight increase in the background count (18.4 ± 3.8 d.p.m./vial). As most experiments resulted in a large difference between sample and background counts, no correction was necessary.

3.1.4. Digestant

A known number of aphids reared on sachets were placed in scintillation vials of known background count and crushed with 6 drops of tissue

B.brassicae Wt/Aphid in µg	
146.0	154.0
183.0	124.0
127.0	117.0
104.5	134.5
178.0	128.5
107.5	142.5
118.0	136.0
115.5	173.5
166.5	148.5
147.0	132.5
133.5	174.0
134.0	123.0
183.0	134.0
109.5	134.0
195.5	158.0
178.0	180.0
198.5	132.0
153.0	165.0
124.0	140.5
127.5	148.0
MEAN = 145.2	
S.D. = 25.1	

M.persicae Wt/Aphid in µg	
102.5	107.0
86.5	95.5
100.0	72.0
106.0	97.0
111.0	101.0
99.5	100.5
74.5	92.0
76.5	73.5
107.5	90.0
110.5	108.5
103.0	80.0
92.5	79.0
78.0	95.0
103.5	70.0
96.0	72.0
110.0	82.0
78.5	73.0
86.0	73.5
99.0	99.0
117.0	101.5
MEAN = 92.5	
S.D. = 13.95	

TABLE 1 Weights of individuals of M.persicae and B.brassicae reared for 7 days from birth on artificial diet sachets

digestant using a glass rod. After neutralisation of the alkaline digestant, 10 ml of Bray's scintillant was added and the vial recounted in the scintillant counter.

The resulting counts (Appendix Table 2), show that the counts per aphid, although slightly higher for B.brassicae, were insignificant when compared with the normally large amounts of radioactivity extracted from the aphids in experiments.

3.1.5. Diet

20µl of artificial diet medium was placed in a scintillation vial of known background count with 10 ml of Bray's scintillant. The vial counts, counts with diet and the differences are shown in (Appendix Table 3). A very slight increase was found, but was small enough to be ignored when analysing experimental data.

3.2. Bioassay

Stock solutions of the pesticides dimethoate and pirimicarb were prepared in acetone and water respectively. The dimethoate was stored at -20°C, and the pirimicarb at 4°C until required. Known volumes of these solutions were added to the artificial diet using Drummond microcaps before completion of the sachet. Because dimethoate was in acetone solution, it was dispersed first onto the lower parafilm membrane and the acetone allowed to evaporate before adding the diet and completing the sachet. This procedure eliminated the antifeedant effects of the acetone reported by Ho (1978). Aphids were allowed to feed on the treated sachets for 48 hours before mortality was assessed. Probit analysis was then carried out on the data.

The response of the two aphid species to dimethoate incorporated in the diet is shown in Appendix Tables 4 and 5 and from pirimicarb in Appendix Tables 6 and 7. Dose/response regressions to dimethoate drawn

for the two aphid species (Fig.2) showed parallel lines (Ratio test for parallelism $p > 0.05$) with a 14 fold difference in the EC_{50} values of B.brassicae (0.372 µg/ml) and M.persicae (4.37 µg/ml).

Dose response regressions were drawn for pirimicarb (Fig.3) and a six fold difference was shown between the EC_{50}'s of B.brassicae (0.048 µg/ml) and M.persicae (0.30 µg/ml). The regression lines for the two species were shown not to be parallel (Ratio test for parallelism $p < 0.05$) however the slopes are not that dissimilar and there is enough separation between the regression lines to consider B.brassicae to be approximately six times more susceptible than M.persicae.

3.3. Excretion rate

20µl of a 5µci/ml stock solution of ^{35}S-labelled sodium sulphate was added to 0.5 m. of artificial diet using a microcap. Aphids were allowed to feed on the labelled diet for 96 hours while their honeydew was collected on glass coverlips, which were changed every 3 hours using the "Aphid Clock". At the end of the experiment the sachets, aphids and honeydew were analysed for radioactivity. The sachet values were then used to standardise the honeydew and aphid values according to whether the sachet on which they had fed was higher or lower than a chosen standard, thus allowing direct comparison between honeydew values and calculation of a mean excretion rate. The standardised honeydew values are recorded in Appendix Tables 8 and 9 for B.brassicae and Appendix Tables 10 and 11 for M.persicae.

The excretion rates of both species of aphids over 48 hours are plotted (Fig.4) and it can be seen that B.brassicae excretes honeydew at a constantly higher rate, twice that of M.persicae (T-test sig. $p < 0.001$, $T = 5.1$) over the 96 hour period. The two depressions in the excretion rate are coincident with a drop in temperature of 3°C occurring when the

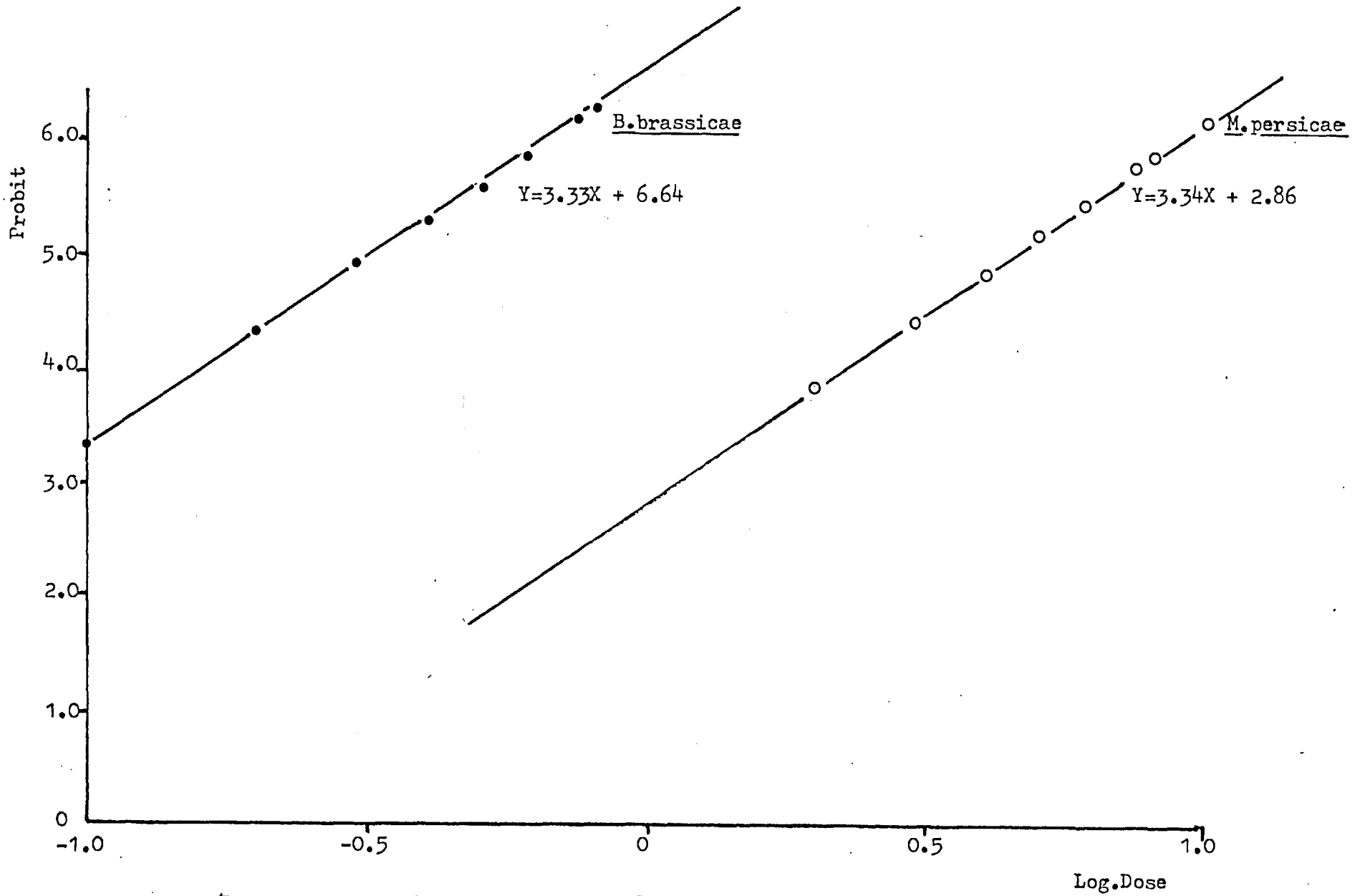


Fig.2 Dose/Response regression for the aphids B.brassicae and M.persicae to the pesticide dimethoate added to diet sachets
(Dose in µg per ml. diet)

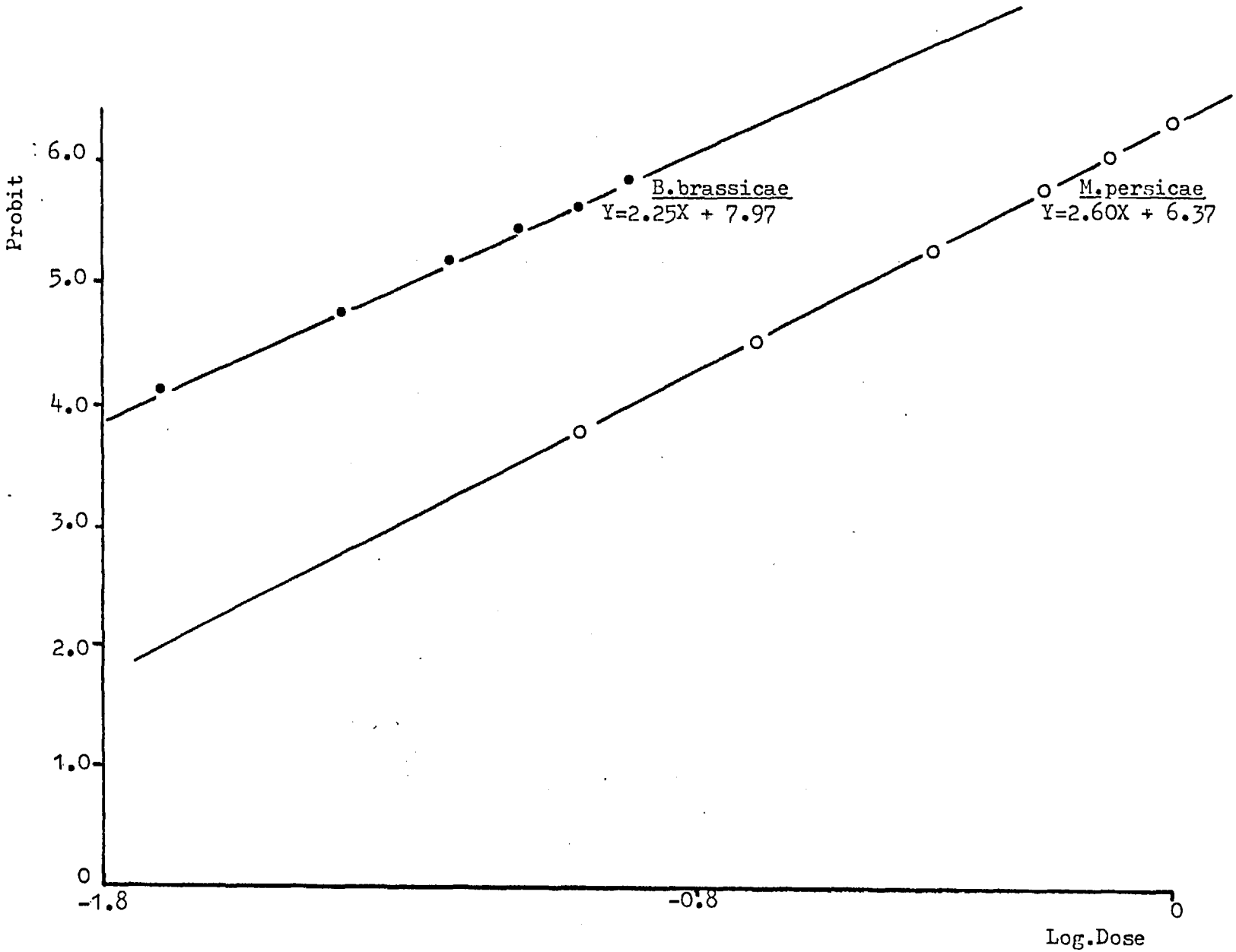


Fig.3 Dose/Response regression for the aphids B.brassicae and M.persicae to the pesticide pirimicarb added to diet sachets
(Dose in μg pesticide per ml. diet)

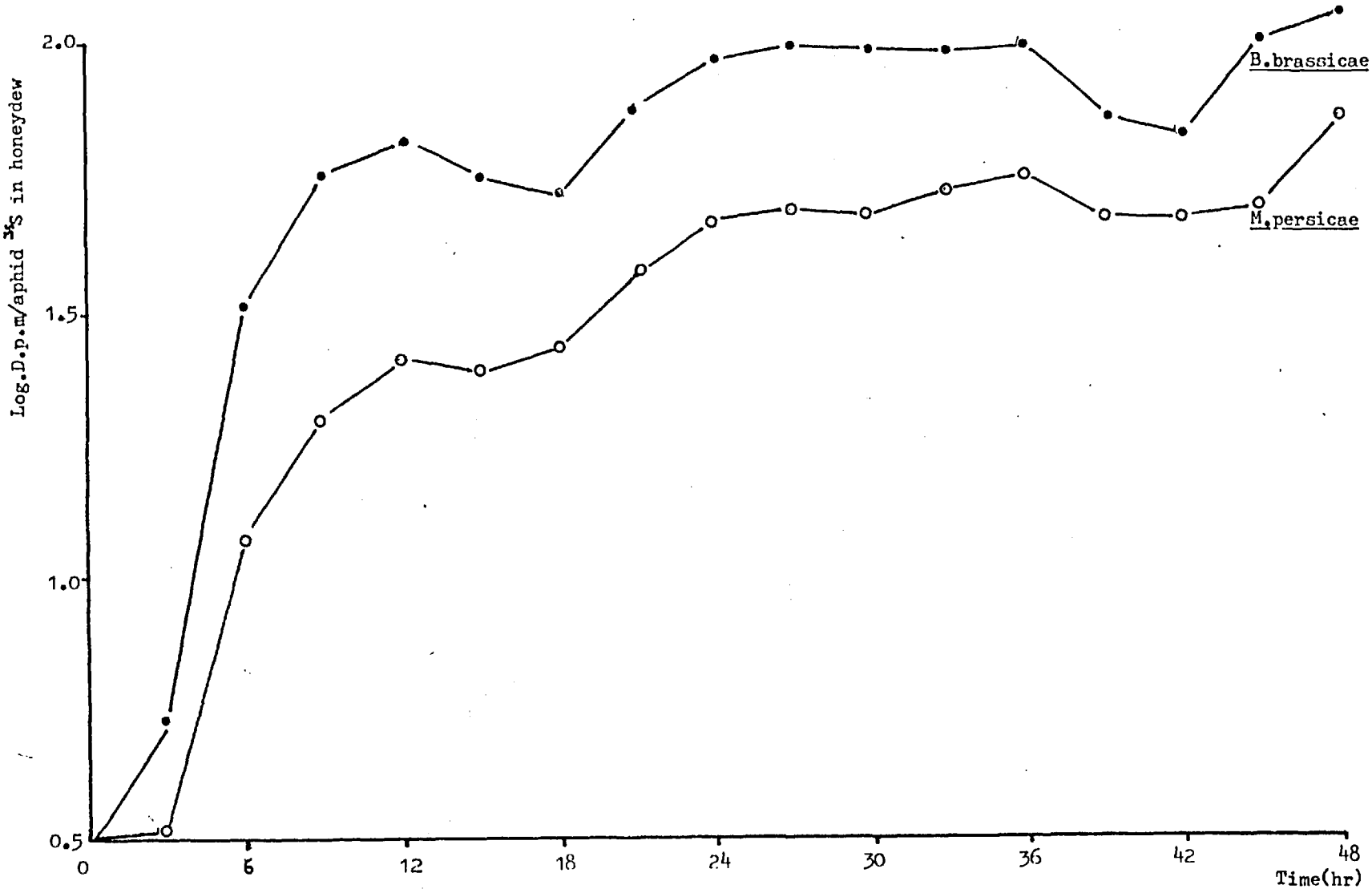


Fig.4 Excretion rates of M.persicae and B.brassicae using ^{35}S labelled sulphate

artificial lighting was switched off during the 8 hours of darkness.

3.4. The effect on excretion rate of changing diet sachets

An experiment was carried out to determine the effect of changing diet sachets on the excretion rate of the two aphid species. After the sachets were changed, the excretion rate experiment was repeated as before, but the honeydew was collected for hourly intervals.

The excretion rates (Appendix Table 12) during the first 6 hours show no significant difference between the two species of aphids (Fig.5), both appearing to settle equally quickly.

3.5. The effect of pesticide on feeding rate and absorption

3.5.1. Dimethoate

Experimental aphids were placed on sachets containing a standard volume of sodium ^{32}P-phosphate, and allowed to feed for 48 hours while the honeydew was collected on glass coverslips. The sachets were then exchanged for others with differing concentrations of dimethoate but a fixed concentration of sodium ^{35}S-sulphate and the honeydew collected on fresh coverslips for a further 48 hours. At the end of each 48 hour period, a sample was taken from the sachet and the concentration of radioactivity determined. The honeydew and aphids were also analysed for radioactivity. The aphids not only contained ^{35}S-sulphate but also ^{32}P-phosphate from the first sachet. Likewise some honeydew samples also contained both isotopes, some overlap occurring when the sachets were changed. The scintillation counter was able to discriminate between the energies of ^{32}P and ^{35}S emission though the counting spectra of these two isotopes overlap to some degree. To facilitate analysis of the data a computer was used to quantify the results. The count of radioactivity in sachets, aphids and honeydew in each period is represented in Appendix Table 13

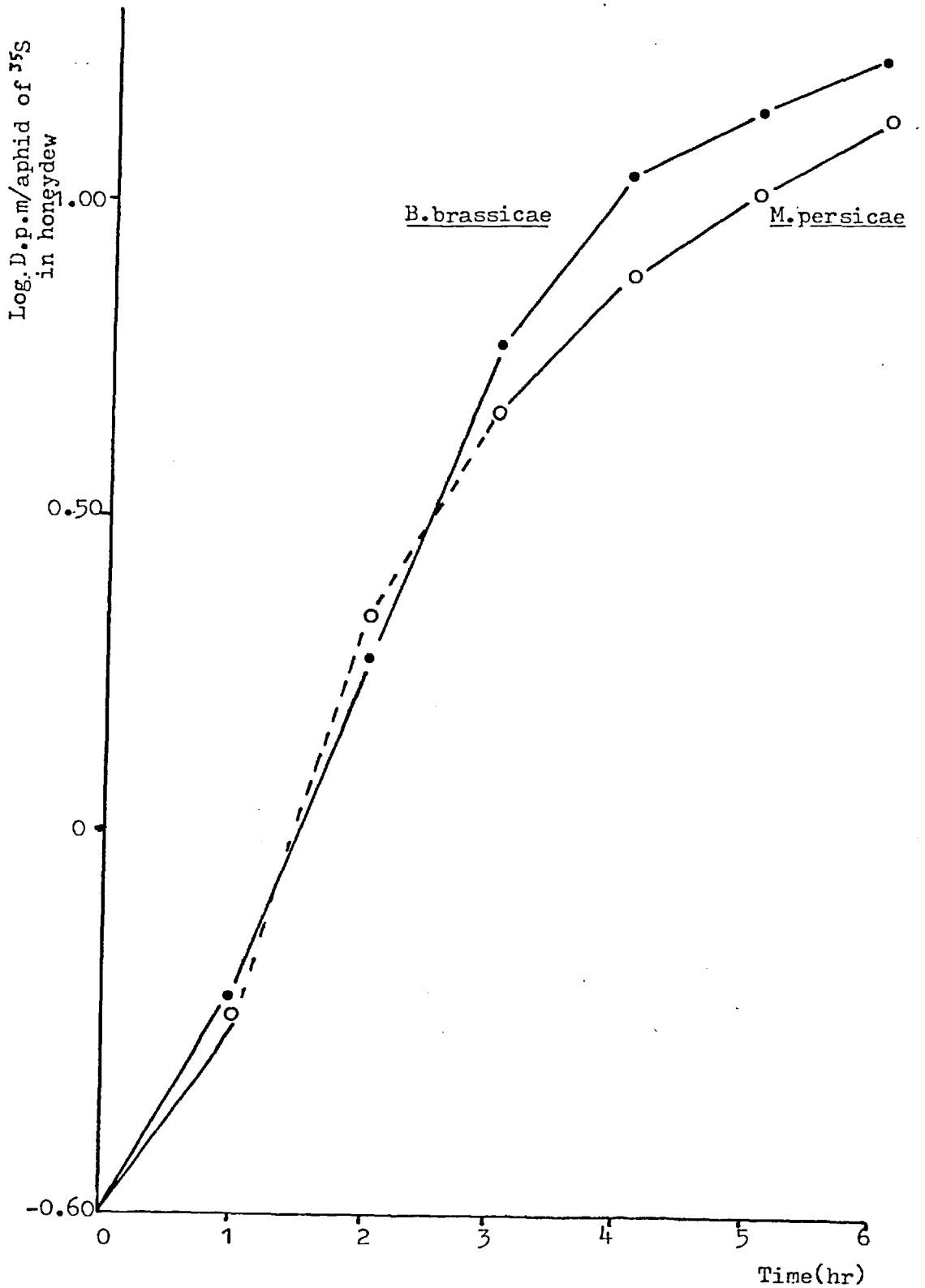


Fig.5 Excretion rates of B.brassicae and M.persicae recorded at 1hr intervals using ^{35}S labelled sulphate added to diet sachets

for M.persicae and Appendix Table 14 for B.brassicae.

