THE ACTION OF SYSTEMIC APHICIDES ON APHIDS AND THEIR ANTHOCORID PREDATORS.

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by

W. M. Elliott, B.Sc. A.R.C.S.

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of the University of London.

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Imperial College of Science
and Technology,
Field Station,
Silwood Park,
Sunninghill,
Ascot,
Berkshire.

ABSTRACT

The work described is a study of the selectivity of three aphicides. The effects of phorate, menazon and dimethoate on *Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum*, feeding on beans (*Viccia faba*), are compared with their effects on two aphid predators. These were *Anthocoris nemorum*, which is associated with a variety of plants and prey, and *Anthocoris confusus*, which occurs mainly on trees, where it feeds on aphids.

Aphid bioassays are described, which establish the effects of the aphicides, applied to the cut roots of beans in the laboratory, thus providing LD50 data for comparison with dosages affecting Anthocorids. The effects of the aphicides on aphids are compared with their different effects on Anthocorid eggs laid in the same plants.

Field Experiments were also done with phorate and menazon, applied to soil at different concentrations, to assess the effects of normal agricultural treatments on Anthocorid eggs laid in bean plants. Differences between egg mortality of *A. nemorum* and *A. confusus*, were compared with the different oviposition sites of the two species.

The distribution of phorate derivatives, from root applications, in beans and other crop plants, was studied, using P32 labelled phorate, and this is compared with the oviposition site of *A. nemorum* in these plants.

The action of dried deposits of the aphicides, on Anthocorids and aphids, is compared with the action via roots.

The uptake of phorate derivatives by Anthocorids, from bean plants and poisoned aphids was demonstrated, using S35 labelled phorate applied to the roots. The effects of the

aphicides, thus recieved, on survival and fecundity of Anthocorids is described.

The different effects of the aphicides, on all stages of Anthocorids, is discussed, with particular emphasis on the effect of phorate on Anthocorid eggs.

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Section 1A Introductory Review.

The research described in this thesis is a study of the selectivity of three aphicides, in relation to their effects on the Aphid predators *Anthocoris nemorum* and *A. confusus*, at all stages in their Life Cycles. This review states the case for selective insecticide studies.

Selective Insecticides and Integrated control: Historical review and definitions

The term "selective insectide" was defined by Ripper (1944) as an "insecticide which would kill a much greater proportion of the pests than the predators and parasites of the pest". The treatment in question was nicotine fumigation of Brussels' sprouts against *Brevicoryne brassicae*. This treatment was shown to kill over 87% of *B. brassicae*, while leaving its coccinellid and Syrphid predators unharmed. The object was to use the surviving predators to "mop up" any aphid survivors, thus preventing a rapid resurgence and the selection and survival of resistant forms, and delaying the need for further treatment. A further example of a selective treatment was the use of the first practical systemic aphicide (Schradan)(Ripper et al 1949).

Ripper (1951) subdivided selective insecticides into: those with "Physiological Selectivity", which killed the pest at concentrations not affecting beneficial insects and another category "Ecological selectivity" where differences in ecology were used as the basis for obtaining selective effects, from chemicals which were physiologically toxic to predators. For example Isopestox killed predators, when applied as a direct spray, but when taken up by the roots, still killed plant feeding insects, but

was harmless to predators.

Potter (1961) distinguished Physiogically selective compounds useful in stored products and Public health, which were selective against insects, but of low mammalian toxicity and those used on Field crops, where low toxicity to beneficial insects was also required. In addition Potter (1961) included ecologically selective formulations of non-physiologically selective insecticides in a category "Physical Selectivity". This also included pesticides of short term toxicity, timed to catch pests at vulnerable stages, the use of attractants to lure particular pest insects into contact with non-selective insecticides and the localization of treatments (e.g. seed dressing or in-row applications of non-selective soil insecticides, as apposed to broadcast treatment). Bartlett (in De Bach 1964) in addition to methods mentioned above, incorporated the use of unsprayed portions of crop as reservoirs of beneficial insects and also the timing of routine spraying to coincide with the most resistant stage in a particular natural enemies' life cycle, into "Physically" selective methods.

Ripper (1958) proposed the integration of biological and chemical control, by means of selective insecticides with two aims, 1. Reduction of pests to sub-economic levels, high enough to retain natural enemics within the crop. 2. Elimination of surviving pest insects by natural enemies, at the end of the growing season, to prevent resistant forms from persisting and spreading. Ripper (1958) showed that *Brevicoryne brassicae* could be controlled in this way with low doses of Schradan, which left sufficient aphids to retain predators and parasites within the crop. Such a system did not work with cotton aphids in the Sudan

(Ripper 1958) since the only important predator (Scymnus marginalis) departed when the aphid population fell after treatment. This system depends upon predators and parasites which can act at relatively low host densities.

Stern et al (1959) gave a full account of the integrated control concept, based on their work on the Alfalfa Aphid which became resistant to parathion Stern (1958). This work emphasised the importance of "Economic thresholds" (aphid levels at which insecticide action must be taken to prevent economic damage), of the need to consider the populations of predators and parasites and of the treatment of growing crops as ecosystems, to which no cultural or pesticidal treatments should be made without considering its influence on all the components. Under these conditions it is not absolutely necessary to "eliminate" the aphid at the end of the season, since resistant forms are not so likely to occur when aphicides are used at low doses and only when outbreaks are expected. This is particularly true in the case of a crop lasting more than one year in an equable climate, where the seasonal fluctuation in predation pressure is not severe. In theory, treatments are applied at low doses to local outbreaks, and the insecticides do not need to be highly physiologically selective, (Stern et al 1960), so long as in general they are more active against the pest than against the predators and parasites and do not have a persistant action against them. The beneficial forms can then re-invade from untreated areas if the aphid population rises.

Apart from the value of selective treatment in integrated control programmes, aimed at particular pests, there is further evidence for the value of predators and

desirability of selective treatments.

The above account traces the origin of some modern ideas on pest control, which are based on the assumption of the value of predators. Evidence for this conclusion is given below (with particular reference to cases involving Anthocorids).

Direct Evidence that predators are important in pest control.

Most of the evidence on the value of predators in biological control comes indirectly from interpretations of the results of non-selective insecticide applications as dicussed below, however some independent direct observations are described here. More detail is given by De Bach (1964) in which apart from the examples described here, he stressed the importance of an adequate programme of sampling to follow the establishment and success of introduced biological control agents. De Bach (1964) also discusses techniques for determining the action of natural enemies e.g. : mechanical exclusion of predators, from infested plants, followed by counts of the increase of the prey species, compared with the fate of the prey species where predators are allowed access, De Bach (1964) points out however, that this may influence the dispersive behaviour of the prey in some cases and modify the micro-climate of the pest.

Hodek et al (1964) used an exclusion technique to evaluate coccinellid predation on Aphis fabae and found the effects of different prey/predator ratios on whether the predators could prevent aphid increases, was related to the temperature at the early stages of natural infestation.

Predatory insects were kept in laboratory conditions

by Nelson and Henderson (1959), Conrad (1959), Hassan (1957), Putman (1955) and Nielson and Currie (1960) and the numbers of prey eaten per day from a large supply, or during development were compared. The searching efficiency of the <u>different</u> species under field conditions are not reflected in these experiments, nor is the time spent searching, suppressing and eating the prey (Holling 1959) which may be more significant under field conditions than the actual capacity for food.

In addition the relative abundance of each predator species is important, when comparing the predators of any prey. Thus Simpson and Burkhardt (1960) found that, while <u>Orius</u> sp. (Anthocoridae) had a <u>lower</u> daily Alfalfa aphid consumption than 9 other predators, when the consumption of each species was multiplied by the field <u>abundance</u> : this species was the 3rd most important predator. A similar result was found by Goodarzy and Davis (1958).

A worthwhile approach is that of Fletcher and Thomas (1943) who seperated and counted eggs and 1st Instar larvae of <u>Heliothis armigera</u> on cotton, which had been eaten by Coccinellids and <u>Orius</u> spp, from infertile or hatched eggs. They showed that the ammount of predation was important, in relation to prediction of damage from egg counts. This method is the nearest approach in <u>predation</u> studies to "percent parasitism" which is relatively easy to obtain.

Huffaker and Kennet (1956) checked the value of <u>Typhlodromus</u> against <u>Tarsonemus pallidus</u> on strawberries, by <u>hand - removal</u> of predators technique (in a greenhouse), before using <u>parathion</u> to eliminate this predator in the field.

Huffaker and Kennet (1956) postulated that newly established strawberry beds suffered _Tarsonemus_ damage because natural invasion by the predator was inadequate and uneven, but after 2 years predator distribution became sufficient. They showed that artificial infestation of predators _prevented_ damage to 2nd year plants in centres of _Tarsonemus_ activity, but damage _did_ occur where no predators were introduced, or where parathion was applied. They also suggested that both prey _and_ predator species should be introduced in new plantings, to create a stable equilibrium and forestall outbreaks. This is comparable to retaining a low aphid population in the integrated control of the Alfalfa aphids (see above).

Indirect Evidence that predators are important in insect Control.

Resurgence phenomena. Ripper (1951) demonstrated that when a non - selective insecticide was used against aphids (for instance parathion on cabbage and strawberry aphids), the kill obtained initially was offset by a very rapid rise in aphid populations to damageing levels, due to annihilation of the predators. Further examples of pest resurgence after treatment are discussed by Ripper (1956) and they are generally associated with the toxicity of the insecticides to natural enemies. De Bach (1964) discusses more recent literature, and Pementel (1961) gave field experimental data on resurgences of aphids on _Brassica_ crops, after non-selective treatments which reduced predators and parasites.

Problems arising when insecticide treatments for a pest, eliminate the natural enemies of another species.

Several well studied examples show how the use of non-

selective insecticides against pest species have created new pests through the destruction of their natural enemies. These are usually taken as evidence of the practical value of natural enemies.

(A) Orchard pests.

Spider mites provide the classical example of this phenomenon. Until modern insecticides were used, they were rarely pests.

In this connection, the toxicity of orchard pesticides to beneficial insects was reviewed by Bessemer (1964) and reveals a complex situation which is discussed here since Anthocorids are involved. Thus Massee and Steer (1929) first associated the increase to pest proportions of *Panonychus ulmi* with the use of tar distillate washes applied against overwintering psyllids, scales and aphids on apples. They attributed this to destruction of hibernating predators (mainly *Anthocoris nemorum* and predacious mites). Subsequently Lord (1949), Massee (1954), Chaboussou and Bessard (1954) and Davis (1952), showed that chlorinated hydrocarbons also killed mite natural enemies, including *Orius* spp.

Collyer and Kirby (1955), Clancy and McAlister (1956), Hukusima et al (1958 and 1959), Reed (1959), MacPhee (1953) and MacPhee and Sandford (1954)(1956), investigated changes in orchard fauna and associated these with insecticides, Acaricides and fungicides and Cakebread (1947) Graham and Higgons (1953) in addounts of D.N.O.C. and chlorbenzide gave special attention to their effects on predators, including Anthocorids.

Pickett and Patterson (1953) reviewded the history of *Panonychus ulmi*, *Spilonota ocellana* and *Lepidosaphes ulmi*

outbreaks in Nova Scotia and associated these with elimination of natural enemies by D.D.T. and by sulphur fungicides. De Bach and Bartlett (1951) reported a similar situation for mites and scales on citrus in the U.S.A. and described the loss of valuable man-made biological control of scales by Vedalia beetles, due to D.D.T. (Bartlett and Lagace 1960). Sugonyaev (1955) described the timing of oil sprays against Coccus hesperidum on citrus to co-incide with the resistant (coccoon) stage of a parasite. Putman and Herne (1960) report similar problems on Peach orchards. High populations of Psylla piricola in commercial pear orchards were associated with the killing of Anthocoris sp. and Chrysopa sp. by D.D.T. (Watson and Wilde 1963) (Bonnemaison and Missonier 1956). Nickel et al (1965) devised low dosage guthion treatments for Codling moth on pears to reduce the effects on these natural enemies.

B. Problems of pest incidence through destruction of natural enemies on Cotton.

This is also discussed since Anthocorids are involved. Gaines (1957) reviews insecticide treatments for cotton pests.

Smith and Fontenot (1942) reported aphid outbreaks on cotton associated with destruction of beneficial insects by arsenicals used for bollweevil and bollworms, and subsequently the use of chlorinated hydrocarbons for Lepidopterous pests, mirids and bollweevils destroyed aphid, mite and Lepidopteran egg predators, one of the most numerous of which was Orius insidiosus (Anthocoridae) (Newsom and Smith (1949)), Campbell and Hutchins (1952), Glick and Lattimore (1954), Gaines (1955) and Pfrimmer (1964).

<u>Predator elimination and other effects of insecticides on pest populations</u>.

Observations that some natural enemies decreased and their prey species increased after use of insecticides led to the development of the "Insecticidal Check" method for evaluating predation (De Bach 1946). This consists of eliminating the predators of a pest, by using an insecticide that does not harm the pest, and then attributing the increases in the pest population to the portion of it, i.e. normally consumed by natural enemies surviving and breeding.

A "biological check" technique for evaluating beneficial insects in the field was also proposed by De Bach et al (1951) comparing the Coccid populations of trees infested by, or kept free of, ants, which tend to reduce natural enemies. These methods can only be applied when the effects of Insecticides or ants on the pest species have been thoroughly studied.

In contrast to the above examples, there is some evidence that insecticide caused pest outbreaks should be examined more carefully thus Kuenen and Post (1958) showed that elimination of predators of <u>Panonychus ulmi</u> was not <u>always</u> followed by outbreaks of this pest and suggested that such mite populations were partly limited by competition and that the mites' reproduction was stimulated, in recorded <u>outbreaks</u>, by the effect of D.D.T. on the leaves and that fertilisation programmes, introduced at the same time as insecticide programmes, might account for the rise of the mite to pest status by a similar nutritional effect Fleschner (1952), showed that individual citrus trees, deprived of predators by <u>hand</u>, varied greatly in the

infestations of Citrus red mites that developed, some having sub-economic populations lower than trees with their complement of predators undisturbed, whereas others, as expected, became overinfested.

Perhaps more important, Fleschner (1952) also showed that Zinc and D.D.T. spray treatments produced a rapid increase in mite populations, which only gradually descended to normal, even on trees which had been kept predator-free

It is interesting that D.D.T. benefited mites through some effect on or via the plants, since it occurred even when the mites were not directly treated.

However the bulk of evidence on the subject of insecticide induced outbreaks supports the idea that predators or parasites are important.

Work on Selective toxicity of insecticides.

Way (1958), Bartlett (1958), Shorey (1963), Heathcote (1963) and Zeleny (1965) have all published work on selective pest control with emphasis on finding insecticides non-toxic to predators and parasites. Bartlett (1958) proposed an "index of selectivity" the ratio between the LD5% of an aphid predator and the LD95% of the aphid prey. Substances with a high selectivity index could thus be used with confidence, whereas those with a low index would preferably not be used when the predator in question was numerous.

A more general approach was used by Carlson (1959), Reynolds et al (1960) and Shorey et al (1962) in which all predatory insects (not only aphid predators characteristic of a crop were counted after insecticide treatments. In addition Harries and Valcarce (1955) and Bartlett (1958)

examined effects of Insecticides on predators of general importance (i.e. on many different crops) and from such work the results of a new programme on a crop can be predicted before field trials.

Value of Anthocoridae in biological control and biology of the species used in this Thesis.

Apart from examples mentioned in the above and A.nemorum (see below), examples of this family associated with pests are frequent thus : Madsen (1961) regarded Anthocoris melanocerus as the most important predator of Psylla pyricola in British Columbia. Iglinsky and Rainwater (1950) postulated that Orius insidiosus was an important cotton mite predator in the U.S.A. Wille (1951) stated that Paratriphleps laeviusculus killed 70% of eggs and first instar larvae of Heliothis virescens on cotton in Peru, and organised interplanting of cotton with maize to increase this predator and give biological control. Ito et al (1960) recorded an Orius species as the most important predator of Pieris rapae crucivora Bois duval on cabbage in Japan.

The non-specific tendencies of some Anthocoridae reduce their effectiveness as predators in some cases thus, although the non-specific A. nemorum was shown to leave fruit trees when large mite populations were reduced by chlorbenzide and was thus shown to be dependent on this food supply (Van de Vrie and de Fluiter 1958), Ewing and Ivy (1943) investigating the association of Heliothis armigera outbreaks with heavy aphid populations on cotton, found that the relatively non-specific Orius insidiosus which feeds on mites, aphids and Lepidopterous eggs, when caged on aphid infested plants, destroyed far fewer H. armigera eggs than

they did on un-infested plants: thus 47/100 eggs hatched and 15 larvae reached 2nd instar on infested plants, compared with 3/100 eggs and 0 2nd instar larvae on un-infested plants. Thus in general this non specific predator may not be sufficiently effective at the right time, to control Lepidopterous eggs even if it is present in the crop at an early stage of infestation. Barber (1943) for instance, showed that while Orius insidiosus increased predation with increasing egg density of Corn Earworm (= Heliothis zea Boddie), a ceiling was reached at 100 plus eggs per plant, after which predation remained around 50% irrespective of egg density. Harrison (1960) showed that O. insidiosus constituted about 50% of the predation of H. zea eggs, and D.D.T. almost halved the percentage of eggs destroyed by predators.

Anthocoris nemorum : Collyer (1953) reviewed the literature on this species as a mite predator in apple orchards. It has the advantage, over Mirid predators, in Orchards, of 2 generations per year, with adults dispersing by flight to new breeding sites in April and July. (Anderson 1961) and this species therefore re-invades a mite infested orchard in the summer, even if previously reduced by spraying, A. nemorum has 3 generations per year in France (Bonnemaison and Missonier 1956). Dicker (1952) stated that Tachyporus spp. and Anthocoris nemorum may account for summer declines of populations of Pentatrichopus fragaefolii on strawberries in England. Hill (1957) recorded this species from 36 different plants, including carrot, potato, broad bean, wheat, hop, barley, blackcurrant, strawberry, blackberry and raspberry. A. nemorum has been observed feeding on

Collembola, Psocoptera, Thysanoptera larval Lepidoptera, Cecidomyid larvae and Panonychus ulmi and Tetranychus telarius (Hill 1957). It also feeds on Psyllids : for example Psylla mali and P. pyri and on many important aphids for example Aphis fabae, Eriosoma lanigerum and Myzus persicae (Hill 1957).

A. nemorum may be present on plants before these are attacked by aphids, since it can subsist on other prey and this could be significant at an early stage of aphid invasion. For instance the writer recorded it as out-numbering Microlophium evansii on Urtica in the spring of 1964. Anderson (1962) described the bionomics of this species and six other British species. A. nemorum differs from most of the others in the wide range of host plants and foods. A. confusus is commonly found feeding on aphids on Oak, Sycamore, Lime and Beech. It is used here as a convenient laboratory animal and has not been recorded breeding on field crops. Two individuals were recorded on beans, during this work. The other British Anthocorids are considerably more host-plant and prey specific except A. nemoralis, which has several hosts.

Sands (1957) described in detail the nymphs of British Anthocoridae and Hill (1957) gave an account of the biology and eggs of A. nemorum and of A. confusus (1965). The reproductive systems and fertilisation are variable within the family and highly anomalous viz. other insects, with separate apertures in the female for copulation and egg laying, and fertilisation in the ovarioles. Carayon figures the system for Orius (Carayon 1953), which is similar to that of Anthocoris, and dissections done during this work agree

with his account.

A. confusus and *A. nemorum* differ in oviposition site, and this was found to be very important in this work. Sand(1957), Collyer (1953) and Hill (1965) describe *A. confusus* as inserting eggs mainly in stems and leaf petioles, whereas *A. nemorum* inserts its eggs into leaf margins (Hill 1957), Sands (1957) and Anderson (1962). The smaller *Orius* species lay their eggs in stems, particularly at the bases of inflorescences Sands (1957) (Iglinsky and Rainwater 1950). As discussed above *Orius* species are important in the U.S.A.

Section 1B. Insects used in this study and Insecticides.

1. Experimental aphids. All the aphids used in the bio-assays were reared on Tick bean plants in the apparatus shown in Fig.1., consisting of a Cellulose acetate cage 6 inches (C 15.0 cm) X 3 inches (7.5 cm) and cylindrical in shape.

The bean plants, growing in an inorganic culture solution in a 1 lb Jam Jar, protruded through the base of the cage. The aphids were thus kept free of parasites, fungus diseases and other aphid species. The toxicity of Cellulose acetate sheeting to leguminous plants reported by Kieckhefer and Medler (1960), was not found to be a significant problem, probably because the cages were frequently washed in hot water to remove honeydew, which removed the odour of phthalate, and each had two muslin air vents 2" X 2" (5cm X 5 cm) and a muslin lid which prevented excess condensation. All rearing of aphids for bio-assay was in a room at 20°C, 16 hours artifical light per day.

2. Anthocorids of both species were reared in the cages described above, in the same room as the test aphids. By making use of Anderson's (1962) results it was possible to rear Anthocoris confusus through 16 generations. As reported by Anderson (1962) A. nemorum could not be continuously reared. Females collected from the field would usually lay eggs in large numbers. These developed to male and female adults of normal appearance and activity, but the females' ovaries did not develop despite the presence, in the females, of living sperm. This species was collected in the field when large numbers were needed.

3. Food aphids for Anthocorids. Large quantities of Myzus persicae, Aulacorthum circumflexum and Acyrthosiphon pisum were reared under lights in a basement at about room temperature. The food plant was Broad Beans, grown 4 to a 5 inch pot.

Insecticides. Three systemic organophosphorus Aphicides - phorate, menazon and dimethoate, were used in this work to exemplify compounds of very different selectivity.

OO diethyl S ethyl thiomethyl phosphorodithioate.
Phorate, is not considered to be a physiologically selective insecticide. It has a high mammalian toxicity (LD50 = 3 mg/Kg, rat, Oral) (Edson 1960) and a powerful contact and fumigant action on many kinds of insects. Phorate 95% pure liquid was used, with acetone and cellosolve as intermediate solvents for making solutions.

Methyl Dimethyl dithiophosphoryl acetamide
Dimethoate, has an even more pronounced contact action against some insects but is not reported to have fumigant action. It is less toxic to mammals : (LD50 = about 500 mg/Kg, rat, Oral). Dimethoate 98% crystals were used, with cellosolve as intermediate solvent.

S(4 6 diamino 1.3.5- Triazin 2yl methyl OO Dimethyl dithiophosphate
Menazon, is said to be a physiologically selective compound (Price - Jones 1961) and it has a very low mammalian toxicity (LD50 = 1950 mg/Kg, rat, Oral). Menazon 98% pure crystals were used, with cellosolve as intermediate solvent.

Culture Solution Recipe.

Potassium Nitrate	69 gms.
Magnesium Sulphate	35 gms.
Ammonium Phosphate	17 gms.
Ammonium Nitrate	9 gms.
Calcium Nitrate	9 gms.
Water	100 litres.

Plus 'trace' quantities of Boric acid, manganese sulphate, Zinc sulphate, and Ferric nitrate.

Section II Effects of Systemic aphicides on Aphids using a laboratory Systemic Uptake Technique.

The basis of this thesis is comparison between aphids and their predators; therefore a background of information on the insecticide susceptibility of various aphid species is necessary to put any effects of insecticides on anthocorids in perspective, as well as to aid the design of comparative experiments.

A standard method of treating plants to test the effects of systemic insecticides on aphids was devised, which aimed at satisfying the following requirements:-

(1) A uniform plant subject which did not change in size or form during the tests (possibly thereby altering the concentrations of toxicant in different localities).

(2) A method of applying a known dosage <u>entirely</u> taken up by the plant.

(3) A suitable test aphid and a method of introducing and confirming these on the plant to give prolonged and uninterrupted feeding and a low control mortality, thereby enhancing sensitivity to low doses.

(4) A treatment time for an insecticide dose to kill the maximum number of aphids relative to control and for mortalities to become consistent with dosage rates, before the effects were obscured by significant decline in toxicity of the plants.

The method was derived from that of David and Gardiner (1951), who used bean plants grown in culture solution, and fed a dose of aqueous insecticide solution, proportional to the plants' weight from a small tube around the cut end of the taproot, while the lateral roots, in a separate body of culture solution, kept the plant turgid. This treatment was used to give a dosage rate in mg./Kg. fresh weight (parts per million fresh weight) which was quickly absorbed by the plant.

Fig. 1. Apparatus and Cages.

A) Celluloid cage base.

B) Cage base with seedlings, on 1 lb jam jar
   f. foam plastic pad.
   p. plasticine wad.
   t. tube of insecticide.

C) Funnel and tube for collecting aphids poisoned by insecticides.

D) =(C) in use, held in position by wire: (W).

E) Top Cage, used with (A) for rearing Anthocorids and in all experiments on systemic action on aphids.
   f. foam plastic, making seal with top of cage base (A).

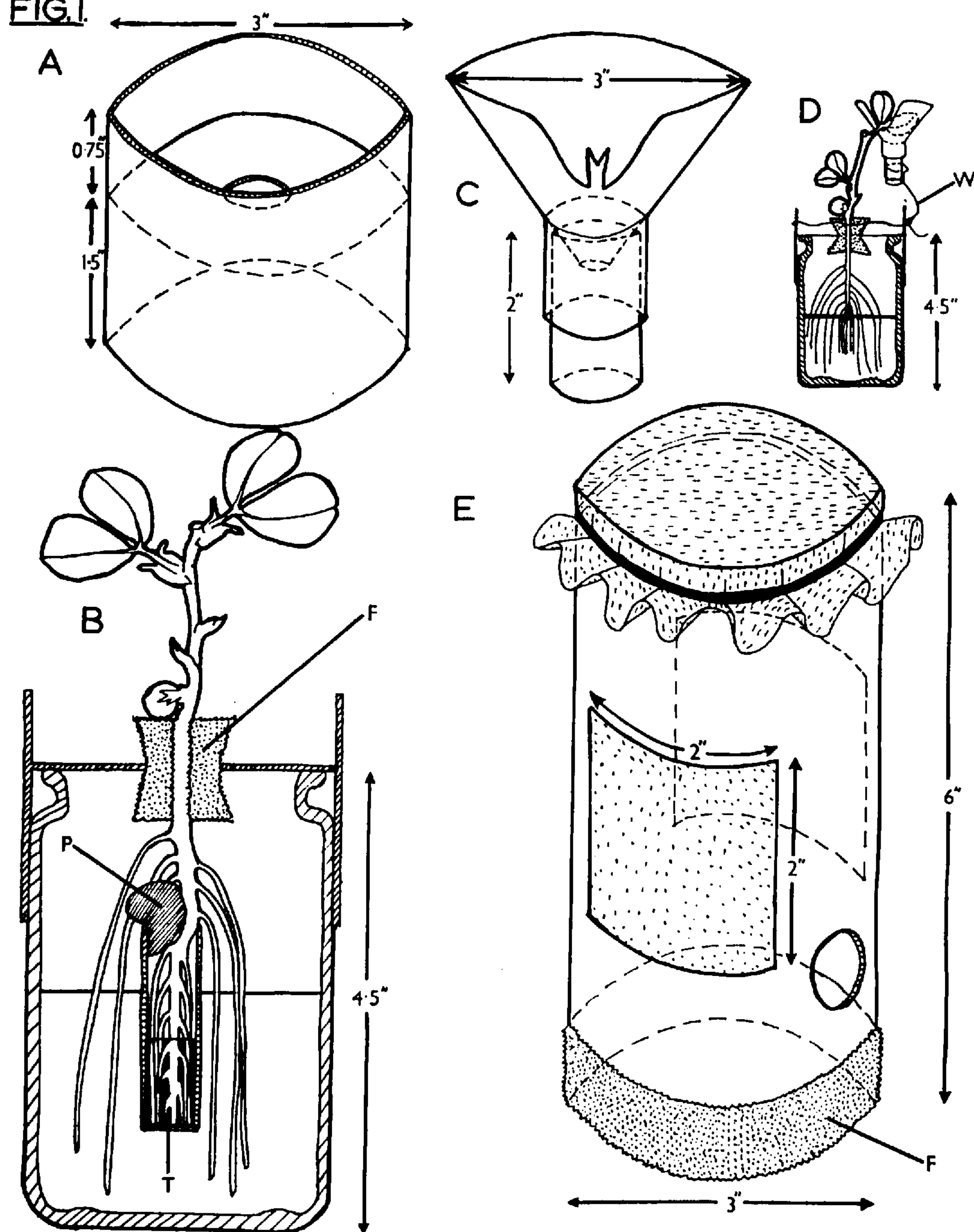


FIG. I.
A
3"
0·75"
1·5"
C
3"
2"
D
W
4·5"
B
F
P
T
4·5"
E
2"
2"
6"
F
3"

Advantages of this method are that there is no difference in the uptake of different insecticides due to their different ability to move through soil and enter roots, and also that no fumes (from volatile insecticides) rising from the soil, could supplement the systemic effects. The effects of differences in mobility in soil, fumigant action and persistance of the chemical and also the effects of natural environmental factors were included in other experiments where seemingly important results of laboratory experiments were tested in the field. No experiments were done with potted plants.

The cut taproot method differed from pot or field experiments in that the dose taken up was not renewed from an insecticide reservoir in the soil and the conditions were constant. Decline in toxicity would be due mainly to chemical change in the plants and transpiration, whereas in the field the toxicity is also affected by the soil reservoir becoming exhausted, leached or chemically changed, by growth and by abscission of leaves and by changes in the weather affecting uptake.

David and Gardiner 1951, traced the fate of Aphis fabae colonies on entire bean seedlings kept in an open glasshouse, but Anthocorids, which are more active than aphids, require confinement, and therefore a small plant that could survive in a cage and could also easily be searched for Anthocorid nymphs and eggs was needed.

The plants used were tick bean seedlings, grown to the equivalent of 4th or 5th leaf stage, but only the first two leaves were kept. (Fig. 1.B).

The other leaves were removed, as they appeared, to prevent the lower 2 becoming senescent, leaving the apical bud (to retain apical dominance) until the lateral roots

were about 3" long. Just before treatment the apical bud was removed to give a miniature plant with two mature leaves and well developed roots, which were cut as shown in Fig 1 B (more precise details given below). Plants cannot be used as soon as the first two leaves are open, since the lateral roots are then too short.

The method used here differs from that of David and Gardiner 1951 in a number of ways and was altered throughout the experiments described and discussed below. The lateral roots (other than those in the main body of culture solution) are shown cut off at their origins in David and Gardiner's 1951 Fig.1, whereas here they were cut off level with the bottom of the tube of insecticide (Fig 1 B), thereby increasing the cross sectional area of root available for uptake. This counteracts the small transpirational area of 2 leaf plants, which were smaller (C. 5gm.) than the intact seedlings (8 - 15 gm.) used by David and Gardiner.

A preliminary test, using the apparatus shown in Fig (1 A and B) showed that fumes rising from 1 cc of 50 p.p.m. culture solution in a 2"x ½" tube suspended below a plant, but <u>not</u> in contact with the roots, did not affect aphids in the top cage, presumably because the fumes did not pass through the cage base and foam pad (Fig 1 A), the same dose taken up by the plant, however, killed all the aphids. This meant that, if desired, the plants could be treated with aphids already on them without these being harmed directly by fumes.

<u>Measurement of doses and fitting of Apparatus</u>.

2 leaf bean seedlings, as described above, were grown with the roots growing through 1" cubes of plastic foam into culture solution in large polythene bowls. When large enough for experiments, each was transferred to a cage base

apparatus of known weight, which was fitted to a 1 lb jam jar of water (Fig 1 B) with the foam making an insect - proof fit below the cotyledons.

The plant plus cage base plus foam pad, were weighed and the plants' weight obtained by subtracting the cage base plus foam pad weight (1gm.) from the total.

Doses were determined in relation to the weight of each plant. Thus for a plant weighing 5 gm., 10 p.p.m. was obtained by uptake of 1 cc of 50 p.p.m. insecticide solution. By adjusting the concentration, further dilutions were obtained and all doses measured out into 2" x ½" flat bottomed glass tubes for uptake by the plants.

The end of the taproot of each plant was cut off and the lateral roots arising up to 2" above the cut end, were trimmed off level with the cut end. Thus a bunch of roots 2" long, cut off squarely, could be pushed into the 2" x ½" tube of insecticide solution, and the tube was then secured, with the cut roots touching the bottom, a wad of plasticine being used to help secure them into the top of the tube. The plasticine was pushed in enough to secure the tube to the roots without crushing them, leaving a passage for air to enter, and replace the solution in the tube as it was drawn up. The roots arising from the taproot <u>more</u> than 2" back from the cut end were left to take up water from the jam jar and keep the plant turgid after uptake of the insecticide dosage. The tubes were always removed from the roots when empty.

<u>Modifications to the method in Experiments 1 - 6.</u>

The technique outlined above was modified in several details to simplify and standardise the procedure and to get more information from each experiment. In addition the handling of the insects and assessment of mortality was

modified as a result of experience. The changes are summarised in Table 1. The most important trend was the reduction in numbers of insects and plants used and the changes in toxicity assessment.

Table 1. Summary of changes made in the cut taproot method of assessing toxicity of Systemic insecticides to Aphids

Experiment	No. Aphids per treatment and stage used.	Insecticide Mortality resultant of:	Method of Assessment
1	2000 apterous all stages	Adult mortality breeding nymph mortality (inseparable)	Counting dead Counting 5 Day survivors
2	240 Adults	Adult mortality breeding nymph mortality separate	Counting Dead and 7 Day survivors
3	60 Nymphs	Nymph Mortality	Counting Survivors 72 hrs.
4	50 Nymphs	"	Counting survivors 24,48,72hr
5	"	"	"
6	"	"	"

Apart from the Experiments (7 & 8) all experiments in other sections, done as in experiment 6

over/...

Table 1. continued.

Experiment	No. plants per treatment & condition	Uptake conditions & Time	Aphids put on	Organic diluent	% control Kill (Ave. all spp)	Mean Square Linearity
1	10 +stipules -Apex	Outdoors uncaged 8 hrs	Before Uptake	Acetone	4 24 hr	-
2	8 + Stipules -Apex	Glasshouse caged 12 hrs	Before Uptake	"	28	8.9
3	6 + Stipules Apex	Glasshouse uncaged 8 hrs	After Uptake	"	15	2.4
4	5 + Stipules -Apex	25 C uncaged 8 hrs	"	Acetone V. Cellosolve	26	2.7
5	-Stipules -Apex	"	"	Cellosolve	8	1.1
6	"	"	"	"	6 48 hr	3.7

Apart from the Experiments (7 & 8) all experiments in other sections, done as in experiment 6

In Experiment 1., roughly 200 A. fabae of all stages were used on each of 10 plants per treatment and the results were recorded as dead aphids each day and survivors at the end. Since the precise number of adult aphids at the outset was not known, no direct count could be made of the death of initial aphids after about the first 24 hours, because breeding obscured mortality at low treatments. Despite the very large numbers used, more aphids were killed at 1 p.p.m. on the first day than at 5 p.p.m. (Table 2) so that the relationship of recorded kill to dosage was not very close.

