Factors affecting activity and persistence of soil applied systemic aphicides

by

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Abstract

The work described is a comparison of four different systemic insecticides against aphids on Brussels sprouts and a study of some factors which influence their toxicity and persistence.

Aldicarb, pirimicarb, menazon and phorate were applied at the rate of 2 lb. a.i. per acre to assess their comparative toxicities and persistence against aphids. The assessment was made by counting natural aphid infestations in the crop and by bioassays which included sleeving aphids on young and old leaves of treated plants in the field and also leaf discs removed for more accurate estimate in standardised laboratory conditions.

The effect of site of placement of the insecticide aldicarb at 1lb./acre with and without fertilizer on its efficiency against Brussels sprouts aphids was also examined.

Aphid bioassays are described which establish the effects of the aphicides on the aphid species M. persicae and B. brassicae, thus providing LC 50 data for the assessment of relative toxicities and the relative resistance of the two aphid species at different temperatures.

Preliminary laboratory experiments were done to investigate the effects of different methods of insecticide application on the relative uptake, translocation and metabolism of aldicarb and phorate in Brussels sprouts, beans and potato.

These were done in an attempt to explain the relative ineffectiveness of phorate against Brussels sprouts aphids. Gas liquid chromatography (G.L.C.) and thin layer chromatography (T.L.C.) methods were used to estimate phorate and aldicarb metabolites.

Factors affecting activity and persistence of soil applied systemic aphicides.

Introduction

Soil application of systemic insecticides offers promis ing scope in integrated control of aphids because this can confer selectivity even to wide spectrum insecticides. A careful study of such chemicals is therefore justified. Soil applied systemic insecticides are known to vary greatly in their toxicity and perristence against insects possibly due to differences in rates of uptake and breakdown in soil as well as in plants. A comparative study of the toxicity and persistence of soil applications of some systemic organo-phosphate and carbamate insecticides against aphids has therefore been made to clarify the factors involved and to distinguish inherent differences in toxicity and persistence from effects caused by the plant, the soil, different rates of application, different placement methods and temperature. Both biological and chemical estimations of toxic residues in different parts of the plant were made in order to provide information on toxicity and fate of the insecticide and its biologically active metabolic products.

REVIEW OF LITERATURE

Aphids are among the most damaging insect pests of crops and have been studied by many workers all over the world to achieve an effective control. Systemic chemicals have been applied either as foliar treatments or through the soil. Soil applications can be made as follows:

(1) Seed treatments, the seed is dressed with a dust or it can be soaked in the insecticide formulation.

(2) Soil treatments,

(a) Side band treatment, the insecticide is applied in a spray, dust or granule band in the seed drill or to the side of the seed drill;

(b) Individual plant treatment, the insecticide as spray, dust or granules is either placed to the side of or below the plant roots after planting, or prior to

planting the plant roots are dipped in a liquid formulation or dust containing the insecticide.

Foliar applications are made either as:

(a) Sprays

(b) Granules

The term "Systemic" is of recent origin, but the concept of such a method for pest and disease control dates back many centuries (Junius Columella A.D.50). The discovery by Schrader (1947) of Systemic insecticidal actions in synthetic organophosphorus compounds gave considerable impetus to the recent large volume of work on Systemic insecticides. The first Systemic insecticides were found as inorganic oompounds taken up in plants from soil where they occurred naturally. The best known chemical of this nature is sodium fluoroacetate. David and Gardiner (1951) showed that this compound could be used as a Systemic insecticide in broad beans but it is not of practical use due to its high mammalian toxicity and now-a-days the only Systemics used are synthetic organic chemicals.

Use of Systemics as seed treatments

A number of workers have shown that Systemic insecticides applied to the seed can be taken up in quantities sufficient to kill insects attacking aerial parts of the plant (Ripper et al., 1950; Ivy, Iglinsky & Rainwater, 1950; Chao, 1950; Andersson & Ossianilsson, 1951; Bronson & Dudley, 1951; Ashdown & Cordner, 1952; Ivy, 1952; Reynolds, Andersons and Swift, 1953; Unterstenhofer, 1953; David & Gardiner, 1955, and Harris & Valcarce, 1957). The main practical advantage of absorption of insecticides by seeds is that protection against insect attacks is afforded from the beginning of the important seedling stage (Parencia, 1955; Parencia et al., 1957 a, 1957 b; Stanley and Breeland, 1957; Reynolds et al., 1957; Parencia et al., 1958; Hopkins et al., 1958; Hanna, 1958; Adkisson, 1958; Dobson, 1958 and Mistric & Spyhalski, 1959). Depending on the relationship between seed and plant size, it was found that plants of cotton, pea and various beans grown fro seeds soaked in a solution of schradan were successfully protected against aphids for about 30 - 50 days (Chao, 1950; Andersson & Ossiannilson, 1951) and the period of protection varied with the weight of the seed(Chao, 1950)

Similar results were obtained on cotton, sugar beet and egg plants (Ashdown & Cordner, 1952; David & Gardiner, 1955; Harries & Valcaree, 1957; Gates, 1959). Schradan and demeton seed treatments could also be successfully used to control A.fabae, A. gossypii, M. persicae and M.pisi (Ivy et al., 1950; Janke, 1951, Lhoste & Leibovice, 1952; Franklin, 1953; Chaudhary, 1956 and Gates 1959). David & Gardiner (1955) stated that seed soaking technique may be an efficient means of getting a systemic insecticide into the plant, but the amount absorbed is often either inadequate for lasting protection or phytotoxic (Way & Needham, 1957). Although seed application of a Systemic insecticide such as demeton at planting time is probably of limited use if a long term protection is required in the aerial parts of the plant, yet relatively small doses of demeton as seed dressing controlled early attacks of S.lineatus and M.persicae on beans and potato respectively (Way & Needham, 1957). Phorate, menazon and disulfoton applied as seed dressings have been shown to give effective control of thrips, green bugs, spider mites and many aphid species on cotton alfalfa, pea, bean, potato, sugar beet for 3 - 9 weeks (Parencia et al., 1957; Anonymous, 1957;

Atkisson, 1959; Hopkins et al., 1958; Gates, 1959; Andres et.al., 1959; Bonnemaison, 1960; PriceJones, 1961; Newman, 1960, 1961; Zaki & Reynolds, 1961) and continued to reduce aphid populations significantly for one growing season on various crops (Hopkins et al., 1959). When applied as seed soaks or directly on seed pieces, phorate, disulfoton, and dimethoate have, however, been reported to cause delayed emergence and marked phytoxicity (Zaki & Reynolds, 1961; Pond, 1963; Matsumoto et al., 1967) but menazon is harmless to most seeds even at relatively high dosage rates.

SOIL TREATMENTS

The most commonly used soil applied Systemic insecticides are phorate, disulfoton, dimethoate, menazon and recently pirimicarb has also shown considerable promise as an aphicide particularly against [illegible] resistant M. persicae. Phorate granules applied to the soil at time of planting, have been shown to give effective control of A. gossypii on cotton, B.brassicae on cabbage and cauliflower, M. persicae on potato and many other crops and M.pisi on peas for about 50 - 150 days (Hopkins et al., 1958;

Andres et al., 1959; Bacon, 1960, 1963; Getzin & Chapman, 1960; Gerbardt & Turley, 1961; Reynolds et al., 1960; Schwartz et al., 1961; Harding, 1962; Savage & Harrison, 1962; Lindley, 1962, 1964; Cook et al., 1963; Shorey, 1963; Linke, 1964; Dominick, 1965; Harrison & Wooldridge, 1966 and Menzer & Ditman, 1968) but failed to protect the crop at less than two pounds per acre (Dobson & Watts, 1957; Bacon, 1960). When applied to the seed drill or seed furrow in the soil phorate has been reported as adequate in providing protection to turnips from turnip aphids upto harvest time (Wilcox & Howland, 1957).

Varying degrees of aphid control by granular application of disulfoton, dimethoate, phorate, menazon, thionazin and vamidothion to the soil at or after planting time have been shown on various crops (Getzin & Chapman, 1959; Burt et al., 1960; Price Jones, 1960; Newman, 1960, 1961; Lindley, 1962, 1964; Linke, 1964; Legge, 1965; Broadbant et al., 1966; Matsumoto et al., 1967; Elliott & Way, 1968; Hopkins and Taft, 1968 and Worthing 1968, 1969). A season long protection was achieved using pirimicarb, aldicarb, menazon and oxydemeton methyl on potato, tobacco and chrysanthemum resulting in greater yield

and high quality potato and tobacco (Gerhardt, 1966; Legge & Shepherd, 1967; Worthing, 1968, 1969; Kearby & Bliss, 1968, 1969 and Way et al., 1969). It was concluded that granules mixed with soil were more persistent than localised treatments with liquids but localised granule treatments were also more effective than those banded in the ridge or applied as side dressings on tobacco against M.persicae (Legge & Shepherd, 1967). At the same time, it was demonstrated that varying amounts of phosphate fertilizer did not affect phorate absorption by the cotton plants as indicated by cotton aphid mortality (Hopkins et al., 1958) but in the case of potato plants individual [illegible] of phorate and aldicarb were not as effective as the same compound applied in conjunction with artificial fertilizers (Burt et al., 1960; Gerhardt & Turley, 1961 and Gerhardt, 1966).

Much work has been done on control of virus vectors by Systemic aphicides. Soil application has proved to be very effective in controlling spread of persistent virus such as leaf roll virus in potato (Burt et al., 1960; Broadbent et al., 1956, Burt et al., 1964 and Broadbent et al., 1966); but it did not prove to be fully successful in controlling non-persistent viruses e.g. rosette virus on groundnuts,

yellows virus on sugar beet (Watson, 1944) and virus on potato (Broadbentet al., 1956; Hull & Sel man, 1964; Burt et al., 1964). This is mainly because immigrating virus carriers feed and can quickly infect tha plants before being killed.

FOLIAR SPRAYS

Absorption of Systemic insecticides from sprays into the leaf is relatively slow and is apparently influenced partly by the nature of the leaf surface, being slower on waxy surface(Price-Jone, 1961). Absorption into the plant by this route is also dependant on the amount of foliage present as shown by green peach aphid control (Price-Jone, 1961). It has also been shown that translocation of schradan and demeton following foliar application is towards the younger leaves and the peripheral growing areas of the treated leaves.

Schradan, demeton, demeton-s-methyl, oxydemeton-methyl, menazon, phorate, thionazin, pirimicarb, carbaryl, vamidothion, dimethoate, formothion, Mevinphos, Morphotion and Phosphamidon have proved very useful Systemics for foliar sprays on a variety of plant species against aphids and other foliage attacking pests (Ivy et al., 1950; David & Gardiner 1951; Jancke, 1951; Ripper et al., 1950, 1951; Dowdy

& Sleesman, 1952; Lhoste & Leibovici, 1952; Wright & Wheatley, 1953; Reynolds et al., 1953; Sleesman, 1953; Schuder, 1953; Way et al., 1954; Davis et al., 1956; Simon, 1956; Saggers, 1956; Austin & Linke, 1957; Dobson, 1958; Motsinger & Morgan, 1960; Price-Jones, 1961; Newman, 1961; Legge, 1965; Worthing, 1968, 1969). When applied in granular form phorate and disulfoton controlled aphids on potato and bean upto harvest time (Knoke, 1959; Hua, 1969).

BEHAVIOUR OF INSECTICIDES IN THE SOIL AND PLANT.

In application of Systemic insecticides to the roots of the plant, the most important factor is the contact between the roots and insecticides. It has been clearly demonstrated that absorption of insecticides by roots from various media is greatest from solution, less from sand and least from soil. David (1952) found that more schradan and dimefox are absorbed from sand than from soil, and it has been shown that while demeton is absorbed freely from solution, the absorption from sand and humus containing soils is a slower process and is related with soil type (Teitz, 1954). It is, therefore, concluded that differences in absorption from various media can be explained on a physical basis, either as a result of the varying contact between insecticide and root, or of the greater affinity of the insecticide for some particles in the soil or soil media (Bennett, 1957).

Soil application depends on absorption of insecticides by the root system, either in solution or in the vapour phase (Burt, Bardner & Etheridge 1965). Their translocation to the aerial parts of the plant depends on some physical or physiological

factors such as -

(i) the physical properties of insecticides e.g. solubility and volatility (Burt et al., 1965; Reynolds & Metcalf, 1962).

(ii) Soil properties e.g. pH, Moisture content and soil type(Reynolds & Metcalf, 1962; Wiese, 1965; Andres et al., 1959; Getzin & Chapman,1959).

(iii) physiology of the plants e.g. translocation path, (David 1951; David & Gardiner, 1952;)

(iv) conditions and age of the plant (Andres, Reynolds & Fukuto, 1959).

Greater insecticide absorption, activity and persistence have been shown to occur in sands and sandy soils than in loams, clays and muck soils (David 1951; David & Gardiner 1951; Davich, 1953; Wallace, 1951; David, 1957; Getzin & Chapman, 1959, 1960; Zaki & Reynolds, 1961; Way & Scopes, 1965; Getzin & Rosefield, 1966; Conrad et al., 1967; Pain & Skrentny 1969). Organic matter and clay content have been found to be important factors related with reduced absorption and faster decline of schradan, demeton, phosdrin and aldicarb (Casida et al., 1952;

Johanson, 1952; Getzin & Chapman, 1959; Abdellatif et al., 1967).

The period of protection is inversely related to the speed the plants grow (Reynolds et al., 1957) and is shortened by larger amounts of organic matter in the soil or excessive leaching (Bardner 1961).

The degree of penetration and the subsequent translocation are functions both of the soil type and the physicochemical properties of the particular compound such as its water solubility, stability within living cells etc. (Teitz, 1954; Lichtenstein, 1959; and Reynolds, 1962). The absorption of Systemic insecticides by the roots shows considerable variation. It has been shown that the roots of broad bean plant selectively absorb dimefox from solution (David, 1952) at rates of transpiration at which Schradan was selectively rejected (David, 1951). The routes of metabolism of Systemics such as schradan, demeton, phorate and other similar compounds and their toxic metabolites are the same in plants, insects and even mammals so that toxic residues in edible produce present no hazards as a result of biochemical activity of the plant (March et al., 1955). The metabolism in the plant leads to very low

residues at harvest time, provided the application has been made sufficiently early or if closer to harvest, at reduced concentration (Davich & Apple, 1955; Metcalf et al., 1955).

The practical usage of an insecticide as a Systemic protectant against aphids depends upon a knowledge of its metabolic fate in the plant. The metabolism of the Systemic insecticide aldicarb has shown that insecticide is readily and completely oxidized to its sulphoxide within 4 - 9 days in cotton leaves at moderate temperatures. The sulphoxide which is more active as a cholinesterase inhibitor is the active metabolite and its long term persistence and relatively slow oxidation to aldicarb sulphone is responsible for the persistent Systemic activity of the compound. Aldicarb sulphoxide is hydrolyzed to the oxime which is the principal degradation product in the cotton plant (Metcalf et al., 1956; Coppedge et al., 1967; Bull et al., 1967; Bull, 1968; Reynolds et al., 1966).

Phorate O,O-diethyl s-ethylthiomethyl phosphorodithioate, when absorbed by plants, is first oxidized at the thioethersulfur to form phosphorodithioate sulphoxide and sulphone and is then oxidized at the thionosulfur to form phosphorothiolate

sulphoxides and sulphone these oxidation products are all more potent anticholinesterase agents than the parent compound and persist in plants for relatively longer periods (Browman & Casida, 1957; Metcalf et al., 1957, 1958, Menzer & Ditman, 1968), but decline rather rapidly during summer (Menzer & Ditman, 1968).

VALUE OF SOIL APPLIED SYSTEMIC INSECTICIDES

Soil applied Systemic insecticides are valuable in integrated control of aphids because they can provide ecological or physical selectivity(Ripper et al., 1951, Potter 1961) and are unlikely to harm soil fertility even if they kill beneficial soil animals (Way & Scopes, 1968). Soil applications have several advantages over more conventional methods such as spraying and dusting, (1) Longer persistence(Reynolds et al., 1957); (2) selectivity, which can introduce ecological or physical specificity by the method of application, while foliar spray make them non-selective, (Ripper et al., 1951; Reynolds, 1958); (3) less toxic hazards at the time of application (Zaki Reynolds, 1961; Reynolds & Metcalf, 1962); (4) special value for seedling plants, because

(a) seedlings are particularly susceptible to pests attack, (b) they have little plant surface to receive and retain direct spray deposits, (c) seedling plants grow relatively very rapidly, so new growth is unprotected by direct spray deposits.

A good historical example of a physiologically selective Systemic is schradan (Ripper, 1952; Verma, 1956) that kills phytophagous insects and is harmless to non-phytophagous ones such as the predators & parasites of insects and pollinators. Most Systemic insecticides are, however, like many other insecticides in being physiologically non-selective and when applied to foliage, are usually more harmful to beneficial insects because these are generally active searching species. Extensive use of such chemicals in the last decade or so has resulted in the common phenomenon of pest resurgence and development of new pests through failure of biological control (Ripper 1952, 1956, Michelbacher et al., 1955). Another drawback of direct application of non-selective chemicals is unnecessary exposure of the whole insect complex to the insecticide, resulting in the development of resistant strains, but with specific insecticides, the entomophagous species, especially the low density dependent ones will be unaffected

by the
chemical and will be able to kill any resistant individuals in the population (Ripper 1956; Michelbacher, 1955).

Selectivity can be improved by way of application as well as formulation of insecticides (Ripper, 1951; Metcalf 1956) e.g. menazon and pirimicarb are intrinsically fairly specific for aphids but phorate and aldicarb are not. Phorate is made more selective by soil application by granular application when it apparently becomes harmless to bees even when applied to foliage.

MATERIALS AND METHODS

INSECTICIDES.

Four Systemic insecticides were used in laboratory and field experiments. Menazon and pirimicarb are relatively inherently selective for aphids while phorate and "Temik" are broadspectrum insecticides in their action against insects.

Aldicarb (Temik): Commercial formulation of 10% w/w of active ingredient in corn cob granules and 88% technically pure chemical.

Pirimicarb. Commercial formulation of 5% w/w of active ingredient in pumice granules and 99% technically pure chemical.

Menazon. 4.1% w/w in Fullers Earth granules and 99% technically pure chemical.

Phorate. 10% w/w in Fullers Earth granules and 98% technically pure chemical.

All the technically pure compounds were stored in deep freeze at about $-12^{\circ}C$ to avoid possible loss of potency. In order to minimize labour and maintain

equality in applying granular treatments to the field plots, weight volume relationships were determined for each granular formulation so that appropriate volumes could be applied to each plant. For the laboratory experiments appropriate concentrations of the technically pure chemical were made up using ethyle cellosolve as solvent.

APHID SPECIES.

Two species of aphids - Myzus persicae (Sulz.) and Brevicoryne brassicae (L.) were reared on potted seedlings of Brussels sprout plants in cages 16" x 16" x 12" in a controlled environment room at 20°C with about 60% relative humidity and 16"34 hours of photoperiod. In order to avoid overcrowding of the aphids plants were changed fortnightly. Young adults and late 4th instar larvae were selected for most of the experiments. Aphids were handled very carefully with a mouth-operated suction tube. Infested leaves were taken from aphid cultures about 24 hours before the insects were needed for experiments. The leaves were allowed to wilt, thus, making the insects withdraw their mouthparts so that they could be collected easily without damaging their mouthparts.

FIELD EXPERIMENTS.

Site and design of field experiments.

Two field experiments were done between 1968 and 1969 to evaluate the Systemic efficacy of granulated formulations of soil applied Systemic insecticides applied at planting out time to control early infestations of immigrating alatae of aphid species and their subsequent population build up on the Brussels sprout plants. The experimental field was situated at Silwood Bottom where the pH value of the soil was found to be 6.1 in 1969 and almost the same as in 1964. The soil was sandy and therefore likely to favour uptake (Kirk & Wilson, 1960; Burtet al., 1965; Raynolds & Metcalf, 1962) and persistent toxicity of the insecticides(Getzin and chapman, 1957).

EXPERIMENT 1968.

Young brussels sprouts plants, Brassica abracea genifera L. variety Irish elegance, were planted out 3 ft. apart in mid May 1968. The experimental field was divided into four parallel blocks 75' long by 15' wide lying across the field. Each block was divided horizontally into 5 randomised plots 15' x 15' ft. square without paths and each containing 5 rows of 5 plants each (25 plants).

INSECTICIDE APPLICATION.

Granular formulations of insecticides were applied by hand at the rate of 2 lbs a.i per acre. The required quantity of each granular insecticide was measured out in a plastic tube of appropriate volume and put into each "dibbling" hole about 3 in. deep and 1-3 in. away from the stem.

FIELD COUNTING.

Five plants per plot were randomly selected and examined almost fortnightly to record alatae, adultapterae and larvae present on treated Brussels Sprout plants. The meanlog (N +1) aphids per plant were calculated for each of the three species of aphids:

(1) Marrosiphum euphorbiae (Thomas)

(2) Myzus persicae (Sulz)

(3) Brevicoryne brassicae (L.)

B. brassicae were relatively very scarce otherwise it would not have been possible to examine populations in whole plants.

BIOASSAY PROCEDURE

Two bioassay methods, depending on the Systemic action of the insecticide, were used to determine the biologically active residues of menazon,

phorate, pirimicarb and aldicarb in plants.

LEAF DISC REMOVAL

The technique described by Galley (1968) was used to test residual toxicity of 1 in. diameter discs, excised four leaves of the experimental plants. Two plants per plot were randomly chosen and marked in order to cut leaf discs from them throughout the period of residual toxicity in the plants. 16 leaf discs were taken from 8 plants of each treatment and 10 M. persicae and 10 B. brassicae were put on each disc. and kept under controlled conditions. At intervals, the aphids were recorded as (1) (N)- those in which no sign of abnormality could be noticed, (2) slightly affected (S) - those in which the effect was noticeable but which could move about or make some progress, (3) badly affected (B) - those which could not leave the place where they were but showed vigorous movements of the various parts of the body, (4) moribund (N) - those which appeared to be dead but on prodding could show some sign of life by feeble movements of some part of the body, (5) dead (D) - those which showed no sign of life under a magnifying lens despite prodding. The criteria of those above categories were almost the same as those of Tattersfield

and Potter (1943). Percentage kills were based on B + M + D (Martin 1940). The levels of residue in the leaves of Brussels Sprouts plants were estimated by comparing the kills obtained with those on similar leaf disc samples from untreated plants dosed with known amounts of the insecticide (Galley 1968).

SLEEVING EXPERIMENTS

Two plants per plot (i.e. 8 per treatment) were randomly selected and 20 (10 M. persicae + 10 B. brassicae) laboratory reared young adult apterae were caged in an organdic sleeve about 15" long and 9" in diameter either on single young leaves or separately on one young and one mature leaf per plant. Sleeved aphids were set up in this way at about fortnightly intervals throughout the season. The effect of a treatment was assessed from the mortality of the adults and from the numbers of live progeny produced introduced during the following 6 - 8 days.
This experiment was done in order to make a general comparison of the Systemic activity and persistence of the four granular soil applied Systemic insecticides.

EXPERIMENT 1969.

The scheme of planting young Brussels Sprouts seedlings was almost the same as in the previous experiment (e.g. planted 3 ft. apart), but the design of the experimental area was slightly changed i.e. treatments replicated into 3 blocks of 6 randomised plots. Plots were 18' x 9' separated by a 3' wide path. Each plot contained only 3 rows of 6 plants each and the middle row (four plants) was treated while the other two rows, one on either side, were used as guard rows.

INSECTICIDE APPLICATION.

A Granular formulation of one insecticide aldicarb was applied at 1 lb. a.i. per acre. The volumetric measurer was again used to put the required amount of insecticide with or without fertilizer (1.2 oz/sq.yard) at different depth levels below or away from the plant. Similar methods of field counting, leaf disc removal and sleeve tests were used in this experiment as described earlier.

This experiment was mainly designed to study the effect of different placement methods on the field efficiency of an insecticide with or without fertilizer.

Fig. No. 1 Placement of pesticide and fertilizer in relation to the plants.

Fig. 1

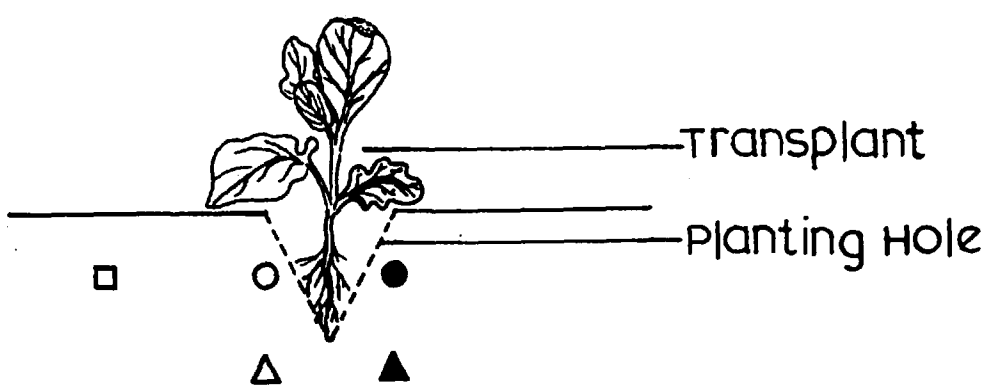
○ 2-3″ deep & 2-3″ away from plant

● 〃 〃 〃 〃 〃 +Fertilizer

△ 9″-1′ 〃 〃 〃 〃

▲ 〃 〃 〃 〃 〃 〃

□ 2-3″ 〃 9″-1′ 〃 〃



The fertilizer used was Nitroform supplied by Imperial Chemical Industries in order to study its effects on the efficiency in uptake and persistence of an insecticide treatment to the soil. Details of the positions applied are given below in fig. 1 and described in field experiment 1969 (page 27)

Laboratory experiments

Laboratory techniques specific to particular experiments will be discussed in the main text. One inch diameter leaf discs were taken from mature leaves of untreated Brussels sprout plants grown at 20°C in a controlled environment room. They were set up in the apparatus described by Galley (1968) in Plate 1 and 5 ul droplets of an appropriate concentration of insecticide in ethyl cellosolve was applied by "Microcap" to the water supply of each disc. 10 M. persicae and 10 B. brassicae were separately caged on each disc. At least 5 concentrations of each insecticide were used and 5 discs treated with each concentration, control discs were treated with ethyl cellosolve.

The air flow in the controlled environment rooms and the relatively low humidity tended to

dessicate the leaf discs so the apparatuses were put in a tray containing a layer of moist sand and was covered with a transparent perspex lid. This maintained a relative humidity of about 85-90% around the apparatus and prevented the leaf discs from dessicating. In experiments at 20°C the state of the aphids was recorded after 24, 48 and 72 hours and even continued for longer periods where an end point was to be determined. The first record was sometimes made decreased to 12 hours in studies of time mortality at higher temperatures. The criteria for recording the state of the aphids was the same as already described.

Experiments with Brussels sprouts grown in nutrient solution (Long Ashton)

Brussels sprouts seeds were sown in sand trays and kept in the controlled environment room at 20°C to accelerate germination and growth of seedlings. When the plants were about 4-5" long they were set in holes in a black polythene sheet over a container of nutrient solution.

Long Ashton stock solution

chemicals in- - - - - - - - - - gm/2 litres of distilled water.

1.	Kno_3	202
2.	$Ca\ (No_3)_2$	656
3.	$Na\ HPo_4\ 2H_2o$	208
4.	$MgSo_4\ 7H_2o$	369
5.	Iron EDTA	77.02
6.	$MnSo_4\ 4H_2o$	4.46
7.	$CuSo_4\ 5H_2o$	0.48
8.	$ZnSo_4\ 7H_2o$	0.58
9.	H_3Bo_3	3.92
10.	$(NH_4)_6Mo_7o_2\ 4H_2o$	0.07

Recipe of the culture solution

Add 2 mls. each of solutions 1, 2, 3 & 4

Add 1 ml. each of solutions 5, 6, 7, 8, 9 & 10

Plus 986 mls. distilled water = 1000 mls. standard solution

The seedlings took almost a month and a half to attain a height of about 8-10". The plants were then put individually into 2 1 lb jam jars painted black and supplied with nutrient solution until they were large enough to be used for experiments. Brussels sprouts were grown in nutrient solution in order to get a supply of well grown uniform plants with their developed root system.

R E S U L T S

FIELD EXPERIMENTS 1968

This experiment was undertaken to compare the field efficacy in toxicity and persistence of soil applications of different Systemic aphicides against aphid on brussels sprout plants.

EFFECTS OF APPLICATIONS ON NATURAL POPULATIONS:

The effects of the granular insecticides applied to the soil two weeks after transplanting the Brussels sprout seedlings were assessed by weekly counts of alatae, adult apterae and larvae of three aphid species, M.persicae, M.euphorbiae and B. brassicae infesting the plants.