Using the data collected, the total volume of diet imbibed for both species over the two 48 hour periods was compared (Tables 2 and 3) and plotted on graphs (Fig.6). Although the doses of pesticide were largely sublethal, the graphs show that B.brassicae is much more sensitive to pesticide in the diet. Linear regression analysis shows B.brassicae to have a slope of -1.17 compared with -0.45 for M.persicae. The sublethal doses in fact appear to have little effect on M.persicae, and it is only at higher concentrations of pesticide, when a small percentage of kill probably accounted for the drop in feeding, that a reduction in the volume imbibed was observed. B.brassicae however shows a clear linear relationship between volume removed from the sachet and increase in pesticide concentration, even at the lowest values.

3.5.2. Ethirimol

Aphids were allowed to feed on sachets containing ^{35}S-labelled sodium sulphate for 24 hours and their honeydew collected every 4 hours using the "Aphid Clock". From a stock solution of the systemic fungicide ethirimol in methanol, sachets were made up containing a known quantity of ^{35}S-sulphate and either 100 or 10 µg/ml of the fungicide. Sachets containing 10 µl of methanol and ^{35}S-sulphate acted as controls. The aphids were fed on the treated sachets for a further 24 hours and analysed with the honeydew for radio-label. Both sachets were sampled for ^{35}S-sulphate after each 24 hour period, and these values used to standardise the aphid and the honeydew values.

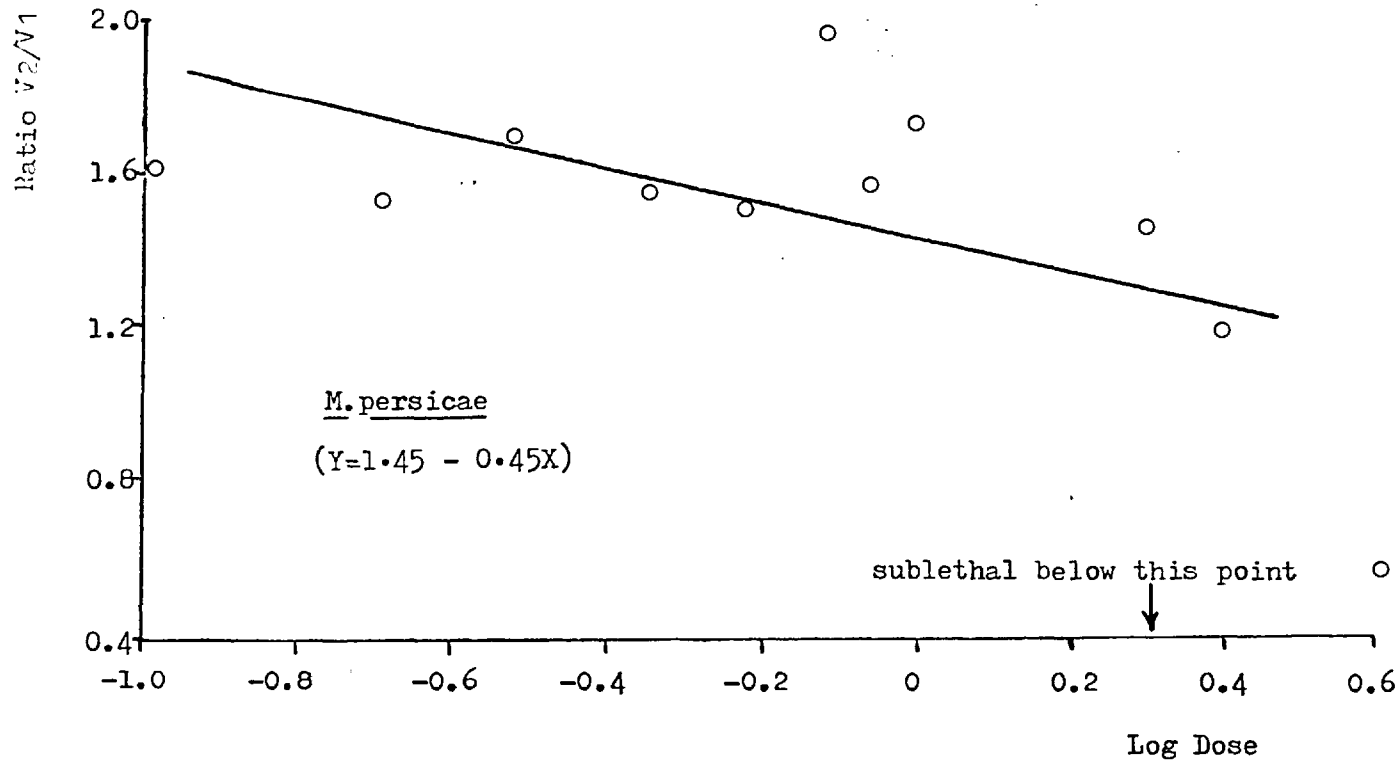
The standardised honeydew values are shown in Appendix Tables 15, 16, 17 for B.brassicae and Appendix Tables 18, 19, 20 for M.persicae. Analysis of variance was carried out on this data using the computer package "Glim" (General linear interactive modelling) written by the

Dose	Vol. Imbibed 1st 48 hours µl/aphid V1	Vol. Imbibed 2nd 48 hours µl/aphid V2	Ratio V2/V1
CONTROL	0.14	0.28	2.0
0.1	0.14	0.23	1.64
0.2	0.16	0.25	1.56
0.3	0.14	0.24	1.71
0.45	0.19	0.30	1.58
0.60	0.17	0.26	1.53
0.75	0.13	0.26	2.0
0.90	0.18	0.29	1.61
1.0	0.17	0.30	1.77
2.0	0.16	0.24	1.50
2.50	0.18	0.22	1.22
4.0	0.21	0.12	0.57

TABLE 2 The effect of various doses of dimethoate on the feeding rate of M.persicae
(Dose in µg/ml of diet)

Dose	Vol. Imbibed 1st 48 hours µl/aphid V1	Vol. Imbibed 2nd 48 hours µl/aphid V2	Ratio V2/V1
CONTROL	0.15	0.49	3.27
0.02	0.25	0.44	1.76
0.04	0.28	0.37	1.32
0.06	0.24	0.34	1.42
0.075	0.31	0.36	1.16
0.10	0.23	0.25	1.09
0.16	0.12	0.11	0.92
0.20	0.11	0.09	0.82
0.30	0.40	0.12	0.30
0.40	0.36	0.08	0.22
0.60	0.69	0.04	0.06

TABLE 3 The effect of various doses of dimethoate on the feeding rate of B.brassicae
(Dose in µg/ml of diet)



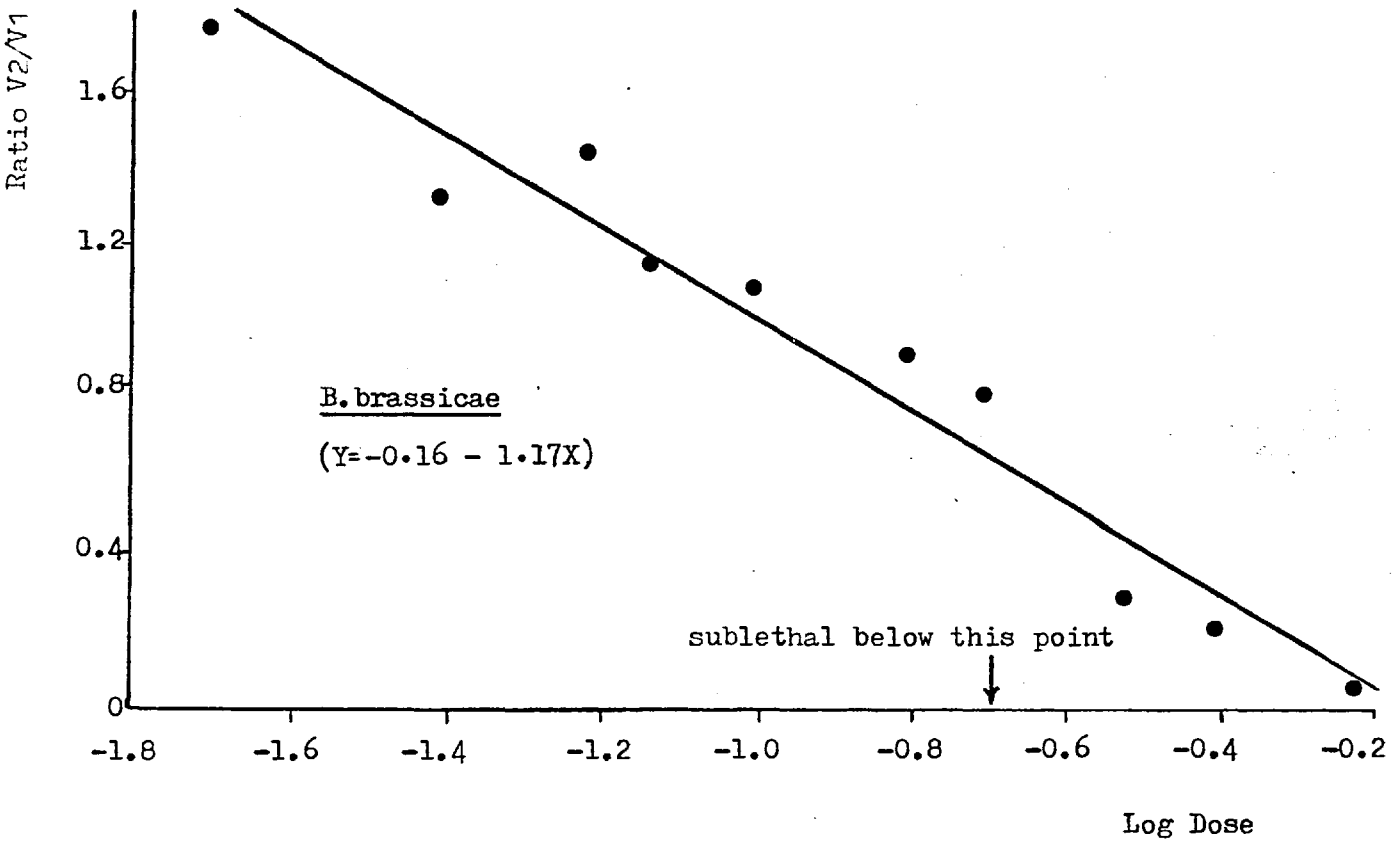


Fig.6 The effect of various doses of dimethoate on the feeding rate of the aphids M.persicae and B.brassicae
(Dose in µg dimethoate per ml. of artificial diet)

Royal Statistical Society of London. Analysis showed that the excretion rate of B.brassicae was significantly reduced by both 10 µg/ml and 100 µg/ml concentrations of ethirimol in the diet (t (10 µg/ml) = 2.17; t (100 µg/ml) = 5.89 . $p < 0.05$). M.persicae however showed no significant reduction in excretion rate when a concentration of 10 µg/ml of ethirimol was added to the diet (t (10 µG/ml) = 0.13 . $p > 0.05$), but a significant decrease did occur when 100 µg/ml of the fungicide was present (t (100µg/ml) = 3.39 . $p < 0.05$).

3.6. Analysis of ^{14}C-phorate residues

Sublethal doses of ^{14}C-phorate were added to diet sachets and aphids allowed to feed on them for 24 hours. The aphids and honeydew were then analysed for radioactivity. Due to the rapid hydrolysis of phorate in aqueous solutions, residue analysis was carried out for both toxic and non-toxic compounds by partitioning into solvent and aqueous soluble fractions.

The quantity of toxic and non-toxic pesticide extracted is expressed as a percentage of the total radioactivity in the sachet (Appendix Tables 21 and 22) and represented in Fig.7. There was 3 times more of both toxic and non-toxic residues in B.brassicae than in M.persicae, whilst the difference in quantities present in the honeydew of the two species was insignificant.

3.7. Absorption on ^{35}S-sulphate by aphids

Aphids were allowed to feed on sachets containing ^{35}S-labelled sodium sulphate for 48 hours. The sachets and aphids were then analysed for radioactivity (Table 4). The results showed an insignificant difference in the amounts of radioactivity present in the two species (T-test sig. $p = 0.32$, $T = 1.04$).

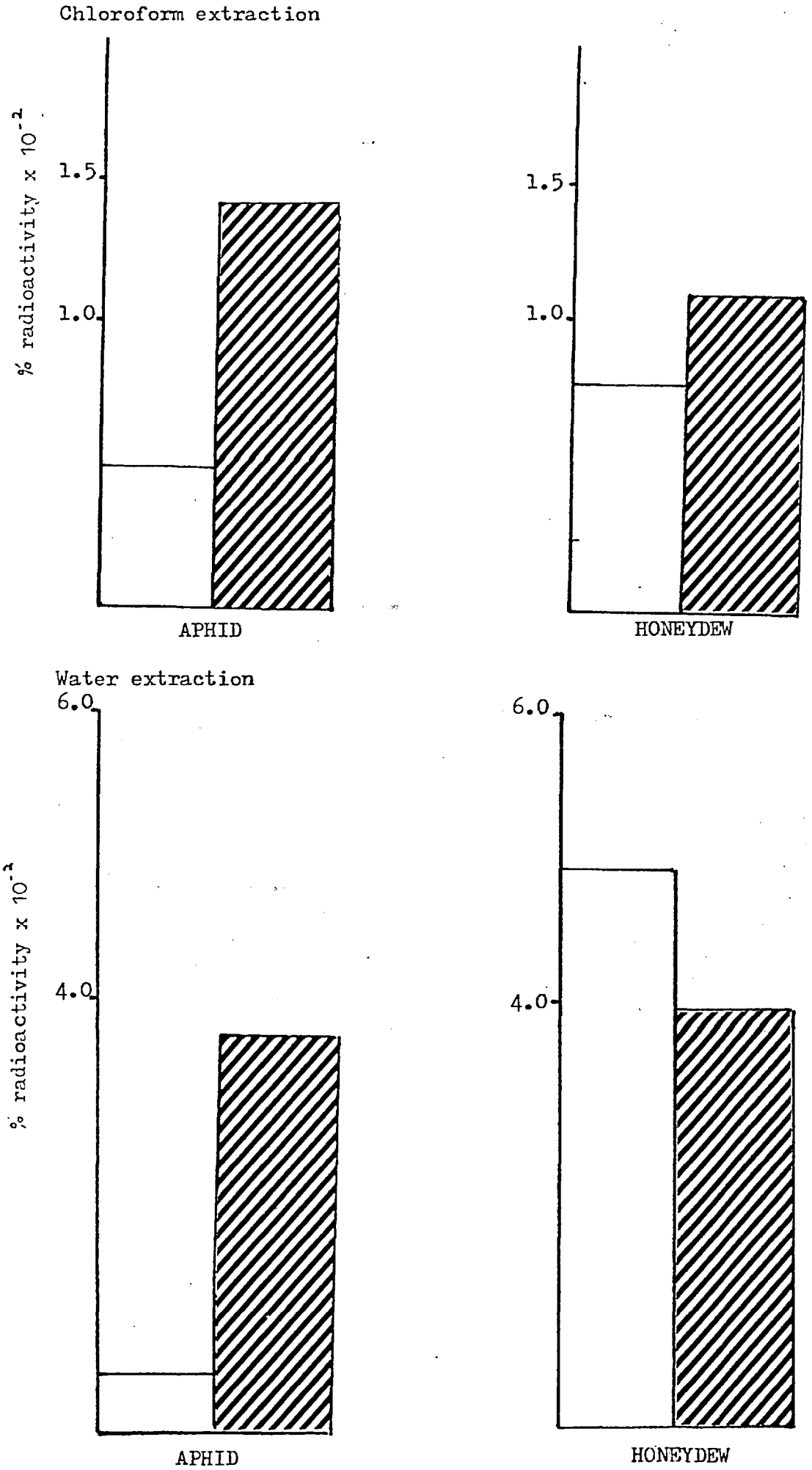


Fig.7 Extraction of ^{14}C-phorate from aphids and honeydew of B.brassicae ▨ and M.persicae □

	B.brassicae	M.persicae
	3007.5	2377.4
	3062.6	2583.8
	2451.7	3233.8
	2654.9	2756.4
	4126.9	3274.4
	3879.9	3942.9
	3920.4	2609.5
	4390.2	3920.8
MEAN ± S.D	3436.8 ± 729.0	3087.3 ± 607.0

TABLE 4 Amount of ^{35}S-labelled sulphate absorbed over a period of 48 hr by the aphids M.persicae and B.brassicae expressed in d.p.m/aphid.