In addition the increase or decrease of the colonies could only be tested by the number of aphids alive at the end of the experiment. Thus the data, while showing that the treatments produced their greatest effects after about 72 hours, made no further use of the numbers recorded dead, since in some treatments the living aphids were increasing rapidly and were uncountable.

Since exactly 130 adult aphids were tested on each of 8 plants in Experiment 2, exact daily cumulative percent mortalities could have been calculated but large numbers of M. persicae were found to be "missing" at the end of the experiment. From the data in Table 3 it was discovered that the number of surviving M. persicae on treated plants gave values of percentage mortality more closely related to dosage than the number of aphids found dead. With Aphis fabae however, as in experiment (7) Section III there were no "missing" aphids in control treatments. From experiment 2 onwards, percentage mortality was derived from counts of living aphids, all those "missing" being considered as dead.

An attempt was made to reduce control mortalities from Experiment 2. onwards, by selecting only large aphid nymphs mainly 4th instar for treatment. These were large enough to count, but would not die of old age, for example during treatment. The main reason for using adults was to compare breeding rates on different treatments, and experiment 2 showed that, for surviving adults, these did not differ significantly.

The reduction in replicate size (from 200 aphids on each of 10 plants per dosage in experiment 1. to 10 on each of 5 plants in experiments 4 - 6) was accomplished without reducing the correlation between dosage and percentage kill (as indicated by the mean squares for deviation from linearity (Table 1. derived from Appendix). Such reduction enabled more interspecific and inter insecticide contrasts to be made.

The smaller numbers of aphids in Experiment 3 onwards were also less damaging to the plants than the large numbers in Experiments 1 and 2, where aphid damage to controls determined when the experiments had to be stopped. The smaller numbers used also enabled all the replicates in each experiment to be done on one day.

The other important modification in the experiments was in the conditions under which the plants took up the dose.

Initially it was thought that the aphids should be feeding on the plants before the insecticide arrived in the leaves, in case the effect on aphids of a slowly increasing dose was different from the effect of starting to feed on a very toxic plant. In Experiment 2, (where the plants were infested, then treated with cages (Fig I E) on and uptake was slow: (12 hours.) it was found that many

Myzus persicae were "missing" despite the cages.

On the other hand, in Experiment 1., the A. fabae were settled and feeding without the top cages (Fig. 1 E) over the plants but therefore had also started to breed, which would be undesirable in critical small experiments.

The results of Experiment 3. however, show that close dosage mortality relationships could still be obtained if the plants were infested after treatment, and so plants were treated before caging the aphids on them from Experiment 2. onward i.e. as Fig. 1 B, and were able to take up the doses in 8 hours.

From Experiment 4. onwards, plants were put in a 25°C illuminated cabinet with a circulator fan, to take up doses of all the insecticides used; this method was pursued in other subsequent experiments involving systemic treatments in other sections of this work except the first phorate versus A. nemorum egg experiment.

Two minor alterations were also made: Experiment 4 showed that Cellosolve, used as an organic diluent for phorate, was as effective as acetone, and more suitable for diluting menazon and dimethoate. Cellosolve was therefore used from experiment 4 onward. Apart from Experimant 3, all experiments were done with 2 leaf seedlings. In Experiment (12) section (3), the rapid action of dimethoate and the close agreement between points and probit lines suggested that the removal of stipules in that experiment might have increased the insecticide effect.

In Experiment 5 and 6 the stipules were removed from the plant before treatment and the LD50 in Experiment 5. for A. fabae (24 hours) was 0.65 p.p.m. compared with 0.49 p.p.m. in 7 days in Experiment 1. so there may have been some effect, although LD50's vary between experiments

even under similar conditions.

The last two columns in Table 1. show that the control mortality and the mean square for departures from linearity of Probit lines, did not increase in later experiments with reduction in replicate size or other simplifying modifications. These changes were justified in that they decreased labour involved and also enabled more complex experiments to be done.

Experiment 1. The action of Phorate on *Aphis fabae* colonies.

The object of the first experiment on systemic action was to find the range of concentrations affecting *A. fabae*, the time taken from treatment to maximum kill and the duration of toxic effects.

Method Small *A. fabae* colonies were started on two leaf bean seedlings (growing through foam pads as in Fig. 1). The seedlings were about 3 weeks old with 3" lateral roots. The aphid colonies were thinned out after 3 days to give about 200 apterous individuals of all stages on each plant, and each plant was then transferred to a cage base apparatus Fig.(1) as described above.

The phorate solution for this experiment was made by diluting 1% phorate in acetone 200 X with distilled water. Part of this formed a colloidal suspension in water, but this remained stable for the few hours required for uptake.

Ten plants were treated with each of four doses of phorate to give 10 p.p.m., 5 p.p.m., 1 p.p.m. and 0.5 p.p.m. Ten control plants were treated with 1 cc of 0.5% acetone in water, equivalent to the concentration of acetone in the 10 p.p.m. doses, thus making sure any effect due to the solvent in phorate treatment also occured in the controls. The plants were placed outdoors during uptake of the dose, the aphids being undisturbed throughout. After uptake the top cages were put on the plants and they were moved to a 20°C room with 16 hours daylight.

Dead aphids below the plants were counted daily (Table 2.). On the 4th day the controls began to wilt through over infestation so all the plants were washed free of living aphids, which were counted (Table 2.).

Table 2. Number of Aphis fabae (all stages) dying and surviving on bean plants treated with 4 levels of phorate (10 plants with 200 Aphids each, per Treatment)

Dosage phorate p.p.m.	Number of Aphids dead						Sur-vivors	No. born	%increase or decrease in living aphids (from 2000)
		(%)				(Total)			
Days	1	1	2	3	4	4	4		
10.0	1797	89.9	259	-	-	2056	0	56	- 100%
5.0	1327	66.4	335	143	51	1856	0	-	- 100
1.0	1515	75.8	471	165	82	2233	694	927	- 65.3
0.5	663	32.2	326	224	86	1299	3311	2610	+ 65.5
Control	72	3.6	40	100	59	275	4398	2669	+ 119.9

These results show that 10 and 5 p.p.m. completely eliminated the colonies and 1 p.p.m. caused a decrease, whereas at 0.5 p.p.m. the numbers increased, despite early deaths. The numbers dying on the lower treatments were highest on the first day and declined towards those on controls after 4 days.

On the first day, when there had been little breeding the kill was greater at 1 p.p.m. than at 5 p.p.m., but there were more survivors in the latter treatment after 4 days. The 0.5 p.p.m. treatment killed more than half the initial number of aphids in 3 days, yet the number of living aphids increased.

After 4 days, 7 out of 10 plants at 1 p.p.m. were still infested, so the plants could be regarded as not very toxic, but all the 10 p.p.m. and 5 p.p.m. plants were free of aphids. A further test was performed on these to see if the toxicity had diminished.

The 10 and 5 p.p.m. plants were infested on the 5th day after treatment with 150 A. fabae about 4th instar on each. Controls (unused plants from the same batch) were similarly infested. The 72 hour mortalities of the 10 p.p.m. 5 p.p.m. treatments and controls were 68.7%, 16.2% and 4.0% respectively which are less than the initial experiment 24 hour mortalities of 90%, 66% and 4%. Thus the plants began to lose toxicity within a few days at 20°C.

The results of Experiment 1. indicated that the most pronounced effects of phorate treatment occured at about 72 hours. The results were obscurred by breeding to a large extent and a further experiment was done to get an estimate of the 3 day mortality of A. fabae adults using exact numbers.

Experiment 2. The action of Phorate on Aphis fabae and Myzus persicae.

The object of this experiment was to compare the tolerance of A. fabae and M. persicae (on the same plants to phorate, using fixed aphid numbers.

Method: Four batches of 10 bean seedlings (like those in Experiment 1.) were treated on successive days in the following way: A. fabae and M. persicae (1 and 2 day old adults) were collected from cultures from which all adults had been removed two days previously. Thirty of each species were put on each experimental plant (Fig 1 B). Cages with large mesh terylene tops were put on each plant and 2 plants were each given 5, 2, 1 and 0.5 p.p.m. phorate. Two were given 1 cc of 0.5% acetone solution. This was done on 4 successive days making a total of 8 plants and 240 of each aphid for each treatment.

The plants were put in a warm glasshouse, with a fan blowing over the cage tops, for about 12 hours. During this

period the insecticide was taken up and the plants were then removed to the 20°C room as before.

Dead aphids were counted each day for a week, after which all the living aphids were washed off and counted. Three and seven day cumulative data is shown in Table 3.

Table 3 Mortality of A. fabae and M. persicae at 4 concentrations of phorate (240 Adults per Treatment) after 3 Days and 7 Days.

Dosage phorate p.p.m.	Aphis fabae 3 Day Dead No.	%	7 Day Dead No. Dead	%Dead**	No. Alive	No. missing or gained
5.0	228	95.0	242	100	0	+ 2
2.0	221	92.1	234	97.5	6	0
1.0	143	59.6	181	75.4	59	0
0.5	156	65.0	194	80.8	43	-3
Control	71	29.6	109	45.4	131	0
	Myzus persicae					
5.0	123	51.3	155	86.7*	32	-53
2.0	76	31.7	106	69.2	74	-60
1.0	78	32.5	117	63.3	88	-35
0.5	80	33.3	113	47.9	125	-2
Control	63	26.3	82	45.4	131	-37

** Percentages derived from No. Dead

* Percentages derived from (Total - No. Alive.)

Results: While A. fabae died in similar proportions to the previous experiment, very few dead Myzus persicae were found under plants with high phorate treatments (e.g. 5 p.p.m. 228 A. fabae, 123 M. persicae at 72 hours) and yet very few M. persicae larvae were born (e.g. 869 at 0.5 p.p.m. after

7 days to 1397 for A. fabae). After 7 days some dead M. persicae adults were found clinging to the plants, whereas aphids killed by phorate normally fall off the plant. Subsequently Verticillium sp. fungus disease was found in the M. persicae cultures. In contrast all A. fabae remaining on the plants were alive and breeding fast and were not diseased. After 7 days all but 5 of the original A. fabae adults were accounted for (Table 3) whereas many M. persicae were missing and others found diseased.

The 7 day mortality derived from "missing" plus "dead" M. persicae adults and "dead" A. fabae (since only 3 missing) were used in probit analysis giving the lines shown in Fig.2. The 3 day mortality data for A. fabae could have been used for probit analysis but the M. persicae mortality at 3 days was inconsistent with dose (Table 3).

In Fig.2. the 7 day probit kills for A. fabae did not show a well defined trend, whereas the M. persicae points were nearly in a straight line. Two provisional parallel lines were drawn, and two parallel lines were calculated (which were very close to the drawn ones). As expected the A. fabae data gave a significant chi squared (34.9) for departures from the line and the M. persicae did not, (Chi squared = 0.7) but the chi squared for departures from parallelism was only 0.162, so the parallel lines were used to derive the 7 Day LD50's as 2.28 p.p.m. for M. persicae and 0.49 p.p.m. for A. fabae.

A factor corresponding to "Relative Potency" was calculated (= 4.7) and its variance was multiplied by a heterogeneity factor (mean square for heterogeneity = 8.9) so that M. persicae was estimated to be 4.7 plus or minus 1.9 times more tolerant than A. fabae to phorate.

Fig. 2. Experiment 2. Section 2.

Action of phorate on *Aphis fabae* and *Myzus persicae* adults by root uptake, on 2 leaf plants.

7 Day Probit lines.
Phorate at: 5.0, 2.0, 1.0 and 0.5 p.p.m.

Fig. 3. Experiment 3.

Action of phorate on *Aphis fabae* and *Myzus persicae* nymphs by root uptake (intact plants).

72 hour Probit lines.
Phorate at: 10.0, 3.98, 2.95 and 1.0 p.p.m.

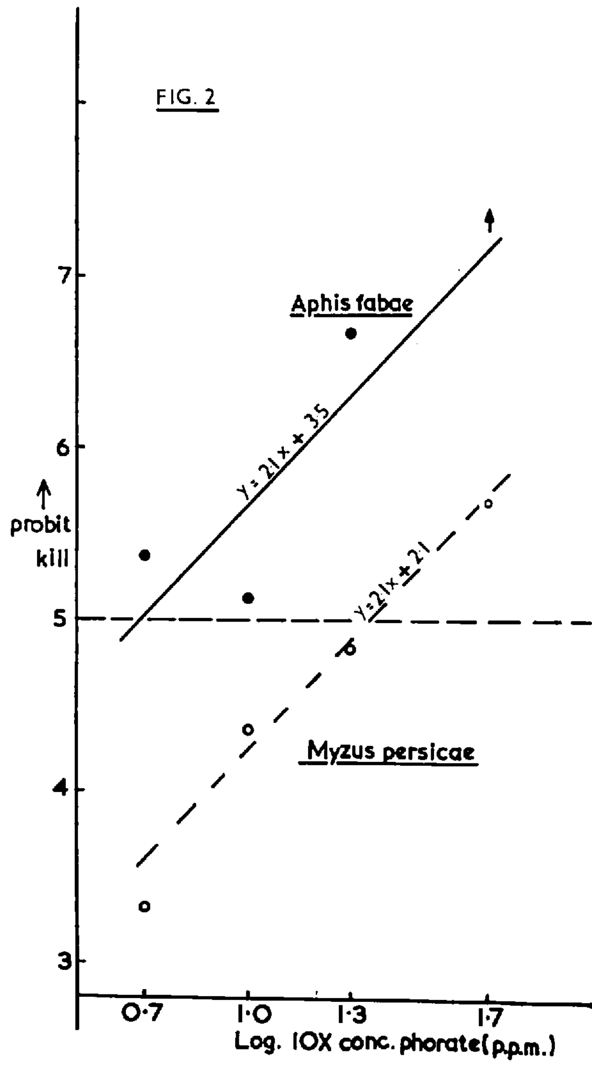


FIG. 2
7
6
5
4
3
probit
kill
Aphis fabae
Y = 21x + 35
Y = 21x + 21
Myzus persicae
0·7
1·0
1·3
1·7
Log. 10X conc. phorate(p.p.m.)

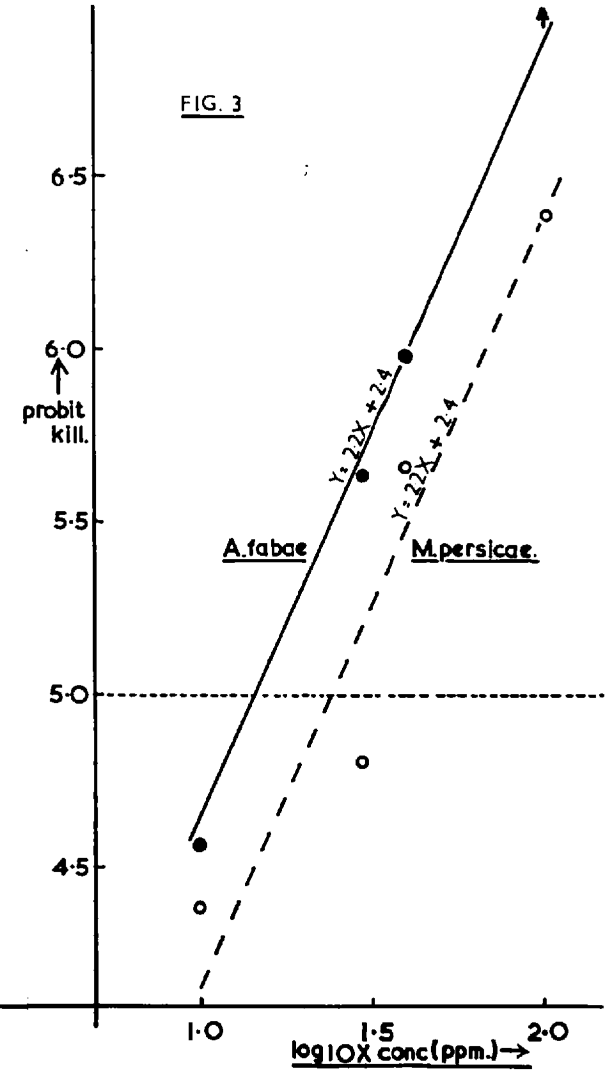


FIG. 3
6·5
6·0
5·5
5·0
4·5
probit
kill.
Y = 22X + 2·4
Y = 22X + 2·4
A.fabae
M.persicae.
1·0
1·5
2·0
log10X conc(ppm.)→

Table 4. Fecundity of Adult A. fabae and M. persicae on phorate treated plants and change in colony size over 7 Days.

Dose p.p.m. Phorate	Total larvae born	Larvae recorded Dead	% Dead	Larvae surviving	Larvae and Adults surviving	% Increase (over 240 initial)
	A. fabae					
5	364	272	76.6	92	92	-62
2	351	212	69.2	139	145	-40
1	745	212	28.5	533	592	+147
0.5	1397	163	11.8	1234	1277	+432
Control	1768	45	2.5	1723	1854	+673
	M. persicae					
5	151	62	41.0	89	121	-50
2	396	49	12.4	347	421	+ 75
1	352	24	6.8	328	416	+ 73
0.5	869	36	4.1	833	958	+299
Control	470	13	4.0	451	582	+143

Breeding Table 4. Although the A. fabae adults were more affected by phorate than M. persicae, the former produced more larvae (except at 2 p.p.m.) (column 1. Table 4) and although these larvae were more affected by phorate (% Kills column 3 Table 4). The larger number of A. fabae born resulted in larger numbers of surviving larvae (column 4 Table 4). In the controls equal numbers of adults of both species survived at 7 days (Table 3) but even so, few M. persicae larvae were born. Since few A. fabae adults were lost, it was possible to derive the number of A. fabae

adults alive each day by subtracting the cumulative mortality (from daily records) from the 240 put on. Summing the number alive over 7 days gave the number of "living aphid days" on each treatment, and dividing the total larvae born on each treatment by this gave the mean number of larvae born per female per day. The breeding rates at 5, 2, 1, 0.5 p.p.m. phorate and control were 2.89, 2.17, 1.33, 2.56, 1.57 per female per day, respectively and this shows no evidence of phorate reducing reproduction in survivors, suggesting that the differences in numbers born on different treatments were due solely to adult mortality.

Survival of Larvae Table 4. gives the percentage kills of larvae born on the treatments and these were proportional to dose for each species. The figures include larvae born over 7 days so that, although larvae appear to be less affected than adults (e,g. Table 4 *A. fabae* 2 p.p.m: larvae 69.2%, Adults Table 3 97.5%) this is probably because larvae were born in the last few days, when the plants had declined in toxicity (see Experiment 1.)

The *A. fabae* results differ from those in Experiment 1 in that the *A. fabae* increased 147% at 1 p.p.m. over 7 days whereas in Experiment 1 they decreased by 34.7% at 1 p.p.m. over 4 days (although they might have increased if left 7 days). In this experiment all the *A. fabae* adults were killed by 5 p.p.m. phorate, but 92 larvae survived, whereas in Experiment 1 no aphids survived this treatment.

Myzus persicae numbers increased on all treatments except 5 p.p.m., and adults and nymphs were more tolerant of insecticide than *A. fabae* which decreased in numbers at both 5 and 2 p.p.m. As stated above *M. persicae* increased less than *A. fabae* on low treatments and control, possibly

because the two species differed in breeding rate, especially as the plants had no young growth which favours M. persicae, whereas mature growth is known not to favour this. The probable interference of Verticillium disease in the kill of M. persicae necessitated confirmation of results of experiments so another experiment was designed to contrast the two species. This was also done to see if the relative tolerance of the two species changed when the M. persicae were able to feed on apical buds and therefore perhaps recieve more insecticide.

Experiment 3 Comparison of the susceptibility of Myzus persicae and Aphis fabae to phorate using intact tick bean seedlings.

Method The plants used in this experiment were seedlings with 3 open leaves plus the intact apical bud. Most had, 4 open leaves by the end of the experiment. The plants were treated by the cut taproot method and took up the doses in a warm glasshouse in front of a fan, without the cage tops in position in 8 hours. (see modifications of method).

The treatments were 10, 3.98, 2.95, and 1 p.p.m. phorate and control (0.5% Acetone solution) with 6 plants at each treatment, the idea being to use one higher dose than in Experiment 2, since the aphids were not put on until after the uptake period and the toxicity might even then have abated slightly. Ten large apterae nymphs of each species were caged on each plant. The plants were put in the 20°C illuminated room and the survivors counted after 3 days (Table 5).

Table 5. Mortality of A. fabae and M. persicae after 72 hours on intact plants treated with 4 doses of phorate. (initially 60 per treatment.)

Dosage of phorate p.p.m.	A. fabae No. of living Aphids	% Dead	Corrected % Dead
10	0	100.0	100.0
3.98	7	88.3	83.3
2.95	11	81.7	73.8
1.0	28	53.3	33.3
Control	42	30.0	-
	M. persicae		
10	5	91.7	91.6
3.98	15	75.0	74.6
2.95	34	43.3	42.3
1.0	43	28.3	27.1
Control	59	1.7	-

Results: The 72 hour mortalities in (Table 5) were used to draw probit lines from which the parallel lines in Fig.(3) were calculated. In Experiment 2. the A. fabae data did not agree closely with a straight line, but here this did, though the M. persicae data was somewhat irregular. Thus chi squared (2D.F.) for deviation from linearity for M. persicae was 8.76 and for A. fabae 0.75, but the chi squared for departures from parallelism was insignificant (1.32, 1D.F.) so the parallel lines were used to give the LD50 for M. persicae as 2.43 p.p.m. and for A. fabae as 1.45 p.p.m., confirming that Myzus persicae was more tolerant. The "relative potency" of phorate to the two species was calculated (as in Experiment 2) with a heterogeneity factor.

The results showed that M. persicae was 1.7 plus or minus 0.43 times more tolerant than A. fabae. The resistance of the two species differed less than in Experiment 2 so perhaps the feeding site factor (P38) might have been important in Experiment 2. Unfortunately the breeding was not recorded in Experiment 3. so it was not known whether the fecundity of M. persicae benefitted from intact plants. The larger control mortality for A. fabae (Table 5) might result from disturbance of the A. fabae when inducing them to withdraw stylets for transfer, whereas M. persicae was easier to transfer.

As stated in the account of modifications to the cut taproot method, it was necessary to see if the intermediate organic solvent, used in making aqueous solutions of insecticides, had any effects on the speed of uptake by the plant or the site of final accumulation, with resultant differences on aphid mortality.

In this experiment acetone (used for the previous three experiments) was compared with cellosolve which preliminary tests showed to be a satisfactory solvent for phorate, menazon and dimethoate. As a 5% aqueous solution, cellosolve proved less phytotoxic than acetone, but it was necessary to compare any effects on toxicity, since de Pietri-Tonielli etal (1961) showed that different organic solvents, used in applying dimethoate via the bark, influenced uptake and phytotoxicity to Citrus plants.

Experiment 4. Comparison of the effects of acetone and cellosolve (used in diluting phorate) on the mortality of Aphis fabae.

Method: This was similar to Experiment 3 : five two-leaf bean plants were treated with 10.0, 1.0 and 0.1 p.p.m. phorate made with each of the solvents acetone and cellosolve,

and also 5 control plants with a 0.5% aqueous solution of each solvent. After 8 hours in a 25°C illuminated cabinet, the uptake was complete in all plants and each plant was infested with 10 A. fabae large apterae nymphs (making 50 per insecticide level per solvent) and then put in the 20°C C.T. room as in Experiments 1. 2 and 3.

The survivors were counted at 24, 48 and 72 hours. The percentage mortalities are shown in Table 6.

Table 6 Mortality of A. fabae nymphs on phorate treated bean plants, using acetone and cellosolve as diluents (50 per treatment)

Treatment phorate p.p.m. (ACETONE)	24 hours % Dead	Corrected % Dead	48 hours % Dead	Corrected % Dead	72 hours % Dead	Corrected % Dead
10.0	44	30.0	84	80.0	100	100.0
1.0	36	20.0	60	50.0	74	67.5
0.1	40	25.0	52	40.0	31	52.5
Control	20	-	20	-	20	-
(CELLOSOLVE)						
10.0	42	14.7	86	79.4	98	97.1
1.0	18	0	72	58.8	88	82.4
0.1	28	0	56	35.3	76	50.0
Control	32	-	32	-	32	-

Results: The control mortality in this experiment all occured in the first 24 hours. The rate of action of phorate made with cellosolve appeared to have been slower, since after 24 hours, only the highest treatment gave a higher kill than control, whereas all treatments with acetone gave kills greater than the control. However by 48 and 72 hours;

the results with the two solvents were similar.

A joint probit line was drawn to fit data from both solvents at 48 and 72 hours and a joint line Fig (4), and LD50, was calculated at each interval (Appendix II 3) and also two regression coefficients and LD50's from the acetone and cellosolve data separately. At 48 hours the joint line had a regression coefficient of 0.57 plus or minus 0.12 (SD). The standard deviation therefore includes the coefficient from acetone data (0.55) and cellosolve (0.59). Likewise at 72 hours the coefficient for the joint line was 0.93 plus or minus 0.14 (SD) and this standard deviation includes the coefficient from acetone data (0.94) and cellosolve (0.92). The LD50 estimated from the joint line at 48 hours was 0.45 p.p.m. and the standard deviation of 'M' included doses from 0.29 p.p.m. to 0.70 p.p.m., thus easily including the value estimated from acetone data alone (0.47 p.p.m.) and cellosolve (0.43 p.p.m.).

The LD50 at 72 hours from the joint line was 0.12 p.p.m. and the standard deviation of 'M' included doses from 0.08 to 0.17 p.p.m. which easily included the value from acetone data alone (0.13 p.p.m.) and cellosolve alone (0.10 p.p.m.). Thus there was no significant difference in LD50's. A goodness of fit chi squared for deviation of the 48 and 72 hour data from the joint lines was calculated and the chi squared (4DF) was 1.83 at 48 hours and 1.75 at 72 hours, so that the deviations from the lines were not significant. It is concluded that in this case the solvent is relatively unimportant as a factor affecting toxicity in this sort of Experiment.

In all experiments done after this, the systemic insecticides were dissolved with cellosolve and then diluted with water.

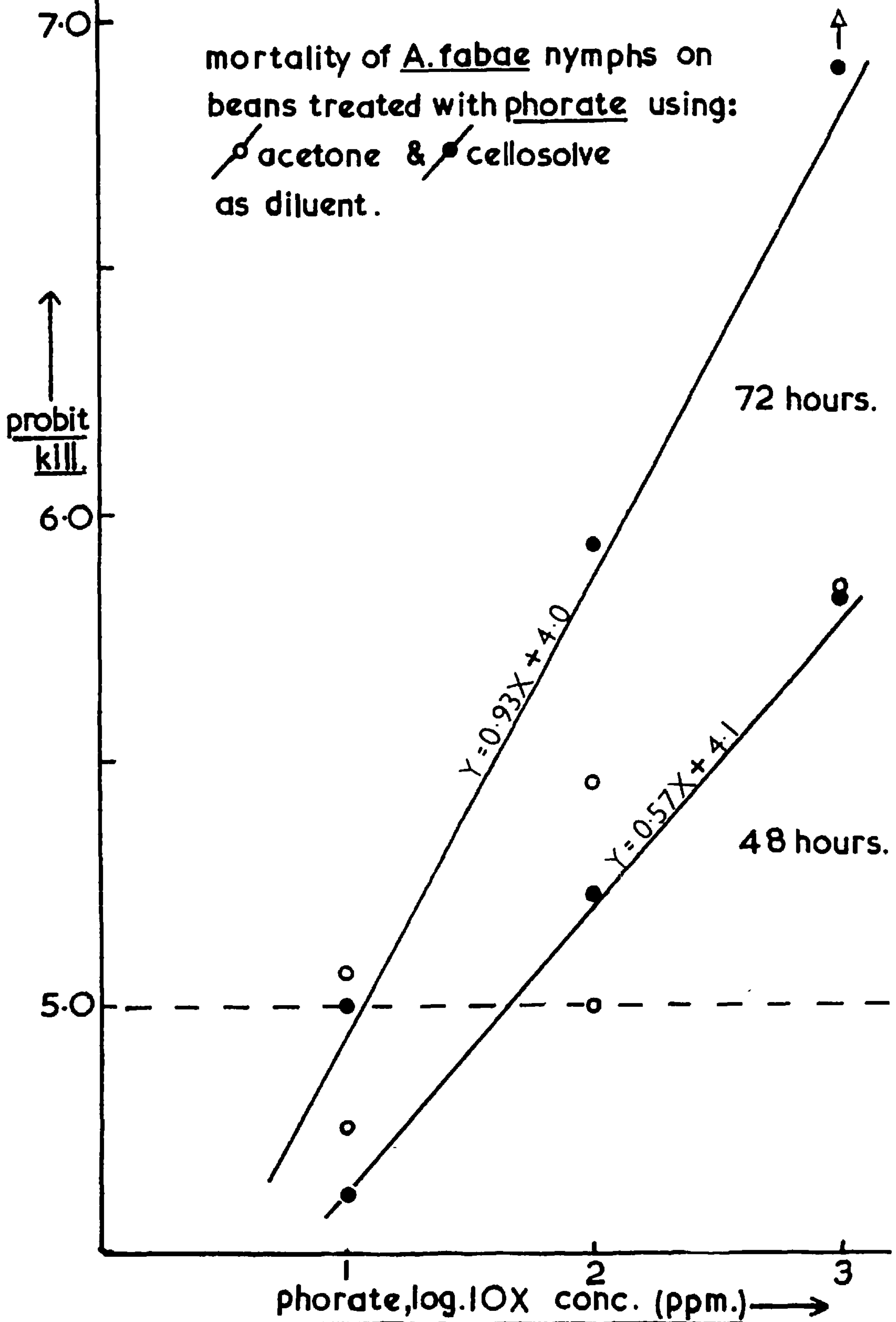


FIG. 4
mortality of A. fabae nymphs on beans treated with phorate using: acetone & cellosolve as diluent.
7·0
6·0
5·0
probit kill.
72 hours.
48 hours.
Y=0·93X+4·0
Y=0·57X+4·1
1
2
3
phorate, log. 10X conc. (ppm.)

Experiment 5. Comparison of Phorate and Menazon on two species of bean aphids.

The object of this experiment was to clarify the results shown in Experiment 9 of Section 3 which suggests that menazon is more toxic to *Aphis fabae* than phorate when applied via the cut taproot method and also to find out if *Aphis fabae* is more tolerant to phorate than *Acyrthosiphon pisum* by systemic treatment as it was in contact tests with dried insecticide films in section (5).

Method: Plants were treated at three concentrations of phorate and menazon (using solutions made with cellosolve) to give: 5 at 5 p.p.m., 5 at 0.5 p.p.m. and 5 at 0.05 p.p.m. Five controls were treated with 0.5% cellosolve solution. The plants were left to take up the insecticide for 8 hours (as in Experiment 4) before treatment, the stipules were removed from the plants as in the last Experiment in section 3, with the object of restricting uptake and feeding sites, and increasing ~~sensitivity~~ reproducibility. This could be done since both *A. fabae* and *Acyrthosiphon pisum* feed readily on stipules as well as on leaves.

The following day the plants were removed to the 20°C room and 10 large apterae nymphs of *Aphis fabae* (mainly 4th instar) and 10 nymphs *Acyrthosiphon pisum* of the same size (1st and 2nd instar) were caged on each. The survivors were counted at 24, 48 and 72 hours and the percentage mortality derived from this data are shown in Table 7.

Results: Table 7 shows that the toxicity of phorate to *A. fabae* was higher than might be expected from Experiment 1. (e.g. 5 p.p.m. in Experiment 1. = LD100 4 days, here only 2 days) but not much greater than found in the results of Experiment 4 for instance. Thus removal of the stipules did not appear to alter absolute toxicity (see modifications of

method) and no complete plants were used for comparison.

Table 7. Percentage mortality of Aphis fabae and Acyrthosiphon pisum nymphs on bean plants treated with 3 concentrations of phorate and menazon. (50 each sp. per treatmént)

Treatment (Day 0) p.p.m.	Percentage Mortality, corrected by Control mortality (bottom line)					
	24 hrs (Day 2)		48 hrs (Day 3)		72 hrs (Day 4)	
Phorate	A.fabae	A.pisum	A.fabae	A.pisum	A.fabae	A.pisum
5.0	89.1	96.0	100	100	-	-
0.5	43.3	64.0	55.6	78.0	72.2	91.6
0.05	8.4	14.0	13.3	18.0	35.6	22.1
Menazon						
5.0	95.6	100.0	97.8	-	100	-
0.5	80.4	100.0	93.3	-	95.6	-
0.05	10.6	18.0	17.8	48.0	22.2	57.9
Control	8.33	-	10	-	10	5

The 24 hour data was used in comparing the potenciés of the two insecticides (Appendix II 4) and even with this, no effective comparison between A. fabae and A. pisum could be made for Menazon since the 5.0 and 0.5 p.p.m. treatments gave 100% mortality of A. pisum. The data as a whole (Table 7) shows, however that A. pisum, at all doses of both insecticides, was always more affected than A. fabae.

The points on Fig (5) for phorate against A. pisum and A. fabae appear to form two parallel lines, with very close agreement whereas the probit lines for A. pisum and A. fabae in Experiment (12) in section 3 for dimethoate

were not parallel.

Analysis of 24 hour phorate data for both species gave a chi squared of only 0.075 for departures from parallelism and, from the parallel lines the LD50 for A. fabae, was 0.65 p.p.m. and for A. pisum 0.29 p.p.m. A. fabae was 2.3 plus or minus 0.48 times more tolerant than A. pisum to phorate. The 24 hour Aphis fabae versus menazon data (Fig 5) did not give such a well defined straight line but to contrast menazon with phorate the line was drawn parallel with the other two lines. The chi squared for departure from parallelism for all 3 lines together was only 0.581, so that the A. fabae line for menazon drawn parallel to the phorate one was used to estimate the LD50 24 hours A. fabae as 0.2177 p.p.m. Thus menazon 3.1 plus or minus 0.95 times more toxic than phorate to A. fabae.

To test the persistance of the insecticides the plants were retained in the 20°C room and, 3 days after the 72 hour mortality was assessed, i.e. on Day 7 the 5.0 and 10.5 p.p.m. plants, which were free, of aphids, were infested as before with 10 A. fabae nymphs, and on day 10 the 72 hour mortality was recorded (in Table 8.). Plants that were free of aphids were re-infested on day 10 with roughly 20 individuals of A. fabae of all stages and the number of plants on which more than 5 of these survived was noted on Days 17, 21 and 31. Further re-infestations of plants having less than 5 individuals were done on these days. The experiment was stopped at 31 days as thd leaves were deteriorating.