Live alatae were recorded separately but apterae and larvae were counted together as it was difficult to make a clear distinction between them.

Separate counts of alatae (table -1) showed that B. brassicae began to colonise the crop about the 4th week after treatment (3rd week in June), but alatae of M.persicae and M.euphorbiae had already arrived by the time the first counts were made in the 2nd week of June. Data indicate that aphids of all species stopped colonising the crop in mid-July.

Table 1 Mean numbers per plant of live alatae of aphids found on Brussels sprout plants treated with different systemic aphicides.

Treatments	Geometric mean no. of live alatae per plant									
	Number of weeks after treatment									
	2	3	4	5	6	7	10	11	14	17
					Myzus persicae (Sulz.)					
Aldicarb	0.04	0.04	0.04	0.04	0.0	0.0	0.0	0.0	0.0	0.0
Pirimicarb	0.24	0.09	0.07	0.04	0.13	0.13	0.0	0.0	0.0	0.0
Menazon	0.15	0.07	0.07	0.07	0.38	0.09	0.0	0.0	0.0	0.0
Phorate	0.82	0.17	0.11	0.61	1.31	0.21	0.0	0.0	0.06	0.0
untreated	2.26	0.63	0.31	1.27	2.54	0.38	0.11	0.0	0.0	0.0
					Brevicoryne brassicae (L.)					
Aldicarb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pirimicarb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Menazon	0.0	0.0	0.0	0.0	0.04	0.0	0.0	0.0	0.0	0.0
Phorate	0.0	0.0	0.0	0.0	0.04	0.0	0.20	0.0	0.0	0.0
untreated	0.0	0.0	0.04	0.13	0.49	0.40	1.00	0.0	0.0	0.0
					Macrosiphum euphorbiae (Thom.)					
Aldicarb	0.07	0.11	0.0	0.04	0.11	0.06	0.0	0.0	0.0	0.0
Pirimicarb	0.26	0.04	0.0	0.0	0.36	0.20	0.04	0.0	0.0	0.0
Menazon	0.23	0.24	0.04	0.27	0.68	0.27	0.04	0.0	0.0	0.0
Phorate	0.46	0.16	0.07	1.65	2.85	0.21	0.00	0.0	0.0	0.0
untreated	0.82	0.81	1.20	2.03	2.10	0.26	0.0	0.0	0.0	0.0

The aldicarb and pirimicarb treatments kept all the aphid species very scarce throughout the period of colonisation. Phorate was the least effective. Aldicarb and pirimicarb also seem to act more quickly against the immigrant alatae as indicated by the counts of live alatae which for the commonest species M. euphorbiae never rose above 0.11 per plant on the aldicarb treated plants compared with 0.36, 0.68, 2.85, 2.10 on pirimicarb, menazon, phorate and untreated plants respectively. The natural populations of all species were relatively small. M.euphorbiae appeared early, reached peak numbers of 27.7 per untreated plant in mid-June but disappeared by mid-July, seven weeks after treatment (Figure 2). M.persicae and B.brassicae remained on the untreated plants throughout the period of the experiment after reaching peak numbers of 58.4 and 19.5 per plant about the end of June and early July (Figure 3 and 4). The comparison of the susceptibility of the three aphid species infesting the crop indicates that B.brassicae was the most susceptible species, since it was completely controlled throughout the season by aldicarb, pirimicarb and menazon. However, some B.brassicae survived on phorate treated plants and they remained

Fig. No. 2 The effect of different soil applied systemic insecticides on the populations of aphid larvae and apterae adults on brussels sprouts plants (1968)

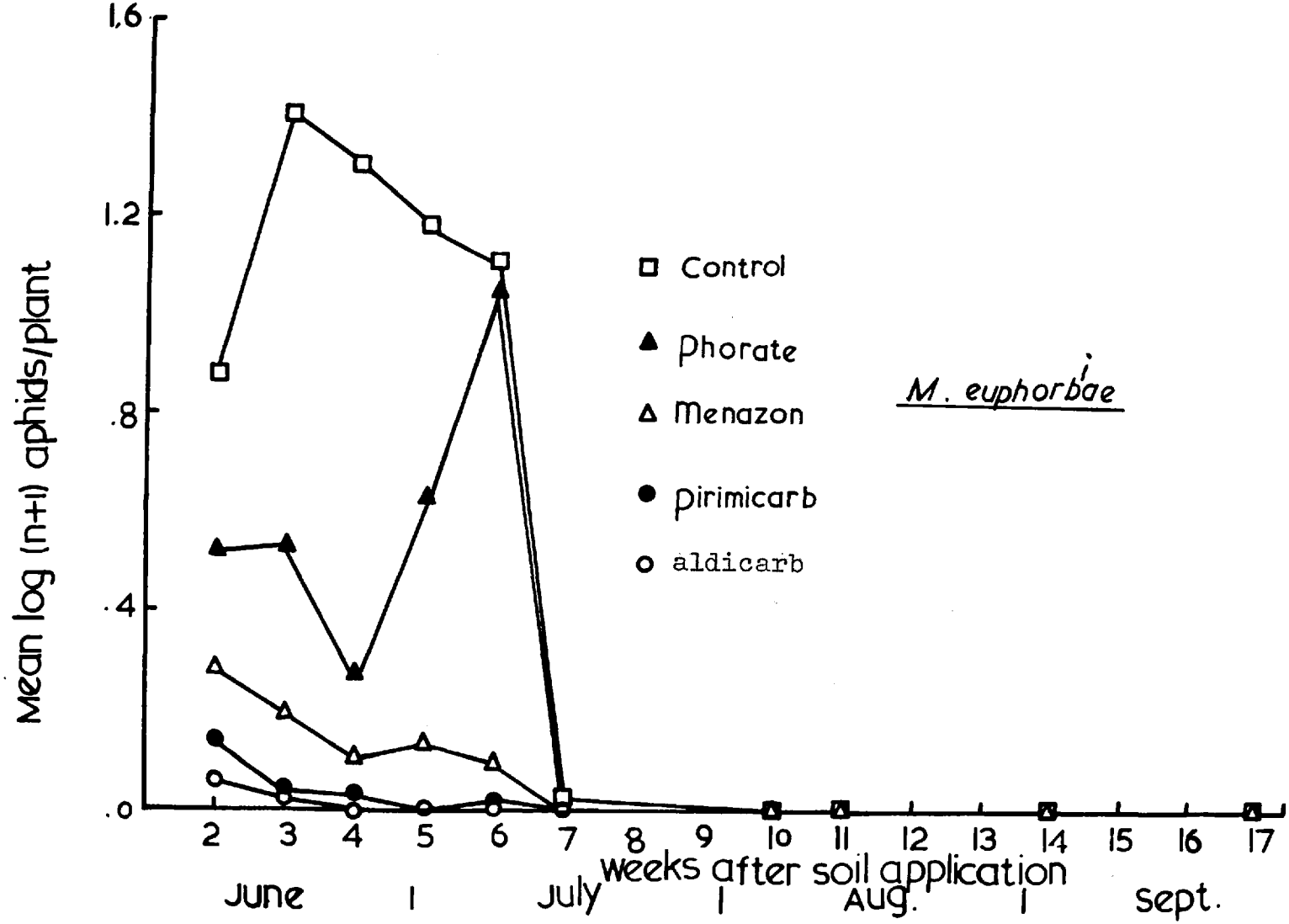

1.6
1.2
.8
.4
.0
Mean log (n+1) aphids/plant
Control
Phorate
Menazon
pirimicarb
aldicarb
M. euphorbae
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
weeks after soil application
June
1
July
1
Aug.
1
Sept.

Fig. No. 3 The effect of different soil applied systemic insecticides on the populations of aphid larvae and apterae adults on brussels sprouts plants (1968)

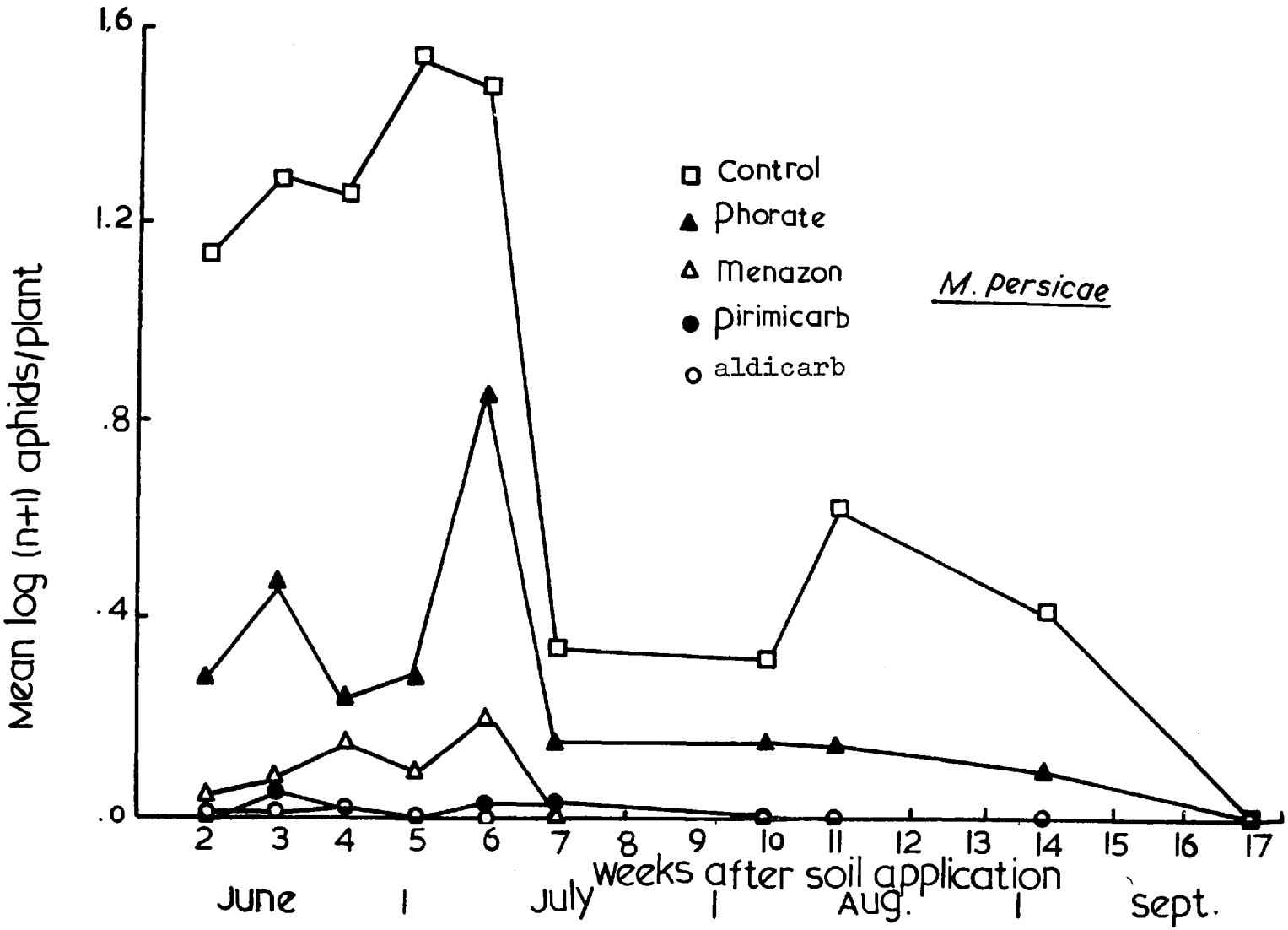
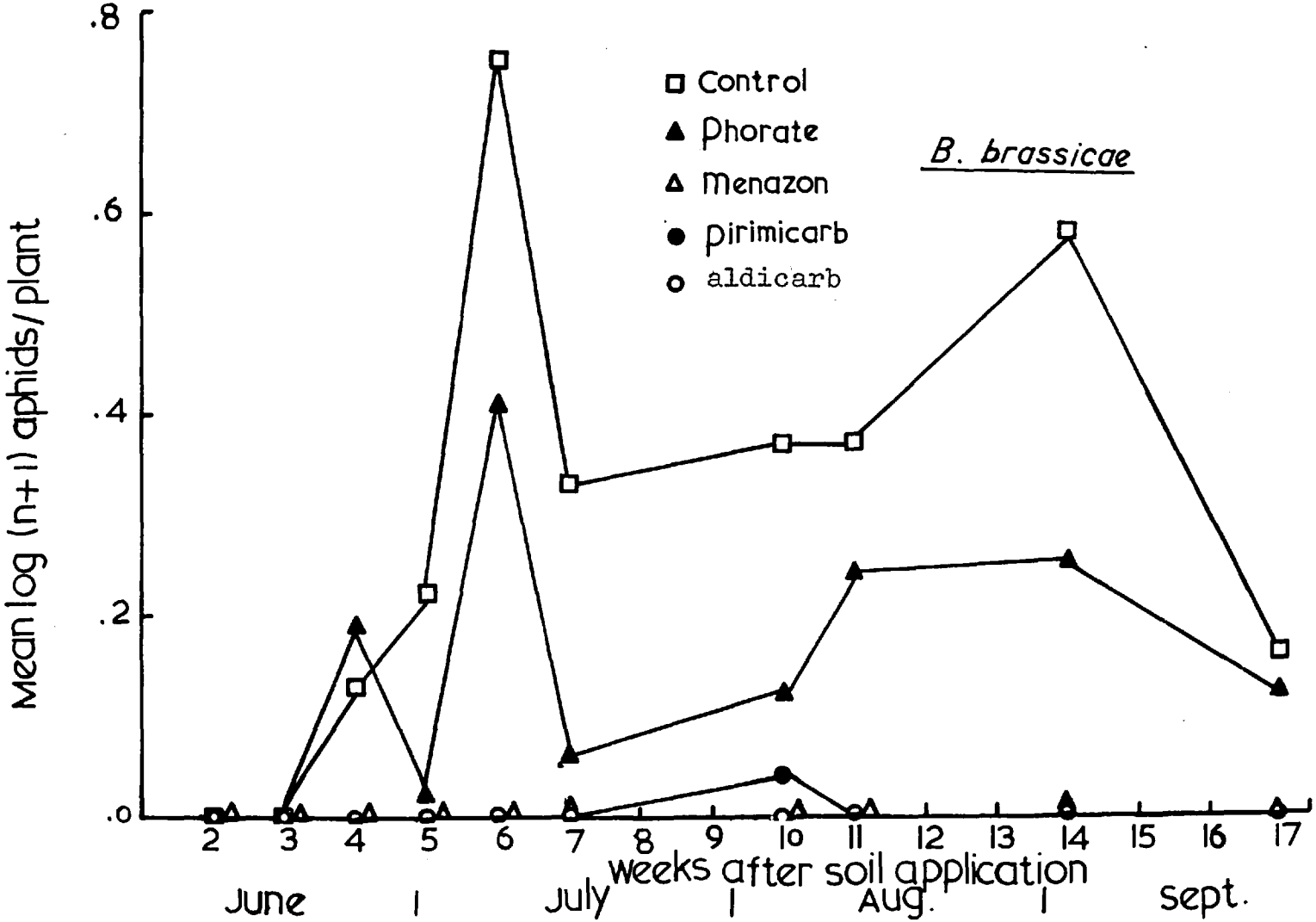

1.6
1.2
.8
.4
.0
Mean log (n+1) aphids/plant
Control
Phorate
Menazon
Pirimicarb
aldicarb
M. persicae
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
weeks after soil application
June
July
Aug.
Sept.

Fig. No. 4 The effect of different soil applied systemic insecticides on the populations of aphid larvae and apterae adults on brussels sprouts plants (1968)


.8
.6
.4
.2
.0
Mean log (n+1) aphids/plant
Control
Phorate
Menazon
pirimicarb
aldicarb
B. brassicae
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
weeks after soil application
June
1
July
1
Aug.
1
sept.

throughout the season reaching a peak number of 2.57 per plant in mid-July compared with 7.08 and 11.22 for M.persicae and M.euphorbiae respectively.

M.persicae and M.euphorbiae were effectively controlled by aldicarb and pirimicarb never reaching more than 0.02 and 0.04 in the last week of June respectively and completely disappeared by mid-July. Menazon was slightly less effective showing peak numbers of 0.21 and 0.28 for M.persicae and M.euphorbiae by mid-July and early June respectively. The M.euphorbiae population in the phorate treatment collapsed suddenly by mid-July (Figure 2) whereas M.persicae and B.brassicae persisted upto the end of the season (Figure 3 & 4). The results suggest that aldicarb, pirimicarb and menazon treatments protected the plants throughout the 20 weeks period of the treatment. The order of effectiveness of the insecticides against the natural populations of aphids was:

aldicarb > pirimicarb > menazon > phorate

while the order of aphid species susceptibility appeared to be B.brassicae > M.persicae > M.euphorbiae. The scarcity of alatae after 7 weeks indicates that no natural reinfestation was occurring at this time. Furthermore the protective ability of the

chemicals was not fully tested because the infestations were small. Further bioassay experiments were therefore done to determine persistence at intervals after treatment. (see next section)

BIOASSAYS

Aphid bioassays were done at regular intervals after treatment and the results were assured as follows:

1. Insect mortality.
   (a) The uptake of the material by Brussels sprout plants as indicated by the time required from soil treatment until maximum mortality occurred.
   (b) The persistence of toxicity in the plants as expressed by the time during which mortality was maintained.

Sleeve tests to determine persistence and degradation of toxicity.

Laboratory reared late instar larval apterae of M. persicae and B. brassicae were sleeved at intervals on young and old leaves of treated and untreated

Table 2 Percent mortality of late instar larval apterae sleeved on young leaves of Brussels sprout plants treated with granules of four different systemic insecticides.

Myzus persicae

Time of examination (dates)	No. of weeks after soil application	Treatment and leaf age: Untreated control	Aldicarb	Pirimicarb	Menazon	Phorate
27.6.68	4	12.5	100.0	100.0	74.3	48.6
11.7.68	6	11.5	100.0	100.0	70.7	21.1
8.8.68	10	0.0	98.7	77.5	28.7	23.7
29.8.68	13	0.0	55.	53.7	33.7	40.0
12.9.68	15	1.5	28.5	25.3	19.8	11.5
28.9.68	17	0.0	13.7	7.5	7.5	3.7
20.10.68	20	0.0	0.0	0.0	0.0	0.0

Brevicoryne brassicae

27.6.68	4	20.0	100.0	100. 0	100. 0	62.5
11.7.68	6	7.5	100.0	100. 0	98.6	50.0
8.8.68	10	o.o	100.0	83.7	50. 0	41.3
29.8.68	13	0.0	81.2	67.5	62.5	46.2
12.9.68	15	2.5	55.5	42.3	39.7	26. 0
28.9.68	17	0.0	23.7	21.2	20.0	11.2
20.10.68	20	0.0	6.0	3.0	o.o	0.0

Table 3 Percent mortality of late instar apterae of aphids sleeved on old leaves of Brussels sprout plants, treated with different systemic insecticides.

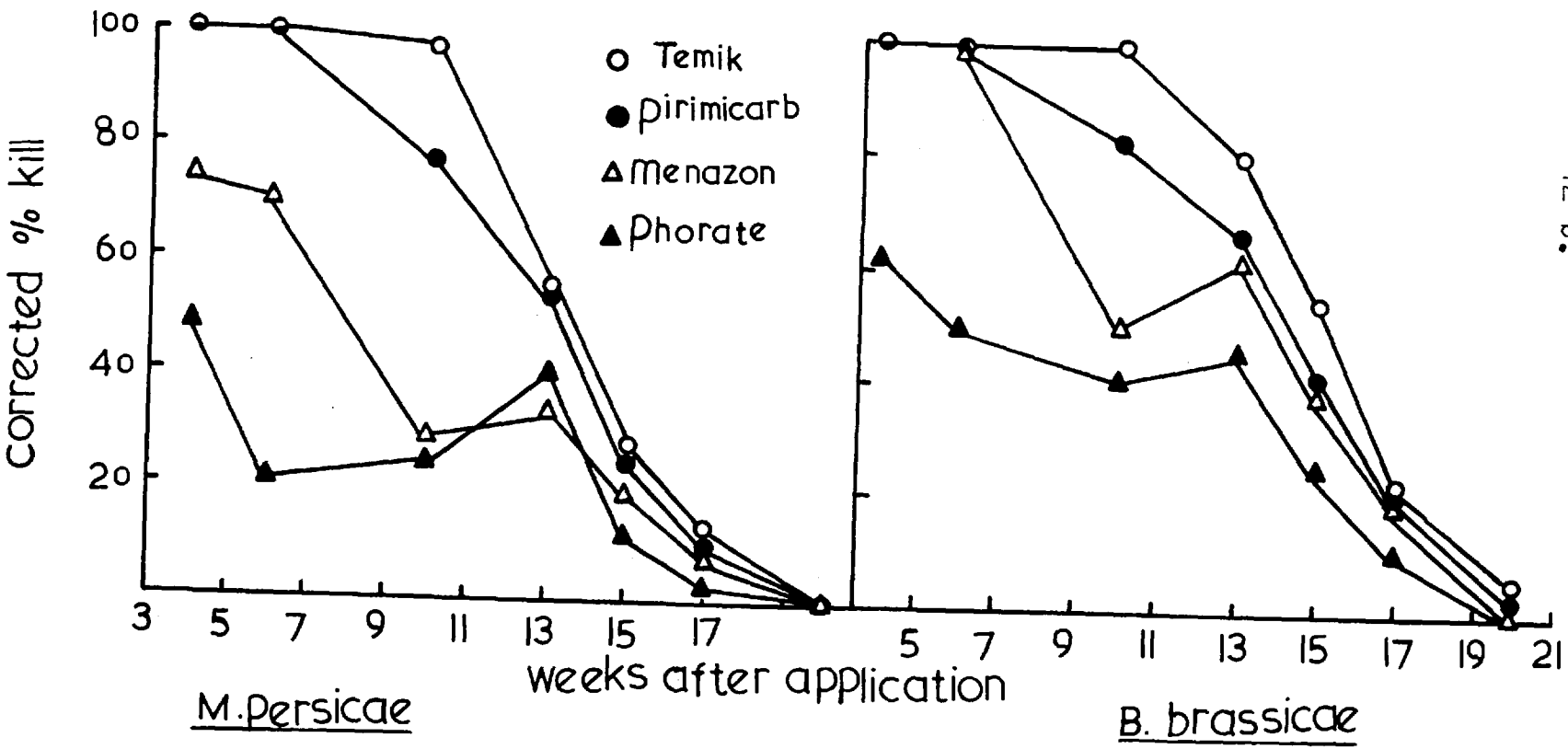
Myzus persicae

Time of examination (dates)	No.of weeks after soil application	Treatment and leaf age				
		Untreated control	Aldicarb	Pirimicarb	Menazon	Phorate
11.7.68	6	12.5	100.0	100.0	98.6	48.8
8.8.68	10	0.0	100.0	73.7	43.7	37.5
29.8.68	13	0.0	95.1	65.0	67.5	41.2
12.9.68	15	0.0	71.0	48.5	37.5	26.4
28.9.68	17	0.0	18.0	16.2	15.0	6.2
20.10.68	20	0.0	3.5	1.8	0.0	0.0

Brevicoryne brassicae

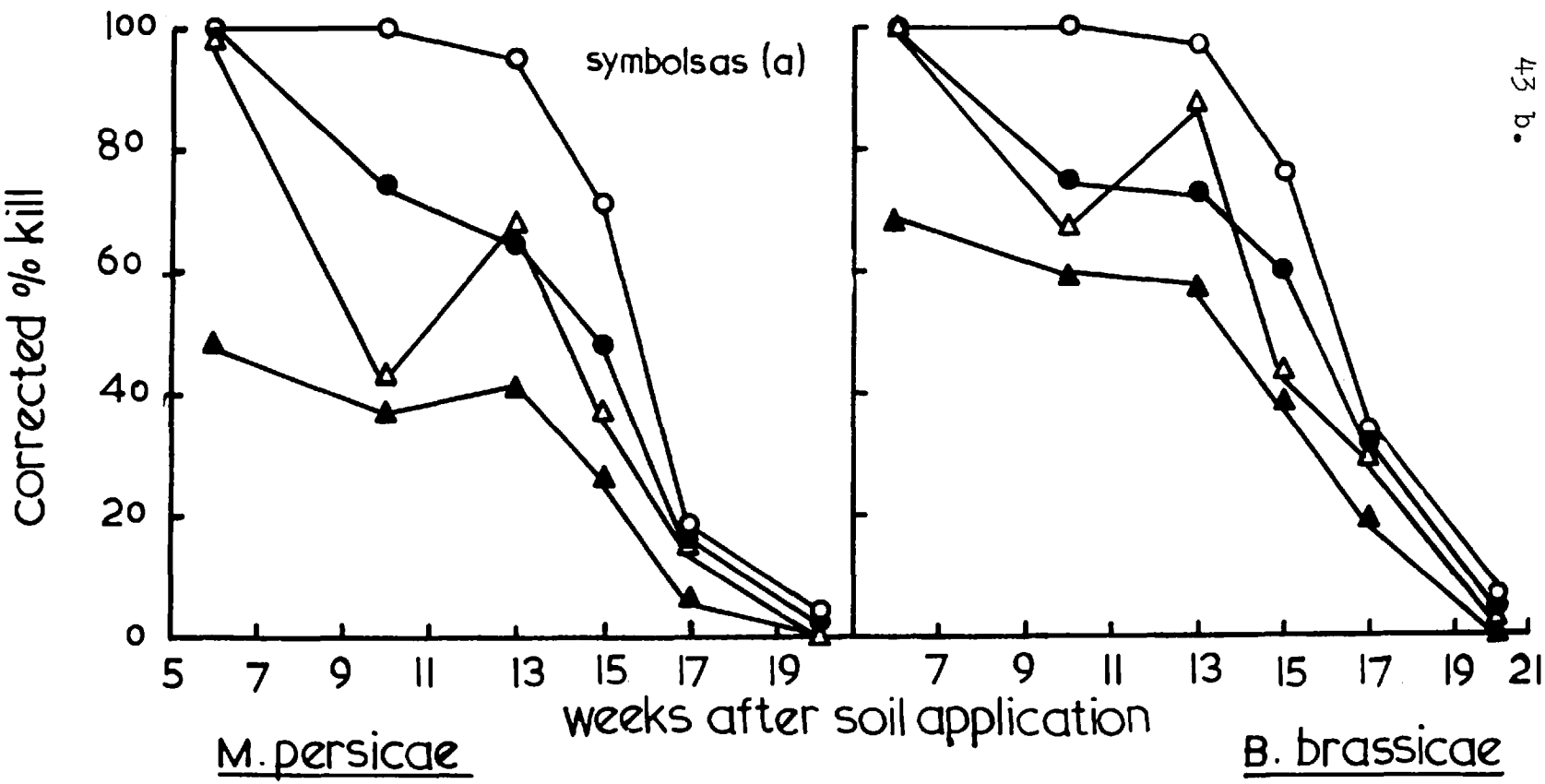
11.7.68	6	22.5	100.0	100.0	100.0	65.2
8.8.68	10	1.3	100.0	75.9	67.0	59.0
29.8.68	13	2.5	97.4	73.1	87.2	57.7
12.9.68	15	0.0	76.5	60.0	43.2	38.5
28.9.68	17	0.0	32.9	32.4	29.1	19.0
20.10.68	20	0.0	5.5	3.8	1.5	0.0

Fig. No. 5 Corrected percent kill of late instar larval apterae of *Myzus persicae* and *Brevicoryne brassicae* sleeved on young leaves of brussels sprouts plants for one week.


100
80
60
40
20
corrected % kill
Temik
pirimicarb
Menazon
Phorate
3 5 7 9 11 13 15 17
5 7 9 11 13 15 17 19 21
weeks after application
M.persicae
B. brassicae

42 b.

Fig. No. 6 Corrected percent kill of late instar larval apterae of *M. persicae* and *Brevicoryne brassicae* sleeved on old leaves of brussels sprouts plants for one week.


corrected % kill
100
80
60
40
20
0
symbols as (a)
5
7
9
11
13
15
17
19
7
9
11
13
15
17
19
21
weeks after soil application
M. persicae
B. brassicae

plants as already described (see Materials and Method). Kills on leaves of treated plants were corrected for kills of M. persicae and B. brassicae respectively in untreated plants. The numbers of dead aphids in each treatment after one week (Figure 5 & 6) show that aldicarb killed all aphids of both species upto 10 and 13 weeks and continued to kill 50% and 70% aphids upto 15 weeks after treatment on young and old leaves respectively compared with pirimicarb which killed all aphids upto 6 weeks and more than 50% upto 13 and 15 weeks after treatment on young and old leaves respectively.

