EFFECTS OF SOME ORCHARD PESTICIDES ON THE PREDATORY MITE *TYPHLODROMUS PYRI* (PHYTOSEIIDAE)

by

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ABSTRACT

From 1979 to 1982 field and laboratory studies were made on the effects of orchard pesticides on the phytoseiid mite Typhlodromus pyri, the key predator of the fruit tree red spider mite, Panonychus ulmi, in the U.K.

In an apple orchard trial, T. pyri tolerated well pre-blossom and post-blossom sprays of carbaryl and diflubenzuron and a mid-July spray of azinphos-methyl, but was almost eliminated by cypermethrin.

In two other field trials, sprays of 25-50 mg permethrin/litre greatly reduced numbers of T. pyri, whether the sprays were timed pre-blossom, from 'bud-burst' to 'late green cluster', or post-blossom, in June. At 'bud-burst' T. pyri were partially protected in hibernating sites, but the lethal effect of the spray was only delayed. In bioassays, toxicity of permethrin residues was negligible on apple bark and leaves by 20 days after spraying. Residues were detectable by G.C. analysis for 3 months or longer. Resurgence of P. ulmi occurred within 2 months after all spray timings and effective predation by T. pyri was fully established only after several months.

T. pyri from isolated unsprayed orchards were reared in the laboratory and used in 'taped-slide' mortality tests to establish base-line susceptibility to azinphos-methyl, parathion, carbaryl and permethrin. Other laboratory stocks of T. pyri from English apple orchards with a spray history of organophosphates and carbaryl

showed resistance of 4 to 6x to azinphos-methyl, c 50x to parathion, >20x to carbaryl and no resistance to permethrin. All stocks from sprayed orchards were resistant to both azinphos-methyl and carbaryl, suggesting cross-resistance; resistance to both these pesticides appeared to be stable in the field when selection pressure was relaxed.

When T. pyri were exposed to pesticide residues for 12 days, carbaryl did not reduce oviposition but azinphos-methyl caused a slight reduction. Sub-lethal residues of permethrin reduced the rate of egg-laying for a week. None of the three pesticides affected egg hatch. A resistant strain of T. pyri had a higher initial oviposition rate, but laid a lower total number of eggs compared to a susceptible strain.

In 'choice chamber' tests T. pyri females avoided sub-lethal deposits of permethrin, at 0.5 mg/litre, but were unable to avoid deposits from discrete droplets containing 1000 mg permethrin/litre. These results indicated that the avoidance reaction was due to irritation rather than olfactory repellency.

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S E C T I O N 1

GENERAL INTRODUCTION

1.1 Apple pest control

Apple orchards are subject to attack by several important diseases including apple scab (Venturia inaequalis), powdery mildew (Podosphaera leucotricha) and canker (Nectria galligena) and by a wide variety of insect and mite pests. Consequently, pest and disease control depends on a complex spray programme of pesticides. Chemical thinning of fruit set, usually achieved with carbaryl, is very widely practised, as is chemical weed control, whilst chemical regulation of shoot growth (Quinlan, 1981) is a rapidly developing field. Apples are a valuable cash crop that will pay for this intensive use of agrochemicals, provided that the quality of the fruit meets market standards. Hudson (1976) expressed this point well when he wrote "it is the fact of being eaten without first being cooked that makes fruit crops a rather 'special case' because appearance — eye-appeal — is an all-important attribute .... in marketing"! To obtain fruit free from minor blemishes as well as free from more serious damage, growers apply a more comprehensive spray programme than would be economic or necessary on other crops. In England, for instance, commercial orchards are usually sprayed weekly for 4-5 months with fungicides, and various insecticides and acaricides are added to the fungicide spray programme on 3-5 occasions. In warmer, drier climates, more insecticides and rather less fungicides are used.

This intensive use of pesticides tends to increase, because the pesticides used to control primary or 'key'

pests and diseases not infrequently stimulate outbreaks of secondary pests and diseases which require additional applications of pesticides. During recent years, progress has been made in the development of 'supervised' and 'integrated' control aimed at a more rational use of pesticides, to take account of natural checks on the levels of pests and diseases. As opposed to traditional or scheduled spray programmes, supervised and integrated control systems both involve regular monitoring of pest and disease levels, and pesticides are applied only when certain 'threshold' levels of abundance are exceeded.

Thresholds are set on the low side so that pest or disease levels causing economic injury are not reached. In supervised control the same broad-spectrum pesticides are used as in calendar programmes. Integrated control, on the other hand, involves a substantial contribution from the effective natural enemies of certain pests and therefore requires selective pesticides that are not harmful to these 'beneficials'. Control of the whole complex of pests and diseases is provided by integrated use of pesticides, natural enemies, cultural measures and any other compatible means of control. Low numbers of insect or mite pests are needed as food for the natural enemies, so integrated control involves a 'management' of pest numbers to keep them below injurious levels. Indeed the term 'integrated pest management' ('IPM') is preferred in North America.

The literature of this subject, even confined to apples, is vast and no attempt will be made to review it here; relevant reviews during the last decade include those

of Kirby (1973), Hoyt and Burts (1974), Croft (1975b), Way (1978), Gruys (1982), Cranham (1982) and Butt and Jeger (1982). The subject of this Thesis concerns the most obvious candidate for biological control on apples, namely the fruit tree red spider mite (or 'European red mite' outside Europe), Panonychus ulmi Koch (Tetranychidae), and its most effective natural enemy in English commercial orchards, the phytoseiid predatory mite Typhlodromus pyri Scheuten (Phytoseiidae) (Cranham, Kapetanakis and Fisher, 1983; Easterbrook, Solomon, Cranham and Souter, 1984). Even this small facet of IPM on apples has received considerable attention in most apple-growing regions of the world, at least in the more developed agricultural economies.

1.2 Biological control of spider mites

There is no better example of a man-made pest than P. ulmi (Cranham, 1979) which first emerged as a serious pest after the introduction of tar-oil winter washes in the 1920s. Since that time, various pesticides in common use have suppressed phytoseiid mites in commercial orchards. The problem has been aggravated by the marked ability of P. ulmi to develop resistance to a variety of chemical groups. Naturally, this evolution under chemical selection has occurred more rapidly in warmer climates and in some countries it has produced critical situations for fruit growers. Generally, however, the impact of resistance

development has been greatly reduced by the multiplicity of acaricides with different modes of action, and because resistance develops specifically to each type.

Field trials in the Netherlands (Gruys, 1982) and in England (Easterbrook, Solomon and Cranham, 1982) showed that it was possible to control most of the major pests and diseases of apple using materials harmless to the native phytoseiid species Typhlodromus pyri (Gruys, 1980; Cranham and Solomon, 1981). The 'selective' insecticides included diflubenzuron and pirimicarb. The former has a novel mode of action: it interferes with moulting in insects by inhibiting chitin synthesis. It controlled larvae of winter moth (Operophtera brumata), codling moth (Cydia pomonella) and certain other tortricid species, apple sawfly (Hoplocampa testudinea) and the leaf-mining apple pygmy moth (Stigmella malella). Pirimicarb was effective on all apple aphids, though not excellent for woolly aphid (Eriosoma lanigerum). Of newer fungicides for mildew control, bupirimate and triadimefon were 'safe' for phytoseiids and some others may prove to be so. Several fungicides (e.g. captan, dodine, dithianon) used for control of apple scab, Venturia inaequalis, are likewise harmless.

In the experimental orchard 'de Schuilenburg' (T.N.O., Lienden) in The Netherlands, phytoseiids were absent for the first four years (1967-1970) of a selective programme. In 1970 they were introduced on cherry foliage to every fifth tree, from which they spread rapidly. A mineral oil spray was applied in 1971 to reduce spider mite numbers;

thereafter the phytoseiids gradually assumed control and maintained them at very low numbers indefinitely (Gruys, 1982). From 1972-77 this work was extended to several trials on commercial holdings with considerable success. Certain other pests were usually well controlled by natural enemies, e.g. Stigmella malella by the parasite Chrysocharis prodice, Eriosoma lanigerum by the parasite Aphelinus mali. Outbreaks of other species of aphids were fewer because of insect predation, notably by mirids and anthocorids. But damage to fruit by the larvae of various moth species tended to exceed commercially acceptable levels, and some minor pests became of increased importance and selective controls for them were lacking.

In England in trials at East Malling, biological control of P. ulmi was established as quickly as the second season after switching to a selective programme based on similar pesticides (Easterbrook, Souter, Solomon and Cranham, 1979) and T. pyri has regulated spider mites at very low numbers, far below a damaging level, for the four seasons 1978-1981. In these trials it was unnecessary to introduce T. pyri. However, as in The Netherlands, caterpillar damage to fruit tended to be rather too high, and increases of mussel scale (Lepidosaphes ulmi), apple sucker (Psylla mali), and larvae of the moth Blastobasis decolorella required the use of non-selective pesticides in the third and fourth years of the trials.

Organophosphates (OPs) have been the most commonly used insecticides on tree fruits since the 1960s. Although spider mites, and certain other secondary pests, developed

resistance to OPs, these insecticides continued to be used because they remained effective for control of most orchard moths and aphids. As a result of this continued use, certain species of phytoseiid mites developed strains resistant to OPs (Croft and Brown, 1975; Croft, 1982). There were thus excellent possibilities for integrating biological control of spider mites by phytoseiids with chemical control of insect pests by OPs. In terms of commercial usage, outstanding progress was made in British Columbia (Madsen and Carty, 1977) and Washington State (U.S.A.) (Hoyt and Caltagirone, 1971) employing the phytoseiid Typhlodromus occidentalis Nesbitt, in Michigan (Croft, 1975b) and North Carolina (Rock, 1972) with Amblyseius fallacis (Garman), and in New Zealand with Typhlodromus pyri (Wearing, Walker, Collyer and Thomas, 1978). Phytoseiids lend themselves to mass-rearing and introductions.

Attempts to establish T. occidentalis and A. fallacis on tree fruits in England and The Netherlands failed (Flegg, 1979), presumably because ecological conditions for these species were unsuitable. An OP-resistant strain of T. pyri from New Zealand was imported into England in 1977 and readily established in two orchards at East Malling Research Station, and later in a commercial orchard. However, in the same year, an indigenous resistant strain was found in a commercial holding at Hadlow, ten miles from East Malling.

When the studies reported in this Thesis commenced in October 1979, research workers at East Malling Research Station (J.E. Cranham, M.G. Solomon, M.A. Easterbrook) were

planning further evaluation of OP-resistant T. pyri in field trials. To provide background information to the practical project, these studies concentrated on the effects of insecticides on OP-resistant T. pyri and its prey. The prey includes not only P. ulmi but also the apple rust mite, Aculus schlechtendali Nalepa (Eriophyidae) and possibly mites of the families Tydeidae and Tarsonemidae. Some observations were also made on the mite Zetzellia mali (Ewing) (Stigmaeidae), a potential predator of P. ulmi and of A. schlechtendali (McMurtry, Huffaker and van de Vrie, 1970; Jeppson, Keifer and Baker, 1975; Santos, 1976).

Azinphos-methyl, probably the most used OP world-wide on apples, was to be the typical organophosphate insecticide studied. The effects of the carbamate carbaryl, widely used in parts of Kent as an insecticide and even more widely as a fruit thinning agent, were of special interest; carbaryl, since it was introduced in the 1950s, had a history of inducing outbreaks of spider mites. In addition, there appeared to be a threat to this form of IPM posed by the increasing usage of synthetic pyrethroids (SPs), of which permethrin was the first to be marketed and widely used. The SPs are popular because of their low mammalian toxicity and high cost-effectiveness for controlling most pests of fruit. However, those in current use (permethrin, cypermethrin, deltamethrin) give little or no control of spider mites whilst they exhibit a strong persistent toxicity to phytoseiids (Aliniazee and Cranham, 1980; Penman, Chapman and Jesson, 1981). Hence the insecticides

chosen for special study were azinphos-methyl, carbaryl and permethrin.

The different facets of this study, the mite fauna on the one hand, and the insecticides on the other, are considered below in greater detail, and the literature of greatest relevance to this study is reviewed.

1.3 Biology of apple mites

Panonychus ulmi (Plate 1.1) diapauses as winter eggs, laid on the bark, which require 100-150 days at temperatures of c -1° to 10°C to break diapause before further development begins in the spring (Lees, 1953). Hatching occurs over a few weeks, beginning in May in England, and development of the immature stages (larva and two nymphal stages) takes 1-3 weeks. Adult males are much smaller and more spidery in appearance than females. There are 4 to 6 overlapping summer generations in England, and up to 10 in warmer climates. Females of the last summer generation lay winter eggs over a prolonged period from the end of August. Diapause is induced by reduced daylength in combination with lower temperatures and poorer nutrition. Spider mites exhibit arrhenotokous parthenogenesis: females develop from fertilised diploid eggs and males from unfertilised haploid eggs. The sex ratio is generally c 2:1 in favour of females. P. ulmi feeds by piercing the leaf epidermis with its stylets, sucking the contents of mesophyll cells. Damage appears first as a speckling and then as a bronzing

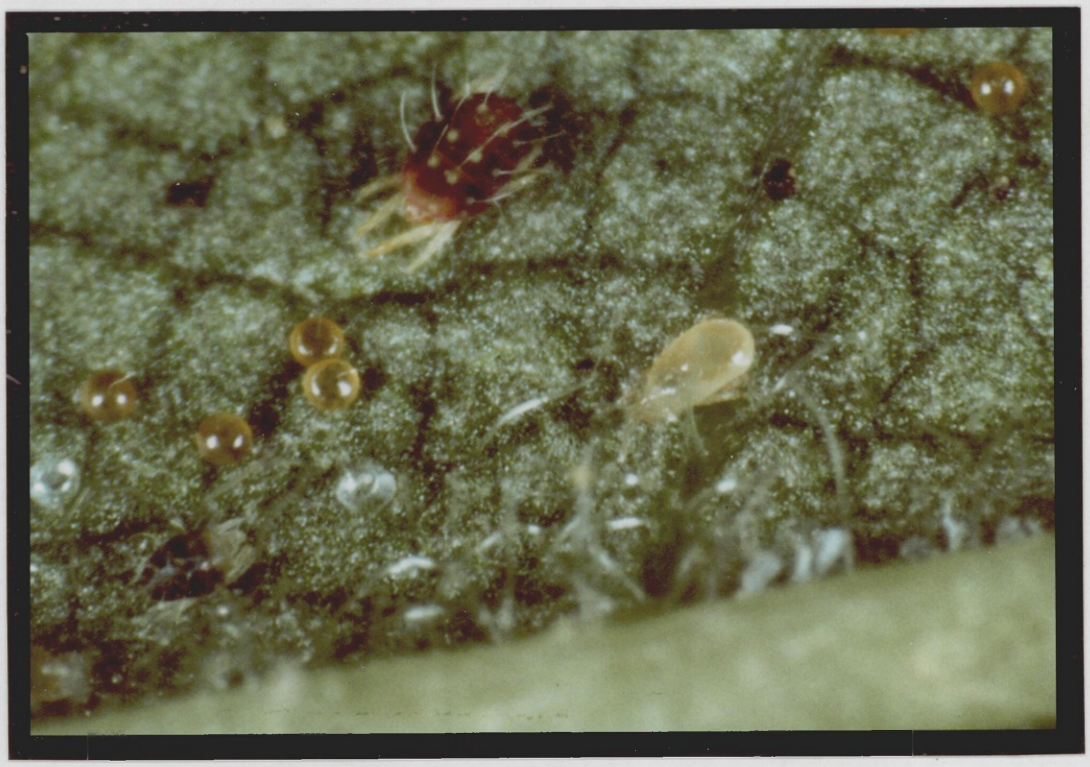


Plate 1.1 Panonychus ulmi adult female (top left), Typhlodromus pyri deutonymph (centre), and P. ulmi eggs



Plate 1.2 Typhlodromus pyri adult females

of the leaves, and photosynthesis is reduced. This has more severe effects if it occurs early in the season; it can then reduce the formation of fruit buds for the following year, and fruit size and colour in the same year. Repeated attacks debilitate trees (Blair, 1951; Avery, 1964; van de Vrie, McMurtry and Huffaker, 1972).

Typhlodromus pyri (Plates 1.1 and 1.2) is native to Britain and Europe but was probably introduced with fruit trees to North America and New Zealand. In England, no other phytoseiid species is common in sprayed commercial orchards (Solomon, 1975, 1982). T. pyri overwinters as fertilised females in reproductive diapause, which shelter in crevices on the bark. They emerge from hibernation a month or more before winter eggs of P. ulmi begin hatching. They lay eggs in the leaf and blossom clusters at around the 'pink bud' stage. With similar life stages to P. ulmi, the quiescent periods between instars are very brief, and the whole duration of development is rather shorter.

Karyological studies on several phytoseiid species showed that females were diploid and males haploid, though both developed from diploid eggs (reviewed by Sabelis, 1981); the haploid condition of males was caused by loss and/or heterochromatisation of half the chromosomes. This genetic system is termed parahaploidy.

After mating, adult females lay 0-2 eggs per day during the reproductive period and a total of c 20-30 eggs/female. They can live, however, for several weeks. Diapause is induced, from August, by reduced daylength and lower

temperatures, but females remain on the leaves almost until leaf-fall. Their preferred prey is P. ulmi, especially immature stages and, secondly, A. schlechtendali, but they will also feed on pollen and can extract some nutrition from leaf cells (Jeppson, Keifer and Baker, 1975). T. pyri may also prey on mites of the families Tydeidae and Tarsonemidae (McMurtry, Huffaker and van de Vrie, 1970; A. van Zon, University of Amsterdam, The Netherlands, personal communication).

Aculus schlechtendali or apple rust mite (ARM) is important on apple in Europe and North America (Hoyt, 1969; Croft, 1975a) but seems to be rare or absent in Australasia. The life history and seasonal occurrence of this free-living eriophyid were studied in England by Easterbrook (1979) and in Nova Scotia by Herbert (1974).

Adult females in reproductive diapause - termed 'deutogynes' - overwinter in bark crevices on spurs and behind buds on one-year-old wood. They move into opening fruit buds from 'bud-burst' to 'pink bud', and into vegetative buds as these begin to swell; they lay eggs on all green parts of these plant organs. Development is rapid, with two nymphal stages. The first generation gives rise to summer females ('protogynes') and males; eggs are then laid on the leaves. After several generations, populations on the foliage usually peak in July/August. At high mite densities, feeding on the epidermal cells can cause 'bronzing' which is easily distinguishable from spider mite

damage on close examination. Renewed production of 'deutogynes' usually begins in July and increases through August/September.

1.4 Interactions between species

A. schlechtendali can provide alternative food for phytoseiid mites, enabling them to survive when spider mite numbers are low. In Washington State, Hoyt (1969) showed that A. schlechtendali developed earlier in the season than spider mites and enabled T. occidentalis to increase to numbers which were better able then to regulate spider mites. This relationship survived the impact of OP insecticides by the development of resistance to OPs in all these mite species. In England, recent evidence points to A. schlechtendali fulfilling the same role in integrated control; therefore, although A. schlechtendali has recently been implicated as a cause of increased fruit surface 'russet' (Easterbrook, 1984), resulting in downgrading of fruit, it remains a beneficial species if managed at low numbers. Croft and Hoying (1977) suggest that it can play another important role in competition with P. ulmi; they found that feeding by A. schlechtendali at levels insufficient to cause economic damage 'preconditioned' foliage so that it was less suitable for development and reproduction of P. ulmi.

1.5 Resistance of phytoseiids to OPs

The first well-documented reports of resistance to azinphos-methyl were in 1970, both for T. occidentalis in the western U.S.A. (Croft and Jeppson, 1970) and for A. fallacis in the mid-west and eastern states of the U.S.A. (Motoyama, Rock and Dauterman, 1970). Field researchers had suspected earlier than 1970 that resistance to certain OPs had developed in both species (Hoyt, 1969; Swift, 1970). Indeed, T. occidentalis would appear to have a high natural tolerance to parathion, evident in 1952 in work on Californian strawberries (Huffaker and Kennett, 1953) soon after parathion was introduced, and later from the comparison of selected and unselected strains made by Croft and Jeppson (1970). In the early 1970s, T. occidentalis populations resistant to several OPs, including azinphos-methyl, diazinon, phosmet, parathion, tepp and phosalone were reported from virtually all the western irrigated apple-growing regions from British Columbia to California, Utah and Colorado. Similarly, OP-resistant populations of A. fallacis were reported from eight eastern and mid-western states of the U.S.A. and the Canadian provinces of Ontario and Quebec. In most of these areas, the OP-resistant strains became the basis for integrated mite control programmes, usually with considerable success in commercial orchards (Croft, 1981).

The most popular OP insecticide for insect control on apples during the 1970s was azinphos-methyl, in North America, Australasia, and in the U.K. Consequently, the

level of resistance to this compound is of special interest. Both T. occidentalis and A. fallacis developed high stable levels of 100-fold or more. T. pyri, on the other hand, at first developed a low level of resistance in New Zealand, 10-fold (Hoyt, 1972) or 14-fold (Collyer and Van Geldermalsen, 1975). In New Zealand, the use of azinphos-methyl for control of tortricid larvae on apple was very intensive, typically eight or more spray applications per season. With a c 10-fold level of resistance T. pyri were unable to survive such a programme, but after 10-15 years' use of azinphos-methyl much higher levels had developed in certain districts of the north island. Penman, Ferro and Wearing (1976) reported 40-fold resistance in some strains. This permitted the development of integrated mite control (IMC) programmes which have been strengthened by importation and releases of OP-resistant strains of A. fallacis, T. occidentalis and Phytoseiulus persimilis Athias-Henriot for control of both P. ulmi and T. urticae on apple.

The other region of the world from which OP-resistant T. pyri were reported in the early 1970s was New York State, U.S.A.; the level of resistance to azinphos-methyl was quantified by Watve and Lienk (1976) as 20-fold when compared with the data of Hoyt (1972). However, workers at Cornell University Agricultural Experiment Station, Geneva, New York regard indigenous A. fallacis as much more effective than T. pyri as a predator of P. ulmi (S. Lienk, New York State, U.S.A., 1981 - personal communication to J.E. Cranham); this probably explains why no further studies on resistance in T. pyri have been published from New York.

1.6 Resistance of phytoseiids to carbaryl

It appears to be well-established that both T. occidentalis and A. fallacis are inherently very susceptible to carbaryl. The development of resistance to OPs in these species did not per se confer cross-resistance to carbaryl (Croft, 1982). OP-resistance rapidly became widespread, to the extent that it was soon difficult to find susceptible populations for experimental study (Morse and Croft, 1981), but resistance to carbaryl in A. fallacis remains localised and develops only under intensive selection. Croft and Meyer (1973) developed a laboratory strain of A. fallacis resistant to both OPs and carbaryl, but attempts to establish this in the field were unsuccessful (Croft and Hoying, 1975). More recently, however, stable levels of resistance to both OPs and carbaryl were reported for some New York field populations of A. fallacis (Croft, 1982).

With IPM programmes in Washington State (Hoyt, 1969), carbaryl when used for fruit thinning is directed at the outer part of the foliage canopy, at a stage of the season when T. occidentalis typically colonises the centre of the canopy. This reduces harm to the predators, but field observations do suggest that resistance has developed (S.C. Hoyt, quoted in Croft, 1982). In California, resistance to carbaryl in this species was quantified by Roush and Hoy (1980); they also selected a laboratory strain resistant to both OPs and carbaryl and successfully established this in Californian orchards.

In contrast, T. pyri may be inherently more tolerant of carbaryl than are the above two species, or it may have a greater potential to develop high, stable levels of resistance. Croft (1982) goes so far as to state that "carbaryl is almost non-toxic to T. pyri but is extremely toxic to A. fallacis and T. occidentalis". From New York State, Watve and Lienk (1976) reported high tolerance or resistance to carbaryl in T. pyri. In New Zealand, Wearing and Ashley (1978) found that OP-resistant T. pyri tolerated the rather high rates of carbaryl used for fruit thinning. Despite these reports, however, at the time that the studies for this thesis commenced, workers at East Malling Research Station had reasons to be apprehensive that carbaryl might prove to be a 'stumbling block' to commercial implementation of integrated control, for the compound had a history of causing outbreaks of P. ulmi. It is also evident that, up to 1979, workers in The Netherlands (P. Gruys, personal communication to J.E. Cranham) had the same experience and opinion. Also van de Vrie (1962) had found that carbaryl caused slight mortality of adult T. pyri but reduced egg production. Outbreaks of P. ulmi caused by carbaryl were seldom documented, however, and they were not analysed. Carbaryl is certainly harmful to some insect predators including mirids, while the evidence for anthocorids is conflicting (Besson, Joly and Touzeau, 1978).

There is also evidence of it stimulating reproduction in spider mites (Dittrich, Streibert and Bathe, 1974, working with Tetranychus urticae). Hence, increases of

P. ulmi after carbaryl were not necessarily due to destruction of T. pyri, which in any event were very scarce in sprayed commercial orchards in England and The Netherlands until the late 1970s.

1.7 The effects of synthetic pyrethroids

Permethrin and cypermethrin have negligible direct toxicity to spider mites or rust mites. Deltamethrin, and especially fenvalarate, have an appreciable direct toxicity to spider mites but are not effective acaricides. Indeed perhaps the major weakness of these SPs, which so effectively control most insect pests, is that they tend to increase problems with phytophagous mites, especially in warmer climates. Even in the cool, maritime English climate they are extremely reliable agents (replacing DDT and carbaryl) for inducing infestations of P. ulmi when these are required for experimental purposes! They also appear to be implicated as one of the causes of an upsurge of A. schlechtendali in commercial orchards (M.A. Easterbrook, East Malling Research Station, U.K., unpublished data). They are very persistently toxic to phytoseiids (Hoyt, Westigard and Burts, 1978; Roush and Hoy, 1978; Rock, 1979; Aliniazee and Cranham, 1980; Gruys, 1980; Penman, Chapman and Jesson, 1981). In addition there is some evidence that SPs may stimulate spider mite resurgence in situations where predators are absent (Hoy, Ross and Rough, 1978; Hoyt, Westigard and Burts, 1978; Hoy, Flaherty, Peacock and Culver, 1979; Riedl and Hoying, 1980). There has so far

been little work on the mechanisms of such stimulation, i.e. whether it occurs by a direct stimulation of mite reproduction, by a trophic effect on host plant nutrition, or by effects on mite behaviour.

Workers in the U.S.A., realising the severe impact that commercial introduction of SPs could have on IPM systems based on OP-resistant phytoseiids (Croft and Hoyt, 1978) have sponsored attempts to develop phytoseiid strains resistant to SPs, with some success. Permethrin resistance was found in field populations of A. fallacis and elevated in laboratory selection experiments (Strickler and Croft, 1981); resistant mites were released and recovered from orchards (Whalon, Croft and Mowry, 1982). Similarly, with T. occidentalis from apple orchards, permethrin resistance was found and selected, and re-establishment in the field appears promising (Hoy, Knop and Joos, 1980). In New Zealand, attempts are being made to develop SP-resistant strains of T. pyri (M.J. Savage, personal communication).

1.8 Cross-resistance patterns

As stated above, T. occidentalis was inherently tolerant to parathion and probably due to this mechanism to phosalone and tepp; later, it developed resistance to azinphos-methyl with cross-resistance to certain other OPs. A. fallacis was not inherently tolerant to parathion, but the development of resistance to azinphos-methyl conferred

cross-resistance to parathion and certain other OPs, but not to phosalone and tepp (Croft, 1982). Similarly, T. pyri was not inherently tolerant of parathion, but development of resistance to azinphos-methyl conferred cross-resistance to parathion and several other OPs (Watve and Lienk, 1975, 1976). Recent field trials at East Malling Research Station suggest that this species has a rather high natural tolerance to phosalone (J.E. Cranham, unpublished data); tepp was not evaluated.

Neither tolerance to parathion in T. occidentalis, nor the developed resistance to azinphos-methyl in this species and in A. fallacis, conferred cross-resistance to carbaryl. Croft (1982) states that "there is no documented cross-resistance between carbaryl and azinphos-methyl in any (phytoseiid) species". Tests by van de Vrie (1962) would suggest that at that time T. pyri were at least more tolerant of carbaryl than the above two species. Such natural tolerance may have been increased by selection (i.e. commercial use of carbaryl) to the higher levels recorded in New York State (Watve and Lienk, 1975, 1976) and New Zealand (Wearing and Ashley, 1978); evidence on the question of cross-resistance is confounded because both azinphos-methyl and carbaryl were in common use.

Both OP-resistant and susceptible strains of phytoseiids are usually very susceptible to SPs, although Croft, Wagner and Scott (1982) consider that in A. fallacis resistance to permethrin may be related to both azinphos-methyl and DDT resistance. On the other

hand, Chapman and Penman (1979) obtained evidence of negative cross-resistance in the spider mite Tetranychus urticae: OP-resistant strains were killed by lower dosages of fenvalarate than were susceptible strains. There is therefore an area of interest in relation to the responses of OP-resistant phytoseiids, including T. pyri, to SPs.

1.9 Aims of the studies reported in this thesis

1. Field studies, to evaluate the effects of azinphos-methyl, carbaryl and permethrin on the mite fauna of apple orchards where OP-resistant T. pyri have been established. Sprays would be applied both pre- and post-blossom in order to investigate whether their effects were influenced by the growth stage of the trees and/or the seasonal biology and behaviour of the mites involved.

2. Laboratory bioassays with Typhlodromus pyri
   (a) to establish 'base-lines' for susceptibility to azinphos-methyl, carbaryl and permethrin,
   (b) to quantify levels of resistance in strains known to be resistant to OPs (New Zealand and Hadlow strains), and
   (c) to quantify levels of resistance to carbaryl and permethrin, if resistance occurs.

Samples of T. pyri would be obtained from
   (i) relatively isolated areas unselected with pesticides,

(ii) from apple orchards of varying spray history (e.g. OPs, no carbaryl; carbaryl, no OPs),

(iii) from orchards in which OP-resistant T. pyri have been established and where subsequently the selection pressure has been relaxed (in relation to the stability of resistance).

Mite stocks would be established in 'plate cultures' (McMurtry and Scriven, 1965). Bioassays would employ the 'taped-slide' technique (Voss, 1961; Anonymous, 1980) since this would permit comparison of the results with those obtained by earlier workers using the same technique with T. pyri (e.g. Hoyt, 1972; Watve and Lienk, 1976; Penman, Ferro and Wearing, 1976). Other techniques of bioassay might be used if appropriate.

3. Laboratory studies on the effects of the same insecticides at sub-lethal dosages on the reproduction and behaviour of T. pyri.

SECTION 2

EFFECTS OF CERTAIN INSECTICIDES ON THE MITE FAUNA OF AN APPLE ORCHARD (1980 FIELD TRIAL)

2.1 INTRODUCTION

The importance of carbaryl as an insecticide and fruit thinning agent on apple was mentioned in the General Introduction (sub-section 1.2). Information concerning the tolerance of T. pyri to this chemical was also reviewed in the same section (sub-section 1.6).

In a field trial in 1980, sprays of carbaryl and diflubenzuron were compared for their effects on the mite fauna of 'Weston_s Field 103' orchard, Rocks Farm, East Malling Research Station. Diflubenzuron was known to be harmless to T. pyri (Cranham, 1978; Wearing and Thomas, 1978; Gruys, 1980) and to other phytoseiids (Bower, 1980).

Three spray applications were made:

(a) once pre-blossom, as applied against winter moth, Operophtera brumata (L.) and

(b) twice post-blossom, in June and July as applied against codling moth, Cydia pomonella (L.) and other tortricids.

Later in the summer the effects of azinphos-methyl and cypermethrin were evaluated in the same orchard.

2.2 MATERIALS AND METHODS

2.2.1 Trial design and spray application

A 2 x 2 randomised block design of two pesticides applied at pre- and post-blossom timings, as shown in Table 2.1, was duplicated on plots of c 0.2 ha, i.e. usually 6 x 7 trees, sampling only from the central six trees (Fig. 2.1). All sprays were applied by a tractor-mounted 'Commandair' airblast sprayer in volumes equivalent to 600 litres/ha. To reduce spray drift, and also mite dispersal between plots over the whole season, it was desirable to use plots of this size and therefore to sacrifice higher replication. The pre-blossom sprays were applied to all trees of the plots to provide insect control. To further reduce spray drift, the post-blossom sprays were applied only on the six sample trees on each plot.

On 16 July, when carbaryl and diflubenzuron were applied for the third time, azinphos-methyl and cypermethrin were also sprayed, each on three separate plots (Fig. 2.1). Formulations and dosage rates were azinphos-methyl as 2.2 litres Gusathion 22% e.c. (Bayer U.K. Ltd), diflubenzuron as 0.6 kg Dimilin 25% w.p. (Midox Ltd, U.K.), cypermethrin as 0.5 litres Ripcord 10% e.c. (Shell Chemicals U.K. Ltd) and carbaryl as 2 kg Sevin 85% w.p. (Union Carbide U.K. Ltd) per hectare. No other insecticides were used in this orchard in 1980 but the fungicides captan and bupirimate were sprayed separately and routinely for control of apple scab and mildew; these fungicides are

Table 2.1

Sprays applied in Westons Field Trial, 1980

Treatment abbreviation (see Fig. 2.1)	Pre-blossom spray at 'late green cluster'* 22 April	Post-blossom sprays 23 June and 16 July
CCC	carbaryl	carbaryl
CDD	carbaryl	diflubenzuron
DDD	diflubenzuron	diflubenzuron
DCC	diflubenzuron	carbaryl

* see Plate 2.2

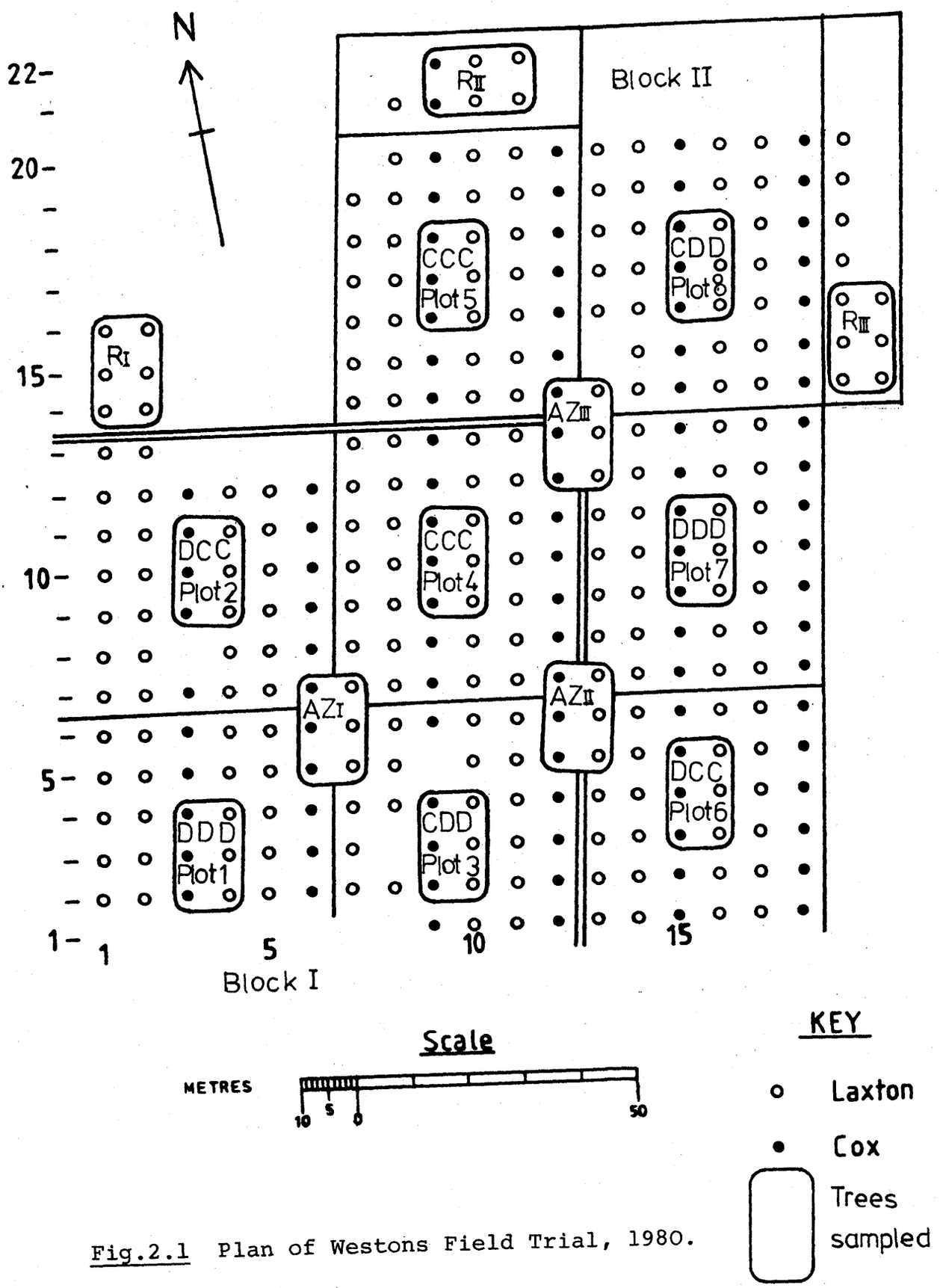


Fig.2.1 Plan of Westons Field Trial, 1980.