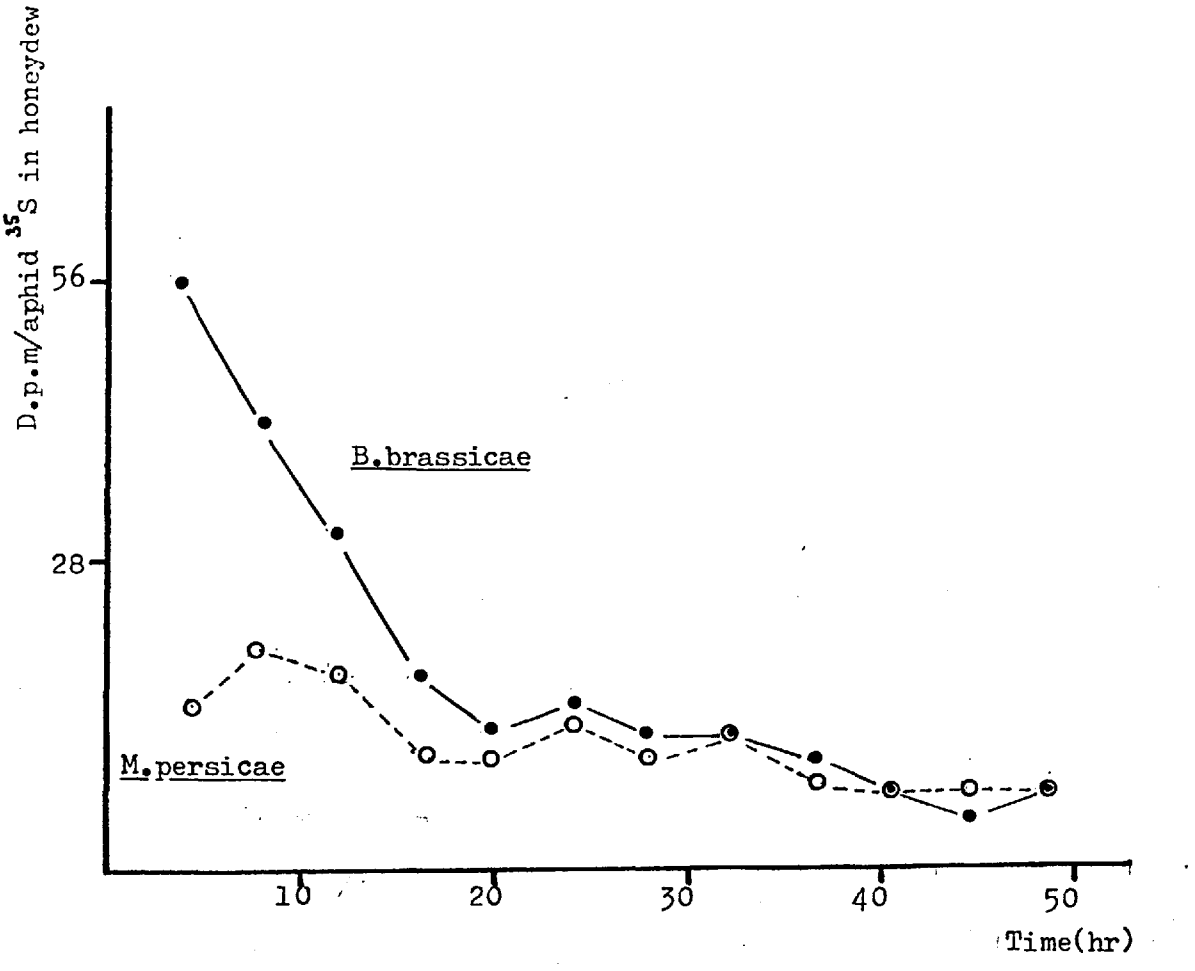


Fig.8 The quantity of ^{35}S-labelled sulphate in the honeydew of B.brassicae and M.persicae at 4-hourly intervals after transfer from radioactive to normal diet sachets

3.7.1. Gut clearance

Aphids were fed on sachets containing ^{35}S-sulphate for 24 hours before exchanging these with similar but non-radioactive sachets. The honeydew was collected every 4 hours over a 48 hour period from the time that the aphids started feeding on the unlabelled sachet and at the end of this time the aphids and honeydew were analysed for radioactivity.

The honeydew values (Appendix Tables 23 and 24) showed that after 20 hours both species were excreting similar quantities of labelled material (Fig.8). Analysis of the aphids (Appendix Tables 25 and 26) also showed no significant difference between the species in the amount of radiolabel present (T-test sig. $p = 0.58$, $T = 0.57$).

3.8. Analysis of water loss from sachets

Aphids were allowed to feed on sachets containing a known amount of ^{35}S-sulphate. The initial concentrations were determined by taking a 20 µl sample of diet before sealing the sachet. A known number of aphids were allowed to feed on these sachets in a Fisons Controlled Environment Cabinet, set at 20°C and 75% R.H. on a 16 : 8 light : dark cycle, for 48 hours. To some sachets a sub-lethal dose of the pesticide pirimicarb was also added, and sachets without aphids acted as controls.

At the end of the 48 hour period the aphids were removed and the sachets sampled again by the concentration of radiolabel. The change in concentration of the radiolabel enabled the volume of water lost by evaporation to be calculated (Appendix Tables 27 to 31). Analysis of variance on the change in concentrations gave $F = 0.12$, showing an insignificant difference between sachets. Likewise analysis of variance on the µl/aphid of water lost by evaporation is also insignificant ($F = 0.31$).

3.9. B-enzyme studies

3.9.1. Electrophoresis

3.9.1. (i) Starch Gel Electrophoresis

Horizontal starch gel electrophoresis was performed on 25 mg of homogenised aphid material for 16 hours at $4^{o}C$ and 5V/cm of gel. The β-esterases were identified using α-naphthyl acetate as substrate and Fast Blues RR (salt) as the dye. The resulting gels are represented in Fig.9 and show both species to possess six different esterases. The most obvious differences were that the most active band from B.brassicae homogenates was approximately 9.5 cm from the origin whereas the most active band from M.persicae was less intense than that of B.brassicae and at 6.0 cm.

3.9.1. (ii) Inhibition of cholinesterases

Gels were incubated in a 10^{-5}M solution of eserine sulphate for 15 minutes in order to inhibit the cholinesterase, before incubation in substrate and dye as before. In both species the two bands nearest the origin were inhibited and did not stain, leaving only four bands on the gel.

3.9.2. Quantitative determination of B-esterases using Hestin's method

3.9.2. (i) Standard curves

Two substrates, acetylcholine chloride and ethyl butyrate, were used to evaluate cholinesterases and carboxylesterases respectively. Standard curves were first prepared by reacting 1 ml of a known amount of substrate with 1 ml of phosphate buffer (pH 7.0, 0.04M) and 2 ml of alkaline hydroxylamine followed by 1 ml of HCl and 1 ml of ferric chloride solution. The optical density was determined using a spectrophotometer set at 540 μm for acetylcholine chloride and 620 μm for ethyl butyrate, and standard curves drawn (Appendix Fig.1.)

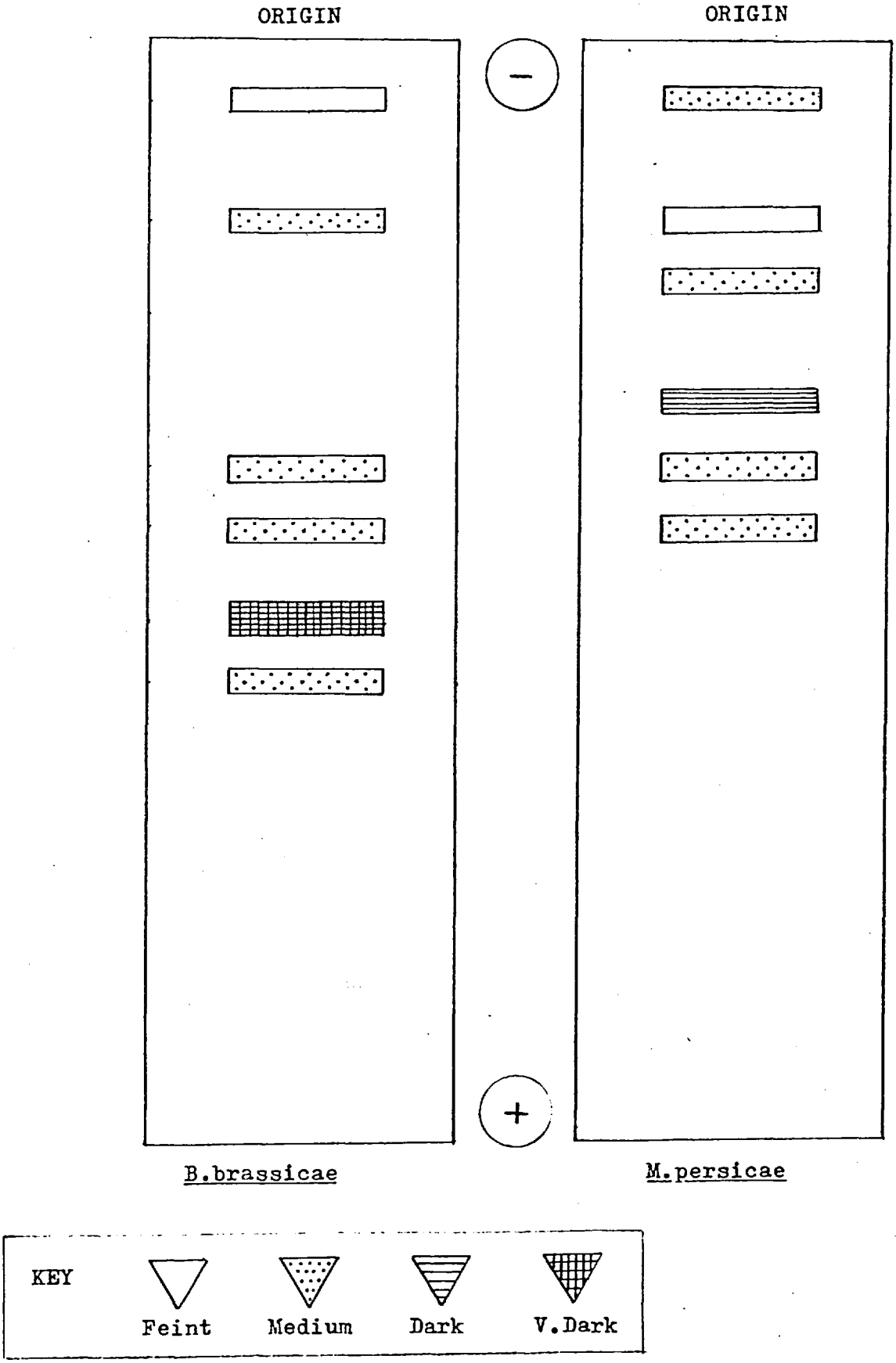


Fig.9 Diagramatic representation of esterases separated by starch gel electrophoresis of the aphids M.persicae and B.brassicae

3.9.2. (ii) Esterase concentrations

Acetylcholine : 30 aphids were homogenised in 1 ml of distilled water at $4^{o}C$ to form the enzyme solution, and various concentrations of substrate were used to determine the activity of cholinesterase present. The results showed there to be approximately 30% more cholinesterase activity in M.persicae than in B.brassicae (Appendix Table 32). The Lineweaver-Burke plot (Dixon and Webb, 1964) for determining the Michaelis Constant (Km) was plotted (Fig.10), and this gave values of 2.64×10^{-3} M for B.brassicae and 3.34×10^{-3} M for M.persicae.

Ethyl butyrate : 1 aphid per ml of distilled water was homogenised at $4^{o}C$. Adding different concentrations of ethyl butyrate substrate showed there to be 40% more carboxylesterase activity in M.persicae than in B.brassicae (Appendix Table II 32). The double reciprocal plot for the carboxylesterases showed the Km value for M.persicae to be 2.18×10^{-3} M and 2.57×10^{-3} M for B.brassicae (Fig.11).

3.9.2. (iii) Inhibition of B-esterases

Cholinesterase : Three cholinesterase inhibitors were used: Dimethoate, pirimicarb and eserine sulphate; their effect on enzyme activity can be seen in Figures 12 and 13. All three inhibitors showed an effect on in-vitro activity of cholinesterase, the enzymes in B.brassicae being more susceptible to different concentrations of inhibitor than those of M.persicae (Log molar I_{50} concentrations for dimethoate : B.brassicae - 4.50; pirimicarb : B.brassicae - 7.13, M.persicae - 7.06; and eserine sulphate B.brassicae - 9.63, M.persicae - 9.56).

Carboxylesterase : The effect of dimethoate and pirimicarb on carboxylesterase activity was also investigated, and again the B.brassicae carboxylesterases were more susceptible than those of M.persicae (Fig.14) The log molar I_{50} concentrations for dimethoate were - 5.70 for B.brassicae and - 5.33 for M.persicae; - 6.60 for B.brassicae and - 4.50 for M.persicae

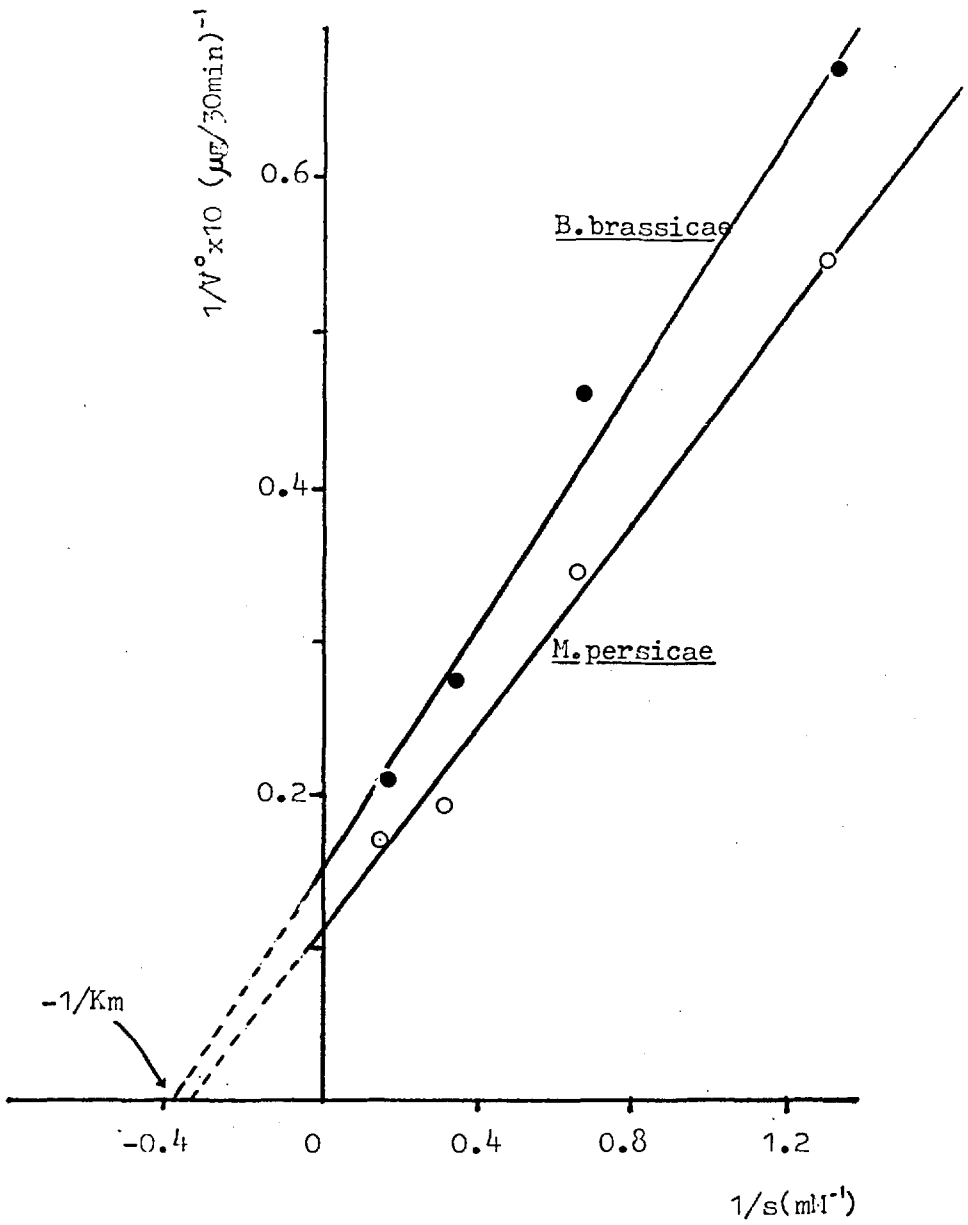


Fig.10 Double reciprocal (Lineweaver-Burk) plot for the reaction catalysed by cholinesterases of B.brassicae and M.persicae (Substrate: acetylcholine chloride)

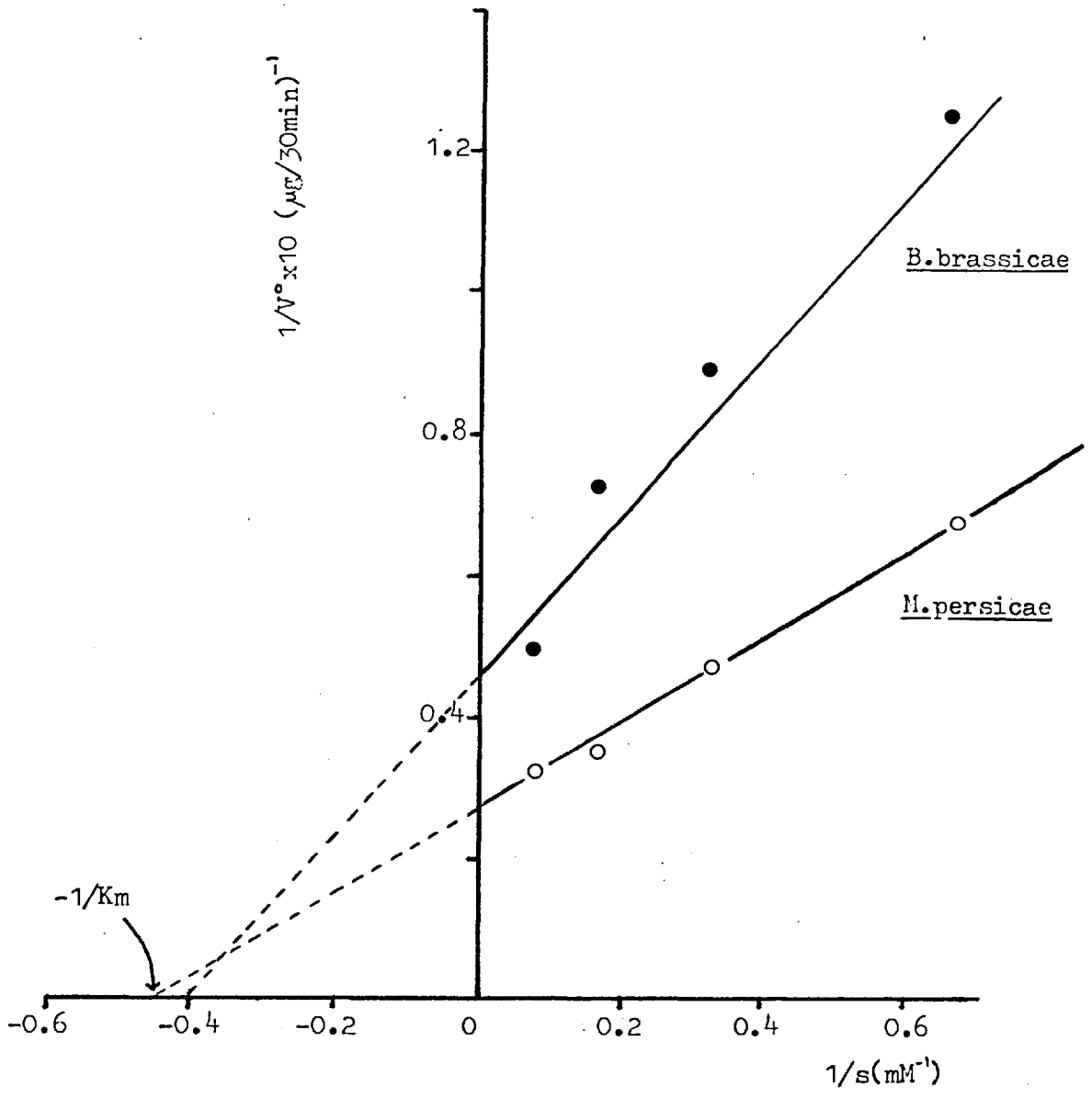


Fig.11 Double reciprocal (Lineweaver-Burk) plot for the reaction catalysed by carboxylesterases of B.brassicae and M.persicae
(Substrate:ethyl butyrate)