The persistence of menazon was almost similar to pirimicarb but with phorate the maximum mortality never rose above 65% and was about 40 - 50% kill 13 weeks after treatment. The rate of loss as indicated by the decreasing toxicity in the sleeves, appears to be relatively faster with phorate. In the other insecticides pattern of loss was almost similar. The complete loss of toxicity occurred after almost 20 weeks in all treatments except phorate where it was lost after 17 weeks. The rate of disappearance of toxicity was slightly greater in young leaves than in old leaves although leaf age seemed

Table 4 Numbers of progeny produced by aphids sleeved on young leaves of Brussels sprouts plants for a week in the field, treated with different systemic insecticides, given as percentages of those in the control.

Myzus persicae

Time of examination (dates)	No. of weeks after soil application	Treatment and leaf age				
		Untreated control	Aldicarb	Pirimicarb	Menazon	Phorate
27.6.68	4	295	0.0	0.0	10.8	63.0
11.7.68	6	197	0.0	0.0	13.9	97.2
8.8.68	10	206	0.0	23.8	60.2	57.7
29.8.68	13	176	72.2	52.8	110.2	104.0
12.9.68	15	156	39.5	58.5	73.5	82.3
28.9.68	17	108	47.2	48.1	85.2	86.1
20.10.68	20	128	59.8	69.0	88.0	96.5

Brevicoryne brassicae

27.6.68	4	61	0.0	0.0	0.0	178.7
11.7.68	6	188	0.0	0.0	0.0	110.6
8.8.68	10	246	20.8	21.0	35.0	31.3
29.8.68	13	94	46.8	87.2	44.1	108.4
12.9.68	15	138	49.0	68.5	59.7	78.5
28.9.68	17	120	55.0	32.5	55.8	58.3
20.10.68	20	138	68.5	72.6	88.0	98.0

Table 5 Numbers of progeny produced by aphids sleeved for a week on old leaves of Brussels sprouts plants, given as percentages of those in the control.

Myzus persicae

Time of examination (dates)	No. of weeks after treatment	No. of progeny as percentages of those in control				
		Untreated control	Aldicarb	Pirimicarb	Menazon	Phorate
27.6.68	4	-	-	-	-	-
11.7.68	6	290	0.0	0.0	0.0	44.1
8.8.68	10	211	0.0	27.0	43.6	39.3
29.8.68	13	143	4.2	40.5	41.9	83.9
12.9.68	15	112	34.4	55.5	76.0	89.5
28.9.68	17	84	63.1	63.1	65.5	104.8
20.10.68	20	93	69.0	75.0	86.0	98.5

Brevicoryne brassicae

11.7.68	6	259	0.0	0.0	0.0	25.8
8.8.68	10	159	30.0	20.7	47.8	61.0
29.8.68	13	96	5.2	19.8	21.9	85.4
12.9.68	15	105	27.4	35.0	39.0	98.5
28.9.68	17	81	45.7	46.9	43.2	123.4
20.10.68	20	110	77.3	85.0	93.5	100.0

to have relatively little effect on toxicity(comparison of figures 5 and 6). The order of toxicity was:

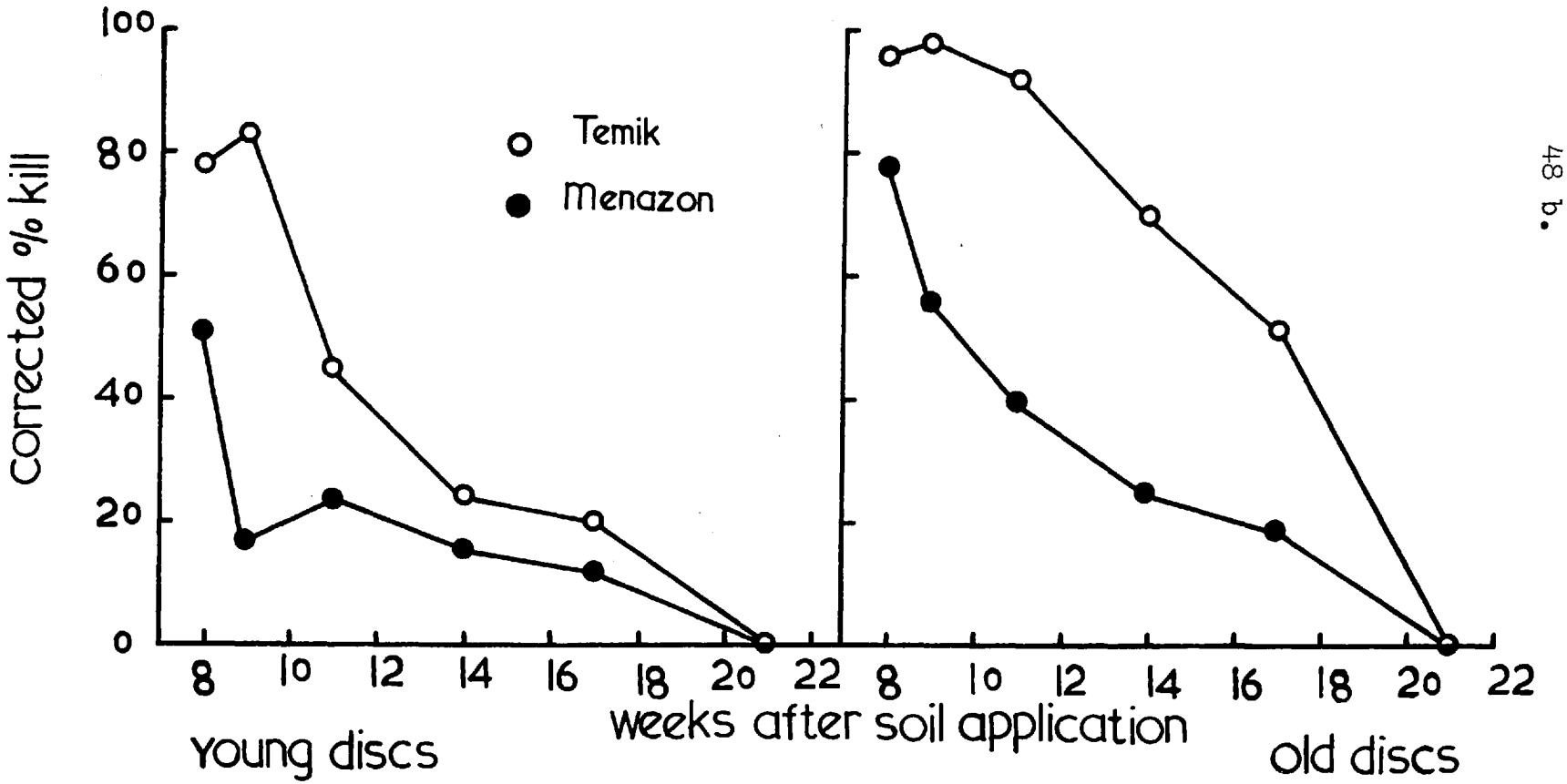
aldicarb > pirimicarb > menazon > phorate, thus reflecting the results with natural populations. It was confirmed that B. brassicae was more susceptible than M. persicae (Table 2 and 3)

Data(in table 4 and 5) indicate that effects of the treatments on reproduction of the two aphid species were related very much to their respective toxicities. Aldicarb ., pirimicarb and menazon kept numbers of progeny very low, but with phorate the numbers often reached those on the untreated controls.

LEAF DISC EXPERIMENTS.

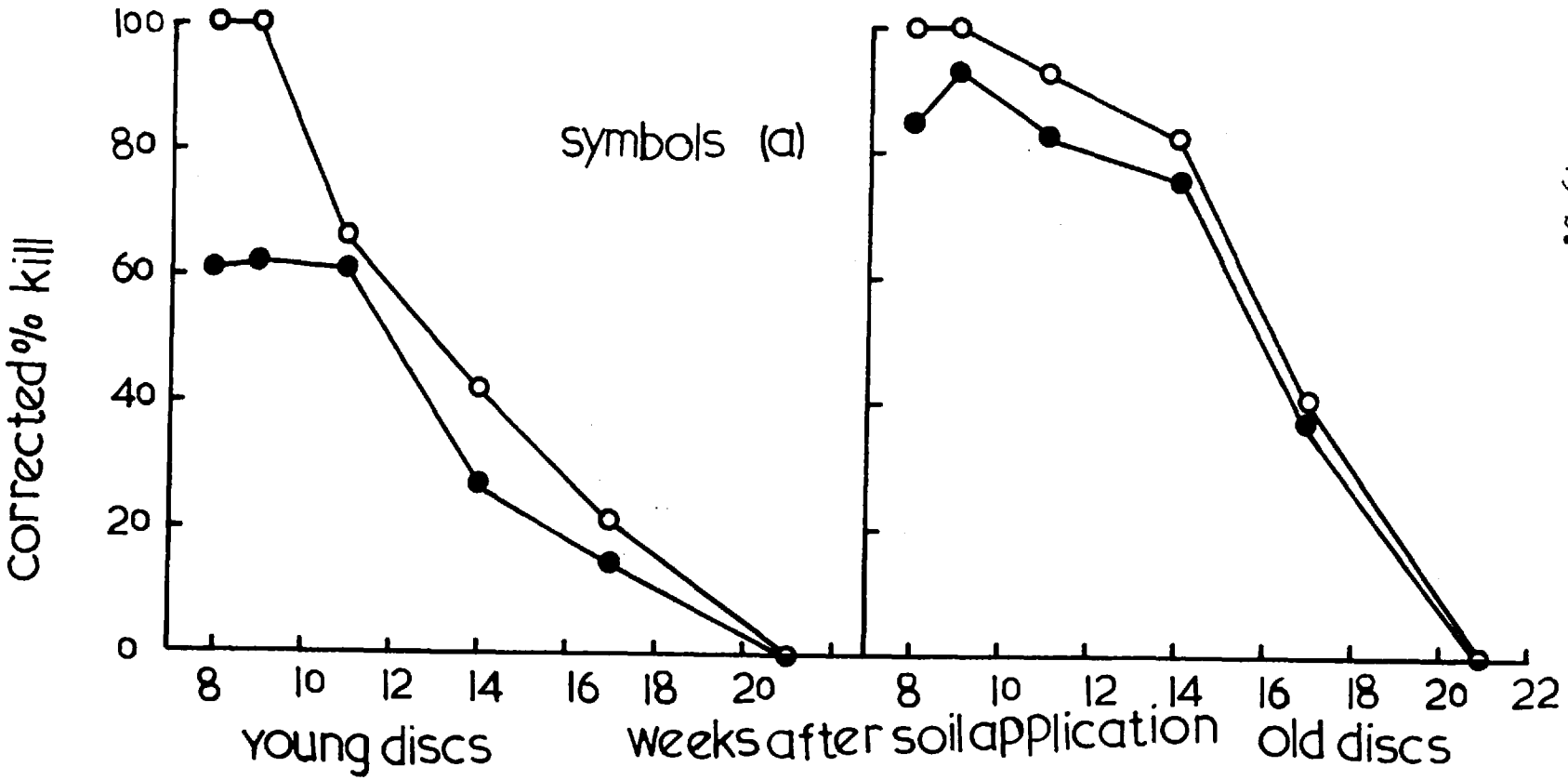
The toxicity of leaf discs removed from the plants treated with one carbamate and one organophosphate insecticide and kept under standardised conditions in the laboratory was used to get a more accurate estimate of uptake and changing persistence than was possible in the variable conditions in the field (see Methods). Mortalities of 4th instar M. persicae and B. brassicae kept on leaf discs from aldicarb and menazon treated plant

Fig. No. 7 Percent mortality of late instar larval apterae of Myzus persicae after 72 hours at 20°C on leaf discs taken from young and old leaves of brussels sprouts plants treated with different systemic insecticides.


100
80
60
40
20
0
corrected % kill
Temik
Menazon
8
10
12
14
16
18
20
22
weeks after soil application
young discs
old discs

48 b.

Fig. No. 8 Percent mortality of late instar larval apterae of Brevicoryne brassicae after 72 hours at 20°C on leaf discs taken from young and old leaves of brussels sprouts plants treated with different systemic insecticides.


corrected % kill
100
80
60
40
20
0
symbols (a)
8
10
12
14
16
18
20
22
young discs
weeks after soil application
old discs

49 b.

for 72 hours at 20°C were corrected for control mortalities of aphids in untreated plants (Figures 7 & 8).

The results (table 6 and 7) show that aldicarb killed all B. brassicae and 80 - 98% of M. persicae on young and old leaf discs upto 9 weeks after treatment compared with menazon which killed 60 - 93% of B. brassicae and 50 - 78% of M. persicae. Both the treatments started losing their toxicity in about 10 weeks after treatment but aldicarb was still killing about 66 - 83% of B. brassicae and 45 - 70% of M. persicae on young and old discs upto 14 weeks after treatment. Menazon was also persisting efficiently and killing about 60 - 70% of B. brassicae and only 25% of M. persicae upto the same period. The rate of degradation of toxicity as assessed by decline in mortality of exposed aphids, appears to be similar in both treatments showing no toxicity left by about 21 weeks after treatment. As before B. brassicae was more susceptible than M. persicae. Furthermore, in these circumstances the older leaves were more toxic than younger leaves.

Data in tables 8 and 9 show the effects of treatments on numbers of progeny produced.

The results indicated that the numbers of progeny produced by aphids on treated leaf discs

Table 6 Percent mortality of late instar larval apterae of Myzus persicae on leaf discs from young and old leaves of Brussels sprout plants treated with granular formulations of different systemic insecticides.

No. of weeks after soil application (dates)	Time of examination (hours)	Treatment and leaf age					
		Untreated control		Aldicarb		Menazon	
		young disc	old disc	young disc	old disc	young disc	old disc
8 22.7.68	24	0.0	0.0	53.7	56.2	2.5	53.7
	48	0.0	0.0	73.7	87.5	26.2	65.0
	72	0.0	0.0	78.7	96.2	51.2	78.7
9 30.7.68	24	0.0	0.0	57.5	88.7	0.0	21.7
	48	0.0	0.0	80.7	96.2	12.5	40.0
	72	0.0	0.0	83.7	98.7	17.5	56.2
11 13.8.68	24	0.0	0.0	11.2	50.0	3.7	3.7
	48	0.0	2.0	13.2	86.0	13.7	7.5
	72	0.0	2.0	45.0	92.5	23.7	40.0
14 3.9.68	24	0.0	2.0	5.0	22.5	2.5	5.0
	48	2.0	4.0	10.0	60.0	3.7	12.5
	72	2.0	4.0	24.0	70.0	15.0	25.0
17 24.9.68	24	0.0	0.0	0.0	15.0	1.5	3.0
	48	0.0	0.0	9.0	45.5	2.8	9.5
	72	0.0	0.0	20.0	52.0	12.5	19.5
21 29.10.68	24	0.0	0.0	0.0	0.0	0.0	0.0
	48	0.0	0.0	0.0	0.0	0.0	0.0
	72	0.0	0.0	0.0	0.0	0.0	0.0

Table 7 Percent mortality of late instar larval apterae of Brevicoryne brassicae on leaf discs from young and old leaves of Brussels sprout plants treated with different systemic insecticides.

No. of weeks after soil application (dates)	Time of examination (hours)	Treatment and leaf age					
		Untreated control		Aldicarb		Menazon	
		young disc	old disc	young disc	old disc	young disc	old disc
8	24	0.0	0.0	80.0	90.0	38.7	63.7
22.7.68	48	0.0	0.0	97.0	100.0	56.2	81.2
	72	0.0	0.0	100.0	100.0	61.2	85.0
9	24	2.0	0.0	87.5	91.2	32.9	80.0
30.7.68	48	2.0	2.5	100.0	100.0	52.5	92.3
	72	2.0	3.75	100.0	100.0	62.0	93.5
11	24	0.0	0.0	25.0	57.5	37.5	56.2
13.8.68	48	1.5	0.0	58.2	88.7	48.1	80.0
	72	2.55	0.0	66.6	93.7	61.1	83.7
14	24	0.0	0.0	12.25	52.5	16.0	32.5
3.9.68	48	0.0	0.0	24.0	70.0	21.0	57.5
	72	0.0	0.0	42.0	83.7	27.5	76.2
17	24	0.0	0.0	4.05	15.5	1.75	11.5
24.9.68	48	0.0	0.0	16.5	38.0	9.20	33.7
	72	0.0	0.0	21.5	41.5	14.00	38.0
21	24	0.0	0.0	0.0	0.0	0.0	0.0
29.10.68	48	0.0	0.0	0.0	0.0	0.0	0.0
	72	0.0	0.0	0.0	0.0	0.0	0.0

Table 8 Numbers of progeny produced by aphids on leaf discs from young and old leaves of Brussels sprout plants treated with granular formulations of four different systemic insecticides.

Myzus Persicae

No. of weeks after soil application (dates)	Time of examination (hours)	Nos. of progeny as percentages of those in untreated control					
		Untreated control		Aldicarb		Menazon	
		young disc	old disc	young disc	old disc	young disc	old disc
8	24	306	256	13.00	11.50	50.90	21.60
22.7.68	48	390	332	5.13	0.00	18.46	13.13
	72	507	512	13.61	5.08	5.90	3.94
9	24	191	132	52.35	36.30	70.68	93.18
30.7.68	48	238	197	23.95	3.55	100.00	55.30
	72	240	136	17.00	2.91	93.75	53.28
11	24	190	203	59.41	34.97	91.58	79.40
13.8.68	48	177	167	58.75	19.16	94.92	73.05
	72	228	155	44.30	19.70	83.68	38.42
14	24	156	119	29.49	53.78	82.05	89.91
3.9.68	48	164	116	84.75	33.62	74.39	111.20
	72	146	96	89.72	27.08	83.56	107.29
17	24	146	125	100.00	95.20	64.38	64.80
24.9.68	48	99	87	91.91	93.10	94.94	79.31
	72	141	126	63.10	49.20	61.00	46.80
21	24						
29.10.68	48						
	72						

Table 9 Numbers of progeny produced by aphids on leaf discs from young and old leaves of Brussels sprout plants, given as percentages of those on untreated control discs.

Brevicoryne brassicae

No. of weeks after soil application (dates)	Time of examination (hours)	Numbers of progeny as percentages of those in control					
		Untreated control		Aldicarb		Menazon	
		young disc	old disc	young disc	old disc	young disc	old disc
8	24	35	24	43.0	33.3	93.0	70.8
22.7.68	48	60	58	0.9	0.0	26.7	17.2
	72	94	126	0.0	0.0	18.0	6.4
9	24	267	22.7	20.0	13.2	68.2	30.4
30.7.68	48	255	311	39.7	1.6	44.3	40.5
	72	216	129	0.0	0.0	38.0	20.2
11	24	185	178	56.7	59.0	100.0	93.3
13.8.68	48	222	239	25.2	15.5	76.5	39.1
	72	259	229	35.1	11.8	47.9	16.2
14	24	193	173	66.3	30.6	66.8	65.3
3.9.68	48	174	150	90.2	34.0	68.4	58.0
	72	161	167	27.1	12.6	61.5	35.9
17	24	179	138	93.3	92.8	39.1	36.2
24.9.68	48	160	129	95.6	93.8	53.1	21.7
	72	209	131	55.0	67.9	45.1	19.0
21	24						
29.10.68	48						
	72						

were usually related to the relative toxicities of both the treatments, menazon and aldicarb. As toxicity decreased with interval after treatment a corresponding increase in numbers of progeny produced was recorded. It was also shown that more progeny were produced by M. persicae on younger than older leaf discs of both treated and untreated plants whereas no marked difference was noted with B. brassicae.

FIELD EXPERIMENTS 1969.

An experimental design of Brussels sprout plants was set up in the field to obtain information on factors which affect absorption, translocation and persistence of the Systemic insecticide aldicarb applied systemically by different methods with and without fertilizer which, it was thought, might attract roots and increase uptake of the insecticide. The rate of application was reduced to one pound active ingredient per acre to make the bioassay more sensitive to effects of possible small differences in toxicity of the different treatments.

INSECTICIDE PLACEMENT METHODS.

A = base of the plant 2-3" deep in the soil

B = base of the plant 2-3" deep + Fertiliser

Table 10 Mean no. of live alatae of aphids per plant found on Brussels sprout plants treated with aldicarb using different methods of application.

Macrosiphum euphorbiae (Thom.)

Treatments	Mean log (n+1) number of live alatae per plant Number of weeks after treatment														
	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20
A	0.03	0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	0.08	0.41	0.07	0.07	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	0.0	0.25	0.0	0.08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	0.12	0.62	0.07	0.13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	0.23	0.62	0.25	0.18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	0.37	0.85	0.52	0.36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Myzus persicae (sulz.)

A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	0.03	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	0.0	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	0.03	0.0	0.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	0.06	0.12	0.05	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	0.06	0.14	0.00	0.13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Brevicoryne brassicae (L.)

A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03	0.29	0.15
B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.04	0.0	0.0	0.28	0.17
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.09	0.24	0.29
D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03	0.06	0.12	0.29	0.25
E	0.0	0.0	0.0	0.08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03	0.22	0.18
F	0.0	0.0	0.0	0.0	0.12	0.05	0.04	0.0	0.0	0.0	0.59	0.63	0.94	1.22	1.15

C = 9"-1' below the plant

D = 9"-1' below + Fertilizer

E = 9"-1' to the side of the plant 2-3" deep in the soil.

F = Untreated.

FORMULATION AND RATE OF APPLICATION.

A - 10% granulated formulation of aldicarb applied at the rate of 1 lb. a - i per acre on 26th June 1969 to Brussels sprout seedlings planted 3 ft. apart. The fertilizer was applied at the rate of 1.2 ounces per square yard (34.05 gms per plant).

CONDITION OF THE PLANTS AT THE TIME OF TREATMENT.

Plants were about 6-9" tall, healthy and well established in the field. Each plant had about 5-6 leaves, weather was sunny and dry making the soil surface also dry but moisture was just below the soil surface. No rain fell until the 4th July.

TREATMENT PROCEDURE.

A soil corer was used to dig soil upto the required depth below or to the side of the plant. A 1½" diameter iron pipe was then inserted and its hole cleared by inserting an iron rod. The

fertilizer and insecticide were then poured down the tube and tapped onto place by means of the iron rod. The pipe was then withdrawn. In the case of B & D treatments, the insecticide was placed by the same method on top of the fertilizer already put in the hole. Each hole was then closed by pouring in loose soil.

EFFECTS OF TREATMENTS ON APHIDS.

A comparison of the effects of the different placement methods was made by counting alatae, apterae including larvae of aphids infesting the crop. As before live alatae were recorded separately but apterae and larvae were counted as a whole because of large numbers and because it was difficult to differentiate between adult apterae and larval instars. Table 10 shows numbers of live alatae present on all plants in each treatment. Immigrant alatae (Figures 9,10 and 11) of M. euphorbiae and M.persicae started colonising the crop just before or soon after the treatment early in July but stopped colonising about 4 weeks after treatment (last week in July) by the time alatae of B. brassicae first arrived. The number of alatae of all aphid species found on the crop was consi-

Fig. No. 9 The effect of different placement methods on the natural populations of aphid larvae and adult apterae on brussels sprouts plants.

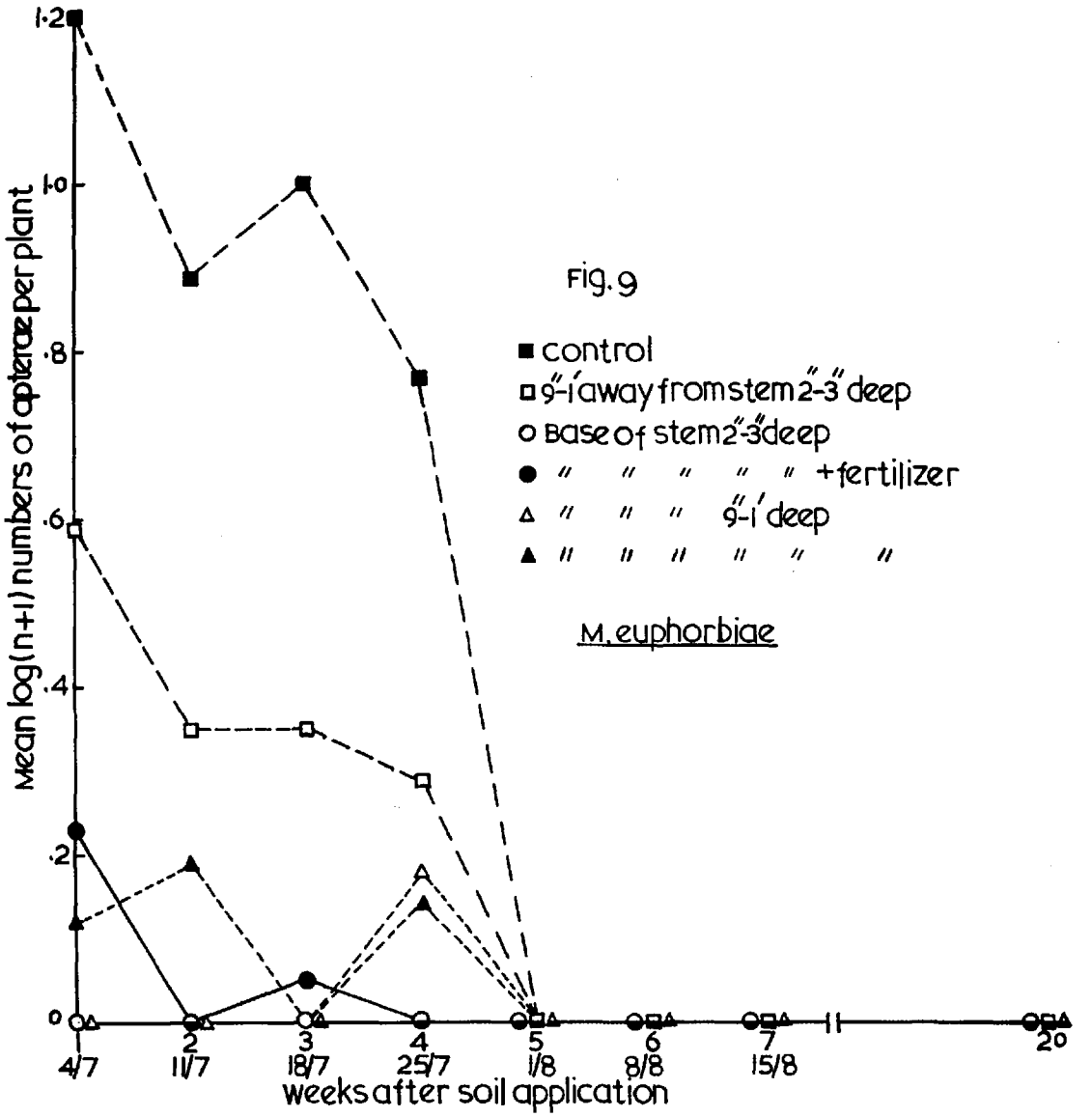

1·2
1·0
·8
·6
·4
·2
0
Mean log(n+1) numbers of apterae per plant
4/7
11/7
18/7
25/7
1/8
8/8
15/8
2
3
4
5
6
7
11
20
weeks after soil application
control
9"-1' away from stem 2"-3" deep
Base of stem 2"-3" deep
" " " " " +fertilizer
" " " 9"-1' deep
" " " " " "
M. euphorbiae

Fig. 9

Fig. No. 10 The effect of different placement methods on the natural populations of aphid larvae and adult apterae on brussels sprouts plants.