The insecticides both declined in toxicity but, whereas in the initial test after 4 days both 5 p.p.m. treatments caused 100% mortality in 72 hours, only the menazon had this effect on the tenth day and the treatment prevented

Fig. 5. Experiment 5. Section 2.

Action of Phorate and Menazon on *Aphis fabae* and *Acyrthosiphon pisum* nymphs, by root uptake.

24 hour Probit lines.
Both insecticides at: 5.0, 0.5 and 0.05 p.p.m.

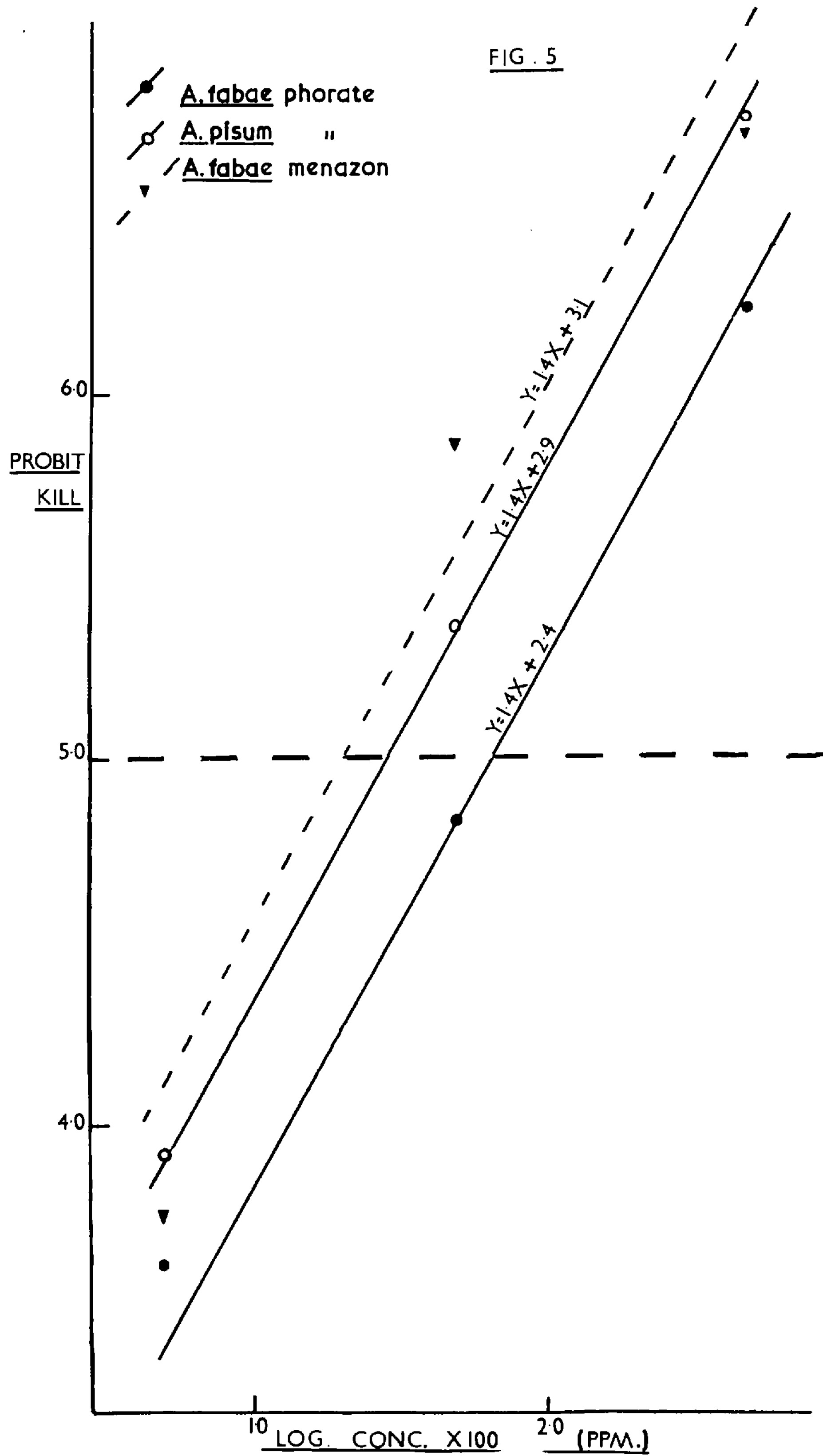


FIG . 5
A. fabae phorate
A. pisum "
A. fabae menazon
Y=1.4X+3.1
Y=1.4X+2.9
Y=1.4X+2.4
6.0
5.0
4.0
PROBIT
KILL
1.0
LOG. CONC. X100
2.0
(PPM.)

infestations developing for the full 31 days of the experiment.

Table 8. Survival of small *A. fabae* infestations on treated bean plants up to 31 days after Menazon and Phorate treatment.

Days after Treatment Day 0	72 hr % Dead 4 (Table 7)	72 hr % Dead 10	Plants (out of 5) with 5 plus *A. fabae* 17	21	31
Treatment:					
Phorate					
5 p.p.m.	100	82.5	1	1	5
0.5 p.p.m.	72.2	55.6	4	4	5
Menazon					
5 p.p.m.	100	100	0	0	0
0.5 p.p.m.	95.6	65.0	1	2	5

In contrast phorate at 5 p.p.m. gave 82.5% kill on day 10 and all 5 plants remained infested with more than 5 individuals of *A. fabae* on day 31.

The conclusion from this experiment is that menazon was more effective than phorate on the two species, of which *Aphis fabae* was more tolerant than *Acyrthosiphon pisum*. Menazon was also more persistant, perhaps due to its non-volatility.

Experiment 6. A comparison of the systemic effects of dimethoate and menazon on 2 age groups of Acyrthosipon pisum nymphs.

The object was to compare the systemic activity of dimethoate with that of menazon and to find out if individuals of different size (and age) differed in their

tolerance to systemic treatments, particularly since interspecific differences in tolerance of individuals of the same *size* would be comparisons of aphids of different physiological *age*, if the adult size of the two species were different. In experiment 2 and 3 *Myzus persicae* and *Aphis fabae* were of almost the same size and stage - adult or about to become adult, but in experiment 5. the chosen *A. pisum* nymphs were much younger than the *A. fabae* nymphs of the same size.

This experiment compares two sizes of one species to find out whether inter-size differences are comparable with inter-specific or inter-insecticide differences found in other experiments.

Method: This experiment used *A. pisum*, since even the very young nymphs are big enough to handle. Menazon was compared with dimethoate so that comparison could be made of the *systemic* toxicity of 3 organic phosphorus insecticides in experiments of the same kind. This was also done with the *contact* toxicity (section 5). The doses used were 1.0 p.p.m., 0.1 p.p.m. and 0.01 p.p.m., since the top doses of menazon in Experiment 5 (5.0 and 0.5 p.p.m.) gave 100% mortality of small *A. pisum* in 24 hours, even so these treatments proved too toxic to give useful 48 hour data. 5 bean plants, without stipules, were treated at each level with dimethoate and menazon, using cellosolve as diluent, and a 0.5% aqueous solution of cellosolve for control. The plants were put in the 25°C cabinet to take up the doses as in Experiments 4 and 5. Afterwards 10 small *A. pisum* larvae and 10 large ones, were caged on each plant and survivors recorded after 24 and 48 hours in the 20°C room. The "small" nymphs were about the size of those used in Experiment 5. (i.e. equivalent to 4th Instar *A. fabae*)

and the "large" ones were 4th instar and about 3.3 times heavier (C 1.4 mg. each)

Results: The percentage mortality of both size groups is shown in Table 9.

Table 9. Percentage Mortality of Large and Small A. pisum larvae at 3 levels of menazon and dimethoate in bean plants (50 per treatment each size)

Menazon p.p.m.	24 hours Large % Dead	Small % Dead	48 hours Large % Dead	Small % Dead
1.0	98.0	100	100	-
0.1	52.0	68.0	64.0	76.0
0.01	32.0	38.0	64.0	68.0
Dimethoate p.p.m.				
1.0	88.0	86.0	100	100
0.1	32.0	68.0	66.0	78.0
0.01	10.0	24.0	14.0	40.0
Control	4.0	0	6.0	6.0

The data in Table 9 shows that menazon killed more aphids than dimethoate (except at 48 hours at 0.1 p.p.m.) and that large nymphs were more tolerant than small ones (except at 24 hours 1.0 p.p.m.). The 24 hour data was analysed (Fig. 6 and Appendix II 5). From probit analysis, it was found that the lines drawn at 24 hours did not depart significantly from parallelism (chi squared 3 D.F.= 0.916) but the points did not agree closely with the lines (chi squared residual heteogeneity, 4 D.F. = 14.86).

The calculated parallel lines confirm that both size

groups were more affected by menazon than by dimethoate: thus the LD50 for menazon = 0.035 and 0.023 p.p.m. for large and small aphids respectively and the LD50 for dimethoate = 0.19 p.p.m. and 0.05 p.p.m. for large and small. Thus even the large nymphs were more affected by menazon than the small ones were by dimethoate. In view of the departures from linearity, the relative pontencies calculated were not very reliable, but menazon was 2.2 times more effective than dimethoate for small aphids and 5.4 times more effective for large ones. (average 3.8 times more effective) Small aphids were 1.5 times more susceptible to menazon than large ones and 3.7 times more susceptible to dimethoate. (average 2.6 times more susceptible) Therefore in this experiment the average inter-insecticide difference (relative potency) was greater than the inter-size groups difference. Nevertheless the inter-size groups differences were large and some inter-insecticide and inter-specific differences in various experiments were small (this will be discussed later)

Fig. 6. Experiment 6. Section 2.

Action of Dimethoate and Menazon on large (c.4th Instar) and small (c.2nd Instar) *Acyrthosiphon pisum* nymphs.

24 hour Probit lines.
Both Insecticides at: 1.0, 0.1 and 0.01 p.p.m.

Thick lines and large points equals large nymphs.
Thin lines and small points equals small nymphs.
Broken lines equal Menazon.
Solid lines equal Dimethoate.

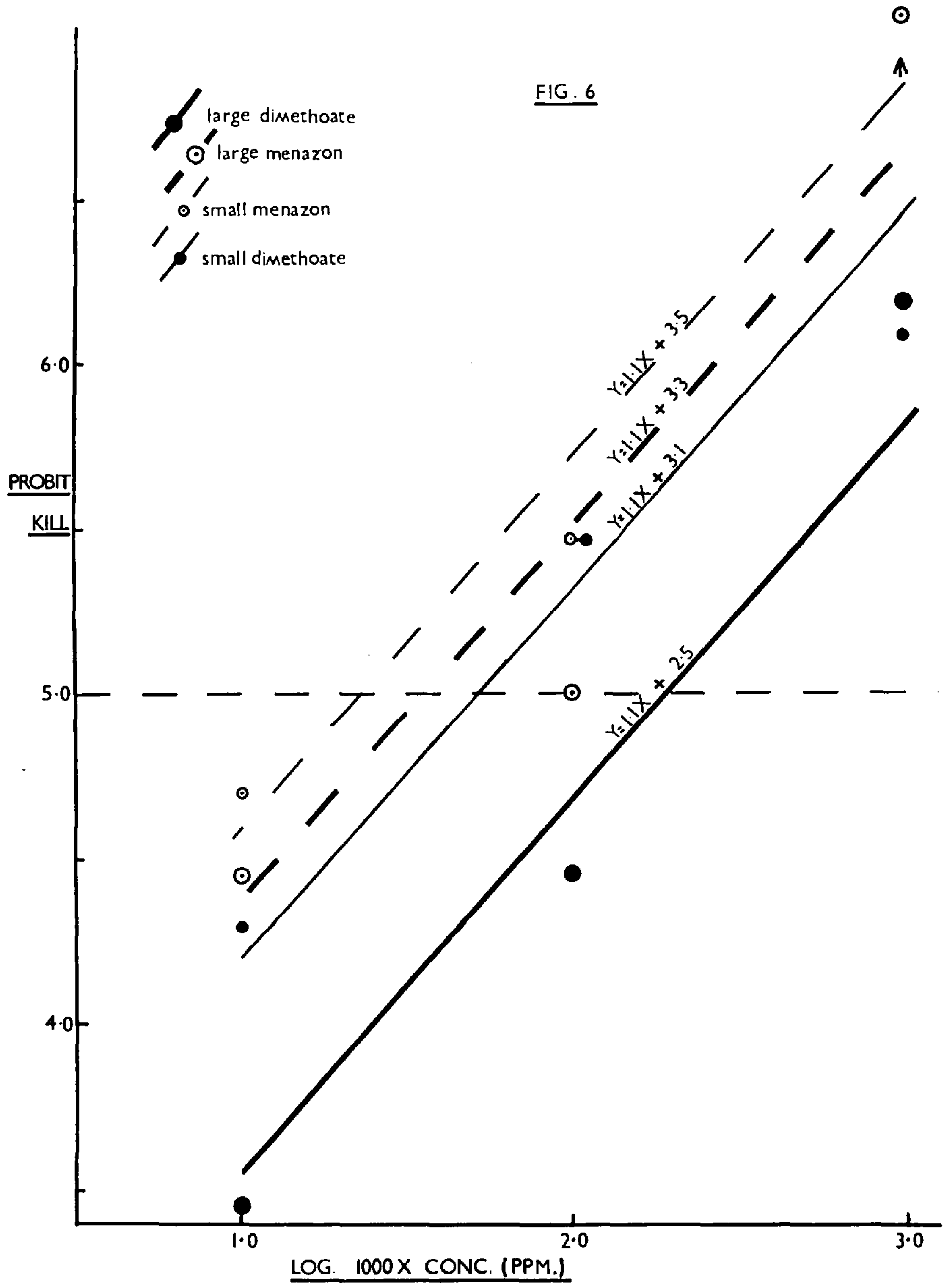


FIG. 6
large dimethoate
large menazon
small menazon
small dimethoate
Y=1·1X + 3·5
Y=1·1X + 3·3
Y=1·1X + 3·1
Y=1·1X + 2·5
PROBIT
KILL
6·0
5·0
4·0
1·0
2·0
3·0
LOG. 1000X CONC. (PPM.)

Section 3 The Effects of Systemic Insecticides on the Eggs of Anthocoris species

The eggs of Anthocoris nemorum and A. confusus have a thin pliable chorion, except in the region of the operculum, and are inserted obliquely into plant tissue by means of the short serrated ovipositor. Once inserted into the plant, only the operculum is visible, the egg forming a characteristic raised blister in the cuticle about 1 mm. long. The displacement or absence of the operculum, leaving a round hole, makes it possible to tell if the egg has hatched successfully, long after the nymph has departed, in contrast to non-viable eggs where the operculum remains in place and the dark remaining egg contents can be clearly seen from outside.

The insertion of eggs within plant tissue, which is not done by Endopterygote aphid predators (Syrphidae, Cecidomyidae, Coccinellidae and Neuroptera) makes the eggs of Anthocorids a vulnerable stage, if an active systemic insecticide, or its toxic metabolite, is present in the plant. Experiments were therefore done, using field beans as a convenient test plant, to find possible effects.

The Oviposition sites on Beans.

The oviposition sites of different Anthocoris species on their wild hosts have been described by Collyer 1953 Sands 1957 and the common Anthocoris nemorum, which breeds on both low vegetation and trees, lays eggs inserted into leaf margins or in the lamina in close proximity to leaf veins. In contrast the more restricted A. confusus lays eggs on deciuous trees, such as oak and sycamore, inserted into green twigs and petioles parallel with the long axis

Data was collected on the oviposition sites used by female Anthocoris of both species when caged on

standardised bean seedlings (Fig. 7.) and also intact bean seedlings, during the course of experiments described below, and is presented in Tables (10&11)

The sites used in captivity are similar to those found in the field for <u>A. nemorum</u> with 50% of all eggs laid in the leaf laminae and 47% laid in the foliaceous stipules. The eggs of <u>A. confusus</u>, are divided between the petioles (40%) the leaf midribs (25%) and the main stem (22.5%) and oviposition sites resemble those found in the field, with a preference for harder tissue. The percentage of <u>A. nemorum</u> eggs laid in <u>A. confusus</u> sites was only 3%, except in crowded conditions on the non-standard plants where it was 6.4% and the percentage of <u>A. confusus</u> eggs laid in <u>A. nemorum</u> sites was only 2.5% so that the division of available sites between the species is virtually complete.

Table (10) Oviposition Sites of Anthocoris nemorum caged on bean seedlings

I Sites on standardised seedlings (Fig. 7)

Experiment No.	7	9.2	9.3	94	12	-	9.1			
No. of plants used	30	42	35	35	27	30	35	Position Total	Position %	% Occurring on:-
No. of female Anthocorids	30	42	35	35	54	30	35			
Position on Fig. (7)										
1	-	0	0	0	-	18	2	20	1.4	Leaves 50.5
2	0	0	2	0	0	3	0	5	0.3	
3	-	54	2	8	-	83	20	167	11.5	Bracts Stipules 46.9
4	0	0	0	0	0	0	0	0	0	
5	-	71	3	9	-	68	49	200	13.8	
6	0	0	1	0	0	0	1	2	0.1	Petioles .3
7	76	30	5	1	-	17	16	145	9.9	
8	0	0	0	0	0	2	0	2	0.1	Internodes 0.5
9	0	15	0	1	6	0	2	24	1.7	
9P	49	14	0	0	119	4	64	250	17.2	Cut Stumps 0.3
10	0	0	2	0	0	0	0	2	0.1	
11	0	2	1	0	0	2	0	5	0.3	Cotyledons 1.4
12	63	30	5	3	-	42	27	170	11.7	
13	1	0	0	0	0	0	1	3	0.2	
14P	133	8	5	6	167	28	102	448	30.8	
14	0	4	0	0	8	0	1	12	0.8	
Total	285	228	26	28	300	267	285	1455	100%	

II Sites on larger intact seedlings

Plants	1	1	1		
Female Anthocorids	12	11	10	Total	%
Leaves	48	119	73	240	47.7
Bracts Stipules	79	79	18	176	35.0
Petioles	4	0	0	4	0.8
Internodes	0	1	0	1	0.2
Cut Stumps	10	5	10	25	5.0
Cotyledons	0	16	9	25	5.0 5.0
Unopened Buds	14	18	0	32	6.4
				503	100%

Table (11) Oviposition Sites of Anthocoris confusus caged on bean seedlings ( )

I Sites on standardised seedlings (Fig. 7)

Experiment No.		Preliminary test	8	9.1	9.2	9.3	9.4	Experiments not descrobed here				Position Total	Position %	% Occurring on:-	
No. of Plants used.		5	18	35	42	35	35	24	21	18	30				
No. of female Anthocorids		5	18	35	42	35	35	24	21	18	30				
Position on Fig. (7)	1	0	0	1	0	0	0	0	1	0	0	2	0.2	Leaves	6.7
	2	0	17	0	10	2	2	4	129	25	24	213	16.8	Bracts	6.7
	3	0	6	0	0	0	0	0	1	0	1	8	0.6	Stipules	6.7
	4	0	23	5	2	3	0	0	78	19	20	150	11.8	Cotyledons	6.7
	5	0	3	0	0	0	0	0	3	1	0	7	5.5	Stumps	6.7
	6	2	17	6	2	0	0	3	81	42	33	186	14.7	etc.	
(MR =	7	0	0	3	0	0	0	0	0	0	0	3	0.2		
Midrib.)	8	13	9	20	3	2	0	18	21	23	29	138	10.9	Midribs	9.2
	9	0	0	0	0	0	0	0	0	0	0	0	0		
	9 MR	0	3	12	0	0	0	9	0	0	0	24	1.9	Petioles	29.0
	10	19	14	14	0	1	0	15	61	57	17	198	15.6		
	11	0	0	0	17	0	0	0	0	0	0	17	1.3	Internodes	59
	12	0	1	1	0	0	0	0	0	0	0	2	0.2		
	13	0	12	14	2	11	10	23	58	63	36	229	18.0		
	14	0	0	0	0	0	0	0	0	0	0	0	0		
	14 MR	17	11	32	1	0	3	24	0	3	1	92	7.3		
Total		51	116	108	37	19	15	96	433	233	161	1269	100%		100%

over/...

Table (11) continued.

Anthocoris confusus sites

II Sites on larger intact seedlings
(Preliminary Tests.)

Position			Total	%
Leaves	0	0)		
Bracts	5	0)	= 11	2.5
Stipules	4	1)		
Cotyledons	1	0)		
Midribs	59	51	110	25.0
Petioles	101	73	174	39.8
Internodes	88	54	142	32.5
Total			437	100%

Fig. 7. Standardised Tick bean Seedling.

As used in Sections 2, 3 and 6, in Laboratory Experiments on effects of root - applied systemic insecticides.

Regions used in describing Anthocorid oviposition sites:

(1): Cotyledons.
(2, 4, 6 and 10): Stem regions.
(3 and 5): Stipules, without leaves but with axial buds.
(7 and 12): True leaf - stipules.
(8 and 13): Leaf petioles.
(9 and 14): Leaf laminae.
(9MR and 14MR): Leaf midribs.
(9P and 14P): Leaf periphery.
(11): Cut stump of main stem.

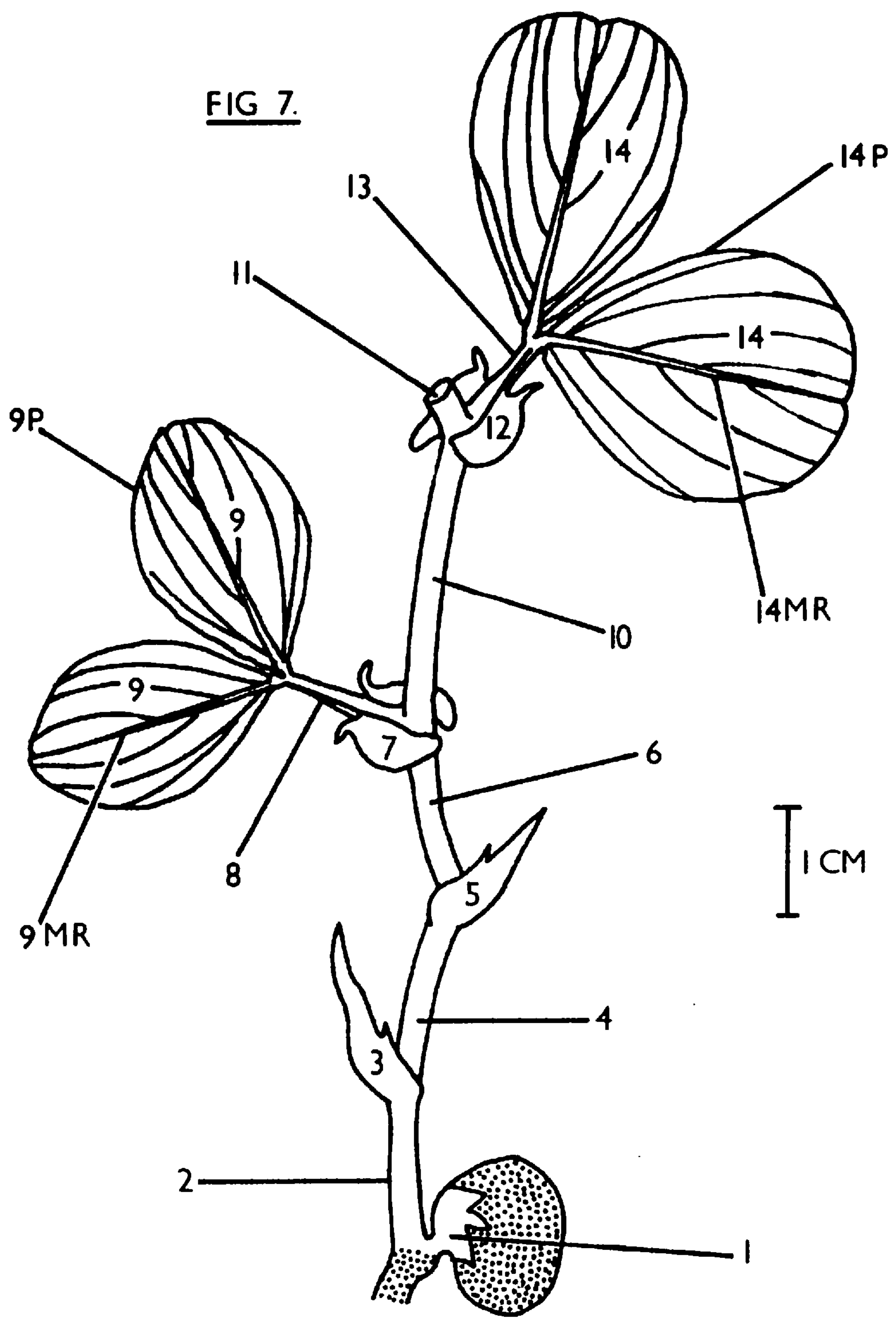


FIG 7.
14
14P
13
11
14
12
9P
9
14MR
10
9
7
6
8
5
1 CM
9 MR
4
3
2
1

Experiments on effects of Insecticides on Eggs, (Exp. 7.)

The taproot method for bioassay of systemic insecticides, when developed to the stage described in Experiment No. 3. section 2, was used to obtain known concentrations of systemic insecticides in standardised bean plants (Fig. 7.). Fertile female A. nemorum and A. confusus were collected in the field or bred in the laboratory and used to provide eggs as required (see sectn. 1B).

In view of the initial lack of evidence on insecticide doses likely to affect Anthocorid eggs, the doses used in the first experiment were based on those required to affect Aphis fabae as indicated by Experiments (1 and 2) in Section 2.

Thus: 10 p.p.m. (expected to give a high kill in the region of 95% in 72 hours), 1 p.p.m. (expected to be more than LD50) and two further dilutions of 0.1 and 0.01 p.p.m. were used. (LD50 for A. fabae from Experiment 2 Section 2 was 0.8 p.p.m.)

At that time (August 1963), the possibility of repulsion of female Anthocoris by treated plants or mortality of adults due to the insecticide or eating systemically poisoned aphids, had not been investigated, and to avoid these effects, the eggs to be used were obtained in advance of treatment in the following way :- The Standardised 2 leaf bean seedlings to be used were divided into three batches of ten, in order to give each batch the egg-laying of all the available females:- the final number of eggs obtained is shown in Table (13)

Each batch of ten plants was infested with Myzus persicae and Aulacorthum circumflexum (to act as food for the Anthocorids) and 6 female A. nemorum were caged on each

plant in an unheated greenhouse for two days. Thus eggs varying from 0 - 48 hours old were obtained. The Anthocorid females were then removed, 10 A. fabae apterous 3rd or 4th instar nymphs were put on each plant and the plants were treated by the taproot method with phorate solutions to give 2 plants containing 10 p.p.m., 2 containing 1 p.p.m. 2 at 0.1, 2 at 0.01 and 2 (controls) treated with 0.5% acetone, which was the intermediate diluent. The plants were transferred to a constant temperature room at 20°C lit for 16 hours per day, and the mortality of A. fabae recorded after 3 days.

The relative merits of different techniques and sequences of operation for the cut taproot method are discussed in Section 2[illegible], but here there was a 2 plant replication of each dosage on each of 3 days making a total of 6 plants at each dosage.

The positions of all the Anthocorid eggs were recorded and they were assessed as hatched or unhatched (Table 13) after 14 days, which allowed a generous hatching period, since the average hatching period of A. nemorum eggs at 20°C is 7 days. The 72 hour A. fabae mortalities are detailed and analysed in appendix III and are summarised in Table 12 where percentage mortality is derived from:-

$$\frac{\text{Dead Aphids at 72 hours X 100}}{\text{Dead plus Living aphids at 72 hours}}$$

since, at the time the "loss" of aphids from experiments was a factor of unknown importance. In later tests this method was discarded and mortality was assessed as:-

$$\frac{\text{(Total Aphids used - Total Surviving) X 100}}{\text{Total aphids put on.}}$$

lost aphids being assumed dead, since there are usually no "lost" aphids on control plants.

Table (12) Mortality of Aphis fabae at 72 hours on bean seedlings treated with 4 dosage levels of phorate.

Dosage of Phorate ppm.	Initial Aphids	No. Dead	No. Alive	No. Missing	% Dead excluding Aphids missing	% Mortality corrected for control Kill	Probit Kill
10ppm.	60	55	3	2	94.9	93.2	6.5
1.0ppm.	60	38	21	1	64.4	52.5	5.1
0.1ppm.	60	22	35	3	38.6	18.1	4.1
0.01ppm.	60	22	37	1	37.3	16.4	4.0
Control	60	15	45	0	25.0	-	-

Table 13

Effects of 4 doses of phorate on the Hatching of Anthocoris nemorum eggs.

Position on plant as in Fig. (7)	Dosage of Phorate (10 plants per treatment) T=Total of Eggs, U=unhatched Eggs.									
	10 ppm.		1.0 ppm.		0.1 ppm.		0.01 ppm.		Control	
	T	U	T	U	T	U	T	U	T	U
7	10	7	15	1	24	3	9	1	18	2
9	7	4	6	2	9	0	6	0	21	4
12	5	1	12	5	26	2	3	1	17	5
13	-		-		1	0	-		-	
14	27	18	18	6	34	6	15	4	38	3
Total	49	30	51	14	94	11	33	6	94	14
% Unhatched	61.2		27.5		11.7		18.2		14.9	
% Kill corrected for control mortality	54		15.0		-		4		-	

Percent mortality Corrected for control (10 and 1.0 ppm. only)	Leaves	Stipules
	45.3%	16.7%

Results

Table (12) shows the A. fabae 72 hour mortality corrected for control mortality and LD50 was estimated (see appendix 3.1.) as 0.55p.p.m. compared with 0.8 p.p.m. in Experiment 2. (section 2) chi squared being 4.25 and not significant.

Effects of Phorate on Eggs

The phorate reduced hatching: thus at 10 p.p.m. 61% of the eggs failed to hatch (54% after Abbott's correction for 15% control mortality) at 1 p.p.m. 15% failed but at 0.1 the percentage failing (11.7%) was lower than in control (15%). A relatively large number of eggs (94) were treated at 0.1 p.p.m. and there were 94 eggs in the control plants also, whereas the corrected kill at 0.01 p.p.m. which was 4% was based on only 33 eggs and is probably less significant.

In view of there being only two doses with a significant kill of eggs and one with a kill of 0%, probit analysis was not done until more data could be obtained from further experiments, but the conclusion from this experiment is that phorate killed some eggs of Anthocoris nemorum, giving lower kills, after 7 days hatching period. than were caused in Aphis fabae over 72 hours. The LD50 for the eggs was about 4 p.p.m. and for the aphids 0.55 p.p.m. The data was recorded in such a way that the kill of eggs in leaves (positions 9 and 14 Table 13) could be compared with that in stipules and bracts (positions 12 and 7 Table 13).

More eggs were killed in leaves than in bracts and stipules (Table 13) which correlates with the relatively heavy accumulations of phorate in leaves from root uptake, as shown by the distribution of P32 labelled phorate see section (4)

Fig. 8. Experiment 7. Section 3.

Action of root applied Phorate on *Aphis fabae* and Eggs of *Anthocoris nemorum* in the same plants.

72 hour Probit line *Aphis fabae* nymphs.

14 Day Probit line *Anthocoris nemorum* eggs (drawn line).

Phorate at: 10.0, 1.0, 0.1 and 0.01 p.p.m.

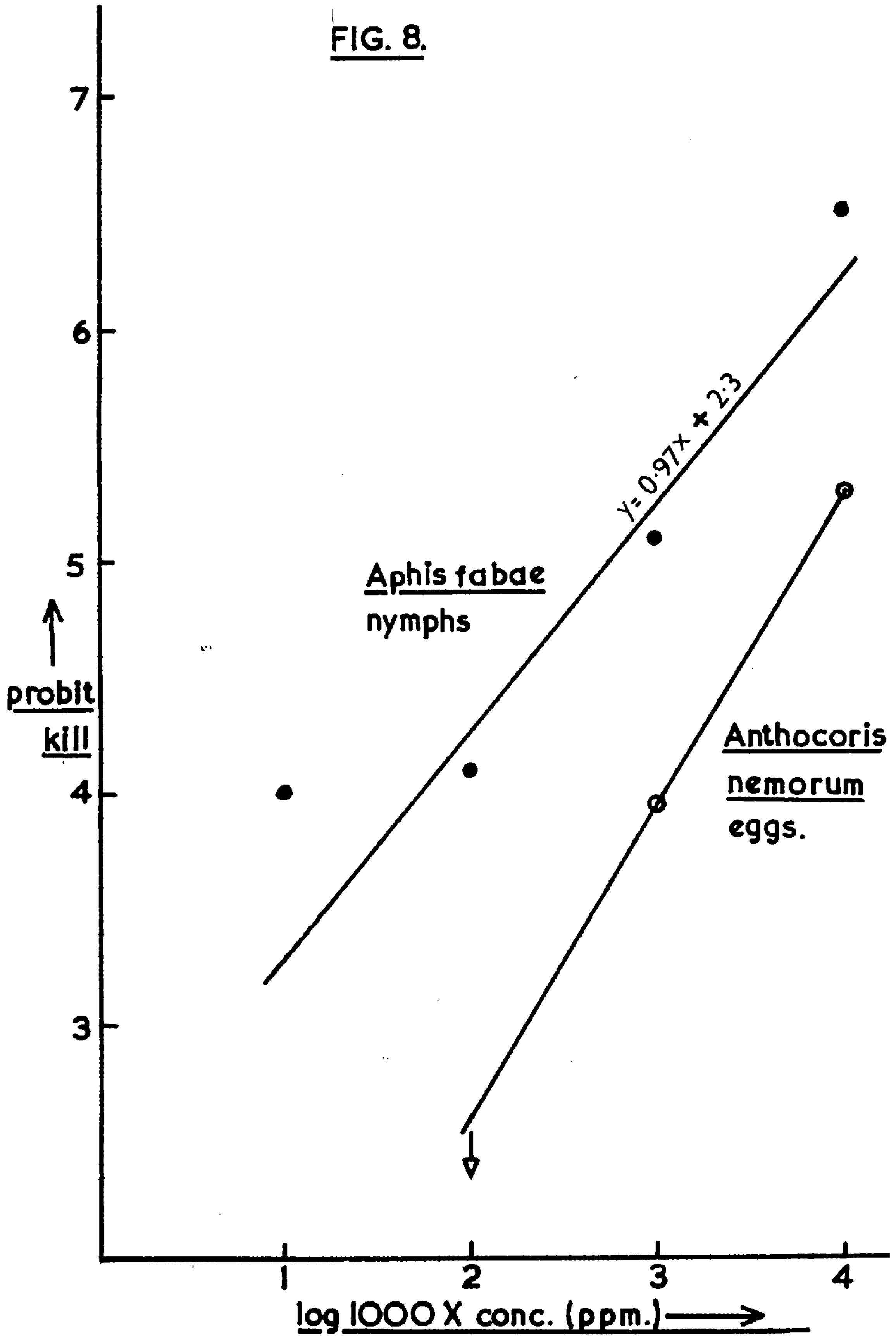


FIG. 8.
7
6
5
4
3
probit
kill
Y = 0·97X + 2·3
Aphis fabae
nymphs
Anthocoris
nemorum
eggs.
1
2
3
4
log 1000 X conc. (ppm.)