Treatments

CCC, CDD, DDD, DCC as in Table 2.1
AZ : sprayed with azinphos-methyl on 16 July
R : " " cypermethrin " " "

known to be harmless to T. pyri (Collyer and Kirby, 1959; Oatman, 1966; Easterbrook, Souter, Solomon and Cranham, 1979; Gruys, 1980).

2.2.2 Sampling

Since the trees were up to 4-5 m high (Plate 2.1) plant samples were taken from the lower part of the foliage canopy on all six trees per plot. The first, pre-spray sample on 17 April, at 'early pink bud' (Plate 2.2), consisted of 36 spurs per plot, c 5 cm long with developing buds. The second sample on 19 May, at 70-80% 'petal fall', consisted of six leaf and blossom clusters per plot. From 9 June onwards only leaves were sampled, as they were fully expanded. Leaf samples consisted of 24 leaves/plot on 9 June and 48 leaves/plot thereafter for carbaryl and diflubenzuron, and 33 leaves/plot for azinphos-methyl and cypermethrin. The samples were carried in sealed polythene bags to a constant temperature room at 7-8°C. T. pyri, P. ulmi and tydeid mites were counted under the microscope within 24 hours. Numbers of A. schlechtendali were visually estimated.



Plate 2.1 Apple orchard WF 103 at Westons Field, Rocks Farm, East Malling Research Station

Plate 2.2 Stages of fruit bud development on apple.

Note. Because of climatic variations, the stages of fruit bud development, and not calendar dates, are used as a reference in relation to development periods of fruit pests and application of plant protective measures.

When plant growth starts in the spring the apple fruit bud passes through 'dormant', 'swelling', 'bud-burst' and 'mouse ear' stages. Then follows the 'green cluster' stage, when rosette leaves have opened, but the five to seven blossom buds forming the cluster still have the sepals tightly closed. At 'pink bud' the swelling of the flower buds causes the typical pink colour of the petals to be exposed. When the flowers are opened, the 'full blossom' stage is reached. 'Petal fall' is the stage at which all, or nearly all, the petals have been shed.

These stages refer to the average state of one variety in one orchard. There is often considerable variation between varieties and between areas.



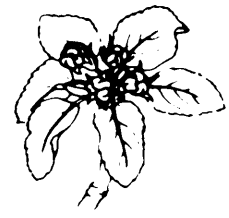
(a) dormant bud



(b) bud burst



(c) green cluster



(d) pink bud



(e) full blossom



(f) petal fall

2.3 RESULTS

2.3.1 Effects on T. pyri and P. ulmi

Carbaryl sprayed pre-blossom (Table 2.2 and Appendix 2.1).

Analyses* of variance of the two post-spray counts (19 May, 9 June) using the pre-spray count (17 April) as covariate showed that (a) the covariate was not significant ($P = 0.05$) and (b) carbaryl did not reduce T. pyri numbers compared to diflubenzuron. Since diflubenzuron was known to be harmless, carbaryl appeared to be equally so.

It was not possible to sample the same 'unit of plant growth' on the three dates because buds on the spurs unfolded into leaf and flower clusters, and foliage then expanded rapidly; consequently the counts do not necessarily indicate a decline in numbers of T. pyri per tree.

When the first sample was taken on 17 April winter eggs of P. ulmi had not started to hatch. Presence or absence of eggs was recorded: 32/144 spurs from carbaryl, and 42/144 spurs from diflubenzuron plots carried eggs. On 19 May some immature active stages of P. ulmi were present on foliage, and on 9 June summer eggs (first generation) formed the major part of P. ulmi numbers (Table 2.3), which were very low. Analyses of variance of the counts on

* Mite numbers in this section have been analysed and illustrated as log (1 + x), where x = mite count per sample.

Table 2.2

Effects of carbaryl and diflubenzuron sprayed on 22 April on numbers of *Typhlodromus pyri* and *Panonychus ulmi*.

For sample sizes see text 2.2.2.

Treatment	Mite numbers - Means per plot		
	Date of sampling		
	Pre-treatment	Post-treatment	
	17 April	19 May	9 June
	T. pyri		
carbaryl	43.5	6.0	7.7
diflubenzuron	19.5	2.0	3.2
	P. ulmi, all stages		
carbaryl	-	4.2	16.0
diflubenzuron	-	1.2	3.2

Table 2.3

Effects on *Typhlodromus pyri* and *Panonychus ulmi* of four insecticides sprayed in the summer; mite numbers per 100 leaves/treatment

Mite (stage)	Mite numbers: Date of sampling				
	9 June	30 June	15 July	4 Aug.	9 Sept.
a. *carbaryl* (sprayed 23 June and 16 July)					
P. ulmi					
eggs	40	36	14	158	92
active stages	0	3	6	17	14
total	40	39	20	175	106
T. pyri	20	29	18	61	78
RATIO					
P. ulmi active stages/*T. pyri*	0	0.1	0.3	0.3	0.2
b. *diflubenzuron* (sprayed 23 June and 16 July)					
P. ulmi					
eggs	39	15	15	137	171
active stages	2	1	13	10	12
total	41	16	28	147	183
T. pyri	26	15	15	38	87
RATIO	0.1	0.1	0.9	0.3	0.1
c. *azinphos methyl* (sprayed 16 July)					
P. ulmi					
eggs			23	348	692
active stages			9	19	63
total			32	367	775
T. pyri			12	23	142
RATIO			0.8	0.8	0.4
d. *cypermethrin* (sprayed 16 July)					
P. ulmi					
eggs			5	112	675
active stages			2	14	168
total			7	126	843
T. pyri			19	0	5
RATIO			0.1	-	34

19 May and 9 June (Table 2.2) showed that there was no difference in the effects of carbaryl and diflubenzuron on P. ulmi numbers (P = 0.05).

Carbaryl sprayed post-blossom (Table 2.3 and Appendices 2.2 and 2.3).

If the pre-blossom carbaryl spray had reduced T. pyri there could have been effects carried over to the summer on these plots; for such an event the four combinations of the two insecticides were planned (Table 2.1). Since this was not so, the effects of post-blossom sprays can be considered as a separate experiment with four-fold replication. Trends in the numbers of T. pyri and P. ulmi, illustrated in Fig. 2.2, a and b, indicate that the predator maintained the spider mite at very low numbers throughout the season on both carbaryl and diflubenzuron plots. Analyses of variance of the counts on 30 June, 15 July, 4 August and 9 September using the count on 9 June as covariate showed that (a) the covariate was not significant and (b) there was no difference in the effects of carbaryl and diflubenzuron on either mite species.

Azinphos-methyl and cypermethrin, sprayed 16 July (Fig. 2.2 c and d, Table 2.3 and Appendix 2.4).

There was no indication that azinphos-methyl reduced numbers of T. pyri over the eight-week period until the end of the season, but it appeared to cause a marked increase of P. ulmi by comparison with the carbaryl and diflubenzuron plots.

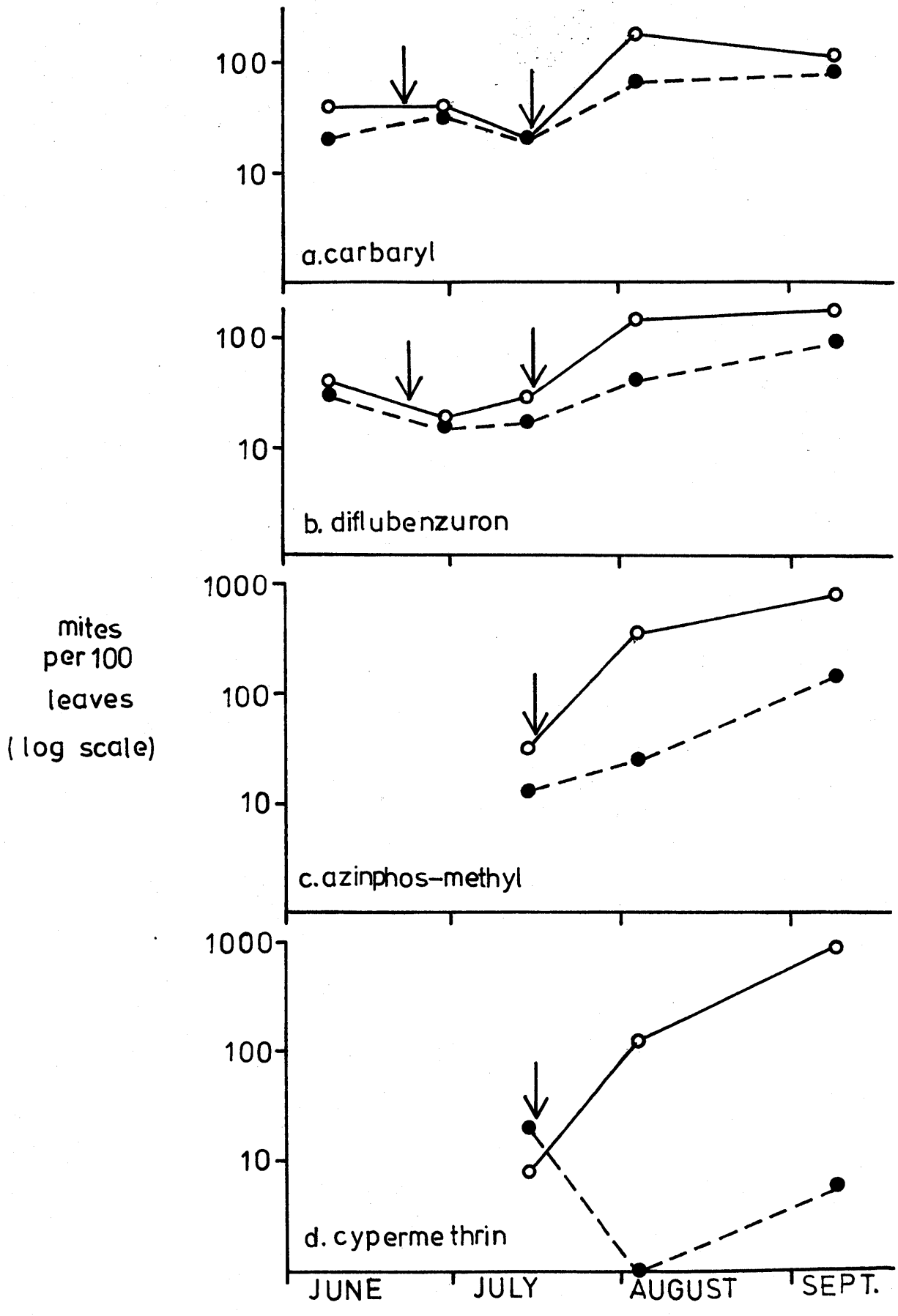


Fig. 2.2 Effects on Typhlodromus pyri and Panonychus ulmi of four insecticides sprayed in the summer; numbers of T. pyri (●--●) and P. ulmi, all stages (o—o) per 100 leaves.
Arrows (↓) indicate dates of spray application.

Cypermethrin reduced T. pyri numbers very greatly; these began to recover by 9 September. On these plots P. ulmi increased 100-fold over the eight-week period.

Ratios

The total numbers of T. pyri and P. ulmi and their ratios on leaf samples, in relation to the effects of the summer sprays of all four insecticides are summarised in Table 2.3. Recent experience at East Malling Research Station has shown that if on a leaf sample the ratio of P. ulmi active stages to T. pyri numbers is lower than 5:1, T. pyri will control P. ulmi at low, non-damaging numbers for the rest of the season. In this trial the 'threshold' ratio was exceeded only on the cypermethrin plots.

2.3.2 Effects on other mite species

On 19 April, 9/144 spurs from carbaryl and 5/144 spurs from diflubenzuron plots were infested with a few rust mites, Aculus schlechtendali; very few were found on these plots for the rest of the season. On the plots used for sprays of azinphos-methyl and cypermethrin there were, similarly, very few A. schlechtendali in July, but by 9 September a substantial increase in numbers had occurred.

Numerous tydeid mites (Table 2.4), mostly hibernating in crevices in the wood, were found on 17 April; a few were found on the clusters sampled on 19 May after the pre-blossom sprays, but none at all on leaves taken on 9 June. After the end of June numbers increased at a similar rate on carbaryl and diflubenzuron plots (Fig. 2.3

Table 2.4

Effects of carbaryl and diflubenzuron sprayed on 22 April on numbers of tydeid mites.

For sample sizes see text 2.2.2.

Treatment and Variety	Tydeid numbers	
	Date of sampling	
	17 April	19 May
carbaryl		
total (4 plots)	56	1
Cox sub-total	53	1
Laxton sub-total	3	0
diflubenzuron		
total (4 plots)	141	7
Cox sub-total	90	5
Laxton sub-total	51	2

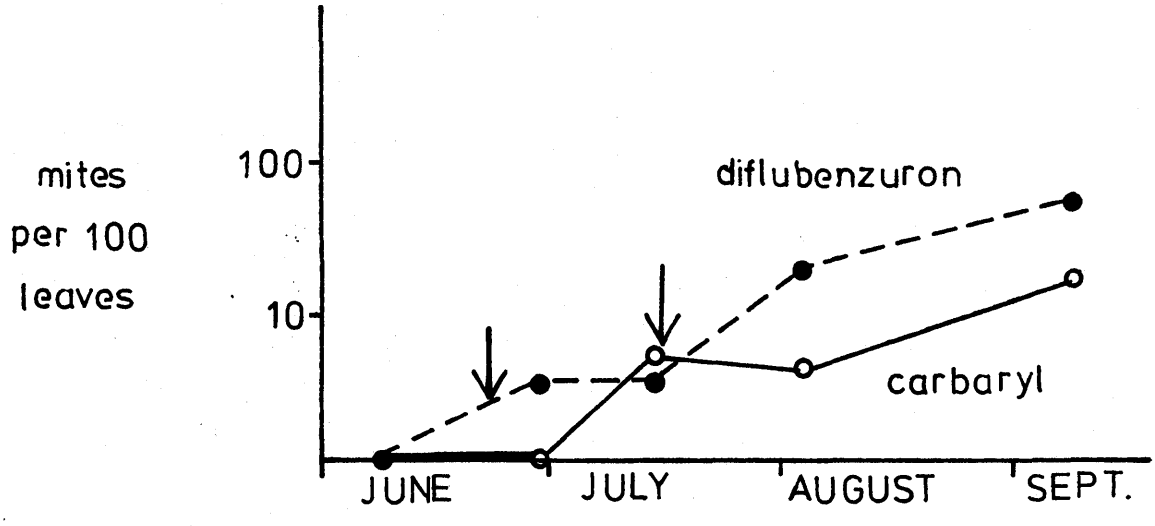


Fig.2.3 Effects on numbers of tydeid mites of carbaryl and diflubenzuron sprayed on 23 June and 16 July.

Arrows (↓) indicate dates of spray application.

and Appendix 2.5). Diflubenzuron has been reported harmless to tydeids (Bower and Kaldor, 1980). A few tydeids were found on the azinphos-methyl plots (four mites/100 leaves on 4 August and three mites/100 leaves on 9 September) but none on the cypermethrin plots after the spray on 16 July. Another pyrethroid, permethrin, has been reported toxic to tydeids (Hoy, Flaherty, Peacock and Culver, 1979).

2.3.3 Varietal differences

Comparison of the two apple cultivars, Cox's Orange Pippin and Laxton's Fortune, present in each plot of the trial apart from the three cypermethrin plots (Fig. 2.1), showed that in most samples the numbers of *P. ulmi* were higher on Laxton's Fortune (Appendix 2.3), numbers of tydeids were higher on Cox (Table 2.4 and Appendix 2.5), whilst the relative numbers of *T. pyri* did not differ consistently between the two cultivars.

2.4 DISCUSSION

2.4.1 Effects on T. pyri and P. ulmi

Westons Field orchard was purchased by East Malling Research Station in 1977 and the spray history before then is unknown. No T. pyri were found during the 1977 growing season. A strain of T. pyri, collected on foliage from a commercial apple orchard at Hadlow, Kent, was released on Westons Field in large numbers in August 1977 and again the following spring. Laboratory and field evidence (Flegg, 1979) showed that this strain was moderately tolerant to organophosphate insecticides (OPs). T. pyri became well established. Tetradifon at 3.5 litres Tedion[1] 8% e.c./ha was applied once at 'petal-fall' in 1978 to reduce P. ulmi; thereafter no acaricides were needed for the rest of 1978 and 1979-80. Pre-blossom insect control was obtained in 1978 with 1 kg Dimilin 25% w.p. plus 0.5 kg Aphox[2] (50% pirimicarb w.p.)/ha, and in 1979 with 4.5 kg Gusathion MS[3] (25% azinphos-methyl + 7.5% demeton-S-methyl w.p.)/ha; no post-blossom insecticides were applied. Programmes of captan and bupirimate were applied for control of apple scab and mildew in the three years 1978-80.

[1] ex Midox Ltd, U.K.

[2] ex Plant Protection Division, Imperial Chemical Industries, U.K.

[3] ex Bayer U.K. Ltd

Effects of azinphos-methyl and carbaryl

The 1980 trial showed that T. pyri were still numerous throughout the orchard and they had evidently controlled P. ulmi since the Tedion spray in spring, 1978. They still tolerated azinphos-methyl although, since the introduction of the 'Hadlow' strain, there had been but slight selection with this or any other OP. The stability of resistance to OPs in T. pyri, under relaxation of selection pressure, will be considered in section 4.

The finding that carbaryl, by comparison with diflubenzuron, had no detectable effect on numbers of T. pyri or P. ulmi was unexpected. Carbaryl, introduced in the 1950s, has a long history of stimulating outbreaks of spider mites either by (a) destruction of insect and/or mite predators, and/or (b) by increasing spider mite reproduction, as a direct effect on mites or through a 'trophic' effect on host-plant nutrition for the mites (Dittrich, Streibert and Bathe, 1974; Hoy, Roush, Smith and Barclay, 1979; Leigh and Wynholds, 1980; Boykin and Campbell, 1982; Maggi and Leigh, 1983; Ahmadi, 1983; other authors reviewed by van de Vrie, McMurtry and Huffaker, 1972, and by Besson, Joly and Touzeau, 1978). Evidence for widespread evolution of resistance to carbaryl in T. pyri in England will be presented in section 4. Since carbaryl was not used in this orchard during 1977-79 it is probable that the Hadlow strain when introduced was already resistant to carbaryl as well as to several OPs. The response to carbaryl was not checked until 1980. It cannot be

concluded from the results of this trial that carbaryl had no effect of type (b) above; such an effect might have occurred but have been completely masked by the efficiency of predation by T. pyri.

One further aspect of the response of the mite fauna to azinphos-methyl requires comment. In spite of the survival of T. pyri after this OP was sprayed, the numbers of P. ulmi increased appreciably more than on carbaryl and diflubenzuron plots (Table 2.3). This may indicate a stimulation of P. ulmi reproduction as recorded by Chaboussou (1962) and other authors reviewed by Besson, Joly and Touzeau (1978).

Effects of cypermethrin

The increase of P. ulmi following the massive reduction of T. pyri after cypermethrin is completely in accord with the responses to pyrethroids seen in other recent trials, e.g. Aliniazee and Cranham (1980), and the trials reported in section 3. The removal of natural control seems sufficient to explain the increase of P. ulmi, but this effect could have masked a direct stimulation of P. ulmi reproduction; this has been recorded for pyrethroid insecticides by Hoy, Ross and Rough (1978); Hoyt, Westigard and Burts (1978); Hoy, Flaherty, Peacock and Culver (1979); and Riedl and Hoying (1980).

2.4.2 Effects on other mite species

Carbaryl usually controls A. schlechtendali quite effectively; diflubenzuron is known to have negligible

toxicity (Easterbrook, 1984). The virtual absence of this species from the diflubenzuron plots of this trial may well have resulted from predation by T. pyri which had very few P. ulmi to feed on. On the azinphos-methyl plots, when P. ulmi increased, probably T. pyri preferred to prey on them rather than the tiny A. schlechtendali; the latter did in fact increase by 9 September. On the cypermethrin plots, where a similar increase occurred, A. schlechtendali, like P. ulmi, were temporarily free from predation.

2.4.3 Varietal differences

Differences between apple cultivars in their liability to severe infestation by P. ulmi, under classic chemical control without predators, are well known. For example, in England, Worcester Pearmain is usually more heavily attacked than Cox's Orange Pippin. On the other hand, some cultivars appear to be less suitable than others for colonisation by phytoseiids (Gruys, 1982) and severe infestation by P. ulmi has sometimes been attributed to this factor (e.g. Downing and Moilliet, 1967). In the Westons Field trial, T. pyri were equally numerous on both the cultivars, and this suggests that the higher numbers recorded on Laxton's Fortune reflect inherent differences between the cultivars in their suitability for P. ulmi.

2.4.4 Insect predators

Observations for insect predators of P. ulmi were not made in this trial, but P. ulmi were too few throughout the 1980 season to attract them; the density-dependence of

insect predation was established by Muir (1965). Various mirid and anthocorid species can be attracted by infestations of apple aphids, but the latter were scarce in 1980. Even if some insect predators were present, Solomon (1982, and personal communication) has shown that mirids, and probably anthocorids, do not prey on T. pyri.

SECTION 3

EFFECTS OF PERMETHRIN SPRAYS ON THE MITE FAUNA OF APPLE ORCHARDS

The experiments of this section were also reported in a publication under the same title submitted to the Annals of Applied Biology in May 1984.

Authors : E.G. Kapetanakis, T.M. Warman and J.E. Cranham

3.1 INTRODUCTION

For the control of most orchard insect pests the synthetic pyrethroids offer excellent cost-effectiveness and low mammalian toxicity, and they are consequently popular with English fruitgrowers. However, the SPs in common use display a high persistent toxicity to natural enemies including phytoseiids, and they tend to cause resurgence of phytophagous mite pests. The studies described in this section involved two trials in apple orchards in 1980 and 1981 which were designed to compare the effects on the mite fauna of permethrin applied at different timings, related to successive growth stages of the host plant. It was known that pre-blossom sprays of SPs were injurious to T. pyri (Aliniazee and Cranham, 1980) but there were two main reasons for more detailed study:

(1) a spray applied very early in the growing season, e.g. at 'bud-burst', might prove rather less damaging (Gruys, 1980) if T. pyri were still in hibernation and partially protected within crevices of the bark from direct spray contact. This might provide a degree of selectivity by spray timing (Niemczyk, 1964);

(2) the effects of sprays applied pre-blossom and post-blossom had not hitherto been directly compared. Such effects might differ, possibly being influenced by the emergence of T. pyri and Aculus schlechtendali from hibernation sites before P. ulmi begins to hatch from

winter eggs, and by the persistent toxicity of the insecticide on bark and leaf surfaces in relation to the rapid growth and expansion of the foliage. The effects might also be influenced by changes in the acarine life stages present and their relative susceptibility to the insecticide.

For the purpose of these studies, there was insufficient information on the spring emergence and pre-blossom location and activity of T. pyri and this aspect was therefore studied. The relative numbers of the main mite species were assessed through the season. In addition, to quantify the effects of sprays, the persistent toxicity was assessed in bioassays on samples of bark and leaves at intervals after spraying. The persistence of permethrin residues was studied, using gas chromatographic analysis, by Mr. T.M. Warman (Plant Protective Chemistry Department, E.M.R.S.), and attempts were made to correlate the decline of biological activity with residue levels.

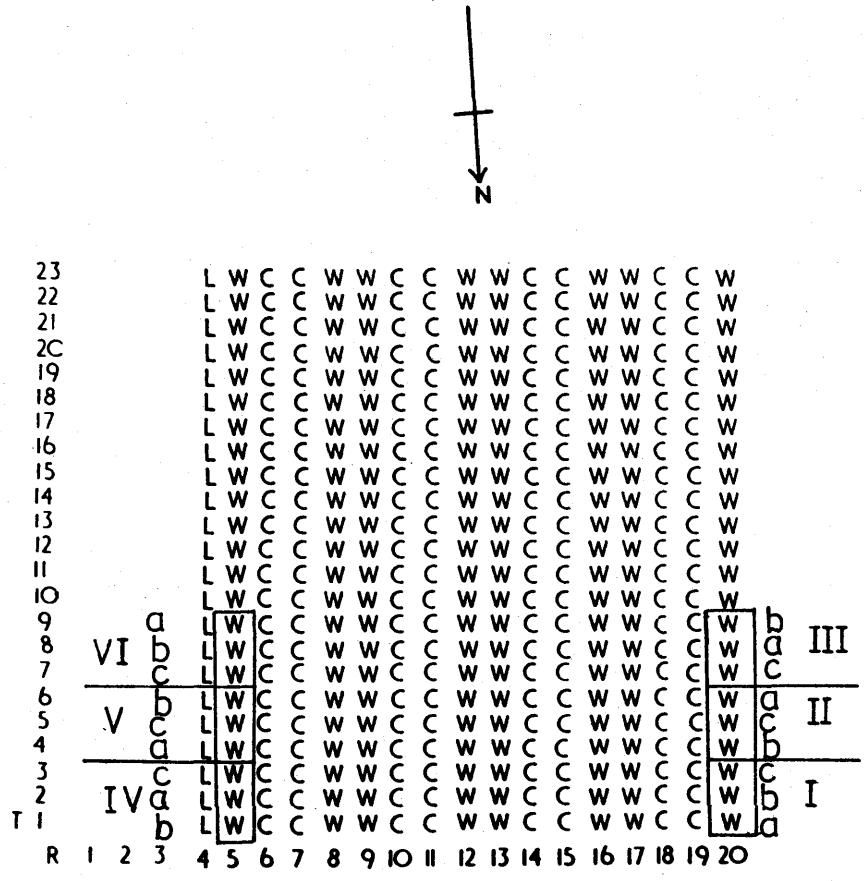
3.2 MATERIALS AND METHODS

3.2.1 1980 Field Trial : Comparison of two pre-blossom spray timings

A single spray of 50 mg a.i. permethrin/litre was applied by hand-lance high volume to 'point of drip' on (a) 25 March at 'late dormant' stage and (b) 5 May at 'late green cluster' stage (Plate 2.2) and compared with (c) unsprayed control trees. Ambush 25% e.c. (ex Plant Protection Division, Imperial Chemical Industries Ltd, U.K.) was the permethrin formulation used. A low spraying pressure was used to minimise spray drift. Plots were single trees of cv Worcester Pearmain replicated in six randomised blocks on the perimeter rows of orchard SP101 at South Park, East Malling Research Station Home Farm (Fig. 3.1, Plate 3.1).

No other insecticides were applied on these trees during 1980. The rest of the trees in this part of the orchard up to tree 12 (Fig. 3.1) received a selective fungicide and insecticide programme (Flegg, 1981). The same selective programme had been applied on all trees of this orchard for the previous three years.

Plant samples were taken haphazardly at a height of 1.6-1.9 m from each tree as shown in Table 3.1. They were carried in sealed polythene bags to a constant temperature room at 7-8°C and examined under the microscope within 24 hours. Numbers of T. pyri, P. ulmi and tydeid mites were counted; infestation of A. schlechtendali was only visually estimated.



Apple varieties		Planting distances
C	Cox	Rows 6.00 m apart
W	Worcester Pearmain	Trees 4.60 m apart
L	Lanes	Planting date: 1951

Fig.3.1 Plan of South Park Trial, 1980 (not to scale).

Treatments : Single sprays of permethrin at 50 mg a.i./litre:

(a) sprayed at 'late dormant' stage, 25 March

(b) sprayed at 'late green cluster' stage, 5 May

(c) unsprayed

Latin numerals, I to VI, indicate blocks



Plate 3.1 Apple orchard SP101 at South Park, East Malling Research Station Home Farm

Table 3.1

Samples for mite counts, South Park Field Trial, 1980

Sampling date	Growth stage of apple	Sample
13 March	Late dormant	'Pre-spray' sample, twigs c 15 cm long bearing leaf and flower buds (total 18 twigs/treatment)
20 May	Full blossom	Leaf and blossom clusters, each with c 5 cm wood (total 18 clusters/ treatment)
2 June) 16 June) 28 July)	Fully expanded leaves	(total 120/treatment)

3.2.2 1981 Field Trial : Comparison of pre-blossom and post-blossom timings

3.2.2.1 Trial design and spray application

The effects were compared of a single spray of 25 mg a.i./l permethrin/litre (Ambush 25% e.c.) applied:

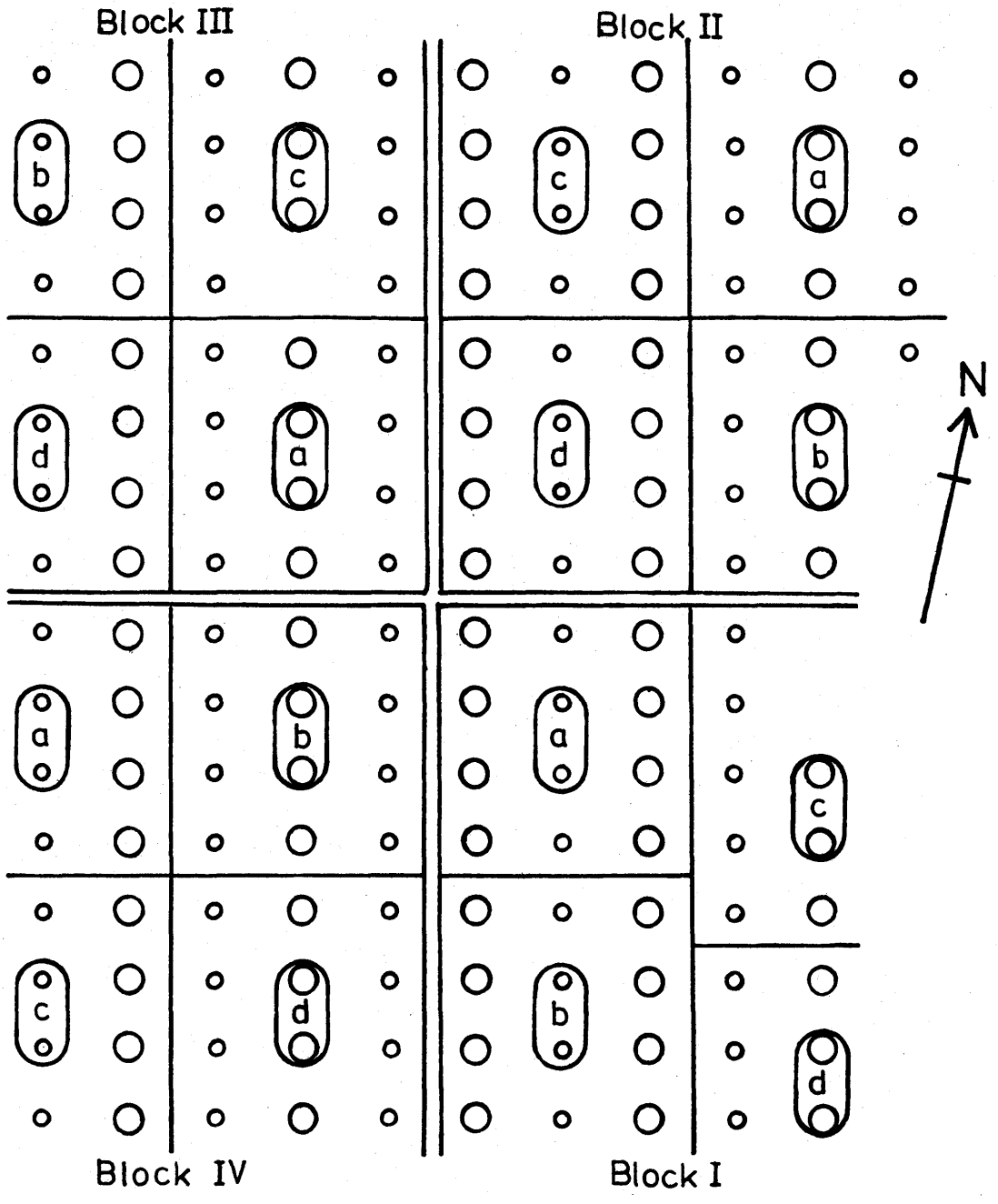
(a) 17 March at 'bud-burst' stage;

(b) 6 April at 'mouse-ear' stage (Plate 2.2), and

(c) 23 June, c one month after the blossom stage when the first spray for control of codling moth is usually applied.

Comparison was made with unsprayed check plots. Details of the trial are shown in Fig. 3.2: plots were of 8-12 trees (Plate 3.2) replicated four times in a randomised block design. Sprays were applied by hand-lance to 'point of drip', using a low pressure in order to minimise spray drift. The 'bud-burst' spray was originally applied on 12 March but heavy rainfall, c 8 mm (Fig. 3.3), started before spraying was completed and before spray deposits had dried. It was considered that much of the deposit would have been removed by the rain and therefore these plots were resprayed on 17 March. Subsequently, residue measurements suggested that between 12 and 17 March 30% or more of the initial deposit (12 March) was removed by weathering and other processes.

For the control of apple sucker, Psylla mali Schm., it was necessary to apply HCH (Gamma-Col, Plant Protection Division, Imperial Chemical Industries Ltd) over all plots on 13 April. In a field trial in 1980,



Planting distances:

Rows 4.25 m apart

Trees 4.25 m apart

Planting date: 1961

KEY

o James Grieve

Egremont Russet

Trees sampled

Fig.3.2 Plan of Middle Park Trial, 1981 (not to scale).