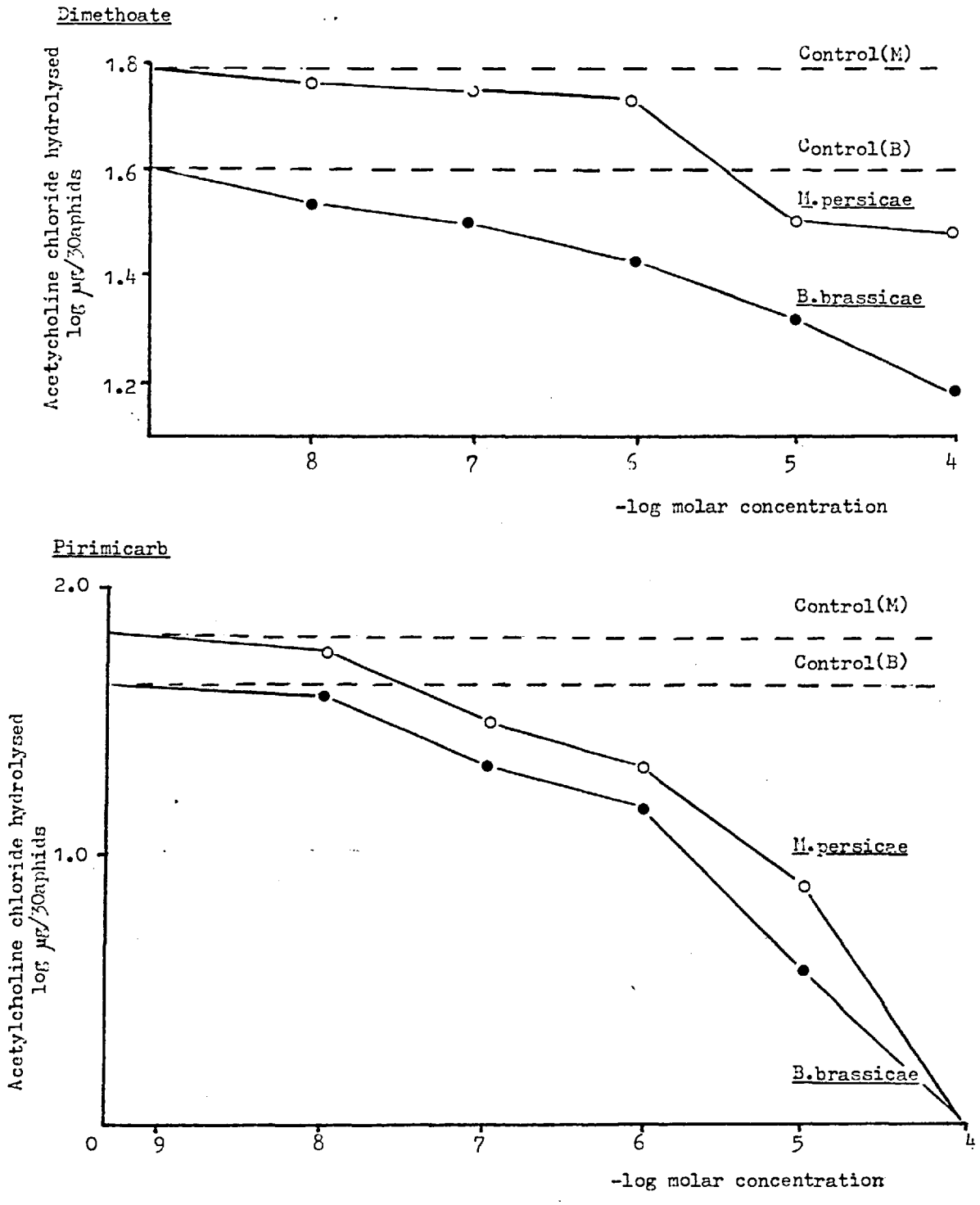


Fig.12 In vitro inhibition of whole aphid cholinesterase activity by different concentrations of pirimicarb and dimethoate.
(Substrate-acetycholine chloride: 3×10^{-3}M at 37°C)

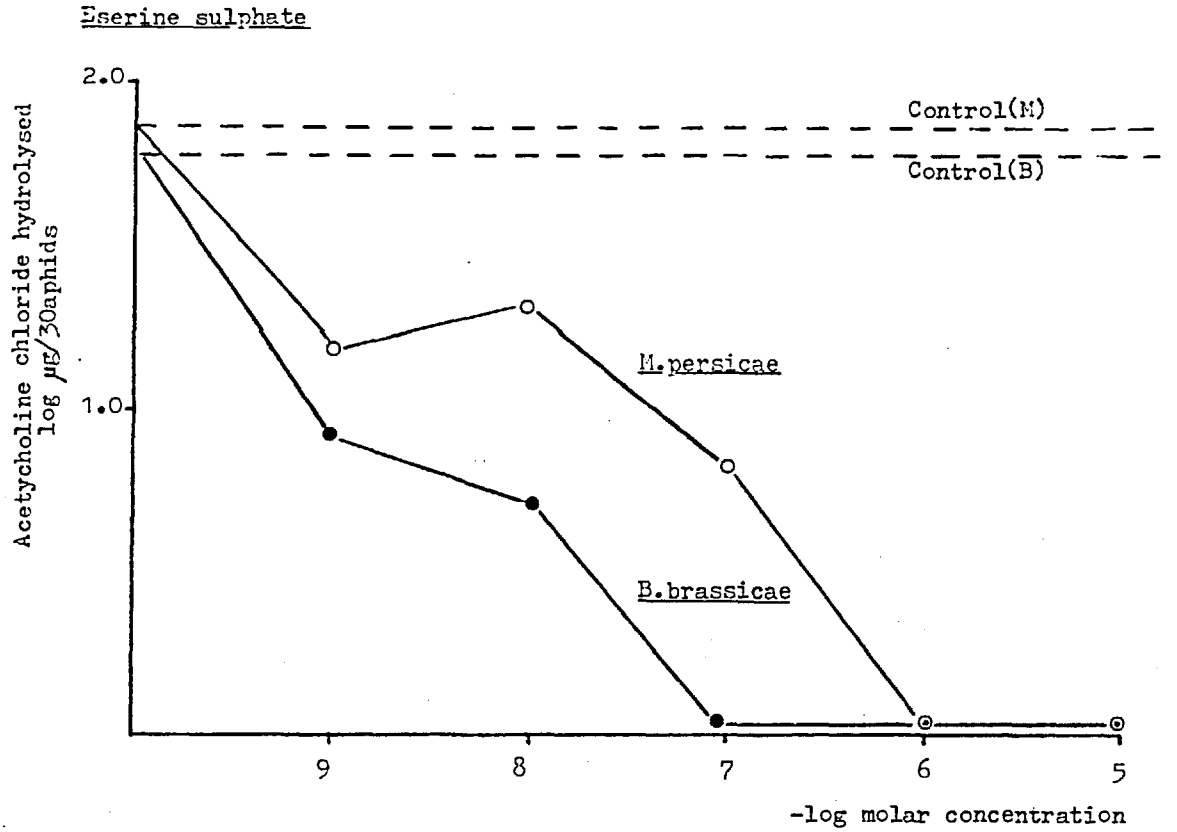


Fig.13 In vitro inhibition of whole aphid cholinesterase activity by different concentrations of eserine sulphate.
(Substrate-acetycholine chloride: 3×10^{-3}M at 37°C)

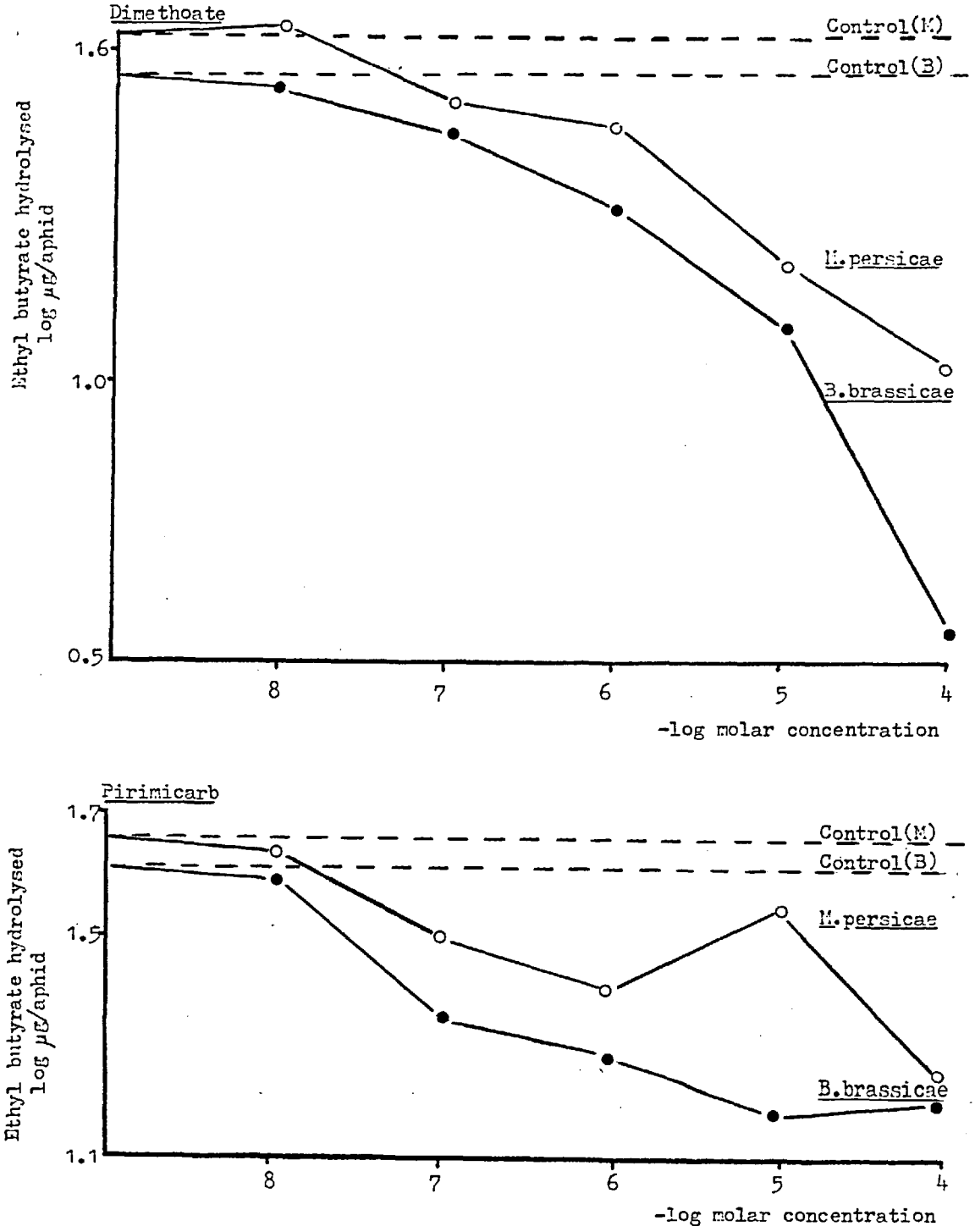


Fig.14 In vitro inhibition of whole aphid carboxylesterase activity by different concentrations of dimethoate and pirimicarb (Substrate-ethyl butyrate: 3.0×10^{-3}M at 37°c)

using pirimicarb.

3.9.3. Quantitative determination of B-esterases using Gomori's method

3.9.3. (ii) Standard curves

These were obtained by reacting 6 ml of α - or β-naphthol solution of appropriate concentration (in 1% acetone and 0.04 M phosphate buffer, pH 7.0) with 1 ml of DBLS solution. The resulting optical densities were plotted against naphthol concentrations to give the standard curves (Appendix Fig.2).

3.9.3. (ii) Esterase activity

To obtain a quantitative determination of carboxylesterase only, cholinesterase activity was blocked by adding 2×10^{-7} eserine sulphate to the reaction. In order to investigate the effect of substrate concentrations on carboxylesterase activity, various dilutions of α and β-naphthyl acetate were reacted with a standard enzyme preparation, consisting of one aphid homogenised in 1 ml of distilled water (Appendix Table 33). The results showed that with α-naphthyl acetate as the substrate the carboxylesterases of B.brassicae are approximately 50% more active than those of M.persicae. The Lineweaver-Burk plot (Fig.15) gives a Km value for B.brassicae of 0.93×10^{-4}M, and 1.87×10^{-4}M for M.persicae.

Using β-naphthyl acetate as substrate the carboxylesterases in M.persicae showed 16% more activity than those in B.brassicae (K_m M.persicae 2.6×10^{-5}M; Km B.brassicae 4.0×10^{-5}M).

3.9.3. (iii) Inhibition of B-esterases

Three inhibitors were used: Dimethoate, pirimicarb and eserine sulphate. The effect of the inhibitors on enzyme activity using α-naphthyl acetate as the substrate can be seen in figures 16 and 17. All inhibitors were more active against enzymes in M.persicae than in B.brassicae. I_{50} values for dimethoate and pirimicarb could not be

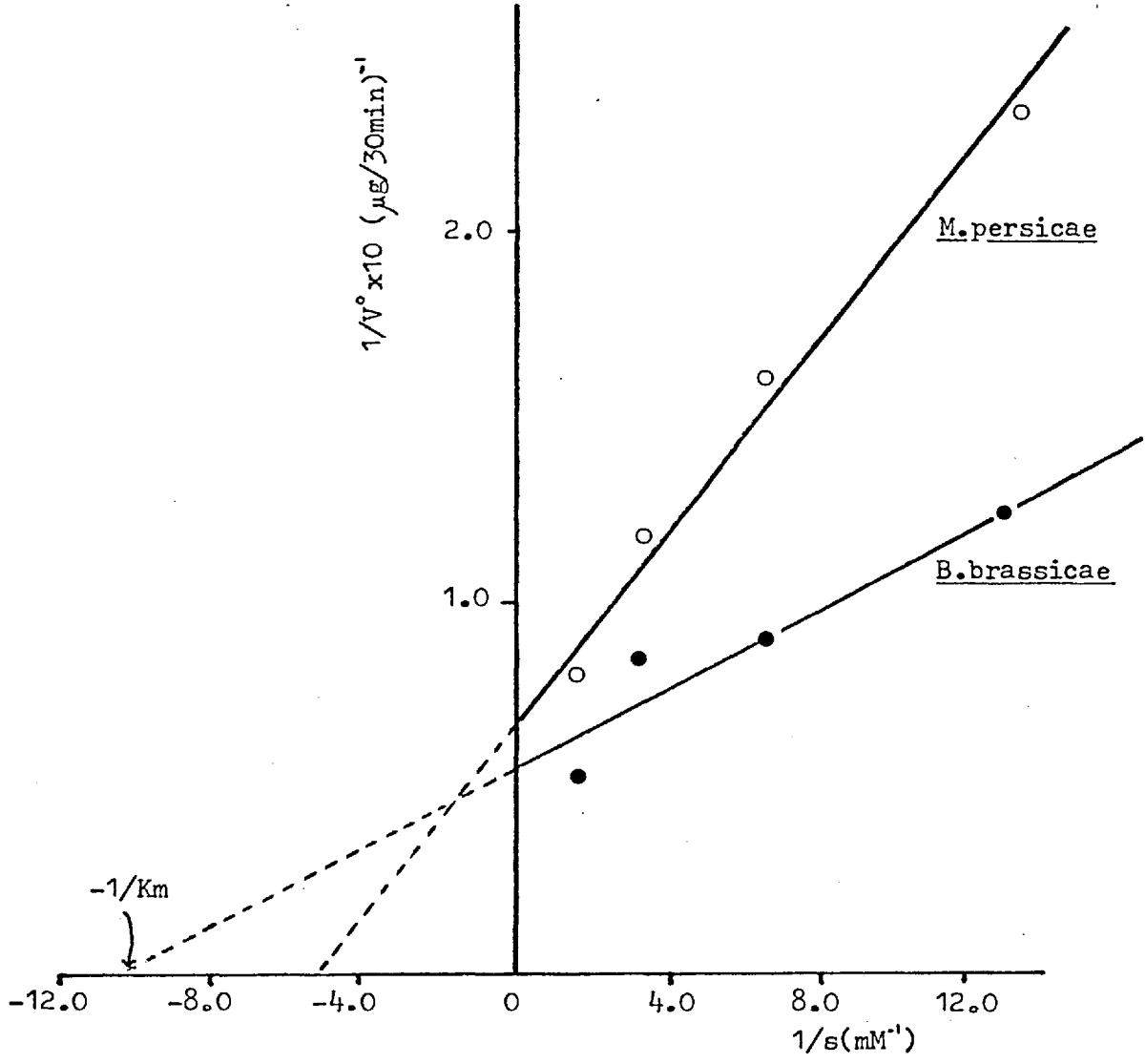
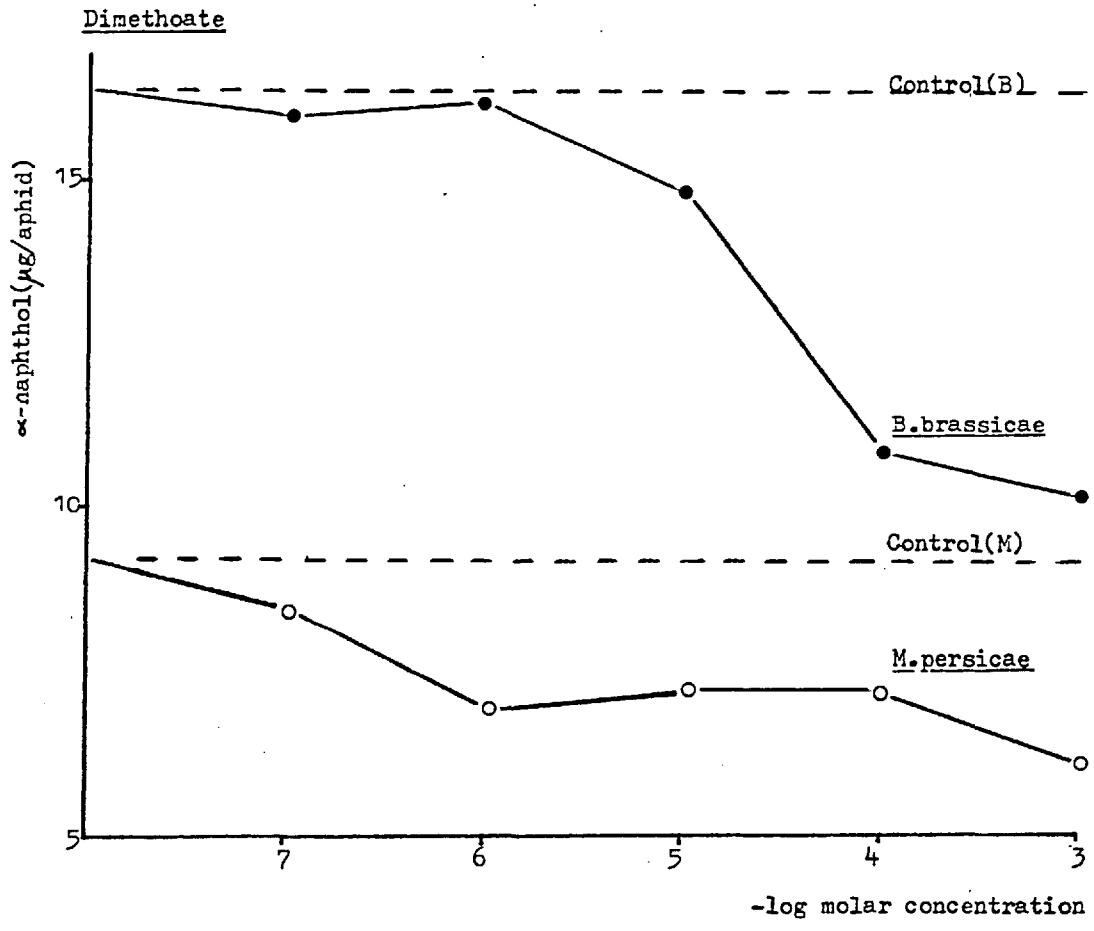
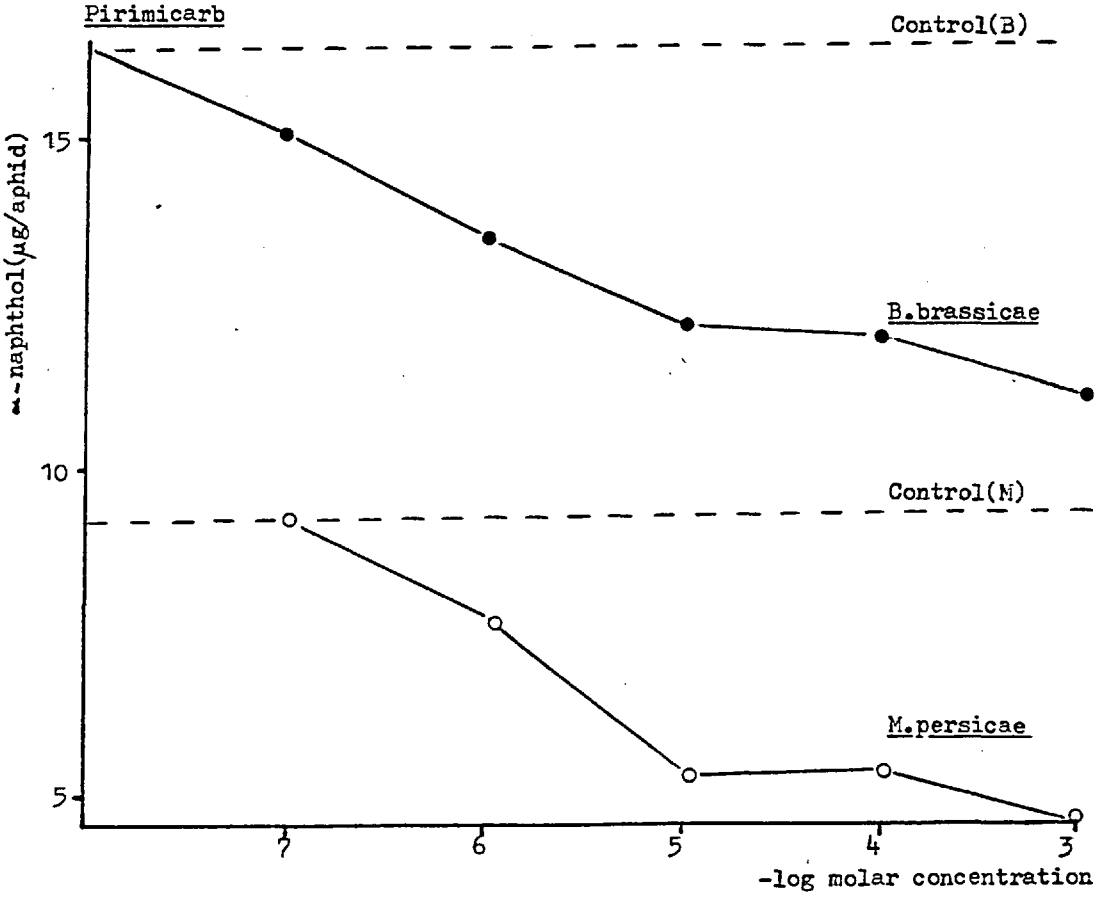


Fig.15 Double reciprocal (Lineweaver-Burk) plot for the reaction catalysed by carboxylesterases of B.brassicae and M.persicae (Substrate: ∝-naphthyl acetate)

Fig.16 In vitro inhibition of whole aphid carboxylesterase by different concentrations of pirimicarb and dimethoate.