Experiment 8. <u>The effect of phorate treated plants on oviposition behaviour and on egg viability of Anthocoris confusus</u>. The object of this experiment was to find out if the eggs of <u>A. confusus</u>, which are laid in different sites from those of <u>A. nemorum</u> (Table11), are differently affected by phorate treatment, also to find out whether phorate inside plant tissue and vapour diffusing out by transpiration are repellent to female <u>Anthocoris confusus</u> (thereby reducing the number of eggs laid) or cause premature death of the adult. Female <u>A. confusus</u> were given a choice of a phorate treated, and an untreated plant, in which to lay eggs. Nine pairs of similar standardised bean seedlings were slected and one plant from each treated at 5 p.p.m. with phorate in aqueous acetone solution by the cut taproot method. The other plant of each pair was treated only with the aqueous acetone diluent. The dose was chosen as being within the range which affects <u>A. nemorum</u> eggs, but not so high as to risk phytotoxicity to the phorate treated plants, since Anthocorid females were to be left on the plants for as long as possible.

After treatment with insecticide or diluent, each pair of plants was labelled and placed in a cage (Fig.1E), the two foam sponges making a tight fit. Care was taken not to bruise the stems.

Two mature mated female <u>Anthocoris confusus</u> from the laboratory culture were put in each cage, care being taken to make sure that, while in the culture, they had been provided with ample aphids for food. No living aphids were supplied in the treatment cages since these would have infested the control plant but been killed by the treated plant. Thus the aphid colonies on the control plants might have stimulated oviposition on these plants and upset

the experiment as a means of determining whether the insecticide itself was repellent. Food was supplied, however, in the form of wounded Drosophila adults and also aphids killed by exposure to temperatures just below 40°C, supplies being renewed every other day. Side growths on the plants were prevented from growing, since these would probably have provided sites with a lower content of phorate than that of the main stems and petioles.

The cages were kept for 6 days at a constant temperature of 20°C under 16 hours artificial light per 24 hours. The female A. confusus were then removed and the plants kept in the same room for a further 10 days to allow the eggs to hatch. The hatched and unhatched eggs were then counted on each plant, their positions being determined as before (Fig.7.)

Results 3 out of 18 females died during the experiment. No eggs were laid on one pair of plants (not associated with the dead females) leaving 8 pairs of plants, the data from which is presented in Table (14).

The number of eggs on individual plants varied from 0 - 21 and the maximum on any pair was 30. No consistent differences in numbers of eggs laid on treated and untreated plants can be seen from the results. The hypothesis that equal numbers of eggs occurred on treated and untreated plants was examined by the chi squared test. This showed that numbers did not differ significantly, chi squared being 11.1 with 7 degrees of Freedom. Since these were more non-viable eggs on the controls (27%) than on the treated plants (11%) it was inferred that phorate at this dose did not harm the eggs of A. confusus. The conclusion from this experiment is that cutroot treatment with phorate does not make bean plants unattractive as an oviposition site to female Anthocoris confusus or harm their eggs.

Table (14) Oviposition and egg viability of female Anthocoris confusus caged with a phorate treated and an untreated bean plant.

Plant Pair No.	Eggs on Control Plants H.	U.	T. (C)	Eggs on 5ppm. Phorate Treated Plants H.	U.	T. (O)	Total Eggs Treated Control T=C +O	Hypothesis:- Expected Eggs on Phorate Plants $E=\frac{1}{2}T$	O-E	$(O-E)^2$	$\frac{(O-E)^2}{E}$
(1)	2	0	2	-	-	0	2	1.0	1.0	1.00	1.000
(2)	0	1	1	2	0	2	3	1.5	0.5	0.25	0.167
(3)	16	5	21	8	1	9	30	15.0	6.0	36.00	2.400
(4)	12	3	15	13	0	13	28	14.0	1.0	1.00	0.071
(5)	12	1	13	16	0	16	29	14.5	1.5	2.25	0.155
(6)	2	4	6	0	0	0	6	3.0	3.0	9.00	3.000
(7)	-	-	0	3	4	7	7	3.5	3.5	12.25	3.500
(8)	2	3	5	5	1	6	11	5.5	0.5	0.25	0.818
Total	46	17	63	47	6	53	116 (Used in table 11.)	Chi squared (7.D.F.)		Total	11.111
% Unhatched 26.98%				% Unhatched 11.32%							

H. Hatched U. Unhatched T. Total.

Experiment 9. Comparison of the effects of phorate and menazon treated plants on the viability of *Anthocoris* eggs.

The previous two experiments showed that phorate, at doses which controled *Aphis fabae*, harmed *Anthocoris nemorum* eggs, laid in leaves and stipules, but it did not affect those of *A. confusus* laid in stems, midribs and petioles. It was therefore ~~necessary~~ desirable to test the eggs of the two insects laid together in the same phorate treated plants and to use as high a dose as possible against *A. confusus* eggs; also to see whether all the *A. nemorum* eggs on a plant can be killed at a dose which does not kill the plant or the *A. confusus* eggs. In addition the experiment was extended to include a systemic insecticide with an inherent selectivity to aphids: menazon.

Aphids were also tested in the experiment to give an independent guide on how any particular treatment was acting on aphids relative to its effects in other experiments.

The experiment and subsequent repetitions were done using the same diluent "cellosolve" (=ethylene glycol monoethyl ether) for both insecticides since this gave a similar uptake to acetone, without phytotoxic effects at the concentrations needed to give high concentrations of phorate. The maximum dosage was fixed at 15 p.p.m. and care was taken to use plants with mature leaves, which were thought to be relatively resistant to insecticide induced phytotoxicity. This was usually satisfactory but sometimes at 15 p.p.m. a lower leaf withered and then the eggs on it were not counted. Dosages of 1 p.p.m. and 0.1 p.p.m. were also chosen as being comparable with ones used in previous experiments, giving 3 concentrations X 2 insecticides +control. Aphid bioassays in section 2 indicated that, for consistent results, at least 5 plants per treatment were required with 10 aphids on each and, in order to get consistent egg

results, the experiment was done 3 times with 5 plants per treatment each time and once with 6 plants per treatment: thus a total of 21 plants was used per treatment.

In each experiment the plants were treated with phorate and menazon solutions by the cut taproot method and allowed to take up the insecticides over a period of 8 hours in a cabinet at 25°C with a circulator fan.

On the day following uptake of the insecticide, 2 Anthocoris nemorum females collected from the wild (overwintering females in Reptitions 1 and 2, summer generation in 3 and 4) and 2 A. confusus females from the culture were caged on each plant in the 20°C 16 hours light per day room with supplies of Myzus persicae and Acyrthosiphonpisum as food. The Anthocorids were removed after 2 days and 10 Aphis fabae apter nymphs were caged on each plant. The survivors from these were recorded after 24, 48 and 72 hours (Table 15). Live aphids were then removed to prevent them from multiplying and damaging the leaves and, after 9 days, the hatched and unhatched Anthocorid eggs were counted and ascribed to positions as in Fig.7 as shown in Table 16.

Aphid Kills: The kills of A. fabae by the two insecticides show that menazon was slightly more effective, though slower acting than phorate. Plants containing 15 p.p.m. of insecticides were very poisonous to the aphids and caused almost complete mortality after 24 hours but, 24 hour data is somewhat unreliable in experiments with systemics, probably because initally uneven doses are recieved by individuals wandering to a variable extent before feeding.

Table (15) Mortality of <u>Aphis Fabae</u> on 3 concentrations of phorate and menazon in bean plants. (4 repetitions of same experiment)

Repetition No.	1			2			3			4		
No. of plants	35			42			35			35		
(No. of Aphids per Treatment)	50			60			50			50		
<u>Treatment</u>	Dead Aphids at:											
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
Phorate												
15.0ppm.	49	50	-	57	60	-	45	50	-	50	-	-
1.0ppm.	26	30	32	18	27	27	20	31	33	14	15	21
0.1ppm.	13	15	15	18	18	19	24	26	36	5	6	10
Menazon												
15ppm.	48	50	-	56	58	60	46	50	-	49	50	-
1.0ppm.	40	48	50	54	58	60	35	40	40	40	46	49
0.1ppm.	16	24	24	27	27	30	16	33	40	7	7	12
Control	5	10	12	6	6	9	5	5	5	6	7	7

<u>Repetitions 2, 3 and 4 combined</u> (used in Fig. 9)

<u>Treatment</u>	<u>Percent Kill</u> (control corrected)		
	24 Hrs.	48 Hrs.	72 Hrs.
15ppm. Phorate	94	100	-
1.0ppm. "	25	36	43
0.1ppm. "	21	23	32
15ppm. Menazon	94	99	100
1.0ppm. "	78	89	92
0.1ppm. "	25	35	44

Table 16 Effects of 3 concentrations of phorate and Menazon in bean plants on the hatch of <u>Anthocoris</u> eggs.

Repetition	1				2				3				4			
No. plants used	35				42				35				35			
Species *	N		C		N		C		N		C		N		C	
Eggs **	T	U	T	U	T	U	T	U	T	U	T	U	T	U	T	U
Treatment																
Phorate 15 ppm.	50	42	7	1	48	39	3	0	6	5	1	0	3	3	9	0
" 1.0 "	49	9	17	1	42	16	8	0	3	1	1	0	4	3	0	0
" 0.1 "	47	23	11	0	42	4	8	0	6	4	0	0	7	1	0	0
Menazon 15 ppm.	18	5	32	2	22	5	2	0	4	0	0	0	0	0	0	0
" 1.0 "	52	24	9	1	21	3	1	0	1	0	4	0	0	0	0	0
" 0.1 "	14	1	17	1	15	3	5	1	4	0	12	1	8	0	0	0
Control	55	8	15	0	38	0	10	0	2	0	1	0	6	0	6	0

Repetitions 2, 3 and 4 combined (used in Fig. 9. and Probit Analysis)

Treatment	Percent kill (Control 0) A. nemorum	Percent kill (Control 0) A. confusus
Phorate 15.0 ppm.	83.0	0
" 1.0 "	40.8	0
" 0.1 "	16.4	0
Menazon 15.0 ppm.	19.2	0
" 1.0 "	13.6	0
" 0.1 "	11.1	10.7

* N = nemorum
C = confusus

** T = Total Eggs
U = Unhatched Eggs.

Numbers of Eggs laid   Few A. confusus eggs were laid in the first two experiments compared with A. nemorum eggs. This was probably because the A. confusus females were from a continuous breeding culture at 20°C, where females tended to oviposit for a long time at a low rate.

The A. nemorum in contrast were collected from the wild and were feeding and ovipositing rapidly when captured, having matured rapidly on resuming feeding after hibernation. They could, therefore, be considered as being at a "peak" of reproduction which would not normally be reproduced in a C.T. room culture.

The second repetition (16/6) used A. nemorum females of the same generation as the first but they had already laid many eggs, so they were not ovipositing as quickly as before.

The 3rd and 4th repetitions were done using A. nemorum females of the summer generation, becoming adult in July. These laid very few eggs, although this generation was used successfully in field Experiments (10).

The first set of Results (Repetition 1 Tables 15&16)

The first time this experiment was done the 15 p.p.m. phorate killed 84% of A. nemorum eggs, but the 1 p.p.m. only killed 18% whereas the 0.1 p.p.m. killed 49%. In the case of A. fabae the phorate kill was proportional to dose (98% at 15, 52% at 1.0 and 26% at 0.1 p.p.m.). Also in this experiment 15 p.p.m. menazon killed 28% of A. nemorum eggs and 1.0 p.p.m. killed 46% and this high mortality for menazon never occurred again. The Aphid kills for menazon were also consistent with dose, so that there is no evidence that either treatment was incorrectly applied, but the eggs did not respond consistently to treatments and there was also a 15% control mortality for A. nemorum eggs which did not recur.

The first results are presented here, but the experiment was repeated and these results used in analysis.

The kills of A. nemorum eggs in the second repetitions were consistent with the dosage for phorate; from Table16 : 81% at 15 p.p.m., 38% at 1 p.p.m. and 9.3 at 0.1 p.p.m. with a control kill of 0%. The 23% kill by menazon at 15 p.p.m. appeared to be a real effect though much less than that of phorate. Repetitions 3 and 4, with small numbers also gave phorate kills of eggs consistent with dose.

Probit analysis was done on the A. nemorum egg data from repetitions 2, 3 and 4 only see appendix (3.2.) in these the 15 p.p.m. treatments of both insecticides killed 94% of aphids in 24 hours and completely eliminated them in 48 hours (except 2 on 15 p.p.m. menazon). All three concentrations of phorate killed a larger proportion of aphids in 72 hours than of A. nemorum eggs during the 7 days incubation period but menazon killed even more aphids and had very little effect on A. nemorum eggs eg. 19% unhatched at 15 p.p.m., 11% on control.

The menazon versus A. nemorum egg data (Table16) was subjected to a chi squared test on the hypothesis that this insecticide did not affect the kill, and unhatched eggs could be expected to occur in equal numbers at all levels of menazon treatment and in the control (Table17). The chi squared was 7.6 which was insignificant at the 5% level, therefore the results obtained despite their linear relationship (Fig 9.) are within the limits of chance variation.

Table 17 chi squared test on Mortality of <u>A. nemorum</u> Eggs on Menazon treated bean plants. Hypothesis that 11/121 of eggs at each treatment are killed regardless of insecticide treatment.

Dose	Observed unhatched Eggs (U) = (o)	Total Eggs Laid (T)	Expected no. unhatched = (e)	(o-e)	$(o-e)^2$	$\frac{(o-e)^2}{e}$
15 ppm.	5	26	2.4	2.6	6.8	2.8
1.0 ppm.	3	22	2.0	1.0	1.0	0.5
0.1 ppm.	3	27	2.5	0.5	0.25	0.1
Control	0	46	4.2	4.2	17.64	4.2

chi squared (3) = 7.6

Maximum permissible value 7.8

Fig. 9. Experiment 9. Section 3.

Action of root - applied phorate and Menazon, on the eggs of *Anthocoris nemorum* and on *Aphis fabae* nymphs.

72 hour Probit lines (Drawn) for Menazon and phorate against *A. fabae* (15 p.p.m. equals LD100)

9 Day Probit line (calculated) for Phorate V. *A. nemorum* eggs.

9 Day Probit line (drawn) for Menazon V. *A. nemorum* eggs (Found to be not significantly different from fluctuations in Natural mortality.)

Phorate and Menazon at: 15.0, 1.0 and 0.1 p.p.m.

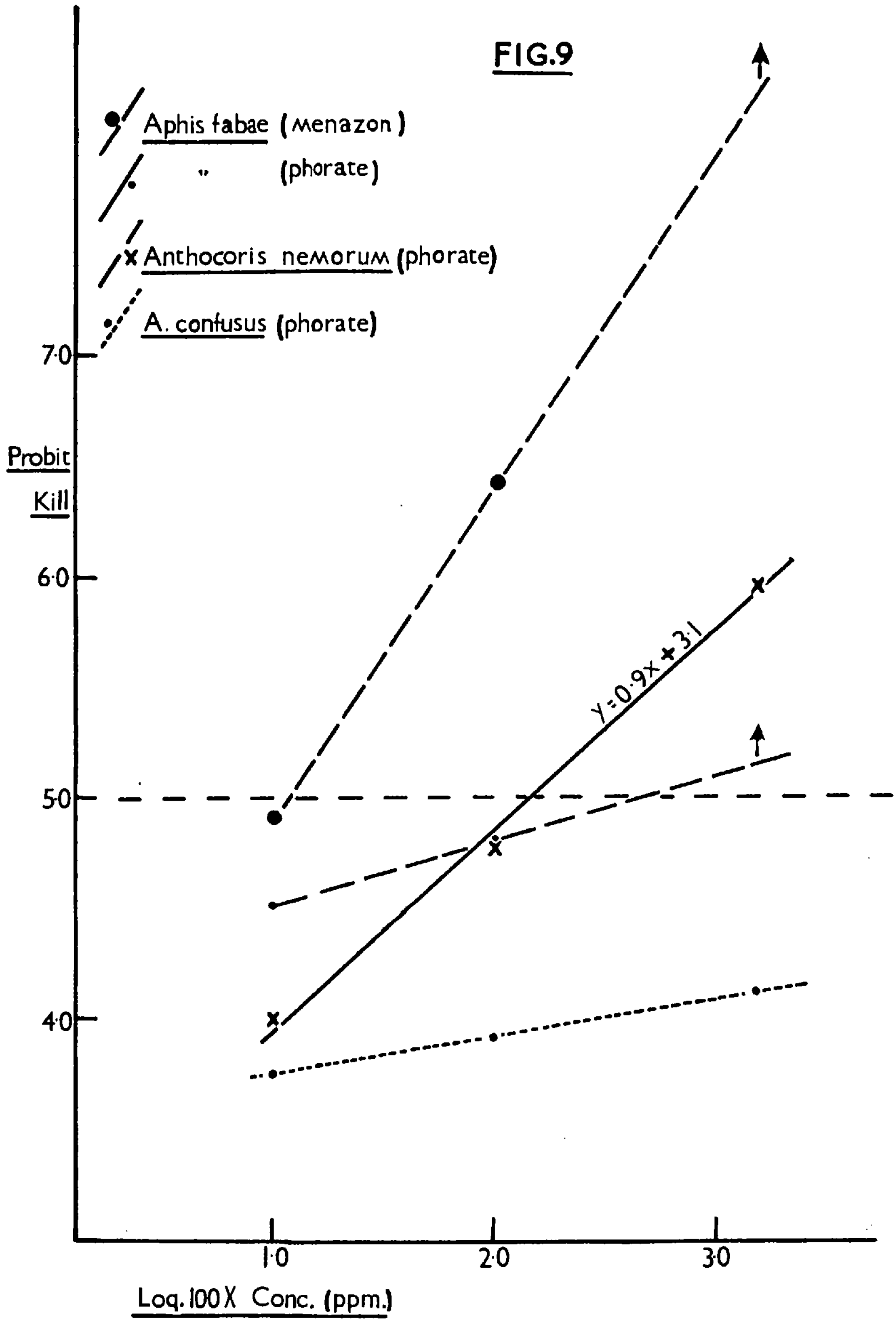


FIG.9
Aphis fabae (menazon)
" (phorate)
Anthocoris nemorum (phorate)
A. confusus (phorate)
Probit Kill
7·0
6·0
5·0
4·0
Y=0·9X+3·1
1·0
2·0
3·0
Loq. 100X Conc. (ppm.)

In contrast the relationship obtained between the kill of *A. nemorum* eggs and concentration of phorate was very close and very striking (Fig: 9.) with LD50 of 1.47   0.13 p.p.m. based on the calculated line (appendix 3.2) but, unlike the previous *A. nemorum* egg experiment, more eggs were killed in stipules than leaves.

The aphid data was not analysed since, despite large numbers used, the phorate mortality was not in close agreement with dose (Fig 9.). The 72 hour kills of Aphids by phorate were all higher than the kills of *A. nemorum* eggs by equivalent dosages of phorate. Since the top dose was an overdose for aphids, being LD94 by 24 hours, probit analysis would only have been fruitful at 24 hours which was considered too short an interval as stated above, apart from which the 72 hour aphid data was the main interest, since this showed the complete aphid kill and demonstrated that for given doses of both insecticides this was higher than the kill of *A. nemorum* eggs, even though not much higher with phorate.

The conclusion from this experiment was that both phorate and menazon at 0.1 - 15 p.p.m. killed *A. fabae* nymphs on the plants, but neither affected the eggs of *Anthocoris confusus* laid in stems, petioles and midribs, whereas phorate killed the eggs of *A. nemorum* laid in leaves and stipules, but menazon had little or no effect.

Studies on the Effects of Systemic insecticides on Anthocoris eggs in the field.

In 1964 the summer generation of egg-laying female Anthocorids was used to find out if plants, treated with soil-applied systemics in spring at normal agricultural rates, were toxic to Anthocorid eggs. Effects on the earlier generation were determined in 1965 and are described later.

The usual practice with phorate is to drill it into soil with the seeds in the form of 10% A.I. W.W. Fuller's Earth (or similar) granules at rates between 1 and 3 lbs A.I. Acre, depending on the soil and crop.

Phorate is normally applied to field or broad beans to control, *Aphis fabae* Scop. which is a major pest in most seasons, migrating into crops in May and June and reaching peak numbers in July and generally declining in August. *Megoura vicciae* Buckt. and *Acyrthosiphon pisum* Harris are less damaging because they are scarce in most years, but they are more likely to bring virus diseases into the crop.

Anthocoris nemorum females disperse from hibernacula during March and April to feeding and breeding sites, initially flowering *Salix* and then to *Urtica* and *Crataegus* and other plants, where early aphid supplies build up. They will move to bean plants when these are infested with aphids but in 1964 even untreated plants in the field trial had very low aphid infestations.

The summer generation of egg-laying females become adults in July and also disperse by flight and start laying eggs. By then, in most season, (including 1965) *Aphis fabae* is numerous on beans and it is eggs from this generation which are crucial in determining the subsequent populations of *Anthocoris* nymphs which are active, at a time when aphids are just past their peak numbers in late July and August,

also Numbers of Anthocorids overwintering may well depend on numbers which become adult in August. It is important therefore to determine the effects of the insecticides on the females of the summer generation and on their eggs.

Experiment (10) <u>The effect of 3 concentrations of soil applied phorate on Anthocoris eggs in Tick bean plants.</u>

This experiment was done in July using a field trial sown in April 1964 as follows:

The trial consisted of 8' x 8' plots in a randomised block design with 4 replicates of each treatment. Each plot was sown with 4 rows of seeds 2' apart at 4 tick bean seeds per foot of row (giving 128 plants per plot) 10% W.W. phorate, in granules of Füller's Earth, was applied by hand in the drills with the seeds (in April 1964) to give 6 lbs, 3 lbs and 1.5 lbs A.I./Acre, and untreated control with 4 plots of each.

The seed drills were then filled in and were kept weeded, but not otherwise cultivated, fertilised or irrigated from the sowing date (20/4/64) onward, this being normal practice.

Nearly all the seedlings appeared above the soil by 5/5/64 and by 25/5/64 were about 20 cm tall, with the 6th leaf unfolding. From the 6th leaf onward most leaves had more than two leaflets and the 9th leaf et seq. were pinnate. By 29/6/64 the plants were about 75 cm tall and 4 - 5 flower trusses from the 9th leaf axil upward, were open with more developing flowers in the higher axils.

At the begining of the experiment the plants were about 100 cm tall with about 20 leaves and the first 4 pod trusses set.

In this experiment there was no obvious aphid damage, because few were present (compared with 1965). The 6th and 7th leaf were used in the experiment, these had been

open and presumably taking up phorate for about 50 days. These leaves bore no aphid colonies, even on the controls, the scattered colonies which did occur being on younger leaves. The 6th and 7th leaves were chosen because they were fully developed and healthy the others being undeveloped or liable to senesce before completion of an experiment.

On 13/7/64 the 6th or 7th leaf on 4 plants in each plot (using outside rows because the plants were too tall to get between rows) was chosen and one leaflet enclosed in a 6" x 3" muslin sleeve. One adult female Anthocoris nemorum, collected from the wild and, as far as possible, one female A. confusus, either from the wild or from the culture, were put in each sleeve for 7 days and were fed every other day with the aphid Microlophium evansii from Urtica dioica. At the time, it was thought that this aphid did not feed on beans and would not be affected by phorate; certainly the aphids on control treatments were dead, as well as those on phorate treatments, when the food was replenished, but in contrast this aphid was found to die out more quickly on treated plants in field experiments in 1965, so there may have been some feeding or probing. Another reason for using a non-bean-feeding aphid was so that the food supplied on treated and control plants would remain the same (i.e. starving M. evansii) rather than that on the controls should live and breed, whereas those on phorate treatments would be less nutritious because they soon died - as well as being potentially poisonous. In practice about equal numbers of A. nemorum females survived on all treatments (Table 18). The number of eggs laid on each was not consistent with a dose effect i.e. 103 at 6 lbs, 200 at 3 lbs, 373 at 1.5 lbs and 169 on the controls. (138 of the 373 at 1.5 lbs/Acre were on 2 leaves alone

where 2 females were supplied by mistake). The Anthocorids and sleeves were removed after the 7 day oviposition period and the leaves labelled and left for a further 10 days for the eggs to hatch.

The leaves were stored in a refrigerator until the distribution of the eggs and the number hatched and unhatched (Table 19) could be recorded with reference to a leaf diagram (Fig. 11).

Assessment of Toxicity to Aphids.

At the same time as the above experiment was set up, 6th and 7th leaves from 4 other plants were taken from each plot, the dust washed off, and put in groups of 4 in tubes of water. Each group of 4 was placed in a 1 lb jam jar, with a muslin lid and 25 last instar *Aphis fabae* introduced giving a total of 100 aphids per treatment.

The leaves were kept at 20°C with 16 hours daylight per day for 72 hours and the mortality of *Aphis fabae* was then recorded (Table 19).

On 20/7/64 an attempt was made to get further information by transferring the previously used *Anthocoris* females to the 9th leaf of the plants, but from 13/7/64 to the end of the month there was no rain and most of the 9th leaves abscissed or withered. Data on survival of the sleeved *Anthocoris nemorum* females is given in (Table 18).

The precise number of *A. confusus* put in on each occasion was not recorded (in error) so the surviving numbers are not given.

Table (18) Mortality of Anthocoris nemorum females on plants treated with 3 concentrations of phorate, in granule form at sowing time.

Treatment (phorate lbs A.I. per Acre)	Period of Caging:- 13/7 - 20/7 Females:- Supplied	Dead	Escaped	20/7 - 28/7 Females:- Supplied	Dead	Escaped
6 lbs	16	3	1	16	2	0
3 lbs	16	4	1	16	1	1
1.5 lbs	18	1	0	16	5	0
Control	16	1	0	16	3	0

Table (18) shows a possible slight effect of phorate on 13/7 - 20/7 but no large differences in mortality between female A. nemorum on control and treated plants.

Table (19) Mortality of Anthocorid eggs in the field and Aphis fabae at 20 C on the 6th and 7th leaves of bean plants treated with different concentrations of phorate when sown (20/4/64).

	Dose of Phorate in lbs/Acre A.I.			
	0(Control)	1.5lbs	3lbs	6lbs
Anthocoris nemorum.				
Total Eggs laid	169	373	200	103
Number of Unhatched Eggs	26	150	115	76
% of Eggs killed	17.0	40.2	57.5	73.8
% corrected for Control Kill	-	28.0	48.8	68.4
Anthocoris confusus.				
Total Eggs laid	17	16	30	9
Number of Unhatched Eggs	0	0	2	0
% of Eggs killed	0	0	6.6	0
Aphis fabae.				
Total aphids used	100	100	100	100
Number of aphids dead 72hrs.	15	20	27	94
% of Aphids killed	15.0	20.0	27.0	94.0
% corrected for Control Mortality	-	5.9	14.1	92.9

As stated above the number of eggs laid by *A. nemorum* and *A. confusus* do not relate closely to the dosage of phorate. The small number of *A. confusus* eggs is probably due partly to the lack of suitable oviposition sites (in sleeves there was only the midrib and a small length of petiole). However the successful hatch, except for 2 eggs out of 30 at 3 lbs/Acre, confirmed the laboratory conclusion that the eggs of this species are not affected by the insecticide.

Probit lines for the *A. nemorum* and *Aphis fabae* data from table (19) are shown in Fig. 10 . The *A. fabae* mortality did not agree closely with dosage, but the chi

squared for deviation (1 D.F.) was still only 3.65 which was not significant. In contrast the line for A. nemorum eggs was drawn by eye, agreement between the points and the line being so close that analysis was unnecessary. The LD50 for eggs of A. nemorum was estimated as 3.2 lbs/Acre and for A. fabae 3.9 lbs/Acre.

These results do not concur with the relationship between egg kill and Aphis fabae 72 hour kill obtained in the laboratory (Experiments 7 and 9) where the A. fabae phorate line was heterogeneous but roughly parallel and definitely above the egg line, so that the mortality of Aphids was greater than that of eggs.

Here the lines seemingly cross at a point just above Probit 5. This indicates that more aphids are killed at high doses and more eggs at low doses.

The true explanation of the difference between the laboratory and field results probably lies in the different conditions of the two experiments. The cut taproot treatment delivers an immediate dose of phorate to the leaves and the supply is not renewed. By contrast in the field the supply of phorate or phorate derviatives from the soil may continue for a long time (in this case the 6th leaf may have accumulated phorate during the previous 50 days). They therefore probably contained a quite different spectrum of phorate derivatives both in quality and quantity possibly with a different distribution in the leaves.

Effects of A. nemorum egg location on Mortality

Experiments using P32 labelled phorate or derivatives showed that phorate taken up by roots, concentrates in mature leaves especially at the margins (Experiment13). Unexpanded leaves and stems contained relatively small amounts of labelled material.

Eggs of A. nemorum and A. confusus laid in different parts of the leaf were ascribed to the different areas shown in Fig. 11 , namely the stem and petiole (region 1 and 2), an approximately 2 mm band around the margin (region 3 and 4) and the central regions (5 and 6). The leaf was divided in half across its long axis to give a proximal (4 and 6) and distal (5 and 3) region.

The radio tracer experiment (13) indicated that regions 5 and 3 contain more phorate than regions 6 and 4 and far more than the petiole (2). The midrib (1) stands out on the radiographs e.g. plate (2C) but is thicker than surrounding tissues so that the concentration is not necessarily great.

The distribution of eggs in the different areas of 43 leaves is shown in Table (20) and the distribution of A. nemorum eggs between the upper and lower surfaces of 34 leaves is shown in table (21), with data on the percent kills.

Unfortunately the 6 lb/Acre leaves could not be included as they had decomposed in storage too much for the eggs to be ascribed to positions.

Table (20) shows that A. nemorum eggs were most numerous in region 3 - the distal marginal area and most scarce and thinly scattered in region 5 (there being only A. confusus eggs in regions 1 and 2).

Region 4 included a much used site - the point where the midrib and veins join the petioles, with eggs lying between veins. Here 76 eggs were laid in an area averaging 0.52 cm^2 on each leaf (=3.4 eggs per cm^2).

Fig. 10. Experiment 10. Section 3.

Action of Phorate applied to soil in April on *Anthocoris nemorum* eggs laid in July, and *Aphis fabae* nymphs.

Probit lines:

Solid line *Anthocoris nemorum* eggs Probit line at 10 Days in field.

Broken line *Aphis fabae* nymphs 72 hours at 20°C.

Phorate at: 6.0, 3.0 and 1.5 lbs/Acre.

Fig. 11. Experiment 10. Section 3.

Regions of tick bean leaflet used in defining positions of Eggs in Table 20.

(1) Midrib.
(2) Petiole.
(3) Distal Margin (2mm. from edge).
(4) Proximal Margin (" " ").
(5) Distal central region.
(6) Proximal central region.

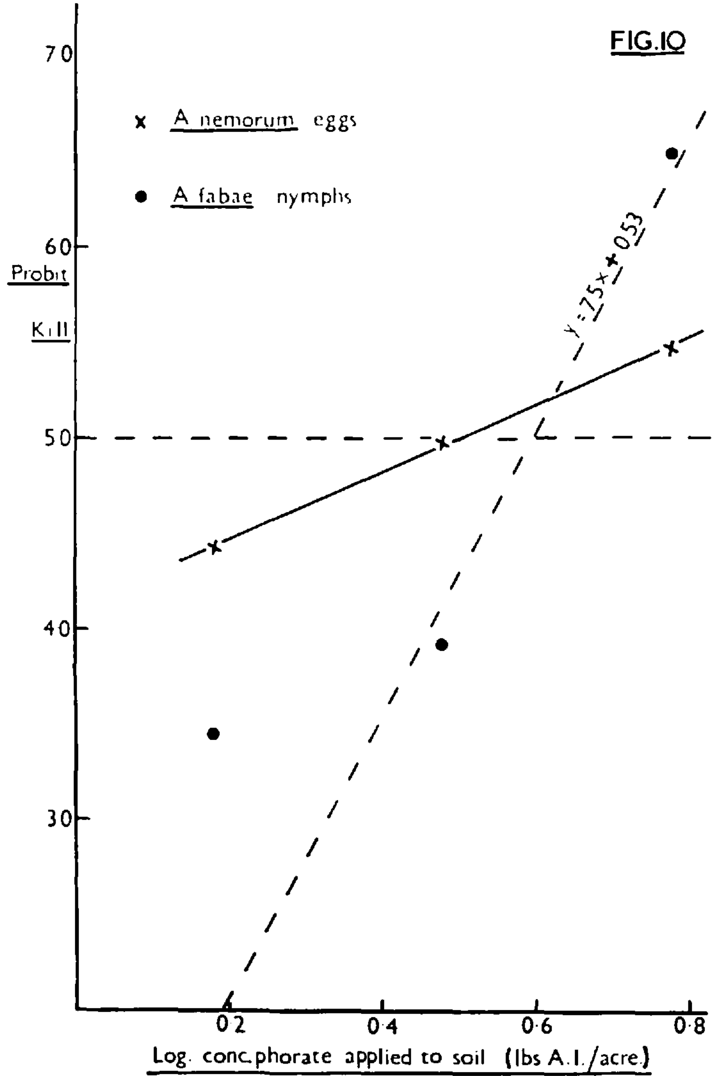


FIG 11 tick bean leaflet showing regions used in defining positions of eggs

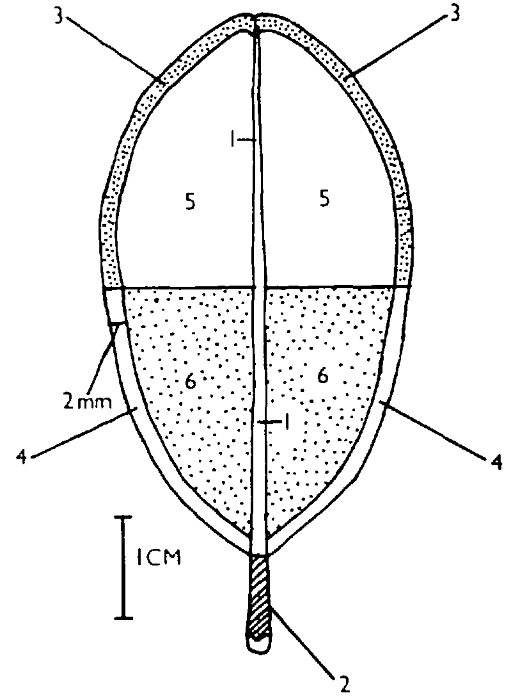


Table (20) Distribution and mortality of Eggs of _Anthocoris_ spp. in Tick bean leaves classified according to areas shown in Fig. 11 (based on 43 leaves: 3lbs, 1.5lbs and control)

Region No.	No. Eggs (43 leaves)	Area of Region both sides (cm^2)	Eggs per cm per leaf	% unhatched 3lb+1.5lb	Total Eggs Control only	% Un-hatched control	% Kill corrected for control
A. confusus							
1	23	-	-	-	-	-	-
2	46	-	-	12.5	-	0	12.5
A. nemorum							
3	248	3.12	1.85	53.9	30	23.0	40.2
4	199	3.54	0.55	50.7	45	17.8	39.1
5	126	17.94	0.16	49.0	16	0	49.0
6	145	15.10	0.22	29.5	22	4.6	26.1
Total	718	39.70					

In terms of percentage unhatched in phorate treatments, the highest mortality (54%) was in region 3, followed by 4, (51%) (both are marginal areas) then region 5, and finally 6, with 29.5%. This fits well with predictions based on autoradiographs of phorate in plants. However, if Abbotts' correction for control is applied to the mortality in each region, using the control mortality in each region, then the corrected kills indicate that most eggs are killed in region 5, followed by the others in the same order as before. The regional control mortalities are based on small numbers of eggs but, on the other hand are probably more relevant than a general control correction based on all eggs.