Treatments: single sprays of permethrin at 25 mg a.i./litre:

a sprayed at 'bud-burst' stage, 17 March

b sprayed at 'mouse-ear' stage, 6 April

c sprayed post-blossom, 23 June

d unsprayed



Plate 3.2 Apple orchard MP102 at Middle Park, East Malling Research Station Home Farm

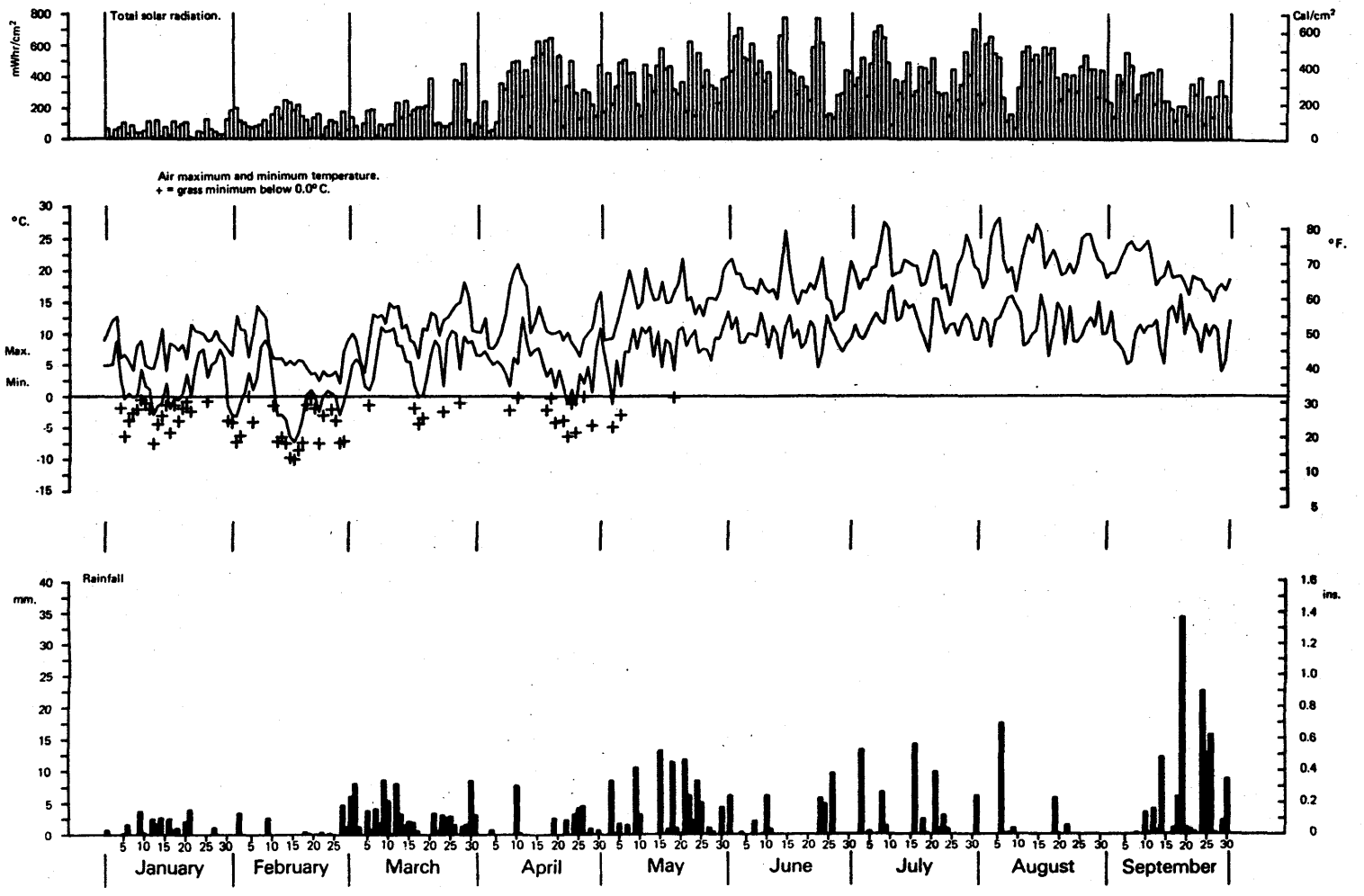


Fig. 3.3 Meteorological data, East Malling Research Station, 1981 (After Wickenden, 1982)

HCH had not reduced the numbers of T. pyri (Flegg, 1981). No other insecticides were used in 1981 on this plot; control of apple scab and mildew was achieved by routine blanket sprays of captan and bupirimate; these fungicides are known to be harmless to T. pyri (Collyer and Kirby, 1959; Oatman, 1966; Easterbrook, Souter, Solomon and Cranham, 1979; Gruys, 1980).

3.2.2.2 Sampling

Plant material was sampled at intervals:

(a) for mite counts in relation to the emergence and distribution of T. pyri and the effects of sprays on the mite fauna, and

(b) for residue bioassay and for chemical analysis tests to estimate the persistence of the permethrin deposits.

Sampling for mite numbers and distribution

Samples were taken at a height of 1.6-1.9 m in the foliage canopy of two trees per plot as shown in Table 3.2.

Hibernating female T. pyri are quickly aroused by warmth and leave their shelters (Putman, 1959). Therefore, to obtain valid data on their emergence and distribution, all plant material was sealed in polythene bags and carried in a cooler box (internal dimensions 33 x 22 x 11 cm, Plate 3.3), at a temperature of 6-10°C, to a cold store maintained at 7-8°C. On collection dates up to and including 11 May, samples were sub-divided

Table 3.2

Samples for mite counts, Middle Park Field Trial, 1981

Sampling date	Growth stage of apple	Sample
12 March 30 March 6 April	Late dormant) Late bud-burst) Mouse-ear)	Twigs c 20 cm long bearing leaf and flower buds (total 16 twigs/treatment)
19 April 11 May	Pink bud) Petal-fall)	Leaf and blossom clusters, each with c 5 cm wood (total 16 clusters/ treatment)
1 June) 23 June) 2 July) 22 July) 6 August) 25 August)	Fully expanded leaves	(total 200 leaves/ treatment)



Plate 3.3 Cooler boxes used for maintaining plant samples at low temperatures during transport and examination

in the field into (a) wood and (b) buds, leaf and flower clusters,and these were sealed separately in polythene bags. All twig and cluster samples were examined by dissection under the microscope within 24 hours of collection. Each bag from the cold store was emptied into a small cooler box (internal dimensions 22 x 11 x 6 cm, Plate 3.3) maintaining c 5°C. A small part of the sample was removed from the box at a time and was examined within a few minutes under cool microscope lighting.

Leaf samples, from 1 June onwards, were also bagged and cold-stored, and examined within 24 hours by brushing (Henderson and McBurnie, 1943; Morgan, Chant, Anderson and Ayre, 1955). A mite brushing machine made by Leedom Engineering, California, U.S.A. was used (Plate 3.4). Mites were brushed onto paper cards (Plate 3.5) covered with a thin layer of 'Tangle-trap', a non-drying adhesive (ex The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.). The cards were scanned under the microscope within one hour of brushing.

Numbers of T. pyri, P. ulmi, Zetzellia mali, tydeid and tarsonemid mites were counted. Numbers of A. schlechtendali were visually categorised. The scoring systems followed for A. schlechtendali when (a) twig and cluster samples and (b) leaf samples were examined, are explained in Tables 3.10 and 3.11 respectively. The numbers of P. ulmi eggs on the wood of the first three samples (12 March, 30 March and 6 April) were also visually categorised, as explained in Table 3.7.

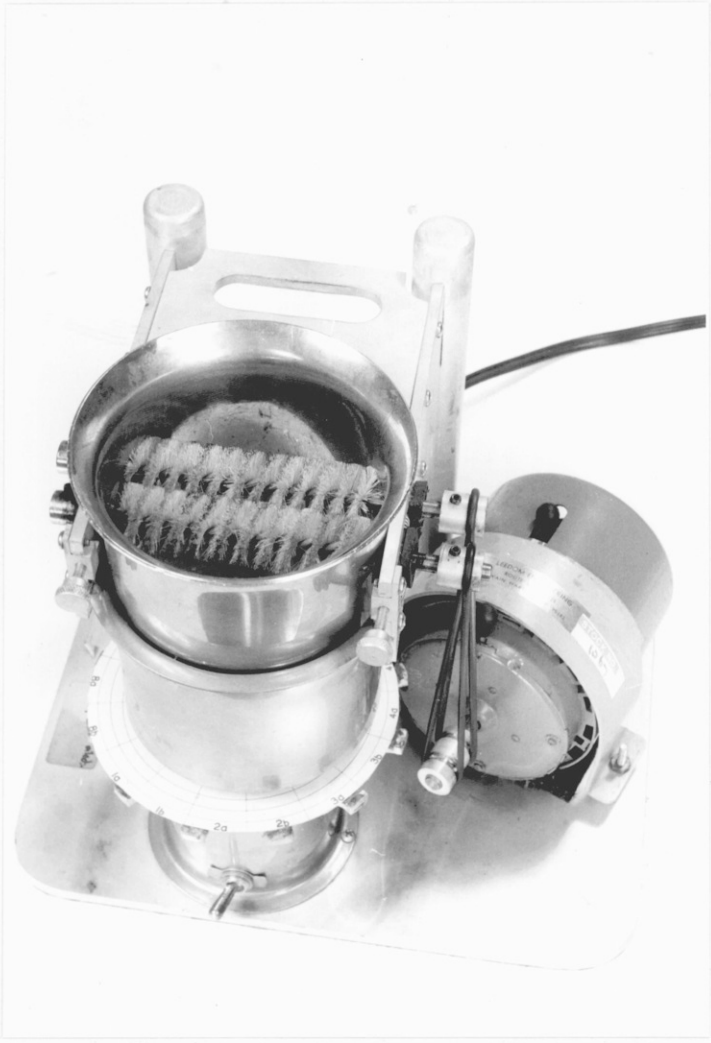


Plate 3.4 Mite brushing machine used for examining apple leaf samples for mite numbers (Leedom Engineering, California, U.S.A.)

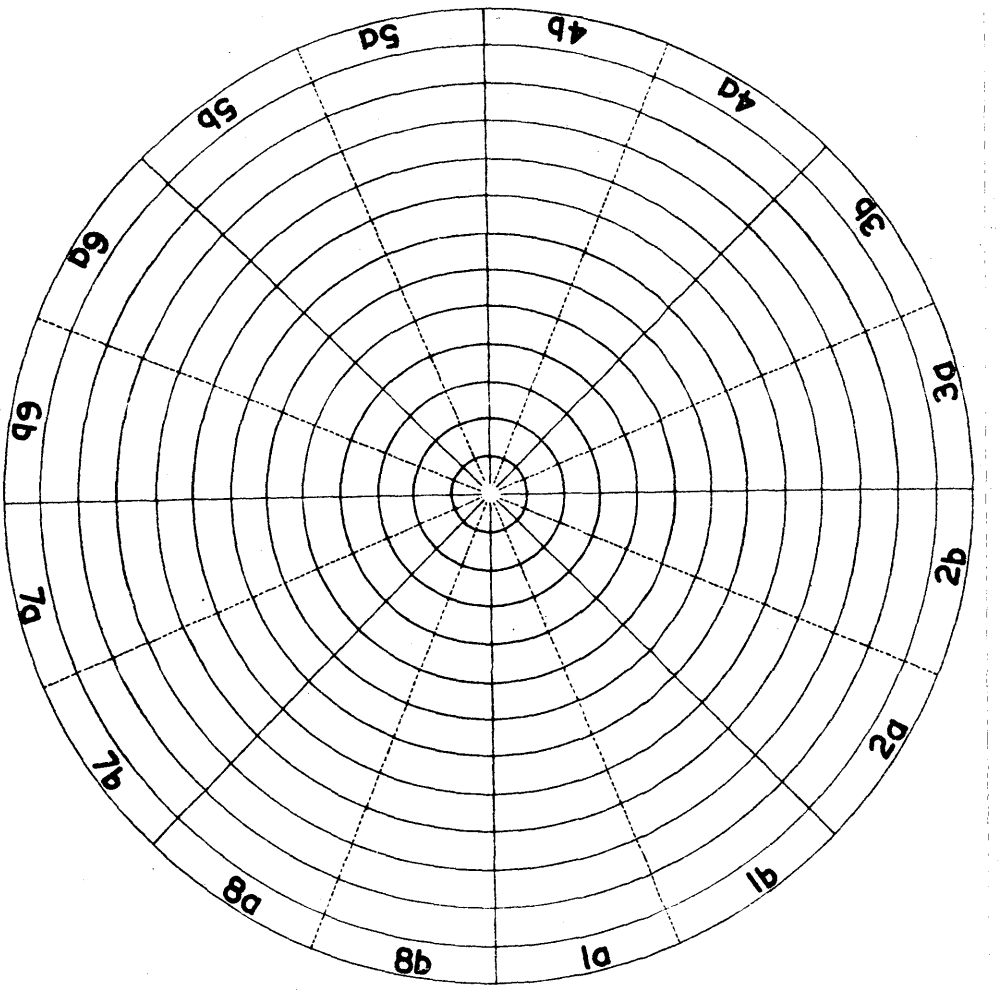


Plate 3.5 Paper card used on the mite brushing machine

Mite counts were subjected to analysis of variance for each sampling date using the transformation log (1 + x), where x = number of mites per plot sample. Mite counts on pre-spray samples were used as covariates.

Sampling to estimate persistence

After the pre-blossom sprays T. pyri were active on the bark surface, whilst after the spray on 23 June their activity was largely confined to leaves. Therefore, after the two pre-blossom sprays, samples of 4-year-old wood (15-20 mm diameter) and, after the June spray, fully expanded leaves were taken at intervals. The sample from each plot was divided for use in bioassays and chemical analysis.

3.2.2.3 Chemical analysis

Four 10 cm shoots (with side shoots and buds removed) or 20 leaves (with petioles removed) were soaked overnight in 100 ml of 1:1 acetone/petroleum spirit (60-80°C boiling range). The extract was decanted off into 175 ml distilled water and the liquid from rinsing the sample with three washes of 5 ml petroleum spirit and 5 ml acetone was added to it. After the addition of 2 g sodium sulphate the whole volume was shaken for three minutes and when separation was complete the aqueous layer was discarded. The petroleum spirit layer was washed with distilled water (2 x 175 ml) and then dried over anhydrous sodium sulphate. This extraction procedure is based on

those described by Williams (1976) and Fujie and Fullmer (1978).

A 1 ml aliquot of the extract was cleaned up by column chromatography using a procedure based on that described by Chapman and Harris (1978). For very low residues, a larger aliquot was taken and reduced to 1 ml by evaporation under nitrogen. The column consisted of a pasteur pipette plugged with cotton wool on to which one 5:1 mixture of 'Florosil' (deactivated with 3% water) and activated charcoal (Nuchor C 190 N) was added to give a 6 cm layer of adsorbent. The loaded column was eluted with 3 ml petroleum spirit and 1 ml 4:1 benzene/hexane mixture and the permethrin was then eluted with a further 2.5 ml of the benzene/hexane mixture. The eluate was evaporated to dryness and taken up in 0.5 ml hexane.

Determination of permethrin was by gas chromatography (Pye 104) using a 1.5 m x 4 mm glass column packed with 2% QF-1 on 80-100 mesh Gas Chrom Q. Column temperature was 205°C and gas flow (nitrogen) was 1.94 litre/h. The electron capture detector was maintained at 270°C. *cis*- and *trans*-isomers of permethrin were resolved under the conditions used (compare work by Chapman and Simmons, 1977) with retention times 4.6 min (*cis*) and 5.4 min (*trans*), and the concentrations of each were determined by comparison of peak heights with those of standards.

Concentrations in solution down to 0.005 $\mu g\ ml^{-1}$ of *cis*- or *trans*-permethrin could be detected and 0.01 $\mu g\ ml^{-1}$ determined, leading to a limit of determination

(total permethrin) of 0.006 $\mu g\ cm^{-2}$ of sample surface. Where concentration was carried out prior to clean-up, down to 0.0006 $\mu g\ cm^{-2}$ could be determined on bark (10-fold concentration) and 0.001 $\mu g\ cm^{-2}$ on leaves (6-fold concentration). Results were corrected for the recovery efficiency of the method. Recovery of permethrin added to unsprayed twigs at levels of 0.01-0.1 $\mu g\ cm^{-2}$ averaged 86.7%. The corresponding recovery for foliage was 81.9%.

3.2.2.4 Bioassays of residues on bark

A disk of bark, 27 mm diameter, was cut from each sample shoot and held flat on the lid of a rigid plastic box (125 x 80 x 22 mm) by double-sided sticky tape. The construction of the type of cell devised to confine mites on a bark surface is shown in Plate 3.6 and in cross-section in Fig. 3.4. Wet filter paper above the double-sided tape acted as a barrier and an essential supply of drinking water. A hole, 16 mm diameter, was cut in both tape and filter paper (before wetting) to form a circular arena. A ring of 'Tangle-trap' was syringed on top of the wet filter paper as an additional 'fail-safe' barrier. It was necessary to prevent water creeping over the bark surface and this was achieved with an annulus of 'Sellotape', with the non-sticky side uppermost, placed below and projecting 1.5 mm within the filter paper walls of the arena. Two bark cells were placed on each plastic box.

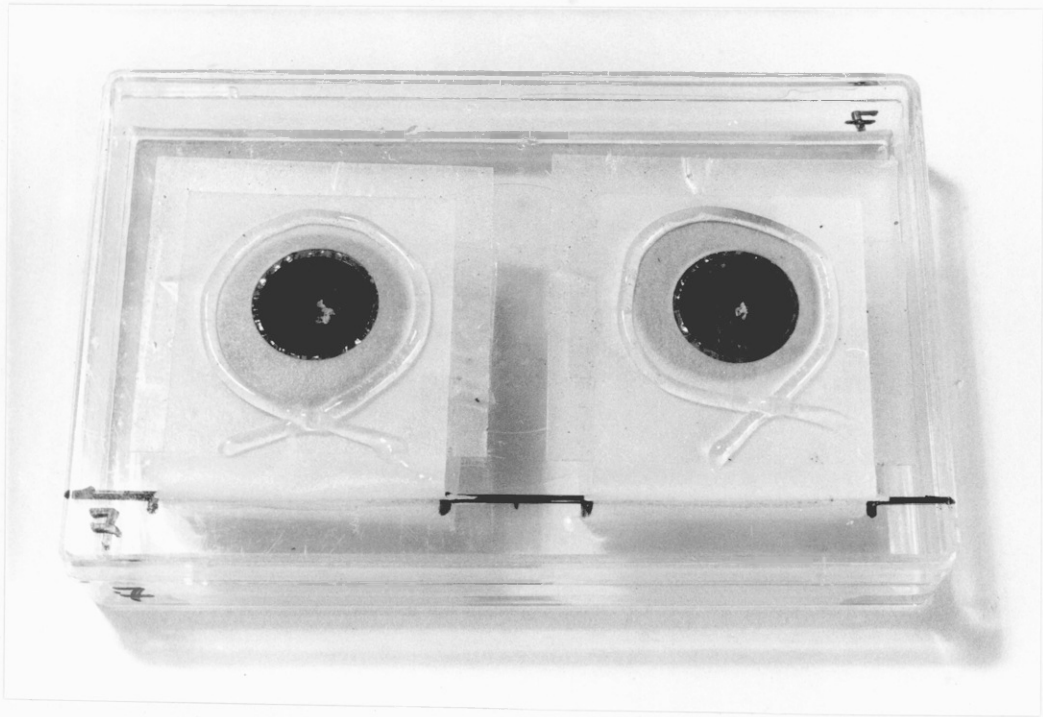


Plate 3.6 Arenas devised for testing the persistence of permethrin residues on apple bark with *Amblyseius potentillae*

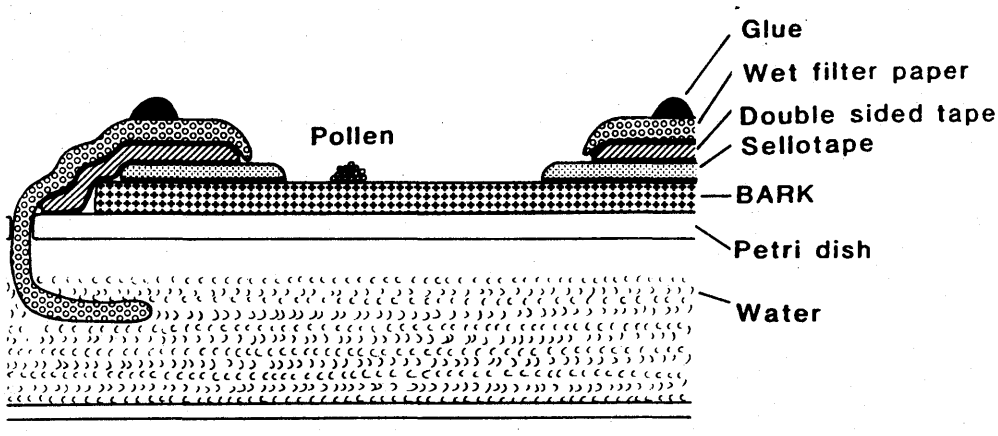


Fig. 3.4 Diagrammatic cross-section of cell devised for testing the persistence of permethrin residues on apple bark with Amblyseius potentillae

The phytoseiid Amblyseius potentillae (Garman) was reared on 'plate' cultures (Kapetanakis and Cranham, 1983; see also section 4 of this Thesis for more details on laboratory cultures of phytoseiids) with pollen of Vicia faba as food. To produce immature mites of known age, adult female A. potentillae were placed on a clean culture plate (Day 1) to lay eggs for 24 hours and were removed (Day 2). On Day 4 any unhatched eggs were removed from the plate. On Day 5 five young mites (larvae and protonymphs) were transferred with a fine paintbrush to each test arena on bark and a trace of pollen was added as food. There were eight replicates per treatment/occasion. Tests were stored in a constant environment room at 20°C, 50-60% r.h. and 17 h daily photophase. Mortality was assessed after 24 and 48 h; mortality was virtually complete in 24 h (see also Appendix 4.4.A of this Thesis). Mites within each test arena, or in the edge of the water barrier, which did not show signs of movement when prodded with a fine brush were recorded as dead.

3.2.2.5 Bioassays of residues on leaves

Disks 16 mm diameter were cut and placed with their abaxial surface uppermost on wet filter paper on the lids of 113 ml plastic cartons (D.G. Containers Ltd, Liverpool), which acted as water reservoirs (Plate 3.7). The subsequent procedure and replication was the same as for the bioassays on bark.



Plate 3.7 Leaf disks and plastic carton used for testing the persistence of permethrin residues on apple leaves with Amblyseius potentillae

3.3 RESULTS

3.3.1 1980 Field Trial

3.3.1.1 Emergence and distribution of T. pyri in the spring

Observations showed that on 13 March all adult female T. pyri were hibernating in crevices on the bark of spurs and older wood. None were found in swelling buds. On samples taken from unsprayed trees at 'bud-burst', on 19 April, c 25% of the T. pyri were found in buds and the rest in bark crevices. On 12 May nearly all adult females were found in the leaf and blossom clusters and had started to produce eggs.

3.3.1.2 Effects of sprays on T. pyri and P. ulmi

Plots sprayed on (a) 25 March, (b) 5 May and (c) unsprayed had pre-spray counts on 13 March of 56, 3 and 48 T. pyri adult females/sample respectively, and post-spray counts on 20 May of 1, 0 and 11 T. pyri per sample. Counts on leaves are shown in Table 3.3; T. pyri was virtually eliminated by permethrin at either spray timing.

The three treatments were very similar in their P. ulmi infestation early in the growing season: on 13 March, when presence or absence of P. ulmi winter eggs on samples was recorded, 4/18 twigs from (a) (Fig. 3.1), 3/18 from (b) and 3/18 from (c) carried winter eggs. The detrimental effect of permethrin on T. pyri at either spray timing persisted until late July leading to a marked increase of P. ulmi on certain replicates; however,

Table 3.3

1980 trial: effects of a single spray of 50 mg permethrin/ litre on numbers of *Panonychus ulmi* and *Typhlodromus pyri* per 100 leaves

Date of spray (growth stage of apple)	Date of sampling		
	2 June	16 June	28 July
	P. ulmi, active stages (eggs)		
25 March (late dormant)	37(10)	38 (736)	239(2705)
5 May (late green cluster)	315(23)	54(1551)	421(4378)
unsprayed	12 (5)	6 (193)	18 (286)
	T. pyri		
25 March	0	0	2
5 May	0	0	0
unsprayed	13	7	7

Note : There were no significant differences (at $P = 0.05$) among the three treatments in any sample

the differences were not significant due to high variation between replicates (Table 3.3).

3.3.1.3 Effects on other mite species

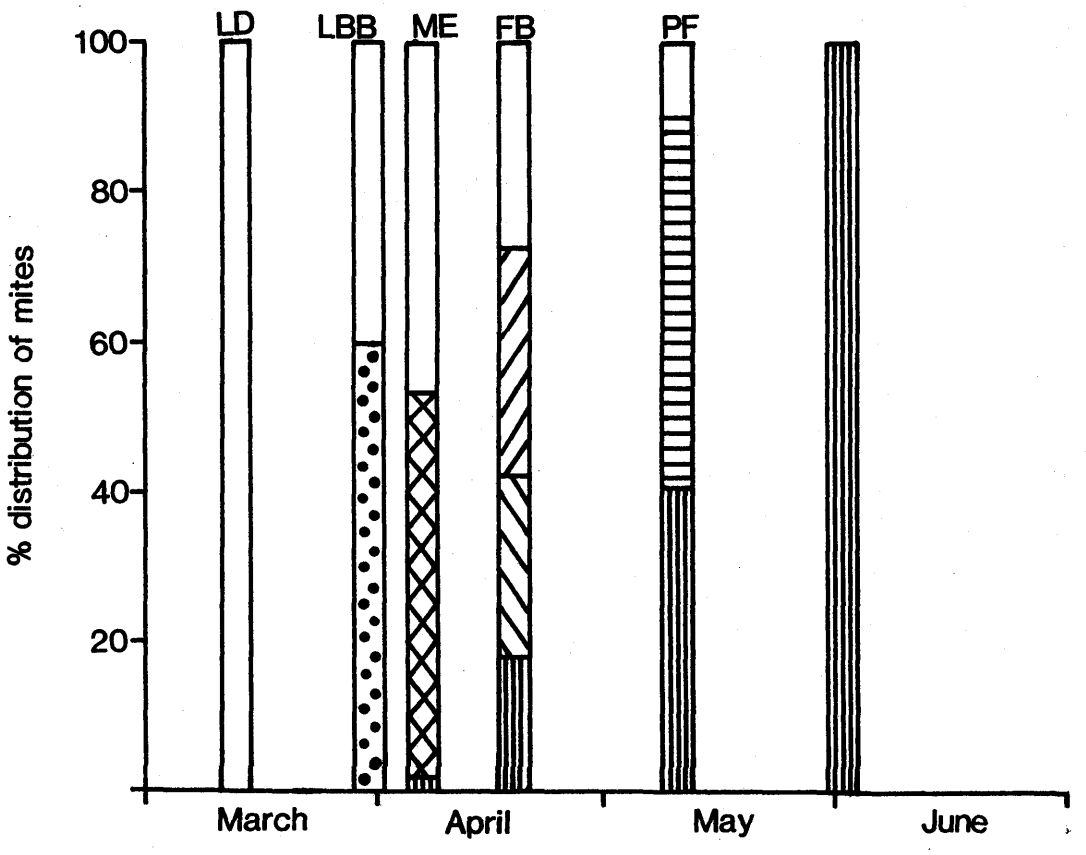
A. schlechtendali was in very low numbers on all three treatments in June. A slight increase was noticed on 28 July but the three treatments were estimated as very similar in infestation.

Tydeids were very common in bark crevices in the pre-spray samples, 13 March, of all treatments (on average 24 mites/twig), and in a sample taken only from unsprayed trees on 9 April (19 mites/twig). None were found on leaf and blossom clusters on 20 May, or on fully grown leaves for the rest of the sampling period, until the end of July, on any of the three treatments.

3.3.2 1981 Field Trial

3.3.2.1 Emergence and distribution of mites in the spring (unsprayed plots)

Fig. 3.5 summarises data on the distribution of T. pyri between overwintering shelters on the bark and the developing leaf and flower clusters, from March to June on unsprayed trees. On 12 March overwintering females of T. pyri were found only in shelters on the bark of spurs, branches and trunk. Cankers, localised areas of rotting wood, scars of removed shoots, loose bark and algae were the most common sites. Most of the mites were found in groups of 3 to 10 individuals. Larger groups, of 10 up to



KEY

T. pyri found:

- on bark
- in buds
- in flower clusters
- at bases of floral pedicels
- on flowers
- on fruitlet clusters
- on leaves

Apple bud development stages

LD	late dormant
LBB	late bud-burst
ME	mouse-ear
FB	full blossom
PF	petal-fall

Fig.3.5 Distribution of Typhlodromus pyri between overwintering shelters on bark and the developing leaf and flower clusters of apple trees, from March to June 1981.
Data from unsprayed plots only.

20 mites or more, were also found, causing a large variation between the counts for replicate plots early in the season (Appendix 3.1).

Tydeid mites (species undetermined) were found in the same shelters with T. pyri, most of them a dark grey colour, a few yellowish green. Some T. pyri females (c 4%) had dark coloured guts, an indication that they had been feeding on tydeids. The stigmaeid mite Zetzellia mali and tarsonemids were also found occupying similar, and quite often the same, shelters as T. pyri.

Active T. pyri were first found on 30 March ('late bud-burst') under bud scales, or in protected sites among hairs at the bases of emerging flower buds. Most looked well fed and their gut colour, light to dark brown, suggested they were feeding on apple rust mite, Aculus schlechtendali, numbers of which had moved already to the new buds. Some tydeids (c 20%) of a pale green colour had moved to the new growth but most Z. mali and all tarsonemids were still found in bark crevices.

On 6 April, c 50% of T. pyri were still in shelters on the bark, although many of these appeared well fed, an indication of movement from the shelters in search of prey, and back in again for protection against spring frosts (Fig. 3.5). Z. mali and most tydeids (c 75%) were still in hibernating sites on the bark whereas a large proportion of tarsonemids were active on the new growth.

On 19 April only about 30% of T. pyri, but over 60% of tydeids were found in shelters. Most A. schlechtendali were on leaves and on flower sepals in the clusters. Very low numbers of Z. mali and tarsonemids were found on this occasion. A few larvae of P. ulmi were recorded.

On 11 May, most T. pyri were on the new growth in protected sites including young unfolded leaves and the bases of young fruit. Many had fed on immature P. ulmi. A large number of T. pyri eggs were counted. Tydeids, over 95%, were on the young leaves.

On 1 June no T. pyri were found on bark and only trace numbers on young fruitlets; their main niche for the rest of the season was the abaxial surfaces of leaves.

3.3.2.2 Effects of sprays on T. pyri and P. ulmi

Pre-blossom sprays (Table 3.4 and Appendix 3.1)

The full effect of the March spray was delayed; reductions in T. pyri numbers, compared to unsprayed plots, were not significant on 30 March and 6 April, but were large and significant on 19 April and 11 May ($P = 0.001$ and $P = 0.01$ respectively). This delay probably resulted because mites were partially protected within shelters. In samples taken 30 March from plots sprayed on the 17th 94% of the T. pyri found were in shelters in contrast to 41% in samples from unsprayed plots (Table 3.5 and Fig. 3.5); on 6 April the proportions were 69% and 48% respectively. It may be that mites on the sprayed plots were temporarily deterred by the spray residues from

Table 3.4

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of *Typhlodromus pyri* up to blossom

Date of spray (growth stage of apple)	Numbers of *T. pyri* - Means per plot*				
	Date of sampling				
	12 March	30 March	6 April**	19 April	11 May
17 March (bud-burst)	24.2 ⟹	21.2	7.2	1.0b	5.7a
6 April (mouse-ear)	20.0	22.5	18.7 ⟹	0a	1.0a
Unsprayed	25.2	30.0	30.0	12.7c	27.0b

* For sample sizes see Table 3.2

** Sample taken before application of April spray

⟹ Spray applied between sample dates

Note. Values followed by the same letter in each column do not differ significantly ($P = 0.05$)

Table 3.5

1981 trial: distribution of *Typhlodromus pyri* numbers between overwintering shelters on bark and the developing leaf and flower clusters of apple trees in March and April 1981

Treatment	Niche	Numbers of *T. pyri* Means per plot*		
		Date of sampling		
		12 March	30 March	6 April
Plots sprayed 17 March	bark	24.2	20.0	5.0
	clusters	0	1.2	2.2
	total	24.2	21.2	7.2
Unsprayed plots	bark	22.6	10.7	11.7
	clusters	0	15.5	12.6
	total	22.6	26.2	24.3

* For sample sizes see Table 3.2

leaving shelters and/or that most of those mites that had left shelters were killed.

The spray on 6 April eliminated T. pyri quickly; none were found on 19 April, when the numbers on March-sprayed plots were also very low (P = 0.001) compared to the unsprayed (Table 3.4). By 11 May, and in the leaf samples of 1 June, T. pyri had begun to increase again in both sprayed treatments but they were still fewer (P = 0.01-0.001) than on unsprayed plots (Fig. 3.6, Table 3.6 and Appendix 3.2). From 23 June until 25 August the differences in T. pyri numbers between the pre-blossom sprayed and unsprayed plots were non-significant.

Numbers of T. pyri appeared higher in the March-sprayed than in the April-sprayed plots only on 19 April.

There was no difference between the two pre-blossom sprays for the rest of the season.

The four treatments were very similar in their initial infestation of P. ulmi winter eggs (Table 3.7) and in the numbers of the first generation of spider mites up to 11 May (Table 3.8). Fig. 3.6 shows that, compared to unsprayed plots, on 1 June the pre-blossom sprayed plots had c 10x more P. ulmi active stages, and the ratio of P. ulmi to T. pyri was 35:1 and 63:1 on the March-sprayed and the April-sprayed plots respectively compared to 1:1 on the unsprayed (Table 3.9 and Appendix 3.3). Nevertheless, from this stage onward T. pyri numbers gradually increased, P. ulmi declined and the ratio P. ulmi to T. pyri was restored to c 6:1 or less from 22 July to 25 August.

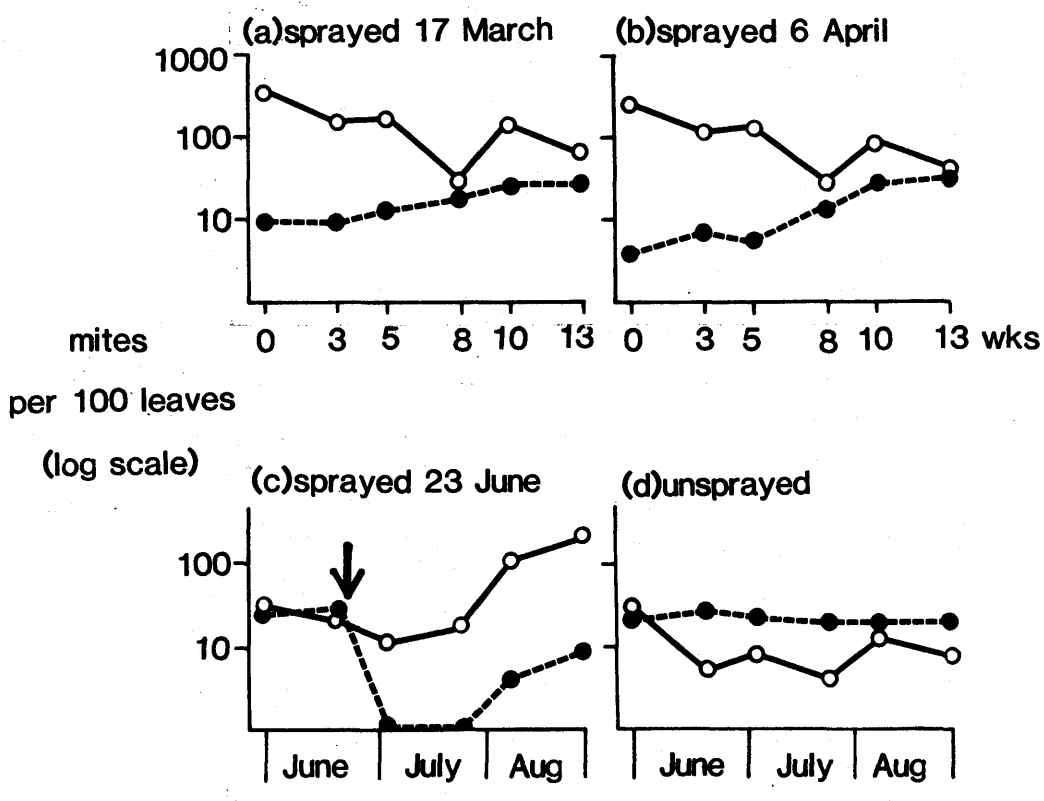


Fig.3.6 Effects of 25 mg permethrin/litre sprayed (a) 17 March, (b) 6 April, and (c) 23 June 1981, compared with (d) unsprayed apple trees; numbers of Typhlodromus pyri (●------●) and Panonychus ulmi active stages (o——o).
Arrow (↓) indicates spray application.