(Substrate-α-naphthyl acetate : 3×10^{-4}M at 28°C)


Dimethoate
Control(B)
B.brassicae
Control(M)
M.persicae
∝-naphthol(µg/aphid)
15
10
5
7
6
5
4
3
-log molar concentration


Pirimicarb
Control(B)
B.brassicae
Control(M)
M.persicae
∝-naphthol(µg/aphid)
15
10
5
7
6
5
4
3
-log molar concentration

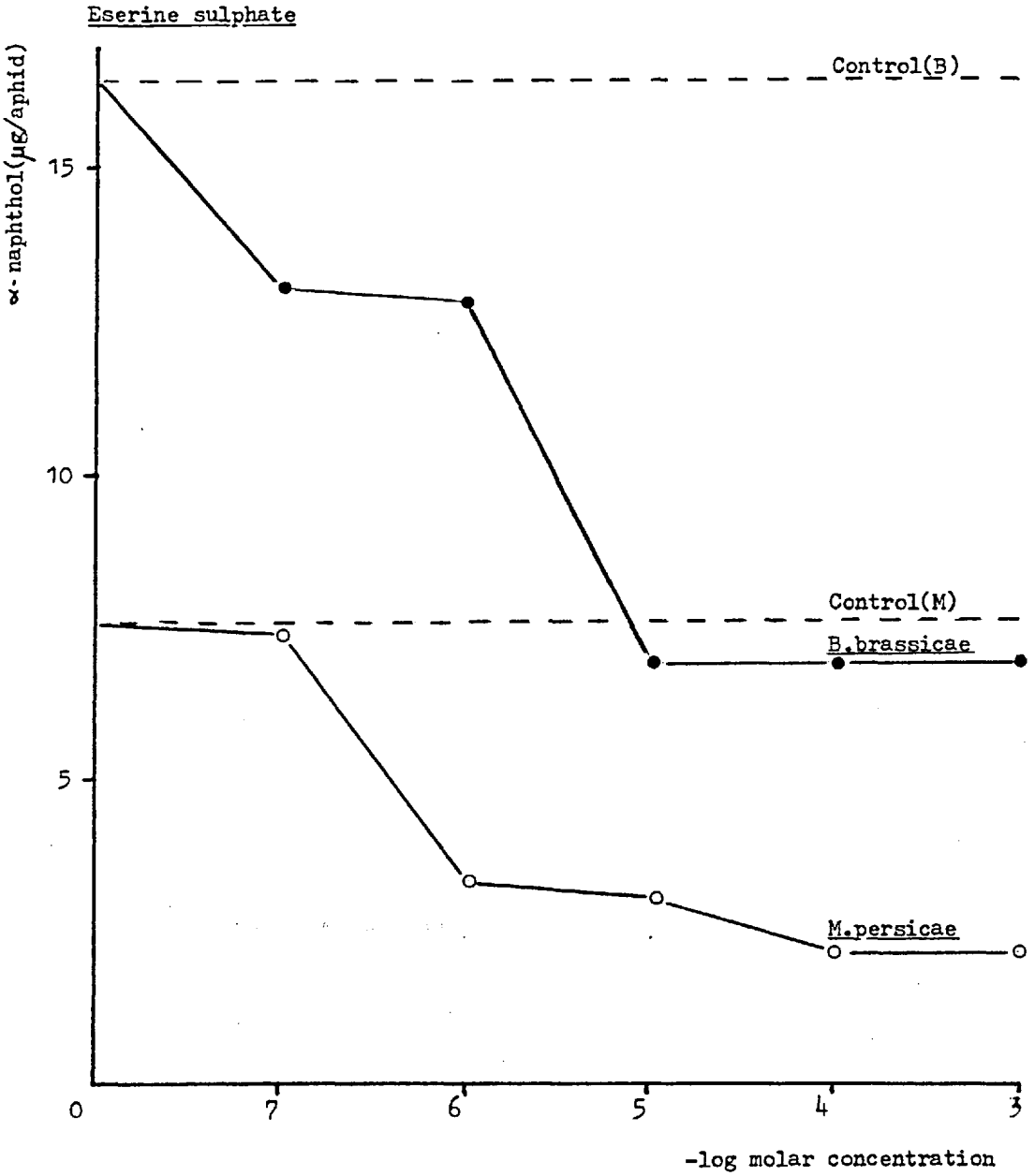


Fig.17 In vitro inhibition of whole aphid carboxylesterase by different concentrations of eserine sulphate.
(Substrate-∝-naphthyl acetate : 3×10^{-4}M at 28°C)

calculated due to the failure of obtaining 50% inhibition of esterase activity at the doses of inhibitor used. The log molar I_{59} using eserine sulphate was -5.23 for B.brassicae and -6.13 for M.persicae.

Using β-naphthyl acetate as the substrate the carboxylesterases in M.persicae were less sensitive to dimethoate and pirimicarb than those of B.brassicae (Fig.18). However eserine sulphate showed a reverse effect (Fig.19) and had a greater effect on enzymes of M.persicae than those of B.brassicae (Log molar I_{50} for eserine sulphate : B.brassicae - 4.33; M.persicae - 6.26). I_{50} values were again unobtainable for dimethoate and pirimicarb.

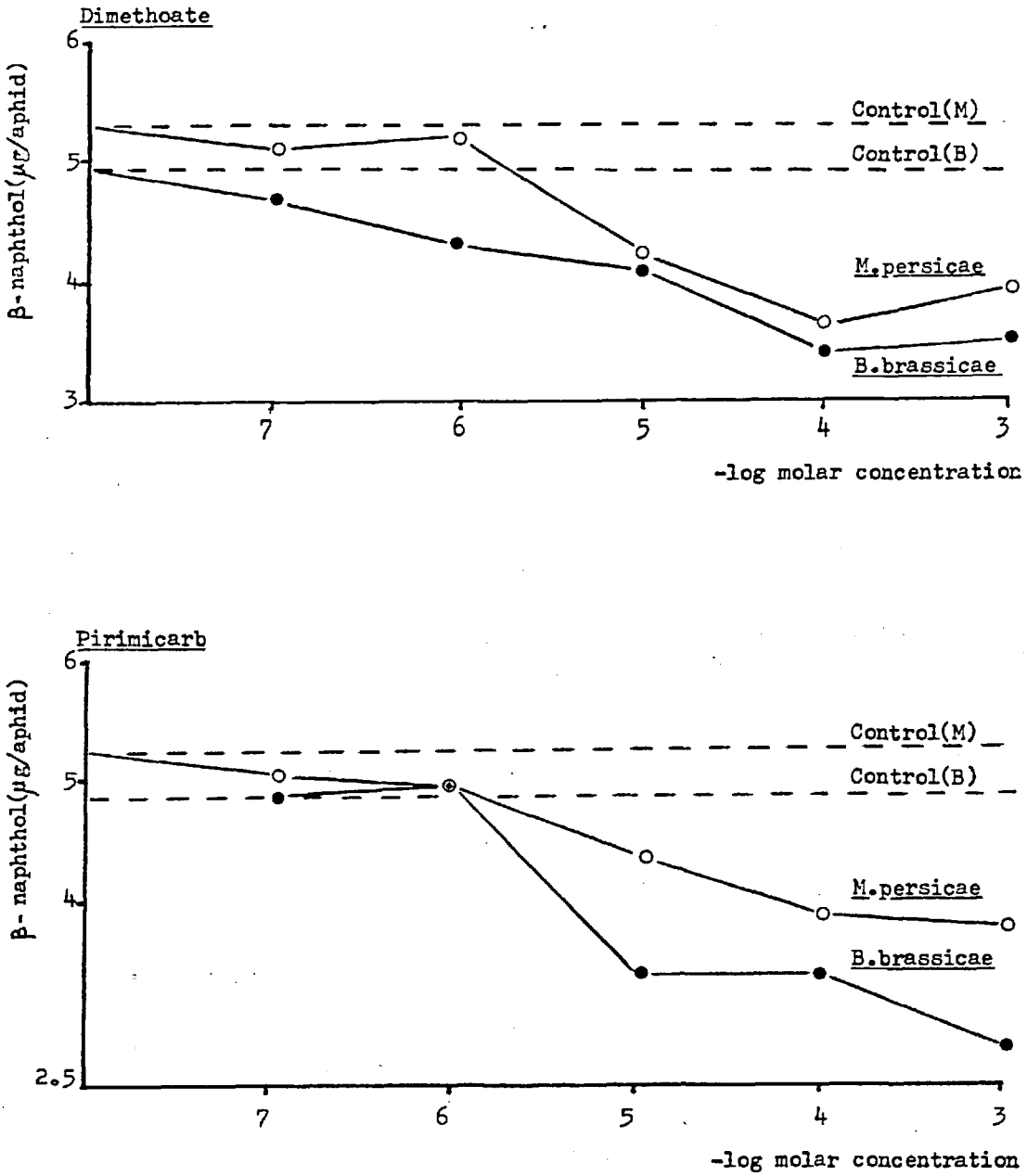


Fig.18 In vitro inhibition of whole aphid carboxyesterase by different concentrations of pirimicarb and dimethoate.
(Substrate-β-naphthyl acetate : 1.2×10^{-5}M at 28°C)

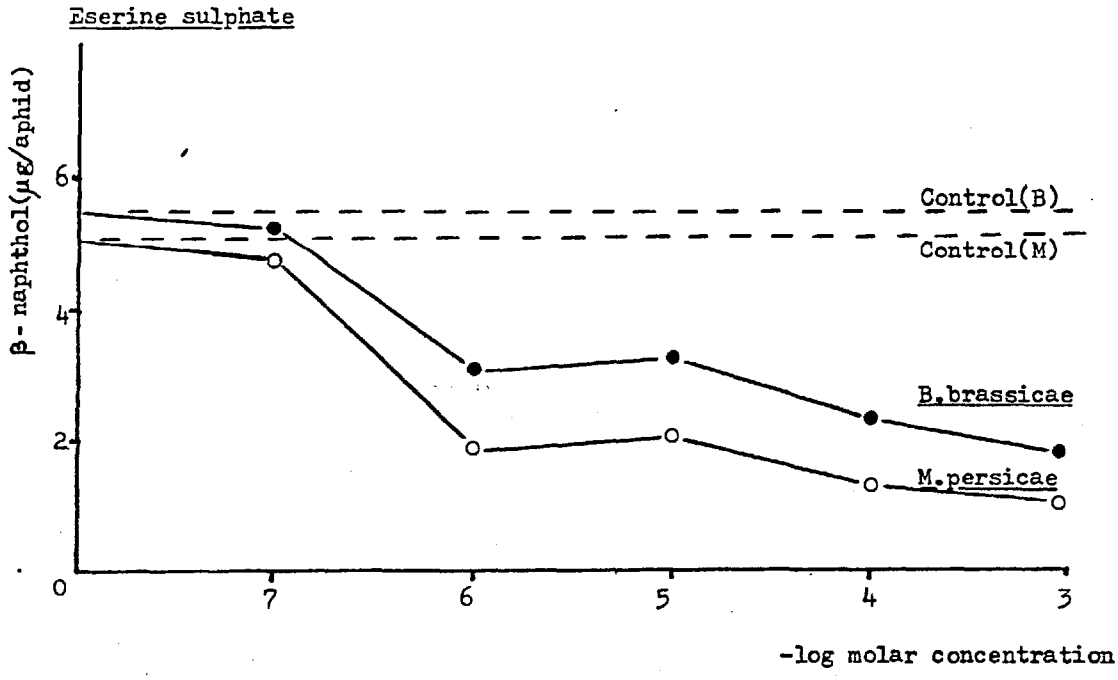


Fig.19 In vitro inhibition of whole aphid carboxylesterase by different concentrati of eserine sulphate
(Substrate-β-naphthyl acetate : 1.2×10^{-5}M at 28°C)

4. DISCUSSION

4.1. Toxicity of pirimicarb and dimethoate added to artificial diet sachets

Early work evaluating the toxicity of systemic insecticides involved caging insects on leaves but variables such as site application, rate of absorption, translocation, oxidation and hydrolysis made the assessment of toxic material available to the insects very unreliable. The use of artificial diet sachets overcomes some of the variables and allows accurate doses to be applied and more controlled bioassays to be performed. There are of course problems associated with the use of artificial diets which should be considered. Firstly the aphids reared on diet medium are smaller than those of similar age feeding on plants. Dadd and Mittler (1966) suggested that this size difference was due to nutrient imbalance, and later work by Llewellyn and Leckstein (1978) demonstrated that Aphis fabae used only 20% of the available energy content of artificial diet for growth, compared to 50% of that available in plants. Smaller size has also been attributed to longer instar duration (Tsitsipis and Mittler, 1976). Problems also arise from adding pesticides to sachets as their lipophilic nature can result in absorption into the sachet membrane. As much as 75% of toxic phorate was absorbed into membranes in experiments carried out by Ho (1978). In an attempt to reduce this tendency, the pesticides used in this study, as well as being systemic, were also reasonably soluble in water (Pirimicarb 2.7 g/l at 25°C; Dimethoate 25 g/l at 21°C). It must be remembered however that the partition coefficients for each pesticide will be the governing factor in determining exactly how much of each insecticide is absorbed into the membrane. Finally, contact toxicity from pesticide in the diet has been suggested, but this is unlikely as only the stylets are in direct

contact with the diet and sachet membrane, the aphids themselves being separated by a feeding membrane. Experiments by Mittler and Pennell (1964) ruled out any contact or fumigant toxicities from pesticides incorporated in diet sachets.

This study showed that there was a considerable difference in the susceptibility of the two aphid species to both pirimicarb and dimethoate incorporated in the diet, particularly to the latter insecticide where B.brassicae was thirteen times more susceptible than M.persicae. Similar susceptibility differences were recorded by Galley (1974) who found B.brassicae to be six times more susceptible than M.persicae feeding on leaf discs treated with dimethoate, and Handique (1977) who found a twenty fold difference when phorate was fed to the aphids in artificial diet.

In contrast B.brassicae and M.persicae were equally susceptible to topical application of dimethoate (Galley, 1974). A possible explanation for this may be a difference in penetration of insecticide through the cuticle, a resistance mechanism reviewed by Ebeling (1974). The cuticle of B.brassicae is covered with a thick grey waxy layer giving the aphid its characteristic mealy appearance, and it is this layer which may reduce the quantity of insecticide penetrating the cuticle.

It would seem therefore that either of the two species of aphid are equally susceptible but with different feeding rates perhaps resulting in the more voracious aphid being exposed to greater quantities of pesticide, or that they are not equally tolerant due to physiological and biochemical differences.

Excretion and feeding rates can have a significant effect on the susceptibility of aphids to pesticides (Parry and Ford, 1967; 1969; 1971). There are two methods for determining excretion rate or honeydew production,

either by measuring the dry weight of honeydew produced, or by the use of radiotracers. In preliminary work the dry weight method produced highly variable results, so a non-toxic radiolabelled salt was used, sodium ^{35}S-sulphate. Many earlier workers used ^{32}P-phosphate (Day and Irzykiewicz, 1953; Watson and Nixon, 1953; Banks and Nixon, 1959), however the short half life and high energy of the β-emission make this isotope less easy to use than the lower β-energy ^{35}S-sulphate which is accordingly more convenient to handle and has the added advantage of being less metabolically active than phosphate.

The excretion rate studies showed that over the 96 hour period studied B.brassicae excreted twice as much radiotracer as M.persicae. The total uptake of diet was determined by adding the amount of radiolabel excreted to the quantity found in the bodies of the aphids. Comparison of the total uptake after 48 hours shows B.brassicae to have removed 25% more diet from the sachets than M.persicae. Although uptake differences were less pronounced than the excretion rates, B.brassicae clearly feeds at a greater rate than M.persicae and would therefore be exposed to more insecticide. Indeed this species was the more susceptible of the two, though the differences in tolerance were much greater than those in their feeding rates.

Physical size must also be considered in excretion rate studies. B.brassicae weighed 50% more than M.persicae at the same age when feeding on artificial diet. This is consistant with the excretion rate study, the larger aphid excreting the greater quantity of honeydew. Increased body weight has been related to resistance (Way, 1954) but no correlation was apparent here, for the bigger aphid (B.brassicae) was the most susceptible. Body weight is likely to be more important when comparisons are made between resistant strains of the same species but between

different species more complex physiological and biochemical factors may be involved.

The feeding rate of M.persicae was relatively unaffected by different sublethal doses of dimethoate in the diet, whereas a marked reduction occurred in the feeding rate of B.brassicae at even the lowest concentration. Other workers have shown the feeding rate of M.persicae to be reduced by adding phosphamidon to artificial diet (Parry and Ford, 1967; 1969; 1971; Halmie and Ford, 1972), and concluded that a repellent or sublethal effect was responsible. Clearly the effect on B.brassicae was far more marked than any effect on M.persicae.

The excretion rate of both species was unaffected by the fungicide ethirimol in the diet except at very high doses. The significance of the results of this experiment is twofold; Firstly although ethirimol is not toxic enough to be considered an insecticide there is evidence to suggest that high doses do affect the aphids and as with insecticides B.brassicae was less tolerant than M.persicae. Secondly, the use of a non- or less-toxic complex molecule could provide a more useful tool in absorption studies than simple compounds. Labelled pesticides are expensive and often have low specific activities making them difficult to detect at low concentrations. The advantage of using labelled fungicides rather than salts for uptake and absorption studies is that molecular weights, partition coefficients and other physical and chemical properties are more closely related to those of other pesticides with the result that their distribution and behaviour in a biological system will be more representative than the simpler organic or inorganic compounds.

Devonshire (1973) suggested that M.persicae could eliminate toxic compounds in the honeydew and that up to 60% of a topically applied sublethal dose of dimethoate was excreted. However B.brassicae and

M.persicae fed on sublethal doses of ^{14}C-phorate in the diet showed no difference in the percentage of toxic material excreted. Analysis of aphids fed on diet containing labelled pesticide showed that B.brassicae contained a greater percentage of toxic material than M.persicae. This perhaps was to be expected in view of the higher feeding rate of B.brassicae, but also offers evidence suggesting absorption or metabolism differences between the two species.