Table (21) The effect of phorate on survival of eggs of Anthocoris nemorum inserted into the upper and lower surfaces of tick bean leaves.

Treatment lbs/Acre	No. leaves used	Upper Adaxial Surface			Lower Abaxial Surface		
		Eggs laid	Eggs Unhatched	% Kill	Eggs laid	Eggs Unhatched	% Kill
6	5	16	15	93.7	25	19	76.0
3	11	79	47	59.5	88	53	60.2
1.5	7	134	59	44.0	81	38	46.9
Total		229	121	= 52.8	194	110	= 56.7
% Kill corrected for control.			49%				50%
Control	11	51	4	7.8	63	9	14.3
Total all treatments	34	280			257		

The mortalities of A. nemorum eggs laid in the upper and lower surfaces of the leaves are shown in table (21).

From table (21) it appears that roughly equal numbers of eggs are laid in each surface (280 and 257) and that mortality is not affected by the surface in which they are laid.

Sleeving made the leaves hang vertically in this experiment. Where leaves are horizontal, most eggs laid in the upper surface e.g 110 out of 155 eggs laid in potatoe leaves were recorded in the upper surface, whereas in this

experiment the difference was not marked.

Conclusion. This experiment shows that phorate applied at sowing time in April at practical doses killed appreciable numbers of *Anthocoris nemorum* eggs in July when female *Anthocorids* might still be laying eggs in beans and other crop plants. Eggs of *A. confusus* were not affected.

In these circumstances the LD50 of phorate for *A. nemorum* eggs was estimated as equivalent to 3.2 lbs A.I./Acre applied at sowing time, which is lower than the equivalent LD50 estimated for *A. fabae* which was 3.94 lbs/ Acre.

The eggs of *A. nemorum* were less readily killed in region 6, (Fig. 11) of the leaf than in other regions. This could be explained by the relatively lower concentrations of phorate in this region indicated by autoradiographs of plants treated with P32 labelled phorate (Experiment 13)

No difference was found between the mortality of eggs inserted into the upper and lower surfaces of the leaves.

Experiment (11) Effects of phorate applied at agricultural rates on two generations of Anthocorid eggs 1965.

The 1964 field experiment was done in July, when bean plants were mature, having been treated with soil applied phorate granules when sown the previous April. There is, however, an egg laying period by overwintered females begining in May during which overwintered females may lay eggs on bean plants in May and June, if these are infested with aphids, but it is also of general interest to determine the toxicity of treated plants to eggs, when the phorate is newly applied.

The objects of this experiment were (1) To find the effect of recently applied phorate in young plants, on eggs laid by overwintered females. (2) To compare the mortality of eggs laid in early June with that of eggs laid in late July. (3) To extend comparisons of the egg mortality on the plants with the mortality of aphids. (4) To confirm that A. confusus eggs are not affected by phorate and to determine whether A. nemorum eggs are affected by menazon, in young plants recently treated. (5) To compare mortality of eggs in leaves on different parts of the plants.

Methods:- Design of Field Experiment: This was similar to the one done in 1964 and consisted of 16 micro-plots of Tick beans, each of four 6 foot rows of plants 2 feet apart with 12 seeds per row. The plot layout was 4 x 4 Latin square design and each block contained 4 treated plots, one at each treatment. The treatments were 6 lbs and 1½ lbs A.I. phorate per acre, 1½ lbs menazon per acre and control. Both insecticides were 10% W.W. of insecticide in granules of Fuller's earth in the seed drills. The plots were treated and sown by hand, on 30/4/65, no further cultivation, other than weeding took place.

Method of assessing aphid toxicity: The first plants appeared above the soil on 12/5/65, and on 16/5/65 most of them were about 1½" tall with the first leaf open, almost flat on the soil. The first aphid toxicity test was made on this date, by caging 2 batches of 10 Aphis fabae nymphs from an outdoor culture on the underside of a leaf, on 2 randomly selected plants from inside rows in each plot. The cage was a padded Clip-on leaf cage (Fig. 15) using 8 cages & 80 aphids per treatment. Kills were assessed after 24 and 48 hours (Table 22). Subsequently the 3rd, 4th, 5th, 6th, 11th, and 17th leaves were tested in the same way on 28/5, 19/6, 8/7, 13/7, 26/7 and 26/7 respectively.

The outside Aphis fabae culture and the natural Aphis fabae population on the control plants, were affected by fungus disease at the time of the test on 26/7 so in subsequent tests Acyrthosiphon pisum nymphs, from a 20°C room culture, were used until the toxicity became neglible in August.

Tests of toxicity to Anthocoris nemorum eggs in June 1965: The first four leaves of the plants on all the insecticide treatments proved very toxic to aphids (Table 22). As soon as the plants had 4 expanded leaves (on 2/6/65) Anthocoris nemorum females, collected on the same day from nettles, were sleeved on the whole plants above the second leaf; using the 6" x 3" muslin sleeves used in 1964. 4 sleeves were used per plot (one per row) and 4 female Anthocorids were put in each sleeve giving 16 per treatment. Microlophium evansii from nettles was again supplied as food and renewed every 2 days, but unfortunately not from 6/6/65 until 10/6/65, when the sleeves were removed. This perhaps affected survival of female A. nemorum as described

in section (6) but fortunately the females laid many eggs before they died.

The leaves which had been sleeved with A. nemorum were labelled and the egg hatch was assessed by removing groups of 8 plants in turn (2 from each treatment on various dates from 24/6 to 15/7). All counts were made at least 14 days after the eggs were laid, thereby giving ample time for hatching (results in Table 23).

Test of toxicity to Summer generation eggs of A. nemorum.

Samples of field collected Anthocoris nemorum females were dissected on various dates in July, to find out when oviposition would start, the eggs from such females being one generation later than the eggs in the June experiment. On 24/7 most collected females had ovulated the first few eggs and were therefore suitable for oviposition experiments. The bean plants in the field experiment were then more than 1 metre tall, except the A. fabae infested controls which were shorter. All had about 21 expanded leaves and were flowering. Randomly selected plants were sleeved with 14" x 8" sleeves with 0.1" mesh, tied on above the 16th leaf and above the 20th leaf. Each leaf was reduced to one large leaflet and the stipules and flowers removed to facilitate subsequent egg counting.

The menazon treatment was not included, since it had been proved to have no effect in June. One plant was sleeved on each plot of the 6 lbs and 1.5 lbs phorate and control, making 4 plants at each treatment and 3 A. nemorum females put in each 14" x 4" sleeve, giving 12 females per treatment. In addition, on the same plants, the 11th leaves of the 1.5 lbs phorate and control plants were sleeved with 3" x 6" muslin sleeves, in each of which was put one female A. nemorum, giving 4 females per treatment. This was done to compare the toxic effects of the older 11th leaves with

that of the 16th and younger leaves.

As before Microlophium evansii was supplied as food; also Drepanosiphon platinoides from Sycamore, supplies being renewed every other day. The sleeves were removed after a week (31/7) and the eggs counted after a further 17 days. Table (24) shows the hatch of the eggs in the different leaves on the two phorate treatments and control.

Test of toxicity to A. confusus eggs.

Autoradiographs (section 4) showed that small quantities of P32 labelled phorate accumulated in stems and petioles where A. confusus lays eggs, compared with at the edges of leaves where A. nemorum lays eggs. The 1964 field results, showed that A. confusus eggs in petioles and midribs were unaffected by phorate so a repeat experiment was done in July 1965, using the same region of the stem as was used in the June experiment with A. nemorum: namely the section between the 3rd leaf and the 7th. The sleeves were fitted to include leaves up to the 10th. The experiment was set up on 13/7. Wire frames were arranged around the stems of the plants and 14" x 8" sleeves were fitted over these, tied tightly at the 3rd and 10th internodes.

Two plants were used at 6 lbs Acre phorate and 2 controls. 6 A. confusus females from the 20°C room culture were caged on each plant. D. platanoides (one of the natural prey of A. confusus) were supplied as food every other day.

On 20/7 the females were removed and on 16/8, the hatched and unhatched eggs were counted on the stems, midribs and petioles. The results are given in (Table 25).

Table (22) Percent mortality of Aphis fabae and Acyrthosiphon pisum caged on phorate and menazon treated bean plants in 1965. (48 hours Kill, 80 per treatment.)

Aphis fabae.						24hr	24hr
Date.		17/5	23/5	29/5	20/6	8/7	13/7
Leaf used.		1	1	3	4	5	6
Phorate							
6lbs/Acre	%Dead	98.8	97.5	98.8	100	85.0	87.5
	Corrected %	98.5	96.7	97.8	100	80.7	83.3
1.5lbs/ Acre	%Dead	82.5	98.8	92.5	83.8	47.5	50.0
	Corrected %	78.5	98.4	86.4	69.1	32.3	33.3
Menazon 1.5lbs/ Acre	%Dead	83.8	68.8	83.8	82.5	41.3	57.5
	Corrected %	80.1	59.1	70.6	66.7	24.3	43.3
Control	%Dead.	18.8	23.8	45.0	47.5	22.5	25.0

over/...

Table (22) continued.

Aphis fabae.				A. pisum.		
Date.		27/7	26/7	8/8	8/8	23/8
Leaf used.		11	17	11	17	23+
	Fungus infection.					
Phorate 6lbs/Acre	%Dead	-	-	-	-	38.1
	Corrected %					0
1.5lbs/ Acre	%Dead	77.5	73.8	38.8	26.0	36.7
	Corrected %	0	36.4	29.8	15.6	0
Menazon 1.5lbs/ Acre	%Dead	--	-	-	-	41.3
	Corrected %					0
Control	%Dead	25.0	58.8	12.8	12.3	45.7

Results I The toxicity of the insecticides to aphids Table (22)

Tests on the 1st, 3rd, 4th, 5th and 6th leaves with 6 lbs/Acre phorate, all gave corrected kills of A. fabae of 80 - 100% after 48 hours. This effectively prevented aphids from surviving on them. This was confirmed by the fact that Aphis fabae infested all the control plants, when they had about 4 leaves open, and continued to breed on them until July, when the numbers declined ,whereas all the treated plants remained free of aphids, except between 29/6/65 and 4/7/65, when a heavy invasion of Aphis fabae alatae produced a temporary infestation of apterae nymphs, which

died out before, or shortly after, becoming adult.

Plants of the 1.5 lbs/Acre phorate treatment remained free of aphids, but gave lower kills than 6 lbs/Acre treatments, except in the 2nd test on the 1st leaf, when 99% were killed after 48 hours compared with 97% at 6 lbs/Acre, and at this level of toxicity the difference was not significant.

The tests on the 5th and 6th leaves gave 48 and 50% mortality in 24 hours compared with 80 plus % for leaves (1 - 3) from which it might be predicted that the former leaves would be less toxic to A. nemorum eggs.

The 1.5 lbs/Acre menazon treatment also kept the plants as free from aphids as the phorate treatments. The kill of aphids in toxicity tests was generally lower than that by 1.5 lbs phorate perhaps because menazon acts more slowly.

The toxicity tests on the 11th and 17th leaves were spoilt by a fungus disease of the A. fabae. The test was repeated with Acyrthosiphon pisum on 7/8/65. This indicated that the 11th leaf was possibly more toxic than the 17th leaf but more detailed comparison was needed. The test on 23/8/65 using the topmost open leaf (normally about the 23rd) of healthy plants on each treatment indicated that all treatments were non-toxic to aphids. The large mortalities on the control plants, and the decline in relative toxicity of the treated ones, was probably affected by heavy aphid damage to the control leaves making them unsuitable for introduced aphids, and by bean virus infections, in all treatments, from July onwards, although badly diseased plants were avoided in tests.

Table (23) Toxicity of insecticide treatments to eggs of A. nemorum laid in Tick bean plants (2/6 - 10/6/65) (Treatments applied in April when crop sown)

Treatment	T	U	% Unhatched	Corrected % Kill
Phorate 6lb/Acre	110	101	91.8	89.5
1.5lb/Acre	134	119	88.8	85.5
Menazon 1.5lb/Acre	274	56	20.4	0
Untreated control	157	37	23.6	-

T = Total U = Unhatched.

II Effects of phorate and menazon on eggs laid in June

Table (23) shows that both 6 lbs and 1.5 lbs/Acre phorate killed many eggs of A. nemorum (89.5 and 85.5%) whereas fewer died on 1.5 lbs/Acre menazon than on control. Of the 15 hatched eggs on 1.5 lbs phorate treatment, 4 were on leaves and stipules 5, 6 and 7, and 11 on leaves 3 and 4, mortality of eggs being 90% on leaves 5, 6 and 7 and 88% on leaves 3 and 4, so that there was no reduction in toxicity towards the top of the plant. The mortality in stipules was 90% compared with 96% in leaves. In general there were too few hatched eggs to show clear differences in toxicity in different places, the whole plant at 1.5 and 6 lbs/Acre being highly toxic to eggs of the overwintered generation of A. nemorum and completely lethal to aphids.

Table (24) Toxicity of Phorate treatments to the second generation of Eggs of A. nemorum laid in tick bean plants. (24/7 - 31/7/65)

Treatment	Leaf	T	U	U%	Corrected % Kill
Phorate 6lbs/Acre	11	-			-
	17	29	24	83	
	18	47	39	83	
	19	76	54	71.1	
Total		152	117	77	73.9
Phorate 1.5lbs/Acre	11	53	19	36	
	17	52	17	33	
	18	55	34	62	
	19	62	20	32	
	20	20	4	20	
	21	54	18	33	
Total		243	93	38	29.8
Control (untreated)	11	84	9	11	
	17	44	5	11	
	18	52	6	12	
	19	37	4	11	
	20	8	2	33	
Total		141	17	12	

U = Unhatched T = Total laid.

III Toxicity of phorate to Summer generation eggs of A. nemorum

From table (24) it can be seen that there was a high kill (74%) of A. nemorum eggs in August by 6 lbs/Acre phorate

applied 4 months earlier. Unfortunately no test of toxicity to *A. fabae* was done at this time, though all treatments ceased to be toxic to aphids by 23/8 (Test on top leaf 23/8 Table 22). The 30% kill of eggs by 1.5 lbs/Acre phorate is higher than the 15% kill of *A. pisum* on leaf 17, on 8/8/65. These results suggest that toxicity of phorate to *A. nemorum* eggs late in the Summer may be higher than its toxicity to aphids. This agrees with the result obtained in July 1964 (Experiment 10) when 3 lbs and 1.5 lbs/Acre phorate treatments killed more *A. nemorum* eggs than *Aphis fabae*, but the precise relation between the two effects at high doses from the 1965 data (because no test of aphid toxicity was done at 6 lbs/Acre) was lost.

No test was done because it was thought that aphid mortality and egg mortality would both be so high in this treatment that it would be best to concentrate the available insects, on the 1.5 lbs/Acre treatment.

Table (25) Mortality of *A. confusus* eggs laid on 6lbs/Acre phorate treated bean plants and Untreated controls.

Treatment	Midribs T	Midribs U	Petioles T	Petioles U	Internodes T	Internodes U	Total all sites T	Total all sites U
6lbs phorate per Acre								
Plant 1	34	2	28	6	4	0	66	8
Plant 2	28	3	18	0	4	1	50	4
					Treatment total =		116	12
					=		10.3% Unhatched	
Control								
Plant 1	16	0	4	1	3	0	23	1
Plant 2	7	1	2	1	5	0	14	2
					Treatment Total =		37	3
					=		8.1% Unhatched	

IV Effects of phorate on A. confusus Eggs.

From table (25) the mortality of Anthocoris confusus eggs on the high (6 lbs/Acre) phorate treatment was 10.3% compared with 8.1% on the controls, which agrees with the results of the laboratory and field experiments in 1964.

Fewer eggs were laid in the main stem and more in the midribs than might be predicted from the data in Table (11) at the begining of this section. Perhaps this was because the mature main stems are very hard relative to the seedling stems used in laboratory experiments.

Conclusions to 1965 Field Experiments: Phorate killed very many A. nemorum eggs of the first generation in June, at both levels (6 and 1.5 lbs/Acre) and aphids did not establish themselves. In August, when the effects of aphid invasion would be insignificant, the bean plants were seemingly more toxic to A. nemorum eggs than to aphids. Menazon at 1.5 lbs/Acre did not affect the eggs in June or August.

Eggs laid in stems, midribs and petioles, in July, by A. confusus were not killed by phorate at 6 lbs/Acre.

Effects of dimethoate on A. nemorum eggs.

Dimethoate was shown to be more toxic to Anthocorid nymphs than phorate when used as a contact insecticide (section 5). An experiment was therefore done on its toxicity to the eggs.

Experiment:-12 To compare the toxicities of root applied dimethoate to Anthocoris nemorum eggs and aphids.

Method.

The cut taproot method as used in Experiment (6) was used to obtain standardised bean seedlings with 5 p.p.m., 1.0 p.p.m. and 0.1 p.p.m. dimethoate treatments. The largest dose applied was 5 p.p.m., because preliminary trials showed that 10 p.p.m. was phytotoxic and caused leaf fall within

one week.

The stipules were removed to make the *Anthocoris* females lay only on leaves and so concentrate the eggs in uniform sites. This was not done in experiment (9.) as it was then intended to find out possible differences in kill in leaves and stipules, since the relative distribution of *phorate* in plants had been studied.

Plants were treated on 6/4/65 as follows: 5 at 5 p.p.m., 6 at 1 p.p.m., 5 at o.5 p.p.m., 6 at o.1 p.p.m. and 5 with 0.5% cellosolve solution (controls). The following day two gravid female *A. nemorum*, from the wild population, were caged on each plant and supplied with *Myzus persicae*, as food which was chosen since survivors were unlikely to be confused with species to be used in the aphid bioassay. From then on the plants were in the 20°C room.

After 48 hours the female Anthocorids were removed and ten 3rd or 4th instar apterae *A. fabae* nymphs were put on each plant. As an additional guide to aphid toxicity, *Acyrthosiphon pisum* nymphs were introduced at the same time, as far as possible using nymphs of the same size as the *A. fabae*. The final numbers of each species used is shown in Table (29).

The 24, 48 and 72 hour mortalities were assessed by counting living aphids and are given in table (29).

Thereafter surplus aphids were removed to protect plants with surviving aphids, from unnecessary aphid damage. The hatch of *A. nemorum* eggs was assessed on 12/4/65, 9 days after oviposition. The hatched and unhatched eggs were counted and ascribed to the particular leaf, or leaf edge in which they occurred, since mortality might vary according to position on the leaf (Table 26).

Table (26) Mortality of A. nemorum eggs on bean plants treated with 4 levels of dimethoate and control.

Dosage (p.p.m.)	5.0		1.0		0.5		0.1		Control	
Eggs *	T	U	T	U	T	U	T	U	T	U
Upper leaf	3	0	0	0	4	0	0	0	1	0
" " Edge	54	10	47	11	11	2	10	2	45	2
Lower leaf	0	0	2	0	3	0	1	0	0	0
" " Edge	32	7	35	4	13	2	16	2	23	4
Total	89	17	84	15	31	4	27	4	69	6
% Kill		19.1		18.0		13.0		19.0		~~9.0~~ 8.7.
% Kill (edges only)		19.6		18.2		6.3		15.4		8.8

* T = Total Eggs U = Unhatched Eggs.

Results I A. nemorum eggs: The results in Table (26) show the percentage mortality at 5 p.p.m. was 19% compared with 9% in the untreated controls. Similar kills were obtained at the other dosages though the results using 0.5 p.p.m. and 0.1 p.p.m. dimethoate were based on too few eggs to carry much weight. Since phorate at 5 p.p.m. killed 69% of eggs (after correction for control mortality) the toxicity of dimethoate to eggs was relatively small.

Chi squared tests Tables (27&28) were done to determine whether the kills for dimethoate differed significantly from the untreated control mortality. The results showed no significant differences, even when the data was restricted to eggs on the leaf edges where all unhatched eggs (both treated and controls) occured.

Table (27)
Chi squared test on Hypothesis <u>all</u> Egg Mortality due to chance.

Dosage p.p.m.	U "O"	Expected U (=15.3%T) "E"	$\frac{(O - E)^2}{E}$
5.0	17	13.6	0.83
1.0	15	12.9	0.35
0.5	4	4.8	0.12
0.1	4	4.1	0.01
Control	6	10.6	1.98
		Chi squared (4DF) =	3.29

(Maximum permissible value = 9.5 at 5% level)

Table (28)

Chi squared test on Hypothesis that mortality of eggs on leaf <u>edges</u> <u>only</u> due to chance.

Dosage p.p.m.	U "O"	Expected U (= 14%T) "E"	$\frac{(O - E)}{E}$
5.0	17	12.0	2.04
1.0	15	11.5	1.08
0.5	4	9.0	2.75
0.1	4	3.6	0.04
Control	6	9.5	1.30
		Chi squared 4DF =	7.204

(Maximum permissible Value = 9.5 at 5% level)

Table (29) Mortality of A. fabae (A.f.) and A. pisum (A.p.) on bean plants treated with 4 levels of dimethoate.

Dosage p.p.m.	No. Plants	No. Aphids A.f.	A.p.	24hr. %Dead A.f.	A.p.	48hr. %Dead A.f.	A.p.	72hr. %Dead A.f.	A.p.
5.0	5	50	45	70	100	90	-	98	-
1.0	6	60	50	45	94	62	96	85	100
0.5	5	50	45	58	73	68	76	80	93
0.1	6	60	50	22	10	33	30	42	38
Control	5	50	40	18	0	24	2.5	28	5.0

II Aphid Bioassay Table (29) Fig. (12)

Table 29 shows that an anomaly in A. fabae mortality at 24 hours, since 1.0 p.p.m. killed 45% and 0.5 killed 58%. This was less pronounced at 48 hours and disappeared at 72 hours. The anomaly may have been because not all aphids fed when introduced, and was not due to an error in doses because A. pisum responded as expected.

Probit analysis was done on the 48 hour data and the LD50 was 0.611 p.p.m. for A. fabae (S(m)0.546 - 0.684 p.p.m.) and 0.170 p.p.m. for A. pisum (S(m)0.161 - 0.180 p.p.m.).

Both calculated probit lines fitted the points well (chi squared 2DF A. fabae 4.083, A. pisum 1.831). The lines were not constrained to parallel, since a preliminary analysis, assuming parallelism, gave a chi squared for departures from parallelism (1DF) of 18.1 Appendix 3.4.

The 72 hour mortality was not used since, although by then the anomaly in A. fabae mortality had disappeared, 2 dosages gave LD100% of A. pisum, leaving only 2 other points to use. Probit lines for 72 hours, drawn by eye are shown in Fig(12) and are consistent with the 48 hour

data.

These results showed that dimethoate had an effect on aphids, by systemic action, comparable with menazon and phorate but did not kill *A. nemorum* eggs as did phorate.

The *A. fabae* were more tolerant (see above) than the *A. pisum* of the same size. This interspecific difference was also found with phorate in Experiment 5 (Section 2).

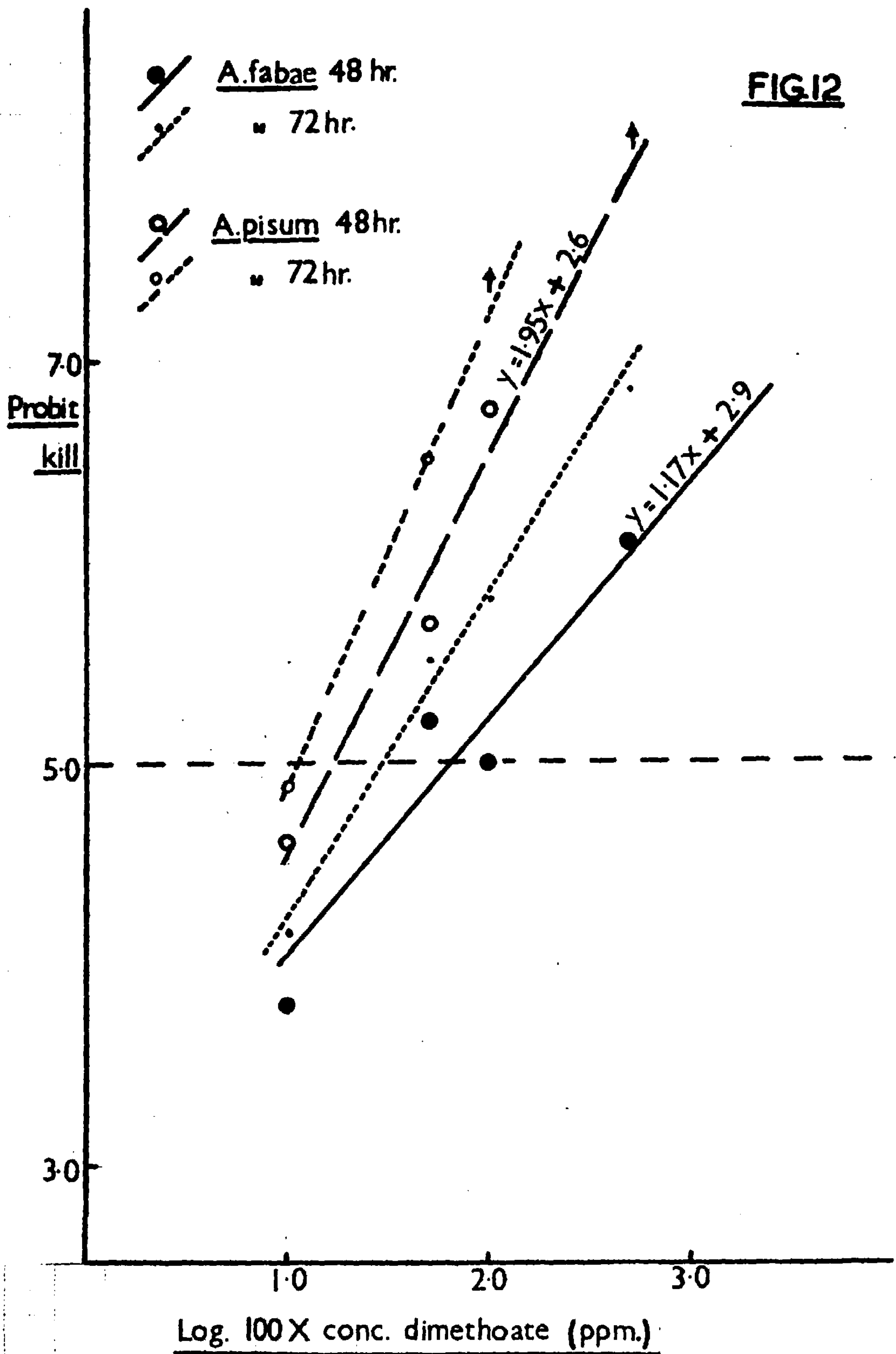
Fig. 12. Experiment 12. Section 3.

Action of root applied Dimethoate on *Acyrthosiphon pisum* (mainly 2nd instar nymphs) and *Aphis fabae* (mainly 4th instar nymphs). This treatment did not affect *A. nemorum* eggs.

Probit lines.
48 hours (calculated) *A. fabae*.
A. pisum

72 hours (drawn) *A. fabae*.
A. pisum.

Dimethoate at: 5.0, 1.0 , 0.5 and 0.1 ppm


A.fabae 48 hr.
" 72 hr.
A.pisum 48 hr.
" 72 hr.
FIG.12
Y=1·95X+2·6
Y=1·17X+2·9
7·0
Probit kill
5·0
3·0
1·0
2·0
3·0
Log. 100X conc. dimethoate (ppm.)

Section1V The distribution of phorate in plants in relation to the oviposition sites of Anthocoris species.

Laboratory and field experiments in Section 3 of this thesis showed that phorate killed the eggs of Anthocoris nemorum, laid in the leaf laminae and stipules of bean plants, without harming those of A. confusus, laid in stems, petioles and midribs. This difference occurred even at the agricultural rate of 1.5 lbs per acre of phorate A.I.

The aim of the experiment in this section was to find the parts of the plant in which phorate accumulated when applied to the roots, which might perhaps account for the difference in egg toxicity in different parts of the plant, assuming that the eggs of the two species do not differ greatly in tolerance to insecticides, but only in oviposition sities. Anthocoris confusus 2nd and 3rd instar nymphs are about 2.3 times more tolerant than A. nemorum to phorate as a contact insecticide (Section 5), but this order of difference would not account for the large differences found with their eggs e.g. 6 lbs A.I. phorate per acre killed 90% of A. nemorum eggs, compared with 2% of A. confusus eggs (1965 Field Experiment).

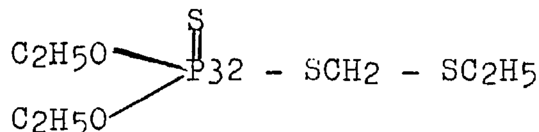
Autoradiography was used to assess accumulation of isotope labelled phorate in beans, other crop plants and some species of deciduous trees in which Anthocoris species are known to breed. The autoradiographs actually show only the position reached by P32 in the plants, and this might not have been in the phorate molecule, but in some derivative from it. As stated below the plants were toxic to aphids, so that they must have contained active toxicant from the phorate, but this is not proof that this is the same material which contained the P32. In this account the

radioactive material is referred to as "phorate derivative".

Experiment 13. The accumulation of phorate in plants from applications of P32 Labelled phorate granules.

The object was as discussed above, with particular emphasis on seeing if the pattern of accumulation in standardised bean seedlings (Fig. 7) was similar to that in other plants, such that data on phorate toxicity to eggs in beans could reasonably be extrapolated to other plants.

Materials used (1) Insecticide and dosage: Phorate was synthesised using P32 i.e.



The insecticide had been absorbed on granules after mixing with unlabelled phorate, to give 1 lb of Fuller's Earth granules containing 8.8% A.I. phorate, with a total activity of 4 millicuries at the time of manufacture of the granules (C 19/5/64). The ½ life of the isotope was 14 days, so that the material used on 1/6/64 had lost nearly ½ of the initial activity. It was thus desirable to use a large dosage in order to be sure of getting a picture of the variation in distribution, since the time it would take for the insecticide to accumulate in different parts of the plant was not known. Two rates were chosen : 100 p.p.m. and 25 p.p.m. in the growing medium. For comparison the normal agricultural rate (2 lbs/Acre in drills) is about 2 p.p.m. in the top 4" of soil.

(2) Growing Medium : Casida, Chapman and Allen 1952, showed that Schradan uptake by plants was fastest in sandy soils, slower in loam and slowest in clay so, in these experiments the plants were grown in silver sand watered with an

inorganic culture solution.

(3) Plants The experimental plants were transplanted, from various sources, into ordinary 3" porous clay flower pots, containing 300 gm silver sand, mostly on 24/5/64, and tended in a greenhouse until established.

When treated (1/6/64) they were as follows:

1) Tick beans sown 3/5/64 (grown in culture solution until 24/5/64)tall, with 4 open leaves (kept in Greenhouse).

2) Tick bean as above but with apex and lateral growth removed (to give only 2 open leaves as Fig. 7) just before treatment (i.e. corresponding to experimental plants in Egg experiments in Section 3)(kept in Greenhouse).

3) Tick beans, sown 26/4 with only first two leaves allowed to develop, so that, when treated, these leaves were about 3 weeks old and very thick and mature, as used for high phorate treatments in Section 6. (kept throughout in Greenhouse).

4) Tick beans, sown on 26/5/64, with the plumule just visible above the sand (i.e. treated at an earlier stage than the other beans used) (kept in Greenhouse).

5) Broad beans sown about 8/5/64 in soil in greenhouse, 4" tall with 2 or 3 open leaves.

6) Potatoes grown in soil in a greenhouse with original tubers attached about 12" tall with 6 leaves.

7) Winter Oats (Var. Aberystwyth.) sown in soil in greenhouse about 8/5/64, with 4 leaves up to 7" long, tillering at base.

8) Brussels sprout (Glossy variety), about 5" tall with 4 or 5 fully developed leaves and 2 or 3 smaller senescent leaves, probably 2 months old, originally sown in soil.

9) Deciduous Twigs:- approximately 12" long twigs of the current years growth, cut from apple, sycamore and beech trees.

Treatment of plants.

Appropriate weights of the phorate granules were put into 2" x 3/8" open ended tubes. These were inserted into slanting holes in the sand in each pot, so that the phorate was delivered just below the point of origin of the roots. The tubes were left in position, so that the granules could be seen by removing them, if necessary. The plants were kept in a glasshouse without temperature control. On the same day the cut deciduous twigs were put in 1 lb Jam jars of distilled water, containing granules calculated to give 100 p.p.m. and 25 p.p.m. in water (although the solubility of phorate is only 50 p.p.m. in water). The jam jars were put in the same glasshouse as the plants, and were topped up with water when the potted plants were watered:- i.e. nearly every day, since the weather was hot. Every fifth day, culture solution was used instead of water.

Rate of Uptake Four days after treatment, 2 batches of 10 aphids were caged, in clip-on cages (Fig. 15), on each species of plant treated with 100 p.p.m. phorate. *Myzus persicae* was used on sprouts and potatoes, *Rhopalosiphon* sp. on oats, and *Aphis fabae* on beans.