Table 3.6

1981 trial: effects of a single spray of 25 mg permethrin/litre on numbers of *Typhlodromus pyri* in the summer

Date of spray (growth stage of apple)	Numbers of *T. pyri* Means per plot (50 leaves) Date of sampling 1 June	23 June*	2 July	22 July	6 August	25 August
17 March (bud-burst)	4.7	4.7	6.5b	9.5b	13.2b	15.0b
6 April (mouse-ear)	2.0	3.5	3.0a	7.2b	14.7b	15.7b
23 June (1 month after petal-fall)	11.9	12.5	0.5a	0.5a	2.0a	4.5a
Unsprayed		12.7	10.7b	8.2b	9.5b	10.0b

* Sample taken before application of June spray

Note. Values followed by the same letter in each column do not differ significantly ($P = 0.05$)

Table 3.7

1981 trial: infestation of Panonychus ulmi winter eggs on apple twigs in orchard MP102, East Malling Research Station

Numbers in the table represent results of scoring of apple twigs as follows:

1 = a few eggs, very difficult to find on a twig scanned under the microscope

2 = a few, but easy to find

3 = many in patches

4 = many all over the twig

5 = twig covered with eggs

Treatment	Scoring of infestation of P. ulmi winter eggs Means per plot* Date of sampling 12 March	30 March	6 April
Sprayed 17 March	2.4	2.3	2.3
Sprayed 6 April	- **	- **	2.1
Sprayed 23 June))	2.9	2.1	2.4
Unsprayed)			

* For sample sizes see Table 3.2

** No observation

Table 3.8

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of Panonychus ulmi up to blossom

Date of spray (growth stage of apple)	Numbers of P. ulmi active stages - Means per plot*	
	Date of sampling	
	19 April	11 May
(1) 17 March (bud-burst)	5	295
(2) 6 April (mouse-ear)	2	224
(3) 23 June (1 month after petal-fall)	1.5	180
(4) Unsprayed		

* For sample sizes see Table 3.2

Note. There were no significant differences (at $P = 0.05$) among the two sprayed treatments (1) and (2) and the unsprayed plots of (3) and (4) in either sample

Table 3.9

1981 trial: effects of a single spray of 25 mg permethrin/litre on numbers of *Panonychus ulmi* in the summer

Date of spray (growth stage of apple)	Numbers of *P. ulmi*, all stages Means per plot (50 leaves)					
	Date of sampling					
	1 June	23 June*	2 July	22 July	6 August	25 August
17 March (bud-burst)	952	1266b	608b	504b	167b	161b
6 April (mouse-ear)	1097	1427b	597b	440b	122b	107b
23 June (1 month after petal-fall)	81	95ab	16a	220b	138b	466b
Unsprayed		31a	8a	46a	11a	21a

* Sample taken before application of June spray

Note. Values followed by the same letter in each column do not differ significantly ($P = 0.05$)

June spray

This virtually eliminated T. pyri and numbers did not begin to recover for six weeks; even by 25 August they were still lower (P = 0.05) than on the pre-blossom sprayed and unsprayed plots (Fig. 3.6, Table 3.6 and Appendix 3.2). This resulted in a marked upward trend in P. ulmi numbers to thirty times those on the unsprayed plots by 25 August (Fig. 3.6 and Appendix 3.3); on that occasion the ratio of P. ulmi to T. pyri was 23:1 and subsequently P. ulmi numbers continued to rise.

3.3.2.3 Effects on other mite species

On all plots from March to May the numbers of A. schlechtendali were low (Table 3.10). In leaf samples on 1 June they were estimated at <16/leaf (Table 3.11). Numbers rose slowly reaching >64/leaf on 6 August on all sprayed plots but were markedly lower on unsprayed plots (P = 0.01-P = 0.05). Differences between sprayed treatments were non-significant.

Numbers of tydeid mites were reduced (P = 0.05) by both pre-blossom sprays (Table 3.12) though, as for T. pyri the full effect of the March spray was delayed. On 1 June leaf samples showed only 15-30 tydeids/100 leaves on sprayed and unsprayed plots alike and the numbers continued to decline until August (Table 3.13).

There was no clear evidence that permethrin was harmful to Z. mali and to tarsonemid mites. These were fairly common on twig samples early in the season

Table 3.10

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of Aculus schlechtendali up to blossom

Numbers in the table represent results of scoring apple twigs as follows:

1 = none or very few rust mites per twig or cluster scanned under the microscope

2 = moderate numbers

3 = numerous

Date of spray (growth stage of apple)	Scoring of A. schlechtendali Means per plot* Date of sampling 12 March	30 March	6 April**	19 April	11 May
17 March (bud-burst)	1.3	1.2	1.3	1.2	1.5
6 April (mouse-ear)	-***	-***	1.3	1.5	1.2
Unsprayed	1.6	1.6	1.4	1.2	1.0

* For sample sizes see Table 3.2

** Sample taken before application of April spray

*** No observation

Table 3.11

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of *Aculus schlechtendali* in the summer

Mites were brushed onto paper cards (see text 3.2.2.2) and their numbers were visually categorised under the microscope as 0-800 or 800-3200 or over 3200 mites per 50 leaves/plot. For statistical purposes these three categories were scored with 1, 2 and 3 respectively.

Date of spray (growth stage of apple)	Scoring of *A. schlechtendali* Means per plot (50 leaves) Date of sampling					
	1 June	23 June*	2 July	22 July	6 August	25 August
17 March (bud-burst)	1.0	1.5	1.5	1.7	3.0b	3.0
6 April (mouse-ear)	1.0	1.7	1.2	2.2	3.0b	3.0
23 June (1 month after petal-fall)	1.0	1.2	1.0	1.2	2.2b	3.0
Unsprayed	1.0	1.5	1.0	1.2	1.2a	2.0

* Sample taken before application of June spray

Note. Values followed by the same letter in each column do not differ significantly ($P = 0.05$)

Table 3.12

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of tydeid mites up to blossom

Date of spray (growth stage of apple)	Numbers of tydeid mites - Means per plot*				
	Date of sampling				
	12 March	30 March	6 April**	19 April	11 May
17 March (bud-burst)	65.7	25.7	14.2	4.7ab	2.5a
6 April (mouse-ear)	40.5	31.2	28.7	2.2a	1.5a
Unsprayed	44.2	34.5	35.7	13.0b	13.0b

* For sample sizes see Table 3.2

** Sample taken before application of April spray

Note. Values followed by the same letter in each column do not differ significantly ($P = 0.05$)

Table 3.13

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of tydeid mites in the summer

Date of spray (growth stage of apple)	Numbers of tydeid mites Means per plot (50 leaves) Date of sampling					
	1 June	23 June*	2 July	22 July	6 August	25 August
17 March (bud-burst)	8.0	1.0	1.0	1.0	2.5	0.7
6 April (mouse-ear)	13.7	1.0	2.2	1.2	2.7	1.2
23 June (1 month after petal-fall)	15.2	2.7	1.7	1.0	3.0	3.5
Unsprayed	10.0	3.5	7.0	10.0	7.5	2.0

* Sample taken before application of June spray

Note. There were no significant differences (at $P = 0.05$) among the four treatments in any sample

(Tables 3.14 and 3.15) though both were very highly aggregated in distribution. Few Z. mali (Table 3.16) and no tarsonemids were found in leaf samples from June to August.

3.3.2.4 Varietal differences

In the leaf samples, P. ulmi numbers were on average, through the summer, four times higher on Egremont Russet than on James Grieve ($P = 0.01$-$P = 0.05$); but no varietal preference was apparent for T. pyri or other mite species found.

3.3.2.5 Differences between blocks

Only P. ulmi showed significant variation among the four blocks in most leaf samples ($P = 0.01$-$P = 0.05$). Considering all samples Block IV had the highest numbers and Block I the lowest, with Blocks II and III in between (Appendix 3.3). Differences were possibly due to the recent pesticide history of the experimental orchard. For the previous two years a trial on the effects of azinphos-methyl on apple mites had been carried out. Blocks II, III and IV had received 1, 2 and 3 azinphos-methyl sprays respectively, whereas Block I had been left unsprayed (Flegg, 1980 and 1981).

3.3.2.6 Residual toxicity and chemical persistence

Fig. 3.7 and Appendix 3.4 show that after all three spray timings the percentage mortality of young

Table 3.14

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of *Zetzellia mali* up to blossom

Date of spray (growth stage of apple)	Numbers of *Zetzellia mali* Means per plot (50 leaves) Date of sampling				
	12 March	30 March	6 April**	19 April	11 May
17 March (bud-burst)	41.2	24.2	4.2	0	1.5
6 April (mouse-ear)	-***	-***	18.0	0	0
Unsprayed	4.0	4.5	15.5	0	0.2

* For sample sizes see Table 3.2

** Sample taken before application of April spray

*** No observation

Note. There were no significant differences (at $P = 0.05$) among the three treatments in any sample

Table 3.15

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of tarsonemid mites up to blossom

Date of spray (growth stage of apple)	Numbers of tarsonemid mites Means per plot* Date of sampling 12 March	30 March	6 April**	19 April	11 May
17 March (bud-burst)	3.0	26.5	7.2	0.2	0
6 April (mouse-ear)	-***	-***	23.0	0.7	0
Unsprayed	19.7	1.5	11.0	1.7	0

* For sample sizes see Table 3.2

** Sample taken before application of April spray

*** No observation

Note. The only sample with a significant difference between the sprayed and the unsprayed treatments was that of 30 March ($P = 0.05$)

Table 3.16

1981 trial: effects of a single spray of 25 mg permethrin/ litre on numbers of *Zetzellia mali* in the summer

Date of spray (growth stage of apple)	Numbers of *Zetzellia mali* Means per plot (50 leaves) Date of sampling					
	1 June	23 June*	2 July	22 July	6 August	25 August
17 March (bud-burst)	2.0	0.5	1.2	1.0	0.5	2.0
6 April (mouse-ear)	3.7	1.2	1.0	2.5	1.0	2.7
23 June (1 month after petal-fall)	8.5	3.5	2.5	2.0	2.0	5.7
Unsprayed	5.0	0	0.5	0.2	0.2	0

* Sample taken before application of June spray

Note. There were no significant differences (at $P = 0.05$) among the four treatments in any sample

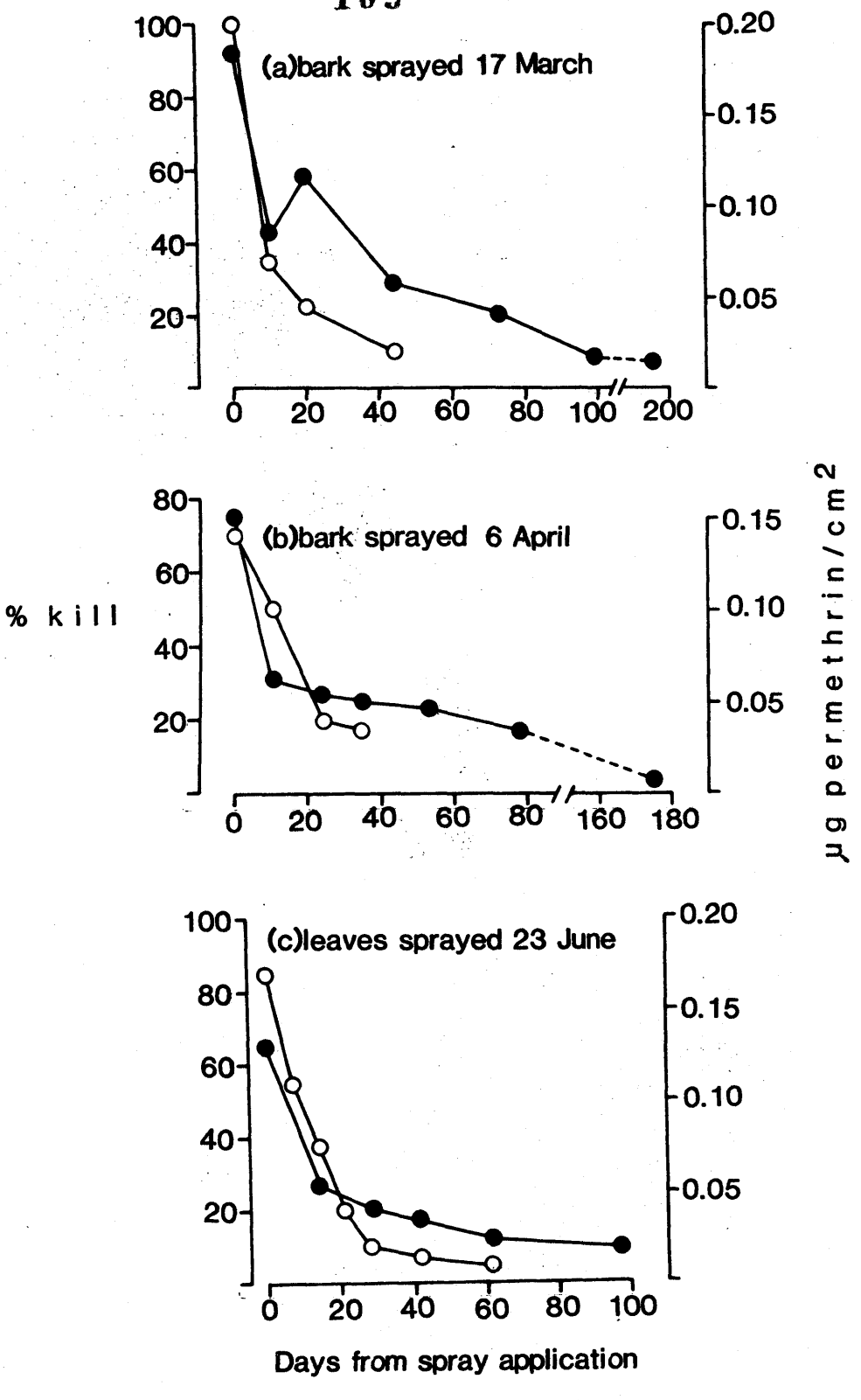


Fig.3.7 1981 trial: persistence of field-weathered residues of permethrin sprays on apple bark and leaves.

o——o % kill of young Amblyseius potentillae in bioassays;

●——● permethrin residues, µg/cm^2, determined by GC analysis

A. potentillae in bioassays, and the levels of permethrin residues determined by G.C. analyses, declined within 10-14 days to about half the initial values recorded on day '0'. Thereafter, mortality declined to negligible levels within 40 days, but low-level residues were detectable for considerably longer. On samples from the unsprayed plots, there were no detectable residues (<0.002 µg total permethrin cm^{-2} on bark and <0.001 µg cm^{-2} on leaves) and less than 5% mortality in bioassays. Fig. 3.8 shows the correlation between the percentage kill obtained in bioassays and the residue levels determined by analyses; there were no large differences between the effects of residues on leaves and on bark, but the results suggest that residues on leaves were on average slightly more toxic. More detailed studies would be needed to reach firm conclusions on this point.

3.4 DISCUSSION

In both years T. pyri became active and began to move out of winter shelters at the end of March, more than a month before winter eggs of P. ulmi began hatching. Observations suggest that, at first, many of the mites were making foraging excursions in search of prey (including A. schlechtendali and tydeids) and returning to shelters on the bark rather than staying in the opening buds. In the 1981 trial, there was evidence that the shelters provided temporary protection from the effects of the spray at 'bud-burst' (17 March), but residues were still present at toxic levels when the mites became more active

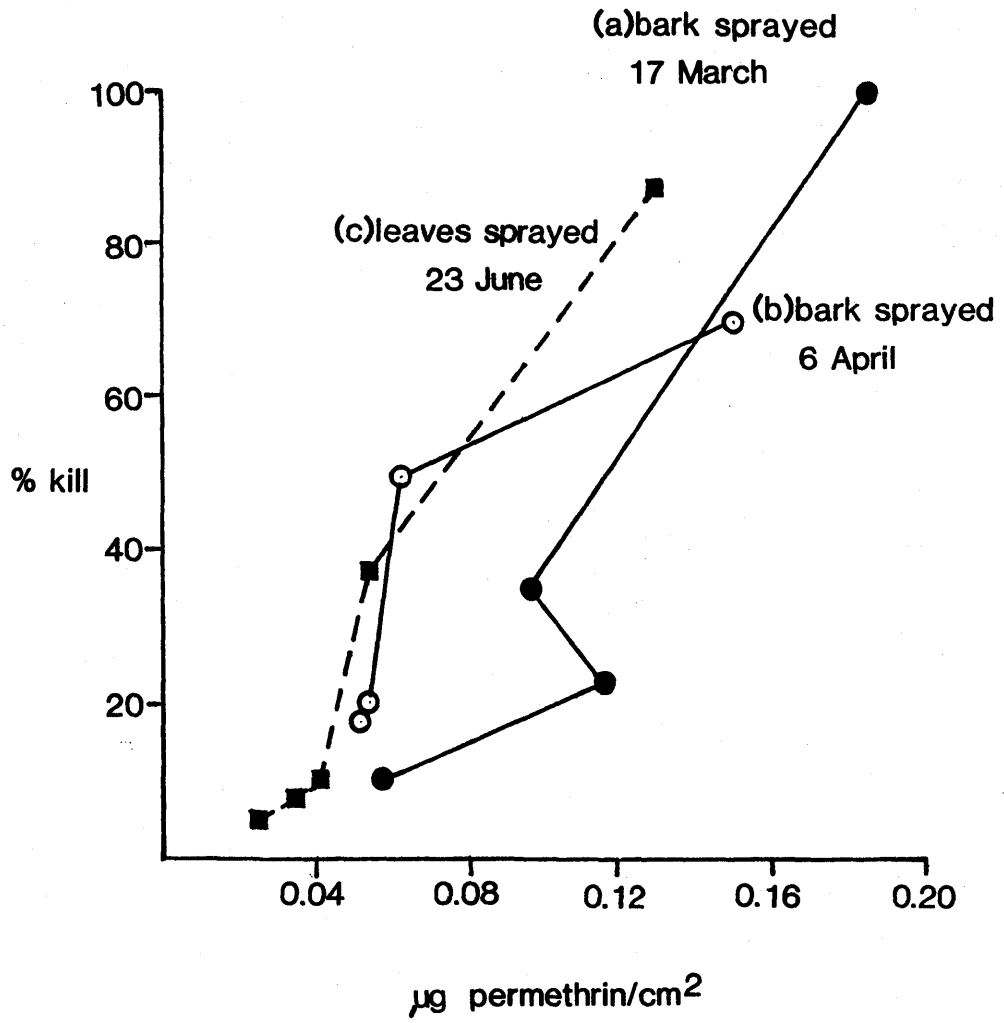


Fig.3.8 Correlation between the percentage kill of Amblyseius potentillae by permethrin residues on apple as shown by bioassays, and the permethrin residue as determined by chemical analysis.

during April. In the results for both trials (Tables 3.3, 3.4, 3.6, 3.8 and 3.9, Fig. 3.6, and Appendices 3.1, 3.2 and 3.3) the disruptive effect of a permethrin spray in March at the 'late-dormant' to 'bud-burst' stages was not appreciably less than that of sprays at 'mouse-ear' (6.4.81) or 'late green cluster' (5.5.80).

3.4.1 Residual toxicity and chemical persistence

The half-life values for permethrin residues determined in this study are in accord with data reported by Ruscoe (1977) and by Brown, Johnson, Hopkins, Durant and Montefiori (1982).

The toxicity of permethrin residues both on bark and on leaves declined to a low level within 20 days, when in the bioassays c 20% of larvae and protonymphs of A. potentillae were being killed. This species was chosen for the bioassay work because T. pyri has a much lower fecundity (Overmeer, Doodeman and van Zon, 1982), and cultures would not provide the numbers of eggs required. Subsequent bioassays employing the taped-slide technique (Kapetanakis and Cranham, 1983) showed that T. pyri was 2-3 times more tolerant than A. potentillae to permethrin. Immature mites are less tolerant than adults (van Zon and Wysoki, 1978). Hence it is likely that permethrin residues in this trial ceased to be toxic to T. pyri in rather less than 20 days, especially pre-bloom when the population consisted only of adult females. However, related tests (reported in section 6 of this Thesis) showed that T. pyri adult females tended to avoid apple

leaf surfaces bearing permethrin residues at sub-lethal levels; when confined on such surfaces egg production was reduced. Therefore it seems likely that the persistent effects of permethrin residues to T. pyri may extend somewhat beyond the period when they cause direct mortality, as determined for example by Ruscoe (1977) for larvae of Plutella xylostella.

Solvent extraction before G.C. analysis would presumably recover permethrin not only from surfaces of bark and leaves where mites would be exposed to it, but also from micro-crevices, leaf waxes and plant tissues where they would not be. The difference between the persistent toxicity and the persistence of total chemical residues, in relation to 'biological availability' was stressed in a review paper by Chiba (1970). In a study comparing bioassay techniques on adult T. pyri (Kapetanakis and Cranham, 1983), fresh residues of permethrin on a glass surface were more toxic than on a leaf surface, so that the ratio of LC_{50}s leaf/glass was 6.6. This suggests that even with fresh residues on a leaf surface only a small fraction of the total permethrin present is available to the mites. In the current study, the correlation between percentage kill and residue levels (Fig. 3.8) suggests that the size of the available fraction did not change greatly with time after spraying. There is an indication from this data that the available fraction on leaves, after the June spray, was rather higher than that on bark after the March spray, though the results for the April spray were not consistent with this conclusion.

3.4.2 Disruptive effect of sprays

After pre-bloom sprays of 50 mg permethrin/litre in 1980, T. pyri numbers had not recovered by late July and control of P. ulmi was lost for the whole season. After pre-bloom sprays of 25 mg permethrin/litre in 1981, the persistent toxicity was judged to be negligible by the end of April. Nevertheless, it took nearly four months, until late July, before the ratio of P. ulmi:T. pyri had recovered to 6:1 or less (Fig. 3.6). With the June spray this ratio was 23:1 at the end of August; subsequently P. ulmi laid many winter eggs so that the disruptive effect was carried over to spring 1982. It is probable that on these small plots immigration of T. pyri from adjacent unsprayed trees boosted the rate at which the population recovered, so that recovery would have occurred rather faster than when whole orchards are sprayed. Weather conditions during 1981 were not conducive to a rapid build-up of P. ulmi; May was one of the wettest on record, and mean temperatures in June were 2°C cooler than average (Fig. 3.3). In 1981, permethrin was tested at the lowest recommended concentration. The conditions of the trial therefore tended to minimise rather than maximise the disruptive effect of permethrin. Nevertheless, resurgence of P. ulmi occurred after all three spray timings.

In contrast to the report by Gruys (1980) in The Netherlands, but in agreement with the results of another pre-blossom trial of pyrethroids in England (Flegg, 1982),

the results do not suggest that the disruptive effect of pre-blossom, even of 'bud-burst', sprays of permethrin, was markedly less than that of post-blossom sprays; both are unacceptable for the practice of 'mite management' (Cranham and Solomon, 1981).

SECTION 4

LABORATORY EVALUATION OF RESISTANCE TO INSECTICIDES IN *TYPHLODROMUS PYRI* FROM ENGLISH APPLE ORCHARDS

The studies of this section were also reported in a paper under the same title published in the Annals of Applied Biology (1983) 103, 389-400.

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4.1 INTRODUCTION

The literature on the effects of some insecticides on phytoseiid mites has been reviewed in the General Introduction (sub-sections 1.5, 1.6, 1.7 and 1.8). Insecticide resistance in particular was also reviewed by Croft and Strickler (1983) for a variety of orchard-inhabiting phytoseiid mites world-wide. Relevant to the studies reported in this section,in New Zealand T. pyri has developed marked resistance to azinphos-methyl (Hoyt, 1972; Penman, Ferro and Wearing, 1976), permitting the development of 'integrated mite control' programmes in which azinphos-methyl is used to control orchard tortricids (Wearing, Walker, Collyer and Thomas, 1978). Resistance of T. pyri to various organophosphates (OPs) was also reported from New York State (Watve and Lienk, 1975, 1976). In England, the accumulated field evidence from 1977 indicates that OP resistance has become common in commercial sprayed orchards (Cranham, Tardivel and Kapetanakis, 1984; Flegg, 1983) and that T. pyri usually tolerates high rates of carbaryl. Similar evidence for high tolerance to carbaryl in this species was reported from The Netherlands (Gruys, 1980), New Zealand (Wearing and Ashley, 1978) and New York State, U.S.A. (Watve and Lienk, 1976). Resistant strains of two other phytoseiid species, Amblyseius fallacis and Typhlodromus occidentalis, have been widely exploited in integrated mite control programmes in N. America and Australasia (Croft, 1981).

When resistance to OPs developed in these two species it did not *per se* confer cross-resistance to carbaryl (Croft, 1982); it was desirable therefore to study this aspect for *T. pyri*.

An OP-resistant strain of *T. pyri* was imported to England from New Zealand in January 1977 (courtesy of Dr. H. Wearing, D.S.I.R., Auckland); it was mass-reared on potted plum trees under glass and released in August 1977 in Middle Park orchard, East Malling Research Station (E.M.R.S.). In the same year, *T. pyri* in a commercial apple orchard at Hadlow, Kent (12 km south of E.M.R.S.) were observed to survive repeated OP sprays; mass releases of this population were made in Westons Field orchard, Rocks Farm, E.M.R.S. in August 1977 and spring 1978. With the apparent development of resistance in native *T. pyri* to several commonly-used effective pesticides (certain OPs, carbaryl, HCH) (Solomon and Cranham *in* Flegg, 1982, pp. 100-101) and the commercial use of several non-acaricidal fungicides, 'integrated mite management' was demonstrably practical in commercial orchards (Cranham and Solomon, 1981). However, a major threat to this development stems from the current popularity of synthetic pyrethroid (SP) insecticides, of which permethrin was the first to be marketed; all SPs sold for orchard use proved persistently toxic to *T. pyri* (Aliniazee and Cranham, 1980; J.E. Cranham *in* Flegg, 1982, p. 101).

The aims of the bioassay studies reported in this section were (1) using stocks from isolated, unsprayed

orchards, to establish levels of 'base-line' susceptibility in T. pyri to azinphos-methyl, parathion, carbaryl and (2) using stocks from orchards with various spray histories of OPs and/or carbaryl, to quantify the levels of resistance occurring. Three different bioassay techniques were compared. In addition, it was hoped that these studies would shed light on the relation between resistance to OPs and to carbaryl in this species, the stability of such resistance and whether the acquisition of resistance to OPs/carbaryl influenced the response to permethrin. To add to available data (reviewed by Croft, 1982) on the comparative toxicological responses of important orchard phytoseiid species, assays were also run on Amblyseius potentillae (Garman), a species considered important in The Netherlands.

4.2 MATERIALS AND METHODS

4.2.1 Provenance of stocks of T. pyri

Table 4.1 lists details of the stocks of T. pyri reared in the laboratory and used in bioassays. The four 'unselected' stocks either had no known history of pesticide exposure, or were known to have been exposed to very few spray applications. Other phytoseiid species were present in these collections, and particular care was taken to start cultures with T. pyri only. Immediately after collection from the field all adult females were individually isolated on plastic plates

Table 4.1 Laboratory stocks of *Typhlodromus pyri* used in bioassays

Mite stock code name	Site of collection; spray history	Date of collection	Initial no. of females
'Unselected' stocks			
Amsterdam	Lab. of Experimental Entomology, University of Amsterdam, Netherlands; unsprayed apple trees	March 1981	20
Bangor	near Bangor, N. Wales; unsprayed apple trees	October 1981	6
Bristol	Bristol, Avon; ADAS apple orchard established *c* 1968, minimal use of insecticides *c* 1970, unsprayed since	July 1982	35
Evesham	near Evesham, Worcs.; plum orchard; one DDT spray/year for 20 yr, no OPs or carbaryl recorded	October 1981	50
OP-selected stocks			
MP4	Middle Park Orchard, EMRS; New Zealand stock established 1977, azinphos-methyl four sprays 1979-80, no carbaryl	June 1981	50
MP9	Different section of same orchard; azinphos-methyl nine sprays 1979-81, no carbaryl	June 1982	50
Carbaryl-selected stocks			
Lynsted	Commercial orchard near Faversham, Kent; mainly carbaryl, occasional demeton-S-methyl for many years	October 1981	50
Norton	As Lynsted	October 1981	50
Reduced selection			
WF (Hadlow)	Westons Field orchard, EMRS; few OP and carbaryl sprays 1978-82	June 1982	50

(Plate 4.1), the same as those used for rearing the phytoseiid colonies (see 4.2.6 below). When the progeny reached the adult stage one adult female per isolation cell was mounted and identified under a microscope. All the mites from 'families' that were positively identified as T. pyri were pooled for each stock and transferred on to a rearing plate.

The remainder of the stocks were collected from mature apple orchards with varying histories of spraying over many years. Isolation of progeny and identification showed that in these collections, T. pyri was the only phytoseiid species present.

4.2.2 OP-selected stocks

The OP-resistant T. pyri imported from New Zealand (see above) were released in Middle Park (MP) orchard, Home Farm, E.M.R.S. on 10 August 1977, shortly after two sprays of azinphos-methyl, intended to eliminate susceptible native T. pyri, were applied on 27 July and 4 August. The whole orchard received one spray of azinphos-methyl in 1978.

4.2.3 Carbaryl-selected stocks

Commercial orchards at Lynsted and Norton had received 2-3 sprays of carbaryl per year for many years, and in certain years a single pre-blossom spray of demeton-S-methyl for aphid control. There appeared to be no commercial orchards that had received only carbaryl and no OPs whatever.

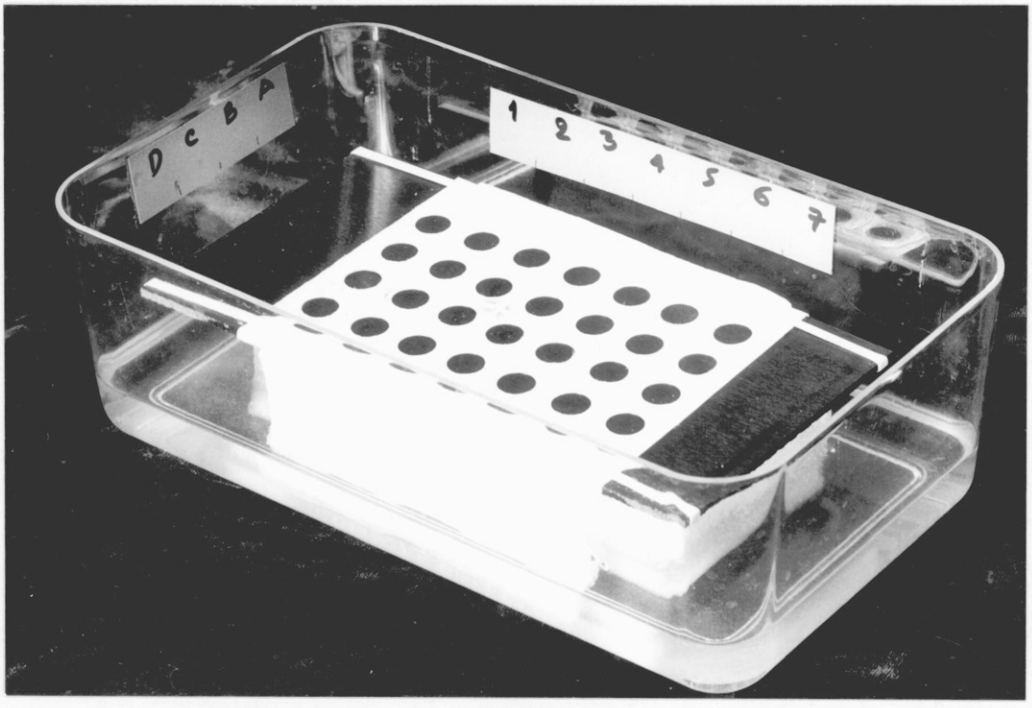


Plate 4.1 Plastic box and plate used for individual isolation of phytoseiid adult females and their progeny

4.2.4 Reduced selection

The OP-resistant Hadlow stock were released in Westons Field orchard, Rocks Farm, E.M.R.S. in 1977-78 (see above). The use of OPs and carbaryl was much lighter than average for commercial orchards: OPs (azinphos-methyl and demeton-S-methyl) were sprayed once pre-blossom in 1979 and 1982, and carbaryl was sprayed three times in 1980 on half the orchard, and once on the whole orchard in 1981.

4.2.5 Amblyseius potentillae (Garman)

A susceptible stock of Dutch origin was supplied by Dr. W.P.J. Overmeer, University of Amsterdam: it had been maintained as a standard laboratory stock for some years.

4.2.6 Rearing

Both species of phytoseiids were reared in a constant environment room at 25°C, 50-60% r.h. and 17-h daily photophase with 750 lux fluorescent lighting, on plate cultures without plant leaves; the method was modified from McMurtry and Scriven (1965). They were fed with either Tetranychus urticae every second day or with pollen of dwarf broad bean (Vicia faba) once per week. T. urticae was reared on potted dwarf broad bean (Plate 4.2) in a heated glasshouse.

The use of pollen as the only food for A. potentillae was standard practice at the University of Amsterdam (Overmeer, 1981) and tests showed that T. pyri would also



Plate 4.2 Potted dwarf broad bean (Vicia faba) used for pollen production, or for rearing Tetranychus urticae, for feeding colonies of phytoseiid mites

reproduce at a satisfactory rate on fresh pollen. Pollen remained in acceptable condition when the r.h. of the room was 50-60%; below this humidity the pollen dried out too quickly and at over 70% r.h. it 'solidified' and became unsuitable. The use of pollen was less time-consuming, individual culture plates could be maintained for longer and mites recovered more easily for bioassays. The bioassay results reported here are for mites fed on pollen, except when otherwise noted.

The rectangular plastic plates, 20 x 10 cm, had a black, matt, non-reflective surface which aided observations under a microscope. Each plate was contained in a clear plastic box, 25 x 15 x 10 cm (Plate 4.3) with distilled water to a depth of 3-4 cm. A thoroughly soaked plastic foam sponge, 17 x 8 x 5 cm, rested in the water with the plate on top. Paper tissues folded twice and thoroughly wetted were draped from all four sides of the plate to cover a border strip 2-3 cm wide and to act as a wick, thus providing a water barrier and a source of drinking water. Because some mites occasionally walked on to the water barrier a complete ring of 'Tangle-trap' non-drying glue (The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.) was syringed on top of the wet tissues as a 'fail-safe' barrier. Each culture plate was provided with a few small pieces of clear gelatin sheet, 2 x 2 cm, folded to form inverted V-shaped 'roofs': these provided shelter and acceptable oviposition sites.

The same boxes and plates were used for the initial isolation of the field-collected phytoseiids (see 4.2.1).