Analysis of aphids after feeding on artificial diet with ^{35}S-sulphate, showed both species contained the same amount of radioactivity. Although obviously the labelled salt would not be expected to behave in exactly the same way as a pesticide, it suggests that both species had similar absorption rates. Aphids allowed to feed on a diet containing ^{35}S-sulphate for 24 hours, then transferred to unlabelled diets and analysed for radioactivity after 48 hours, showed that there was again no significant difference in the amount of radioactivity found in each species. It appeared that the radioactivity was contained in the body tissue, and that none of this label remained in the gut.

These experiments support the earlier findings using labelled insecticide, but with ^{25}S-sulphate both species contained similar amounts, whereas with insecticide more toxic residues were found in B.brassicae. Possible explanations are that either B.brassicae absorbs the insecticide more readily or that M.persicae contains a more efficient detoxifying system.

When considering the susceptibility differences between insects some mention should be made of behavioural effects caused by the insecticides. M.persicae is a polyphagous aphid not restricted to one host plant as is B.brassicae feeding exclusively on cruciferae. M.persicae feeds almost entirely on the underside of leaves (Cababrese and Edwards, 1976)

and prefers lower leaves whereas B.brassicae feeds at the growing tips and on younger maturing leaves. Wearing (1972) concluded that M.persicae responds to nutrients and will walk about in order to find the best feeding site, but B.brassicae will not. Many workers have described increased walking of M.persicae when in contact with insecticide (Chapman, 1951; McEwen, 1953; Roland, 1953; Mittler and Pennell, 1964). This is an important consideration in controlling aphids, not only might the aphid be able to detect and avoid treated areas (Evans, pers. comm.) but increased mobility may result in a wider spread of virus infections.

Observations made on aphids feeding on sachets containing pesticide indicated that M.persicae exhibited greater mobility than B.brassicae, the latter continuing to feed even at the higher doses. When confined on artificial diet sachets the aphids have no alternative but to feed on treated diet, however on a plant the situation is different, and it is likely that M.persicae would attempt to move to an area where there was less toxic material. It was noted by Lowe (1976) that whereas B.brassicae fed mainly on the phloem, M.persicae spent a large proportion of time feeding from the spongy mesophyll. Being polyphagous, M.persicae has an even wider choice of alternative plants than B.brassicae, and therefore has a much better chance of avoiding toxic material.

As aphids probe through the sachet membrane to reach the diet they form holes through which water can evaporate (Mittler, 1970). Increased probing by aphids would cause more holes and therefore greater evaporational loss. An experiment attempting to show greater probing activity when a sublethal dose of pirimicarb was added to the artificial diet sachets yielded inconclusive results. Although differences did occur, studies with other insecticides using more replicates may be able to quantify behavioural observations and relate activity to the

presence of insecticide.

If a colony of M.persicae is disturbed whilst feeding, a proportion of the aphids fall off from both sachets and plants whereas B.brassicae does not detach itself unless considerable disturbance occurs. Ho (1978) noted that changing diet sachets interrupts feeding, an observation also made here. In order to investigate any differences in settling behaviour between the species, the excretion rate was monitored at hourly intervals after a sachet change. Both species settled equally quickly after the sachet was changed and it could be assumed that this disturbance would not influence relative honeydew production results.

There are several other topics that could be investigated which follow on from the work done here. A clear difference existed between excretion rate and absorption in B.brassicae and M.persicae although the role of the gut is difficult to evaluate. Apart from a good review by Forbes (1964), the morphology and physiology of the aphid gut is still poorly documented. Isolation of the gut to enable permeability studies similar to those performed by Shah and Guthrie (1971) and Shah et al (1972) is difficult due to the small size and fragile nature of the gut tissues. These constraints make sampling very difficult and the resulting fluid volumes so small that analysis becomes inaccurate.

A resistance mechanism put forward by Eastop and Banks (1970), working on M.persicae could also prove worth studying in relation to the differing susceptibilities of B.brassicae and M.persicae. These workers noted that the siphunculi of a resistant strain of M.persicae were relatively longer than those of the susceptible strain, and proposed that excretion of insecticide by these structures, in association with wax or lipid, could be a resistance mechanism.

Further behavioural studies could be carried out in choice-chamber

experiments like those used by Mittler and Dadd (1964b) and Parry (1966). It would then be possible to determine the sensitivity of each species to insecticide in the diet and relate this to its toxic effect.

4.2. Enzyme studies

The horizontal starch gel electrophoresis of whole aphid esterases showed distinct patterns for each aphid species. The esterases of B.brassicae appeared to be the most mobile and were characterised by one very dark band, the bands resulting from M.persicae being generally less pronounced. The cholinesterases of both species follow the general pattern described by Augustinnson (1958) and move the least distance from the origin. The electrophoresis experiments used α-naphthyl acetate as the substrate which as well as being split by carboxylesterases is also affected by arylesterases (Menzel et al, 1963) and acid or alkaline phosphatases (Cook and Forgash, 1965). The electrophoreses experiment demonstrated a qualitative difference between esterases in the two species which warrants further examination and it would therefore be of interest to repeat these experiments with other substrates and characterise the bands by selective inhibition.

The most obvious result from the quantitaive activity studies was the characteristic behaviour of aphid esterases that combine high or low carboxylesterase activity depending on the substrate being hydrolysed. This suggests the presence of several isoenzymes, each acting differently on a variety of substrates (Sudderuddin, 1972). The activity studies showed the carboxylesterases of M.persicae to be more active than those of B.brassicae with ethyl butyrate and β-naphthyl acetate as substrates, the reverse situation occurred when α-naphthyl acetate was used. Van Asperen and Oppenoorth (1959) and Van Asperen (1962; 1964) found

resistant houseflies exhibited lower activity towards α-naphthyl acetate than susceptible strains. Devonshire (1975a; 1977), however, found a good correlation between increased carboxylesterase activity and resistance in M.persicae, as did Ozaki (1969) working on leafhoppers. The differences in carboxylesterase activity between M.persicae and B.brassicae was small using ethyl butyrate and β-naphthyl acetate, but large using α-naphthyl acetate. This may be significant in that the activity differences between resistant and susceptible strains are normally large, twenty fold differences being reported by Devonshire (1975a; 1977). Devonshire however expressed his values per unit weight of aphid, whereas in this study the values have been expressed as activity per aphid. M.persicae was smaller than B.brassicae when reared on artificial diet and thus a correction factor applied to express activity on a weight basis would result in a greater activity variation using ethyl butyrate and β-naphthyl acetate but reduce the α-naphthyl acetate differences. However as the bioassay was expressed in terms of numbers rather than weights of aphids, the uptake and absorption too have been related to individual aphids, it was decided to express activity values per aphid.

There appears therefore to be a good correlation between increased carboxylesterase activity and greater tolerance of M.persicae. The activity difference using α-naphthyl acetate is probably a result of the non-specificity of this substrate, which may be further accelerated by an increased arylesterase activity in B.brassicae.

Greater cholinesterase activity was observed in M.persicae, with the specific substrate acetylcholine chloride. It is believed however that increased cholinesterase activity alone is unlikely to affect susceptibility and it is sensitivity to inhibition that is the most

important consideration. Two factors are believed to influence the susceptibility of insects to pesticides: Firstly the susceptibility of the cholinesterases and carboxylesterases to inhibition, and secondly the ability of carboxylesterases and phosphatases to degrade the pesticide (Matsumura, 1975).

The results of the experiments using specific enzyme substrates (acetylcholine chloride and ethyl butyrate for choline- and carboxylesterases respectively) showed that the quantity of insecticide needed to give 50% inhibition of enzyme activity was greater for M.persicae than B.brassicae. The I_{50} values correspond with the bioassay results obtained earlier, M.persicae needing a higher concentration of insecticide than did B.brassicae for the same percentage kill. The greatest difference in LD_{50} values occurred with dimethoate and likewise the in-vitro inhibition of enzymes with this insecticide resulted in the largest differences in I_{50} values.

Some workers, for example Main and Iverson (1966), believe that determining the I_{50} value (the biomolecular rate constant (O'Brien,1960)), may not be the most reliable criterion for measuring the inhibitory powers of an insecticide on esterases. They suggest that the affinity constant (Ka) and the phosphorylation constant (Kp) need to be compared. In order to calculate these values the time course of the inhibition of esterases must be determined using a modified colourimetric technique such as that described by Ellman et al (1960). Using this method, Tripathi and O'Brien (1973) and Devonshire (1975b) have attributed insensitivity of acetylcholinesterase to insecticide resistance in Musca domestica.

The mechanism involved in the inhibition of acetylcholinesterase by organophosphorus and carbamate insecticides has been reviewed by

Aldridge and Reiner (1972), the essential feature being the inhibition produced by both organophosphorus and carbamate compounds is brought about by them reacting with the enzyme in a manner precisely analogous to that of the normal substrate. The inhibitory effect resulting from the relatively long life of the phosphorylated or carbamylated enzyme compared with the acetylated enzyme in the normal physiological reaction. Calculation of the Ka and Kp values has been described by Main and Iverson (1966) and this type of study would provide the most logical step forward from the simple inhibition studies carried out here.

The enzyme extracts used in these in vitro studies were simple preparations and may not have given a true picture of enzyme affinities for which specific or several non-specific substrates should be used (Sudderuddin, 1973). The enzyme extract can be purified by various physiological techniques into isoenzymes before kinetic studies are performed, Devonshire (1975b) for example described a method of extracting acetylcholinesterase from houseflies. However their inconvertibility and conditional detection by some techniques only, suggests that they may arise as a product of in vitro manipulation, their existance in vivo being uncertain.

Another problem with this type of enzyme study lies in determining the exact location of the enzyme. Acetylcholinesterase is generally regarded as being confined to the central nervous system of insects, but the location of other enzymes is less certain and many less specific esterases may be more widely distributed in insect tissues. In particular those involved with digestive mechanisms and other degradation processes are closely associated with food uptake and its absorption either within the gut lumen or in the gut wall itself. The gut is obviously a critical area for systemic pesticides because metabolism here is going

to be important in determining the quantity of toxic material reaching the nervous system. Work done by Krieger et al (1971) showed that caterpillars feeding on a wide range of plant species had greater midgut oxidase enzyme activity than those limited to feeding on only one or two species. The polyphagous feeding habits of M.persicae might require a wider range of gut enzymes than the monophagous B.brassicae, thus affording greater protection to ingested pesticide. In view of the similar susceptibilities of B.brassicae and M.persicae to topically applied pesticide, this is a strong possibility. As mentioned earlier the aphid gut is difficult to work with, but it may eventually be possible to isolate whole aphid guts to provide comparisons in enzyme activity between species.

4.3. Appraisal

The difference is susceptibility of B.brassicae and M.persicae to systemic insecticides appears to be a result of differential feeding and absorption rates coupled with differences in the activity and sensitivity of B-esterases. No single factor differs between the species to the same extent as the bioassay results, and thus a combined effect seems likely. It is popular to attribute insecticide susceptibility entirely to enzymes but other physiological mechanisms may be equally important and until the significance of each factor is properly understood, all options both biochemical and physiological must be considered before their interactions and exact contributions to different susceptibilities can be formulated.

SUMMARY

Dimethoate and pirimicarb included in artificial diet sachets were bioassayed with aphids previously reared on untreated sachets. Probit analysis on mortality data obtained 48 hours later showed that, with dimethoate, the EC_{50} for M.persicae was thirteen times greater than that for B.brassicae. A sixfold difference occurred when pirimicarb was used.

These differences were investigated further with feeding, absorption and excretion rate experiments, supplemented with enzyme activity studies.

Preliminary experiments showed that the normal precautions taken to reduce interference from chemiluminescence in the radio-assay determinations were adequate. Thus no additional correction factors were necessary following the extraction of radio-labelled compounds from aphids, honeydew and diet.

The feeding rates of the two species were compared using artificial diet sachets containing ^{35}S-labelled sodium sulphate, followed by analysis of the radio-label in the aphids and honeydew. B.brassicae was found to feed at twice the rate of M.persicae. Comparison of body weights of the two species showed B.brassicae to be approximately 50% heavier than M.persicae.

The effect on feeding rate of different concentrations of dimethoate in the diet was investigated. M.persicae showed no alteration of feeding rate until the dose was increased to toxic levels. B.brassicae however exhibited a significant reduction in feeding rate at very low sublethal doses of pesticide. Varying concentrations of the fungicide ethirimol fed to the aphids in the diet had no significant effect on the feeding rate of either species.

When sublethal doses of ^{14}C-labelled phorate were fed in the artificial diet, analysis of the aphids 24 hours later, indicated a higher proportion of toxic and non-toxic residues in B.brassicae than in M.persicae.

Horizontal starch gel electrophoresis on whole aphid esterases showed no difference between M.persicae and B.brassicae in the number of bands present, but slight variations between their distribution and intensity were found.

In vitro studies of the esterases in whole aphid homogenates showed differences in the activity of the cholinesterases and carboxylesterases from the two species. Using acetylcholine chloride as substrate, the cholinesterase activity of M.persicae was greater than that of B.brassicae. The carboxylesterases of M.persicae were also more active when β-naphthyl acetate and ethyl butyrate were used as substrates. However the enzyme activities were reversed when α-naphthyl acetate was the substrate, the carboxylesterases of B.brassicae being the more active.

The effect of various concentrations of three inhibitors: eserine sulphate, dimethoate and pirimicarb, on enzyme activity was investigated. Cholinesterase and carboxylesterase in B.brassicae was inhibited by lower concentrations than were those of M.persicae, but when α-naphthyl acetate was the substrate for carboxylesterases, those of M.persicae were more readily inhibited.

The difference in susceptibility between M.persicae and B.brassicae to systemic insecticides is discussed in relation to these experimental results.

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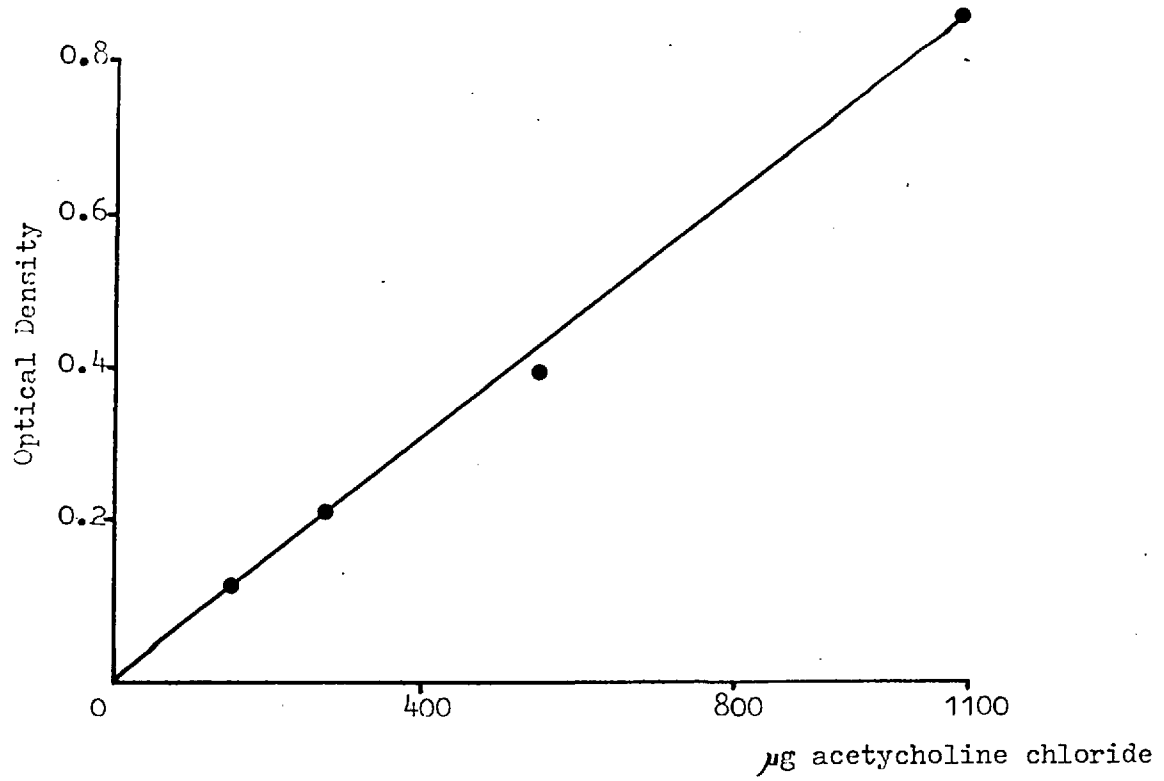
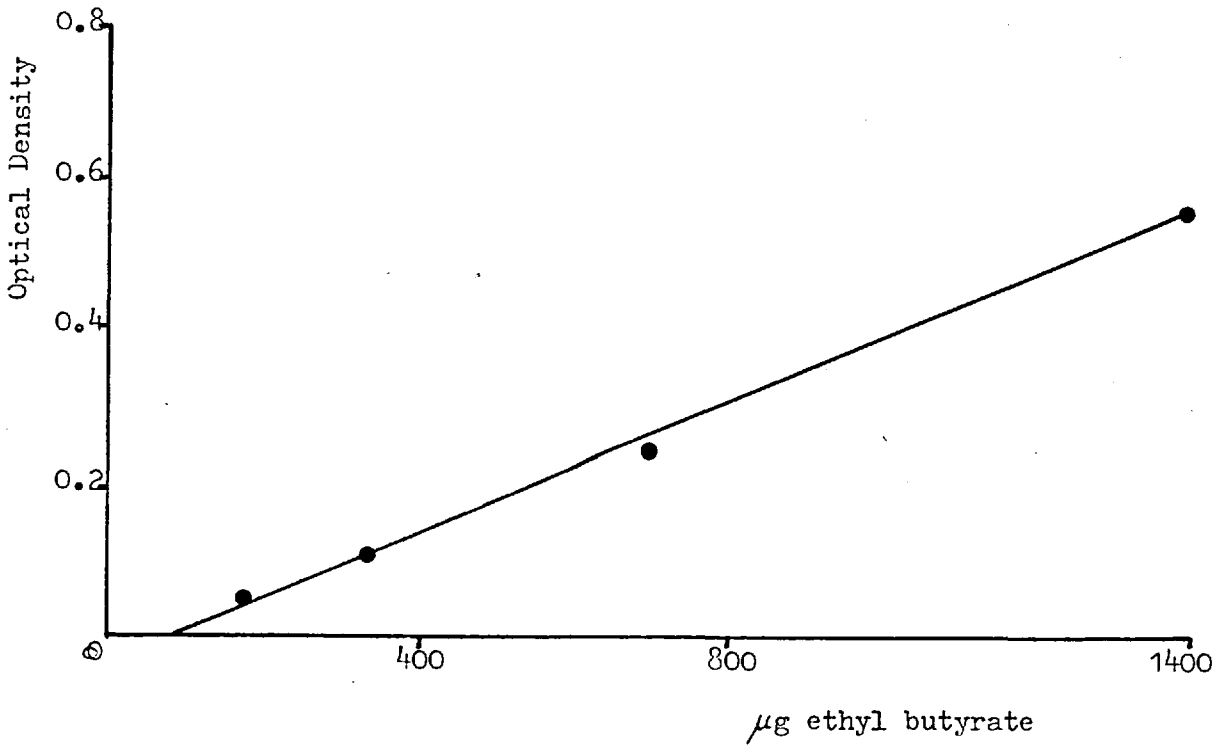
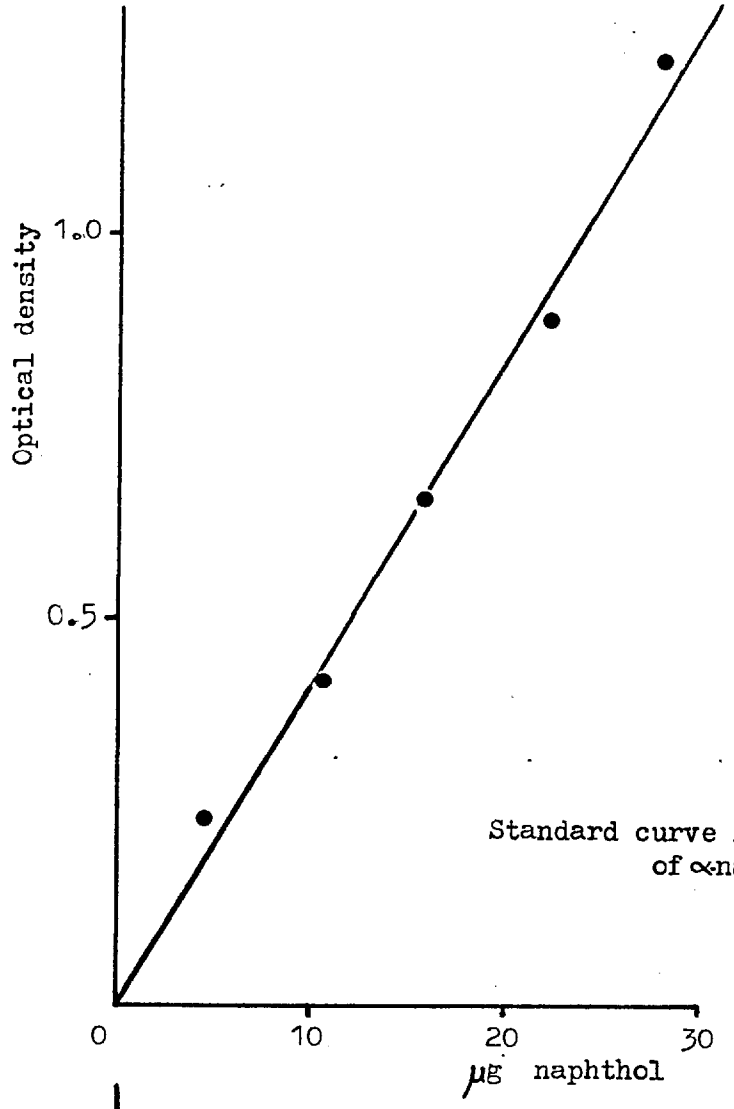