All the aphids were dead after one day (6/6/64).

On 7/6/64 the first signs of phytotoxicity appeared and the first autoradiographs were taken on 8/6/64.

Autoradiography of Plant Material 8" x 10" Ilfex E Xray plates were exposed to the plant material mounted on 8" x 10" typing paper. The leaves and stems were cut from the plants and stuck to the paper by small pieces of "Sellotape". The thicker stems were flattened so that neighbouring parts would not be kept away from close proximity to the plate. Since fresh plant material may contain materials which sensitize Xray plates, and fats and oils from plants may

cause the latent image to fail to develop (Boyd 1955), an extremely thin film of terylene "Melanex", was fixed over all the plant and insect material. Control plates were set up with non - radioactive leaves from untreated plants, and one treated leaf for comparison, to show artifacts that might occur despite the terylene, such as marks due to the pressure of the plants against the plates. The twigs and plants were jointed and the leaves and stems laid out as shown in plates 1 - 6.

Xray plates were exposed by inserting the mounted plant material (covered with terylene) in a light proof lead - lined Xray holder with an Xray plate facing it. The lead lined plate holders protected each Xray plate from external radiation. The plate holders were put in a light proof box, tightly packed to ensure close proximity of the plates to the subjects.

The box of plate holders was then placed in a deep freeze to "fix" the sap in the plants and to prevent decay.

<u>Choice of Exposure Period</u> The first Xray plates were exposed to plants treated one week previously with 100 p.p.m. phorate in sand. It appeared that 48 hours (plate 1.2) was an adequate exposure period for bean plants, being preferable to 24 hours (1.1) in that the outlines and veins were clearer, without excessive blackness or blurred outlines from diffusion of radiation from high concentration areas. The 24 hour radiographs of oat leaves gave poor traces and a 4 day exposure was chosen for them. Four days was also used for second autoradiographs of the same subject and also for all 25 p.p.m. treatments. Ten days was used for the broad bean flowers (3.2.D) and for the Control plate which was completely blank for untreated leaves. (The single treated leaf also on the control plate gave a normal trace, therefore showing the Xray plate used was correctly developed.

<u>Results</u>.

Plates 1 - 6 are photographs of sections of Xray plates showing the accumulations of phorate derivative in different plants. In some, drawings of the leaves etc. accompany the autoradiographs.

Plate 1. shows two tick bean leaves, taken from affected plants, showing phytotoxicity from treatment at 100 p.p.m. 1.1. is the trace of one after 24 hours exposure 1.2. is of the other after 48 hours exposure.

The 24 hour trace shows phorate derivative at the edge of the distal half of the leaf with regions of local concentration. The proximal part of the leaf is only just visible and no veins can be seen. The 48 hour exposure Plate 1.2. shows the entire margin, and very feintly the petiole. The regions of local concentration correspond with the brown lesions, characteristic of phorate damage, both for beans.(plate1.3 and Potatoes Plate 4.) These do not show on undamaged leaves 2a.

Plate 2 shows the different parts of an entire bean plant (see "plants" no.1.) treated at 100 p.p.m. The bottom leaf 2d, which is always small, and the larger middle leaves 2c have pronounced local concentrations. A drawing of the leaves, showing the paths of the midribs and the lesions, drawn by tracing the outlines of the actual leaves and lesions etc. with an illuminated tracing screen, shows that the local high concentrations of phorate derivatice correspond with the lesions. The upper leaves 2a showed no lesions or local concentrations of phorate derivative, and generally lesions did not appear on leaves until they reached the maximum size and were fully mature.

The pieces of stem 2.f, g, h. showed concentrations corresponding to stem thickness and decreasing up the stem

Plate 1.

1.1 Tick bean 100 p.p.m. 24 hour exposure.

1.2 Tick bean " " 48 " "

1.3 Tick bean shortened as in Experiments (2 leaves).

a) Upper leaves.

b) Lower leaves.

c) Upper stipules.

d) Lower stipules.

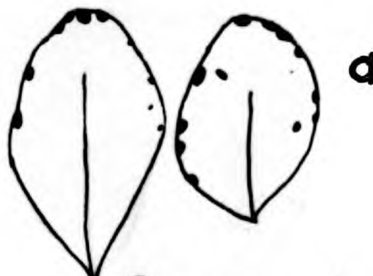
e) Stem sections.

1

1.1.

1.2.

1.3.



a

b

c.

d.

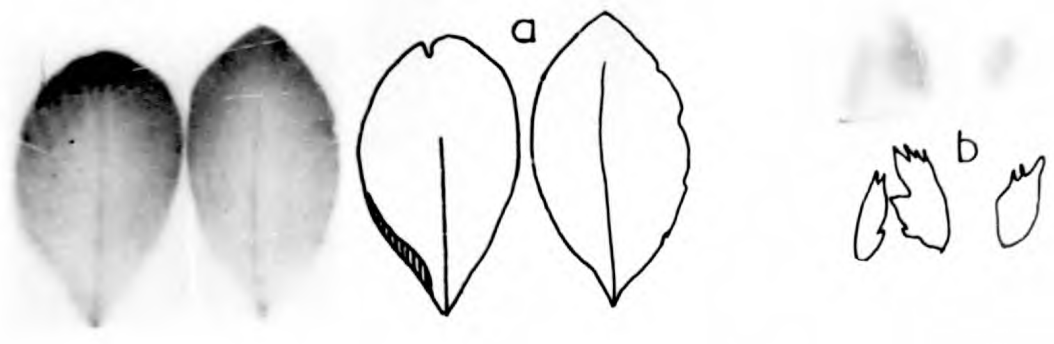
e.

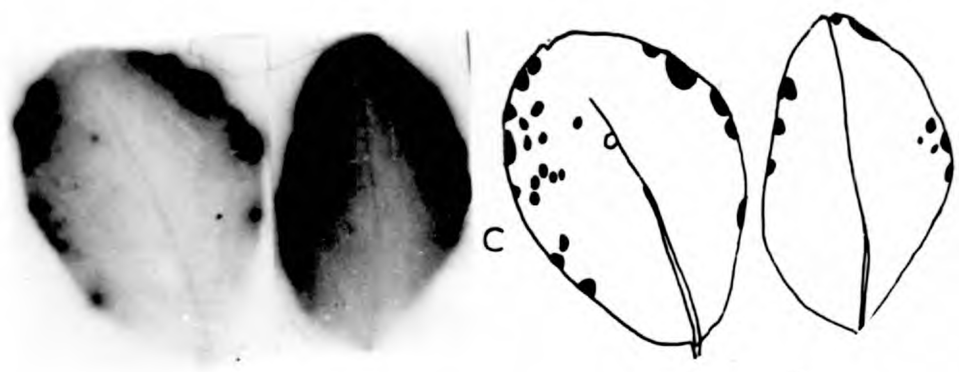
Plate 2.

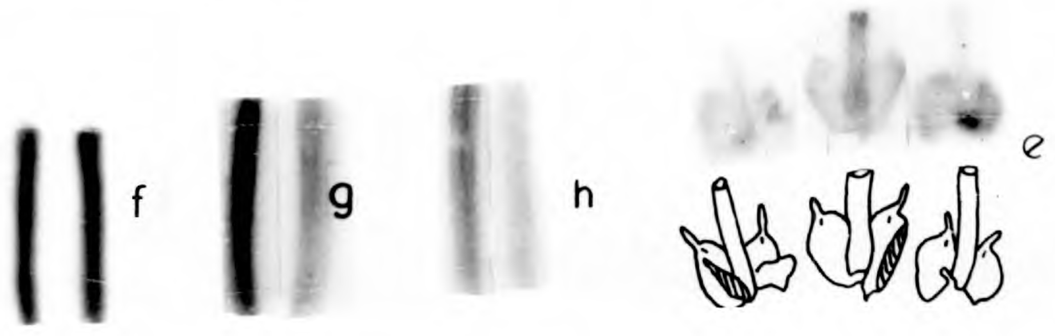
Tick beans 100 p.p.m.; 48 hour Xray (as in plate 1, but intact plants.)

a) Upper immature leaves.
b) Apical buds.
c) Mid - stem mature leaves.
d) Lower senescent leaves.
e) Stipules.
f) Lower (thick).)
g) Mid.) stem sections.
h) Upper (hollow).)

II







and, considering their thickness relative to leaves (possibly counteracted by absorbtion of the radiation to some extent) they probably contained less phorate derivative.

The apical buds 2b gave very feint traces, considering their thickness, whereas the very thin stipules 2e gave almost equally strong traces as young leaves.

Plate 2 shows that phorate derivatives accumulate at the edges of leaves, eventually forming local concentrations in mature leaves (2c) associated with lesions, whereas little is found in unexpanded leaves.2a.

The distribution of stomata differs over the surfaces of leaves and stipules. A count showed that the periphery of the leaf had 11. per unit area underneath and 8 on top, compared with 9 and 6 near the centre at the base. If this represents differences in transpiration rates, it hardly accounts for the increasing concentration towards the edges. The stipules had about 10 stomata on the abaxial surface and 8 on the adaxial (in the same unit area), and were thus similar in this respect to leaves. No stomata occurred within about 5 cells distance from the edge of the leaf, which might account for the centres of phytotoxic lesions being mostly just away from the edge.

Plate 1.3. shows the leaves of a tick bean plant essentially the same as used in Sections 2 and 3 (i.e. with the apex removed before treatment). The pattern of uptake is similar to that for the entire plant (plate 2.)

Plate 3.2. is a Garden Broad bean (listed as 5 under "Plants" above) treated at 100 p.p.m. but not showing phytotoxicity symptoms, the large leaves 2f and the compound leaves 2e show an even concentration at the edge of the leaves. A flower was dissected and exposed for 10 days. No traces were given by any parts except the calyx 2d indicating

Plate 3.

3.1 <u>Brussels' sprout</u> 100 p.p.m. 48 hour Xray.

a) Outside leaf.

b) Half grown leaf.

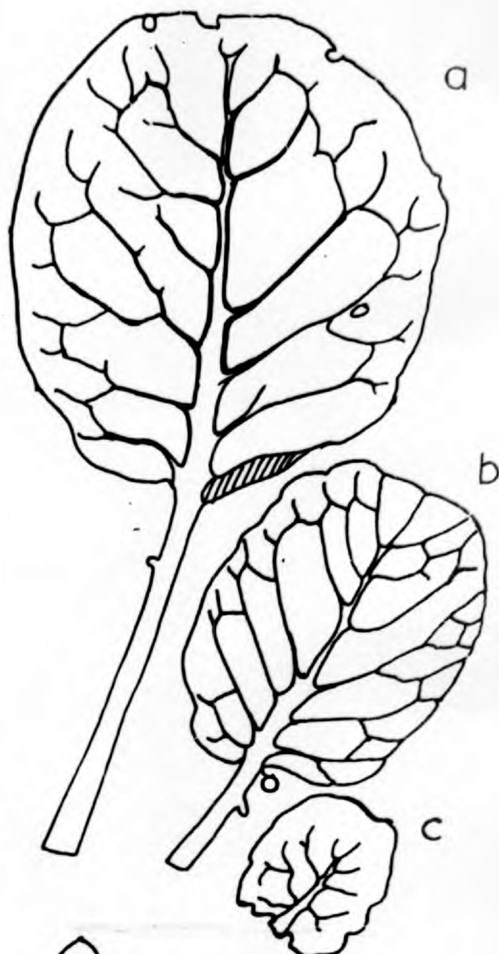
c) Developing leaf.

3.2 <u>Broad bean</u> 25 p.p.m. 4 Day Xray.

d) Calyx.

e) Compound leaf.

f) Simple leaf.


III.1
a
b
c

III.2
e
cm.
d
f

that these may present little danger to Anthocorids or bees, feeding from them. Extrafloral nectars occuring in the centre of each stipule (plates 1 and 2) also do not show up as accumulating phorate derivatives.

Potatoes treated at 100 p.p.m. (plate 4) showed lesions 7 days after treatment, which coincided with the local accumulations of phorate derivative (plate 4d.). The pattern was similar to that for beans, except that marginal accumulations and phytotoxicity were more pronounced. The undeveloped leaves 4b contained less phorate than the mature ones 4c, d. The accumulations on each side of the tips of the large leaflets coincide with the normal guttation points.

In Oats (7.) treated at 100 p.p.m. (plate 5e) phorate derivative accumulated in the tips (guttation points)of the mature leaves which withered back from the tip about ½". The fact that this withering occurred in treated plants in leaves formed after transplantation, but only in the first leaf of controls, indicates that the damage was phytotoxicity.

Brussels sprouts (8) treated at 100 p.p.m. (plate 3.1.) did not show phytotoxic damage and the traces from them show that mature leaves 3.1.a had even concentration gradients towards the edge, with the veins showing, where these faced the Xray plate. The immature leaves contained little phorate (3.1.c).

All plants treated at 25 p.p.m. (plate 6.c.d.e.) gave lighter traces than those at 100 p.p.m. even when exposed for 4 days. The distribution appears to be similar to that at 100 p.p.m., and since there were no phytotoxic symptons, this would probably be the distribution derived from a correctly applied field treatment. The 25 p.p.m. material, and undamaged 100 p.p.m. material sometimes showed local concentrations which may be the early stages of the very

Plate 4. <u>Potato</u> <u>100 p.p.m</u>. 48 hours Xray.

a) Stem (flattened).
b) Youngest leaves.
c) Mid - stem leaf.
d) Mature lower leaf.

4

pronounced concentrations associated with lesions.

Young bean leaves could take a heavy accumulation of phorate derivative, without being damaged; thus the leaves of the beans which had not appeared above the sand, when treated (listed under "Plants"4) gave extremely black traces 76.a. without damage, although damage appeared when they matured.

The deciduous twigs, from Anthocorid host trees, treated at 25 and 100 p.p.m. phorate gave similar pictures to the rooted plants, with the veins standing out clearly (5a, c, d). The accumulations were large, without phytotoxicity, but, since none of the other plants were treated via cut stems, this might be due to either treatment or the leaf structure.

The leaves of the old bean plants (listed as "Plants"3), which had been prevented from senescing by removing the upper leaves, gave strong traces, without pronounced phytotoxic damage. This ability of leaves, artificially protected from senescence to survive heavy treatments, was made use of in Section 6.

Conclusion on autoradiographs and their significance in relation to Anthocorid oviposition sites; The mature leaves of all plants accumulated large quantities of phorate derivative, the immature leaves less and the stems relatively little, considering their bulk.

The accumulation of phorate around the edges of tick bean leaves and stipules coincides exactly with the oviposition site of *Anthocoris nemorum* (Table 10.), eggs of which are killed by phorate treated plants, as shown in the previous section. The stem, petiole and midrib tissue, which is the oviposition site of *A. confusus*, however contains less phorate, which might explain the evidence that

Plate 5.

a) Mature Sycamore leaf 100 p.p.m. 48 hours Xray.
b) Sycamore twig " " " "
c) Immature Sycamore leaf" " " "
d) Apple leaf " " " "
e) Oat tiller " 4 Day "

V

Plate 6.

a) Tick bean leaf from plant treated prior to emergence (100 p.p.m.) 48 hours.

b) Tick bean leaf protected from senescence by upper leaves being removed (sown 26/4/64) (100 p.p.m. 48 hours.)

c) Brussels' sprout 25 p.p.m. 4 Day Xray.

d) Broad bean " " "

e) Sycamore leaf " " "

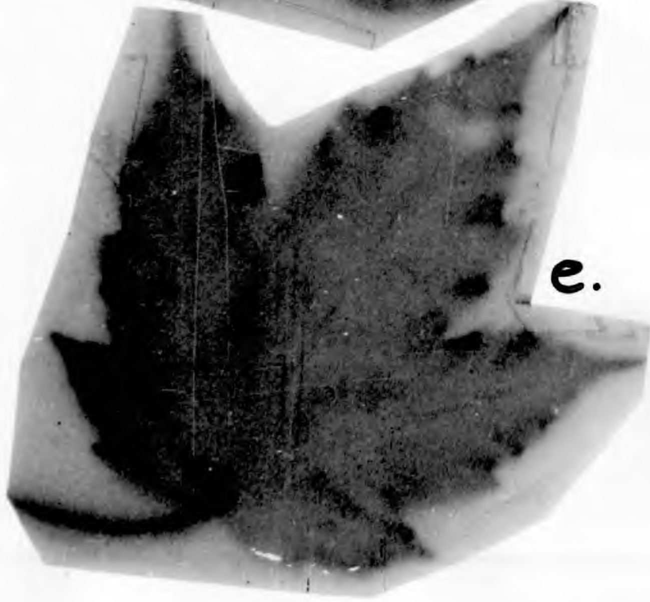
6

a.

b.

c.

d.



e.

A. confusus eggs are unharmed. The two species overlap by less than 2.0% in oviposition site (Tables 10 and 11), and could not be forced to lay in each others oviposition sites in experiments designed to do this.

It was decided to find out if *A. nemorum* females laid most of their eggs at the leaf edges of some of the plants (other than beans) in which the distribution of phorate had been demonstrated in Experiment 13. This would indicate whether the results obtained for the Toxicity of phorate to *A. nemorum* eggs, laid in beans, could reasonably be extrapolated to other crops.

Experiment 14. Oviposition Sites of *Anthocoris nemorum* in Crop Plants.

Method; Female *A. nemorum* were caged on foliage of the plants in question, using the cage in Fig.1. for potatoes, Brussels' sprouts, and apple leaves, with the stems of the foliage extending into water below the cages, and a cylindrical cellulose acetate cage fitted into a flower pot with the plants growing in it, for oats. After about 7 days the Anthocorids were removed and the eggs counted. The eggs were ascribed to positions on a standard diagram in the case of potatoes (Fig.13.) and the tip or side of the leaf in the case of oats. The small number of eggs on sprouts and apples were not classified.

Potatoes: Fig 13. shows a leaf typical of the potato used (Variety not recorded). There were usually 7 large leaflets, these being 3 lateral pairs (1, 2 and 3) and a single large terminal leaflet (4). Between the large lateral leaflets there were very much smaller lateral leaflets (5, 6 and 7). Table 30. shows the distribution of eggs on the various leaflets, on the sides and tip of each leaflet and also on

the upper and lower surfaces, based on 17 leaves of varying size.

Table 30. Distribution of *Anthocoris nemorum* eggs on Potato leaves as in Fig. 13 from 17 leaves (opposite paired leaflets amalgamated).

Leaflets large	Tip of Leaflet		Side of Leaflet		Total per leaflet
	Upper	Lower	Upper	Lower	
1	13	15	1	2	31
2	31	10	3	1	45
3	17	5	2	0	24
4	18	7	1	0	26
Total all large leaflets	79	37	7	3	126
Leaflets small					
5	2	0	0	0	2
6	5	0	4	1	10
7	12	4	1	0	17
Total of small leaflets	19	4	5	1	29
Positon Totals	Total at tip of leaflets 139		Total in sides of leaflets 16		155
Surface Totals	Total Upper surface 110		Total lower surface 45		155

Fig. 13. Experiment 14. Section 4.

Standard potato leaf, typical of those used in Experiment 14, showing leaflet arrangement as in Table 30.

(1. 2, 3): Large lateral leaflets.
(4): Terminal leaflet.
(5, 6, 7): Small lateral leaflets.

Fig. 14. Experiment 14. Section 4.

Distribution of 118 *Anthocoris nemorum* eggs over 112 oat seedlings (first leaf only).

Histogram equals observed number of Leaves (f) with observed number of eggs (x).

Curve equals expected number of leaves at each value of x, from Poisson distribution.

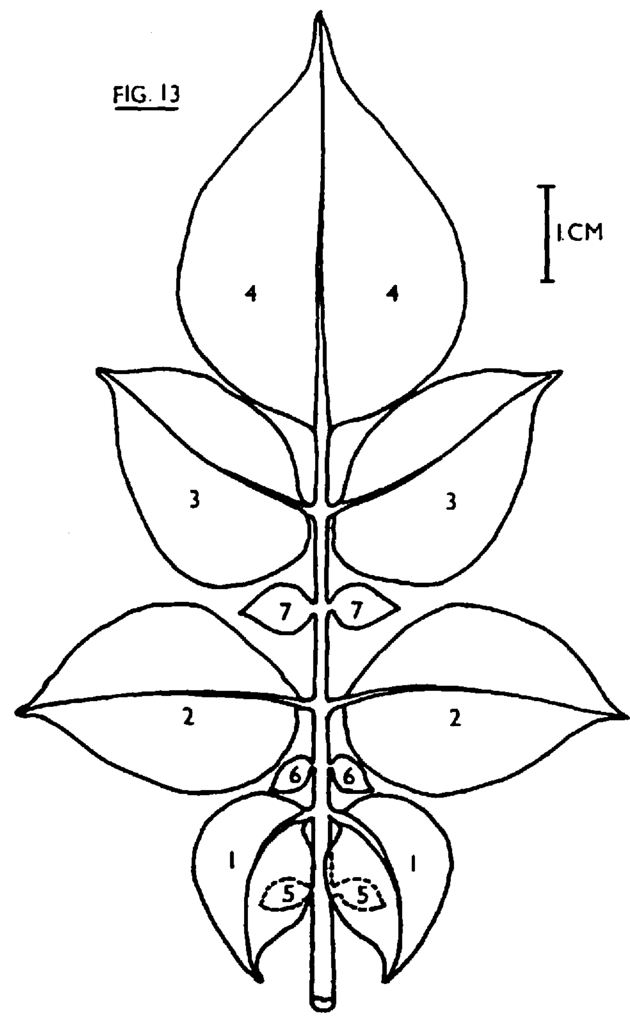


FIG. 13

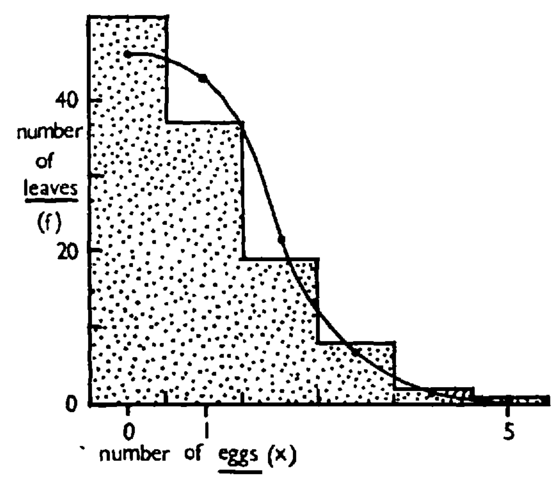


FIG. 14.

Most of the 155 eggs were in the upper surface (110)V 45 in the lower surface. This difference did not occur in tick beans in Experiment 10, where the leaves hung vertically in sleeves. Without exception the eggs were laid at the edges of the leaflets, and 139 were laid in the tips of the leaflets whereas only 16 were laid in the sides. This tip preference was not found in bean leaves, which do not have such pronounced tips. The most favoured sites were the tips of the large lateral leaflets (2. Fig.13)

There was no evidence of selection of any particular age of potato leaf from the foliage in the cages - the number of eggs per leaf being roughly proportional to the size. The terminal leaflet (4) was the usual site in very young leaves, this being proportionally larger than the others at this stage.

In potatoes the eggs were surrounded by blackened leaf tissue, in their immediate vicinity, which was not found with other plants.

From these results it seems probable that eggs laid in phorate treated potato leaves (Plate 4.) would be killed by phorate concentrating at the edges of the leaflets.

Distribution of *A. nemorum* eggs in Oats.

Ten female *A. nemorum* caged on 118 oat seedlings, as described above, laid 142 eggs. The seedlings were about 1½" tall, with the first leaf protruding from the coleoptile, when the females were put on, and after 7 days, when the females were removed, the first leaves were 5" long and the second leaves about 2" long. The former contained more eggs (135; Table 31) probably because the second leaves (with only 7) were only available at the end of the oviposition period.

<u>Table 31</u>. Distribution of <u>Anthocoris nemorum</u> eggs in the sides and tips of the leaves of 118 oat seedlings.

	First leaf		Second leaf	
	Tip	Side	Tip	Side
	112	23	7	0
Total	135		7	

From Table 31. it is obvious that the tip of the leaf is the preferred site and this is the place where phorate accumulates (Plate 5e.).

The distribution of the 112 eggs in the tips of the first leaves of the 118 plants (Table 32) is of interest, since this is the only example in which large numbers of eggs were laid in a large number of nearly identical sites. Eggs often occurred clustered together in leaf tips in other plants, but this could be explained by there being more eggs laid than tips available. Alternatively single females may have laid several eggs in succession or females, on finding an egg, may have been stimulated to lay one of their own. Here there were more tips available than eggs laid, so the number of plants (f) in which 1, 2, 3, and x eggs were laid, was recorded (Table 32 Fig.14). A Poisson distribution was calculated (Appendix 4.1. and Fig. 14.) to find the expected numbers of (f) plants at each value of x eggs, if eggs were laid entirely randomly, and this fitted the data in Table 32 (chi squared departures = 2.5). From this it appears that eggs were laid without relation to others present.

Table 32. Distribution of 112 Anthocoris nemorum eggs (laid in the tips of the first leaves) in 118 Oat seedlings (Fig.14.)

No. of Eggs = x	No. of Plants with x eggs = f	Expected f. (Poisson Distribution)	fx
0	51	46.1	0
1	37	43.3	37
2	19	20.4	38
3	8	6.4	24
4	2	1.5	8
5	1	0.3	5
Total	118 plants	118.0 plants	112 Eggs

Twenty two Anthocoris nemorum eggs laid on Brussels' sprouts were all on the leaf margins. Fifteen eggs laid on apple foliage were at the edges of leaves and 5 next to large veins. All the results in this section indicate that A. nemorum eggs are at risk when phorate is used, because of the preference for leaf edges as an oviposition site.

Section V. Effects of Insecticides applied to leaves on Anthocorids and Aphids.

So far, a comparison has been made of insecticides acting systemically, after root uptake. Thus only the insect stages in close obligate intimacy with the plant tissues:- the Anthocorid egg and the feeding aphid, have been directly exposed to the insecticides.

Section 6. deals mainly with the indirect effects of the root applied insecticide on Anthocorids, and the results of this work indicates the extent to which ecological selectivity succeeds.

In contrast the possibilities of ~~intrinsic~~ physiological selectivity are best demonstrated by applying the insecticides as a film on the plant foliage, so that both predator and aphid recieve the insecticide in the same way, i.e. by contact action from dried residues.

The prey/predator differences in contact tests are still subject to interpretation, for instance activity level of insects, size differences and cuticle properties, may intervene in insecticide uptake.

Phorate, dimethoate and menazon were compared in experiments where the interspecific and inter-insecticide differences were measured in a situation as natural as possible, and presumably the insecticide effects interacted with the effects mentioned above, as they might in the field.

Application of Insecticides: All three insecticides were applied in the same way. Broad bean leaves, cut from plants (as in Fig.15A) were inserted in a cage base apparatus, mounted on a jam jar of water, and thereby kept turgid.

Fifty microlitres of solution, using insecticides dissolved in cellosolve and diluted with water, or just cellosolve in water for controls, (at the same conc. as

maximum insecticide dose) were deposited as a single drop in the centre of each leaf with a syringe - operated micro - pipette, each drop being about 5 mm across. The leaves were put in a 25°C cabinet (as in Section 2 and 3) for the deposits to dry, before leaf cages were put on. No spreaders were used, the idea being to contain all the insecticides in a small area and surround this with a leaf cage. The different dosages used were made by altering the concentration of the solutions.

The drops were much larger than those obtained by agricultural spraying, but the technique enabled the quantity of insecticide to be fixed exactly in advance and the insects to be confined over all of it. The dosages were expressed as Mg. active ingredient per cm^2 (actual % concentration used of solutions are shown in Table 33.). The area referred to is only the area of the drop (about 20 mm^2, D. Fig.15).

Test Situation: A clip on leaf cage was used (Fig.15.C.) which confined the insects over the drop of insecticide (Fig.15.D.) in the centre of the floor formed by the upper surface of the leaf. The walls of the cage were formed by a transparent "Evolite"ring padded at the base with plastic foam, which gave a total leaf area of 177 mm^2. The ceiling of the cage formed by another evolite ring lid, with muslin stuck across it about 10 mm above the leaf. A thin strip of cellulose acetate, stuck round it, formed an insect-proof overlap round the cage wall. (Fig.15.B.)

The aphids settled on the cage floor and fed, soon after being put in the cage, whereas the Anthocorids usually settled on the upper parts of the cage away from the insecticide drop. This was counteracted somewhat by the greater activity of the Anthocorids, when in search of prey.

Fig. 15. Section 5. (Also Experiment 11. Section 3.)

(A) Clip on leaf cage in use.

(B) Lid of Clip on leaf cage.

(C) Cage opened out (side view).

(D) Drop of Insecticide.

(E) (Black) Spring which holds cage shut.

(F) Foam padding.

(G) Cage seen from above (Lid removed).

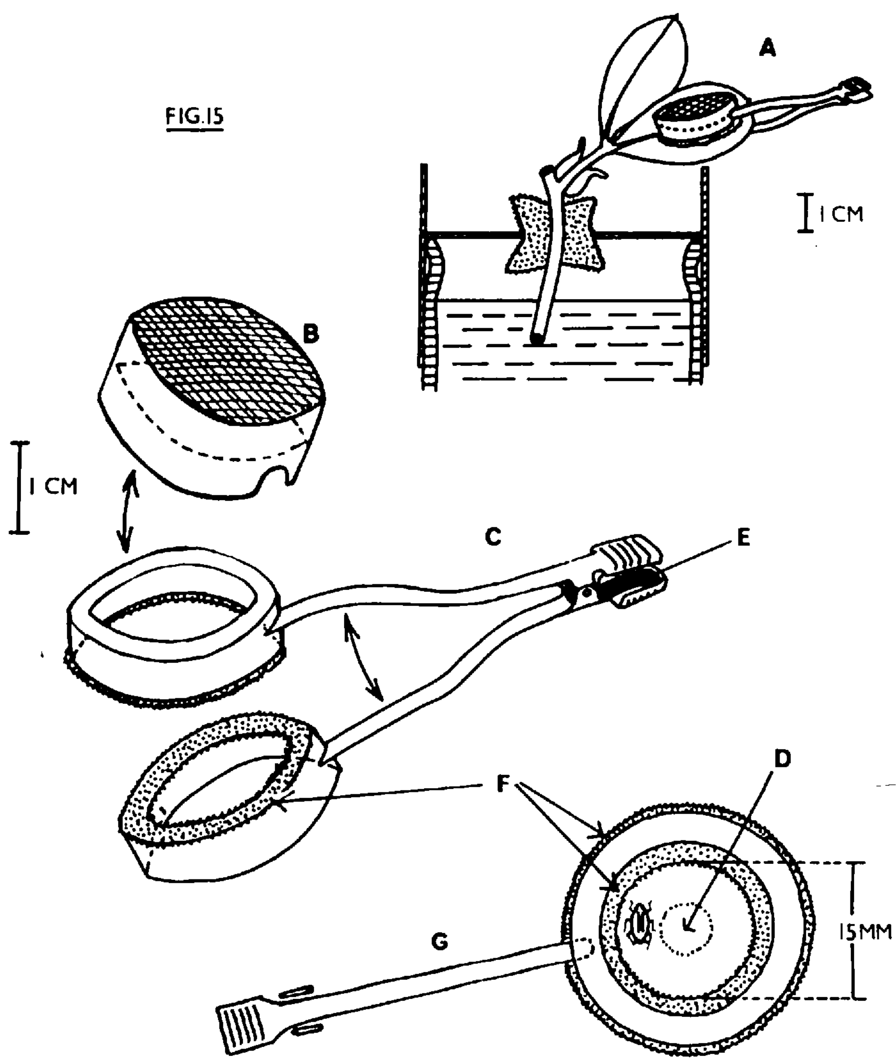


FIG.15
A
1 CM
B
1 CM
C
E
F
D
G
15MM

They then walked about the cage floor more than the aphids. In tests done using a living leaf as a substrate, the aphids would be affected by that part of the dose systemically taken up by the leaf as well as by direct contact, whereas the Anthocorids would only be effected by direct contact, unless they fed on the leaf (see section 6.). In these experiments the Anthocorids were supplied with Acyrthosiphon pisum nymphs as food mainly to reduce cannabalism, and starvation. The field situation these experiments reproduce is one where an aphid infestation has been sprayed and Anthocorids nymphs are searching over treated leaves for dead aphids and pockets of survivors, which would be feeding on the plants. Thus the Anthocorids are in contact with the insecticide but not necessarily starving. The aphids they find might be affected by insecticide as in these experiments.

Caging of Insects: The Anthocorids were caged in groups of 3 or less to reduce cannabalism. 2nd and 3rd instar nymphs of about the size of the aphids were used. All the individuals in one cage were the same instar to reduce cannabalism. The aphids were mainly 4th instar Aphis fabae or 2nd instar Acythosiphon pisum these being about the same size. They were caged in groups of 10.

Design of Experiments: Except in Experiment 15, results presented in the same tables were obtained at the same time. All experiments took place at 20°C and 16 hours daylight per day. Adjacent doses differed by a factor of 4 X. in all experiments (expressed to 3 significant figures in Tables).

Experiment 15. Comparison of the Effects of phorate and dimethoate on Anthocoris nemorum and A. confusus nymphs.

The object was to compare the toxicity of the two insecticides, and, at the same time, to compare the two

species to see if they differed sufficiently to account for the difference in toxicity of phorate to their eggs (Section 3). Preliminary tests using 50 microlitres of 0.1% solutions ($=25.5 \times 10^{-5}$ mg A.I./cm^2) showed that menazon applied as above was non - toxic at this dose, whereas phorate and dimethoate were LD 100 to A. nemorum nymphs.

Table 33 and Fig.16 show the numbers used, dosages and results which were obtained in three separate experiments in which the technique was carefully standardised. This was necessary because too few Anthocorids were available at any one time. In addition the relevant results of preliminary tests and Experiments 16 and 17 were added on.

Parallel probit lines Fig.16. were drawn from the 72 hour data and analysis showed these were a good fit (chi squared departures from parallelism equals 0.61, for heterogeneity, 8 DF, 5.85).

Dimethoate was more toxic than phorate to both species and A. nemorum was more susceptible to both insecticides than A. confusus. Calculated LD50's were: dimethoate : A. nemorum = 31.1×10^{-5} mg/cm^2 A. confusus = 61.2×10^{-4} mg/cm^2, Phorate : A. nemorum : 20.2×10^{-3} mg/cm^2 A. confusus : 46.4×10^{-3} mg/cm^2. Calculated relative potencies showed that dimethoate was 6.5 plus or minus 3,0 times more toxic than phorate to A. nemorum and 7.5 plus or minus 5.1 times to A. confusus (S.D.'s overlap).