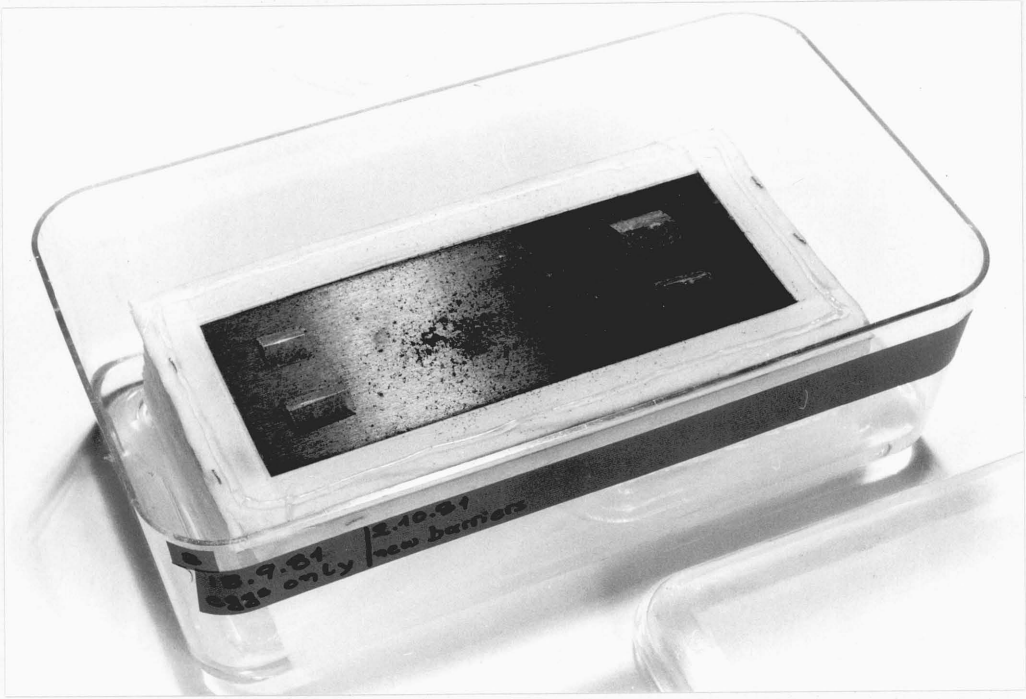


Plate 4.3 Plastic plate and box used for rearing phytoseiid mites

Each plate was covered with a wet sheet of thick filter paper with 12 mm diameter holes forming the individual cells (Plate 4.1). 'Tangle-trap' was also used for the 'safe' isolation of each cell.

4.2.7 Bioassay techniques

All bioassays were on adult female mites and mortality was assessed after 24 and 48 h. Most of the tests employed the taped-slide technique (Voss, 1961) which has been standardised and widely used for spider mites and phytoseiids (Anon., 1980), but slightly modified in that mites on slides were sprayed with serial concentrations of each toxicant under a Potter tower (Fig. 4.1). Of each serial concentration of toxicant, 3 ml were sprayed at 48 kPa (7 p.s.i.) to give a standard deposit close to 4 mg wet spray/cm^2. Ten adult female phytoseiids were used per slide. After spraying, slides were allowed to dry for up to 1 h and then stored at 25°C over wet capillary matting in boxes to maintain a high humidity (Plate 4.4). Mortality was assessed after 24 h and 48 h by prodding each mite with a fine brush.

Certain T. pyri stocks were also assayed using pesticide residues on apple leaf and glass surfaces. Plastic cartons, 112 ml capacity with lids, were used as water reservoirs, with filter paper on the carton lid kept moist by paper wicks. Leaf disks, 25 mm diameter, were sprayed in the Potter tower (deposit c 4 mg/cm^2), allowed to dry and placed on the carton lids. A filter paper with

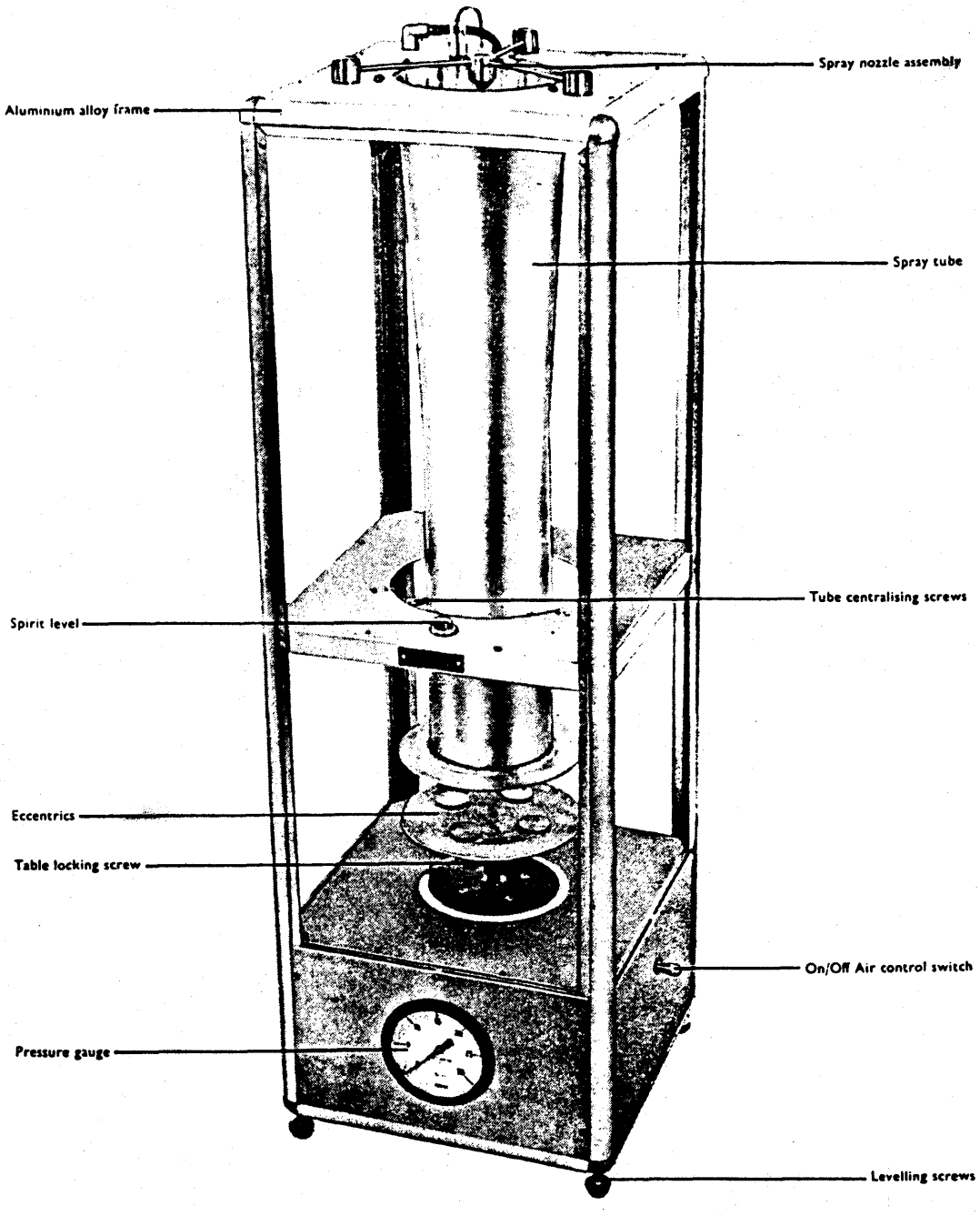


Fig. 4.1 Potter Spray Tower

(Burkhard Technical Information)

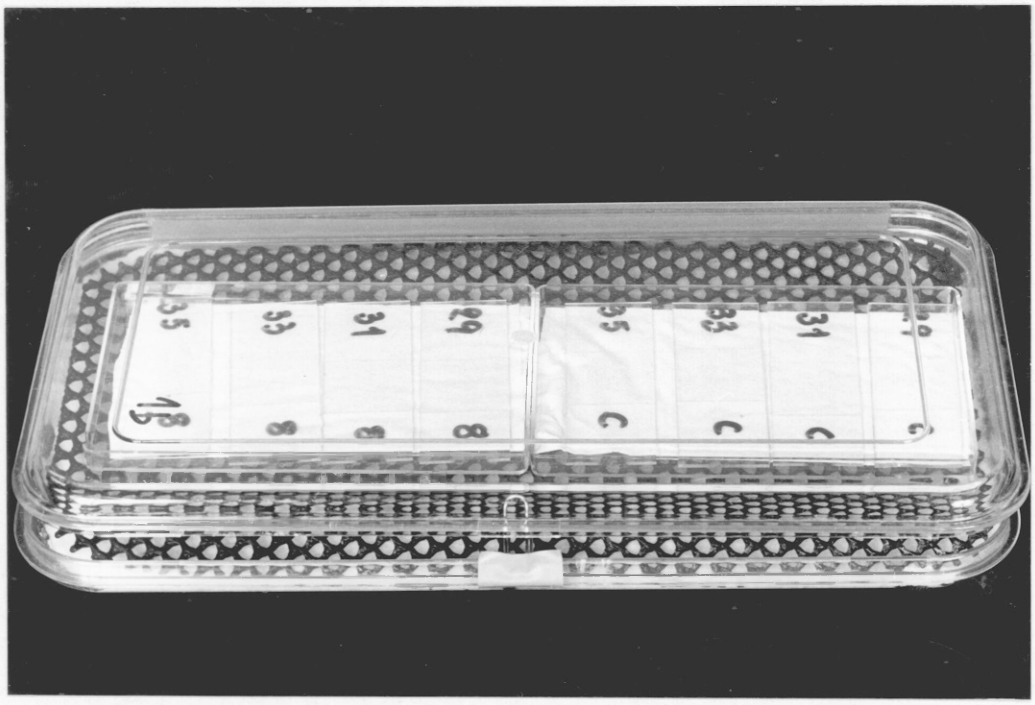


Plate 4.4 Clear plastic box used with the taped-slide technique for storing the sprayed slides with the phytoseiid mites in high humidity

an 18 mm diameter hole was fitted over each leaf disk, in contact with the wick, to provide both a barrier and drinking water. As with the rearing method, an annulus of 'Tangle-trap', c 40 mm diameter, was syringed on top of the wet paper as an additional 'fail-safe' barrier (Plate 4.5). Five mites were moved to each arena and a little pollen was added as food. The technique with glass (microscope slides) was similar except that a 5 x 8 mm gelatin roof (sprayed on both sides) was also added to each arena. Part of the carton lid under the glass slide was covered with black adhesive tape to aid observation under the microscope (Plate 4.6). As with the taped-slide technique, mortality was assessed after 24 h and 48 h by prodding each mite with a fine brush, as the total of mites found dead in the arenas and 'drowned' in the wet paper barriers.

Tests with each toxicant and mite stock were replicated and repeated two or three times and results were pooled. Assay data were subjected to probit analysis, with the assistance of G.P. Barlow (Statistics Department, East Malling Research Station) using a computer programme.

Commercial formulations of pesticides were used:

(1) azinphos-methyl 22% e.c. ('Gusathion' ex Bayer UK Ltd);
(2) carbaryl 85% w.p. ('Sevin' ex Union Carbide UK Ltd);
(3) permethrin 25% e.c. ('Ambush' ex Plant Protection Division, Imperial Chemical Industries, UK) and
(4) parathion 20% e.c. (also ex Imperial Chemical Industries, but no longer marketed in the UK).

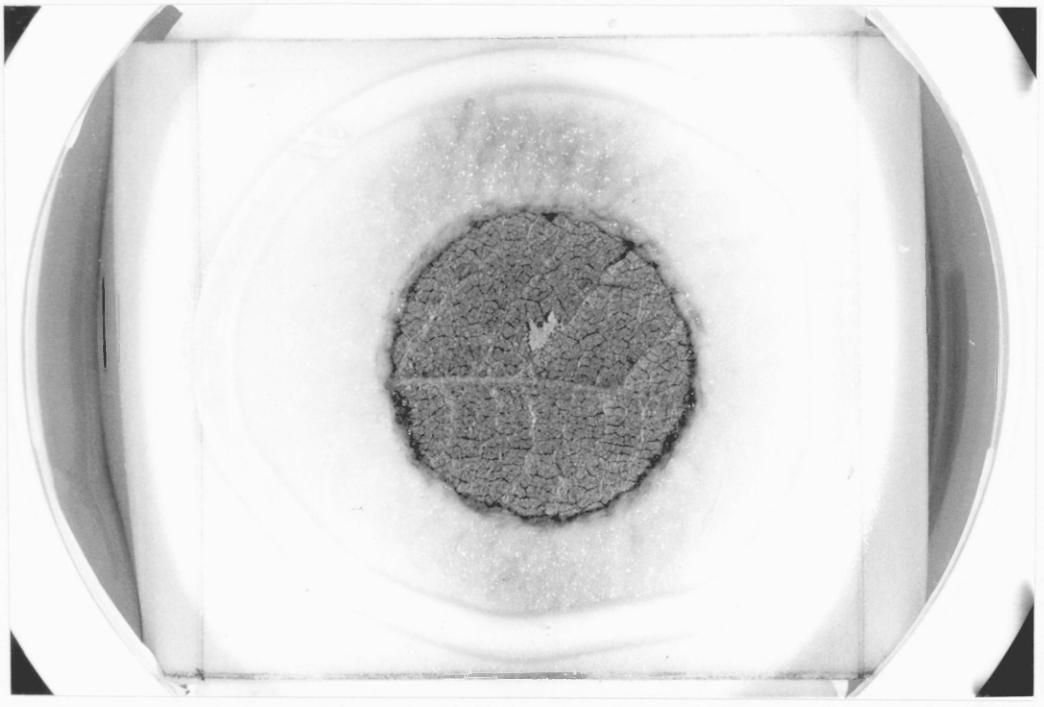


Plate 4.5 Arena used for bioassays with pesticide residues on apple leaf surface



Plate 4.6 Arena and plastic carton used for bioassays with pesticide residues on a glass surface

4.3 RESULTS

4.3.1 Responses of T. pyri to OPs and carbaryl in taped-slide tests (Table 4.2, Fig. 4.2a and b, and Appendices 4.1 and 4.2).

The site of collection of the Bangor stock (North Wales) was the most remote from sprayed orchards; sites for Bristol and Evesham stocks were isolated but closer to commercial fruit-growing areas. Even the collection site for Amsterdam in the Netherlands was not so remote from sprayed orchards as was Bangor. The latter was the most susceptible stock to both azinphos-methyl and carbaryl; at 48 h Bristol and Amsterdam also gave LC_{50}s close to 100 mg azinphos-methyl/litre but they were rather more tolerant of carbaryl than was Bangor.

Amsterdam stock fed on (a) pollen and (b) Tetranychus urticae gave very similar responses to azinphos-methyl providing evidence that a diet confined to pollen did not affect the level of response to this pesticide.

With Bangor as a measure of base-line susceptibility, MP9 (of New Zealand origin) and native stocks (Lynsted, Norton, WF) exhibited a 4-6 fold* resistance to azinphos-methyl and a >20-fold* resistance to carbaryl, except for a slightly lower level in WF (Hadlow). Evidence that the rather low level of increased tolerance to azinphos-methyl

* These 'resistance factors' refer to LC_{50}s for 24 h

Table 4.2 Results for taped-slide bioassays of OPs and carbaryl on *Typhlodromus pyri*: LC_{50}s (mg/litre) and slope values from probit analyses

Mite stock	Azinphos-methyl			Carbaryl			
	LC_{50}		Common slope	LC_{50}		Slope	
	24 h	48 h		24 h	48 h	24 h	48 h
Amsterdam	220	110	4.87	2 900	800	1.58	2.36
Amsterdam (fed *T. urticae*)	230	120	4.21	-	-	-	-
Bangor	120	100	6.13	990	320	1.44	2.73
Bristol	190	100	4.48	3 100	1 700	4.38	2.75
Evesham	270	170	5.16	4 800	420	1.41	2.18
MP4	360	240	3.75	>20 000	13 000	*	2.65
MP9	710	540	5.38	>20 000	>20 000	*	*
Lynsted	460	360	4.72	>20 000	>20 000	*	*
Norton	560	370	3.97	>20 000	>20 000	*	*
WF (Hadlow)	740	580	4.56	9 900	7 100	2.46	2.20

	Parathion		
	LC_{50}		Common slope
	24 h	48 h	
Bangor	37	27	3.95
MP9	2 300	1 800	4.76
Norton	1 800	1 400	5.32

1. The 95% fiducial limits of LC_{50}s for azinphos-methyl and parathion were in the range of 8-22% of the values; those for carbaryl, when estimable, in the range of 19-71% of the values.
2. S.E.s of slopes for azinphos-methyl and parathion were in the range 9-17% of the values and those for carbaryl in the range 12-32% (* = not estimable; - = not tested).

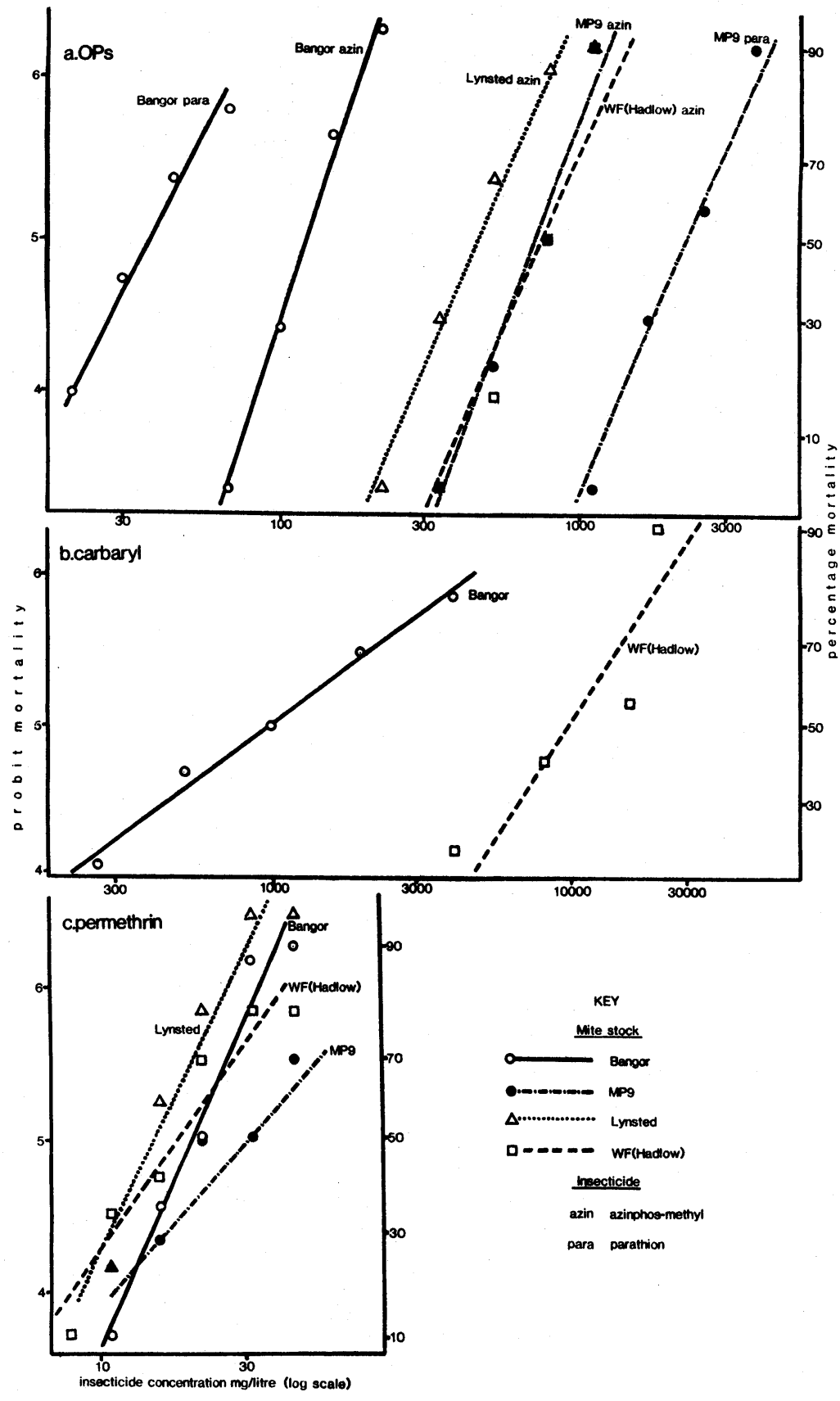


Fig.4.2 Mortality responses to four insecticides of four stocks of T. pyri (24 h assessment). See Table 4.1 for provenance of these stocks. Responses of only one stock per group were plotted here to show differences.

represents a development of resistance to OPs was seen in the responses to parathion, i.e. a 49- and 62-fold* resistance in Norton and MP9 respectively as compared with Bangor.

In spite of the very limited use of OPs in the WF (Hadlow) orchard this stock gave LC_{50}s for azinphos-methyl as high as those obtained for MP9 (nine sprays, 1979-81). However, MP4 (four sprays, 1979-80) was appreciably less tolerant of azinphos-methyl than MP9; this accords with the effects of azinphos-methyl sprays recorded in the orchard in 1978-80 (J.E. Cranham, unpublished) and it is possible that MP4 was not an homogenous resistant stock. Purity was not checked in the laboratory.

With azinphos-methyl, kills at 48 h were higher than at 24 h, although a common slope for the log-concentration (lc-p) lines could always be fitted. With assays of carbaryl on the four 'unselected' stocks, the increased kill at 48 h was more marked than it was for azinphos-methyl and the slopes of the lc-p lines increased, except that for Bristol. For stocks from sprayed orchards, however, carbaryl was virtually innocuous; it was not practicable to test concentrations above 2% carbaryl, which except with WF (Hadlow) gave much less than 50% kill. The parameters for lc-p lines could not be estimated.

The same very high resistance to carbaryl was found with MP4 and MP9 which received no sprays of carbaryl in

* These 'resistance factors' refer to LC_{50}s for 24 h

1977-82, as for the Lynsted and Norton stocks which received two to three sprays/year for a longer period; thus, resistance to carbaryl, once developed, appears to be stable when selection is relaxed.

Fig. 4.3 shows the relationship between the 24 h LC_{50}s for azinphos-methyl and carbaryl for the four 'unselected' stocks, WF (Hadlow) and the range of values obtained for the other resistant stocks. Since for the latter, LC_{50}s for carbaryl could be quantified only as >20 000 mg/litre there are too few data for a correlation coefficient to be meaningful. However, the data suggest a positive correlation between the resistance to both pesticides. No stock was susceptible to azinphos-methyl and highly resistant to carbaryl, or vice versa.

4.3.2 Responses of T. pyri to permethrin in taped-slide tests (Table 4.3, Fig. 4.2c, and Appendix 4.3A)

None of the stocks had a spray history involving synthetic pyrethroids and the main purpose of the tests was to establish the level of 'base-line' susceptibility to permethrin, and to look for any influence of resistance to OPs and carbaryl on the response. MP9 was slightly more tolerant to permethrin than OP-susceptible or native OP-resistant stocks which gave LC_{50}s in the small range 15-25 mg/litre. The New Zealand mites from which MP9 was developed were received in 1977, i.e. almost certainly before T. pyri in New Zealand could have received any exposure to synthetic pyrethroids. For the range of

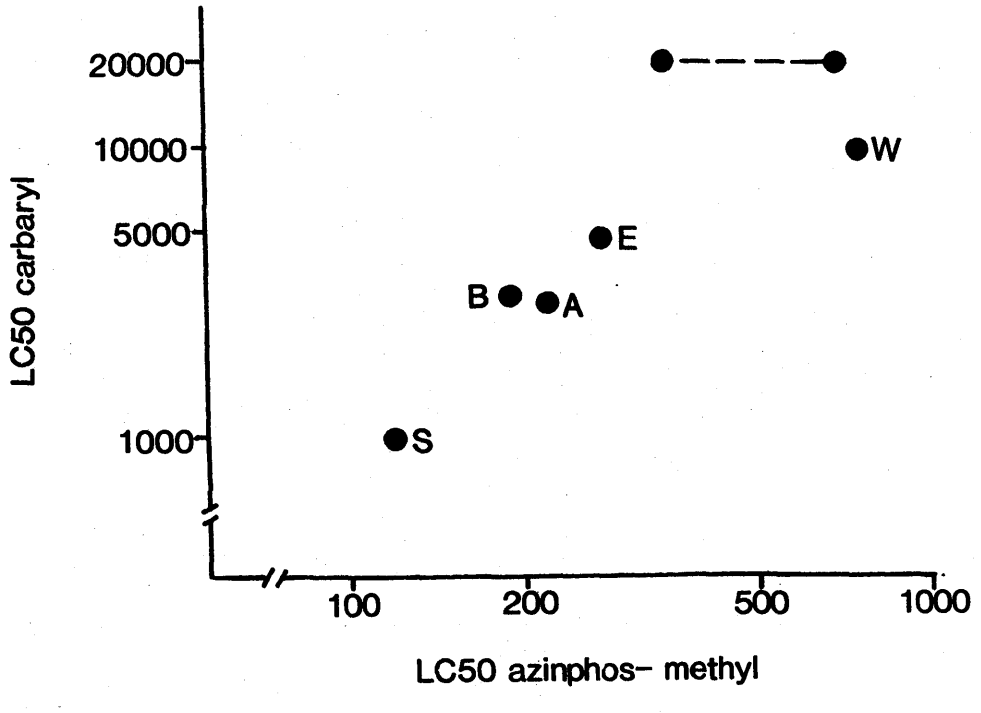


Fig. 4.3 Relation between LC_{50}s (24 h, mg/litre, log scale) for carbaryl and azinphos-methyl: S, Bangor; B, Bristol; A, Amsterdam; E, Evesham; W, WF (Hadlow); ●---●, range of values for other stocks of Typhlodromus pyri

Table 4.3

Results (24 h) for taped-slide bioassays of permethrin on *Typhlodromus pyri*: LC_{50}s (mg/litre) and slope values from probit analyses

Mite stock	Azinphos-methyl	Permethrin	
	LC_{50}	LC_{50}*	Slope*
Bangor	120	20	4.81
Bristol	190	20	2.80
Evesham	270	25	4.01
MP9	710	31	2.24
Lynsted	460	15	4.11
Norton	560	18	3.84
WF (Hadlow)	740	18	2.87

* 95% fiducial limits of LC_{50}s were in the range of 18-44% of values; S.E.s of slopes in the range of 13-22% of values.

Note. Only data for the 24 h assessment are shown in this Table, as mortality was virtually complete in the bioassays of permethrin within 24 h (see Appendix 4.3.A)

samples tested there is no indication that resistance to OPs and carbaryl affects the level of response to permethrin.

Kills at 48 h were not significantly higher than at 24 h and a common slope for the lc-p lines could always be fitted.

4.3.3 Responses of T. pyri in residue bioassays (Table 4.4, Fig. 4.4, and Appendix 4.4)

The Amsterdam and MP4 stocks of T. pyri which, in taped-slide tests, showed a small difference in response to azinphos-methyl and a large difference with carbaryl, were assayed by the alternative techniques described under '4.2.7 Bioassay techniques', involving residues on (1) leaf surfaces and (2) glass. Table 4.4 compares the LC_{50}s and slope values (24 h kills) obtained by probit analyses. Comparison of the 48 h values gives much the same picture (Appendix 4.4). With all three pesticides, mites were killed by lower concentrations in leaf residue assays, and by much lower concentrations in glass residue assays, than were required in taped-slide tests (Fig. 4.4): in Table 4.4 the ratio R1 compares the three techniques for each stock/pesticide. The ratio R2 compares LC_{50}s for the two stocks of T. pyri obtained by each technique and the results show a rather small variation in these estimated 'resistance factors'.

Increased activity of the mites was observed in the permethrin treated, which resulted in 'run off' and drowning in the wet paper barriers of an appreciable

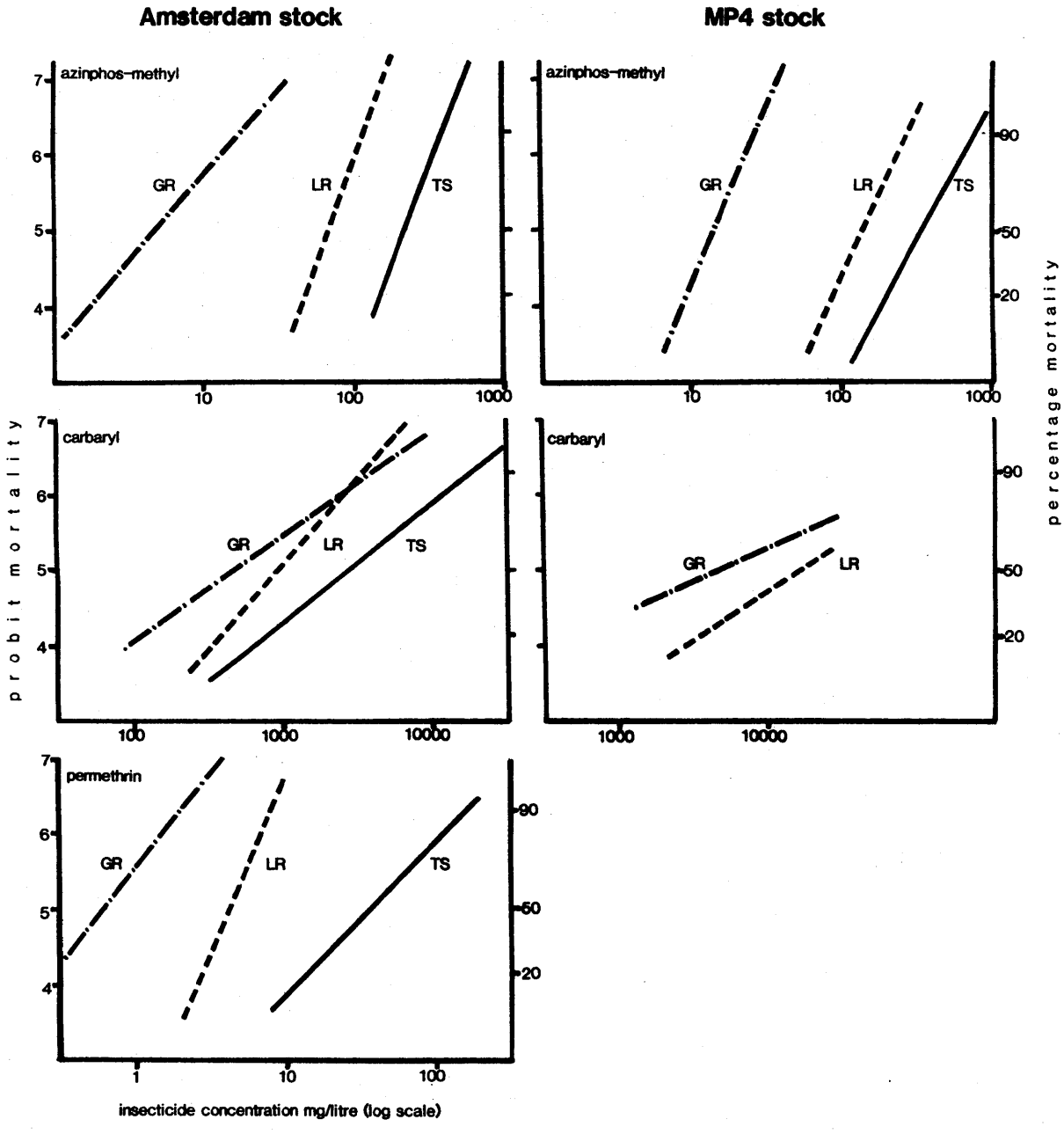
Table 4.4 Comparison of three techniques of bioassay on two stocks of Typhlodromus pyri: 24 h LC_{50}s (mg/litre) and slope values

Pesticide:technique	Amsterdam stock LC_{50}	(R1)	Slope	MP4 stock LC_{50}	(R1)	Slope	(R2)
azinphos-methyl:							
taped-slide	220	(46)	4.87	360	(26)	3.75	(1.6)
leaf/residue	67	(14)	5.32	150	(11)	4.46	(2.2)
glass/residue	4.8	(1)	2.24	14	(1)	4.92	(2.9)
carbaryl							
taped-slide	2 900	(6.2)	1.58	>20 000	(>3.9)	n.s.	(>6.9)
leaf/residue	920	(2.0)	2.19	17 000	(3.3)	1.27	(18)
glass/residue	470	(1)	1.36	5 100	(1)	n.s.	(11)
permethrin							
taped-slide	37	(58)	1.95	not tested			-
leaf/residue	4.3	(6.7)	4.73	not tested			-
glass/residue	0.64	(1)	2.18	not tested			-

R1 : Ratio of LC_{50} to that obtained by the glass/residue technique with the same insecticide and mite stock

R2 : Ratio of LC_{50} MP4/LC_{50} Amsterdam for the same insecticide and bioassay technique ('tolerance factor')

n.s. = Not significant



KEY

TS = taped-slide technique

LR = leaf/residue technique

GR = glass/residue technique

Fig.4.4 Comparison of three bioassay techniques for assessment of mortality responses to three insecticides of two stocks of Typhlodromus pyri

number of individuals, especially at the higher concentrations tested (see also sections 5 and 6).

4.3.4 Responses of Amblyseius potentillae to three pesticides (Table 4.5 and Appendix 4.3.B)

In taped-slide assays, this species proved to be more susceptible than the Bangor stock of T. pyri to azinphos-methyl and carbaryl and considerably more susceptible to permethrin than all stocks of T. pyri.

4.4 DISCUSSION

4.4.1 Responses of T. pyri to OPs and carbaryl

Several authors used the taped-slide bioassay technique to assess tolerance levels in phytoseiid mites; usually the mites were dipped, not sprayed as in the present study, in serial concentrations of the pesticides. This probably makes little difference to the mortality obtained for any given concentration, since in both spraying and dipping the mites are thoroughly wetted. The time between treatment and assessment - 24 or 48 h - affected the response recorded, as shown in the present study.

Information from the literature concerning bioassays on T. pyri has been collated in Table 4.6; LC_{50} values are for 24 h at a temperature of 25-27°C. Only the present study and that of Hoyt (1972) report tests on OP-susceptible (OP-S) stocks of T. pyri, and LC_{50} values

Table 4.5

Results for taped-slide bioassays on Amblyseius potentillae: data from probit analyses

Pesticide	24 h		48 h	Common slope ± S.E.
	LC_{50}	95% fiducial limits	LC_{50}	
azinphos-methyl	56	(46-65)	46	5.31 ± 0.87
carbaryl	410	(300-550)	260	2.50 ± 0.36
permethrin	7.9	(6.2-10)	6.4	2.40 ± 0.24

Table 4.6 Comparison of LC_{50}s (mg/litre) from the literature on the responses of *Typhlodromus pyri* to azinphos-methyl and carbaryl in bioassays using the taped-slide technique, 24 h assessment, except when noted otherwise

Authors:country	OP-susceptible	OP-resistant	Resistance factor
		azinphos-methyl	
Hoyt (1972):			
New Zealand	72	700	10
Penman, Ferro and Wearing (1976):			
New Zealand	-	800-3 000	11-42
Wong and Chapman (1979):			
New Zealand	-	990	14
Watve and Lienk (1976):			
New York State, U.S.A.	-	840, 1 150, 1 404	12-20
Kapetanakis and Cranham (1983):			
U.K.			
MP (New Zealand origin)	-	360-710	5-10
English stocks	120	460-740	6-10
Overmeer and van Zon (1983):			
(glass/residue, 4 days)			
The Netherlands	0.8	1.8	2
		carbaryl	
Watve and Lienk (1976):			
U.S.A.	-	>18 000	-
Kapetanakis and Cranham (1983):			
U.K.	990	>20 000	>20
Overmeer and van Zon (1983):			
(glass/residue, 4 days)			
The Netherlands	42	>2 000	>50

were of the same order. Estimations of the resistance factor for azinphos-methyl by Penman, Ferro and Wearing (1976) and by Wong and Chapman (1979) in New Zealand, and by Watve and Lienk (1976) in New York State, were based on the LC_{50} value (72 mg/litre) obtained by Hoyt (1972) for an OP-S stock; this has therefore been adopted for uniform comparisons in Table 4.6. This evidence points to similar levels for both 'base-line' susceptibility and for resistance in New Zealand in 1970-71 (Hoyt, 1972) and from the present study in England in 1981-82. This relatively low level of 10-fold or less was insufficient for T. pyri to survive the eight or more sprays of azinphos-methyl common in certain apple-growing districts of New Zealand. However, by 1976 Penman, Ferro and Wearing (1976) confirmed that levels as high as 40-fold had developed after 10-15 yr of intensive use. This permitted the development of an integrated mite control system in commercial orchards at least for certain important districts where such high levels were found (Wearing, Walker, Collyer and Thomas, 1978).