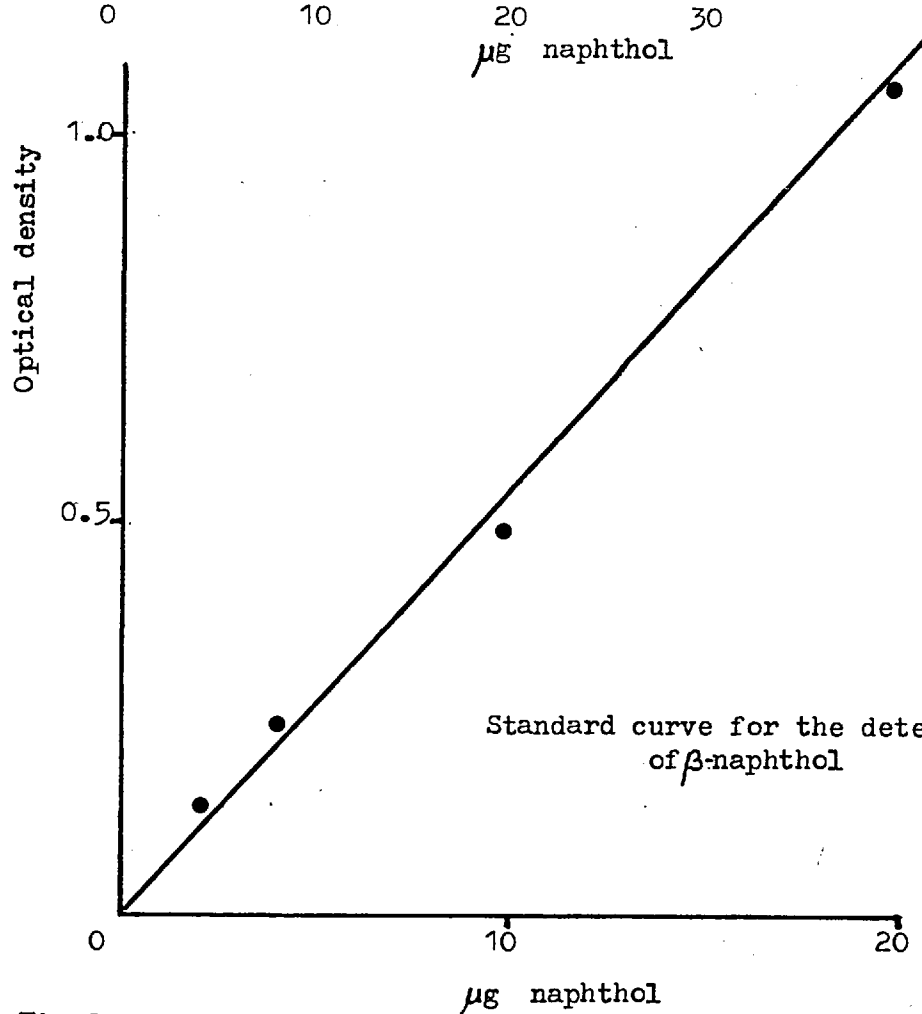


Appendix Fig.1

Standard curves for the determination of acetylcholine chloride and ethyl butyrate



Standard curve for the determination of ∝naphthol



Standard curve for the determination of β-naphthol

Appendix Fig.2

VIAL No.	VIAL COUNT D.p.m.	VIAL COUNT + GLASS D.p.m.	DIFFERENCE D.p.m.
1	48.1	64.7	16.6
2	74.2	103.8	29.6
3	47.7	65.0	17.3
4	46.7	64.0	17.3
5	47.7	67.6	19.9
6	46.9	66.5	19.6
7	48.8	62.1	13.3
8	45.8	65.0	19.2
9	54.6	71.3	16.7
10	55.1	76.3	21.2
11	48.6	59.4	10.8
12	51.2	72.2	21.0
13	56.6	71.9	15.3
14	44.5	62.0	17.5
15	47.9	68.8	20.9
16	54.1	71.0	16.9
17	64.0	85.0	21.0
18	47.3	62.1	14.8
19	45.2	65.5	20.3
20	59.8	78.9	19.1
		MEAN ± S.D.	18.4 ± 3.8

APPENDIX TABLE 1 The effect of crushed coverslips on the background count in scintillation vials containing Bray's scintillant.

NUMBER OF APHIDS	BACKGROUND (B) D.p.m.	APHIDS + DIGESTANT (D) D.p.m.	D-B D.p.m./aphid
brassicae			
238	50.2	302.8	1.06
251	58.1	228.5	0.68
121	46.1	90.8	0.37
137	53.4	96.6	0.32
127	49.9	70.6	0.16
		MEAN ± S.D.	0.52 ± 0.36
persicae			
186	49.0	71.3	0.12
103	46.2	64.7	0.18
178	46.9	72.5	0.14
171	47.2	70.3	0.14
161	48.6	64.8	0.10
		MEAN ± S.D.	0.14 ± 0.03

APPENDIX TABLE 2 The effect of adding tissue digestant and aphids on the background count in scintillant vials containing Bray's scintillant

REPLICATE	BACKGROUND COUNT/VIAL D.P.M.	BACKGROUND COUNT + 20µl DIET	DIFFERENCE IN D.P.M.
1	46.0	46.9	0.9
2	46.8	48.2	1.4
3	46.4	48.7	2.3
4	47.4	47.0	-0.4
5	46.8	47.4	0.6
6	46.9	47.9	1.0
7	49.6	48.7	-0.9
8	45.3	45.8	0.5
9	47.5	50.3	2.8
10	46.0	43.3	-2.7
		MEAN ± S.D.	0.55 ± 1.6

APPENDIX TABLE 3 The effect of 20.1 of artificial diet on the background count in scintillation vials

DOSE	REPLICATES										MEAN % MORTALITY
	1		2		3		4		5		
	AR	AN	AR	AN	AR	AN	AR	AN	AR	AN	
10.0	41	43	33	38	76	80	32	37	29	40	88.7
8.0	51	59	72	86	21	27	25	33	24	30	82.1
7.5	43	49	50	60	75	101	35	55	14	25	74.8
6.0	36	52	17	27	20	27	72	111	19	31	62.1
5.0	29	51	24	40	25	30	22	47	19	34	58.9
4.0	29	41	29	51	19	50	21	73	39	55	50.7
3.0	6	17	8	20	5	19	5	18	4	19	30.1
2.0	6	92	14	62	11	116	15	78	11	154	11.4
CONTROL	0	50	0	42	0	33	0	77	0	65	0.0

APPENDIX TABLE 4 Mortality of the aphid M.persicae at various concentrations od dimethoate added to diet sachets (Dose in µg pesticide per ml. of artificial diet)

(AR = Numbers responding to treatment)
(AN = Total number of animals treated)

DOSE	REPLICATES										MEAN % MORTALITY
	1		2		3		4		5		
	AR	AN	AR	AN	AR	AN	AR	AN	AR	AN	
0.8	37	39	42	45	42	46	48	52	33	34	93.5
0.75	18	21	45	53	28	32	35	38	29	31	88.6
0.60	41	58	116	124	88	101	49	52	53	64	87.0
0.50	17	36	29	36	19	37	53	58	27	53	65.9
0.40	29	40	11	17	20	47	13	30	45	63	59.9
0.30	22	77	21	32	35	96	35	78	14	27	41.0
0.20	8	25	5	38	4	15	15	45	13	102	20.0
0.10	5	21	3	26	6	28	4	24	0	39	13.0
CONTROL	0	51	0	39	1	40	0	73	0	61	0.0

APPENDIX TABLE 5 Mortality of the aphid B.brassicae at various concentrations of dimethoate added to diet sachets (Dose in µg pesticide per ml. of artificial diet).

(AR = Numbers responding to treatment)
(AN = Total number of animals treated)

DOSE	REPLICATES						MEAN % MORTALITY
	1		2		3		
	AR	AN	AR	AN	AR	AN	
0.1	3	50	8	70	6	35	11.2
0.2	63	182	148	344	98	358	35.0
0.4	118	173	15	42	68	130	55.8
0.6	32	55	56	75	22	38	65.5
0.8	119	133	82	96	135	151	88.4
1.0	196	201	329	353	34	39	94.3
CONTROL	0	57	0	91	0	199	0.0

APPENDIX TABLE 6 Mortality of the aphid M.persicae at various concentrations of pirimicarb added to diet sachets.
(Dose in µg pesticide per ml. of artificial diet)

(AR = Numbers responding to treatment)
(AN = Total number of animals treated)

DOSE	REPLICATES						MEAN % MORTALITY
	1		2		3		
	AR	AN	AR	AN	AR	AN	
0.01	12	47	6	74	2	64	10.9
0.02	30	187	19	165	24	94	16.4
0.04	126	241	63	150	44	114	46.1
0.06	35	71	125	234	121	188	57.0
0.08	81	114	130	212	99	153	64.1
0.10	183	228	237	312	201	284	75.4
0.12	69	76	197	231	102	122	87.8
CONTROL	0	76	0	54	0	153	0.0

APPENDIX TABLE 7 Mortality of the aphid B.brassicae at various concentrations of pirimicarb added to diet sachets.
(Dose in µg pesticide per ml. of artificial diet)

(AR = Numbers responding to treatment)
(AN = Total number of animals treated)

TIME (hr)	HONEYDEW d.p.m/aphid				MEAN ± S.D.
	1	2	3	4	
3	6.34	5.38	2.83	6.88	5.36 ± 1.8
6	20.04	39.8	29.4	38.5	32.0 ± 9.2
9	41.9	67.9	50.0	63.1	55.7 ± 11.9
12	52.7	70.9	51.6	80.9	64.0 ± 14.3
15	43.9	63.1	52.5	67.1	56.6 ± 10.5
18	40.5	61.0	45.9	61.9	52.3 ± 10.8
21	69.7	90.0	60.5	84.1	76.1 ± 13.4
24	83.1	92.5	86.6	103.0	91.3 ± 8.7
27	90.2	113.4	75.7	109.3	97.1 ± 17.5
30	84.6	117.0	77.3	103.7	95.6 ± 18.1
33	77.9	110.7	86.9	110.1	96.4 ± 16.6
36	87.6	110.1	84.7	109.3	97.9 ± 13.7
39	72.0	83.5	53.1	75.3	71.0 ± 12.9
42	67.5	75.8	54.0	64.5	65.4 ± 9.0
45	94.9	126.0	69.2	106.7	99.2 ± 23.8
48	109.6	117.9	100.4	122.2	112.5 ± 9.6

APPENDIX TABLE 8 Excretion rate of B.brassicae using ^{35}S labelled sulphate added to diet sachets.

TIME (hr)	HONEYDEW d.p.m./aphid				MEAN ± S.D.
	1	2	3	4	
51	16.7	5.7	6.8	8.6	9.5 ± 5.0
54	90.9	58.5	46.6	68.0	66.0 ± 18.7
57	146.3	110.3	81.2	113.6	112.9 ± 26.7
60	156.8	138.4	94.8	136.7	131.7 ± 26.2
63	126.6	108.7	79.0	92.8	101.8 ± 20.5
66	110.0	97.7	86.4	96.6	97.7 ± 9.7
69	173.9	154.1	118.9	142.4	147.4 ± 23.0
72	203.9	190.0	129.9	162.8	171.7 ± 32.7
75	174.8	166.5	127.0	152.0	155.1 ± 21.0
78	170.5	153.5	129.0	128.8	145.4 ± 20.3
81	150.6	132.5	117.7	133.9	133.7 ± 13.5
84	140.4	121.5	121.6	120.6	126.0 ± 9.6
87	103.4	73.0	77.0	76.5	82.5 ± 14.1
90	93.3	68.2	80.3	83.6	81.3 ± 10.4
93	137.5	116.2	123.9	133.2	127.7 ± 9.6
96	147.7	133.8	119.6	117.6	129.7 ± 14.0

APPENDIX TABLE 9 Excretion rate of B.brassicae using ^{35}S labelled sulphate added to diet sachets

TIME (hr)	HONEYDEW d.p.m/aphid				MEAN ± S.D.
	1	2	3	4	
3	3.76	3.52	3.10	2.81	3.30 ± 0.42
6	13.19	13.8	10.7	9.5	11.8 ± 2.0
9	20.7	22.3	18.2	16.4	19.4 ± 2.6
12	26.4	27.3	24.0	21.9	24.9 ± 2.5
15	24.5	24.2	25.1	22.9	24.2 ± 0.9
18	26.7	27.0	27.5	25.1	26.6 ± 1.0
21	41.3	43.3	33.1	30.5	37.1 ± 6.2
24	47.6	51.7	42.6	39.3	45.3 ± 5.5
27	50.5	54.9	45.1	41.7	48.1 ± 5.8
30	53.0	56.3	48.4	35.4	48.3 ± 9.2
33	50.7	58.2	51.9	46.5	51.9 ± 4.8
36	58.5	59.9	55.6	49.1	55.8 ± 4.8
39	46.1	45.7	50.7	43.7	46.5 ± 2.9
42	46.8	45.4	52.2	42.3	46.7 ± 4.2
45	64.3	65.1	55.1	48.8	58.3 ± 7.8
48	74.1	91.7	67.1	56.1	72.3 ± 14.9

APPENDIX TABLE 10 Excretion rate of M.persicae using ^{35}S labelled sulphate added to diet sachets.

TIME (hr)	HONEYDEW d.p.m/aphid				MEAN ± S.D.
	1	2	3	4	
51	4.1	6.2	5.7	7.7	5.9 ± 1.5
54	20.3	24.3	21.7	30.6	24.2 ± 4.6
57	36.4	39.8	36.7	57.8	42.7 ± 10.2
60	40.2	41.5	44.5	72.4	49.7 ± 15.3
63	39.3	37.8	43.8	68.5	46.4 ± 15.2
66	39.4	40.2	46.4	73.6	49.9 ± 16.1
69	55.2	59.3	54.0	90.9	64.9 ± 17.5
72	58.8	66.8	71.2	113.8	77.7 ± 24.7
75	62.6	71.8	78.0	124.3	84.2 ± 27.5
78	63.4	74.6	76.5	126.8	85.3 ± 28.2
81	67.0	78.6	77.2	126.5	87.3 ± 26.6
84	61.2	72.1	82.3	132.0	86.9 ± 31.3
87	52.9	56.4	67.5	94.4	67.8 ± 18.8
90	54.0	57.8	63.9	94.5	67.6 ± 18.4
93	70.5	83.9	79.9	103.1	84.3 ± 13.7
96	73.6	88.9	89.7	112.2	91.1 ± 15.9

APPENDIX TABLE 11 Excretion rate of M.persicae using ^{35}S labelled sulphate added to diet sachets

TIME (hr)	HONEYDEW d.p.m/aphid				MEAN ± S.D.
	1	2	3	4	
M.persicae					
1	0.46	0.56	0.63	0.30	0.50 ± 0.13
2	1.67	2.94	2.2	1.9	2.2 ± 0.6
3	2.6	5.98	4.90	4.9	4.6 ± 1.4
4	4.93	9.88	7.9	8.3	7.7 ± 2.1
5	7.34	12.5	9.90	11.4	10.3 ± 2.2
6	10.6	15.7	12.9	14.1	13.3 ± 2.2
B.brassicae					
1	0.4	1.1	0.3	0.3	0.5 ± 0.4
2	1.0	4.0	0.4	2.3	1.9 ± 1.6
3	4.0	8.7	2.1	8.6	5.8 ± 3.3
4	7.7	21.2	3.1	12.1	11.0 ± 7.7
5	6.3	19.8	5.8	24.6	14.1 ± 9.5
6	11.8	27.8	7.4	21.3	17.0 ± 9.2

APPENDIX TABLE 12 Excretion rates of B.brassicae and M.persicae at 1 hr intervals using ^{35}S-labelled sulphate added to diet sachets

Dose µg/ml	Concentration ^{32}P/Sachet d.p.m.	^{32}P Aphid d.p.m/aphid	^{32}P Honeydew d.p.m/aphid 0-2	2-4 days	Concentration ^{35}S/Sachet d.p.m.	^{35}S Aphid d.p.m/aphid	^{35}S Honeydew d.p.m/aphid
CONTROL	423586.0	17.7	92.4	12.1	1024520.0	470.2	96.7
0.1	407254.0	15.2	81.6	9.9	1084145.0	425.9	79.3
0.2	410034.0	17.4	105.5	11.9	1119822.0	463.1	90.4
0.3	426702.0	15.2	96.1	11.2	1097520.0	431.6	88.1
0.45	378406.0	17.1	116.7	11.8	1040567.0	538.1	89.9
0.6	415434.0	18.7	109.5	14.3	1078613.0	472.9	94.9
0.75	405799.0	16.7	80.2	11.3	1088452.0	480.4	82.7
0.9	370009.6	17.6	105.9	11.8	1034915.0	509.2	81.6
1.0	413656.8	18.5	110.1	12.8	1096245.0	564.6	92.1
2.0	400745.6	17.2	101.6	11.9	1092143.0	453.0	78.4
2.5	371010.8	15.2	106.1	11.1	1044471.0	392.3	71.5
4.0	375589.6	18.0	125.4	12.9	1074886.0	234.3	33.3

APPENDIX TABLE 13 The effect of various doses of dimethoate on feeding rates and absorption in M.persicae using ^{35}S and ^{32}P-labelled salts added to diet sachets