Interspecific relative potencies were calculated and A. nemorum was 2.0 plus or minus 1.1 times more susceptible to dimethoate than A. confusus and 2.3 plus or minus 1.3 times more susceptible to phorate (S.D.'s also overlap). The interspecific difference in susceptibility of the nymphs to phorate is much smaller then the difference between the susceptibility of their eggs as shown in section3.

Table 33. Effects of dimethoate and phorate on <u>Anthocoris nemorum</u> and <u>Anthocoris confusus</u> nymphs.

(N equals A.nemorum, C equals A.confusus)

Treatment: % conc. solution	Mg. A.I./cm² (3 sig. figs.)	Initial No. N	Initial No. C	Control corrected % Kills (from bottom line) at: 24hrs. N	24hrs. C	48hrs. N	48hrs. C	72hrs. N	72hrs. C
(A) Dimethoate									
1×10^{-1}	25.5×10^{-2}	7 *	-	86	-	100	-	100	-
4×10^{-2}	10.2×10^{-2}	5 *	4	60	74	100	100	100	100
1×10^{-2}	25.5×10^{-3}	36	23	53	50	71	64	79	73
25×10^{-4}	63.7×10^{-4}	35**	18	26	13	41	24	57	42
62.5×10^{-5}	15.9×10^{-4}	42**	14	14	3	41	25	42	33
15.6×10^{-5}	39.8×10^{-5}	4**	-	0	-	22	-	20	-
(B) Phorate									
1×10^{-1}	25.5×10^{-2}	4 *	-	100	-	100	-	100	-
4×10^{-2}	10.2×10^{-2}	43	23	63	27	71	54	78	64
1×10^{-2}	25.5×10^{-3}	39 *	18	33	24	41	30	45	36
25×10^{-4}	63.7×10^{-4}	36**	22	17	19	25	24	38	24
62.5×10^{-5}	15.9×10^{-4}	12**	-	8	-	4	-	11	-
(C) Control		49	22	0	5	4	5	6	5

* Data from Prelimary tests included

** Data from Experiments 16 and 17 included

Fig. 16. Experiment 15. Section 5.

Contact Action of Dimethoate and Phorate on *Anthocoris nemorum* and *A. confusus*.

72 hour calculated Probit lines.

Dimethoate at 10.2×10^{-2}, 25.5×10^{-3}, 63.7×10^{-4}, 15.9×10^{-4} and 39.8×10^{-5} mg/cm^2

Phorate at 25.5×10^{-3}, 63.7×10^{-4}, 15.9×10^{-4} and 39.8×10^{-5} mg/cm^2

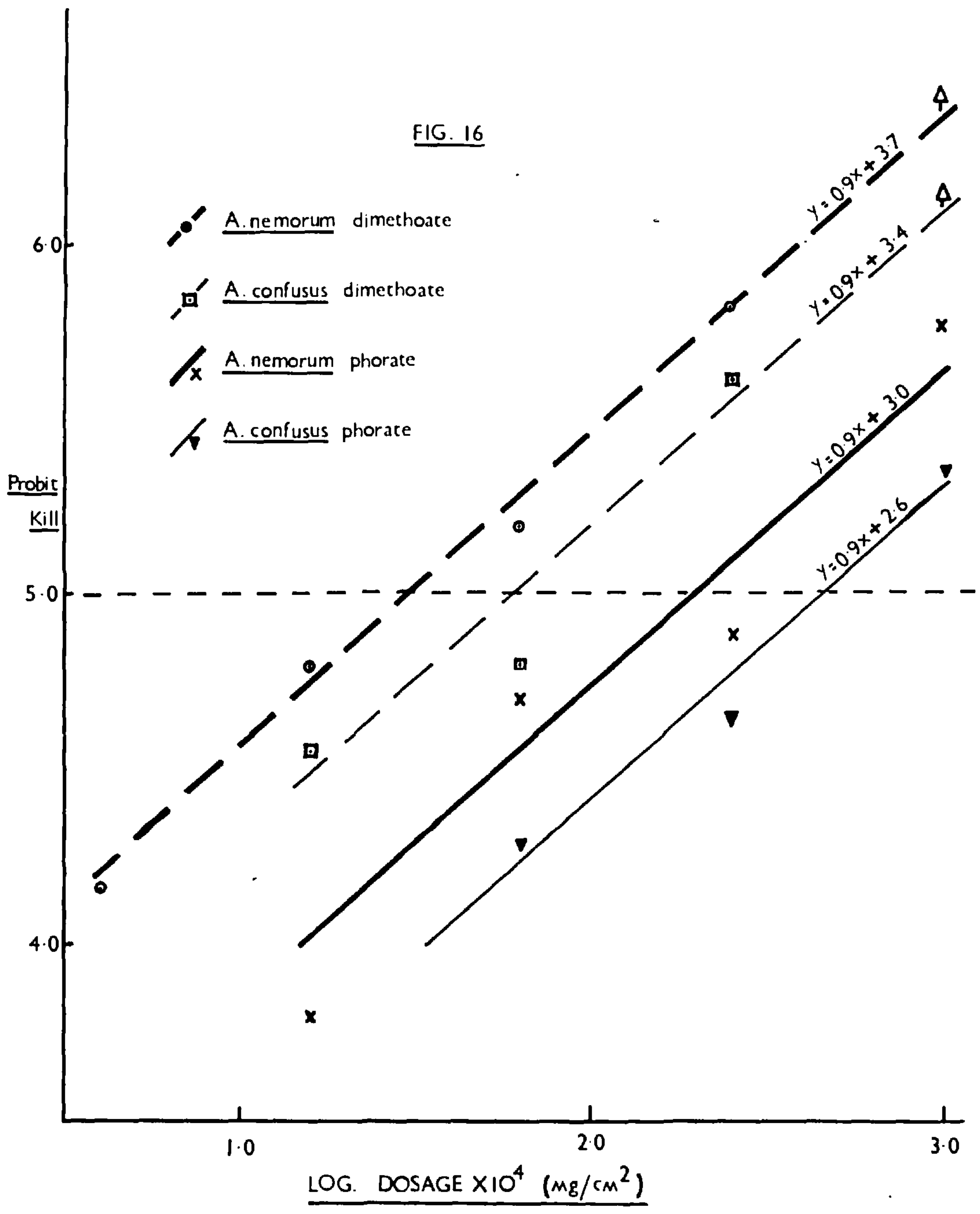


FIG. 16
A. nemorum dimethoate
A. confusus dimethoate
A. nemorum phorate
A. confusus phorate
Y=0.9x+3.7
Y=0.9x+3.4
Y=0.9x+3.0
Y=0.9x+2.6
Probit Kill
6.0
5.0
4.0
1.0
2.0
3.0
LOG. DOSAGE X10⁴ (mg/cm²)

An experiment was then done to find out if aphids were more susceptible to phorate than Anthocoris nemorum (the more susceptible predator).

Experiment 16. Comparison of Susceptibility of Aphis fabae, Acyrthosiphon pisum and Anthocoris nemorum to 4 concentrations of Phorate.

Broad bean leaves were treated at 10.2×10^{-2}, 25.5×10^{-3}, 63.7×10^{-4} and 15.9×10^{-4} mg/cm^2 phorate and with the control medium and 3 A. nemorum 2nd instar nymphs caged on each of 4 leaves at each treatment with A. pisum larvae for food. (making 12 per treatment.)

Groups of ten A. fabae and A. pisum nymphs (sizes equal) were caged on further leaves at the same time giving the initial numbers shown in Table 34. Mortalities of each species were recorded at 24, 48 and 72 hours, in Table 34.

Table 34 shows that at all doses, both aphid species were considerably more affected then were the Anthocorids. It was not possible to deduce the relative susceptibility of the two species because at 63.7×10^{-4} mg/cm^2 A. pisum was more affected, but at 15.9×10^{-4} mg/cm^2 A. fabae was more affected.

The Anthocoris nemorum data was used in Table 33.

Table 34. The Effects of 4 concentrations of phorate on Anthocoris nemorum 2nd instar (N), Acyrthosiphon pisum (P) and Aphis fabae (F) nymphs.

Concentration of Phorate (mg/cm^2)	Initial number of Insects	Control Corrected % Kills (from Bottom line) at: 24hrs.	48hrs.	72hrs.
10.2×10^{-2}	12N	66.7	66.7	63.7
	60P	100	-	-
	60F	100	-	-
25.5×10^{-3}	12N	25.0	33.3	27.3
	60P	100	-	-
	60F	100	-	-
63.7×10^{-4}	12N	8.3	16.7	18.2
	20P	100	-	-
	40F	94.4	100	-
15.9×10^{-4}	12N	8.3	8.3	9.2
	20P	85.0	85.0	88.9
	40F	97.2	100	-
Control	12N	0	0	8.3
	20P	0	0	10.0
	30F	10	10	10.

A similar experiment was done with dimethoate to see whether a similar predator/prey difference in susceptibility existed.

Experiment 17. Comparison of Susceptibility of Aphis fabae, Acyrthosiphon pisum and Anthocoris nemorum to 4 concentrations of dimethoate.

In this experiment the concentrations used were 25.5×10^{-3}, 63.7×10^{-4}, 15.9×10^{-4} and $39.8 \times 10^{-5} mg/cm^2$

and control.

This was done to get an aphid kill in the region of LD50. The initial numbers of each insect and the kills are shown in Table 35, the method being the same as that of Experiment 16. The results for A. nemorum were added to Table 33.

Table 35. The Effects of 4 concentrations of dimethoate on Anthocoris nemorum 2nd instar nymphs (N), Acyrthosiphon pisum (P) and Aphis fabae (F) nymphs of the same size.

Concentration of Dimethoate (mg/cm^2)	Initial Number of Insects	Control corrected % Kills (from Bottom line) at: 24hrs.	48hrs.	72hrs.
25.5×10^{-3}	10N	40	68.4	79.0
	60P	100	-	-
	60F	90.6	98.0	98.0
63.7×10^{-4}	12N	16.7	21.1	47.4
	60P	93.3	100	-
	60F	86.8	88.5	92.3
15.9×10^{-4}	12N	0	13.4	13.4
	60P	86.7	100	-
	60F	54.7	73.1	86.5
39.8×10^{-5}	4N	0	21.1	21.1
	60P	84.4	89.4	100
	60F	45.3	78.9	88.5
Control	20N	0	5.0	5.0
	60P	25.0	36.7	38.3
	60F	11.7	13.3	13.3

Table 35. Shows, as with phorate, that Anthocorids are far less susceptible than aphids to dimethoate. It

appears that dimethoate may have been less effective than phorate against *A. fabae* at 24 hours (55% kill at 15.9×10^{-4} mg/cm^2 and 97% for phorate Table 34.) but the 72 hour kills were similar.

The kills at 72 hours were too high for probit lines to be drawn. The 24 hour data, however, shows that *A. fabae* was consistently less susceptible to dimethoate than was *A. pisum* of the same size. This difference was also found in Experiment 12. where dimethoate was applied *systemically* to individuals of the same size, of the two species.

The previous three experiments show that deposits of dimethoate and phorate are toxic to Anthocorids and more so to aphids. A proportion of deposited insecticide is taken into plants after spraying, and this may persist, when the deposit is washed off by rain, or may be translocated to sites not reached by the spray, such as the undersides of leaves. An experiment was done to see if Anthocorids and aphids were killed when kept on the *opposite* side of leaves from that to which high doses of insecticide, were applied

Experiment 18. *The Effects of high dosages of dimethoate and phorate on Anthocoris nemorum and Acyrthosiphon pisum on the untreated side of the treated leaves.*

The treatments were 10.2×10^{-2} mg/cm^2 dimethoate and phorate and control, applied on the upper (adaxial) leaf surface as in previous experiments. When drops were dry, the leaf petioles were gently twisted so that the drops were underneath and secured in this position with plasticine. The abaxial surface was then uppermost and the Anthocorids and the *A. pisum* larvae were caged on this surface.

The insecticide was thus absorbed through the same surface as before. The number of insects used and their mortality at 24, 48 and 72 hours is shown in Table 36.

Treatment (mg/cm^2)	No of Insects		Control Corrected % Kills (from bottom line) at:							
			24hrs.		48hrs.		72hrs.		96hrs.	
	N	P	N	P	N	P	N	P	N	P
(a) Dimethoate 10.2×10^{-2}	18	60	0	100	0	-	0	-	0	-
(b) Phorate 10.2×10^{-2}	18	60	0	98.2	0	100	0	-	0	-
(c) Control	18	60	5.6	6.7	5.6	10.0	5.6	11.7	5.6	11.7

Table 36. Mortality of Anthocoris nemorum (N) and Acyrthosiphon pisum (P) 2nd instar nymphs, caged on the untreated side of bean leaves treated with dimethoate and phorate.

Table 36 shows that, over 4 days, no A. nemorum were killed by treatments which gave kills of 100% and 78% for dimethoate and phorate (Experiment 15) when the insects were caged on the same side.

In contrast all the aphids were killed in 48 hours on both insecticides. A further experiment was done in the field (Section 6) in which Anthocoris nymphs were starved on plants toxic to aphids and the results were similar.

As stated in Experiment 15, preliminary tests showed that menazon was non-toxic to Anthocorids at doses giving LD100 by phorate and dimethoate. An experiment was done to confirm this and to see if aphids were controlled by

similar doses of menazon to those giving control by dimethoate.

Experiment 19. Comparison of the effects of Menazon on Anthocoris nemorum 2nd instar nymphs and Acyrthosiphon pisum nymphs.

Leaves were treated with 15.9×10^{-4} mg/cm^2 of menazon and others with the control medium. Anthocoris nemorum nymphs and A. fabae and A. pisum were caged separately on the treated sides of the leaves. Some aphids were also caged on the untreated lower leaf surface to show results of translocation. The numbers of each species used and mortality at 24, 48, 72 and 96 hours are shown in Table 37.

Table 37. Mortality of Anthocoris nemorum 2nd instar nymphs and Acyrthosiphon pisum nymphs caged on plants treated with menazon at 15.9×10^{-4} mg/cm^2.

Treatment		Initial No.	Control Corrected % Kills (From bottom line) at: 24hrs.	48hrs.	72hrs.	96hrs
(A) Menazon 15.9×10^{-4} mg/cm2	A.nemorum	9	0	0	0	0
	A.pisum on treated side	40	59.4	83.3	90.0	93.3
	A.pisum on untreated side	40	40.6	50.0	56.3	65.6
(B) Control	A.nemorum	10	20.0	20.0	20.0	20.0
	A.pisum	40	4.0	5.0	5.0	5.0

Table 37 shows that no A. nemorum nymphs had died after 4 days on the menazon treatment. In contrast the A. pisum were quickly affected, with 59% dying on the

treated side, and 41% on the opposite side in 24 hours.

After 48 hours 83% of the A. pisum on the treated side were dead whereas the same dosage of dimethoate in Experiment 17. killed 100% in 48 hours. From this result it seemed probable that menazon might be less toxic to A. pisum, by this method of treatment, than dimethoate and this was tested in a further experiment.

Experiment 20. Effects of dimethoate and menazon on Acyrthosiphon pisum.

Second instar A. pisum nymphs were tested at 39.8×10^{-5}, 10.0×10^{-5} and 24.9×10^{-6} mg/cm^2 of dimethoate and menazon on the treated side of the leaf, and at 39.8×10^{-5} mg/cm^{-5} on the untreated side of the same leaves. Controls were set up as before. The numbers used and the percent mortalities at 24, 48 and 72 hours are shown in Table 38.

Table 38 shows that menazon kills were not entirely consistent with dosage.

Probit lines were drawn from the 24 hour data, but these showed considerable heterogeneity and diverged significantly. The 24 hour LD50 for dimethoate was about 6.9×10^{-5} mg/cm^2 and for menazon about 33×10^{-5} mg/cm^2.

The aphids caged on the untreated side of the leaves were less affected than those caged directly on the drops, and dimethoate was more effective than menazon.

The greater toxicity of dimethoate contrasted with the result obtained in Experiment 6. when the two insecticides were compared by systemic root uptake.

Table 38. Effects of 3 concentrations of Dimethoate and Menazon on 2nd Instar _Acyrthosiphon pisum_ nymphs.

Treatment mg A.I./cm^2	Initial Number	Control corrected % Mortality (from bottom line) at: 24hrs.	48hrs.	72hrs.
(A) _Dimethoate_				
39.8×10^{-5}				
(a) treated side	50	100	-	-
(b) untreated side	50	8.4	79.7	100
10.0×10^{-5}	50	41.0	65.4	79.7
24.9×10^{-6}	60	30.8	43.0	65.4
(B) _Menazon_				
39.8×10^{-5}				
(a) treated side	50	59.3	73.6	81.7
(b) untreated side	50	29.2	57.3	67.5
10.0×10^{-6}	50	18.6	24.7	28.8
24.9×10^{-6}	60	16.6	35.6	37.2
(C) _Control_				
(a) Medium treated side	50	1.7	1.7	1.7
(b) Untreated side	50	2.0	2.0	2.0

In conclusion: This series of experiments shows that phorate and dimethoate were not intrinsically selective and killed the predators as well as the aphids, although the latter were more susceptible.

Phorate and dimethoate did not affect the predators caged on the untreated side of the leaf, whereas they still affected aphids by systemic trans-lamina action.

Menazon was intrinsically selective being completely nontoxic to the Anthocorids but only about 4 times less toxic to aphids than dimethoate (at LD50, *A. pisum*).

Section 6. The Uptake of aphidides by Anthocorids from systemically treated plants and prey and effects on survival and fecundity.

Sections 3 and 5 showed the direct effects of aphicides on *Anthocoris* eggs and nymphs. Anthocorids could also recieve the insecticides, when sucking sap from plants (discussed by Anderspn 1961), although they can be reared on exclusively insect food.

In addition, where systemically affected aphids are present, the predators might feed on them and thereby accumulate the insecticide, or its derivatives. Experiments were done to show whether S35 isotope, from labelled phorate, is ingested by Anthocorids, from plants alone and from aphids. The effects, on Anthocorids, of prey killed systemically by phorate, menazon and dimethoate were also studied

Experiment 21. The uptake of S35 isotope from phorate in plants and prey by Anthocorids.

Material and Method. The phorate for this experiment was labelled with S35 isotope with a ½ life of 87 days, and formulated as 0.28 gm. phorate in 3 ml. acetone solution with a specific activity of 11.4 millicuries per gram. Application was made to "standard plants" (Fig.7.) by the cut taproot method as perfected in Section 2, giving 10 p.p.m. in the tissues of 2 leaf tick bean seedlings (equivalent to 0.5 microcuries in each 5gm. plant).

These plants were used to obtain radioactive aphids.

Intact 4-leaf seedlings were also used to test for plant feeding, giving a wider choice of feeding site.

Autoradiography Insects to be autoradiographed were killed, stuck to "Sellotape" and covered with "melanex" film ..

They were then exposed to Xray plates for 24 days and these were developed as in section 4. Plate 7. is a negative photograph, made by projecting light through both the insects themselves, and their Xray traces, onto Photographic paper, with a photographic enlarger.

Uptake of phorate from plants. Ten *A. nemorum* females, ten *A. nemorum* nymphs and ten *A. confusus* nymphs were caged without aphids, on intact tick bean seedlings treated at 10 p.p.m. with phorate. Three similar batches were caged on treated plants with aphids and three batches on untreated plants without aphids. After 17 days at 20°C only 1 nymph and 1 adult survived in the unfed batches. (Survival on treated plants is discussed in Experiment 23.)

All three groups were autoradiographed.

As expected, the Anthocorids on untreated plants, gave no trace at all (not shown) whereas the series supplied with aphids on treated plants gave pronounced traces (some of which are shown Plate 7. E and F). The Anthocorids on treated plants without aphids gave feint traces (Plate 7 C, D). These must have been derived from the plants themselves, presumably when the Anthocorids fed in them.

Accumulation of Phorate from Prey. Two-leaf bean seedlings, after treatment at 10 p.p.m., were fitted with the apparatus shown in Fig.1.C., held in position by a wire loop below the top leaf (Fig.1.D.). The funnel part of the apparatus was coated with fluon (Polyletrafluorethane) on the inside, as was the upper end of the 2" x 1" flat bottomed glass tube below.

Aphids from cultures, on small pieces of leaf, placed on top of the top leaf, moved round to the lower surface, fed on it, and were killed by the phorate. In a moribund

state, they fell from the leaf and slid down the funnel into the tube.

Most of the aphids were dead, and in the tube below, in just over 24 hours after infestation.

Each Anthocorid was confined, in its tube, by the fluon at the top. It thus fed on the fallen aphids.

"Control" Anthocorids were fed with live aphids, which had not been phorate treated (this is discussed below).

The phorate treated plants were renewed every 7 days, since otherwise toxicity began to decline and aphids began to survive on them. The plants were re-infested and dead aphids were removed every other day, otherwise they would rot and might endanger the Anthocorids. The bottom of each tube was lined with plaster of Paris to a depth of ¼", which was moistened at intervals. This prevented dessication of the aphids and Anthocorids. Anthocorids nymphs of both species were treated in this way, but some escaped. Details are given here of 58 which were autoradiographed. The numbers which started as 1st, 2nd, 3rd, 4th and 5th instar nymphs were 10, 8, 5, 13 and 22 respectively. Of these nine died before metamorphosis and ten died as adults. The effects of a phorate - killed diet are discussed later in Experiments 24 and 25. Insects on the radioactive diet which died, were autoradiographed, as well as surviving nymphs and adults after varying intervals, giving 58 in all.

Plate 7.G and H. shows an example of an Anthocorid reared on S35 phorate killed aphids, 4 of the aphids can be seen below it. 7.AA and 7.BB are also radioactive aphids.

Two different aphids were used:- Aphis fabae, a relatively susceptible aphid (as shown in Experiments 2 and 3.) and Myzus persicae (with a few Aulacorthum circumflexum)

which are less susceptible to phorate (Experiments 2 and 3.). A. circumflexum survived longest on the plants. It was thought the dead A. fabae might contain less phorate than the more tolerant M. persicae, since less would be required to kill them, although other factors could easily affect amounts required to kill and also availability of insecticide from a corpse.

The Anthocorids were given code numbers before autoradiography, and the Xray plates were examined without reference to treatment records. The traces given were graded by eye as "-" = no traces at all, '±' very feint mark, + = stronger mark and "++" = very strong mark showing outline of insect. For example in Plate 7. 'F' is a ++ mark, 'H' is a +, and 'D' marks are ±. The grades were then compared with the treatment records as in Table 39. Seven individuals were classed as '++', 25 as +, 14 as ±. In table 39 the same 58 autoradiographed individuals are classified according to (1) species (2) Adults or numphs (3) dead or alive before test (4) prey species.

The number of individuals expected at each radioactivity level, on the null hypothesis that these categories make no difference to the uptake of insecticide, (strictly: only homogenous groups should have been tested but then the expected numbers in each class would be very small) are shown in Table 39, and the only significant differences (chi-squared test) were between individuals tested as adults and as nymphs. (Table 39.2) Since all individuals were put into the experiment as nymphs, this is the expected result. The mean numbers of days lived on the labelled diet is shown for individuals at each radioactivity level, (Table 39.5.) and the 7 "++" individuals (all adults)

had been on the diet about 25 days, whereas the "-" individuals had been on it for about 10 days. Thus more than ten days were required for the predators to take up enough S 35 to give a trace. The A. fabae supplied as food did not themselves show weaker traces, than M. persicae

During the experiment, excrement was collected from some Anthocorids, by replacing the 2" x 1" tubes with plaster of Paris at the bottom, by similar tubes with the floun coating extended down to the bottom and a 22 mm. glass coverslip on the floor. The bugs were thus only able to excrete on the coverslip. Humidity was provided by running a drop of water under the coverslip. The excrement was autoradiographed and found to be radioactive (Plate 7 A and B). However Plate 7 E and F. show that the entire body and even the head, becomes radioactive, so that labelled material is obviously widely circulated, as well as excreted.

This also applied to individuals which took the labelled material directly from the plants. Autoradiographs of squashes of different organs indicated that no particular organ accumulated it more than others.

Since Anthocorids take in phorate directly from treated plants in the absence of insect food, they could be affected in the same way as aphids, since the insecticide would not then have passed through another insect.

Table 39.

Division of 58 Anthocorids into 4 levels of radioactivity and between various categories, showing observed number (o) and Expected number (e) on the hypothesis that the categories contain contain similar proportions at each radioactivity level.

Classification	Categories	Radioactivity level: from Auto-radiographs. (++) o	e	(+) o	e	± o	e	(-) o	e	T
1. Anthocorid species	Confusus	5	3.7	15	13.4	6	7.5	5	6.4	31
	nemorum	2	3.3	10	11.6	8	6.5	7	5.6	27
										58
2 Stage* tested	Adults	7	3.9	16	13.2	3	7.7	6	7.2	32
	nymphs	0	3.1	9	10.8	11	6.3	6	5.8	26
										58
3. State before test	Dead	2	2.3	11	8.2	2	4.6	4	3.9	19
	Alive	5	4.7	14	16.8	12	9.4	8	8.1	39
										58
4 Prey Species	M. persicae	5	5.4	22	19.4	10	10.9	8	9.3	45
	A. fabae	2	1.6	3	5.6	4	3.1	4	2.7	13
										58
5 Mean number of Days on diet.		25.3		18.9		10.4		9.9		

Plate 7.

Negative photograph obtained by projecting shadow of subject and autoradiograph onto photographic paper. Traces due to S35 derived from phorate.

N.B. white lines and streaks are artifacts due to folds and edges of melanex and Sellotape catching the light.

A. Excrement of *A. nemorum*, on diet of aphids killed by 10 p.p.m. phorate.

B. Radiograph of same.

AA. *M. persicae*, food of above.

BB. Radiograph " " "

C. *Anthocoris nemorum*, confined on treated plants *without* aphids.

D. Radiograph " " " " " " "

E. *A. confusus* confined on treated plants *with* aphids.

F. Radiograph " " " " " " " "

G. *A. confusus* plus 4 *Myzus persicae*, from apparatus under treated plant.

H. Trace from same " " " " "

A
B
AA
BB
C
D
E
F
G
H

Experiment 22. Field Effects of phorate in plants on Anthocorids in the absence of insect food.

This experiment was done with plants grown in the field trial in Section 3. Experiment 11.

On 4/6/65, sixteen fourth and fifth instar A. confusus nymphs were caged on the 4th leaf of tick bean plants, which had been treated at 6 lb phorate/Acre in April. These leaves killed 100% of A. fabae in 48 hours when tested on 20/6/65 (Table 22). Sixteen similar nymphs were caged on control leaves. Clip-on cages (Fig. 15) were used and no food was supplied. Deaths were recorded on alternate days, but many escaped perhaps because starvation increased the insects attempts to escape. The survival records are summarised in Table 40 and show no deaths attributable to phorate. Three of the fifth instar nymphs on control and 4 on phorate treated plants became adult and one fourth instar nymph became 5th instar.

Table 40. Deaths and escapes among 32 A. confusus nymphs caged on 6 lb phorate treated and untreated bean plants for 10 days without food.

Days	Control		6lb/Acre Phorate	
	Dead	Escaped (out of 16)	Dead	Escaped (out of 16.)
2	0	5	0	4
4	2	6	1	7
6	4	6	4	8
8	6	7	6	8
10	9	7	8	8

In Experiments 10 and 11 in Section 3., female Anthocoris nemorum were sleeved on phorate treated and

untreated plants, supplied with aphids. In Experiment 10 (Table 18) mortality was almost the same (5/32) on 6 lbs/Acre phorate as on control (4/32). However in June 1965, using younger plants 13/16 females died at 6 lbs/Acre and 7/16 at 1.5 lb/Acre phorate compared with 4/16 and 0/16 on controls and 1.5 lb menazon/per Acre respectively,(Expt.11 (Table 41 A). As suggested in Experiment 11 this may have been due to the aphid food dying out more quickly on the phorate treatments, since even the 1.5 lb/Acre phorate was slightly more toxic to A. fabae than 1.5 lb/Acre menazon (Table 22.). Phorate vapour,collecting in the small 6" x 3" sleeves ,may have killed the females however, and further tests were done using the larger open mesh sleeves on wire frames, described in Experiment 11, in which it is unlikely that vapour will collect.

Experiment 23 The Effects of Systemically applied phorate on survival of Anthocorids in the field, when supplied with aphids.

The method used was always the same:- counted groups of Anthocorids (listed in Table 41.) were caged on phorate or menazon treated plants and control plants, and fed on alternate days with Microlophium evansii or Drepanosiphon platanoides, which feed on beans less readily than bean aphids and therefore last longer on treated plants, but do not breed on control plants. The sleeves used were emptied and the Anthocorids counted after 7 days. (Table 41) (Some of these were the females providing eggs for experiments 10 and 11.) The results with A. nemorum females (Table 41 B) except those described on (Table 41 A) showed a possible slight effect due to phorate, but departures from randomness were not siginificant at the 5% level (chi squared test).

Table 41 Effects of field applications of Systemic insecticides on survival of sleeved Anthocoris spp. supplied with food. P = control corrected % Dead, T = total put on, D = Dead, X = escaped, L = large sleeve open mesh, S = small muslin sleeve.

Species	Stage	Period	Type of sleeve	Phorate 6lb/ Acre. T D X	Phorate 1.5lb/ Acre. T D X	Menazon 1.5lb/ Acre T D X	Control T D X
(A) Nemorum	♀	2/6/65 -10/6/65	S	16 13 2	16 7 5	16 0 0	16 4 2
				P = 75.1%	P=25.1%	P=0	
(B)						Phorate 3lb/ Acre	
Nemorum	♀	13/7/64 20/7/64	S	16 3 1	18 1 0	16 4 1	16 1 0
	♀	20/7/64 28/7/64	S	16 2 0	16 5 0	16 1 1	16 3 0
	♀	11/6/65 21/6/65	L	8 4 4	-	-	7 3 0
	♀	24/7/65 31/7/65	L	12 0 0	16 0 0	-	16 1 0
				P =3.2%	P =0	P= 1.2%	
(C) Confusus	♀ & ♂	13/7/65 20/7/65	L	20 0 5			19 0 7
(D) Nemorum	4th 5th instar	24/6/65 2/7/65	L	50 11 2			50 3 4
		11/6/65 21/6/65	L	20 4 4			16 2 5
				P=14.9%			

The corrected % mortality at 6lb/Acre phorate was only 3.2%. A. nemorum nymphs (41 D) however, showed a 15% corrected mortality at 6lb/Acre phorate and the results could not be explained by random variation (chi squared (IDF) equals 4.7). A. confusus adults (41 C) appeared to be unaffected. The possibility remains therefore, that some of the females recorded in Table 41 A were affected by phorate vapour in the small sleeves and also that A. nemorum nymphs feed sufficiently on plants under natural conditions to be affected by phorate. The latter is important, since A. nemorum is widespread on crops and is more susceptible to phorate than A. confusus. An experiment was done next, to distinguish possible effects of poisoned prey from the effects of treated plants.

Experiment 24 The effects of a continuous diet of aphids killed by root-applied phorate, menazon and dimethoate, on the survival of Anthocorid nymphs.

The same apparatus and method was used as in Experiment 21., using mainly 3rd instar numphys, feeding on the falling aphids from plants treated at 10 p.p.m. with phorate, menazon and dimethoate. Two-leaf bean plants with over mature leaves, known to tolerate large dosages of phorate shown in Section 4. were used, and changed whenever toxicity to aphids began to fail. Myzus persicae was used as food for all treatments, since this was good for rearing Anthocorids and did not fall from the plants when disturbed. "Control" Anthocorids were kept in similar apparata without plants. They were fed with aphids killed by warming to 40°C, in order that the control aphids, like the poisoned ones, would be dead, thereby isolating insecticide effect from dead food

effects.

The control food tended to go stale, whereas the supply of aphids under treated plants was erratic but fresh, since aphids fell off as soon as dead, but took varying times to die.

The experiment was done at 20°C.

Table 42. shows the total of each species treated with each insecticide, and the number emerging as adults. The proportion surviving in all treatments was similar to that in controls, so it is concluded that the insecticide had no detectable effects on survival.

Table 42. Effect of phorate, menazon and dimethoate killed aphids on survival to adult stage of Anthocoris spp. nymphs (mainly from 3rd instar). (all treatments at 10 p.p.m.=LD 90+ aphids) T = Total tested, D = No. Dead, P = % Successfully emerged.

Species	Phorate		Menazon		Dimethoate		Control	
	T	D	T	D	T	D	T	D
Nemorum	61	14	47	9	15	3	24	7
P	77%		81%		80%		71%	
Confusus	24	5	23	6	-		28	5
P	79%		74%				82%	

A further experiment was done to see if the reproduction of Anthocoris females differed on diets of aphids killed by different insecticides and from reproduction of females fed on untreated aphids.

Experiment 25. The effects of aphids killed by phorate, menazon and dimethoate on the fecundity, fertility and oviposition period of female *Anthocoris confusus*.

A. confusus was used in this experiment since nullipar mated females of known age were available from the culture. The apparatus and method were similar to those used in previous experiments, except that the controls were given abundant untreated living *Myzus persicae*. In addition the plaster of Paris lining to the bottom of each tube, had a small cylindrical well cut in it and a 1" lenght of bean stem was fitted into this for the females (Anthocorids) to lay eggs in. The stems were changed on alternate days, when the aphid supplies were renewed, and subsequently the hatched and unhatched eggs were counted. Males were kept with the nullipar females until oviposition started, and were put in whenever less than 3 eggs were found. Summarised data from 40 females is given in Table 43. Females which eventually escaped, or laid *no* fertile eggs were not included.

From the data for each individual analysis of variance was done to find significant effects, appendix 6. There was no significant difference from random variation in longevity, oviposition period and percentage hatch of the eggs, and for the total number of eggs laid.

Table 43. Longevity, oviposition period, fecundity and fertility of 40 A. confusus females on diets of aphids killed by 10 p.p.m. phorate, menazon and dimethoate and living aphids.

Insecticide	No. of females tested	Mean No. of days lived in apparatus (days)	Oviposition period (first to last day Eggs laid) (days)	No. of Eggs laid per female	Percent Hatch of Eggs.
Phorate	10	31.2	18.5	40.4	62
Menazon	11	29.8	26.1	72.3	63
Dimethoate	9	29.7	19.1	35.3	72
Control	10	32.9	24.0	58.9	66

Section 7. Summary and Discussion of Results.

Systemic action of phorate, menazon and dimethoate on aphids.