Recent trial results in England show that 5-10-fold resistance to azinphos-methyl will allow T. pyri to survive up to four sprays per season (two pre- and two post-blossom) without disrupting the effective predation of P. ulmi (J.E. Cranham, personal communication). More intensive insecticide spraying is very seldom required in England; average commercial use is three to five insecticide sprays per season.

The results obtained by Watve and Lienk (1976) show a variation in the level of resistance through the season. Mites in reproductive diapause, collected October 1974, showed 20-fold resistance to azinphos-methyl; after emergence in the spring (collected March, 1975, tested when out of diapause) the factor was 16-fold. The same population collected in August 1975 showed 12-fold resistance. Pesticide usage in New York State is more intensive than in England, reflecting higher summer temperatures and more species of 'key' pests which can cause direct damage to fruits (Glass, 1975) but usage is probably less intensive than in New Zealand. The levels of resistance found appear to reflect the intensity of use.

In the present study, it is notable that the MP9 stock (of New Zealand origin) and native stocks had similar levels of resistance. The MP stock (imported in 1977) was taken from a district in New Zealand where high levels of resistance had been recorded but the level of resistance for the sample was not assessed either before despatch or after arrival in England. It is, however, known that 2 yr after the releases in 1977 the population on Middle Park orchard had a relatively low resistance; Aliniazee and Cranham (1980) recorded 80% reduction in numbers in 1979 from a single pre-blossom spray of azinphos-methyl + demeton-S-methyl sulphone. In the post-blossom period it was evident (J.E. Cranham _in_ Flegg, 1980) that two to three sprays of azinphos-methyl disrupted predation of _P. ulmi_. It seems probable that the released mites had

hybridised with susceptible native T. pyri and/or that, under relatively low selection pressure, the level of resistance had declined. Numerous genetic studies of OP-resistance in spider mites, reviewed by Helle and Overmeer (1973) and similar studies on the phytoseiid A. fallacis (Croft, Brown and Hoying, 1976), showed that resistance to OPs was controlled by a single major gene, dominant or semi-dominant in expression. Also, in spider mites, the level of resistance to individual OPs can usually be increased, under specific selection, by minor gene effects. A reasonable hypothesis, therefore, for the genetic background to resistance in T. pyri is that a single major gene confers low-level resistance to azinphos-methyl and a high level to parathion (recent work of Overmeer and van Zon, 1983, involving selection in the laboratory and genetic analysis, showed that parathion resistance in T. pyri is based on a single semi-dominant major gene which confers a high level of resistance to parathion and a very low level to azinphos-methyl), but that the level to azinphos-methyl can be increased by intensive selection with that pesticide. If selection is relaxed, such high levels may not be 'stable', but the low level due to the major gene probably is. The results obtained for the WF stock suggests such stability.

Further to this study Cranham, Kapetanakis and Fisher (1983) reported the results of laboratory tests and field trials, involving nine OPs and carbaryl, on the tolerance of T. pyri from unsprayed orchards, and the

resistance and cross-resistance pattern of T. pyri from commercial orchards.

4.4.2 Comparisons with other phytoseiid species

The comparisons developed for T. pyri in Table 4.6 have been extended to other species in Table 4.7.

As Croft (1982) noted: 'interspecific differences within the family (Phytoseiidae) are as variable as those of their tetranychid prey'.

With T. occidentalis, Croft and Jeppson (1970) compared two stocks from unsprayed and two from sprayed orchards (ex Washington, Utah and California); the two unselected strains susceptible to azinphos-methyl (Az-S) and the two strains resistant to azinphos-methyl (Az-R) were equally tolerant to parathion, and susceptible to carbaryl. This species appears to be inherently tolerant to parathion (Croft, 1982), as observed by Huffaker and Kennett (1953) working on strawberry in California, when the use of parathion and other OPs first began. The data of Croft and Stewart (1973) (Table 4.7) also confirm that high resistance to azinphos-methyl does not per se confer resistance to carbaryl. Roush and Hoy (1980) obtained a stock of this species with increased resistance to carbaryl by laboratory selection; the resistance level is not easily quantified from their results but was probably less than 10-fold. Croft and Aliniazee (1983) tested (1) T. occidentalis and the equally common Typhlodromus arboreus Chant from a commercial apple orchard in Oregon

Table 4.7 Comparison of LC_{50}s (mg/litre) from the literature on the responses of five species of phytoseiids to two OPs and carbaryl in bioassays using the taped-slide technique, 24 h assessment, except when noted otherwise

Species:authors	Resistance status	Azinphos-methyl	Parathion	Carbaryl
Typhlodromus occidentalis:				
Croft and Jeppson (1970) (48 h)	Az-S	20,30	5100,5400	310,350
	Az-R	1760,2031	5400,5800	210,520
Croft and Stewart (1973) (leaf disks, 48 h)	Az-R	10 920	-	78
Croft and Aliniazee (1983) (48 h)	OP-R	1150	-	1150
Typhlodromus arboreus:				
Croft and Aliniazee (1983) (48 h)	OP-S	110	-	270
	OP-R	800	-	550
Amblyseius andersoni:				
Croft and Aliniazee (1983) (48 h)	OP-S	22	-	150
Amblyseius fallacis:				
Croft and Hoying (1975)	OP-R	-	-	54-68
	OP-R	-	-	295*
Rock and Yeargan (1971)	OP-S	42	48	-
	OP-R	1205,3495	2658,2766	-
Croft, Brown and Hoying (1976) (48 h)	OP-S	11	4	-
	OP-R	810	670	-
Croft, Wagner and Scott (1982) (48 h)	OP-S	70	-	90
	OP-R	1100-2100	-	72-196
Amblyseius potentillae:				
Kapetanakis and Cranham (1983)	OP-S	56	-	410

* From an orchard with a history of heavy carbaryl spraying
A = azinphos-methyl; OP = organophosphate; R = resistant; S = susceptible

with a history of several sprays per year for 20 yr of OPs and carbamates (including azinphos-methyl and carbaryl), and (2) T. arboreus and Amblyseius andersoni Chant from an unsprayed orchard. T. occidentalis and T. arboreus from the commercial orchard showed similar levels of resistance to azinphos-methyl and carbaryl (Table 4.7). Differences between azinphos-methyl LC_{50} values for T. arboreus from the commercial orchard vs the unsprayed orchard were c 7- to 8-fold, indicating a moderate level of resistance to this compound; but the authors were not sure whether the response of T. arboreus from the unsprayed orchard constitutes a susceptibility 'base-line' for this mite. A. andersoni from the unsprayed orchard was 30- to 60-fold more susceptible to azinphos-methyl than either resistant Typhlodromus species and 5-fold more susceptible than the susceptible strain of T. arboreus. These authors found that the curves for responses to carbaryl were very flat, in agreement with curves shown in this Thesis (Figs 4.2 and 4.4) and with data of Roush and Hoy (1980).

With A. fallacis, the data of Rock and Yeargan (1971) and that of Croft, Brown and Hoying (1976) show that this species is not inherently tolerant of parathion and the development of OP-resistance in the field resulted in a high level to both azinphos-methyl and parathion. Resistance to OPs did not confer cross-resistance to carbaryl (Croft, Wagner and Scott, 1982), but where there was intensive use moderate resistance developed to this pesticide (Croft, 1982). Croft and Hoying (1975) tested

several OP-resistant stocks from orchards in Michigan and were able to develop a moderate level of resistance after prolonged selection.

No developed resistance has been recorded for A. potentillae and tests in the present study confirmed the stock was susceptible to azinphos-methyl and to carbaryl.

No other reports were found which compared stocks of T. pyri susceptible and resistant to carbaryl. The data of Watve and Lienk (1976) for an OP-resistant stock show an extremely high resistance to carbaryl (Table 4.6). A review by Croft (1982) states that carbaryl 'is almost non-toxic to T. pyri'. If the result obtained with the Bangor stock represents 'base-line' susceptibility, T. pyri is indeed rather more tolerant than the above three species but it has been shown (J.E. Cranham, unpublished) that the level of tolerance in native OP-susceptible T. pyri did not prevent some reduction of a field population after a carbaryl spray.

It would appear that T. pyri has easily developed very high levels of resistance to carbaryl, and that this has occurred in England, New York State, New Zealand (Wearing and Ashley, 1978) and The Netherlands (Gruys, 1980). Croft (1982) further states that 'there is no documented cross-resistance between carbaryl and azinphos-methyl in any phytoseiid species'. The results reported here for T. pyri accord with a hypothesis of cross-resistance between OPs and carbaryl; however, they do not prove it,

since both chemical groups have been widely used in English commercial orchards for c 25 yr, as they have been more widely in Europe, N. America and Australasia. Recent work of Overmeer and van Zon (1983) on genetic analysis of parathion resistance in T. pyri indicated that resistance to parathion confers cross-resistance to carbaryl in this mite. Further investigation of this topic might require more genetic and/or biochemical studies.

4.4.3 Responses of T. pyri to permethrin

In the bioassays with permethrin in the present study the LC_{50} values for OP-resistant and OP-susceptible stock of T. pyri were of a similar order, i.e. there was no clear indication of cross-resistance, positive or negative.

Some English orchards have been sprayed with synthetic pyrethroids for 4-5 yr; on these plots very few T. pyri, often none, can be found (M.G. Solomon in Flegg, 1983). Hence, field observations so far do not suggest the development of resistance to SPs in England.

The status of development and management of pyrethroid resistance in other orchard-inhabiting phytoseiids were reported by Hoy, Westigard and Hoyt (1983), and Croft (1983).

4.4.4 Comparison of three bioassay techniques

Comparing results with the three bioassay techniques (Table 4.4, Fig. 4.4 and Appendix 4.4) there were large

differences between the LC_{50}s, in the same order for all three pesticides: taped-slide> leaf/residue> glass/residue. Experimental work to seek explanation for these differences was not within the scope of this study. Hartley and Graham-Bryce (1980) have reviewed the topic of pick-up of pesticides from residues on different surfaces. It seems probable that on glass virtually all the pesticide would remain 'available' for pick-up by mites walking over the surface. On a leaf surface, some of the pesticide would be 'unavailable' in micro-crevices of the surface structure and some absorbed in the waxy components of the leaf cuticle (see also section 3, '3.4 Discussion' of this Thesis). It is more difficult to suggest possible explanations for the higher concentrations required in the taped-slide tests. The rate of penetration of the pesticide to the site of toxic action may be reduced if mites are relatively inactive, i.e. held on their backs as in the taped-slide tests; alternatively, penetration through the tarsi may be an exceptionally efficient route of entry which is minimised in this test. Experiments could be devised to investigate such aspects.

Possibly the leaf/residue technique approximates to field conditions more closely than either the taped-slide or glass/residue techniques; however, no bioassay technique is fully adequate for predicting field results and the information must be extrapolated with considerable caution and experience. In the field, weather conditions, the complex of life stages present, and behavioural aspects, may all affect the result. The purpose of bioassays was

to compare the responses of different stocks to pesticides and it is notable that this comparison (R2, Table 4.4) was of the same order for all three techniques.

'Run-off' of mites when exposed to pyrethroid residues, as observed on T. pyri in this study, was reported by Penman, Chapman and Jesson (1981): when they exposed A. fallacis, T. occidentalis and T. urticae to fenvalerate residues, mites 'ran off' and were trapped in the arena barriers in increasing numbers with increasing pesticide concentrations. Further observations on this effect are also reported and discussed in sections 5 and 6 of this Thesis.

SECTION 5

EFFECTS OF THREE INSECTICIDES ON CERTAIN ASPECTS OF REPRODUCTION IN *TYPHLODROMUS PYRI*

5.1 INTRODUCTION

The effects of sub-lethal dosages of insecticides on arthropods were reviewed by Moriarty (1969), but almost exclusively for pest species. Giles and Rothwell (1983) reviewed the sub-lethal effects of amidine insecticides on insects and acarids. Franz (1974), Croft and Brown (1975), and Croft (1977) reviewed the limited amount of existing information on the effects on arthropod natural enemies of agricultural pests. All these authors stressed that the reports they reviewed referred largely to the most obvious effects, because the sub-lethal effects of insecticides are often subtle in expression and may require special test procedures for evaluation. Sub-lethal effects on natural enemies are of four types: (a) on reproduction, (b) on developmental rates, (c) on longevity and (d) on behaviour related to predation or parasitism.

This section (5) is concerned with the effects on certain aspects of reproduction. Many authors have reported that certain pesticides can stimulate the reproduction of phytophagous pests, including spider mites (Dittrich, Streibert and Bath, 1974; Hoy, Roush, Smith and Barclay, 1979; Leigh and Wynholds, 1980; Boykin and Campbell, 1982; Maggi and Leigh, 1983; Ahmadi, 1983; other authors reviewed by Bartlett, 1968, van de Vrie, McMurtry and Huffaker, 1972, and by Besson, Joly and Touzeau, 1978), and various insect pests (Abdel-Salam and Nasr, 1968; Parry and Ford, 1971; Esaac, El-Grogary, Abdel-Fatah and Ali, 1972; El-Lakwah and Abdel-Salam, 1974; Ball and Su, 1979; Chelliah,

Fabellar and Heinrichs, 1980; Gordon and McEwen, 1984). With natural enemies, although stimulation of reproduction was sometimes observed (Fleschner and Scriven, 1957; Attalah and Newson, 1966), most authors reported inhibition, e.g. DDT on Syrphus spp. (Way, 1949), carbaryl on Bracon hebetor (Grosch, 1975), malathion on Menochilus sexmaculatus (Parker, Sook Ming, Peng and Singh, 1976). With phytoseiids, depression of oviposition rates associated with sub-lethal doses was reported by Swirski, Amitai and Dorzia (1967), Nakashima and Croft (1974), and Hislop and Prokopy (1981).

The importance of the effects of pesticides on the reproduction of beneficial arthropods was realised by the member-researchers of the Working Group 'Pesticides and Beneficial Arthropods' of the International Organisation for Biological Control/Western Palaerctic Regional Section (IOBC/WPRS). Records of egg production constitute a major part of the standardised test methods currently developed by the Group for the evaluation of the adverse effects of pesticides on natural enemies of pests (Hassan et al., 1983). Of these workers van Zon and Wysoki (1978), van Zon and van der Geest (1980), Overmeer and van Zon (1981, 1982) and Samsøe-Petersen (1983) estimated the effects of many pesticides on various phytoseiid species. The reduction in total egg production can be used as a measure of the overall harmful effect of a pesticide, because it has been established that egg production in phytoseiids closely reflects their predatory 'capacity' (Bravenboer and Dosse, 1962; Ford and Giboney, 1980; Sabelis, 1981).

The studies reported here concentrated on certain effects on the reproduction of T. pyri of residues of azinphos-methyl, carbaryl and permethrin on glass and on apple leaf surfaces. A pesticide-resistant and a susceptible stock of T. pyri were exposed to the pesticide residues and fecundity (egg production) and egg hatch were determined. The two stocks were also compared for fecundity and egg hatch under pesticide-free conditions to determine whether the resistant stock was at a 'selective disadvantage' (Georghiou and Taylor, 1977a and 1977b). McEnroe and Naegele (1968) reported a case of reduced fitness linked to pesticide resistance; Ascher (1960) and Keiding (1967) reviewed similar reports. In some cases reduced fitness was due to lower reproductive rates.

5.2 MATERIALS AND METHODS

5.2.1 Stocks of T. pyri

Stocks described in section 4 were used: 'MP4', with low resistance to azinphos-methyl and high resistance to carbaryl, and 'Amsterdam', the second most susceptible stock to these two pesticides. 'Amsterdam', but not 'MP4', had been tested previously with permethrin; all stocks reported in section 4 showed similar susceptibility to this pyrethroid. 'Amsterdam' females reared on T. urticae produced c 3 times as many eggs as those reared on broad bean pollen (see 5.3.1 in 'Results'). Therefore, only mites previously reared on T. urticae were used in all experiments on reproduction, but during these experiments they were fed on pollen. When T. urticae are used as food, their webbing and debris render observation and counts very difficult.

5.2.2 Insecticides

Unformulated insecticides (technical grade) were kindly provided by the following: azinphos-methyl by Bayer UK Ltd, carbaryl by Union Carbide UK Ltd, and permethrin by Rothamsted Experimental Station, Harpenden, Herts, U.K.

To determine the highest sub-lethal level of residues, a small range of concentrations was tested for each pesticide. A concentration expected to cause very low mortality (i.e. LC_{10}) over the testing period was used as

the top of each range. This was estimated from residue/mortality data of bioassays reported in section 4. Stock solutions were made in acetone. Dispersions of the appropriate serial concentrations were prepared by diluting stock solutions in water containing 50 mg 'Agral' a.i./litre. ('Agral' contains 90% alkyl phenol ethylene oxide condensate, ex I.C.I., Plant Protection Ltd, UK). The dispersions were sprayed using a Potter tower; controls were also sprayed with pesticide-free 'Agral'-water/acetone dispersion.

5.2.3 Determination of fecundity

T. pyri adult females were continuously exposed to pesticide residues. The materials, experimental procedure, and conditions were the same as for the residue mortality bioassays described in section 4. Five or ten young females were transferred from a young colony to each test arena. Broad bean pollen was provided as food (see 5.2.1 in 'Materials and Methods'). Every second day the number of surviving females and the eggs laid were recorded. The eggs were removed and new pollen was provided. Experiments were replicated and on a few occasions repeated to cover additional parts of the pesticide concentration range, usually with a total of 20-30 females per concentration.

The experiments for comparison of egg production of the two stocks under pesticide-free conditions lasted c 6 weeks, when the females stopped egg-laying. The experiments on the effects of pesticides were discontinued after 12 days.

Wyatt and White (1977) proved for aphids and spider mites that a "logical measure of fecundity" (as far as it affects population increase) is the number of progeny an adult female produces up to the time the progeny start reproducing. They defined this time as the period from birth to first reproduction ('pre-reproductive time'). Their method was utilised here for T. pyri. Approximately 60 eggs, 0-9 h old, from the Amsterdam stock reared on T. urticae were placed on a clean plastic plate (Plate 4.3). They were observed daily, and when the mites hatched, they were fed with broad bean pollen. When they became adult, 15 females were transferred individually to another plastic plate with isolated arenas (Plate 4.1). One male from the original colony was added to each arena. The arenas were observed daily. Fourteen of the 15 females began oviposition before or on the twelfth day, in agreement with data of Overmeer (1981). Hence fecundity was estimated in these experiments over a 12-day period.

5.2.4 Egg hatch

The eggs laid by T. pyri adult females during each period in each arena were removed and transferred to another small arena on a plastic plate (Plate 4.1). This was floating on water in a plastic box with fine gauze to maintain the high level of r.h. (c 80-90%) necessary for successful egg hatch (McMurtry, Mahr and Johnson, 1976; Sabelis, 1981). Arenas were checked at 2-day intervals and the newly-hatched larvae were removed to avoid

cannibalism on the remaining eggs. The number of unhatched eggs was recorded. Over half the eggs had hatched by the second day after their transfer (see 5.3.4 in 'Results') and nearly all of them by the fourth day. Eggs that had not hatched by the tenth day were considered non-viable.

5.2.5 Analysis of data

From the mortality and egg production recorded every two days in the experiments involving pesticide residues, the Net Rate of Egg Production (represented by Re for this study) was computed according to Southwood (1978). An example of the method of calculation of Re is shown in Appendix 5.1.

For comparison of fecundity of the two *T. pyri* stocks, the numbers of eggs laid/mite/day were used and compared using a 't' test.

5.3 RESULTS

5.3.1 Comparison of fecundities of T. pyri reared on T. urticae and T. pyri reared on pollen

The diet on which female T. pyri were reared, before they were used for fecundity experiments, proved very important. Although both kinds of mites were fed on pollen during the egg lay counts, the T. urticae-reared females had a Net Rate of Egg Production (Re) during a 12-day period c 3 times as high (P = 0.05) as the rate for females originally reared on pollen: Re = 9.9 (equivalent to 0.9 eggs/mite/day) and Re = 3.3 (equivalent to 0.3 eggs/mite/day) respectively. Data are shown in a life table form in Appendix 5.1.

5.3.2 Effects of pesticides on the fecundity of T. pyri

Azinphos-methyl and carbaryl. These two insecticides did not reduce the rate of egg production in T. pyri at concentrations which caused low or no mortality. However, Figs 5.1 and 5.2 show that the Net Rate of Egg Production (Re) was lowered by the higher range of azinphos-methyl concentrations, coinciding with reduced survival. To illustrate a possible connection of low Re with low survival, Figs 5.1 and 5.2 (and 5.3, referring to permethrin - see below) include a 'mite survival factor'. This represents live-mite-days during the experiment of 12 days as a percentage of the total possible (i.e. with no mortality), and was calculated from the life tables as

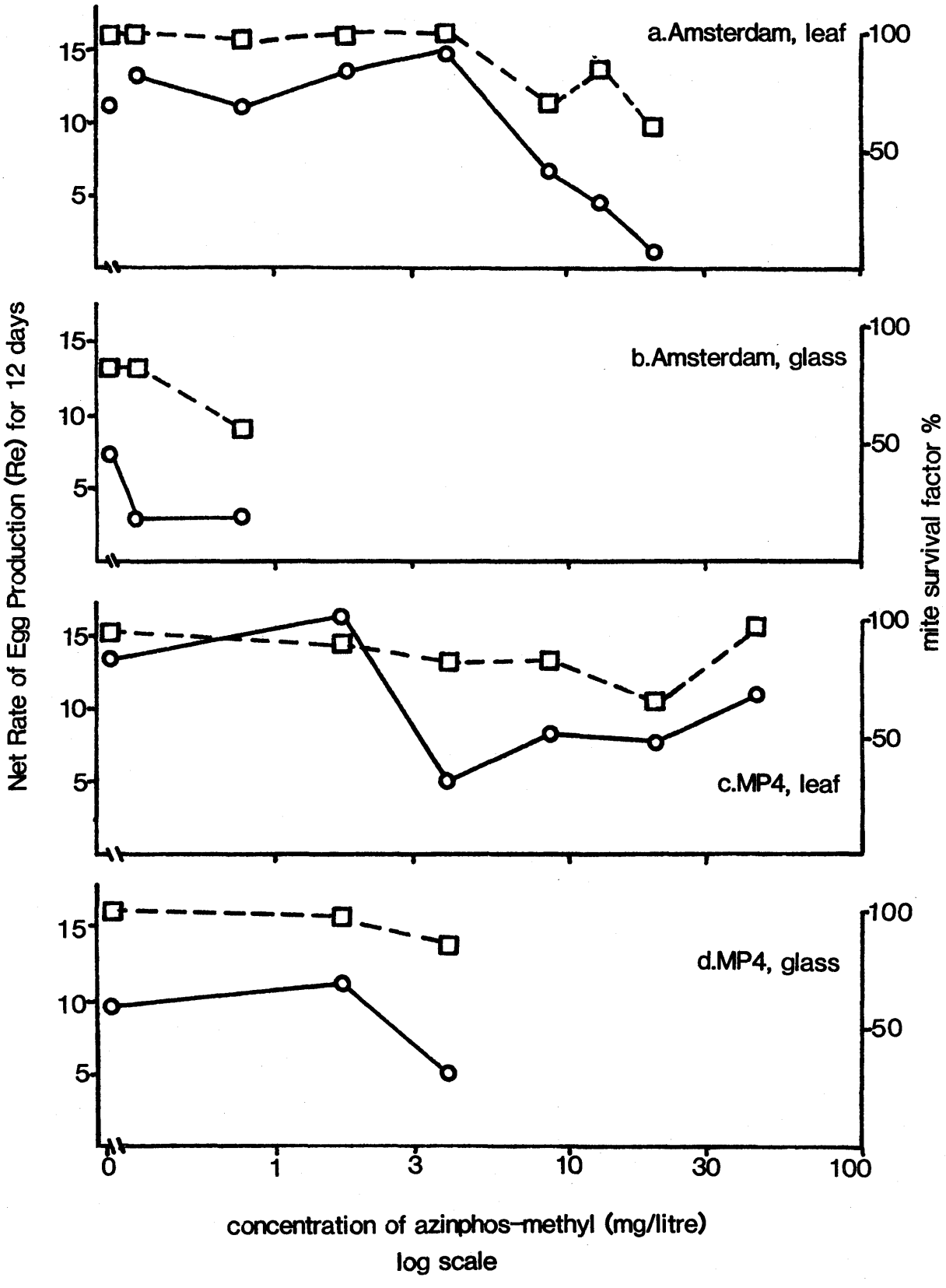


Fig.5.1 Effects of azinphos-methyl residues on the Net Rate of Egg Production of two different stocks of Typhlodromus pyri over a period of 12 days in relation to mite survival during that period.

Net Rate of Egg Production (Re)

Mite survival factor (see text)

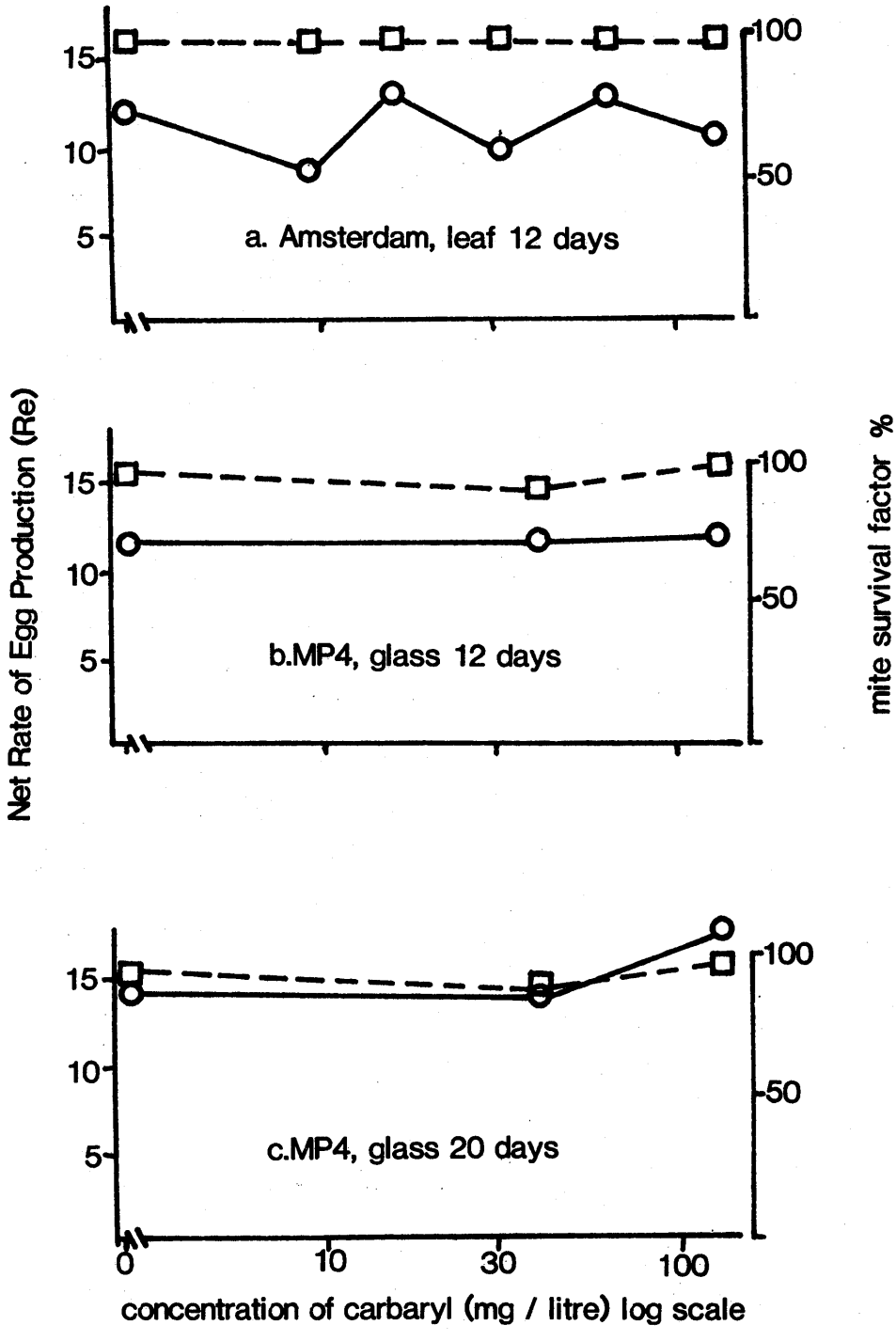


Fig.5.2 Effects of carbaryl residues on the Net Rate of Egg Production of two different stocks of Typhlodromus pyri in relation to mite survival during the oviposition period.

Net Rate of Egg Production (Re)

Mite survival factor (see text)

$[\Sigma 1_x/6]$ x 100 (see Appendix 5.1). For the resistant stock, MP4, mortality and reduction of Re were demonstrated for slightly higher azinphos-methyl concentrations than for Amsterdam, as expected after the results of section 4 (Fig. 5.1). It can be concluded that azinphos-methyl reduced Re only at concentrations causing high mortality. Also, in line with results of mortality bioassays (section 4), residues were more toxic on glass than on apple leaf regarding reduction of oviposition.

Concentrations which caused complete mortality before the end of the 12-day period of observation are not reported here.

Because of shortage of mites the Amsterdam stock was tested with carbaryl only on apple leaf surfaces. MP4 stock was only tested on glass surfaces for the same reason and also because, being highly resistant to carbaryl, it would have to be tested at very high concentrations. It was expected to respond to lower concentrations on glass than on apple leaf surface. Nevertheless the concentrations tested did not cause any reduction of Re either on Amsterdam tested on leaf, or on MP4 tested on glass surfaces (Figs 5.2a and b). The experiment with MP4 on glass was continued to the twentieth day but carbaryl still did not cause any reduction in the Re of this stock (Fig. 5.2c). Mortality of the females both on glass and leaf surfaces was negligible.

Permethrin. Only one stock, Amsterdam, was tested as all stocks of T. pyri available showed similar

susceptibility to permethrin (see section 4). As a total over the 12-day period, permethrin reduced Re at the two higher concentrations on leaf and all concentrations on glass surfaces (Fig. 5.3). Survival was also reduced at 4 mg/litre on leaves and in all treatments on glass surfaces. Higher concentrations than those illustrated in Fig. 5.3 caused total mortality before the end of the observation period. Figs 5.4 and 5.5 show the rate of oviposition vs time in relation to survival for the rest of permethrin concentrations tested. On apple leaf (Fig. 5.4), at 2 and 4 mg/litre, oviposition was decreased at first, but increased again at the end of the first week. Survivors on glass surfaces laid no eggs, apart from a few laid after the end of the first week at the lowest concentration, 0.12 mg/litre.

Other observations. It was noticed that in glass arenas with azinphos-methyl residues, especially at the higher concentrations tested, mite excreta were in a more liquid form than in the controls. Many females were stuck to the glass surface or had many pollen grains adhering to the rear part of their abdomens. This may be connected with the slight reduction in oviposition at those high concentrations.

In contrast, in permethrin-treated arenas there were considerably less excreta spots than in the controls. It was presumed that the permethrin residues had an anti-feedant effect on the mites. Also, in permethrin-treated arenas, mites were always more active, showing a higher locomotion and failure to settle, compared to the controls.

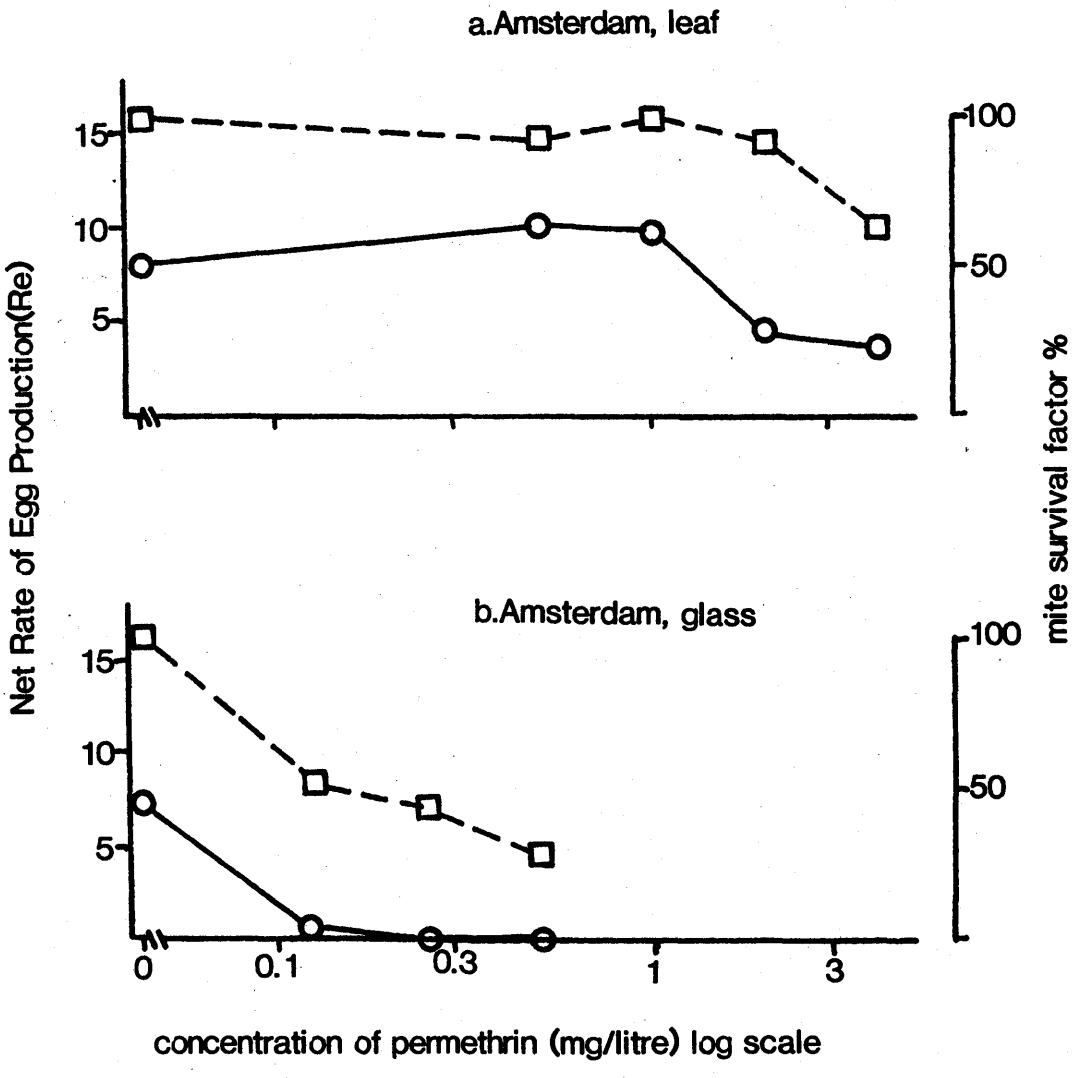


Fig.5.3 Effects of permethrin residues on the Net Rate of Egg Production of Typhlodromus pyri over a 12-day period in relation to mite survival during that period.