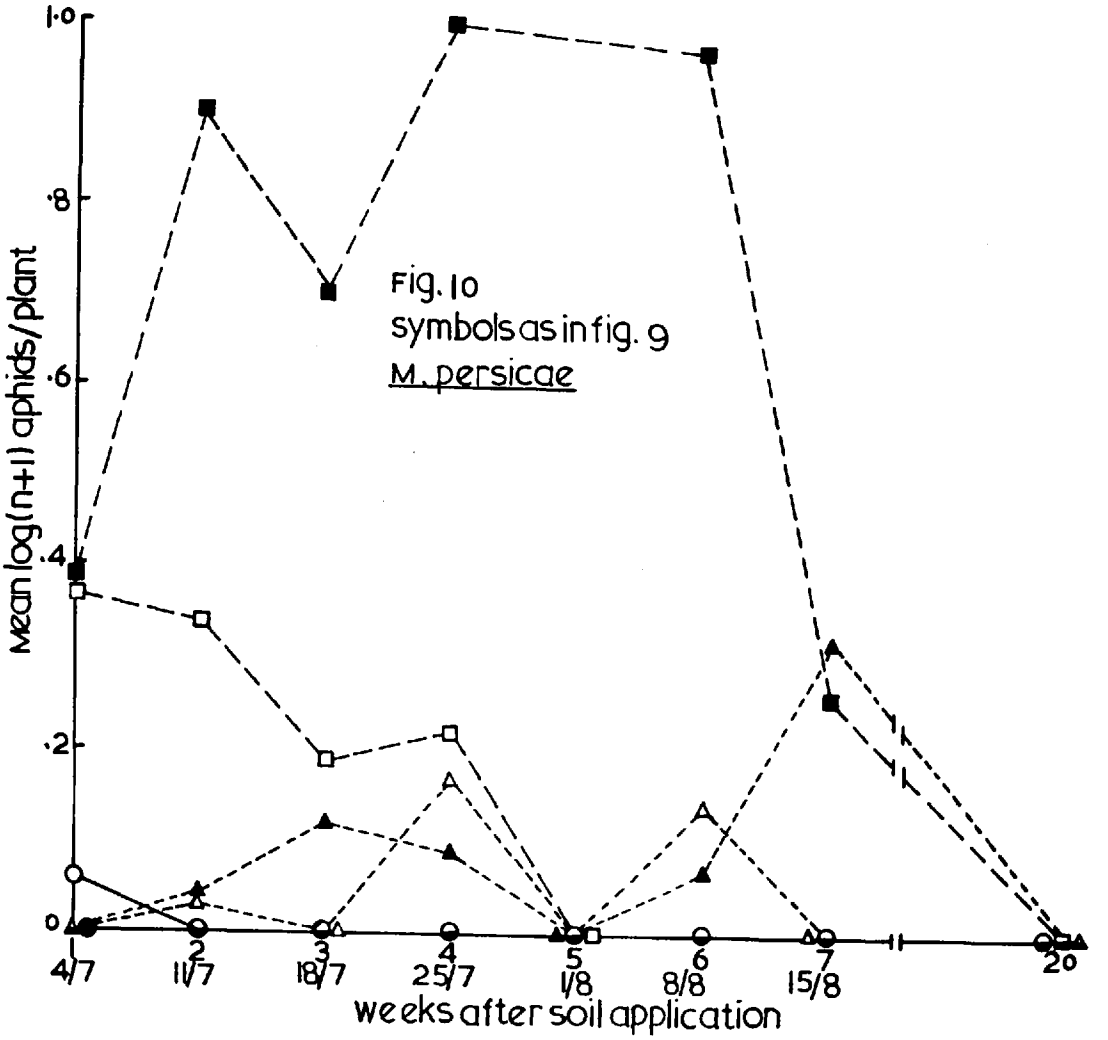


Fig. 10
symbols as in fig. 9
M. persicae

Fig. No. 11 The effect of different placement methods on the natural populations of aphid larvae and adult apterae on brussels sprouts plants.

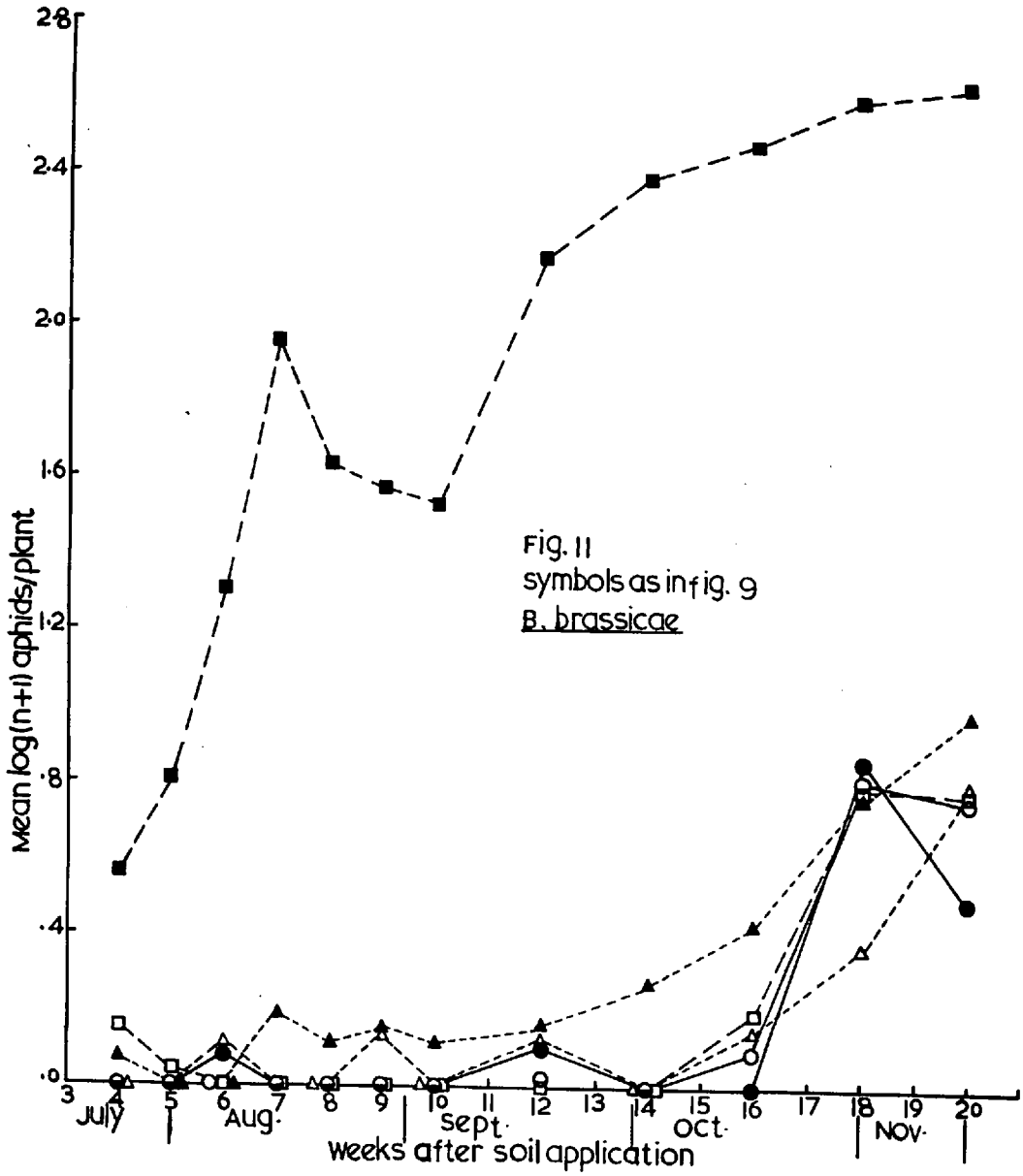


Fig. 11
symbols as in fig. 9
B. brassicae

derably smaller in all treatments than in the control never reaching more than 0.62, 0.12 and 0.11 in mid-July for M. euphorbiae, M. persicae and B. brassicae respectively.

Many alatae of B. brassicae were found on the crop late in the season which were produced by the persisting aphid colonies rather than immigrating in the crop from outside. They were mainly responsible for the enormous build up of B. brassicae populations on the untreated crop towards the end of the season.

M.euphorbiae and M. persicae apterae (Figures 9,10& 11) remained on the plants for 4 and 7 weeks after treatment respectively compared with B. brassicae which arrived later but infested the crop throughout the season. Treatments A, B, C & D kept all aphid species very scarce from the start of the season, but E showed little effectiveness in the beginning when roots were possibly too short to reach the treatment but indicated high efficiency by about 5 weeks after treatment (Ist week in August). The most effective treatments wereA, B & C. The treatment given with fertilizer deep in soil was the least effective (D). The toxicity of C and D treatments were lost comparatively faster

Table 11 Corrected percent mortality of late instar larval apterae of Brevicoryne brassicae on leaf discs from young leaves of Brussels sprout plants treated with granules of aldicarb at the rate of 1lb. a.i. acre using different methods of application.

No.of weeks after soil application (dates)	Time of examination (hours)	Treatment and leaf age					
		Untreated control F	B	E	C	D	A
7 15.8.69	24	0.0	25.0	56.0	40.0	26.0	65.0
	48	0.0	45.0	64.0	50.0	35.0	80.0
	72	0.0	65.0	72.0	60.0	60.0	93.0
8 21.8.69	24	0.0	20.0	46.0	38.0	16.0	56.0
	48	0.0	36.0	56.0	52.0	38.0	68.0
	72	0.0	68.0	70.0	62.0	58.0	86.0
9 28.8.69	24	0.0	16.0	32.0	28.0	14.0	50.0
	48	0.0	28.0	46.0	36.0	30.0	58.0
	72	0.0	56.0	62.0	56.0	48.0	70.0
10 9.9.69	24	8.0	8.7	4.3	13.0	8.7	0.0
	48	8.0	16.0	12.0	19.0	16.0	2.0
	72	8.0	26.0	32.0	22.0	18.0	16.0
11 17.9.69	24	0.0	0.0	0.0	0.0	0.0	0.0
	48	0.0	5.2	3.6	0.0	0.0	0.0
	72	0.0	10.0	18.0	8.0	6.0	2.0

Table 12 Corrected percent mortality of late instar larval apterae of Myzus persicae on leaf discs from young leaves of Brussels sprout plants treated with aldicarb using different methods of application at 20C.

No. of weeks after soil application (dates)	Time of examination (hours)	Treatment and leaf age					
		Untreated control F	B	E	C	D	A
4 25.7.69	24	0.0	66.0	58.0	60.0	10.0	36.0
	48	0.0	80.0	92.0	86.0	20.0	50.0
	72	0.0	90.0	94.0	92.0	26.0	58.0
5 31.7.69	24	0.0	30.0	70.0	14.0	24.0	18.0
	48	0.0	32.0	76.0	22.0	36.0	38.0
	72	0.0	46.0	80.0	22.0	38.0	46.0
6 7.8.69	24	0.0	64.0	30.0	10.0	6.0	12.0
	48	2.0	69.0	40.0	14.0	6.0	26.0
	72	4.0	77.0	40.0	14.0	6.0	26.0
7 15.8.69	24	0.0	15.0	10.0	2.0	0.0	7.0
	48	0.0	17.5	30.0	12.5	10.0	23.0
	72	0.0	30.0	56.0	15.0	10.0	30.0
8 21.8.69	24	0.0	35.0	6.0	0.0	4.0	4.0
	48	0.0	40.0	16.0	0.0	8.0	16.0
	72	0.0	62.0	22.0	2.0	8.0	22.0
9 28.8.69	24	0.0	16.0	6.0	4.0	2.0	4.0
	48	0.0	28.0	18.0	4.0	4.0	4.0
	72	4.0	36.0	18.0	4.0	4.0	6.0
10 8.9.69	24	0.0	0.0	0.0	0.0	0.0	0.0
	48	0.0	0.0	0.0	0.0	0.0	0.0
	72	0.0	18.0	12.0	0.0	2.0	2.0

Table 13 Percent mortality of late instar larval apterae of Myzus persicae on leaf discs from old leaves of Brussels sprout plants treated with granules of aldicarb at the rate of 1lb./acre using different methods of application (Abbott's correction has been applied.)

No. of weeks after soil application (dates)	Time of examination (hours)	Treatment and leaf age: Untreated control F	B	E	C	D	A
2	24	0.0	54.0	2.0	98.0	60.0	84.0
10.7.69	48	0.0	66.0	14.0	100.0	86.0	92.0
	72	0.0	78.0	34.0	100.0	94.0	100.0
3	24	0.0	98.0	34.0	80.0	74.0	90.0
17.7.69	48	0.0	100.0	62.0	100.0	86.0	100.0
	72	0.0	100.0	68.0	100.0	92.0	100.0
4	24	0.0	100.0	98.0	100.0	94.0	100.0
25.7.69	48	0.0	100.0	100.0	100.0	96.0	100.0
	72	0.0	100.0	100.0	100.0	96.00	100.0
5	24	0.0	80.0	86.0	98.0	32.0	60.0
31.7.69	48	2.0	89.0	100.0	100.0	59.0	91.0
	72	2.0	91.0	100.0	100.0	60.0	93.0
6	24	0.0	78.0	48.0	36.0	16.0	54.0
7.8.69	48	0.0	100.0	68.0	60.0	42.0	80.0
	72	4.0	100.0	69.0	64.0	42.0	88.0
7	24	0.0	46.0	58.0	46.0	22.0	28.0
15.8.69	48	0.0	58.0	66.0	52.0	46.0	84.0
	72	0.0	66.0	72.0	62.0	62.0	88.0
8	24	0.0	48.0	66.0	58.0	34.0	32.0
21.8.69	48	0.0	58.0	78.0	68.0	44.0	50.0
	72	0.0	72.0	84.0	72.0	56.0	78.0
9	24	0.0	56.0	44.0	36.0	12.0	4.0
28.8.69	48	4.0	57.0	72.0	42.0	40.0	16.0
	72	6.0	60.0	74.0	44.0	40.0	22.0
10	24	0.0	19.0	30.0	20.0	0.0	12.0
9.9.69	48	0.0	38.0	45.0	40.0	16.0	28.0
	72	0.0	57.0	65.0	40.0	20.0	36.0

Table 14 Percent mortality of late instar larval apterae of Brevicoryne brassicae on leaf discs from old leaves of Brussels sprout plants treated with granules of aldicarb at the rate of 1lb./acre using different methods of application (Abbott's correction has been applied.)

No. of weeks after soil application (dates)	Time of examination (hours)	Treatment and leaf age					
		Untreated control F	B	E	C	D	A
7	24	0.0	48.0	64.0	72.0	76.0	96.0
15.8.69	48	0.0	80.0	80.0	80.0	88.0	100.0
	72	0.0	92.0	88.0	80.0	96.0	100.0
8	24	0.0	40.0	60.0	62.0	66.0	80.0
21.8.69	48	0.0	76.0	78.0	76.0	82.0	88.0
	72	2.0	90.0	84.0	82.0	90.0	92.0
9	24	0.0	70.0	70.0	44.0	28.0	8.0
28.8.69	48	0.0	80.0	75.0	60.0	60.0	62.0
	72	4.0	86.0	80.0	61.0	65.0	62.0
10	24	0.0	40.0	42.0	24.0	24.0	32.0
9.9.69	48	0.0	66.6	62.0	36.0	40.0	50.0
	72	2.0	80.0	80.0	41.0	58.0	52.0
11	24	5.0	10.0	18.0	12.0	19.0	6.0
17.9.69	48	10.0	50.0	60.0	22.0	32.0	24.0
	72	12.0	58.0	71.0	34.0	36.0	30.0
12	24	0.0	2.0	14.0	2.0	4.0	2.0
25.9.69	48	0.0	14.0	32.0	4.0	10.0	2.0
	72	0.0	20.0	32.0	10.0	14.0	2.0
13	24	0.0	0.0	4.0	2.0	0.0	0.0
4.10.69	48	0.0	8.0	14.0	4.0	4.0	0.0
	72	0.0	8.0	18.0	6.0	10.0	4.0
15	24	0.0	4.0	10.0	4.0	0.0	6.0
21.10.69	48	0.0	4.0	18.0	10.0	0.0	10.0
	72	0.0	12.0	28.0	16.0	0.0	12.0
18	24	0.0	4.0	0.0	6.0	4.0	0.0
18.11.69	48	0.0	6.0	0.0	6.0	8.0	0.0
	72	0.0	6.0	0.0	8.0	10.0	2.0

than others. All treatments seem to have lost their toxicity by about 20 weeks after treatment. The order of toxicity was A > B > C > E > D and the order of aphid susceptibility to each treatment was B.brassicae > M. persicae > M.euphorbiae.

LEAF DISC REMOVAL EXPERIMENTS.

The evaluation of uptake, persistence and degradation of toxicity of the insecticide was made by exposing aphids on leaf discs removed from two randomly selected treated plants per plot and kept under standardised conditions at 20°C. Mortalities of late instar larval apterae of M.persicae and B. brassicae kept on leaf discs from young and old leaves of treated plants for 72 hours were corrected for control mortalities of aphids on untreated plants. Treatments A, C and D (Fig. 12)killed almost all M.persicae on old leaf discs 2 weeks after treatment compared with B and E which killed 78% and 34% respectively. Toxicity increased gradually to its highest level killing almost 100% and 26 - 94% of M. persicae in all treatments on old and young discs respectively by about 4 weeks after treatment (4th week in July). Decline in toxicity started

Fig. No. 12 Persistence of activity of soil applied systemic aldicarb applied at the rate of 1 lb a.i./acre, as shown by kills of late instars larval apterae of Myzus persicae kept on excised leaf discs for 72 hours at 20°C from young and old leaves of brussels sprouts plants.

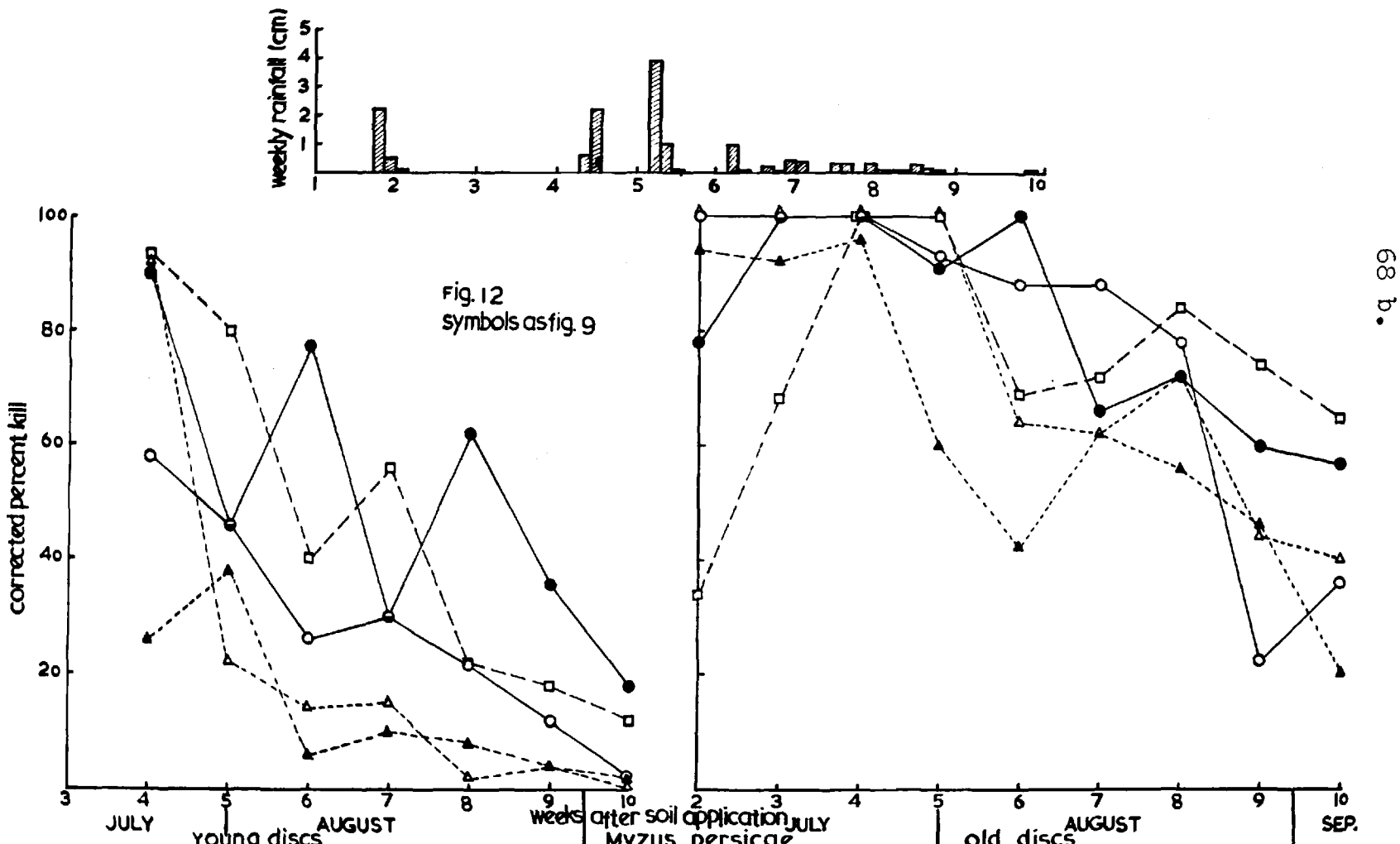


Fig. 12
symbols as fig. 9

Fig. No. 13 Persistence of activity of 1 lb/acre of aldicarb applied about two weeks after transplanting, as shown by kills of Brevicoryne brasicae kept on excised leaf discs from young and old leaves of brussels sprouts plants for 72 hours at 20°C.

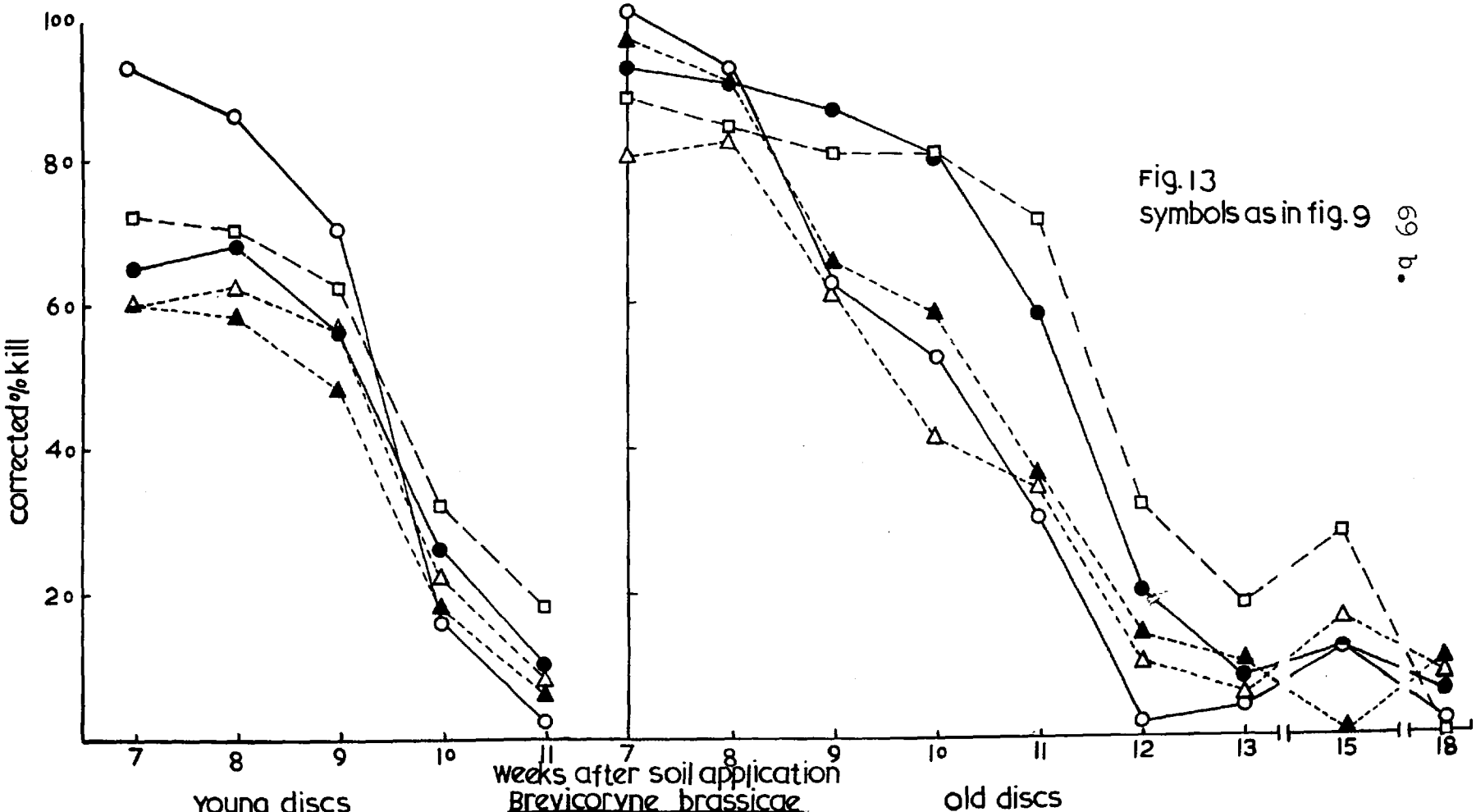


Fig. 13
symbols as in fig. 9

69 b.

first in treatment D (deep treatment + fertilizer). (Tables 11 & 12) show#that young leaves were the first to lose their toxicity (by about 9-10 weeks weeks after treatment), compared with older leaves which showed longer persistence and gave more consistent results with both M. persicae and B. brassicae. Treatment A, B and C (tables 13&14, Fig13) gave 70 - 80% kill of M. persicae and 90% of B. brassicae on old leaf discs upto 8 weeks after treatment. Treatment E killed almost 65% M. persicae and 71% of B. brassicae on old leaves upto 10 and 11 weeks after treatment which probably was the most persistent treatment compared with b which killed 58% of M. persicae and B. brassicae upto 10 and 11 weeks after treatment. Toxicity seemed to have been lost competely by about 18 weeks after treatment (mid-November). Fertilizer treatments (B and D) did not show any marked difference in their toxicity from treatments without fertilizer (A and C).

SLEEVE TESTS.

The method was the same as already described in field experiment 1968 (page 44). The numbers of dead aphids in each treatment after one week (table 15 & 16) show that all treatments

Table 15 Corrected percent kill of late instar larval apterae sleeved on young leaves for a week in the field.

Myzus persicae

Time of examination (dates)	No. of weeks after soil application	Treatment and leaf age Untreated control					
		F	B	E	C	D	A
7.8.69	6	4.0	96.0	100.0	96.0	83.0	100.0
21.8.69	8	6.0	80.0	50.0	66.0	46.0	76.0
11.9.69	11	6.0	14.0	24.0	29.0	42.0	8.0
25.9.69	13	2.0	8.0	12.0	10.0	15.0	2.0
		Brevicoryne brassicae					
11.9.69	11	10.0	59.0	40.0	48.0	84.0	18.0
25.9.69	13	10.0	14.0	33.0	3.0	33.0	17.0
9.10.69	15	5.0	4.0	22.0	6.0	12.0	8.0

Table 16 Corrected percent mortality of late instar larval apterae sleeved on old leaves for a week in the field.

Myzus persicae

Time of examination (date)	No. of weeks after soil application	Treatment and leaf age					
		Untreated control					
		F	B	E	C	D	A
24.7.69	4	4.0	100.0	43.0	100.0	83.0	100.0
7.8.69	6	6.0	100.0	100.0	100.0	95.0	100.0
21.8.69	8	2.0	92.0	86.0	100.0	76.0	96.0
11.9.69	11	6.0	78.0	85.0	50.0	60.0	61.0
25.9.69	13	0.0	18.0	30.0	22.0	26.0	10.0

Brevicoryne brassicae

11.9.69	11	13.0	86.0	92.0	72.0	100.0	95.0
25.9.69	13	16.0	59.0	71.0	11.0	67.0	47.0
9.10.69	15	6.0	28.0	36.0	8.0	18.0	20.0

Fig. No. 14 Persistence of activity of aldicarb applied at 1 lb/acre, as shown by kills of Myzus persicae sleeved for a week in the field on young and old leaves of brussels sprouts plants.