Dose µg/ml diet	Concentration ^{32}P/Sachet d.p.m.	^{32}P Aphid d.p.m/aphid	^{32}P Honeydew d.p.m/aphid		Concentration ^{35}S/Sachet d.p.m.	^{35}S Aphid d.p.m/aphid	^{35}S honeydew d.p.m/aphid
CONTROL	48114.8	2.65	8.6	3.5	328772.0	251.9	71.6
0.02	351986.0	35.7	109.2	29.0	736402.0	478.4	172.6
0.04	318295.0	32.3	118.2	29.0	978008.0	538.0	193.0
0.06	356737.2	34.1	108.1	28.7	995624.0	489.6	192.7
0.075	348936.4	35.9	147.4	34.8	1009167.0	514.7	212.2
0.10	399697.8	27.8	136.0	21.3	897447.5	338.1	110.3
0.16	363885.5	21.1	54.3	13.9	935785.8	170.7	38.0
0.20	382547.2	21.9	53.3	10.8	934442.0	147.4	25.5
0.30	354399.2	41.4	223.4	16.6	874405.0	177.1	32.2
0.40	365219.8	37.5	215.5	12.9	868781.0	116.7	17.1
0.60	370171.4	36.5	458.2	15.1	869416.0	62.9	10.7

APPENDIX TABLE 14 The effect of various doses of dimethoate on feeding rates and absorption in B.brassicae using ^{35}S and ^{32}P-labelled salts added to diet sachets

TIME (hr)	HONEYDEW d.p.m/aphid 1	2	3	MEAN ± S.D.
4	27.2	15.4	15.7	19.4 ± 6.7
8	72.7	55.7	61.4	63.3 ± 8.7
12	77.7	69.2	80.8	75.9 ± 6.0
16	70.9	60.6	63.9	65.1 ± 5.3
20	71.0	55.9	54.3	60.4 ± 9.2
24	99.9	83.0	90.7	91.2 ± 8.5
Fungicide added				
28	82.8	38.8	44.3	55.3 ± 24.0
32	106.5	32.9	29.6	56.3 ± 43.5
36	92.5	31.7	25.6	49.9 ± 37.0
40	65.1	20.2	15.0	33.4 ± 27.6
44	50.6	15.6	18.4	28.2 ± 19.5
48	66.1	32.2	35.9	44.7 ± 18.6

APPENDIX TABLE 15 The effect of the fungicide ethirimol on the excretion rate of the aphid B.brassicae when added to diet sachets at the concentration of 100 µg/ml diet

TIME (hr)	HONEYDEW d.p.m/aphid 1	2	3	MEAN ± S.D.
4	8.7	4.0	9.2	7.3 ± 2.9
8	35.3	16.4	28.9	26.9 ± 9.6
12	38.2	22.1	25.0	28.4 ± 8.6
16	31.4	18.6	25.3	25.1 ± 6.4
20	52.3	31.8	43.8	42.6 ± 10.3
24	62.3	40.8	47.2	50.1 ± 11.0
Fungicide added				
28	35.2	32.1	23.9	30.4 ± 5.8
32	51.8	53.8	30.2	45.3 ± 13.1
36	50.9	55.5	20.2	42.2 ± 19.2
40	37.1	38.8	13.6	29.8 ± 14.1
44	57.3	57.2	27.2	47.2 ± 17.4
48	78.8	80.5	42.7	67.3 ± 21.4

APPENDIX TABLE 16 The effect of the fungicide ethirimol on the excretion rate of the aphid B.brassicae when added to diet sachets at the concentration of 10µl/ml diet

TIME (hr)	HONEYDEW d.p.m/aphid 1	2	MEAN ± S.D.
4	7.05	3.7	5.4 ± 2.4
8	33.7	18.8	26.3 ± 10.5
12	28.5	28.5	28.5 ± 0.0
16	39.6	25.0	32.3 ± 10.3
20	36.4	25.3	30.9 ± 7.9
24	53.7	33.0	43.4 ± 14.6
methanol added			
28	44.9	41.8	43.4 ± 2.2
32	66.4	69.7	68.1 ± 2.3
36	75.7	67.9	71.8 ± 5.5
40	55.9	49.3	52.6 ± 4.7
44	51.2	50.0	50.6 ± 0.9
48	83.4	59.0	71.2 ± 17.3

APPENDIX TABLE 17 The effect on the excretion rate of B.brassicae when 10 µl/methanol is added to diet-sachets

TIME (hr)	HONEYDEW d.p.m/aphid 1	2	3	MEAN ± S.D
4	10.9	20.9	9.1	13.6 ± 6.4
8	30.6	52.6	27.1	36.8 ± 13.8
12	38.4	61.7	46.5	48.9 ± 11.8
16	38.1	59.9	44.3	47.4 ± 11.2
20	40.6	52.5	45.9	46.3 ± 6.0
24	57.3	76.7	56.8	63.6 ± 11.4
Fungicide added				
28	16.7	45.2	21.7	27.9 ± 15.2
32	24.7	54.7	43.5	41.0 ± 15.2
36	21.7	46.8	35.9	34.8 ± 12.6
40	14.2	27.1	20.4	20.6 ± 6.5
44	9.0	17.6	12.4	13.0 ± 4.3
48	11.0	18.1	15.4	14.8 ± 3.6

APPENDIX TABLE 18 The effect of the fungicide athirimol on the excretion rate of the aphid M.persicae when added to diet sachets at the concentration of 100 µg/ml diet

TIME (hr)	HONEYDEW d.p.m/aphid			MEAN ± S.D
	1	2	3	
4	2.4	2.5	2.1	2.3 ± 0.2
8	9.6	8.0	6.1	7.9 ± 1.8
12	14.4	12.0	9.0	11.8 ± 2.7
16	14.0	12.3	9.6	12.0 ± 2.2
20	14.0	14.1	10.7	12.9 ± 1.9
24	21.9	21.2	17.3	20.1 ± 2.5
Fungicide added				
28	10.0	10.7	16.0	12.2 ± 3.3
32	20.8	18.1	22.5	20.5 ± 2.2
36	25.4	19.4	20.1	21.6 ± 3.3
40	19.2	16.3	16.8	17.4 ± 1.6
44	14.3	16.0	18.3	16.2 ± 2.0
48	20.6	22.2	22.5	21.8 ± 1.0

APPENDIX TABLE 19 The effect of the fungicide ethirimol on the excretion rate of the aphid M.persicae when added to diet sachets at the concentration of 10µg/ml diet

TIME (hr)	HONEYDEW d.p.m/aphid 1	2	MEAN ± S.D.
4	2.6	1.8	2.2 ± 0.6
8	8.4	5.1	6.8 ± 2.3
12	14.6	10.7	12.7 ± 2.8
16	15.5	12.7	14.1 ± 2.0
20	15.5	14.0	14.8 ± 1.1
24	21.5	18.5	20.0 ± 2.1
Methanol added			
28	15.8	13.2	14.5 ± 1.8
32	21.6	19.8	20.7 ± 1.3
36	23.5	22.3	22.9 ± 0.9
40	20.5	19.9	20.2 ± 0.4
44	23.6	16.3	20.0 ± 5.2
48	21.8	20.9	21.4 ± 0.6

APPENDIX TABLE 20 The effect on the excretion rate of M.persicae when 10µl of methanol is added to diet sachets

Brevicoryne brassicae

Non-toxic Residue		
REPLICATES	APHID %/aphid	HONEYDEW %/aphid
1	0.070	0.080
2	0.040	0.034
3	0.034	0.029
4	0.025	0.033
5	0.029	0.030
6	0.024	0.026
MEAN ± S.D	0.037 ± 0.017	0.039 ± 0.021
Toxic Residue		
1	0.021	0.014
2	0.018	0.012
3	0.0075	0.0089
4	0.016	0.0080
5	0.012	0.0110
6	0.012	0.0130
MEAN ± S.D	0.014 ± 0.0049	0.0110 ± 0.0023

APPENDIX TABLE 21 Analysis of ^{14}C-phorate, toxic and non-toxic fractions absorbed and passed out in the honeydew of the aphid B.brassicae, when added to diet sachets (expressed in % of total radioactivity in the sachet)

Myzus persicae

Non-toxic Fraction		
REPLICATES	APHID %/aphid	HONEYDEW %/aphid
1	0.017	0.042
2	0.018	0.034
3	0.014	0.044
4	0.014	0.040
5	0.011	0.053
6	0.012	0.079
MEAN ± S.D	0.014 ± 0.003	0.049 ± 0.016
Toxic Fraction		
1	0.0038	0.0037
2	0.0045	0.0058
3	0.0044	0.0035
4	0.0065	0.0026
5	0.0062	0.0170
6	0.0039	0.0150
MEAN ± S.D	0.0049 ± 0.0012	0.0079 ± 0.0064

APPENDIX TABLE 22 Analysis of ^{14}C-phorate, toxic and non-toxic fractions, absorbed and passed out in the honeydew of the aphid M.persicae, when added to diet sachets (expressed in % of total radioactivity in the sachet)

TIME (hr)	HONEYDEW d.p.m/aphid				MEAN ± S.D
	1	2	3	4	
4	15.1	11.9	14.0	11.9	13.2 ± 1.60
8	18.8	18.6	22.2	20.2	19.95 ± 1.66
12	17.8	18.9	14.4	15.6	16.7 ± 2.05
16	10.3	10.4	7.1	7.6	8.85 ± 1.75
20	6.5	6.8	9.2	7.7	7.55 ± 1.21
24	8.0	15.8	10.6	8.4	10.7 ± 3.59
28	9.2	13.2	7.5	8.1	9.5 ± 2.57
32	7.6	16.9	6.8	8.2	9.9 ± 4.72
36	7.1	6.8	5.3	6.1	6.3 ± 0.80
40	7.6	8.6	4.5	3.3	6.0 ± 2.51
44	3.5	7.3	3.5	3.1	4.35 ± 1.98
48	5.9	4.1	6.7	6.7	5.85 ± 1.23

APPENDIX TABLE 23 The quantity of ^{35}S-labelled sulphate in the honeydew of M.persicae at 4 hourly intervals after transfer from radioactive to normal diet sachets.

TIME (hr)	HONEYDEW d.p.m /aphid				MEAN ± S.D
	1	2	3	4	
4	40.6	71.4	66.1	43.5	55.4 ± 15.6
8	28.2	32.5	60.4	52.4	43.4 ± 15.5
12	18.2	23.2	37.0	45.3	30.9 ± 12.5
16	9.8	11.3	19.5	24.7	16.3 ± 7.03
20	7.4	9.2	14.4	15.2	11.5 ± 3.8
24	8.7	6.3	17.5	21.5	13.5 ± 7.19
28	8.1	8.3	15.6	16.4	12.1 ± 4.52
32	7.3	7.7	12.1	13.0	10.0 ± 2.9
36	4.3	7.5	11.1	10.3	8.3 ± 3.1
40	2.6	2.4	6.8	6.4	4.6 ± 2.4
44	2.2	1.7	5.3	4.7	3.5 ± 1.8
48	3.4	4.4	7.9	5.1	5.2 ± 1.9

APPENDIX TABLE 24 The quantity of ^{35}S-labelled sulphate in the honeydew of B.brassicae at 4 hourly intervals after transfer from radioactive to normal diet sachets.

APHID NO.	COUNT/APHID d.p.m.	SACHET COUNT d.p.m/20µl	STANDARDIZED d.p.m.
105	621.0	83076.8	523.3
84	615.0	83707.3	514.3
85	673.4	84049.2	560.8
85	599.9	90527.6	463.9
162	616.5	82781.6	521.3
201	491.3	49794.4	690.7
147	628.2	45858.3	958.9
103	849.7	62933.6	945.1
114	751.8	63449.7	829.4
		MEAN ± S.D	667.5 ± 196.0

APPENDIX TABLE 25 Quantitative analysis of ^{35}S-labelled sulphate residues in the aphid M.persicae 48 hours after transfer to standard diet sachets.

APHID NO.	COUNT APHID d.p.m.	SACHET COUNT d.p.m/20µl	STANDARDIZED COUNT APHID d.p.m.
280	645.4	82733.2	546.1
161	831.6	73357.2	793.5
212	819.6	77654.8	738.8
314	564.7	81125.2	487.3
184	778.3	81125.1	671.6
123	479.2	49059.2	683.8
149	461.4	49037.1	658.6
245	316.0	53487.1	413.6
240	455.0	51595.2	617.3
		MEAN ± S.D	623.4 ± 121.5

APPENDIX TABLE 26 Quantitative analysis of ^{35}S-labelled sulphate residues in the aphid B.brassicae 48 hours after transfer to standard diet sachets.

REPLICATES	CONCENTRATION d.p.m/20µl t = 0	CONCENTRATION d.p.m/20µl t = 48	APHID NO'S	µl/aphid EVAPORATED
1	22657.8	23505.0	193	-0.19
2	25317.6	20723.8	64	+2.83
3	24455.3	27066.4	172	-0.62
4	21618.7	26042.8	135	-1.52
5	25695.5	26955.3	153	-0.32
6	14607.3	14285.9	132	+0.17
7	12740.5	14406.5	92	-1.42
8	11861.6	13231.9	182	-0.64
9	12196.7	14965.1	101	-2.25
10	12784.3	12689.1	95	+0.07
11	12387.1	14773.9	59	-3.27
12	14649.0	13959.8	67	+0.70
13	13345.6	15154.0	11	-12.36
14	13109.2	13533.6	26	-1.23
15	14488.3	13808.1	12	+3.92
16	13534.6	14705.4	83	-1.05
17	14598.5	13402.1	145	+0.57
18	14476.1	14695.1	105	-0.14
19	13341.8	12756.9	100	+0.44
20	14238.9	11306.2	54	+3.82
21	15249.9	12483.1	37	+4.89
22	12461.9	14764.1	49	-3.78
23	15151.5	11839.6	84	+2.61
24	34261.2	38094.1	203	-0.55
25	36605.7	35776.5	290	+0.08
26	30157.8	40203.4	208	-1.60
27	33784.2	36201.7	206	-0.35
28	35438.6	33154.9	166	+0.39
29	33651.7	32170.4	87	+0.51
30	33627.7	32910.3	144	+0.15
+ water gain - water lost			MEAN ± S.D	-0.36 ± 2.97

APPENDIX TABLE 27 Water loss from artificial diet sachets expressed in µl per feeding aphid, for the species B.brassicae

REPLICATES	CONCENTRATION d.p.m/20µl t = 0	CONCENTRATION d.p.m/20µl t = 48	APHID NO'S	µl/aphid EVAPORATED
1	15183.7	14197.9	95	+0.68
2	12404.3	14804.9	113	-1.72
3	13233.4	14243.4	151	-0.50
4	13687.7	14799.6	89	-0.91
5	14102.7	13680.0	55	+0.55
6	13625.9	13883.9	130	-0.15
7	13537.5	14931.2	167	-0.62
8	13843.5	14416.1	94	-0.44
9	13154.1	15137.0	89	-1.70
10	14635.0	15175.1	31	-1.19
11	14064.7	15034.1	50	-1.38
12	13355.8	12335.8	79	+0.96
13	12974.2	12647.0	65	+0.39
14	13027.1	13052.8	64	-0.03
15	12904.8	13293.5	75	-0.40
16	9981.1	11912.2	47	-4.13
17	11674.2	11106.7	109	+0.45
18	19072.2	19501.1	97	-0.24
19	13740.6	14130.8	89	-0.32
20	14516.8	14031.6	103	+0.32
+ water gain - water lost			MEAN ± S.D	-0.52 ± 1.15

APPENDIX TABLE 28 Water loss from artificial diet sachets containing a substantial dose of pirimicarb, for the species B.brassicae expressed as µl oer feeding aphid.

REPLICATES	CONCENTRATION d.p.m/20µl t = 0	CONCENTRATION d.p.m/20µl t = 48	APHID NO'S	µl aphid EVAPORATED
1	28049.0	27756.0	150	+0.07
2	29343.7	28531.8	177	+0.16
3	22942.9	24185.0	143	-0.38
4	26628.6	24322.1	82	+1.06
5	23991.6	26882.6	100	-1.21
6	28361.0	27335.6	116	+0.31
7	23172.3	25135.1	101	-0.84
8	26735.4	28689.4	127	-0.58
9	25939.2	26048.7	149	-0.03
10	30100.4	27857.7	187	+0.40
11	25665.4	27530.8	122	-0.60
12	21579.6	25917.0	128	-1.57
13	25727.7	22832.1	105	+1.07
14	26968.7	22479.2	146	+1.14
15	25787.1	18408.5	104	+2.75
16	29668.3	26477.6	104	+1.03
17	28154.3	26446.0	135	+0.45
18	26740.8	22816.5	125	+1.18
19	24924.0	30770.8	112	-2.10
20	32297.4	24864.8	122	+1.89
21	13688.6	17320.6	178	-1.49
22	28365.4	30775.3	114	-0.75
23	18223.8	27084.2	233	-2.09
24	22868.5	26973.1	64	-2.81
			MEAN ± S.D	-0.12 ± 1.35

+ water gain
- water lost

APPENDIX TABLE 29 Water loss from artificial diet sachets expressed in µl per feeding aphid, for the species M.persicae.

REPLICATES	CONCENTRATION d.p.m/20µl t = 0	CONCENTRATION d.p.m/20µl t = 48	APHID NO'S	µl aphid EVAPORATED
1	26611.9	27784.9	31	-1.42
2	28134.4	28016.3	135	+0.03
3	28999.0	27572.7	121	+0.41
4	27344.0	28089.6	83	-0.33
5	24575.9	25256.3	104	-0.27
6	25155.5	25830.6	50	-0.54
7	20414.9	22419.4	53	-1.85
8	20040.3	21732.8	113	-0.75
9	24282.6	26368.7	85	-1.01
10	24074.6	26547.5	39	-2.64
11	25015.1	24555.5	55	+0.33
12	23483.9	22274.6	44	+1.16
13	23339.0	24657.7	58	-0.98
14	21123.0	21644.5	75	-0.33
15	21215.1	24576.9	59	-2.70
16	23482.3	25179.7	127	-0.57
17	24936.7	25348.2	41	-0.42
+ water gain - water lost			MEAN ± S.D	-0.70 ± 1.02

APPENDIX TABLE 30 Water loss from artificial diet sachets containing a sub-lethal dose of pirimicarb, in µl per feeding aphid for the species M.persicae.

REPLICATES	CONCENTRATION d.p.m/20µl t = 0	CONCENTRATION d.p.m/µl t = 48	APHID NO'S	µl EVAPORATED
1	12712.4	13320.6		-48
2	12854.2	14013.3		-90
3	14579.7	14909.0		-23
4	12820.9	12949.7		-10
5	13330.3	14481.8		-86
6	12845.6	13181.6		-26
7	14493.1	14452.8		+3
8	14625.1	15620.7		-68
9	13695.2	13715.4		-2
10	15141.4	15066.3		+5
11	25605.5	27901.7		-90
12	24074.5	24535.2		-19
13	25614.0	25425.2		+7
14	19963.2	24753.3		-240
15	25577.7	23916.8		+65
			MEAN ± S.D	-41 ± 70

+ water gain
- water lost

APPENDIX TABLE 31 The total volume of water lost from artificial diet sachets without aphids feeding upon them, expressed in µl

(i) Substrate: acetylcholine chloride		
Concentration (M)	Activity µg/30 aphids	
	B.brassicae	M.persicae
6.0×10^{-3}	46.7	58.3
3.0×10^{-3}	36.7	52.3
1.5×10^{-3}	21.7	28.2
0.75×10^{-3}	15.0	18.3

(ii) Substrate: ethyl butyrate		
Concentration (M)	Activity µg/aphid	
	B.brassicae	M.persicae
1.2×10^{-2}	20.0	30.0
0.6×10^{-2}	13.8	27.5
0.3×10^{-2}	11.3	21.3
0.15×10^{-2}	8.0	15.0

APPENDIX TABLE 32 The effect of substrate concentrations on esterase activities of M.persicae and B.brassicae.

(i) Substrate: α-naphthyl acetate		
Concentration	Activity µg/aphid	
	B.brassicae	M.persicae
6×10^{-4}	18.3	12.8
3×10^{-4}	11.5	8.5
1.5×10^{-4}	11.1	6.2
0.75×10^{-4}	8.0	4.3

(ii) Substrate: β-naphthyl acetate		
Concentration	Activity µg/aphid	
	B.brassicae	M.persicae
2.4×10^{-5}	4.9	6.3
1.2×10^{-5}	3.2	3.1
0.6×10^{-5}	1.7	1.9
0.24×10^{-5}	0.6	0.7

APPENDIX TABLE 33 The effect of substrate concentrations on esterase activities of B.brassicae and M.persicae