Net Rate of Egg Production (Re)

Mite survival factor (see text)

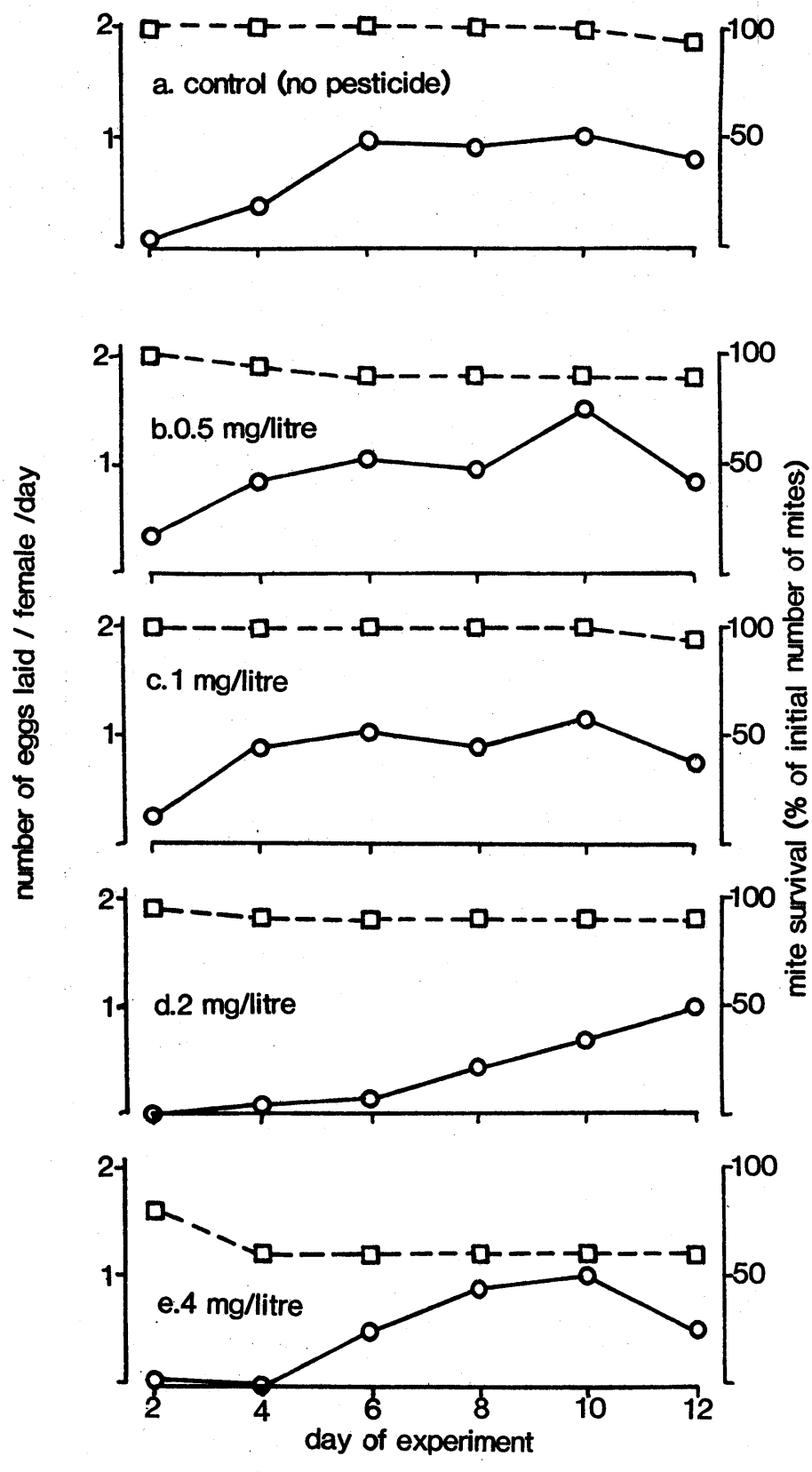


Fig.5.4 Effects on the rate of egg production and survival of Typhlodromus pyri of permethrin residues on apple leaf surface.

Number of eggs laid per mite/day

Mite survival as a percentage of the initial number of mites

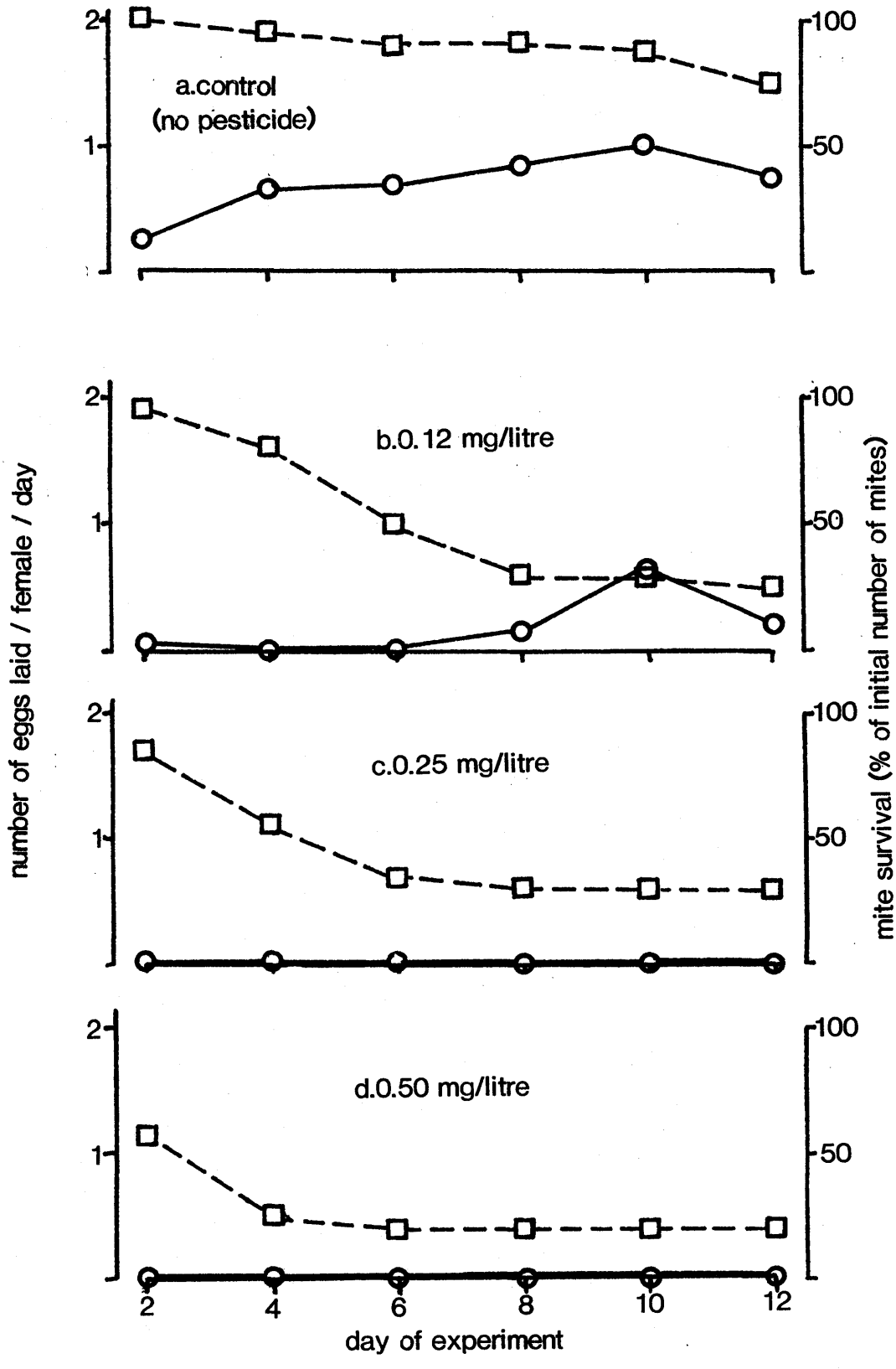


Fig.5.5 Effects on the rate of egg production and survival of Typhlodromus pyri of permethrin residues on a glass surface.

Number of eggs laid per mite/day

Mite survival as a percentage of the initial number of mites

In the case of carbaryl, mites exposed to residues on a glass surface showed a tendency to cannibalise their eggs. Many eggs were eaten in the experiment of MP4 on glass with concentrations of carbaryl 40 and 130 mg/litre, but only a few in the experiments involving azinphos-methyl and permethrin. Detailed counts were not taken.

5.3.3 Egg viability

Under the experimental conditions in this study, egg hatch was virtually always complete. In the course of all these experiments, with a total of c 3500 eggs counted, only seven had not hatched on the tenth day after collection. Individually, these eggs belonged to different treatments, in no case constituting more than 2% of the eggs laid for any one treatment.

5.3.4 Comparison of the two stocks

Fecundity. Fig. 5.6 shows the average daily oviposition per female for the two stocks of T. pyri (pooled results of two experiments). As observed in other experiments of this study, the oviposition rate increased during the first week, perhaps indicating an adaptation period of the mites to their change of diet, i.e. from T. urticae to broad bean pollen (Fig. 5.6, also Figs 5.4a and 5.5a). A peak after the end of the first week was followed by a decline (Fig. 5.6), which occurred earlier with MP4 (resistant) than with 'Amsterdam'. MP4 ceased ovipositing after the end of the fourth week in

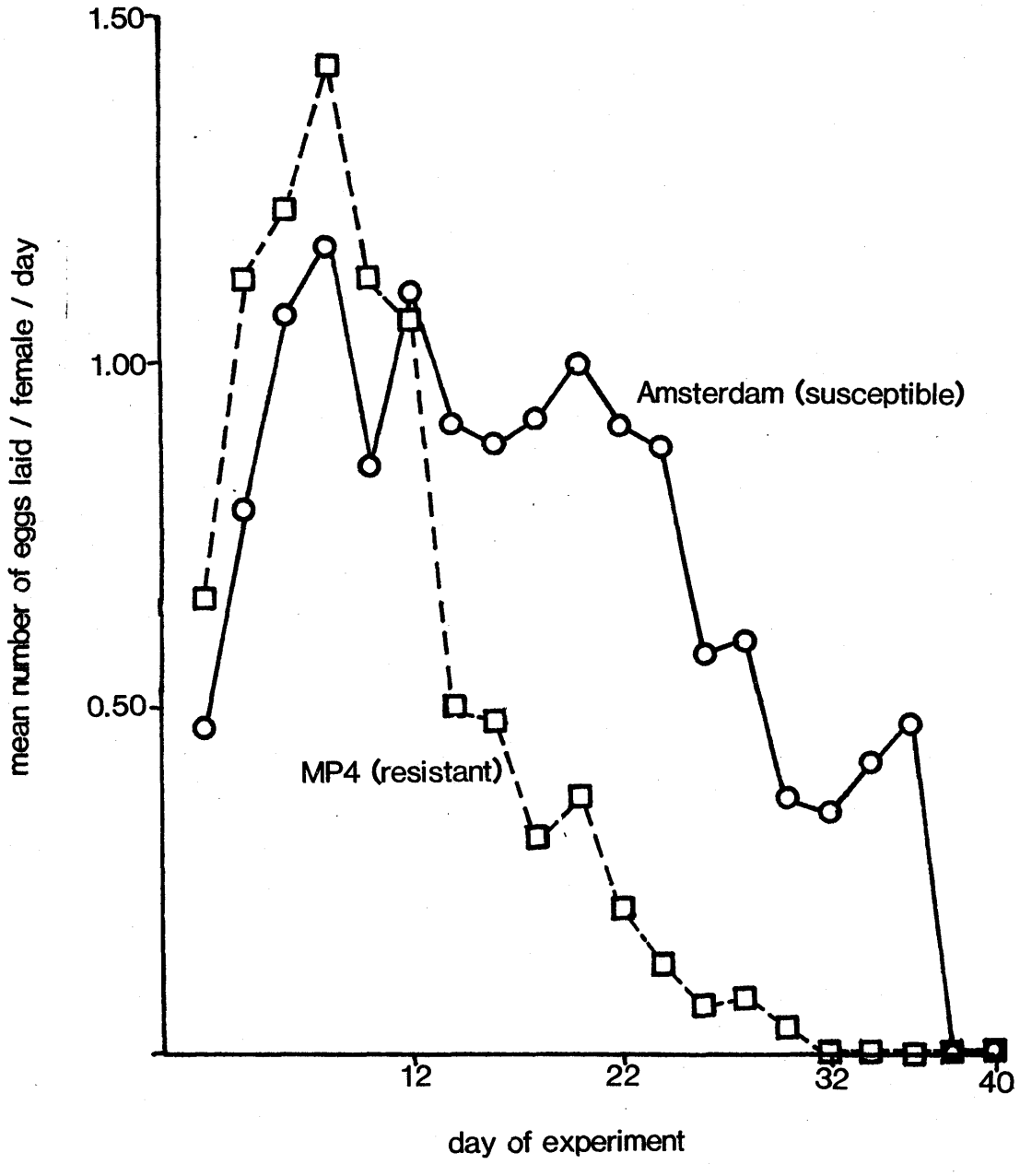


Fig.5.6 Comparison of rates of egg production of a pesticide susceptible and a resistant stock of Typhlodromus pyri.

Pooled data from two different experiments totalling 30 T. pyri females for each stock

both experiments, whilst the susceptible continued laying for a further week. However, the resistant stock had a 22% higher rate of egg production ($\underline{P}$ = 0.05) compared to the susceptible for the first 12 days: 1.1 ± 0.31 and 0.9 ± 0.33 eggs/female/day respectively (means of six replicates ± 95% confidence intervals). Ten days later the average rates for the 22-day period were very similar: 0.8 ± 0.19 and 0.9 ± 0.18 eggs/female/day for the resistant and the susceptible respectively, because the rate of the resistant had steadily declined. By the thirty-second day the susceptible had laid an average of 0.80 (± 0.13) eggs/female/day, 33% more ($\underline{P}$ = 0.01) than the resistant stock (0.6 ± 0.16 eggs/female/day). The total egg production per female, estimated as the sum of age specific fecundities m_x (see Appendix 5.1), was 26.2 ± 4.66 eggs for the susceptible and 17.6 ± 3.20 eggs for the resistant stock ($\underline{P}$ = 0.01), i.e. 49% higher for the susceptible.

Egg incubation. The percentage of eggs hatched two days after they were collected was taken as an indication of the egg incubation period. The eggs of the susceptible hatched slightly earlier ($\underline{P}$ = 0.05) as Table 5.1 shows. The ratio of hatched eggs after two days was approximately 11:10 susceptible to resistant.

Table 5.1 Comparison of the rates of egg hatch of two stocks of *Typhlodromus pyri* (see text for details)

Egg batch no.	Amsterdam stock			MP4 stock		
	Total number of eggs	Number of eggs hatched after 2 days	Percentage eggs hatched	Total number of eggs	Number of eggs hatched after 2 days	Percentage eggs hatched
1	26*	15*	58	37*	20*	54
2	37	20	54	46	24	52
3	44	32	73	54	39	72
4	44	25	57	58	32	55
5	33	28	85	39	29	74
6	40	27	68	41	24	59
7	24	14	58	17	7	41
Totals	248	161		292	175	
Means ± 95% confidence intervals			64.6 ± 10.29			58.2 ± 10.75

* The numbers of eggs are totals of four replicates.

Note A paired 't' test showed that the percentage egg hatch for the susceptible stock was significantly higher for $P = 0.05$

5.4 DISCUSSION

5.4.1 Fecundities of T. pyri reared on two different diets

When the two diets were compared, T. pyri reared on pollen produced on average 0.3 eggs/female/day for the first 12 days of oviposition, the same as reported by Overmeer (1981), whereas T. pyri reared on T. urticae (and fed on pollen during the oviposition experiment) had an egg production rate three times higher, laying 0.9 eggs/female/day ('Results', 5.3.1). The same value was also obtained in the two experiments reported in 'Results', 5.3.4. This high rate, slightly higher than the value reported by Overmeer (1981) for T. pyri reared and also fed on T. urticae throughout the oviposition period (0.7 eggs/female/day), made possible the use of pollen as food for all subsequent experiments on the reproduction of T. pyri. It was not necessary to cope with the low oviposition rate of females reared entirely on pollen or, alternatively, feed the ovipositing females with T. urticae, which would cause difficulties in the counts because of its webbing and debris.

5.4.2 Effects of pesticides on oviposition of T. pyri

Field experiments of van de Vrie (1962) indicated that carbaryl reduced egg production in T. pyri, but the author is not sure about that result now (M. van de Vrie, personal communication). In laboratory tests of Penman, Chapman and Jesson (1981) carbaryl at 1000 mg/litre did

not have any effect on survival or egg production of T. urticae.

In this study the sub-lethal concentrations of carbaryl tested (40 and 130 mg/litre) also did not reduce oviposition of T. pyri, but azinphos-methyl caused a slight reduction at concentrations that also caused a slight mortality. Survivors of permethrin residues in tests that caused high percentage mortality did not lay any eggs. At lower permethrin concentrations oviposition was also low for the first few days but mites then resumed egg laying.

Reissig et al. (1983) tested the effects of azinphos-methyl residues on the oviposition of the apple maggot, Rhagoletis pomonella. They developed a mathematical model showing that the percentage reduction in oviposition was proportional and always slightly higher in comparison to the percentage mortality in the females caused by various dosages of the insecticide. Hislop and Prokopy (1981) also found that residues of azinphos-methyl on bean leaves caused a slight reduction in oviposition of A. fallacis. Overmeer and van Zon (1981) reported that residues of azinphos-methyl at 100 mg/litre on bean leaf surface caused a 50% reduction in the "total reproduction" of T. pyri and A. potentillae, but their results are not directly comparable with those of this study because they included the mortality of the egg-laying females and their progeny in the "total reproduction" assessment. The same authors (1982), testing various pesticides on A. potentillae, reported 100% reduction in the reproduction

of females exposed to residues of permethrin and fenvalerate (another pyrethroid) on glass surface. Again their results are not directly comparable with those of this study, because the concentrations they used, recommended for field application, were very high: 200 mg/litre for permethrin and 750 mg/litre for fenvalerate. Obviously the reduction in oviposition by 100% is the result of mortality of the ovipositing females. With their method and the dosages used any sub-lethal effects of pesticides on oviposition cannot be detected.

The same result, 100% reduction of oviposition by the same two pesticides, was reported by Samsøe-Petersen (1983) for Phytoseiulus persimilis using a leaf residue method. Similarly to Overmeer and van Zon, she used very high concentrations, 50 mg/litre for permethrin and 225 mg/litre for fenvalerate.

The results of Penman, Chapman and Jesson (1981) with leaf/residue tests on T. urticae were similar to those reported here on T. pyri: azinphos-methyl caused only a slight reduction in oviposition of T. urticae but fenvalerate showed a marked inhibitory effect. This effect was only temporary. Their conclusion about fenvalerate appears to conflict with reports on cases of stimulation of spider mite reproduction by pyrethroids (Hoyt, Westigard and Burts, 1978; Riedl and Hoying, 1980). A possible explanation for these seemingly conflicting reports is that pyrethroids may cause an initial reduction in oviposition, which lasts only for a short period, and

is followed by a reproductive rate higher than normal as residues decline. A case of inhibitory and stimulatory effects caused by different sub-lethal doses of insecticides was reported by Mansour (1978) on the cotton leaf worm, Spodoptera littoralis. This aspect needs further investigation.

It was observed in this study that reduced egg laying by T. pyri in permethrin-treated arenas coincided with increased activity and, possibly, reduced feeding of the females. Ruscoe (1977) also reported antifeedant effects of sub-lethal residues of permethrin on the diamond-back moth, Plutella xylostella. Neville (1979) also reported that sub-lethal doses of permethrin reduced oviposition in the rice brown plant hopper, Nilaparvata lugens. Tan (1981) found that permethrin and cypermethrin residues had an irritant and antifeedant effect on larvae of the large white butterfly, Pieris brassicae, which resulted in reduced pupal weight. They concluded that this could affect the fecundity of the females that would subsequently emerge. In bioassays J.E. Cranham found (unpublished data - personal communication) that pyrethroids caused hyperactivity in P. ulmi, resulting in failure to settle for feeding. Penman, Chapman and Jesson (1981) reported that fenvalerate residues had an antifeedant effect on A. fallacis and also caused 'run-off' and trapping of A. fallacis, T. occidentalis and T. urticae in arena barriers. Permethrin residues caused hyperactivity and 'run-off' in T. pyri in residue bioassays reported in section 4 of this Thesis. These observations lead to

the conclusion that the reduction in oviposition of T. pyri exposed to permethrin residues, as shown in this study, may be the result of hyperactivity and reduced feeding caused by this pyrethroid.

5.4.3 Differences between the two stocks of T. pyri

The two laboratory stocks tested showed only slight differences in their oviposition response to azinphos-methyl. This was probably due to their small difference in tolerance to this pesticide (see section 4). Comparison of mite strains with a greater difference in tolerance (resistance) may show reduction in oviposition at concentrations differing considerably for the two strains; but it is likely that for all strains azinphos-methyl will reduce oviposition only at concentrations within the lethal range for each strain.

Under pesticide-free conditions the resistant stock of T. pyri had a higher rate of egg production for the first 12 days of oviposition, i.e. the equivalent of the "pre-reproductive time", which, according to Wyatt and White (1977), is the important part of the reproductive life span. The daily rate of egg production for the resistant decreased steadily after the twelfth day compared to the susceptible, and by the time they both stopped egg laying the total fecundities were at a ratio of 3:2 for the susceptible and the resistant respectively. Also the eggs of the susceptible hatched slightly earlier than the eggs of the resistant.

Hocart (1978) compared an OP-resistant and a susceptible strain of T. pyri for certain aspects of reproduction. Similarly to this study, she found that the resistant had a higher fecundity compared to the susceptible for the first 12 days, but, unlike the results of the respective comparisons here, she reported a slightly higher total fecundity and also a very low viability of eggs (50-80%) for the resistant strain. These contrasting results indicate the danger in drawing general conclusions from results obtained for one or two resistant strains. Results may not prove consistent when several resistant and susceptible strains of different provenance or mode of selection are compared (Ascher, 1960).

Increased reproductive rates have also been found in OP-resistant aphids: by Eggers-Schumacher (1983) in a detailed study comparing several susceptible and resistant clones of the peach-potato aphid, Myzus persicae, and by Lorriman and Llewellyn (1983) comparing susceptible and resistant biotypes of the hop aphid, Phorodon humuli.

A case of reduced reproductive rate was reported by Inoue (1981) working with a dicofol resistant strain of the citrus red mite Panonychus citri (McG.). Computer simulation analysis showed that this resistant strain had a lower value of fitness compared to a susceptible strain.

These differences in reproductive characteristics, being results of laboratory experiments, need confirmation

in the field. Their impact under field conditions may be lower than expected, although even small differences in reproductive potential may result in considerable differences in population levels after many generations, at the end of a growing season. On the other hand, laboratory differences may be invalidated or even reversed under field conditions as in the case (involving two different phytoseiid species) reported by Downing and Moillet (1974). In the laboratory A. fallacis consumed more prey and reproduced faster than T. occidentalis, but when A. fallacis was released in British Columbia orchards it decreased in numbers while the endemic population of T. occidentalis increased.

5.4.4 Implications for Integrated Pest Management

The reduction in fecundity of T. pyri by permethrin and azinphos-methyl, as shown by this study, must be taken into account for assessing the total impact of these pesticides in the application of IPM programmes.

Regarding field releases of resistant strains and the selective spray required before the release to prevent dilution of the resistant genes in the local susceptible population, a reduced reproductive potential in a resistant strain would be an additional reason for the application of the pre-release selective spray and the maintenance of the selection pressure after the release.

5.4.5 Further research

The present study on the effects of three insecticides on the reproduction of T. pyri is by no means conclusive. With reference to topics investigated, it would be interesting to expose T. pyri to higher rates of carbaryl. It was shown in the course of the study that the rates tested for carbaryl were well below the highest sub-lethal dosages that could be tested.

As regards the experimental procedure, (a) a larger number of females of the same age per treatment would produce less variation in the results; (b) individual isolation of the females would make possible a more detailed study of reproductive characteristics; (c) the presence of males in the isolation arenas with T. pyri females is necessary, if the full reproductive potential of the mites is to be expressed, especially in long-term experiments. [After the end of this study, Overmeer, Doodeman and van Zon (1982) reported that during the reproductive life span of T. pyri several copulations are required.]

Aspects of reproduction that were not investigated include possible effects of insecticides on developmental rates and sex ratios in T. pyri.

Such detailed studies on effects of pesticide residues on reproduction, including all the above features, although even more demanding than the present study due to the long duration of experiments and handling of a large number of mites and eggs, would provide valuable

information to complement existing knowledge on acute toxicology of pesticides and predatory mites. But as regards comparison of the reproductive potential of individual resistant and susceptible strains, such studies may yield results of short-term and local interest rather than of general importance. Different resistant strains in different areas may or may not have a low reproductive potential depending on their past history and the time period over which resistance was developed and maintained. Fitness, including reproductive performance, can be improved by natural selection in resistant genotypes, which were initially at a disadvantage compared to the susceptible population (Keiding, 1967).

SECTION 6

BEHAVIOURAL RESPONSES OF TYPHLODROMUS PYRI TO INSECTICIDE DEPOSITS

6.1 INTRODUCTION

In the Introduction of section 5 the sub-lethal effects of pesticides on natural enemies of phytophagous pests were briefly reviewed. Effects on behaviour related to predation or parasitism were mentioned as important.

This section is concerned with avoidance reactions elicited by pesticides, as a result of what is often called 'repellency'. There seems to be some confusion in the literature on the meaning of terms referring to insect behaviour. Many authors have adopted the relevant terms proposed by Dethier, Browne and Smith (1960): (a) arrestant, (b) locomotor stimulant, (c) attractant, (d) repellent and (e) feeding, mating and oviposition stimulant.

The repellent properties of insecticides have long been of interest to economic entomologists because repellency can be of considerable importance in increasing or decreasing the effectiveness of an insecticide against phytophagous pests under field conditions. An insecticide which under laboratory conditions is highly toxic to an insect may prove to be ineffective in the field because of its high repellency which causes the insect to leave the pesticide deposit before it has taken up a lethal dose. On the other hand an insecticide which may be only moderately toxic to a certain pest, or slow in its action, may prove highly effective under field conditions if the absence of repellency permits the insect to pick up a lethal dose before it leaves the pesticide-treated surface. Uniformity of deposit is in general of greater importance in an insecticide with high

repellency characteristics than in one which is relatively non-repellent (Rogoff, 1952).

It is not surprising that there is only a restricted amount of information on the subject of behavioural responses of arthropods to insecticides because of the difficulty of assessing precisely a single behavioural reaction which may be elicited by a variety of stimuli, each of which can produce varying qualitative and quantitative reactions under different sets of conditions (Dethier, Browne and Smith, 1960).

A brief review follows of literature on behavioural effects of pesticides on insects and mites.

6.1.1 Pest species

Published reviews on behavioural responses include those by (a) Perry and Agosin (1974), with relation to insecticide resistance, (b) Giles and Rothwell (1983) referring to sub-lethal activity of amidine insecticides, and (c) Pluthero and Singh (1984) concerned with the evolutionary significance of behavioural responses regarding insecticide resistance, adaptation, speciation and problems of insect control.

With reference to phytophagous mites, Fisher and Hansell (1964) suggested that spider mites may avoid discontinuous deposits of dicofol, particularly where they are widely spaced. Later, Fisher and Morgan (1968) showed that T. urticae was able to avoid large droplets of dicofol and laid eggs between them.

Pyrethroids

Almeida (1967) reported that larvae of the Indian meal moth, Plodia interpunctella, avoided distinct droplets containing natural pyrethrins. Later the synthetic pyrethroids were reported by many authors as irritant, repellent and antifeedant from their early stages of development. Ruscoe (1977) reported that the cotton leaf worm, Spodoptera littoralis, laid more eggs on untreated than on permethrin-treated foliage, the boll weevil, Anthonomus grandis, showed an avoidance reaction following initial contact with permethrin-treated foliage, and sub-lethal residues of permethrin had an antifeedant effect on the diamond-back moth, Plutella xylostella. Jollands (1979) also reported that cypermethrin, but not pirimiphos-methyl (an OP insecticide), had a similar repellent/antifeedant effect on P. xylostella. In experiments of Neville (1979) the rice brown planthopper, Nilaparvata lugens, strongly avoided plants treated with permethrin or deltamethrin. Moore (1980) also found that deltamethrin residues had a repellent effect on A. grandis causing (a) avoidance of or departure from sprayed cotton fields, (b) an antifeedant effect and (c) reduction in oviposition. Tan (1981) reported that residues of permethrin and cypermethrin on leaf surfaces had an antifeedant and irritant effect on larvae of the large white butterfly, Pieris brassicae. Sawicki and Rice (1978) found that the peach-potato aphid, Myzus persicae, avoided contact with potato leaf disks treated with various pyrethroids including permethrin, cypermethrin and deltamethrin. In later tests of Rice, Gibson and Stribley (1983),

more M. persicae walked or flew from excised potato leaves pre-treated with deltamethrin than from untreated leaves. Ferrari (1980) found that field rates of fenvalerate, cypermethrin and permethrin, and to a lesser extent fenpropathrin and deltamethrin, were repellent to M. persicae and the black bean aphid, Aphis fabae. Virgona, Holan and Shipp (1983) reported that pyrethrum, bioresmethrin and permethrin were repellent to many different strains of houseflies.

Glasshouse experiments of Aerts (1978) showed that deltamethrin had a strong repellent effect on T. urticae and kept fully-grown tomato plants practically free of spider mite infestation. Hall (1979) found that pyrethroids had an antifeedant and 'lethargic' effect on the plum curculio, Conotrachelus nenuphar, and elicited an avoidance reaction in this insect as well as on A. grandis; and in choice tests in the laboratory T. urticae fed less and laid a lower number of eggs on the fenvalerate-treated half of a leaf disk compared to the untreated half. In bioassays of Penman, Chapman and Jesson (1981) T. urticae ran off leaf disks and was trapped in barriers around them when the disks were treated with fenvalerate, but not with azinphos-methyl or carbaryl, and avoided fenvalerate-treated surfaces as oviposition sites. In later tests of Penman and Chapman (1983) T. urticae showed a 'spin down' reaction when transferred on fenvalerate-sprayed leaves of bean plants and in another choice test it did not colonize sprayed leaves. Azinphos-methyl did not elicit these effects.

6.1.2 Beneficial species

Authors reporting on integrated control trials often imply that the avoidance of pesticide residues by natural enemies explain the otherwise unaccountable decreases of their numbers, or the poor control of pests despite the presence of a large number of natural enemies after pesticide application. Walker and Penman (1978), and Penman, Wearing, Collyer and Thomas (1979) attributed the erratic control of P. ulmi by T. pyri in apple orchard trials to distributional imbalances in T. pyri numbers caused by sprays of azinphos-methyl. Flaherty and Huffaker (1970) had previously come to a similar conclusion, working with T. occidentalis on vines.

However, little work has been done to check whether spraying phytophagous pests, their natural enemies and their environment, either with non-toxic materials or with toxic materials at non-toxic levels, leads to the prey becoming either less or more readily available and/or acceptable to the natural enemy through possible behavioural changes. Bartlett (1965) estimated the repellency of 60 pesticides to adults of 2 hymenopterous parasites and 2 coccinellid predators. Dabrowski (1969) reported several pesticides to be repellent to the phytoseiid predators Typhlodromus finlandicus (Oud.), Phytoseiulus macropilis (Banks) and T. pyri, but his results were not confirmed by Hobart, Ford, Kirkby and Jackson (1970). Jackson and Ford (1973), investigating some aspects of the feeding behaviour in Phytoseiulus persimilis, found that eggs of T. urticae treated with malathion were strongly repellent to the

predator, and also eggs treated with captan were less acceptable than pesticide-free eggs. Halstead (1970) did similar work but failed to confirm some of their results.

In laboratory tests of Hislop, Auditore, Weeks and Prokopy (1981) residues from sprays at field rates of phosmet, azinphos-methyl, captan and, to a lesser extent, endosulfan had repellent effects on A. fallacis females, including reduced consumption of T. urticae eggs, reduced frequency of A. fallacis presence and/or reduced oviposition on treated sites. Penman, Chapman and Jesson (1981) also found that A. fallacis consumed less T. urticae eggs treated with fenvalerate than untreated eggs. Following feeding on fenvalerate-treated eggs, all the predators were trapped and killed in the restricting barriers. Similarly, T. occidentalis avoided fenvalerate but not azinphos-methyl residues on glass surfaces and a high proportion of the mites were trapped in the barriers.

Many questions remain unanswered, but sufficient is known to suggest that pesticide applications at sub-lethal rates may have important effects on predator-prey interactions through behavioural changes in the presence of pesticide residues. These may simply retard satisfactory spread of the predators, whereas broken spray deposits may hinder searching ability. However, it is highly probable that in many instances the effect is complex, altering in subtle ways both the environment and the behaviour of interacting organisms (Hussey and Huffaker, 1976).

The studies reported in this section are concerned with behavioural effects of insecticide deposits on T. pyri. Most tests involved permethrin, a member of the synthetic pyrethroid (SP) group of insecticides, which are known to have pronounced effects on the behaviour of various arthropods. Azinphos-methyl, with conflicting reports on its behavioural effects (see above), and carbaryl were also tested. T. pyri females were tested:-

(i) for their ability to avoid deposits from discrete droplets of lethal permethrin concentrations and

(ii) in 'choice tests' for their preference between
(a) glass surfaces treated with permethrin concentrations that caused partial or no kill and
(b) untreated surfaces.
Sub-lethal concentrations of azinphos-methyl and carbaryl were also tested with this technique.

(iii) In other 'choice tests' on apple leaf disks, permethrin-treated and control surfaces were compared for the distribution of T. pyri females and the number of eggs laid.

6.2. MATERIALS AND METHODS

6.2.1 Mites

T. pyri adult females from a pesticide-susceptible stock, Amsterdam, bred on broad bean pollen were used (see section 4, 'Materials and Methods').

6.2.2 Insecticides

Unformulated active ingredients of technical grade were used for azinphos-methyl, carbaryl and permethrin. Their origin and preparation of solutions (dispersions) were described in section 5 'Materials and Methods'. To ensure consistent deposits 'Agral' was added to all solutions at 200 mg a.i./litre for glass and 50 mg a.i./litre for leaf surfaces.

6.2.3 Experimental procedure

Four different tests were carried out to investigate different aspects of behavioural effects. The methods are summarised in a comparative form in Fig. 6.1. In all tests observations were made under a binocular microscope with two cool light sources at diametrically opposite positions over the circular test arenas. All tests were carried out under room temperature, c 20 ± 2°.

6.2.3.1 Test 1

Objective: to test the repellency of permethrin deposits to T. pyri females.

Method. The type of glass arena devised for the residue/mortality bioassays was also used here (section 4, 'Materials and Methods'). One µl droplet of a permethrin dispersion

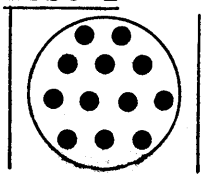
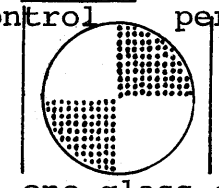
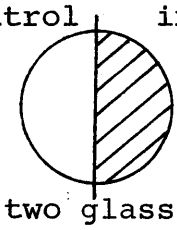
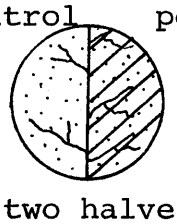
DIAGRAM	SUBSTRATE	BARRIERS	PESTICIDE/APPLICATION
Test 1  one glass slide discrete large droplets 2.2 mm diam.	glass	wet filter paper	Permethrin 1000 mg/litre 'Agral' 200 mg/litre Methyl blue 1mg/litre Droplets of 1 $\mu\ell$ delivered by micro-applicator, one per 25 mm^2
Test 2 control permethrin  one glass slide covered with small droplets 0.85 mm diam.	glass	wet filter paper	Permethrin 2 mg/litre 'Agral' 200 mg/litre Droplets of 0.1 $\mu\ell$ delivered by micro-applicator, one per mm^2
Test 3 control insecticide  two glass slides sprayed separately	glass	talc	Permethrin 0.5 mg/litre or azinphos-methyl 0.3 mg/litre or carbaryl 20 mg/litre 'Agral' 200 mg/litre Sprayed in Potter tower
Test 4 control permethrin  two halves of a leaf disk sprayed separately	apple leaf	wet filter paper	Permethrin 0.5 mg/litre 'Agral' 50 mg/litre Sprayed in Potter tower

Fig. 6.1 Summary of the experimental methods used in tests on the effects of insecticide deposits on behaviour of Typhlodromus pyri.

at 1000 mg a.i./litre (LC_{50} for 24 h for this stock of T. pyri was 0.64 mg/litre - see section 4, 'Results') were delivered on a 19 mm circular glass arena using an Arnold Microapplicator, ex Burkhard Manufacturing Co. Ltd., Rickmansworth, Herts., U.K. The dispersion also contained 1 mg methyl blue/litre to make the dry deposits visible under the microscope. In preliminary tests methyl blue did not have any effect on T. pyri even after long exposure to higher concentrations. One droplet of the permethrin dispersion was delivered per 25 mm^2 (Fig. 6.2) leaving a dry deposit of c 2.2 mm diameter. The distance between the circular deposits was 2.8-3.4 mm; the dimensions of T. pyri females were 0.40 x 0.25 mm. Therefore, the mites had ample space to manoeuvre between the deposits.