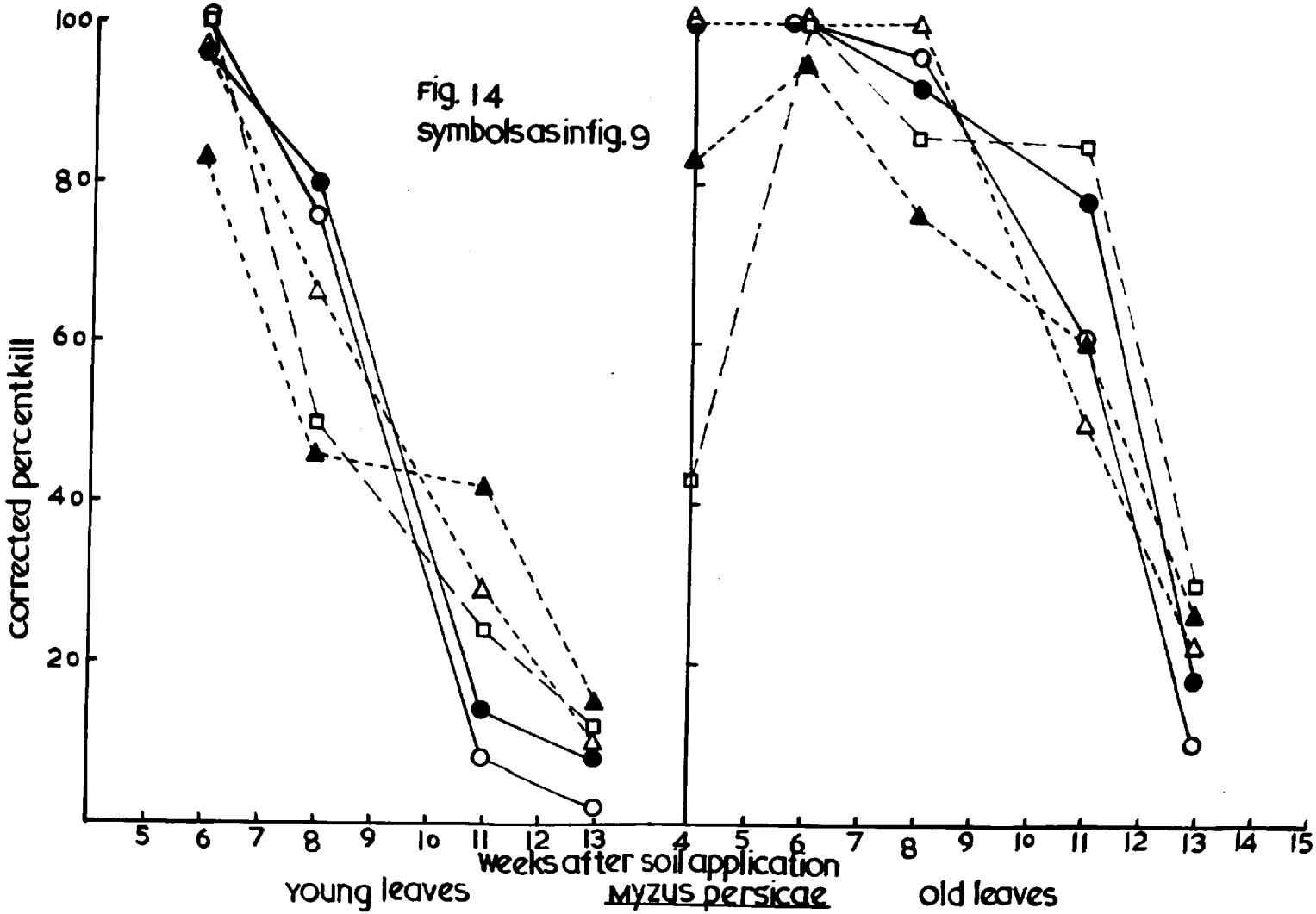


Fig. 14
symbols as in fig. 9
corrected percent kill
100
80
60
40
20
5 6 7 8 9 10 11 12 13
4 5 6 7 8 9 10 11 12 13 14 15
weeks after soil application
young leaves
Myzus persicae
old leaves

killed 83 - 100% and 95 - 100% of M. persicae 6 weeks after treatment on young and old leaves respectively and continued to kill 50 - 78% on old leaves after 11 weeks (Fig.14). B. brassicae being more susceptible as shown earlier (page 47) were used later to assess the toxicity i.e. at a time when M. persicae might no longer be much affected. The results (tables 15 & 16) show that all treatments killed about 18 - 84% and 70 - 100% B. brassicae upto 11 weeks after treatment on young and old leaves respectively. More than 60% of B.brassicae were killed in treatments B, D and E on old leaves upto 13 weeks after treatment compared with M. persicae where kill did not rise above 30% at 13 weeks.

Laboratory experiments

Laboratory bioassays were done to compare the systemic action of the four organophosphorus and carbamate insecticides on the two aphid species at different temperatures. Leaf discs were treated with different dosages of insecticide and then kept in constant environmental conditions either at 15°C or 20°C or 25°C. Laboratory reared adult apterae of M. persicae and B. brassicae were separately exposed on treated leaf discs (Galley 1968) and mortalities were recorded at regular intervals of 24 hours and continued up to 72 hours after treatment. In some experiments at 15°C and 25°C mortalities were also recorded 12 hours after treatment and continued up to the time no obvious mortalities occurred or as long as leaf discs did not wilt. Dosage mortality curves were obtained with the range of known concentrations of insecticides and were analysed by the method of probits (Finney, 1952). Typical examples of probit lines are shown in figures 15&16 and 17&18. From the calculated lines it is possible to read off the doses of insecticide corresponding to the kill obtained in the unknowns, as shown in the figures described above. In practice the estimate of concentration of the unknown was calculated from the formula:-

Fig. No. 16 Action of menazon on Myzus persicae through wick uptake at 20°C.

24, 48 and 72 hours Probit lines

Menazon at 0.934%, 0.26%, 0.0043%, 0.0007%

Fig. No. 15 Action of menazon on Brevicoryne brassicae by wick application at 20°C.

24, 48 and 72 hours Probit lines

Menazon at 0.0043%, 0.0007%, 0.00012%, 0.00002%, 0.000003%

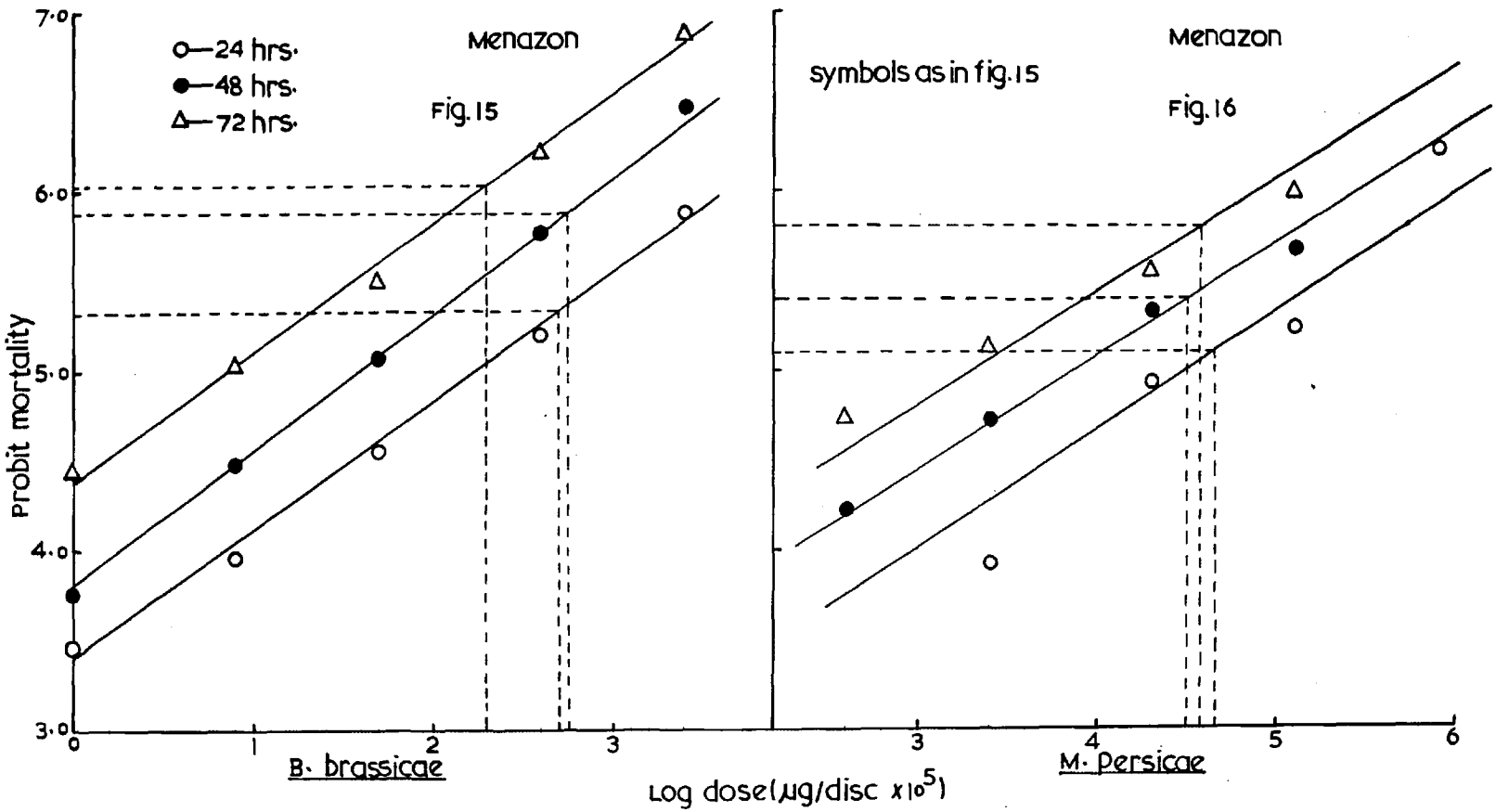

7·0
6·0
5·0
4·0
3·0
Probit mortality
o—24 hrs.
●—48 hrs.
Δ—72 hrs.
Menazon
Fig. 15
0
1
2
3
B. brassicae
Menazon
symbols as in fig. 15
Fig. 16
3
4
5
6
M. persicae
Log dose(µg/disc x10⁵)

Fig. No. 18 Action of aldicarb on Myzus persicae through wick uptake at 20°C.

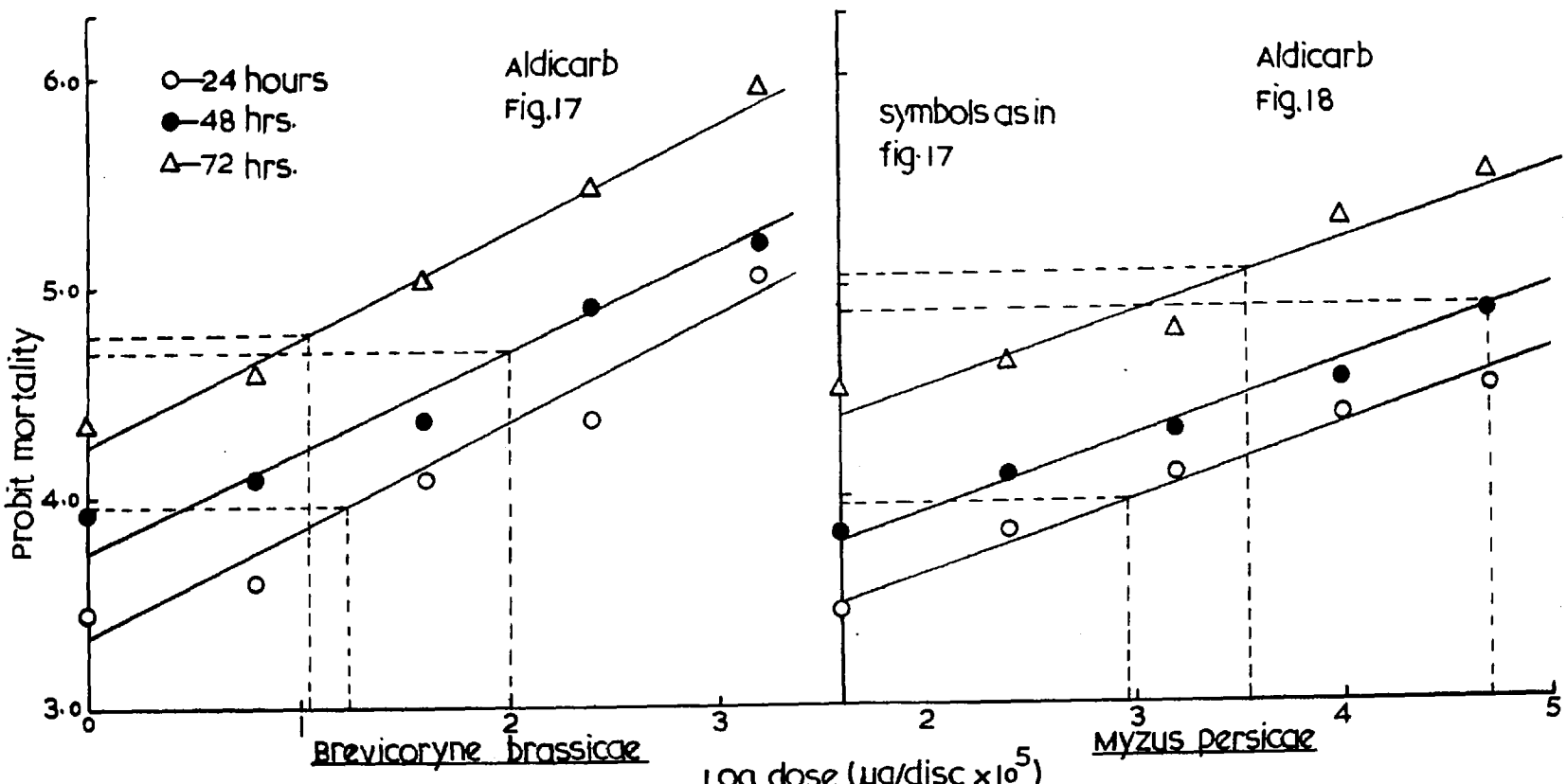
24, 48 and 72 hours Probit lines

Aldicarb at 0.05%, 0.0083%, 0.0014%, 0.00023%, 0.00004%

Fig. No. 17 Action of aldicarb on Brevicoryne brassicae through wick uptake at 20°C.

24, 48 and 72 hours Probit lines

Aldicarb at 0.0014%, 0.00023%, 0.000007%, 0.0000011%


o—24 hours
●—48 hrs.
Δ—72 hrs.
Aldicarb
Fig.17
symbols as in
fig·17
Aldicarb
Fig.18
Probit mortality
6·0
5·0
4·0
3·0
0
2
3
4
5
Brevicoryne brassicae
Myzus persicae
Log dose (µg/disc x 10^5)

$$\text{concentration} = \text{Antilog } 10 \frac{y-5}{b} + m$$

where y = corrected probit kill of the test aphid.

b = slope of regression line calculated from known concentrations of insecticide.

m = LD50 calculated from the regression line.

Some sources of error were evident when calculating the unknown concentration, firstly the error of position and slope that may be found on the standard regression line and secondly the error in determining the kill of aphids caused by the unknown sample. An approximate estimate of standard error from an unknown (i.e. a percentage kill based on either 24, 48 or 72 hours counts) was calculated from the formula

$$\frac{1}{b} \sqrt{\frac{1}{n_o w_o} + \frac{1}{n_1 w_o} + \frac{(x-\bar{x})^2}{Sxx}}$$

where b = slope of regression line calculated from known concentrations of insecticide.

n_o = numbers of insects from sample to give estimated ratio.

n_1 = number of insects tested at each known concentration.

w_o weighting coefficient corresponding to no_o or n1.

x = log 10 concentration.

$\bar{x}$ = weighted mean of log 10 concentration.

sxx = estimates of weighted sums of squares of x.

The object of the first five bioassay experiments was to find the range of concentrations of aphicides affecting M. persicae and B. brassicae, their relative susceptibility and the reproducibility of the results, 'a' and 'b' of the following bioassays were done simultaneously.

Bioassay no. 1

a M. persicae (adult apterae) on leaf discs treated with menazon at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	2.99 ± 0.085	2.6 ± 0.09	2.18 ± 0.106
Formula of the line	0.33 + 1.2x	1.0 + 1.1x	1.47 + 1.11x
Slope + S.E.	1.17 ± 0.113	1.11 ± .02	1.11 ± .12
Chi. sq.	1.92	2.11	2.23
D.F.	3	3	3

b B. brassicae (adult apterae) on leaf discs treated with menazon at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	1.66 ± 0.164	0.87 ± 0.14	1.80 ± 0.182
Formula of the line	3.47 + 0.53x	3.84 + 0.62x	4.51 + 70x
Slope + S.E.	0.53 ± 0.084	0.62 ± .12	0.696 ± .01
Chi. sq.	2.28	3.52	5.7
D.F.	3	3	3

Bioassay no. 2

a *M. persicae* on leaf discs treated with menazon at 20°C to show reproducibility of results.

	24 hours	48 hours	72 hours
LD50 + S.E.	3.61 ± 0.103	2.7 ± 0.13	2.17 ± 0.199
Formula of the line	1.08 + 0.85x	2.27 + 0.72x	3.03 + 0.62x
Slope + S.E.	0.85 ± .06	0.72 ± .05	.62 ± .02
Chi. sq.	2.1	2.32	1.93
D.F.	3	3	3

b *B. brassicae* on leaf discs treated with menazon at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	1.28 ± 0.144	0.60 ± 0.13	1.85 ± 0.165
Formula of the line	3.4 + 0.7x	3.75 + 0.78x	4.41 + 0.69x
Slope + S.E.	0.703 ± .12	.78 ± 0.03	0.694 0.05
Chi. sq.	1.98	2.11	3.44
D.F.	3	3	3

Bioassay no. 3

a *M. persicae* on leaf discs treated with pirimicarb at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	2.7 ± 0.01	1.7 ± 0.11	1.2 ± 0.15
Formula of the line	1.19 + 0.9x	1.36 + .99x	1.98 + 0.96x
Slope + S.E.	0.9 ± 0.04	0.99 ± 0.13	0.96 ± 0.12
Chi. sq.	6.42	2.07	3.65
D.F.	3	3	3

b *B. brassicae* on leaf discs treated with pirimicarb at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	1.44 ± .09	1.9 ± 0.06	1.34 ± 0.24
Formula of the line	1.56 + 0.99x	3.82 + 0.63x	4.03 + 0.73x
Slope + S.E.	0.99 ± 0.07	0.63 ± 0.05	0.73 ± 0.09
Chi. sq.	2.06	3.39	1.41
D.F.	3	3	3

Bioassay no. 4

a *M. persicae* on leaf discs treated with aldicarb at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	4.9 ± 0.48	4.02 ± 0.49	2.5 ± 0.25
Formula of the line	3.01 + 0.33x	3.3 + 0.32x	3.83 + 0.35x
Slope + S.E.	0.33 ± 0.11	0.32 ± 0.07	0.35 ± 0.07
Chi. sq.	2.20	0.14	1.56
D.F.	3	3	3

b *B. brassicae* on leaf discs treated with aldicarb at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	2.6 ± 0.33	1.6 ± 0.26	0.5 ± 0.18
Formula of the line	3.27 + 0.52x	3.84 + 0.41x	4.26 + 0.51x
Slope + S.E.	0.52 ± 0.02	0.41 ± 0.13	0.51 ± 0.14
Chi. sq.	1.51	1.34	0.44
D.F.	3	3	3

Bioassay no. 5

a *M. persicae* on leaf discs treated with phorate at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	4.1 ± 0.08	3.3 ± 0.07	2.3 ± 0.11
Formula of the line	2.38 + 1.45x	-1 + 1.39x	1.56 + .98x
Slope + S.E.	1.45 ± 0.13	1.39 ± 0.01	0.98 ± 0.05
Chi. sq.	1.7	2.11	4.99
D.F.	3	3	3

b *B. brassicae* on leaf discs treated with phorate at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	3.7 ± 0.19	2.7 ± 0.12	1.7 ± 0.17
Formula of the line	1.32 + 0.75x	2.22 + 0.78x	3.5 + 0.54x
Slope + S.E.	0.75 ± 0.01	0.78 ± 0.06	0.54 ± 0.11
Chi. sq.	0.42	3.98	0.40
D.F.	3	3	3

Relative toxicities of insecticides to the two aphid species at 20°C.

In bioassay experiment no. 1 the estimated LD50 values of menazon after 72 hours were 0.0015% and 0. 0.0000063% for M. persicae and B. brassicae respectively and with a fiducial probability of 95%, the true values for the median lethal dose would be expected to lie between 0.0025% and 0.00091% for M. persicae and 0.000014% and 0.0000028% for B. brassicae. In bioassay no. 2 i kills of aphids 72 hours after treatment were very similar to those of bioassay no. 1 (LC50's 2.17 and T 85 for M. persicae and B. brassicae respectively) thus showing the reproducibility of the results under similar laboratory conditions (compare bioassay 1 and 2). The concentrations required to get a probit line for pirimicarb were about 9 times less than for menazon and in experiment 3, 72 hour kills rose to about 94-100% in the top three doses (Appendix table 1). Aldicarb surprisingly in view of field results was less effective than pirimicarb, menazon and phorate.

The relative resistance of the two aphid species remained different. Thus LC50's indicated that at 20°C after 72 hours M. persicae was about 4 times more resistant than B. brassicae to phorate, about 65 times to pirimicarb, about 100 times to aldicarb and 190 times to menazon.

Bioassay no. 6

a *M. persicae* on leaf discs treated with menazon at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	2.98 ± 0.09	2.50 ± 0.14	2.18 ± 0.12
Formula of the line	2.1 ± 0.97x	2.65 ± 0.94	2.60 ± 1.07x
Slope + S.E.	0.97 ±	0.94 ±	1.07 ±
Chi. sq.	2.01	1.93	2.14
D.F.	3	3	3

b *M. persicae* on leaf discs treated with pirimicarb at 20°C.

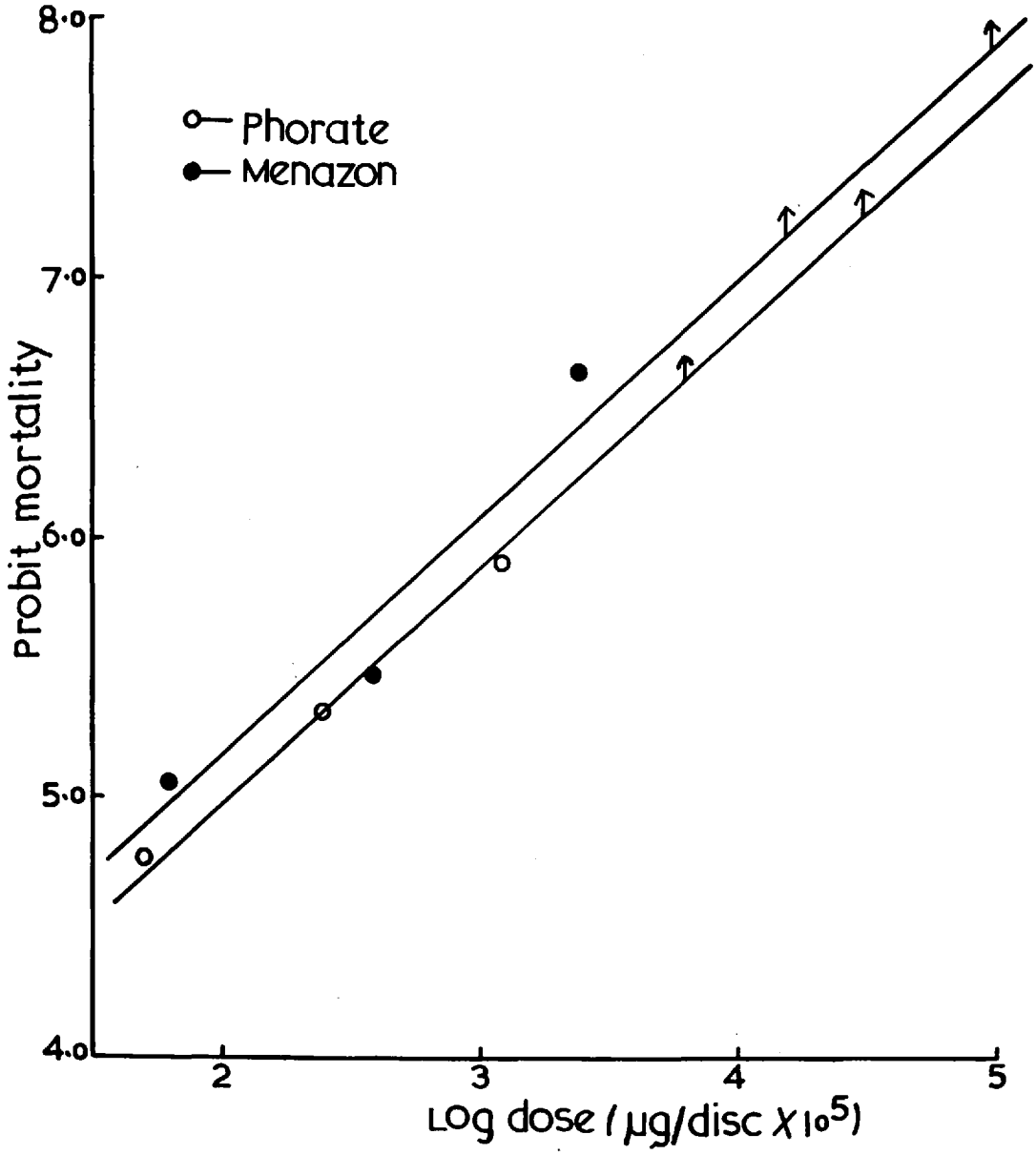
	24 hours	48 hours	72 hours
LD50 + S.E.	2.73 ± 0.08	1.32 ± 0.30	1.18 ± 0.34
Formula of the line	1.8 ± 1.2x	3.85 ± 0.87x	3.89 ± 0.93x
Slope + S.E.	1.19 0.09	0.87 ± 0.10	0.93 ± 0.04
Chi. sq.	2.49	1.79	2.23
D.F.	3	3	3

Bioassay no. 7

a *M. persicae* on leaf discs treated with menazon at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	2.91 ± 0.1	2.30 ± 0.15	2.0 ± 0.15
Formula of the line	2.44 ± 0.95x	3.50 ± 0.78x	3.17 ± 0.98x
Slope + S.E.	0.95 ± 0.04	0.78 ± 0.02	0.98 ± 0.03
Chi. sq.	0.48	2.35	6.66
D.F.	3	3	3

Fig. No. 19 Dose-response regression lines for Myzus persicae to the systemic action of menazon and phorate at 20°C.


8·0
7·0
6·0
5·0
4·0
Probit mortality
Phorate
Menazon
2
3
4
5
Log dose (µg/disc X 10^5)

b M. persicae on leaf discs treated with phorate at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	3.99 ± 0.02	2.99 ± 0.03	2.22 ± 0.11
Formula of the line	0.34 + 1.41x	1.92 + 1.16x	2.71 + 1.12x
Slope + S.E.	1.41 ± 0.01	1.16 ± 0.08	1.12 ± 0.07
Chi. sq.	14.84	5.3	0.96
D.F.	3	3	3

Bioassay no. 8

a M. persicae on leaf discs treated with menazon at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	3.27 ± 0.06	2.32 ± 0.13	2.10 ± 0.15
Formula of the line	0.05 + 1.51x	2.94 + 0.89x	2.88 + 1.01x
Slope + S.E.	1.51 ± 0.04	0.89 ± 0.05	1.01 ± 0.05
Chi. sq.	1.79	0.79	2.11
D.F.	3	3	3

b M. persicae on leaf discs treated with aldicarb at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	4.95 ± 0.43	4.00 ± 0.49	2.55 + 0.19
Formula of the line	2.01 + 0.62x	3.68 + 6.33x	3.9 + 0.43x
Slope + S.E.	0.62 ± 0.06	0.33 ± 0.13	0.43 ± 0.11
Chi. sq.	1.19	2.17	2.62
D.F.	3	3	3

Bioassay no. 9

a *M. persicae* on leaf discs treated with phorate at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	4.08 ± 0.1	3.25 ± 0.08	2.24 ± 0.12
Formula of the line	1.17 + 1.07x	2.07 + 1.03x	3.03 + 0.91x
Slope + S.E.	1.07 ± 0.04	1.03 ± 0.01	0.91 ± 0.07
Chi. sq.	3.48	7.57	5.34
D.F.	3	3	3

b *M. persicae* on leaf discs treated with aldicarb at 20°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	5.00 ± 0.48	4.02 ± 0.26	2.50 ± 0.21
Formula of the line	2.27 + 0.59x	2.7 + 0.63x	4.04 + 0.4x
Slope + S.E.	0.59 ± 0.02	0.63 ± 0.11	0.4 ± 0.11
Chi. sq.	3.25	2.16	3.49
D.F.	3	3	3

On the basis of LC50's after 72 hours at 20°C, relative toxicities of insecticides to *M. persicae* are: pirimicarb > menazon > phorate > aldicarb. Based on LC50's (Appendix 2) pirimicarb was about 10 times more toxic than menazon, about 13 times more toxic than phorate and about 26 times more toxic than aldicarb.

Table 17 Effect of time interval after treatment on the order of toxicity of insecticides against M. persicae at 20°C.

Aphid species	Time interval (Hours)	Insecticides
M. persicae	24	pirimicarb 1.78 > menazon 11.8 > phorate 3.7 > aldicarb
	48	pirimicarb 14 > menazon 4.6 > phorate 10 > aldicarb
	72	pirimicarb 10 > menazon 1.3 > phorate 2 > aldicarb

The results after 24, 48 and 72 hours (table 17) indicate no change in the order of toxicity, 72 hour kills were more consistant than 24 hours e.g. in five experiments (Bioassay nos. 1 - 5) the LC50 of menazon for M. persicae in ppm of leaf ranged from 0.30 - 0.38 after 72 hours compared with 2.03 - 10.0 after 24 hours.

The data in appendix table 2 was used to calculate the order of toxicity of insecticides to B. brassicae at 20°C (table 18).

pirimicarb > menazon > aldicarb > phorate

Table 17 Effect of time interval after treatment on the order of toxicity of insecticides against M. persicae at 20°C.

Aphid species	Time interval (Hours)	Insecticides
M. persicae	24	pirimicarb 1.78 > menazon 11.8 > phorate 3.7 > aldicarb
	48	pirimicarb 14 > menazon 4.6 > phorate 10 > aldicarb
	72	pirimicarb 10 > menazon 1.3 > phorate 2 > aldicarb

The results after 24, 48 and 72 hours (table 17) indicate no change in the order of toxicity, 72 hour kills were more consistant than 24 hours e.g. in five experiments (Bioassay nos. 1 - 5) the LC50 of menazon for M. persicae in ppm of leaf ranged from 0.30 - 0.38 after 72 hours compared with 2.03 - 10.0 after 24 hours.

appendix

The data in table 2 was used to calculate the order of toxicity of insecticides to B. brassicae at 20°C (table 18).

pirimicarb > menazon > aldicarb > phorate

Table 18 The order of toxicity of insecticides to *B. brassicae* at 20°C.