The results of root application experiments (5) and (9) show that menazon, acting systemically, is about 3 X more toxic than phorate to *Aphis fabae* and 2 - 5 X more toxic than dimethoate to *Acyrthosiphon pisum* (Expt.6.). In contrast, in the field, soil applied phorate, at 1.5 lb/ Acre, was slightly more toxic after 48 hours than the same dose of menazon to *Aphis fabae* on Tick beans. For example, on seedlings with only 3 leaves expanded, phorate killed 93% of *A. fabae* in 48 hours and menazon killed 84% (Table 22.).

This compares with the results of Burt et al (1965) who showed that all three compounds were of similar toxicity (phorate slightly less toxic than menazon) when applied to the roots of wheat seedlings as aqueous solutions, but in dry seasons in the field, phorate and disulfoton were more effective than menazon and dimethoate, against potato aphids. Burt et al (1965) attributed this to the ability of phorate and disulfoton to diffuse, as a vapour, through dry soil and to their uptake as a vapour by the roots of plants.

In the present work menazon was shown to be more toxic than dimethoate by the cut-taproot systemic application technique, whereas Burt et al (1965) found these two insecticides almost equally toxic when applied to the intact roots of wheat seedlings. Menazon was more persistent than phorate, in toxicity, when applied by the cut taproot method; thus bean seedlings receiving one application at 5 p.p.m. kept free of infestation for at least 31 days, compared with 21 days for phorate (Expt.5.).

Experiments 2 and 3 showed that _Aphis fabae_ was 2 - 4 X more susceptible than _Myzus persicae_, when treated with phorate by the cut-taproot method. _Acyrthosiphon pisum_ was about 2.3 X more susceptible to phorate, and about 4 X more susceptible to dimethoate than _A. fabae_ , based on parallel probit lines.

The cut-taproot bioassay method showed significant kills, by the systemics, at concentrations as low as 0.01 p.p.m. of fresh weight of plant (Expt. 6.). The highest LD50 recorded, after 72 hours, was 2.4 p.p.m. phorate, for _Myzus persicae_ (Expt. 3.).

The values of LD50 for a particular species and insecticide varied between experiments, but the orders of toxicity between insecticides and between species remained similar.

In Experiment 2, the calculated daily larviposition rates of surviving _A. fabae_ adults, at 4 levels of phorate, did not decrease with increases in phorate concentration and were not lower than on untreated plants. These results do not confirm those of Cook et al (1963) who found that sub-lethal _systemic_ phorate treatments in the field, reduced the fecundity of _Acyrthosiphon pisum_.

Experiment 6. shows that small _A. pisum_ larvae were more affected by dimethoate and menazon than large ones.

Systemic Effects of phorate, menazon and dimethoate on Anthocorids.

In Experiment 21, plant feeding and uptake of phorate (or its derivatives) by both _A. nemorum_ and _A. confusus_, was demonstrated with S35 labelled phorate. No mortality, attributable to phorate, was found, when _A. confusus_ nymphs

were caged on aphid-free, one month old bean plants, in the field, treated with 6 lb A.I. phorate/Acre at sowing time. Similar numbers of nymphs survived for over 8 days, whereas at this time 100% of A. fabae were killed in 48 hours on the treated plants.

However, when A. nemorum females and nymphs, were sleeved on phorate treated plants, with food, for a week (Table 41), there was no significant mortality in 4 batches of females, but in one batch of females (Table 41 A.) and among the nymphs (41 D), there were control corrected kills of 75 and 15% by 6 lb/Acre phorate.

The female A. nemorum which died, were confined in small muslin sleeves with a mass of closely folded leaves (owing to the plants' rapid growth). The sleeves were also only about 5 inches above the soil, so the females may have been unduly affected by vapour from the soil or from the plants.

In contrast, these conditions were avoided in the test with A. nemorum nymphs (Table 41D). Large wire framed sleeves of about 1.0 mm. terylene mesh were used, which, permitted free air circulation round the plants, and adequate food was supplied. The 15% mortality of nymphs under these conditions is attributed to uptake of phorate from the plants, rather than from prey. This is discussed later.

Contact action of aphicides on aphids and Anthocorids.

All three insecticides killed aphids, when these were caged on insecticide deposits on leaves. Probit analysis was not possible, but in Experiment 16, phorate at 16×10^{-4} mg/cm^2 was 100% toxic to Aphis fabae in 72 hours and in

Experiment 17 dimethoate at 40 X 10^{-5} mg/cm^2 killed 89% of A. fabae. Dimethoate was about 4 X more toxic than menazon to Acyrthosiphon pisum. This is the opposite of the result for root-applied systemic action (Expt. 6.) where menazon was 2 - 5 X more toxic than dimethoate.

After 24 hours, A. pisum nymphs were generally more affected, by dimethoate and phorate, than A. fabae of the same size. It should be noted that the A. fabae nymphs would have been physiologically older. This difference agrees with results obtained with root applied dimethoate, against nymphs of the same size (Expt. 12.).

Dimethoate and phorate were toxic to Anthocorid nymphs by contact action, whereas menazon was not. Dimethoate was about 6.5 X more toxic than phorate to A. nemorum and 7.5 X more to A. confusus (Expt. 15.). Menazon had no contact action against A. nemorum at 16 X 10^{-4} mg/cm^2, but killed 90% of Acyrthosiphon pisum in 72 hours (Expt. 19.) and was thus physiologically selective as stated by Price-Jones (1961).

In contrast dimethoate killed 42% of A. nemorum nymphs (Expt. 15.) at this concentration and also 87% of A. pisum (Expt. 17.) in 72 hours. Phorate killed 11% of A. nemorum nymphs (Expt. 15.) and 89% of A. pisum nymphs (Expt. 16.), at this concentration in 72 hours. Since both dimethoate and phorate killed more aphids than Anthocorid nymphs (which were of the same size) they are slightly physiologically selective but not nearly as selective as menazon. Dimethoate and phorate were ecologically selective when 16 X 10^{-4} mg/cm^2 was applied to the upper side of the leaves, with the predators and aphids caged on the underside (Expt. 18.). Under these conditions the A. pisum were dead

in 48 hours and none of the Anthocorids had died after 4 days. This might have application, when crops are sprayed, but, it must be pointed out that the Anthocorids and Aphids were forced to stay in position by the cages. Under natural conditions the Anthocorids would probably wander widely and be affected by deposits on the leaf upper surface, even though the aphids/prey would be on the lower surface. They might thus be more affected than aphids.

Effects on Anthocorids of prey from plants treated systemically via the roots.

No mortality of either species of Anthocorid nymphs, attributable to phorate, menazon or dimethoate, was found when nymphs were reared exclusively on a diet of dead or moribund aphids, falling from plants treated via the roots, at 10 p.p.m. (Expt. 24.).

Experiment 21, showed that phorate, or at least its sulphur metabolite, was present in the bodies of these nymphs after about ten days on the diet. Metcalf et al (1957) showed that the oxidation products of phorate in plants, occuring in the first few days, inhibited choline-esterase, so presumably the phorate metabolite present in dead prey, or as accumulated by Anthocorids must be different, or if active, present in sub-lethal quantities.

Various authors have tested the toxicity of aphids, killed by root applications of systemic insecticides, to predators feeding on them. None of these refer to Anthocorids or the insecticides used here. Ripper (1949) found that aphids killed by schradan were non-toxic to coccinellids, when eaten. Ahmed (1955) confirmed this result for schradan, but showed that cotton aphids killed by demeton were very toxic to Syrphid larvae, less so to

coccinellids and harmless to chrysopids. Bonnemaison (1962) confirmed the effect of demeton on syrphids, using Brevicoryne brassicae, and also showed that aphids sprayed with phosdrin, diazinon, methyl demeton and dimethoate, were toxic by ingestion, presumably, due to residues of active ingredient on the bodies of the aphids. Zeleny (1965) showed that aphids killed by contact action with Malathion and ekatin, were poisonous to coccinellids, but aphids killed systemically by ekatin were not.

Action of Aphicides on Anthocorid eggs.

Experiments 7 and 9 showed that root-applied phorate killed the eggs of A. nemorum, laid in the leaves of Tick bean seedlings just after uptake. Thus 15 p.p.m. killed 83% of eggs in 9 days and 100% of Aphis fabae in 48 hours. This result was subsequently confirmed in the field with some differences, thus plants at 1.5 lb/Acre phorate, (applied at sowing time in April) killed 86% of eggs laid in June and also killed 100% of A. fabae in 48 hours.

In July the same plants killed 30% of A. nemorum eggs but only 15% of A. pisum. (Control corrected kills.).

Perhaps phorate metabolites occurring later in the season were non-toxic or un-available to aphids, but still toxic to eggs.

Menazon, by root uptake, was non-toxic to A. nemorum eggs, even at 15 p.p.m., which killed 99% of A. fabae in 48 hours. It was also non-toxic at 1.5 lb/Acre in the field, under the same conditions as described for phorate above.

Dimethoate, at 5 p.p.m. by root uptake, killed 11% of A. nemorum eggs (after correction). Analysis of the uncorrected data showed this did not differ significantly from

mortality in untreated controls. At the same concentrations dimethoate killed 90% of A. fabae in 48 hours.

Eggs of A. confusus laid in the phorate treated plants at the same time as those of A. nemorum (Expts. 9, 10 and 11.) were not killed. Data in Tables 10 and 11 show that A. confusus eggs were laid in stems, midribs and petioles of beans, whereas A. nemorum eggs were laid mainly in leaf and stipule edges.

In Experiment 13 the P32 isotope, from labelled phorate was shown to accumulate mainly at the edges of leaves. Stipules, petioles and midribs contained less P32 and stems, in most cases, apparently also contained less. This is advanced as possible explanation of the difference, in phorate of effects between A. confusus and A. nemorum eggs, although there may also be intrinsic differences between the species, as there were in contact effects of dimethoate and phorate on the nymphs, for instance.

In Experiment 13 the distribution of P32 from root applied phorate, was shown to be similar, in other plants, to that in beans, and oviposition sites for A. nemorum were also similar (Expt. 14.). This implies that phorate might have a similar ovicidal action against A. nemorum in other crops and, if this were true, it would mean that this species would not breed successfully on treated crops in the May - June oviposition period and many eggs laid in July - August would also be killed, at a time when the plants are begining to lose toxicity to aphids. As a result, the only Anthocorids on treated crops would be migrating adults or wandering nymphs from nearby vegetation.

The comparison with A. confusus is somewhat artificial, as this species is not common on field crops, but Orius

species like *A. confusus* lay eggs in large leaf veins and in petioles (Sands 1957). These are sometimes important predators of field crops (e.g. Fletcher and Thomas 1943) and it would be valuable if their eggs, like those of *A. confusus*, were un-affected by phorate and similar chemicals.

Possible Modes of Action of phorate on *A. nemorum* eggs in leaves.

Of the 3 insecticides tested, phorate was the only one affecting *A. nemorum* eggs. If toxicity to eggs was a matter of non-selectivity, then dimethoate could be expected to be more ovicidal than phorate, since it was certainly more toxic to nymphs, but dimethoate was non-toxic, like menazon. Perhaps phorate acts as a fumigant, in the air spaces of the leaf's mesophyll (Way, Private Communication) just as it travels as vapour through soil air spaces to plant roots (Burt et al 1965). In this context Judson et al (1962) showed that phorate vapour was toxic to *Aedes aegypti* eggs, and, after comparison with other organophosphorous compounds, suggested that differences in their ovicidal action, as fumigants, was largely related to the different vapour pressures of each compound.

There is also the possibility that the eggs absorbed phorate in water from the plants. Water uptake is reported by Johnson (1937) to force the operculum off the eggs of *Notostira* at hatching, but the solubility of phorate in water is only 50 p.p.m., whereas dimethoate is more soluble in water (2.5%) and has no systemic effect on eggs.

Stage of development when eggs were killed.

Eggs of *A. nemorum*, killed by phorate, were dissected from leaves, 2 weeks after they would normally have hatched, and were usually found to contain fully developed embryos, generally well preserved and still enclosed within the egg membranes.

Lord and Potter (1951) and Potter et al (1957) showed that TEPP. at *high* concentrations killed Lepidopterous eggs at an *early* stage of development, but, at normal toxic concentrations, the embryo is not killed until *fully* developed or just after hatching. The ovicial action has been attributed to inhibition of choline-esterase, which forms towards the end of embryonic development (Mehrotra and Smallman 1957)(Salkeld 1961). Where compounds kill larvae immediately *after* hatching (e.g. parathion Zschintzch et al 1965) this is perhaps because the insecticide does not penetrate the embryo, but accumulates in the chorion or membranes, and contaminates the hatching larva. The egg of *A. nemorum* is normally laid in plants and the chorion does not tan, remains pliable, and dessicates easily in air. It might therefore be particularly easy for an insecticide to enter the embryo at an early stage, but none the less the embryos develop fully before dying. This might implicate cholinesterase inhibition as the cause of death.

Other Effects of Systemics on Anthocorids.

The studies on toxicity of systemics to Anthocorid eggs laid in plants are perhaps the most important part of this work. Apart from the toxicity of phorate to *A. nemorum* eggs, and the contact action of the non-selective

dimethoate and phorate, the only other undesirable side effect of systemic treatment found, was the small mortality from systemic phorate, probably picked up by direct feeding on the plants.

Otherwise there were no undesirable side effects, even from doses of phorate which were about 4 - 10 X more than those needed to give complete kills of aphids. For example Anthocorids fed on phorate poisoned aphids were unharmed.

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MATHEMATICAL APPENDIX

In this appendix symbols are used as in Finney (1952) and Bailey (1959). Significance is tested at the 5% probability level.

Appendix 2. 1. Experiment 2. Section 2.

Probit lines : toxicity of 4 concentrations of phorate to Myzus persicae and Aphis fabae ,7 days. All doses X10 Table 3. Fig.2

Myzus persicae

Snw= 148.807	Snwx= 204.115	Snwy= 748.362	
	$\bar{x}$= 1.3717	$\bar{y}$= 5.0291	
$Snwx^2$=293.463	$Snwy^2$=3820.710	Snwxy= 1054.904	Chi squared M
Sxx= 13.483	Syy= 57.139	Sxy= 27.584	=0.707

Aphis fabae

Snw= 173.127	Snwx= 175.385	Snwy= 935.175	
	$\bar{x}$= 1.0130	$\bar{y}$= 5.0905	
$Snwx^2$= 191.534	$Snwy^2$=5703.070	Snwxy= 1028 .520	Chi squared A
Sxx= 13.861	Syy= 101.955	Sxy= 30.496	=34.86

Regression Coefficient , parallel lines

$\sum$Sxx= 27.344 $\sum$Syy= 159.094 $\sum$Sxy= 58.080

joint b = 2.1241 joint Chi squared= 35.729

Analysis of Variance

Items	DF	S.Sq.	M.Sq.	F
departures from parallel	1	0.162	0.162	0.018
residual M+A	4	35.567	8.892	
Total	5	35.729		

Equations are M. persicae Y= 2.1241x + 2.1155, m= 1.358, LD50=2.28ppm

A. fabae Y= 2.1241x + 3.5388, m= 0.6879, LD50= 0.49ppm.

Relative Tolerance =4.677 ± 1.9 (heterogeneity factor = 8.892)

Appendix 2.2. Experiment 3. Section 2.

Probit lines: toxicity of 4 concentrations of phorate to *Myzus persicae* and *Aphis fabae*, 72 hours, all dosages X10 from Table 5. Fig 3.

M. persicae

Snw= 111.268	Snwx= 166.401	Snwy= 583.677
	$\bar{x}$= 1.4955	$\bar{y}$= 5.2457
$Snwx^2$=259.483	$Snwy^2$= 3113.437	Snwxy= 894.239 Chi squared M
Sxx= 10.631	Syy= 51.651	Sxy= 21.552 = 8.76

A. fabae

Snw= 54.498	Snwx= 76.447	Snwy= 336.408
	$\bar{x}$= 1.450	$\bar{y}$= 5.6516
$Snwx^2$= 130.408	$Snwy^2$= 1937.601	Snwxy= 501.507 Chi squared A
Sxx= 5.299	Syy= 37.210	Sxy= 13.905 = 0.75

Regression Coefficient , parallel lines :

$\sum$Sxx= 15.930 $\sum$Syy= 88.861 $\sum$Sxy= 35.457

joint b = 2.21 Joint Chi squared = 10.829

Analysis of Variance

Items	DF.	S.Sq.	M.S.	F.
departures from parallel	1	1.319	1.319	0.5546
residual	4	9.510	2.378	
Total	5	10.829		

Equations are :

M. persicae Y= 2.21X + 1.941 , m= 1.385, LD50=2.4[illegible]ppm.

A. fabae Y= 2.21X + 2.44 , m= 1.158, LD50=1.45ppm.

Relative Tolerance,(M) =0.227, S(M) = 0.1125, heterogeneity factor = 2.378 , Relative tolerance = 1.68 ± 0.4338

Appendix. 2. 3. Experiment 4. Section 2.

Probit lines : toxicity of 3 concentrations of phorate to Aphis fabae using 2 different solvents. All doses X 100

A. phorate made with acetone 48hours Table 5. Fig 4.

Snw= 59.303   Snwx= 121.193   Snwy= 308.769

$\bar{x}$= 2.0436 $\bar{y}$= 5.2066

$Snwx^2$= 284.949 $Snwy^2$= 1620.106 Snwxy 651.474 Chi squared A

Sxx= 37.276   Syy= 12.459   Sxy= 20.486   =1.201

Estimate of b = 0.5496 Equation from acetone data alone is :

Y= 0.5496X + 4.0832, m= 1.6681 , LD50 = 0.4656 ppm

B. phorate made with cellosolve 48 hours

Snw = 46.400   Snwx= 96.507   Snwy= 244.195

$\bar{x}$= 2.0799 $\bar{y}$= 5.2628

$Snwx^2$= 229.519 $Snwy^2$= 1295.270 Snwxy= 524.963 Chi squared B

Sxx= 28.795   Syy= 10.115   Sxy= 17.064   = 0.003

Estimate of b = 0.5926 Equation from cellosolve data alone is

Y= 0.5926 X + 4.0302 , m = 1.6365 , LD 50= 0.433 ppm.

Joint line from A and B

ΣSxx= 66.071   ΣSxy= 37.550   ΣSyy= 22.574

Joint $\bar{x}$= 2.0595, joint $\bar{y}$= 5.2313, joint b= 0.5683, V(b)= 0.1513

S(b)= 0.123, joint b = 0.5683 ± 0.123

Equation for joint line = Y= 0.5683X + 4.0609 , m= 1.6525

LD50= 0.4493 ppm , V(m)= 0.03706, S(m)= 0.19 so LD50= 0.2883 -0.70 ppm

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Chi squared test for goodness of fit of joint line to all points

Treatment ppm.	E. from line	O. corrected kill/50	$\frac{(O - E)^2}{E}$
10.0 C	39.26	40	0.01395
10.0 A		40	0.01395
1.0 C	29.55	29	0.01024
1.0 A		25	0.70051
0.1 C	16.2	18	0.20000
0.1 A		20	0.89136

Chi squared = 1.83001

C. phorate made with acetone, 72 hours

Snw= 49.022   Snwx= 86.886   Snwy= 275.238

x̄= 1.7724   ȳ= 5.6146

Snwx^2= 181.399   Snwy^2=1574.818   Snwxy= 515.544 Chi squared C

Sxx= 27.403   Syy= 29.472   Sxy= 25.715   = 5.341 C

Estimate of b = 0.9384, Equation from acetone data alone is

Y = 0.9384X + 3.9514, m = 1.1174 , LD50 = 0.131 ppm

D. phorate made with cellosolve, 72 hours.

Snw= 38.375   Snwx= 68.028   Snwy= 219.103

x̄= 1.7727   ȳ= 5.7095

Snwx^2= 141.252   Snwy^2=1268.631   Snwxy=407.505 Chi squared

Sxx = 20.658   Syy=17.[illegible]57   Sxy= 19.097   = 0.003 D

Estimate of b = 0.9244, Equation from cellosolve data alone is

Y = 0.9244X + 4.0708 , m = 1.0052 , LD50 = 0.1012 ppm.

Joint line from C and D

ΣSxx= 48.061 ΣSyy= 47.129 ΣSxy= 44.812, joint x̄= 1.7725

joint ȳ= 5.6563, joint b = 0.9324, V(b)= 0.0208, S(b)= 0.1442

b= 0.9324 ± 0.1442, equation for joint line is :

Y = 0.9324X + 4.0036, m= 1.0885, LD50= 0.1171ppm, V(m)=0.02502

S(m)= 0.1582 so LD50= 0.0813 - 0.1686 ppm.

Chi squared test for Goodness of fit of joint line to all data

Treatment	E.(from line)	O.(corrected kill/50	$(O-E)^2$
10.0 ppm C	48.2	48.5	0.00187
10.0 A	"	50.0	0.0[illegible]722
1.0 C	4[illegible].5	41.2	0.00[illegible]7
1.0 A	"	35.8	1.4[illegible]868
0.1 C	24.0	25.0	0.04[illegible]67
0,1 A	"	26.3	0.2[illegible]096
		chi squared =	1.75257

Appendix 2. 4. Experiment 5. Section 2.

Probit lines : Toxicity of 3 concentrations of phorate and menazon to A. fabae and A. pisum, Table 7 Fig 5. all dosages x100, 24hrs

A. Phorate v A. fabae

Snw= 49.397	Snwx= 94.959	Snwy= 254.698	
	$\bar{x}$= 1.9231	$\bar{y}$= 5.156	
$Snwx^2$= 202.857	$Snwy^2$= 1350.095	Snwxy= 516.899	Chi squared A
Sxx= 20.245	Syy= 36.357	Sxy= 27.098	= 0.036

B. phorate v A. pisum

Snw= 62.345	Snwx= 94.441	Snwy= 317.020	
	$\bar{x}$= 1.5148	$\bar{y}$= 5.08493	
$Snwx^2$= 179.243	$Snwy^2$= 1672.639	Snwxy= 522.996	Chi squared B.
Sxx= 30.183	Syy= 60.614	Sxy= 42.77	= 0.008

C. Menazon v A. fabae

Snw= 50.706	Snwx= 75.468	Snwy= 266.096	
	$\bar{x}$= 1.4883	$\bar{y}$= 5.2478	
$Snwx^2$= 134.950	$Snwy^2$= 1454.956	Snwxy= 421.469	Chi squared C
Sxx= 22.628	Syy= 58.532	Sxy= 35.426	= 3.070

Parallel regression lines

ΣSxx= 73.056	ΣSyy= 155.503	ΣSxy= 105.294	Chi squared(joint)
joint estimate of b= 1.4413			= 3.745

Analysis of Variance

Items	DF	S.Sq.	M.Sq.
departures from parallelism	2	0.581	0.2905
residual	3	3.164	1.0547
Total		3.745	not significant

Equations are :

A. Phorate v A. fabae: Y= 1.44X + 2.586, m= 1.8153, LD50= 0.6556ppm

B. Phorate v A. pisum: Y= 1.44X + 2.902, m= 1.4572 LD50= 0.2865 "

C. Menazon v A. fabae: Y= 1.44X + 3.103, m= 1.3176 LD50= 0.2078 "

"M" (phorate v menazon)= 0.4977 ± 0.1313, relative potency=3.146 ±0.95

"M" (fabae v pisum)= 0.3[illegible]1± 0.090[illegible], relative tolerance=2.281 ±0.4749

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Appendix 2.5. Experiment 6. Section 2.

Probit lines :toxicity of three concentrations of menazon and dimethoate to large and small Apisum nymphs Table 9 Fig 6.
all dosages x1000

A. menazon v small nymphs

Snw= 61.712 Snwx= 99.974 Snwy= 326.055

$\bar{x}$= 1.6200 $\bar{y}$= 5.2832

$Snwx^2$= 139.5[illegible]0 $Snwy^2$= 1763.352 Snwxy= 559.719 Chi squared A

Sxx= 27.591 Syy= 40.834 Sxy= 31.539 = 4.782

B. dimethoate v small nymphs

Snw= 69.477 Snwx= 130.482 Snwy= 360.543

$\bar{x}$= 1.8781 $\bar{y}$= 5.1894

$Snwx^2$= 282.692 $snwy^2$= 1901.555 Snwxy= 710.151 Chi squared B

Sxx= 37.639 Syy= 30.558 Sxy= 35.029 = 1.574

C. menazon v large nymphs

Snw= [illegible]2.110 Snwx= 116.099 Snwy= 322.767

$\bar{x}$= 1.8693 $\bar{y}$= 5.3577

$Snwx^2$= 249.853 $Snwy^2$= 1725.456 Snwxy= 640.027 Chi squared C

Sxx= 32.835 Syy= 48.113 Sxy= 36.695 = 7.104

D. dimethoate v large nymphs

Snw= 58.942 Snwx= 133.467 Snwy= 293.825

$\bar{x}$= 2.2644 $\bar{y}$= 4.9850

$Snwx^2$= 330.455 $Snwy^2$= 1522.506 Snwxy= 705.094 Chi squared D

Sxx= 28.035 Syy= 57.793 Sxy= 39.763 1.396

Regression coefficient , parallel lines.

$\sum$Sxx= 126.100 $\sum$Syy= 177.298 $\sum$Sxy= 141.026

joint Chi squared= 15.772 , joint b = 1.1184

Analysis of Variance

Items	DF	S.sq	M.Sq	F
departures from parallelism	3	0.916	0.3053	0.082
residual(A+B+C+D)	4	14.856	3.714	
Total	7	15.772		

Equations are:

A. menazon small, Y= 1.118X + 3.472, m= 1.3667,LD50= 0.0233ppm

B. dimethoate small,Y=1.118X +3.090, m= 1.7087,LD50= 0.0511ppm

C. menazon large Y= 1.118X+3.268, m= 1.5494, LD50=0.0354ppm

D. dimethoate large Y= 1.118X+2.453, m= 2.2778, LD50=0.0190ppm

Relative potencies

	menazon	X	dimethoate
small	2.2	x	1
large	5.4	x	1

Relative tolerances

	large	x	small
dimethoate	3.7	x	1
menazon	1.5	x	1

APPENDIX 3

Appendix 3. 1. Experiment 7 Section 3

Probit lines: toxicity of 4 concentrations of phorate to *Aphis fabae*, 72hours. From Table 12 Fig 8. All dosages X 1000

Snw= 57.24	Snwx= 163.07	Snwy= 291.91
	$\bar{x}$= 2.8489	$\bar{y}$= 5.0998
$Snwx^2$= 507.35	$Snwy^2$=1532.97	Snwxy= 873.001
Sxx= 42.76	Syy= 44.30	Sxy= 41.38

Chi squared = 4.25

b= 0.9678, Equation is Y = 0.97X + 2.34,m=2.7422,LD50=0.55ppm

Appendix 3. 2. Experiment 9 Section3.

Probit lines: toxicity of 3 concentrations of phorate to *Anemorum* eggs. Table 16 Fig 9. Alldosages X 100

Snw= 82.1	Snwx= 171.74	Snwy= 405.3
	$\bar{x}$= 2.0918	$\bar{y}$= 4.937

Snwx^2= 419.86 Snwy^2= 2050.02 Snwxy= 902.256 Chi squared

Sxx= 60.61 Syy= 49.19 Sxy= 54.434 =0.32

b= 0.898, Equation is Y = 0.9XX+ 3.06 , m= 2.1556,LD50=1.43ppm. acre

V(m)= 0.01386,S(m)= 0.1178, LD50=(1.313 - 1.549) ppm.

Appendix 3. 3. Experiment 12 Section 3.

Probit lines : toxicityof 3 concentrations of phorate, applied to soil on A.fabae , 72 hour data, Table 19 Fig 10 dosages X 1.

Snw= 71.24 Snwx= 43.908 Snwy= 366.507

$\bar{x}$= 0.6163 $\bar{y}$= 5.145

Snwx^2= 28.788 Snwy^2= 1985.98 Snwxy= 238.814 Chi squared

Sxx= 1.727 Syy= 100.47 Sxy= 12.923 = 3.65

b= 7.485 , Equation is Y= 7.49X + 0.53, m= 0.597, LD50= 3.953lb/acre.

Appendix 3. 4. Experiment 12. Section 3.

Probit lines,toxicity of 4 concentrations of dimethoate to Aphis fabae and Acyrthosiphon pisum, 48 hours data Table 29 Fig 12 , all dosages X 100

A.fabae

Snw= 64.553 Snwx= 127.34 Snwy= 336.498

$\bar{x}$= 1.9727 $\bar{y}$= 5.2128

Snwx^2= 266.348 Snwy^2= 1778.833 Snwxy= 681.488 Chi squared

Sxx= 15.151 Syy= 24.754 Sxy= 17.697 = 4.083

b= 1.168, Equation is Y = 1.17X + 2.9, m= 1.786,Ld50= 0.611

V(m)= 0.002376,S(m)= 0.04874,LD50= (0.546 - 0.684 ppm)

A.pisum

Snw= 62.5 Snwx= 89.13 Snwy= 336.131

$\bar{x}$= 1.1426 $\bar{y}$= 5.3781

Snwx^2= 138.701 Snwy^2= 1853.536 Snwxy= 501.92 Chi squared

Sxx= 11.594 Syy= 45.791 Sxy= 22.57 = 1.831

b= 1.9467, Equation is Y= 1.947X + 2.602, m= 1.231, LD50= 0.17ppm

V(m)= 0.0006224, S(m)= 0.02495,LD50= (0.1607 - 0.1803 ppm)

APPENDIX 4

Appendix 4. 1. Experiment 14. Section 4.

Fitting Poisson Distribution to distribution of 112 *Anthocoris nemorum* eggs in first leaves of 118 2-leaf oat seedlings Table 32 Fig14

Poisson terms : probability of obtaining x eggs in 1 plant $= \frac{e^{-m} m^{x}}{x!}$, where e = 2.718 and m = mean no. eggs / plant = 0.94

x	x!	$\frac{e^{-m} m^{x}}{x!}$	Expected no. of eggs in 118 plants = E		Observed no.f = O		$\frac{(O-E)^2}{E}$
0	0	0.3906	46.09		51		0.5231
1	1	0.3672	43.33		37		0.9247
2	2	0.1725	20.36		19		0.0909
3	6	0.0541	6.38		8		
4	24	0.0127	1.50	8.16	2	11	0.9885
5	120	0.0024	0.28		1		
					Chi squared(3)=		2.5272

APPENDIX 5

Experiment 15. Section 5 toxicity of dimethoate and phorate to *Anthocoris nemorum* and *A. confusus* nymphs Table 33 Fig 16

alldosages X 10,000

A. dimethoate v *A. nemorum*

Snw = 61.568 Snwx= 107.827 Snwy= 321.902

$\bar{x}$= 1.7514 $\bar{y}$= 5.2284

Snwx^2= 210.587 Snwy^2= 1698.730 Snwxy= 580.395 Chi squared A

Sxx=21.744 Syy= 15.699 Sxy= 16.633 = 2.976

B. dimethoate v *A. confusus*

Snw= 30.990 Snwx= 61.425 Snwy= 160.326

$\bar{x}$= 1.9821 $\bar{y}$= 5.1735

Snwx^2= 130.035 Snwy^2= 839.287 Snwxy= 326.375 Chi squared B

Sxx= 8.285 Syy= 9.847 Sxy= 8.594 = 0.932

C. phorate v A. nemorum

Snw= 66.613 Snwx= 157.507 Snwy= 336.608

$\bar{x}$= 2.3645 $\bar{y}$= 5.0532

$Snwx^2$= 393.002 $Snwy^2$=1721.459 Snwxy= 815.574 Chi squared C

Sxx= 20.575 Syy= 20.516 Sxy=19.662 = 1.726

D. phorate v A.confusus

Snw= 31.833 Snwx= 79.222 Snwy= 154.146

$\bar{x}$= 2.4887 $\bar{y}$= 4.8423

$Snwx^2$= 204.944 $Snwy^2$= 752.926 Snwxy= 390.612 Chi squared D

Sxx= 7.786 Syy= 6.50 Sxy= 6.993 = 0.219

Regression Coefficient , parallel lines

$\sum$Sxx= 58.390 $\sum$Syy= 52.5[illegible]2 $\sum$Sxy= 5[illegible].882, joint chi squared=

joint b = 0.8885 = 6.463

Analysis of Variance

Items	DF	S.Sq.
departures from parallelism	3	0.610
residual(A+B+C+D)	8	5.853
Total	11	6.463 not significant

Equations are :

A. dimethoate v A. nemorum Y=0.889X + 3.67,m=1.4943,LD50= 0.00312

B. dimethoate v A.confusus Y=0.889X + 3.41,m= 1.7868,LD50=0.00612

C. phorate v A.nemorum Y= 0.889X + 2.95 ,m= 2.3047,LD50= 0.02017

D. phorate v A confusus Y= 0.889X + 2.63 ,m= 2.6661,LD50= 0.04635 mg/cm^2

Relative Potency "P" Dimethoate X phorate

A.nemorum M=0.8104 ± 0.2012, P= 6.477 ± 2.997

A.confusus M=0.8793 ± 0.2898, P= 7.573 ± 5.048

Relative tolerance confusus X nemorum

phorate "M"= 0.3614 ± 0.2452, P= 2.298 ± 1.296

dimethoate "M" =0.2925 ± 0.2487, P= 1.961 ± 1.122

APPENDIX 6

Appendix 6. 1. Experiment 21. Section 6.

Chi squared tests on differences in radioactivity of Anthocorids Tested as adults and as nymphs : Hypothesis that adults and nymphs equally radioactive. From Table 39 Expected values below 5 are amalgamated.

	Observed O	Expected E	O-E	$(O-E)^2$	$\frac{(O-E)^2}{E}$
Adults					
(++) (+)	23	17.1	5.9	34.81	2.036
(±)	3	7.7	4.7	22.09	2.869
(-)	6	7.2	1.2	1.44	0.200
nymphs					
(++) (+)	9	13.9	1.2	24.01	1.727
(±)	11	6.3	4.7	22.09	3.506
(-)	6	5.8	0.2	0.04	0.007

Chi squared (2) = 10.345

Appendix 6. 2. Experiment 23.

Chi squared test on Hypothesis that equal nos. of A.nemorum females survived on treated plants and untreated plants. From Table 41B, using 6lb/acre data only.

	Dead	Alive	Total
6lb/acre	9	38	47
control	8	47	55
total	17	85	102

Chi squared =

$$\frac{102\,(9 \times 47 - 8 \times 38)^2}{17 \times 85 \times 47 \times 55}$$

=0.387 NS

Chi squared test on hypothesis that equal nos. of A. nemorum Nymphs survived on phorate-treated plants and untreated plants From Table 41D.

	Dead	Alive	Total
6lb/acre	15	49	64
Control	5	52	57
total	20	101	121

Chi squared (1) =

$$\frac{121\,(15 \times 52 - 5 \times 49)^2}{20 \times 101 \times 57 \times 64}$$

= 4.6998 significant