Aphid species	Time of examination (Hours)	Insecticides
B. brassicae	24	pirimicarb 1.3 > menazon 5.8 > aldicarb 20.2 > phorate
	48	pirimicarb 2 > menazon 8.6 > aldicarb 6 > phorate
	72	pirimicarb 3.7 > menazon 5.2 > aldicarb 13 > phorate

Bioassay no. 10

a M. persicae on leaf discs treated with pirimicarb at 15°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	2.83 ± 0.09	2.3 ± 0.09	1.98 ± 0.07
Formula of the line	0.08 + 1.09	-0.32 + 1.24x	-3.18 + 1.95x
Slope + S.E.	0.1.09 ± 0.12	1.24 ± 0.01	0.1.95 ± 0.13
Chi. sq.	3.42	1.04	4.52
D.F.	3	3	3

b M. persicae on leaf discs treated with pirimicarb at 25°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	1.40 ± 0.09	1.8 ± 0.08	1.2 ± 0.23
Formula of the line	2.09 + 0.98x	1.61 + 1.56x	3.69 + 0.94x
Slope + S.E.	0.98 ± 0.03	1.56 ± 0.05	0.94 ± 0.08
Chi. sq.	12.71	6.25	5.31
D.F.	3	3	3

Bioassay no. 11

a B. brassicae on leaf discs treated with pirimicarb at 15°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	1.66 ± 0.08	1.33 ± 0.09	1.06 ± 0.04
Formula of the line	0.97 + 1.09x	1.68 + 1.x	2.37 + 0.88x
Slope + S.E.	1.09 ± 0.01	1.0 ± 0.03	0.88 ± 0.03
Chi. sq.	8.13	6.39	6.25
D.F.	3	3	3

b B. brassicae on leaf discs treated with pirimicarb at 25°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	1.1 ± 0.12	2.8 ± 0.32	2.3 ± 0.32
Formula of the line	3.42 ± 0.47x	4.77 ± 0.44x	4.68 ± 0.54x
Slope + S.E.	0.91 ± 0.04	0.44 ± 0.05	0.54 ± 0.02
Chi. sq.	0.84	1.12	2.05
D.F.	3	3	3

Bioassay no. 12

a M. persicae on leaf disc treated with aldicarb at 15°C.

	24 hours	48 hours	72 hours
LD50 + S.E.	5.51 ± 0.46	4.64 ± 0.23	3.5 ± 0.26
Formula of the line	0.18 ± 0.74x	1.95 ± 0.54x	2.63 ± 0.53x
Slope + S.E.	0.74 ± 0.11	0.54 ± 0.05	0.53 ± 0.11
Chi. sq.	0.33	0.79	1.38
D.F.	3	3	3

b M. persicae on leaf disc treated with aldicarb at 25°C.

LD50 + S.E.	4.6 ± 0.64	2.10 ± 0.30	1.26 ± 0.77
Formula of the line	3.14 ± 0.33x	4.13 ± 0.28x	4.9 ± 0.39x
Slope + S.E.	0.33 ± 0.07	0.28 ± 0.12	0.39 ± 0.06
Chi. sq.	0.34	12.23	7.54
D.F.	3	3	3

Bioassay no. 13

a M. persicae on leaf disc treated with aldicarb at 15°C.

	24hours	48hours	72hours
LD50 + S.E.	5.19±0.34	4.52±0.21	3.48±0.24
Formula of the line	2.84+0.83x	3.26+0.79x	4.29+0.73x
Slope + S.E.	0.83±0.10	0.79±0.13	0.73±0.05
Chi. sq.	2.39	2.13	2.79
D.F.	3	3	3

b M. persicae on leaf disc treated with aldicarb at 25°C.

LD50 + S.E.	4.50±0.29	1.84±0.31	1.29±0.56
Formula of the line	2.13+1.7x	3.25+0.33x	2.94+0.39x
Slope + S.E.	0.37±0.11	0.33±0.05	0.34±0.12
Chi. sq.	1.93	2.41	5.29
D.F.	3	3	3

Effect of temperature on toxicity of aldicarb and pirimicarb to the two aphid species.

The results of bioassay experiments 10-13 show that under the conditions of the technique used toxicities of pirimicarb and aldicarb increased greatly with increasing temperature during treatment (Table 19).

Fig. No. 20 The effect of temperature on the rate of kill by pirimicarb and aldicarb applied systemically against M. persicae and B. brassicae.

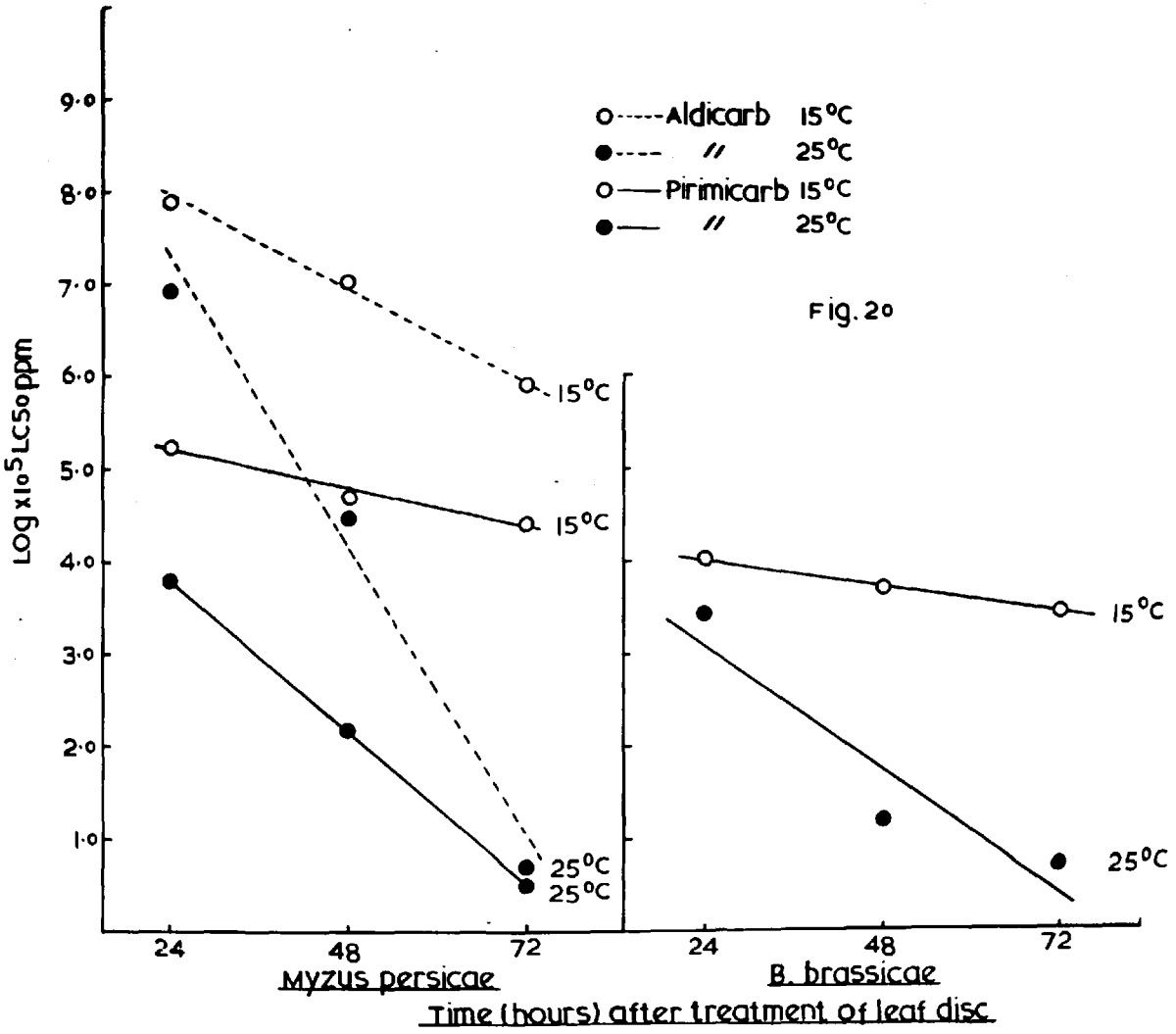

o-----Aldicarb 15°C
●---- // 25°C
o—Pirimicarb 15°C
●— // 25°C
Log x10⁵ LC50 ppm
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
15°C
15°C
25°C
25°C
15°C
25°C
24
48
72
24
48
72
Myzus persicae
B. brassicae
Time (hours) after treatment of leaf disc

Fig. 2o

Table 19

The effect of temperature on the toxicity of pirimicarb and aldicarb against M. persicae and B. brassicae.

Insecticide	Temperature	Aphid species	
		M. persicae (LC50 ppm)	B. brassicae (LC50 ppm)
Pirimicarb	15°C	0.24	0.028
	25°C	0.0004	0.00005
Aldicarb	15°C	7.9	
	25°C	0.0005	

The results show that with both aphid species an increase of 10°C from 15°C to 25°C increased toxicity of pirimicarb after 72 hours by about 600 times and that of aldicarb by about 15800 times against M. persicae. The time mortality curves (Fig. 20) do not indicate intrinsic diference in toxicity.

This difference could be caused by a number of factors including (a) greater uptake by the leaf at the higher temperature, (b) greater uptake by the aphid due to increased feeding rate at the higher temperature, (c) intrinsically greater toxicity at the higher temperature i.e. positive temperature coefficient of action as is known to be characteristic of many organo-phosphorus insecticides, (d) different effect of temperature on function of metabolites, (e) greater fumigant action at the higher temperature.

Table 20 Effect of change in temperature on toxicity after an initial uptake of aldicarb for 24 hours

Temperature	15°C		15° - 25°C		25°C		25° - 15°C	
conc. percent	0.25%	0.01%	0.25%	0.01%	.00008%	0.000000064%	0.00008%	0.000000064%
Time after treatment								
24 hours	44%	0.0	90.0	18	22	4	0	0
48 "	80%	0.0	100	38	62	36	10	8
72 "	100%	38.0	100	94	84	60	48	32

The possibility that uptake from the "wick" by the leaf disc was an important factor, was investigated by a small experiment in which leaf discs were kept for 24 hours at one temperature while the insecticide was being taken up and were then transferred to insecticide free units and tested at another temperature. Unfortunately the "controls" kept at the same temperature throughout, were not removed to insecticide-free units after 24 hours.

Nevertheless results (table 20) show that for example, a concentration of 0.00008% aldicarb taken up at 25°C and then tested at 15°C killed 48% of aphids compared with 38% by a concentration of 0.01% kept constantly at 15°C in continuous contact with insecticide. Uptake from the wick by the leaf disc was seemingly therefore a major factor increasing the toxicity at the higher temperature. Similarly the 0.00008% concentration kept continuously at 25°C caused 84% kill compared with 0.01% transferred from 15°C - 25°C after 24 hours which killed 94% of aphids. Again greater uptake at 25°C is seemingly a major cause of the difference.

SECTION 4.

LABORATORY STUDIES ON COMPARATIVE UPTAKE FROM SAND AND PERSISTENCE AND TOXICITY OF TWO GRANULAR INSECTICIDES IN BRUSSELS SPROUTS AT 20°C.

This experiment was done as part of a series aimed at understanding the reasons for relative inactivity of phorate in brussels sprout plants after soil application. The relative effectiveness of granular phorate and aldicarb was determined after application to sand in which brussels sprouts were grown. The possible effects of adsorption of insecticide by soil was minimised.

POT EXPERIMENTS USING SAND CULTURE.

Young brussels sprout seedlings grown in nutrient solution (See Materials and Methods) were transferred into 9" pots containing sand. The plants were kept in a constant environment room at 20°C and watered with nutrient solution until they were large enough to be used for experiments.

Granular formulations of phorate and aldicarb were poured around the stem about 1" deep at the

rate of 0.1 lb a.i. per acre (95 mg/plant). Treatments were replicated into 4 pots and "controls" consisted of 4 potted plants with untreated sand.

Leaf discs from young and old leaves of treated and untreated plants were removed at regular intervals and 10 laboratory reared late instar larval apterae of M. persicae were put on each leaf disc.

Mortalities of aphids after 72 hours (table 22) show that aldicarb killed all aphids on young and old discs upto 4 weeks after treatment and killed about 43% and 93% 7 weeks after treatment on young and old discs respectively compared with phorate which killed all aphids upto 2 weeks after treatment on young and old leaf discs and killed 26% and 44% 7 weeks after treatment on young and old leaf discs respectively. Thus loss of toxicity started earlier with phorate treated plants. Some toxicity was still shown in both the treatments upto 11 weeks after treatment when 20% and 8% were killed on old leaf discs with aldicarb and phorate respectively. Old leaves appear to have either taken up large amounts of insecticide and maintain the toxicity for longer periods or they continued to take it more efficiently than young leaves which, although causing higher kill soon after treatment,

Table 22

Percent mortality of late instar larval apterae of M. persicae on leaf discs from young and old leaves of Brussels sprout plants treated with aldicarb and phorate granules in sand at 20°C (Abbott's correction has been applied)

		Treatment & leaf age					
No. of week after sand applic.	Time of examination	Untreated Control		Aldicarb		Phorate	
(Dates)	(Hours)	young disc	old disc	young disc	old disc	young disc	old disc
1 7/2/69	24	0	0	100	94	98	74
	48	0	0	100	100	100	90
	72	0	0	100	100	100	96
2 14/2/69	24	0	0	92	94	94	96
	48	0	0	100	96	100	98
	72	0	0	100	100	100	100
4 4/3/69	24	0	2	56	89	52	75
	48	0	2	96	97	72	89
	72	0	2	96	100	84	93
6 18/3/69	24	0	0	12	70	10	20
	48	0	2	20	89	20	43
	72	2	6	32	93	28	46
7 25/3/69	24	0	0	18	64	8	10
	48	4	2	33	85	16	30
	72	4	2	43	93	26	44
10 15/4/69	24	0	0	0	2	2	2
	48	0	0	6	16	4	10
	72	0	2	18	34	6	11
11 22/4/69	24	0	0	0	0	0	0
	48	0	0	0	8	0	0
	72	0	0	10	20	0	8

lost toxicity more quickly either by distribution of insecticide to other rapidly growing leaves of the plant or from less uptake.

At the rate of application used initial kills were virtually 100 per cent by both chemicals but later the greater kills by aldicarb indicate greater toxicity and (or) greater persistence than phorate. The results therefore show that soil does not affect the relative toxicities of the two chemicals i.e. phorate is still less active in the absence of soil.

SECTION 5.

EFFECTS OF DIFFERENT METHODS OF APPLICATION ON THE RELATIVE UPTAKE, TRANSLOCATION, PERSISTENCE AND TOXICITY OF PHORATE AND ALDICARB IN BRUSSELS SPROUT. PLANTS GROWN IN NUTRIENT SOLUTION AND AS POTTED PLANTS

A series of laboratory experiments were done to study the effects of different sites of application, on the uptake, translocation and persistence of phorate and aldicarb by brussels sprout plants grown in nutrient solution and as potted plants. They were done to indicate whether translocation was interfered within parts of the plant other than the roots. Potted brussels sprout seedlings were grown in constant environment room at 20°C. The plants were later removed to the laboratory for the following methods of application to be made.

INSECTICIDE APPLICATION

Freshly prepared aldicarb dissolved in cellosolve or acetone was injected carefully sloping downwards at 45° into the central parenchymatous cells of pith region of stem of each plant. As

the plants were potted, exact weight of individual plant could not be determined so the exact parts per million treatment could not be made for each plant. An estimated amount of 10 µl /plant (1µl = 10µg) was, therefore, injected and the exact ppm treatment was calculated later from the weights of cut parts of plants. The aerial parts of the plant were used for chemical estimation of the parent compound and its biologically active metabolic products. Several plants were injected of which some were removed to 15°C and cut from the pots at different intervals (as earlier described) to be weighed and then frozen for chemical analysis. The remaining treated plants were taken to the 20°C C.T. room for leaf discs to be removed at different intervals for aphid bioassays. Controls were treated only with solvents. The bioassay results in table 23 show that the translocation to the aerial parts of the plant was not properly completed one day after treatment as indicated by low mortalities of aphid species, but aphid mortality of 50% M. persicae and 80% B. brassicae 4 days after the treatment indicated sufficient translocation and perhaps conversion to more toxic

metabolites. The disappearance of the insecticide or its metabolites started in about 3 - 4 days after treatment as indicated by decreasing aphid mortalities. Toxicity appeared to persist for about 4 weeks after treatment or even later but aphid mortality was considerably lowered 3 weeks after treatment. As shown by the results in table 23 the solvent whether acetone or cellosolve did not make any difference in toxicity or show any adverse effects.

CHEMICAL ANALYSIS

The residue analysis of aldicarb and its metabolites was done by thin layer chromatography (t.l.c.) as described by Skrentny and Ellis (1970) Some of the details of aldicarb analysis are as follows:-

Treatment	Time after treatments (Days)	Wt. of plant material (excluding roots)	Calculated Treatment	Approximate (ppm in green)
Aldicarb	1	26.4 gm	3.85 ppm	3.5 ppm
	3	31.8 "	3.1 "	
	7	27.2 "	3.7 "	

Table 23

Percent mortality of late instar larval apterae of M. persicae and B. brassicae on leaf disc from Brussels sprout plants injected with 3.5 ppm and 1.75 ppm of aldicarb using acetone and cellosdve as solvent respectively at 20°C.

No. of days after injection (Days)	Time of examination (Hours)	Treatment and aphid species					
		Untreated control		Aldicarb (3.5 ppm)		Aldicarb (1.75 ppm)	
		M.p.	B.b.	M.p.	B.b.	M.p.	B.b.
one day 2/1/69	24	0	0	0	0	2	0
	48	0	0	8	8	10	10
	72	0	0	16	14	22	18
4 days 6/1/69	24	0	0	12	34	2	0
	48	0	0	30	46	8	14
	72	0	0	50	84	24	28
7 days 10/1/69	24	0		0		0	
	48	0		0		4	
	72	0		34		16	
14 days 17/1/69	24	0		2		0	
	48	0		16		4	
	72	0		30		12	
21 days 24/1/69	24	0		0		0	
	48	0		6		2	
	72	0		10		4	
28 days 31/1/69	24	0		0		0	
	48	0		0		0	
	72	0		4		0	

Key to the table

M.p. Myzus persicae

B.b. Brevicoryne brassicae Aphid species was not used for bioassay 4 days after the treatment.

Approximate average treatment based on wet weight of 2 whole plants (greens included leaves with their petioles and stem) excluding roots for different intervals.

Table 24

Percent of aldicarb remaining at intervals after injection in brussels sprout plants.

Sample	Period of breakdown and quantity detected			
B. sprouts	Days			
% remaining	1	3	7	
Aldicarb	0	0	0	
Sulphoxide	100	100	88.0	
Sulphone	0	0	2.0	
% loss	0	0	10	

Plant pesticide residue analysis was made by thin-layer chromatography (t.l.c.) and the results indicate that aldicarb was completely broken down 24 hours after treatment because it was readily converted into its sulphoxide. This metabolite stays as such for about 6 days and then starts getting converted into sulphone and at the same time shows loss of about 10% one week after treatment. A comparative analysis by thin-layer chromatography (t.l.c.) of the fate of aldicarb (table 25) injected

into three crop plants shows that brussels sprouts and beans converted 50% of the parent compound into aldicarb sulphoxide after 6 hours and almost all by 24 hours after the treatment compared with 15% and 50% by potato plants. Brussels sprouts and potato plants showed 50% aldicarb sulphoxide and 2% and 0.0% of aldicarb sulfone in about two weeks after the treatment respectively compared with beans which had 20% of aldicarb sulphoxide and 75% of aldicarb sulphone. It has been further shown that 20% aldicarb sulphoxide still persisted in Brussels sprouts for six after the treatment compared with only 5% in beans. Data indicate that brussels sprouts and potato lost about 50% of aldicarb metabolites in 2 weeks compared with only 5% in beans. It was further estimated that 50% of aldicarb in its metabolite forms was still present in bean plants 56 days after injection compared with 30% in brussels sprouts 42 days after injection. The rate of metabolism appeared to be faster and similar in beans and brussels sprouts than potato but the rate of degradation was highest in brussels sprouts and lowest in beans. It was also shown that bean was the first to convert aldicarb sulphoxide to its sulphone (5%) 1 day after treatment and increased to 50% and 75% in 1 and 2 weeks after

Table 25

Percent of aldicarb remaining at intervals after injection into 3 crop plants.

Beans

	Hours		Days after injection							
% remaining	1	6	1	3	7	14	28	42	56	70
Aldicarb	90	50	0	0	0	0	0	0	0	0
Sulphoxide	10	50	95	80	50	20	10	5	2	1
Sulphone	0	0	5	20	50	75	70	65	48	25
% loss	0	0	0	0	0	5	20	30	50	74

Brussels sprouts

	Hours		Days after injection							
% remaining	1	6	1	3	7	14	28	42	56	70
Aldicarb	8	50	0	0	0	0	0	0		
Sulphoxide	20	50	100	100	88	50	30	20		
Sulphone	0	0	0	0	2	2	10	10		
% loss	0	0	0	0	10	48	60	70		

Potatoes

	Hours		Days after injection							
% remaining	1	6	1	3	7	14	28	42	56	70
Aldicarb	100	85	50	1	0	0				
Sulphoxide	0	15	50	90	80	50				
Sulphone	0	0	0	0	0	0				
% loss	0	0	0	9	20	50				

Notes

1 - 50 ug ofaldicarb stem injected at 15°C
2 - plant 3 weeks old
3 - " 4 " "
4 - 100 ug. ofaldicarb injected at 15°C
5 - Plants 4-5 weeks old

treatment respectively compared with brussels sprouts which showed only 2% of aldicarb sulphone in two weeks and none in beans.

Section 5 (iii a, b) Comparison of the relative uptake and toxicity of aldicarb injected into the stem and petiole of the brussels sprout plants.

It was hoped to distinguish possible effects of stem or petiole on efficiency of translocation of the insecticides.

Young brussels sprout seedlings were kept at 20°C C.T. room and when grown to about 8-10" long, were removed to the laboratory for insecticide treatment. Each plant was taken from its pot, washed its roots carefully dried and the whole weight then taken. The exact dose to be made to the plant could then be calculated in a ppm basis. In the case of petiole treatment, leaves of the same size were first weighed and the approximate amount of the insecticide was then injected into individual leaf petioles of each plant. All the treated plants were put separately into narrow mouth flasks at 20°C containing nutrient solution.

Bioassay results (table 26) of aphids kept for 72 hours on leaf disc taken regularly from treated plants

Table 26

Percent mortality of late instar larval apterae of M. persicae and B. brassicae on leaf discs from Brussels sprout . plants injected with 5 ppm of aldicarb in acetone into the stem and petiole of the plant. (i.e. ppm of total weight of plant).

Time after treatment	Time of examination (hours)	Treatment and aphid species					
		Untreated control		Stem treatment		Petiole treatment	
		M.p.	B.b.	M.p.	B.b.	M.p.	B.b.
one day 6/3/69	24	0	0	100	100	60	64
	48	0	0	100	100	80	90
	72	0	0	100	100	92	100
one week 12/3/69	24	0	0	10	50	30	58
	48	0	0	54	74	66	74
	72	0	0	64	84	88	98
2 weeks 19/3/69	24	0	0	0	0	0	0
	48	0	0	0	2	26	28
	72	0	2	12	32	48	85
3 weeks 27/3/69	24	0	0	4	8	12	36
	48	0	0	10	20	54	88
	72	2	0	34	68	83	96
4 weeks 3/4/69	24	0	0	8	10	10	16
	48	0	0	10	16	20	30
	72	2	0	28	44	36	78
5 weeks 10/4/69	24	0	0	2	6	0	4
	48	0	0	8	14	4	14
	72	0	2	16	24	16	20
6 weeks 17/4/69	24	0	0	0	0	0	0
	48	0	0	0	0	0	10
	72	0	0	8	14	10	12

showed that all aphids of the two species were killed by both the treatments one day after the treatment. Stem treatment killed 64% of M. persicae and 84% of B. brassicae one week after the treatment and continued to kill 34% of M. persicae and 68% of B. brassicae up to 3 weeks after the treatment compared with petiole treatment which killed 88-83% of M. persicae and 98-96% of B. brassicae for 1-3 weeks. Aphid mortalities showed little difference between the treatments one day after the treatment but later greater kills by petiole treatment showed greater uptake or greater conversion into toxic metabolites than stem treatment. Toxicity also appeared to have persisted up to 6 weeks after treatments.

Section 5 (iii- a,b, and c)

Evaluation of uptake, translocation and toxicity of aldicarb through the stem, petiole and the root system of brussels sprout plants grown in nutrient solution.

This experiment was undertaken to compare the relative effectiveness of aldicarb applied by the different methods as just described except that all were compared in one experiment, and dosages were based on the part treated rather than in the total weight of the plant. Thus in the petiole treatment, the leaf along with its petiole was removed from the plant and weighed and then treated with exactly 5 ppm

of aldicarb based on the leaf and petiole weight only. Root uptake was probably more efficient in plants grown in nutrient solution than with plants grown in soil as the root system of the latter became damaged while removing and while washing off the clinging particles of soil and dried leaves.

Insecticide application

(a) Stem injection. Each plant was removed from nutrient solution and its roots were dried before the whole plant was weighed and then injected with 5 ppm of the insecticide. The treatment was replicated 3 times. Each treated plant was separately put into flasks having nutrient solution.

(b) Petiole uptake. Leaves of almost equal sizes were removed from the plants and were separately put into 3" x 1" glass tubes having 5 mls of distilled water with 5 ppm of insecticide. The treated leaves as such were put outside to accelerate the uptake which took nearly 2 hours to be completed. Each leaf was given another 5 ml of distilled water to be absorbed in order to make sure that no insecticide was left in the tubes.

(c) Root uptake. The roots of each plant were separately put in 10 mls of distilled water in glass tubes having 5 ppm of insecticide. The uptake was again accelerated by either putting outside or supplying fanned aeriation

Table 27 Percent mortality of late instar larval apterae of M. persicae on leaf disc from brussels sprout plants treated with 5 ppm of aldicarb by different methods. (Abbot's correction has already be applied)

Time after treatment (Days)	Time of examination (Hours)	Treatment and leaf age							
		Untreated controls		Stem injection		Petiole uptake		Root uptake	
		Margin	Centre	Margin	Centre	Margin	Centre	Margin	Centre
1 day 7/8/69	24	0	0	90	60	100	95	40	10
	48	0	0	100	95	100	95	55	35
	72	0	0	100	100	100	100	75	70
4 days 10/8/69	24	0	0	90	55	70	46	65	55
	48	0	0	95	85	90	55	80	70
	72	0	0	95	85	95	75	85	75
7 days 13/8/69	24	0	0	36	36			40	5
	48	0	0	76	70			52	35
	72	0	0	80	76			62	45
14 days 20/8/69	24	0	0	2.5	0			15	7.5
	48	0	0	15	10			17.5	20
	72	0	0	45	32			52	38
21 days 27/8/69	24	0	0	2.5	0			12	0
	48	4	2	12	2			18	0
	72	4	2	16	6			26	4

in the glasshouse which took about 2-3 hours including additional 10 mls of distilled water after the insecticide uptake. All the treated plants were then transferred to 20°C C.T. room and put separately into flasks having nutrient solution.

Leaf disc was removed from the margin and centre of leaves of the treated plants and the results of aphid mortality after 72 hours indicate (table27) that stem and petiole treatments killed all M. persicae on marginal and central leaf discs for 1 day after treatment and continued to kill 95%-85% and 95%-75% on marginal and central discs up to 4 days after treatment respectively compared with root treatment which killed 75%-70% for one day after treatment and 85%-75% up to 4 days after treatment. Kills of 80%-76% on marginal and central discs were still shown up to 1 week after treatment respectively compared with 62%-45% in root treatment. Unfortunately the leaves given the petiole treatment did not survive for a week after treatment and thus did not show the full persistence of aldicarb. Aphid mortality decreased similarly in stem and root treatments below 16% and 26% 3 weeks after treatment respectively but a more rapid decline was shown by disc from the leaf centre than from marginal areas of the leaf.

The uptake and translocation appeared to be more rapid in stem and petiole treatments than in root treatment but the persistence was similar in all the three treatments.

Section 5 (iii-d)

Comparative uptake and toxicity of aldicarb and phorate applied by injection into the stem of brussels sprout plants grown in nutrient solution

This experiment was done to compare the relative effectiveness of phorate and aldicarb injected into the stem of brussels sprout plants grown in nutrient solution. As before dosage was based on the whole weight of plant. A treatment of 5 ppm was injectedd and the treated plants were kept in 20°C C.T. room for aphid bioassays.

The results (table 28) indicated that aldicarb killed about 90% of M. persicae one day after the treatment and still killed more than half of aphids one week after the treatment compared with phorate which killed 60% and 32% 1 and 7 days after the treatment respectively. Aldicarb was still killing about 16% M. persicae 3 weeks after the treatment compared with phorate where kill was almost negligible.

It therefore shows that phorate is either trans-

Table 28

Percent mortality of M. persicae on leaf disc from brussels sprouts treated with 5 ppm of aldicarb and phorate.

Time after treatment	Time of examination	Treatment and aphid species		
		Untreated control	Aldicarb m.p.	Phorate m.p.
1 day 7/7/69	24	0	16	8
	48	0	50	26
	72	0	89	60
4 days 10/7/69	24	0	12	4
	48	0	32	10
	72	0	88	48
one week 13/7/69	24	0	8	2
	48	0	24	8
	72	0	58	32
two week 20/7/69	24	0	12	0
	48	0	16	4
	72	0	32	16
3 week 27/7/69	24	0	4	0
	48	0	10	0
	72	0	16	4

located inefficiently than aldicarb or is metabolised more slowly which confers its inactivity even through injection uptake.

Section 5 (iv-a)

Effect of different methods of application on the efficiency and toxicity of aldicarb

This was done to study the efficiency in uptake of aldicarb applied by injection into the main vein of the leaf disc and by wick application. The results in

Table 29 Percent mortality of late instar of larval apterae of M. persicae on leaf disc treated with different doses of aldicarb by two methods. (Abbot's correction has already been applied)

Insecticide	Method of application	Time after treatment (Hours)	Treatment Dosages in ppm wet weight of leaf disc					
			Untreated control	30 ppm	15 ppm	10 ppm	5 ppm	2.5 ppm
Aldicarb	Injection to the main vein of disc	24	0	94	56	46	28	18
		48	0	100	90	96	68	66
		72	4	100	95	95	84	72
Aldicarb	wick application	24	0	96	84	72	54	14
		48	0	98	100	98	100	50
		72	6	100	100	97	100	70

(Table29)indicated greater kill 24 hours after wick application than injection into the main vein but little difference in mortalities was shown 72 hours after treatment by the two methods of application. Both methods of application indicate efficient uptake of the insecticide by leaf disc.

Section 5(iv-b)

This experiment was done to compare the relative uptake and persistence of toxicity of phorate and aldicarb applied through root system of brussels sprout plants grown in nutrient solution. Each plant was dipped into known amount of insecticide in relation to the treatment (ppm) based on the whole weight of plant and after a complete absorption of insecticide by plants they were separately put into flasks containing nutrient solution and kept in 20°C C.T. room. Aphid bioassay of 72 hours (table30) at two different intervals after treatment indicated that aldicarb killed 54% and 76% of M. persicae at 15 ppm treatment on leaf disc removed 5 and 24 hours after treatment respectively compared with phorate which killed 2% and 40% at 15 ppm treatment. The uptake of phorate appears to have been completed but its subsequent translocation to the aerial parts of plant is either considerably reduced due to blockage in roots or the compound was not metabolised to be effective against aphids. Leaf disc taken after 5 hours was

probably too early for adequate translocation and (or) metabolism of phorate which gave only 2% at 15 ppm and no kill in all other treatments compared with aldicarb which did demonstrate an uptake and translocation killing almost half of aphids at the top dose. The uptake and metabolism of aldicarb and metabolites were much more efficient 24 hours after treatment.

Table 30

Corrected percent mortality of M. persicae on leaf disc from brussels sprout plants treated with phorate and aldicarb at 20°C.

Insecticide	Method of application	Time after treatment	Treatment: Dosages in ppm based on wet weight of whole plant					
			Untreated control	15	10	5	2.5	1.25
Aldicarb	Root dip 5 hours	24	0	20	2	2	0	0
		48	0	50	20	16	2	0
		72	0	54	31	26	4	0
Aldicarb	Root dip 24 hours	24	0	0	0	0	0	0
		48	0	60	48	46	8	4
		72	0	76	72	54	12	8
Phorate	Root dip 5 hours	24	0	2	0	0	0	0
		48	0	2	0	0	0	0
		72	0	2	0	0	0	0
Phorate	Root dip 24 hours	24	0	0	0	0	0	0
		48	0	18	12	8	4	0
		72	0	40	24	16	8	0

Section 5(v-a)

This experiment was also done to study the relative uptake and translocation of phorate in brussels sprouts and beans in the absence of a root system. This might also indicate why phorate is relatively inactive in brussels sprouts. The mature plant leaves of plants grown in a constant environment room at 20°C were removed and their petioles put in 5 ppm of phorate in water with and without DNP at 15°C. Another treatment was selected where DNP was added after an initial uptake of phorate. All the leaves were dipped for 24 hours. The results (table 31) of aphid bioassay on leaf discs from 24 hours of uptake through the petiole of dipped leaves indicate that beans killed about 20-26% and 54-84% of M. persicae after 24 and 120 hours of exposure respectively compared with 0-6% and 8-14% in brussels sprout. leaves.

Unfortunately no chemical analysis was done to estimate the phorate residues in the two different plant species which might have indicated a greater accumulation of phorate or that more has been converted into more toxic metabolites in bean leaves than in brussels sprouts leaves. It appears that although DNP inhibits the energy for active transport phorate is still absorbed and translocated showing

Table 31

Percent mortality of late instar larval apterae of M. persicae on leaf discs from "petiole dip" uptake of phorate for 24 hours at 15°C.

Plant species	Time of examination (Hours)	Untreated control		Treatments		
		DNP	Untreated	Phorate	DNP + Phorate	Phorate, then DNP
Beans	24	2	0	20	26	20
	48	2	0	40	48	32
	72	2	0	48	72	48
	96	2	0	60	84	52
	120	2	0	64	84	54
Brussels sprouts	24	0	0	0	6	2
	48	0	0	2	8	4
	72	2	0	4	14	8
	96	2	0	6	14	12
	120	2	0	8	14	12

Note Freshly collected M. persicae which were not starved were used in this experiment showing less kill in brussels sprouts.

greater toxicity than in control. This could be explained on the basis that DNP is either not effective in the absence of root system or that more is metabolised into toxic compounds. The addition of DNP after an initial uptake of phorate affects slightly showing less kills than in phorate without DNP. Similar patterns of uptake and toxicity have been shown with respective

treatments in brussels sprouts which as before indicated less toxicity than in beans.

The results suggest that the root system is not the only factor responsible for the inactivity of phorate in brussels sprouts but it is either the absence or poorly developed transport mechanism.

Section 5(v-b)

Evaluation of roof dip uptake, metabolism and biologically active residue of phorate and its derivatives in different parts of brussels sprout plants.

This experiment was undertaken in an attempt to explain why phorate worked so poorly by root uptake by brussels sprout plants. Perhaps brussels sprout roots do not contain active transport mechanism that aid uptake and translocation; an active transport inhibitor dinitrophenol (DNP) was therefore used to see whether the absorption and translocation were less affected than with other plants which might contain the active transport mechanism.

Insecticide application

Brussels sprout plants about 3-4 weeks old were dipped into DNP 10^{-4} m solution for 24 hours before being treated with the insecticide. A treatment of 5 ppm in acetone was then given to untreated brussels

sprout plants as well as to the plants already dipped in DNP. The plants were kept in the phorate solution for about 24 and 72 hours at 15°C before being frozen for chemical analysis of the plant parts.

Method of chemical estimation of phorate

The roots and greens (green means stem and leaves with their petioles) of the treated and untreated plants were separated and then frozen in polythene bags for about 24 hours at -20°. The frozen materials were then crushed into smaller pieces and weighed individually before being put into bottles with 100 ml of Hexane: Benzene (1:1 by vol.) solvent. Anhydrous sodium sulphate almost equal to the weight of plant material was added and the mixture homogenised for 10 minutes and left over night to settle completely. The material was filtered off on a Buckner funnel having thin layers of woolly asbestos and Hyflo supercel as a filter. The residue was further extracted with chloroform (100:30:30) in separating funnels, shaken and then allowed to settle for about 15-20 minutes before being decanted.

Concentration

The filtrates were concentrated to 10 ml in rotary film evaporators at 40°C. The concentrated plant extract was further reduced to ½ ml over a warm water

bath supplied with an air stream. This ½ ml was then eluted through a chromatographic column; a glass tube (20.8 cm x 1.4 cm) having a constriction at one end was packed with asbestos wool and about 1" layer of granulated sodium sulphate. This was covered with about 8 cm of A_2IO_3 BDH (chromatographic grade) and finally topped up by sodium sulphate. Another 100 ml of Hexane: Benzene (1:1 by vol.) solvent was eluted through the column. The green colouring (chlorophyll) was removed to a greater extent and the filtrates were again concentrated in a rotary film evaporator followed by warm water bath evaporation reducing from 10 ml to ½ ml which was then ready for G.L.C. injection.

Aphid bioassays

Leaf discs were removed from treated and untreated plants before they were frozen for chemical analysis. This was done to show the presence of biologically active compound in the leaves of brussels sprout plants from 1 and 3 days of dip uptake which could then be related to the chemical analysis data.

The results of concurrent aphid bioassay (table32) indicate that leaf disc taken from plants dipped in phorate for 24 and 72 hours killed 35.5% and 50% of M. persicae respectively compared with DNP + phorate

Table 32

Phorate + its derivatives residue and Bioassay data of Brussels sprout plants dipped for 24 and 72 hours at 15°C.

Sample	24 Hours Dip		72 Hours Dip	
Brussels sprouts	Bioassay	G.L.C.	Bioassay	G.L.C.
Phorate Greens Phorate Roots	35.5% -	2.5 ppm 19.04 "	50.0% -	4.0 ppm 31.5 "
DNP + Phorate Greens " " Roots	2.5% -	0.14 ppm 10.32 "	18.3% -	0.40 ppm 14.0 "
Control Greens " Roots	0.0% -	- -	0.0% -	- -
DNP Control Greens " " Roots	2.0% -	- -	0.0% -	- -

treatments which killed 2.5% and 18.2% after 24 and 72 hours of dip respectively. The chemical estimation of phorate + its derivatives residues in plant extracts (table32) shows that phorate accumulated in roots to as much as 19.04 ppm and 31.5 ppm after 24 and 72 hours of uptake respectively compared with small amounts of 2.5 ppm and 4.0 ppm in greens. The DNP + phorate treatments also indicated similar patterns i.e. about 10.32 ppm and 14.0 ppm in roots and 0.14 ppm and 0.40 ppm in greens after 24 and 72 hours of dip uptake respectively.

Section 5(vi)

This experiment was done to study further the effect of plant species on the differential uptake, rate of metabolism and toxicity of phorate and its metabolites. As has already been indicated in previous experiments, brussels sprouts either absorbed and translocated less phorate or converted it more slowly to its toxic metabolites. Dinitrophenol (DNP) (an inhibitor of energy for active transport) was again used as one of the treatments in order to clarify the factors responsible for inactivity of phorate in brussels sprouts. Two different periods of uptake, one shorter and the other longer were selected to see whether there was any differential activity of phorate in the plant species.

Phorate uptake by the root system of plants

A set of three to four plants (about 4-6 weeks old) of brussels sprouts and beans were dipped in 5 ppm of phorate with and without dinitrophenol for 24 and 96 hours at 15°C.

Chemical analysis of phorate and its metabolites

The extraction and clean up method of Menzer and Ditman (1968) was partly modified to get a more accurate estimate of toxic residues in the plant material. A method of thin layer chromatography (t.l.c.) was used metabolite separation with quantitative estimation of

phorate and its sulphoxide and sulphone products. The method is quite sensitive as a quantity as small as 0.5 to 1 mg of analytical standards of phorate and its metabolites could be detected on silica gel plates. It is however noted that this method is only a semi-quantitative analysis and that an error of about ± 10% could be expected. All plant material collected for analysis of phorate and its metabolites was first frozen for about 24 hours at about -20°. Plant samples of 20-25 g. were homogenised (in a M.S.E. homogeniser) for about 10 minutes with 150 ml of a solution of acetone and water (1:1 by vol.) in a 400 ml glass jar. The solid material was filtered off on a Buckner funnel which had layers of woolly asbestos, Hyflo supercel and anhydrous sodium sulphate. The filtrate was extracted in Chloroform (3:1:1 by vol.) in separating funnels and then concentrated to 25 ml in a rotary film evaporator at 40°C.

Clean up method

The concentrated plant extract was cleaned by adding about 1 g. of charcoal per 5 gm. of plant material. The mixture was evaporated to dryness over a warm water bath by an air stream and then taken up in acetone. The resulting suspension was added to the top of a chromatographic column of sodium sulphate, Florocil and sodium sulphate. Elution with 100 ml of acetone removed

phorate and its metabolites. This was concentrated on a rotary film evaporator to about 5-8 ml followed by a warm water bath with an air stream to complete dryness. This was then made up in 5 ml of acetone. One ml of the concentrated sample was dried by an air stream on warm water bath again and then finally made up in 10-20 ml of acetone for spotting on to glass plates (20 x 20 cm) coated with silica gel and were developed in methanol chloroform (25:4 by vol.). When the solvent front had reached about 30 mm from the top of the plate they were removed from the solution and dried and sprayed with palladiumchloride and sodium hydroxide. Phorate and its sulphoxide and sulphone developed yellowish brown spots at R_f 0.82 , 0.54 and 0.75 respectively. Estimates of the amounts of these compounds were made by comparing the size and intensity of colour of the spots with those of known standards spotted along the side of the sample. Phorate and its 5 metabolite were combined and then spotted at several concentrations to allow valid comparison with several reference spots. The standard separated into the following serial order: Phorate, phorate sulphone, phoratoxin, phorate sulphoxide, phoratoxin sulphone and phoratoxin sulphoxide.

The bioassay results in (table 33) indicated that an uptake of phorate for 24 hours by the roots of plants

killed 38% and 18% of M. persicae on leaf disc after 72 hours by phorate and DNP + phorate treatments respectively in brussels sprouts compared with 48% and 20% in beans, whereas toxicity appeared to have greatly increased from an uptake of 96 hours killing 46% of M. persicae on phorate treatment in brussels sprouts compared with 50% and 28% in phorate and DNP + phorate treatments respectively in beans. Unfortunately the 96 hours DNP + phorate treatment proved phytotoxic in brussels sprouts which completely wilted. The chemical analysis of phorate and its sulphoxide and sulphone products by thin layer chromatography (t.l.c.) demonstrated that a dip uptake of 24 hours accumulated 25 and 10 ppm of phorate in brussels sprouts roots from phorate and DNP + phorate treatments respectively compared with 2 and 1 ppm in beans. The dip uptake of 96 hours showed a corresponding increase having 35 ppm of phorate in phorate treated brussels sprout roots and 5 and 2 ppm in phorate and DNP + phorate treatments respectively in bean roots. The analysis of the green parts of treated plants showed that about 2 and 1 ppm and 0.5 and 0.5 ppm of phorate from phorate and DNP + phorate treatments in brussels sprouts and bean greens respectively. No phorate was detected after 96 hours of uptake by both the plant species.

Table 33 Percent mortality of late instars larval apterae of M. persicae and chemical estimation of phorate and its metabolites in brussels sprouts and beans.

Plant species	Brussels sprouts						Beans					
Treatments	Roots		Greens				Roots		Greens			
	Phorate	DNP + Phorate	Phorate		DNP		Phorate	DNP + Phorate	Phorate		DNP + Phorate	
				Bio-assay		Bio-assay				Bio-assay		Bio-assay
After 24 hours of uptake												
Phorate	25	10	2	38%	1	18%	2	1	0.5	48%	0.5	20%
PSo	3	2	1		0.8		3	1.5	1.25		0.75	
PSo2	-	-	Trace		Trace		-	-	-		-	
Total	28 ppm	12	3	-	1.8		5 ppm	2.5	1.75		1.25	
After 96 hours of uptake												
Phorate	35		0	46%			5	2	-	50%	-	28%
PSo	2.5		2				2.5	1.6	2.5		1.5	
PSo2	-		2				-	-	0.1		-	
Total	37.5		4				7.5	3.6	2.6		1.5	

The oxidative metabolites of phorate (table 33) appeared to be similar in quantity in both the roots of brussels sprouts and beans while showing almost half of quantity of metabolites in DNP treatments. As shown earlier in the case aldicarb metabolism in beans, phorate was also more rapidly metabolised to sulphoxide and sulphone than in brussels sprouts which are indicative of higher toxicity to aphids. It was more obvious in 96 hours of uptake where no phorate was found in greens either in brussels sprouts or beans but gave aphid kills of 46% and 50% which could only be regarded as due to conversion products of phorate to sulphoxide and sulphone.

The results therefore clearly indicate that 2, 4 dinitrophenol inhibited partly the active translocation of phorate from roots to stem and leaves, and the difference in aphid mortality on leaves was due to different rate of metabolism in the two plant species i.e. higher in beans than in brussels sprouts.

Discussion

Field efficacy of soil applied systemic insecticides against aphids on brussels sprouts plants (1968)

Effects on natural aphid populations:

The numbers of live alatae (Table 1) and their progeny including adult apterae (Figs.2, 3 & 4) of three aphid species colonising the crop indicate that aldicarb, pirimicarb and menazon acted effectively not only against immigrant alatae and kept their numbers very small but were also effective in controlling the subsequent build up of progeny which is the cause of damage to the crop. Phorate was surprisingly less effective than the others and was the only treatment where apterae of M. persicae and B. brassicae persisted up to the end of the season (figs 3& 4). In general, however aphid populations were small due to small infestations by migrants.

Sleeve tests:

Fairly natural environmental conditions prevailed in sleeve tests where aphids were caged for a week on leaves attached to the field plants so that there was / continuous uptake of insecticide from the treated soil.

As organophosphorus and carbamate insecticides are known to be changed chemically either in soil or in the plant or in the insect (Ahmed and Casida 1958;

Meun & Hoskins, 1962; Calderbank, 1965 and Bull, 1965) the bioassays of soil applied systemic insecticides therefore indicate the toxicity of possible conversion products as well as that of the original compounds. In the sleeve tests at 2 lb/acre, aldicarb, pirimicarb and menazon treatments showed high toxicity to aphids from the beginning of the season (i.e. 2 weeks after treatment) not only providing effective control of naturally occurring immigrant alatae in July (Table 1) but also persistence against aphids sleeved subsequently on the plants thus providing good protection of the crop for about 15 weeks after treatment. Phorate as shown earlier by natural counts of the populations was less active killing only 50% of aphids from 4-13 weeks after treatment and thus gave a poor protection to the crop (Tables 2 and 3). The order of effectiveness of the above mentioned insecticides against aphids was:

aldicarb > pirimicarb > menazon > phorate

All treatments showed higher kills of both aphid species in old leaves than in young ones and the greater susceptibility of B. brassicae than M. persicae was also confirmed by tests with leaf disc removed from field treated plants. A more accurate estimate of uptake and changing toxicity of one carbamate and one organophosphate insecticide was undertaken to amplify and to

for which
confirm earlier results/ aldicarb and menazon were chosen. Experiments with these showed greater toxicity and persistence of aldicarb than menazon and as well as confirmed dissimilar toxicity of young and old leaves and greatersusceptibility of B. brassicae than M. persicae (Tables 6&7).

The results have demonstrated, as expected, that insecticide toxicity to aphids was related to low reproductive rates which increased as toxicity decreased with time interval after treatment (Tables 8 &9). These results confirm those of Cook et al (1963) who showed that sub-lethal systemic phorate treatments in the field reduced the fecundity of Acyrthosiphon pisum.

silwood conditions
The results therefore show in / brussels sprout. plants may effectively be protected from aphid attack not only at the vulnerable seedling stage but also for prolonged periods throughout the season by an initial localized granular application of aldicarb, pirimicarb and menazon to the soil.

The effectiveness and persistence of aldicarb in giving good protection to brussels sprouts probably throughout the season is comparable with good result with aldicarb on other crops (Gerhardt, 1966; Worthing, 1967 a & b; Legge and Sheperd, 1967; Hopkins & Taff,

1969; and Kearby and Bliss, 1969).

The remarkably effective control of aphids by the above mentioned soil applied granular treatments is no doubt related to soil type, which was a light-medium acidic sandy soil at Silwood of the type that is known to enhance activity and persistence compared with clay or peat soils (Casida, Chapman & Allen, 1952; Way & Scopes, 1968; Getzin and Chapman, 1959; Zake and Reynolds, 1961; Parker and Dewey, 1965; Pain & Skrentny, 1969). This comparatively longer persistence is desirable especially in a season favouring large populations at the time of sprout-formation, because one of the most severe forms of damage is caused by aphids in the sprouts, making them un-marketable.

A care should however be taken to assess the possible hazards of toxic residues in the sprouts, although the time interval between soil treatment and harvesting is so large that it is very unlikely for edible produce to contain toxic residues because of biochemical activity of the plants (March et al., 1955; Metcalf et al., 1955).

Although phorate is relatively ineffective as a soil applied aphicide on brussels sprouts, on the other hand it is regarded generally as a better soil applied

systemic aphicide than, for example, menazon partly because toxic residues can move in the vapour phase and as such can be absorbed directly by roots (Burt et al., 1965). Its inactivity in brussels sprouts was therefore surprising in contrast with its high efficiency in controlling aphids and other related pests of several plant species e.g. potatoes, carrots, cauliflower, beans, sugar-beet, peas, cucumber cotton and tobacco (Hopkins et al., 1959; Bacon, 1960; Genhardt and Turley, 1961; Schwartz et al., 1961; Pond, 1963; Broadbent et al., 1964; Lindley, 1962, 1964; Hofmaster & Dunton, 1961; Patterson & Rawlins, 1964; Libby, 1964).

Brussels sprouts may however have an exceptional effect on the action of phorate placed near its main stem. Its inactivity is either due to low water solubility which might be uniquely affected in brussels sprouts or inefficient uptake and translocation to the leaves or selective specificity of its roots in rejecting phorate and its metabolites. As absorption, translocation and persistence of systemic insecticides depend on the physiology of plants, the latter becomes an essential agent which unfortunately differs from species and species of plants in relation to different insecticides. David (1951) showed that broad bean roots selectively absorbed dimefox from solutions at rates of transpiration at which schradan was selectively rejected.

This may not however be the case with phorate in Brussels sprouts but the phenomenon might be the same and should be investigated. Preliminary results of Kwan (1967) also indicated the inactivity of phorate in Brussels sprouts compared with its activity in beans and potatoes and as well fits in with the findings of Chapman and Noum (1966) who demonstrated that relatively less phorate was taken up by turnips, spinach, cabbage and cauliflower than by peas, squash and tomato plants.

Relative susceptibility of aphid species to insecticides

During field experiments B. brassicae was found to be more susceptible than M. persicae to the same amounts of aldicarb, pirimicarb, menazon and phorate applied in soil to Brussels sprout plants. The difference has been earlier indicated by Way et al (1968) and Galley (1968). This difference is probably due to inherent differences in susceptibility or to differential uptake by the two species, perhaps different feeding rates or sites of feeding, since different levels of tolerance have also been confirmed in other experiments with thionazin, disulfoton and dimethoate where B. brassicae was always the more susceptible species in the field experiments with rape, Brassica napus L. Thus, Bonnemaison (1956) also found that B. brassicae was more susceptible

than M. persicae to the systemic action of schradan.

Anderson, Schulz and Hibbs (1961) demonstrated that almost forty-seven times greater accumulation of cholinesterase inhibitory residues of phorate occurred in the foliage than in other parts of the treated plants. The rate of movement of insecticide from roots to stems and leaves was accelerated by increased transpiration, and greater accumulation of insecticide in leaves seems to occur under environmental conditions favourable to transpiration (Hacskaylo et al 1961). It was observed by Bardner (1964) that old leaves lost their toxicity to insects more slowly and gave higher kills of aphids than young leaves. This compares almost exactly with the results of the present work but contrary to the findings of Cherry and Pless (1969) who showed that as the tobacco-growing season progressed, the upper and sucker leaves of the plant gave a higher mortality than maturing leaves.

The comparatively lower toxicity of young leaves than old leaves may either be due to the short period of uptake or dilution of the insecticide and their derivatives by active growth or by less active transpiration in young leaves than old leaves or by more rapid degradation in the young leaves.

Relative toxicities of insecticides

The results of laboratory bioassays (6-9) show that the relative toxicities of insecticides to M. persicae after 72 hours at 20°C were: pirimicarb > menazon > phorate > aldicarb. Based on LC50's (Appendix2) pirimicarb was about 10 times more toxici than menazon, about 13 times more toxic than phorate and about 26 times more toxic than aldicarb. In contrast, in the field, soil applied systemic aldicarb at 2 lb/acre, was the most effective treatment while the other insecticides were in the same order as found in the laboratory bioassays. The relative inactivity of aldicarb in laboratory conditions is surprising. Possibly aldicarb is not metabolised into its toxic sulphoxide by the time aphids pick up their doses. In the field its high efficiency is perhaps partly related to its complete metabolism in 4-9 days to the most toxic and stable aldicarb sulphoxide.

The results further indicate that time interval after treatment did not change the order of relative toxicities of different insecticides (Table 17) although 72 hours kills were more consistent than 24 hours (Appendix 3 Table 2).

M. persicae was consistently more resistant than B. brassicae and thus M. persicae after 72 hours at 20°C was about 4 times more resistant than B. brassicae to phorate, about 65 times to pirimicarb, about 100 times to aldicarb and 190 times to menazon.

Effect of temperature on toxicity

The results indicate that in the laboratory conditions used a 10°C rise from 15°C to 25°C increased toxicity of pirimicarb to M. persicae and B. brassicae by about 600 times and of aldicarb by about 15800 times against M. persicae. Change in temperature after an initial uptake of 24 hours indicated that much of the large difference in toxicity was not due to intrinsic difference in toxicity but rather to differential uptake by the leaf disc at different temperature.

Effect of placement of insecticide in soil

It was thought that the method of application of an insecticide in soil with relation to plant roots is likely to affect its uptake and persistence. An experiment was therefore done to test the effect of placement of aldicarb for control of aphids on Brussels sprouts. In order to get critical differences in toxicity between different treatments, the rate of application was reduced

to 1lb./acre (i.e. 0.94g of aldicarb/plant).

The results showed that placing the insecticide about 2 - 3" deep and 2 - 3" away from the plants (A) provided effective control of aphids and is comparable with treatment C where although the insecticide was placed 9" - 1' deep at 2 - 3" away from plants but showed high efficiency probably because of moisture in deeper layers of soil which is known to enhance uptake (Reynolds and Metcalfe, 1962; Chapman and Noum, 1966). This in particular is considered to be an effective method of application in soil in dry weather.

Treatment applied 2 - 3" deep but 9" - 1' away from plants was less effective than the others in the beginning of treatment presumably because the root system was too small to reach the treatment and absorb the insecticide efficiently but as the season progressed this treatment gained its full effectiveness in about 4 weeks after treatment.

All the three treatments gave complete protection to the crop from aphid attack for about 5 weeks after treatment (table 13) where A and C treatments were initially active from the beginning of the treatments compared with E which has the disadvantage of being less effective at the important seedling stage when an early

infestation might endanger the crop at the start of the season but has the advantage in being very effective and most persistent treatment (still killing 65% and 71% of M. persicae and B. brassicae after 10 and 11 weeks after treatment respectively (tables 13 and 14).

In an attempt to enhance the activity of the insecticide in the soil the experiment included treatments in which the insecticide was placed in combination with a nitrogenous fertilizer "Nitroform" which might help to attract the root system and thus enhance uptake of insecticide. It is interesting that fertilizer with aldicarb placed 2 - 3" deep and 2 - 3" away from the plant did not improve its initial activity against aphids but increased persistence compared with the same treatment without fertilizer, (table 13). However, placed 9" - 1' deep it appeared to decrease the toxicity of aldicarb.

For practical purposes placing the insecticide 9" - 1' away from the plant would seem to be best for long term toxicity and persistence against insect pests.

Relative effectiveness of aldicarb and phorate in sand.

The relative inactivity of phorate in Brussels sprouts grown in soil was shown also when grown in sand, where as the time progressed, the activity of phorate in terms of toxicity and persistence became much less than aldicarb. Similarly in plants grown in nutrient solution (plus insecticide) phorate was less effective than aldicarb. This indicates that the relative inactivity of phorate was not connected with adsorption factors in soil or movement in soil.

A. Metabolism of aldicarb and phorate

An explanation of the differences between : activity of phorate and aldicarb in beans, potatoes and Brussels sprouts was sought in preliminary experiments by examining uptake and metabolism by chemical means. To eliminate uptake factors the chemicals were initially injected into the plants.

It was found that aldicarb was oxidised completely to sulphoxide twenty-four hours after injection into the stem of Brussels sprout. plants and persisted as such for 3 - 6 days at 15°C, while being converted to sulphone. This pattern is similar to that of aldicarb in cotton plants, (Metcalf et al, 1966; Coppedge et al, 1967) where aldicarb sulphoxide was found to be the major metabolite. Aphid

mortality reached its maximum in about 4 days after treatment which seemed to be related with the rate of metabolism in Brussels sprouts and thus fits in with the results of Metcalf et al (1966) who showed that sulphoxide and sulphone were more active cholinestrase inhibitors than aldicarb but is contrary to the findings of Skrentny and Ellis (1970) who demonstrated less effectiveness of sulphoxide and sulphone to Aphis fabae. Different techniques however make exact comparison difficult. The rate of metabolism appeared to be relatively faster in bread beans and Brussels sprouts than potato in which, for example, 50% of aldicarb (table 25) was converted to sulphoxide twenty-four hours after injection compared with 95% and 100% in beans and Brussels sprouts respectively. The conversion of sulphoxide to sulphone was also relatively much faster in beans (50% in 7 days compared with 2% and 0% in Brussels sprouts and potato respectively) than in other plant species. The decrease in aphid mortality on leaf discs from beans appeared to be related to conversion of sulphoxide to sulphone which is known to be less toxic (Weiden, 1968).

The initial translocation of aldicarb to leaves following injection into stem and petiole appeared to be

similar but its subsequent persistence against aphids was relatively higher in petiole treatment than in stem injection which might be due to nearness to leaf lamina of the petiole.

It has also been demonstrated (table 27) that greater accumulation of insecticide occurred in the leaf margins than in the centre of the leaf. This is very similar to accumulation of phorate in leaf margins of Brussels sprouts and beans (Elliott, 1966).

Chemical estimation indicated absorption of phorate by roots of Brussels sprout plants (perhaps partly superficial even though not removed washing in water) but its subsequent translocation to leaves and stem was very slow thereby explaining its low toxicity to aphids. Thus after 24 and 96 hours uptake by roots, as little as 2 and 5 ppm. of phorate accumulated in roots of bean plants compared with Brussels sprouts which accumulated as much as 25 and 35 ppm. The stems and leaves, however, contained 0.5 and 2 ppm. respectively in beans but only 2 ppm. and 0 ppm. in Brussels sprouts, and the leaves therefore showed less toxicity to M. persicae on Brussels sprouts than in beans. The rate of phorate metabolism was faster in beans (table 33) than in Brussels sprouts, phorate was oxidised to 1 and 2 ppm. of sulphoxide after 24 and 96 hours after treatment and gave 38% and 46% mortality of aphids respectively

compared with beans in which it converted to 1.25 and 2.5 ppm. of phorate sulphoxide in stems and leaves showing aphid mortality of 48% and 50% respectively.

B. Thus the relative in effectiveness of phorate in Brussels sprouts may be caused by decreased translocation from the roots associated with decreased conversion of phorate to its sulphoxide and sulphone.

These results fit in with those of Bowman and Casida (1957) and Blinn (1964) who showed that phorate is a weak anticholinesterase agent but its oxidative metabolites are active cholinesterase inhibitors.

Summarized Discussion

Factors affecting activity and persistence of systemic insecticides

Systemic insecticides applied to soil are influenced by many factors which control their uptake and persistence in plants and their toxicity to insects. Such factors are:

(A) Soil factors
- (1) Soil type
- (2) Moisture content of soil
- (3) Leaching
- (4) Degradation in soil by micro-organisms
- (5) Site of placement of insecticide in soil*
- (6) Amount applied to soil*

(B) Plant factors
- (7) Plant species*
- (8) Rate of metabolism in plant*
- (9) Factors affecting translocation in plants*
- (10) Nature of root growth*

(C) Insecticide factors	(I1) Volatility (I2) Solubility in water (I3) Inherent rate of degradation (I4) Order of toxicity of parent compound and metabolites
(D) Climatic factors	(I5) Temperature* (I6) Rainfall
(E) Insect facors	(I7) Aphid species* (I8) Size and age (I9) Site of feeding on plants* (20) Presence of resistant strains

All may have profound effect. Some of those marked with asterisks, were studied and the main results are summarised as follows:-

(I) The order of effectiveness against two species of brussels sprouts aphids of four systemic pesticides applied to sandy soil in field cnditions at I-2 lb/acre was:- aldicarb > pirimicarb > menazon > phorate. All except phorate gave good protection from aphid attack for I5 weeks.

(2) All the insecticides were more effective against aphids on old than young leaves and were more toxic to Brevicoryne brassicae than to Myzus persicae.

(3) The reproductive rates of the aphids were inversely related to toxicity of the insecticides.

(4) The site of placement of aldicarb in the soil affected its toxicity. The best long-term control were obtained by placing it 2-3 in.deep and 9 in.to the side of the plant though initially this treatment was not so good as placement close to the plant stem or 9 in. below it. The placement of a nitrogenous fertilizer with the insecticide did not enhance its toxicity or persistence.

(5) As from soil, phorate taken up by brussels sprouts plants from sand or water culture was less effective than the other insecticides but applied to the water intake of leaf discs the order of toxicity to aphids was pirimicarb > menazon > phorate > aldicarb.

(6) Phorate was relatively much more effective when taken up by roots of field bean plants than by brussels sprouts and, in an attempt to explain the differences, chemical analyses were made which showed that phorate was less quickly changed to toxic metabolites in brussels sprouts and that it accumulated in roots and was not translocated to leaves so rapidly as in beans.

(7) Aldicarb and its toxic metabolites accumulated more in leaf margins than in the centres of leaves.

(8) Toxicity of aldicarb and pirimicarb increasedvery greatly with increasing temperature when the insecticides were applied to leaf discs. Many factors may be responsible for this, including increased rate of uptake by the leaf disc with increasing temperature.

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Appendix Table 1 Percent Kill of aphids on leaf discs treated with different doses of Insecticides.

Bio-assay Number	Insecticide + Temp.	Aphid species	Time of examination Hours	No. of aphids/dose	Dosages					
					0.934%	0.156%	0.026%	0.0043%	0.0007%	Untreated
1a	Menazon 20°C		24	50	100	92	76	30	12	0
			48	50	100	94	82	36	22	0
			72	50	100	100	92	62	42	4
					0.0043%	0.0007%	0.00012%	0.00002%	0.000003%	untreated
1b	Menazon 20°C		24	50	68	50	36	16	6	0
			48	50	86	72	52	30	20	0
			72	50	100	92	82	60	46	0
					0.934%	0.133%	0.019%	0.0027%	0.0004%	untreated
2a	Menazon 20°C		24	50	90	60	48	16	0	2
			48	50	100	76	64	40	24	2
			72	50	100	86	74	60	46	10
					0.0027%	0.0004%	0.000055%	0.0000081%	0.000001%	untreated
2b	Menazon 20°C		24	50	92	60	36	18	10	4
			48	50	94	80	56	36	18	8
			72	50	98	90	72	56	36	10
					0.104%	0.017%	0.003%	0.0005%	0.00008%	untreated

3a	Pirimi-carb 20°C	24	50	90	64	46	18	8	0
		48	50	100	94	80	44	36	8
		72	50	100	98	94	62	54	14
				0.003%	0.0005%	0.00008%	0.000014%	0.0000023%	untreated
3b	Pirimi-carb 20°C	24	50	82	52	44	6	0	4
		48	50	96	86	84	54	48	10
		72	50	00	96	88	74	64	16

				Dosages					
				0.05%	0.0083%	0.0014%	0.00023%	0.00004%	Untreated
4a	Aldi-carb 20°C	24	50	32	26	18	12	6	0
		48	50	44	32	24	18	12	0
		72	50	72	64	44	38	34	4
				0.0014%	0.0002-3%	0.00004%	0.000007%	0.0000011%	untreated
4b	Aldi-carb 20°C	24	50	52	26	18	8	6	0
		48	50	58	46	26	18	14	0
		72	50	84	70	54	38	30	6
				0.34%	0.056%	0.0093%	0.00155%	0.00026%	untreated
5a	Phorate 20°C	24	50	74	34	0	C	0	0
		48	50	98	78	28	2	0	0
		72	50	100	92	60	34	20	0
				0.0093%	0.0015-5%	0.00026%	0.000043%	0.000007%	untreated

5b	Phorate 20°C	24	50	24	10	4	0	0	0
		48	50	62	36	28	2	0	0
		72	50	74	58	44	28	12	2
				0.934%	0.156%	0.026%	0.0043%	0.0007%	untreated
6a	Menazon 20°C	24	50	100	88	64	30	18	0
		48	50	100	94	86	46	34	4
		72	50	100	100	94	64	42	6
				0.625%	0.125%	0.025%	0.005%	0.001%	untreated
6b	Pirimi-carb 20°C	24	50	100	100	84	52	22	4
		48	50	100	100	96	90	74	4
		72	50	100	100	98	95	78	4
				0.934%	0.156%	0.026%	0.0043%	0.0007%	untreated
7a	Menazon 20°C	24	50	100	92	76	46	22	0
		48	50	100	96	92	66	52	4
		72	50	100	100	96	70	56	6
				0.34%	0.068%	0.0136%	0.0027%	0.0054%	untreated
7b	Phorate 20°C	24	50	100	100	30	8	4	2
		48	50	100	100	68	32	26	6
		72	50	100	100	82	66	46	8

				Dosages					
				0.934%	0.156%	0.026%	0.0043%	0.0007%	untreated
8a	Menazon 20°C	24	50	94	88	66	24	8	0
		48	50	98	98	92	52	36	2
		72	50	100	100	94	60	50	4
				0.05%	0.01%	0.002%	0.0004%	0.00008%	untreated
8b	Aldi-carb 20°C	24	50	28	20	12	0	0	0
		48	50	42	26	20	16	12	0
		72	50	76	50	46	36	26	0
				0.34%	0.068%	0.0136%	0.0027%	0.00054%	untreated
9a	Phorate 20°C	24	50	100	46	18	16	4	0
		48	50	100	100	50	28	16	0
		72	50	100	100	70	58	38	0
				0.05%	0.01%	0.002%	0.0004%	0.00008%	untreated
9b	Aldi-carb 20°C	24	50	22	18	14	0	0	0
		48	50	62	30	20	14	12	0
		72	50	80	56	48	42	36	4
				0.625%	0.104%	0.017%	0.003%	0.0005%	untreated
10a	Pirimi-carb 15°C	24	50	98	94	82	50	8	0
		48	50	100	100	88	62	18	4
		72	50	100	100	94	80	32	8
				0.003%	0.0005%	0.000083%	0.000014%	0.0000023%	untreated

10b	Pirimi-carb 25°C	24	50	96	88	28	24	10	0
		48	50	98	92	80	64	48	8
		72	50	100	94	86	84	68	8
				0.017%	0.003%	0.0005%	0.00008 3%	0.000014%	untreated
11a	Pirimi-carb 15°C	24	50	98	78	32	30	4	0
		48	50	100	86	48	52	14	0
		72	50	100	90	62	56	28	6
				0.0005%	0.0000-83%	0.000014%	0.000 0023%	0.0000004%	untreated
11b	Pirimi-carb 25°C	24	50	56	40	30	14	12	0
		48	50	94	82	78	64	56	8
		72	50	98	90	80	64	60	8
				0.25%	0.05%	0.01%	0.002%	0.0004%	untreated
12a	Aldi-carb 15°C	24	50	20	8	4	0	0	0
		48	50	48	28	20	14	6	2
		72	50	74	54	38	28	24	4
				0.05%	0.01%	0.002%	0.0004%	0.00008%	untreated
12b	Aldi-carb 25°C	24	50	36	32	24	16	10	2
		48	50	74	56	52	50	40	4
		72	50	96	94	88	84	80	6

				0.25%	0.05%	0.01%	0.002%	0.0004%	untreated
13a	Aldi-carb 15°C	24	50	28	4	2	0	0	0
		48	50	62	14	6	0	0	0
		72	50	92	56	28	20	14	0

				0.0004%	0.0000-8%	0.000016%	0.0000032%	0.00000064	untreated
13b	Aldi-carb 25°C	24	50	24	12	12	8	6	0
		48	50	36	30	22	16	14	4
		72	50	92	80	68	58	50	8

Table 2. Details of probit analyses based on aphid mortality by different doses of insecticides.

				LC 50			IC 50 in ppm of wet wt. of leaf disc		
				24 Hours	48 Hours	72 Hours	24 h.	48 h.	72 h.
1a	20°C	M.p.	Menazon	2.99±0.085	2.6 ±0.094	2.18±0.106	2.44	0.99	0.38
1b		B.b.	"	1.66±0.164	.87±0.141	1.80±0.182	0.099	0.02	0.002
2a	20°C	M.p.	Menazon	3.61±0.103	2.70±0.131	2.17±0.199	10.00	1.25	0.37
2b		B.b.	"	1.28±0.144	0.60±0.128	1.85±0.165	0.048	0.01	0.002
3a	20°C	M.p.	Pirimicarb	2.7 ±0.011	1.7 ±0.11	1.2 ±0.148	1.25	0.125	0.039
3b		B.b.	"	1.44±0.099	1.9 ±0.060	1.34±0.244	0.068	0.002	0.00055
4a	20°C	M.p.	Aldicarb	4.9 ±0.486	4.02±0.498	2.5 ±0.250	198.0	26.0	0.79
4b		B.b.	"	2.6 ±0.332	1.6 ±0.260	0.5 ±0.177	0.99	0.099	0.0079
5a	20°C	M.p.	Phorate	4.1 ±0.082	3.3 ±0.069	2.3 ±0.105	31.0	5.0	0.5
5b		B.b.	"	3.70±0.188	2.7 ±0.119	1.7 ±0.168	12.5	1.25	0.125
6a	20°C	M.p.	Menazon	2.98±0.099	2.50±0.144	2.18±0.116	2.38	0.79	0.38
6b		M.p.	Pirimicarb	2.73±0.085	1.32±0.30	1.18±0.335	1.34	0.052	0.038
7a	20°C	M.p.	Menazon	2.91±0.10	2.30±0.15	2.0 ±0.15	2.03	0.52	0.30
7b		M.p.	Phorate	3.99±0.024	2.99±0.026	2.22±0.109	24.0	2.4	0.42
8a	20°C	M.p.	Menazon	3.27±0.059	2.32±0.125	2.10±0.146	4.65	0.52	0.32
8b		M.p.	Aldicarb	4.95±0.427	4.00±0.496	2.55±0.197	222.0	25.0	0.89
9a	20°C	M.p.	Phorate	4.08±0.10	3.25±0.083	2.24±0.115	30.0	4.4	0.43
9b		M.p.	Aldicarb	5.00±0.482	4.02±0.263	2.50±0.210	255.00	26.0	0.79

(Table 2 contd.)

10a	15°C	M.p.	Pirimicarb	2.83±0.091	2.3 ±0.089	1.98±0.068	1.69	0.49	0.24
10b	25°C	M.p.	"	1.40±0.095	1.8 ±0.077	1.2 ±0.230	0.063	0.0015	0.00039
11a	15°C	B.b.	Pirimicarb	1.66±0.085	1.33±0.094	1.06±0.036	0.11	0.05	0.028
11b	25°C	B.b.	"	1.1 ±0.115	2.8 ±0.319	2.3 ±0.322	0.031	0.00015	0.00005
12a	15°C	M.p.	Aldicarb	5.51±0½463	4.64±0.226	3.5 ±0.257	809.00	109.0	7.9
12b	25°C	M.p.	"	4.6 ±0.642	2.10±0.304	1.26±0.769	99.5	0.31	0.0005
13a	15°C	M.p.	Aldicarb	5.19±0.341	4.52±0.210	3.48±0.240	387.2	82.77	7.85
13b	25°C	M.p.	"	4.50±0.293	1.84±0.314	1.29±0.561	79.05	0.17	0.00048

LC 50 Based on Log IO^{5} x concentration (percent)

Table 3 Slope of regression lines based on aphid mortality by different doses of insecticides

Insecticide	Bioassay number	24 Hours Slope ± S.E.	48 Hours Slope + S.E.	72 Hours Slope ± S.E.	24 Hours Chi. sq.	48 Hours Chi. sq.	72 Hours Chi. sq.
Menazon	1a	1.17 ± 0.11	1.11 ± 0.02	1.11 ± 0.12	1.92	2.11	2.23
"	1b	0.53 ± 0.03	0.62 ± 0.12	0.69 ± 0.01	2.28	3.52	5.70
"	2a	0.85 ± 0.06	0.72 ± 0.06	0.62 ± 0.02	2.10	2.32	1.93
"	2b	0.70 ± 0.12	0.78 ± 0.03	0.69 ± 0.05	1.98	2.11	3.44
Pirimicarb	3a	0.9 ± 0.04	0.99 ± 0.13	0.96 ± 0.12	6.42	2.07	3.65
"	3b	0.99 ± 0.07	0.63 ± 0.05	0.73 ± 0.09	2.16	3.39	1.41
Aldicarb	4a	0.33 ± 0.11	0.32 ± 0.07	0.35 ± 0.07	2.20	0.14	1.56
"	4b	0.52 ± 0.02	0.41 ± 0.13	0.51 ± 0.10	1.51	1.34	0.44
Phorate	5a	1.45 ± 0.13	1.39 ± 0.01	0.98 ± 0.05	1.70	2.11	4.99
"	5b	0.75 ± 0.01	0.78 ± 0.06	0.54 ± 0.10	0.42	3.98	0.40
Menazon	6a	0.97 ± 0.08	0.94 ± 0.12	1.07 ± 0.10	2.01	1.93	2.14
Pirimicarb	6b	1.19 ± 0.09	0.87 ± 0.10	0.93 ± 0.04	2.49	1.78	2.22
Menazon	7a	0.95 ± 0.04	0.78 ± 0.02	0.98 ± 0.03	0.48	2.35	6.66
Phorate	7b	1.41 ± 0.01	1.16 ± 0.08	1.12 ± 0.07	14.84	5.30	0.96
Menazon	8a	1.51 ± 0.04	0.89 ± 0.05	1.01 ± 0.05	1.79	0.79	2.11
Aldicarb	8b	0.62 ± 0.06	0.33 ± 0.13	0.43 + 0.010	1.19	2,17	2.62
Phorate	9a	1.07 ± 0.01	1.03 ± 0.04	0.91 ± 0.07	3.48	7.57	5.34

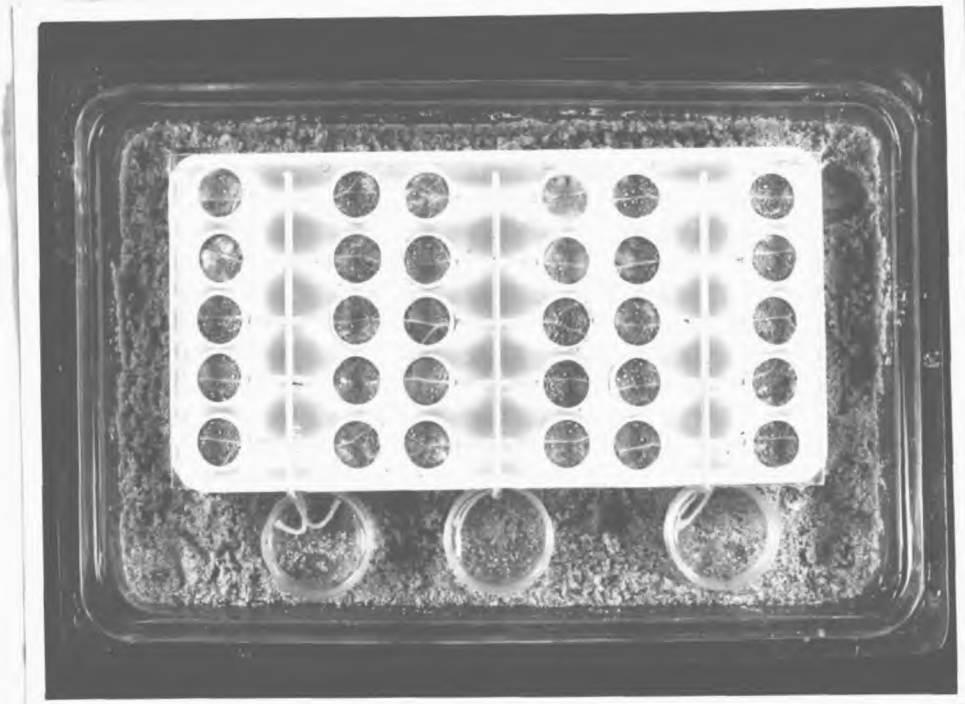
(Table 3 contd.)

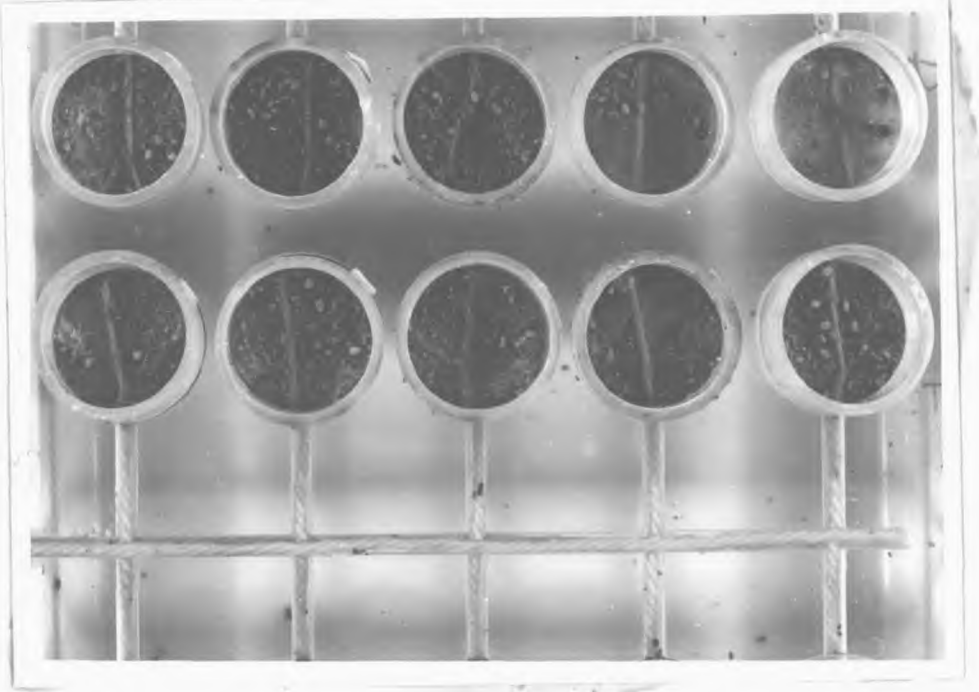
Insecticide	Bioassay number	24 Hours Slope ± S.E.	48 Hours Slope ± S.E.	72 Hours Slope ± S.E.	24 Hours Chi. sq.	48 Hours Chi. sq.	72 Hours Chi. sq.
Aldicarb	9b	0.59 ± 0.02	0.63 ± 0.11	0.40 ± 0.01	3.25	2.16	3.49
Pirimicarb	10a	1.09 ± 0.12	1.24 ± 0.01	1.95 ± 0.13	3.42	1.04	4.52
"	10b	0.98 ± 0.03	1.56 ± 0.05	0.94 ± 0.08	12.71	6.25	5.31
"	11a	1.09 ± 0.01	1.00 ± 0.02	0.88 ± 0.03	8.13	6.39	6.25
"	11b	0.91 ± 0.04	0.44 ± 0.05	0.54 ± 0.02	0.89	1.12	2.05
Aldicarb	12a	0.74 ± 0.11	0.53 ± 0.05	0.42 ± 0.11	0.33	1.38	1.45
"	12b	0.33 ± 0.07	0.28 ± 0.12	0.39 ± 0.06	0.34	12.23	7.54
"	13a	0.83 ± 0.10	0.79 ± 0.13	0.73 ± 0.05	2.39	3.13	2.79
"	13b	0.37 ± 0.11	0.33 ± 0.05	0.39 ± 0.12	1.93	2.41	5.29

Table 4 Estimates of insecticide residues and standard errors (log scale) in plants using aphid bioassays.

Insecticide Aphid species	Date of application	Rate of application	Estimate of Residue 24 Hours conc. percent + S.E.	48 Hours conc. percent + S.E.	72 Hours conc. percent + S.E.
Aldicarb *B. brassicae*	28th May 1968	2 lb a.i. per acre	1.23±0.43	2.0 ±0.31	1.04±0.61
Aldicarb *M. persicae*			2.98±0.29	4.7 ±0.23	3.54±0.53
Menazon *B. brassicae*			2.70±0.31	2.78±0.41	2.3 ±0.27
Menazon *M. persicae*			4.66±0.29	4.58±0.59	4.50±0.33

Plate No. 1 Biological assay technique for the assessment and comparison of systemic insecticide residues.





with fertilizer

Plate No. 2 Effect of placement of aldicarb/2-3" deep and 2-3" away from plants on the condition of plant and quality and quantity of sprouts.

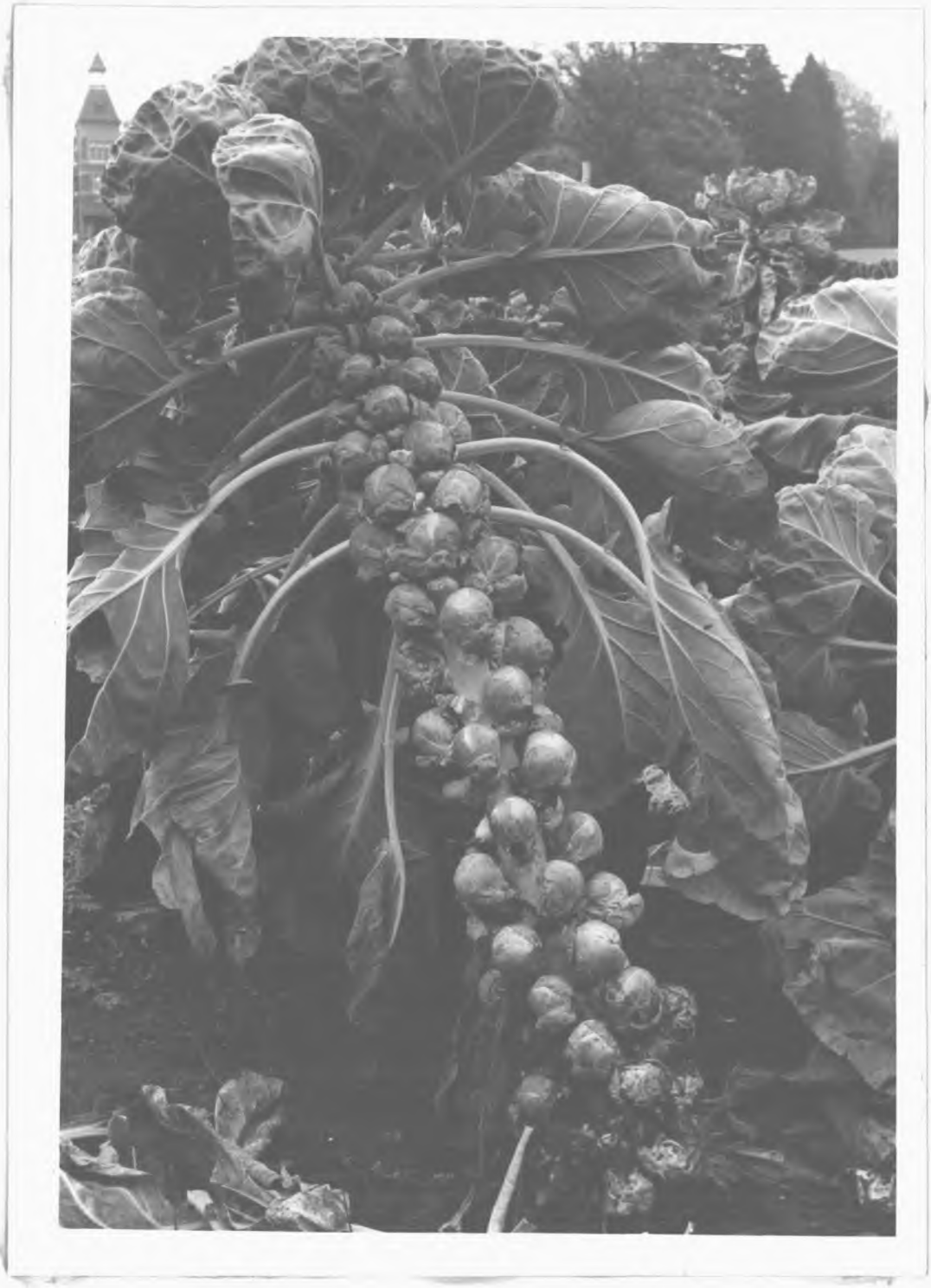


Plate No. 3 Effect of placement of aldicarb 2-3" deep but 9"-1' away from plants as shown by quality and quantity of sprouts.

Plate No. 4 Untreated control plants Brussels sprouts.