After the deposits had dried one T. pyri female was transferred to the centre of the arena. Its behaviour was constantly observed under the microscope and its walking pattern was recorded at a scale of 1:5, using a drawing tube attached to the microscope (Fig. 6.2). The test was repeated 5 times using 5 different mites on the same arena.

6.2.3.2 Test 2

Objective: to test the avoidance by T. puri of a glass surface covered with permethrin deposits at 2 mg/litre.
Method. A 'split arena' (or 'choice chamber') technique (Kennedy, 1977) was employed. Regarding the theoretical background of this technique, Kennedy (1947) stated: "For practical purposes most workers would agree that a surface is repellent if insects are found to spend less time, and so occur in smaller numbers, on it than on other available

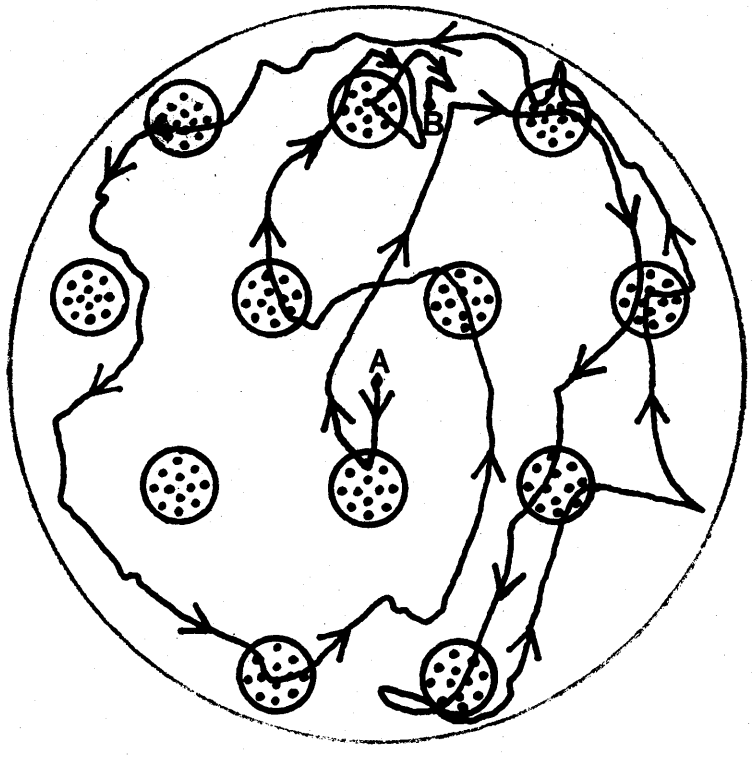


Fig. 6.2 Testing the avoidance of permethrin deposits by Typhlodromus pyri: schematic diagram showing the layout of permethrin droplets in the glass arena and the route a T. pyri adult female followed.

A : point where mite was placed, start of test

B : point where mite stopped walking and died

droplet of permethrin solution, volume 1 microlitre

Magnification c 1:5

and comparable surfaces". Busvine (1971) describes the 'choice chamber' as an arena divided into two halves, one of which is expected to be attractive or repellent and the other neutral, and this is decided by the observed distribution of the insects at intervals. He assumes that the basic response reflected by the distribution of insects is negative orthokinesis, i.e. the insects come to rest on the most favoured (or least unfavourable) part of the chamber.

The particular technique used for this test had been previously employed in a similar form by Chamberlain (1959). Two opposing quarters of a circular glass arena were covered with 0.1 µl droplets of a permethrin dispersion at 2 mg/litre. (In glass/residue tests reported in section 4 this concentration caused 100% mortality in 24 h, being c 3-fold the LC_{50} for this stock of T. pyri). An Arnold micro-applicator was used to deliver 1 droplet/mm^2. This was achieved by placing graph paper under the glass arena and delivering the droplets under the microscope (Fig. 6.1). The remaining 2 quarters of the arena were similarly treated with permethrin-free 'Agral'-water/acetone dispersion. After the droplets had dried (leaving dry deposits of 0.85 mm diameter), 8 T. pyri females were transferred to the centre of the arena and their distribution between the permethrin-treated and the untreated surface was observed and recorded every minute for 20 minutes. Two hours after the transfer, another series of 10 distribution counts started, one count every 25 min for c 4 h. Also 22 h after transfer the distribution of the mites was recorded again 3 times at half-hour intervals. After each count the arena was rotated by an angle of 90° to exclude influence of external factors.

Mites found in water barriers were brought back to the closest arena quarter.

6.2.3.3 Test 3

Objective: to test the avoidance by T. pyri of a glass surface sprayed with permethrin or azinphos-methyl or carbaryl.

Method. 'Split arenas' were used with some improvements compared to Test 2, for better defined boundaries between the treatments and avoidance of mite 'run off' into the wet barriers. One microscope slide, 75 x 25 mm, was sprayed in the Potter tower at 48 kPa (7 p.s.i.) with 2 ml of an 'Agral'-water/acetone dispersion of an unformulated pesticide, producing a wet deposit of c 2.7 mg wet spray/cm^2. One concentration was used from each of the three insecticides, estimated as only slightly lower than the lethal range of concentrations determined in section 4. The concentrations were 0.5 mg/litre for permethrin, 0.3 mg/litre for azinphos-methyl and 20 mg/litre for carbaryl. A second slide was sprayed with the blank (pesticide-free) dispersion. After the two slides had dried they were firmly fixed next to one another, with the sprayed surfaces up, on double adhesive tape at the bottom of a clear rigid plastic box, 125 x 80 x 22 mm (Plate 6.1). A metal ring of 19 mm external diameter was carefully positioned over the slides so that the separating line between the two slides formed a diameter of the ring. Talc ('Talc fine powder', BDH Chemicals Ltd., Poole, England) was put around the outside of the ring with a small spatula and the ring was lifted and removed. With this method an arena was obtained, consisting of one half treated with a pesticide, the other half being pesticide-free. There were

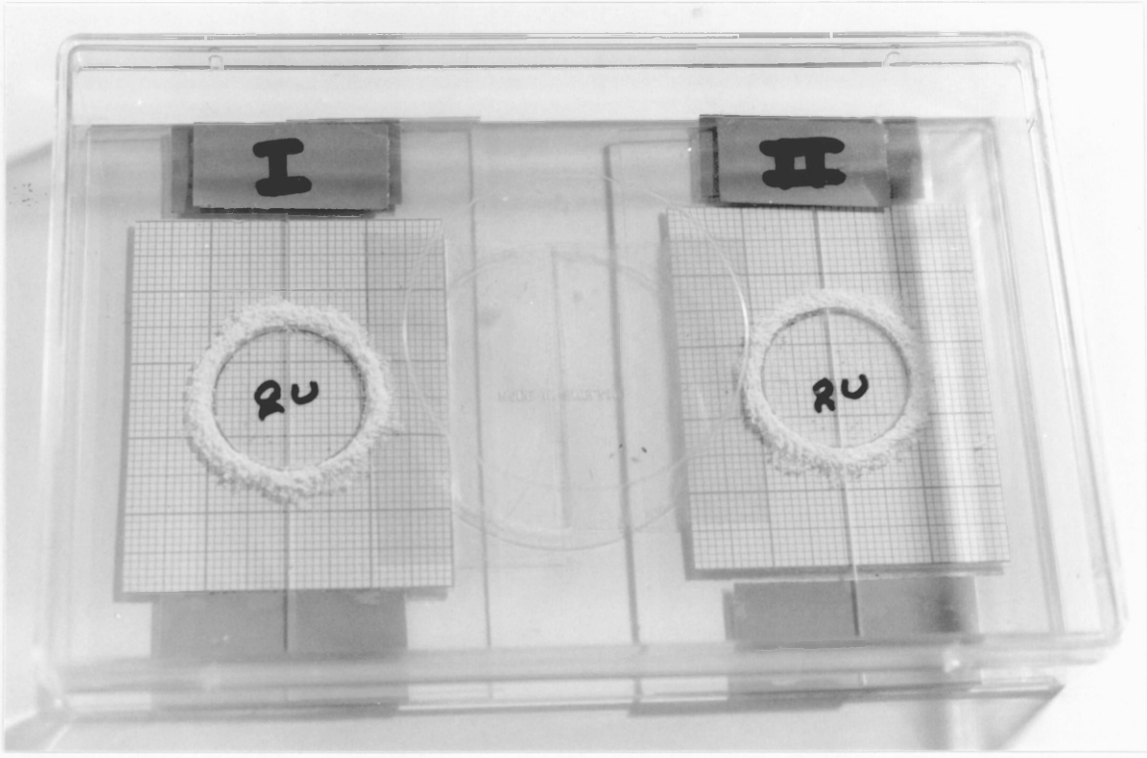


Plate 6.1 Arenas and clear plastic box used for testing the avoidance by Typhlodromus pyri of pesticide deposits on a glass surface.
Approximately actual size.

two arenas - replicates per plastic box (Plate 6.1), one box for each of the 3 insecticides. Ten T. pyri adult females were transferred to the centre of each arena and their distribution between the two halves of the arena was recorded every 10 min for 3 h 20 min. After each count the boxes were rotated by an angle of 180° to avoid influence of external factors. A small amount of pollen was provided at the centre of each arena after this first series of counts and all 3 boxes were kept for observations next day. In the case of permethrin a second series of counts was taken starting c 18 h after the transfer of the mites in the arenas.

6.2.3.4 Test 4

Objective: to test the avoidance by T. pyri females of apple leaf surfaces sprayed with permethrin as resting and oviposition sites.

Method. A similar procedure to Test 3 was followed. A disk, 23 mm diameter, was removed from an apple leaf so that the midrib formed a diameter of the disk. With a surgical blade the disk was dissected along the midrib into two halves of which one was sprayed with a dispersion containing 0.5 mg permethrin and 50 mg 'Agral'/litre, the other with the blank, permethrin-free dispersion. When the two halves had dried they were fixed next to one another, so that they formed a disk again, on double adhesive tape on the lid of a plastic carton of the kind used for leaf/residue and reproduction tests (section 4, Plate 4.5, and section 5).

There were four arenas-replicates. Ten young T. pyri females were transferred in each arena and their distribution

between the two halves of the arena was recorded every 10 minutes for nearly 4 h. After each count the arena was rotated by 180°. Pollen was provided at 4 h. From the next day the distribution of mites and eggs laid on the two halves of each arena were recorded and the eggs were removed. Fresh pollen was provided each day for a week.

6.3 RESULTS

6.3.1 Test 1

All 5 mites used showed the same behaviour and very similar walking patterns in the arena. Fig. 6.2 shows diagrammatically the route followed by one of the mites. None of them seemed to 'hesitate' walking into any deposit patch or make any orientated movements to walk away from them. After a few minutes of observation their speed of locomotion seemed to be lower on the deposits. Their average speed, on and off the deposits, was apparently higher than normal for the first 8-10 minutes of observation and they tended to walk along the periphery of the arena. Later their average speed was constantly reduced until c 30-35 min from the transfer, when their walking co-ordination was lost and signs of poisoning were apparent. This moribund stage lasted c 10-15 min, after which they died.

A few minutes after the start of the test, and especially when each of the mites was on permethrin deposit, they stood on their rear legs, with their body at an angle of c 45-60°, waving the front legs excitedly, which were

straight and extended upwards. This kind of behaviour was observed many times with each mite tested.

6.3.2 Test 2

For the first 20 minutes after the start of the test the mites stayed longer ($P = 0.01$) on the permethrin-treated surface than on the control surface of the arena (Fig. 6.3.A and Appendix 6.1.A). During these counts the mites showed behaviour similar to that observed in Test 1, only to a lesser extent, i.e. increased locomotion at the beginning which decreased later, standing up and moving their extended front legs excitedly.

The two later series of counts, started 2 h and 22 h after the transfer, also indicated that the mites stayed longer on the permethrin-treated than on the control surface of the arena (Fig. 6.3.B and C and Appendix 6.1.B and C); but the differences were not significant due to the small number of mites and observations.

Similarly to Test 1, the mites did not seem to detect the permethrin deposits whenever they walked through the boundary towards the treated surface. Also they did not show any tendency to walk away from the deposit, being rather slower on it, but they tended to walk into the wet barriers. Two mites were found dead at different stages of the Test (see Appendix 6.1). Approximately 24 h from the start of the test no eggs were laid in any part of the arena.

6.3.3 Test 3

Permethrin. In both series of distribution counts, one immediately after the transfer of the mites and the other next day (Fig. 6.4 and Appendix 6.2), a smaller number of

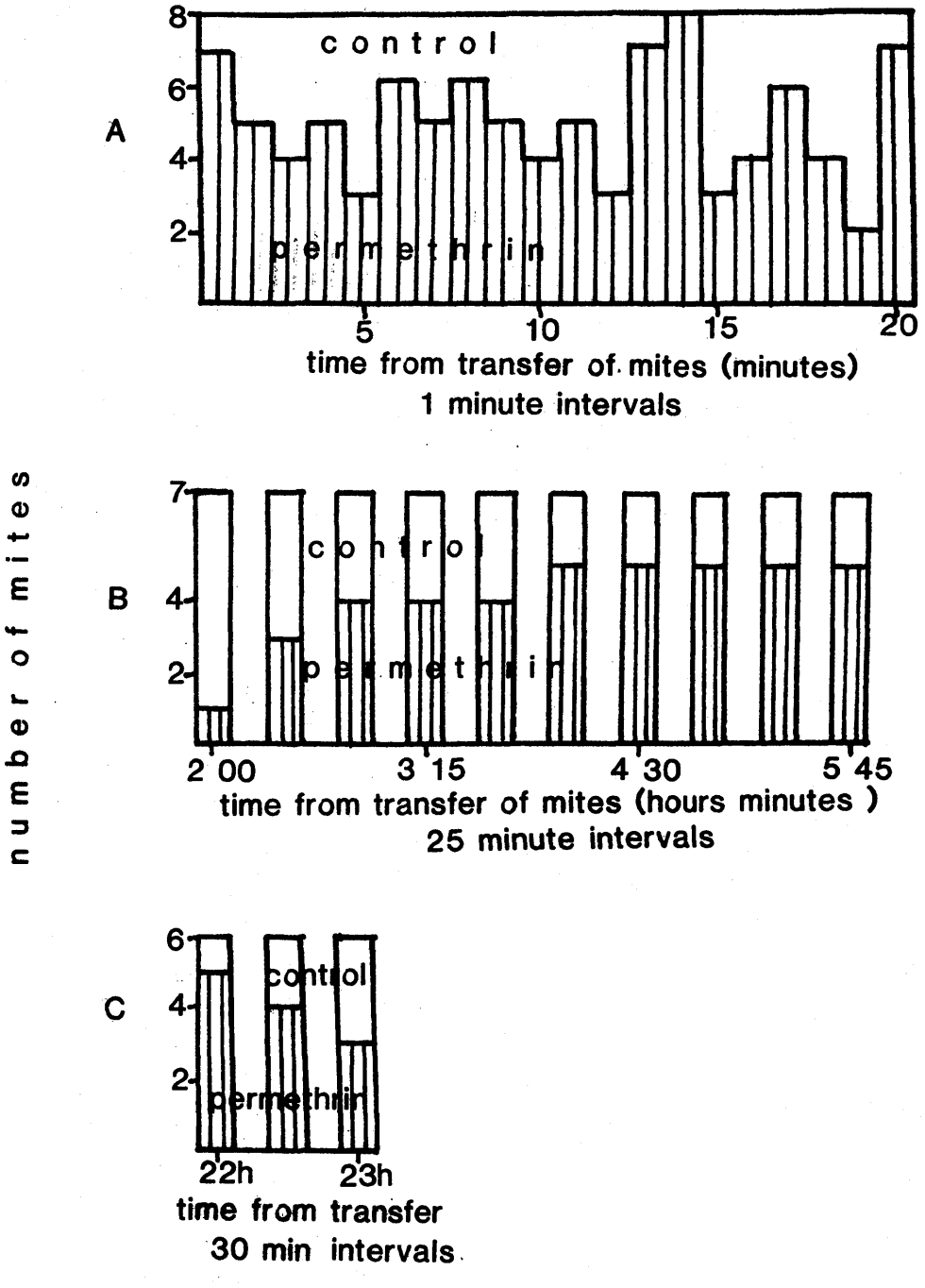
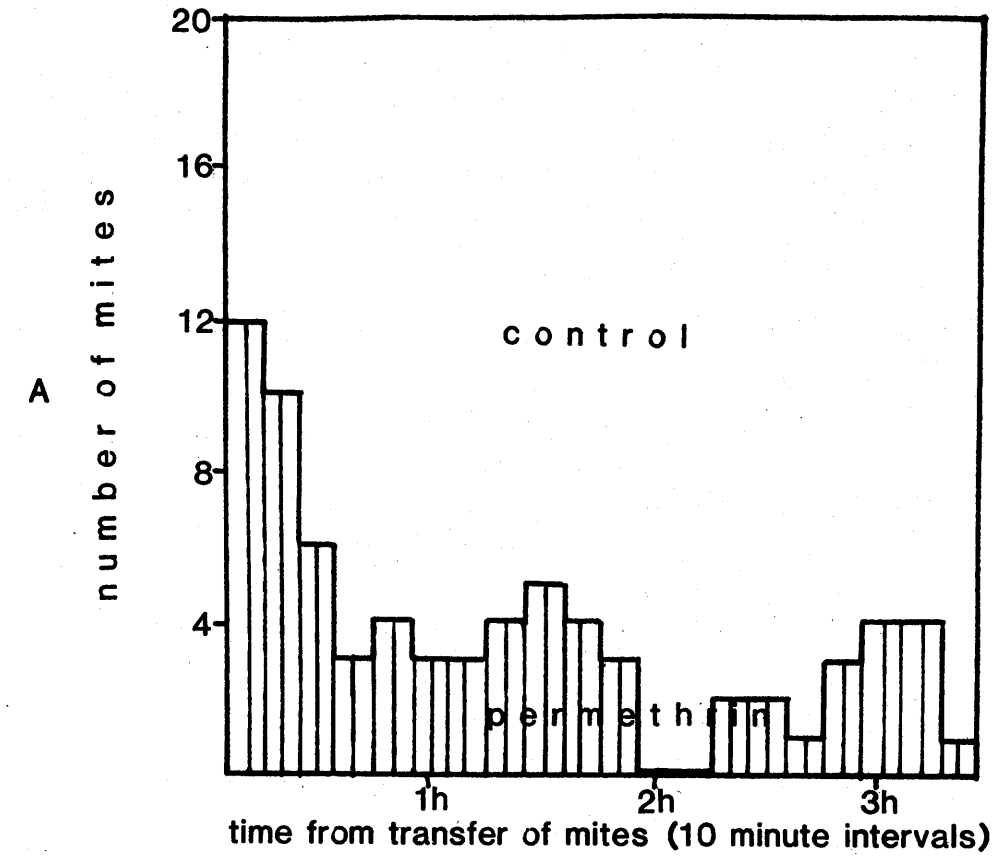


Fig. 6.3 Distribution of Typhlodromus pyri adult females between the permethrin-treated (2 mg/litre) and the control surface of a glass arena



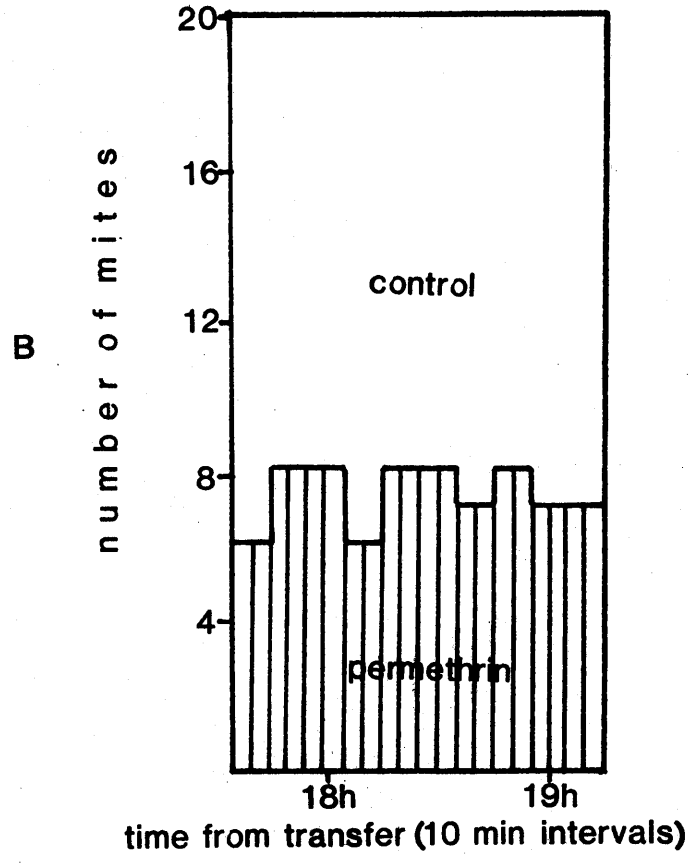


Fig. 6.4 Distribution of Typhlodromus pyri adult females between the permethrin-sprayed (0.5 mg/litre) and the control halves of glass arenas

mites was found on the permethrin-sprayed than on the control surface of the arenas (P = 0.001). Fig. 6.4 shows that the avoidance effect gradually increased to a maximum, so that 2 h after the transfer there were no mites on the permethrin residues. Later, and next day, this effect was less apparent. Approximately 20 h from the start of the test a few mites showed reduced locomotion but there was no mortality and no eggs were found in either of the two arenas.

Azinphos-methyl and carbaryl (Fig. 6.5 and Appendices 6.3 and 6.4) T. pyri did not show a significant preference for either the sprayed or the control halves of the arenas for over 3 h after the mite transfer. All mites looked normal in their behaviour. Approximately 20 h from transfer a total of 8 eggs were laid in the azinphos-methyl arenas and 9 eggs in the carbaryl arenas, randomly distributed over sprayed and control surfaces.

6.3.4 Test 4

In both series of distribution counts, one covering the first few hours after mite transfer, the other consisting of one count per day for a week (Fig. 6.6, Appendix 6.5), a lower number of mites was found on the permethrin sprayed than on the control halves of the arenas (P = 0.001). Also a consistently lower number of eggs was laid on the permethrin halves of the arenas (P = 0.001), the totals of the two treatments being at a ratio of c 1:4, permethrin:control (Fig.6.7 and Appendix 6.6).

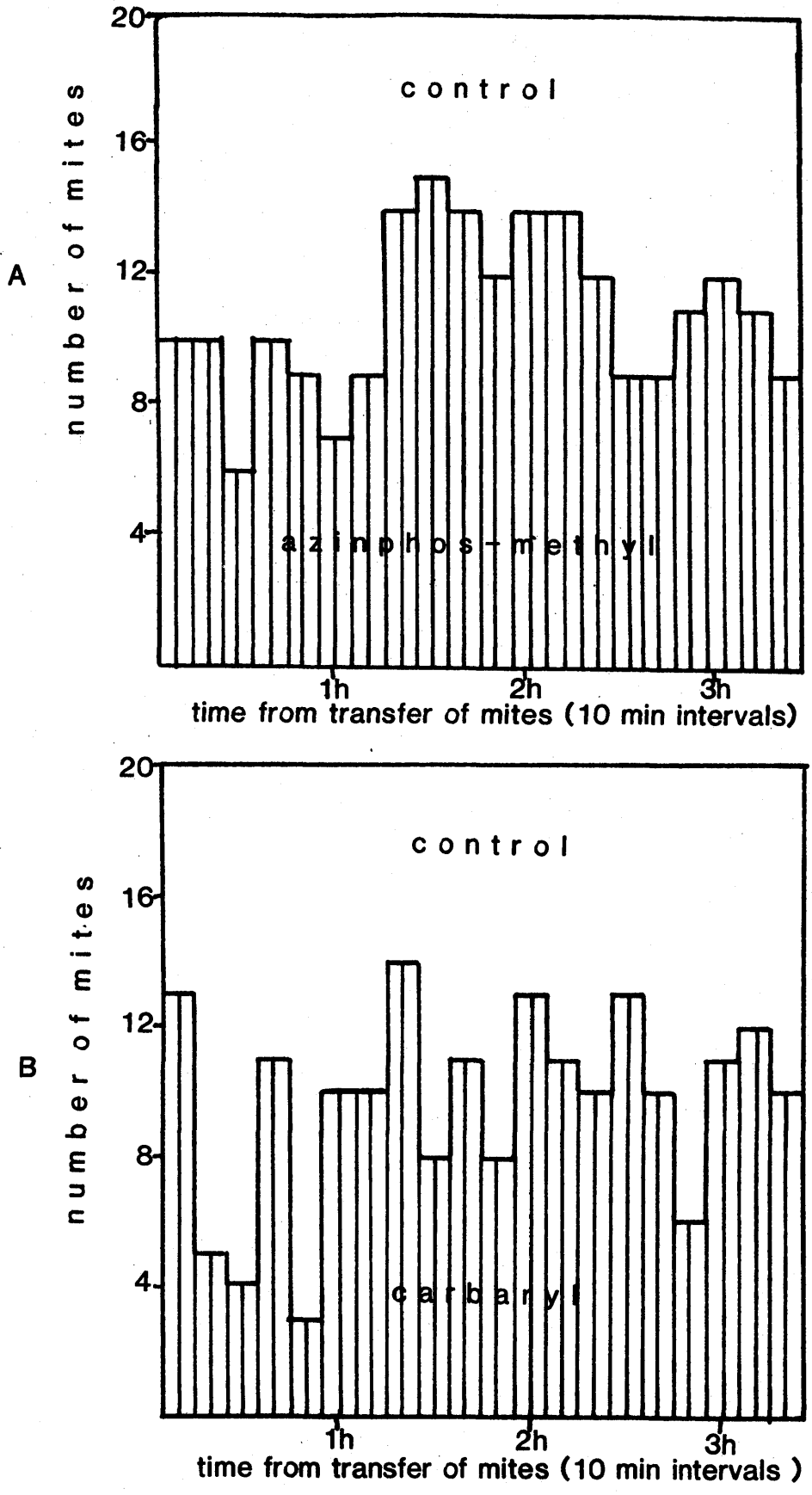


Fig. 6.5 Distribution of Typhlodromus pyri adult females between the pesticide-sprayed and the control halves of glass arenas

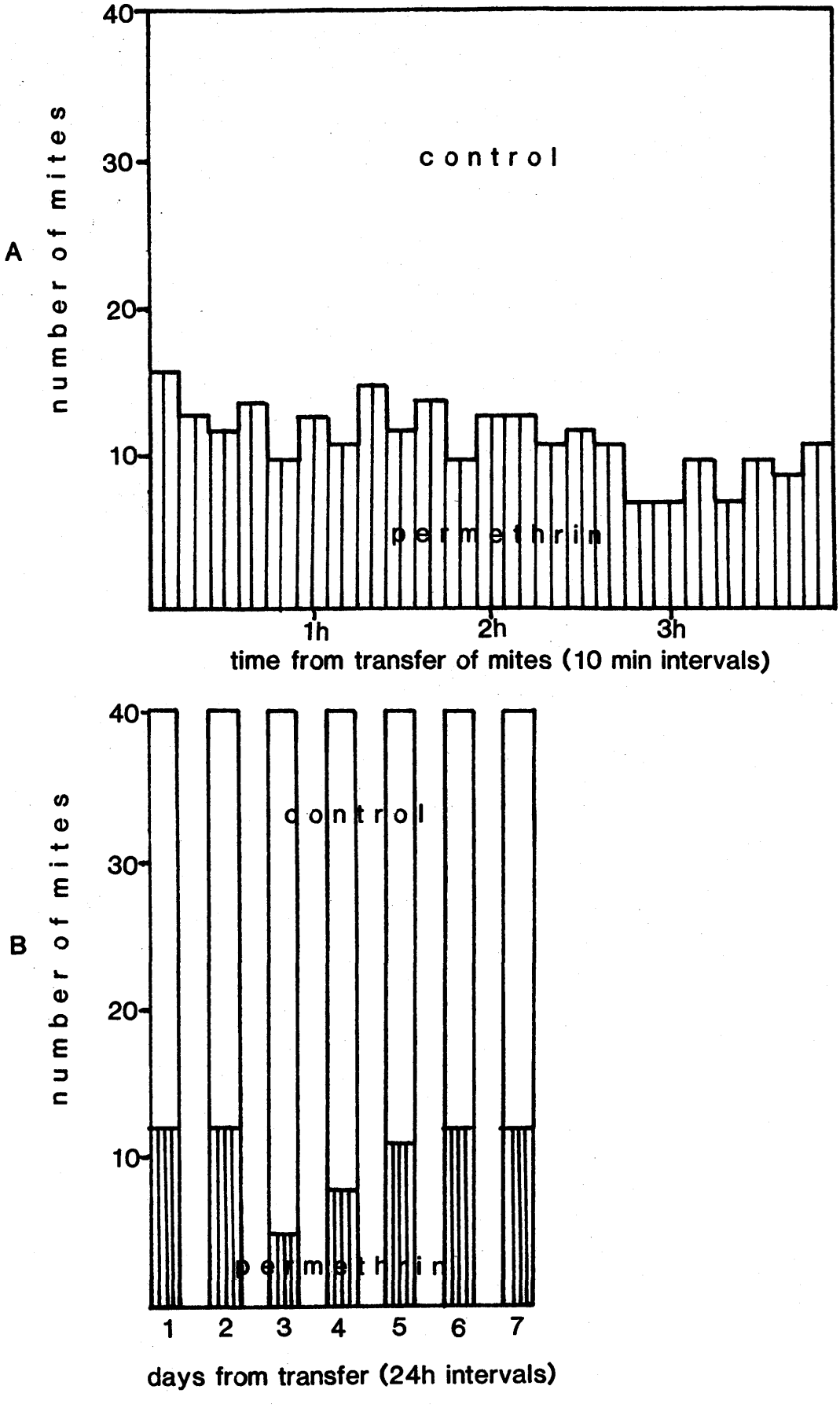


Fig. 6.6 Distribution of Typhlodromus pyri adult females between the permethrin-sprayed and the control halves of apple leaf arenas

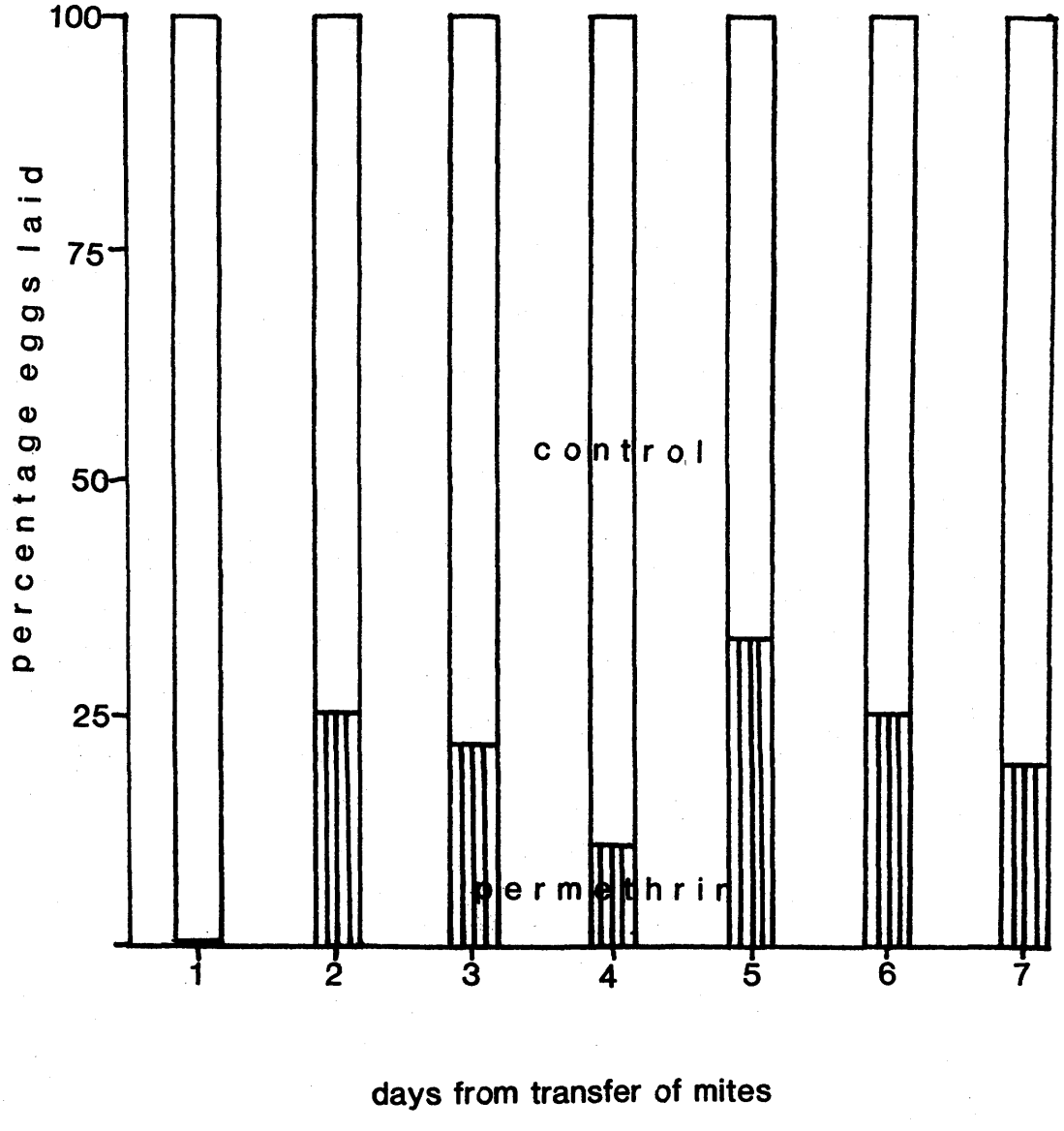


Fig. 6.7 Percentage eggs laid by Typhlodromus pyri females on the permethrin-treated (▥) and the control halves (□) of apple leaf arenas

6.4 DISCUSSION

There is virtually no information in the literature on effects of pesticides on behaviour of T. pyri, and the relevant information for other phytoseiids is very restricted (see 6.1 'Introduction'). As group specificity in repellency is not known and species specificity is not very common (Busvine, 1971), the results of this study are discussed in comparison to other studies on various insect and mite species, including phytoseiids and tetranychids.

6.4.1 Test 1

Observations in this Test showed that T. pyri was unable to detect and avoid permethrin residues by olfaction. This could be due to the low vapour pressure of permethrin (Worthing, 1983) although if permethrin had such a property ('olfactory repellency') it might have been demonstrated because of the high concentration used (1000 mg/litre) and the small size of the mite. In similar tests of Fisher and Morgan (1968) with lethal concentrations of the acaricide dicofol, T. urticae were repelled by deposits of discrete droplets and laid eggs between them.

6.4.2 Test 2

During the distribution counts of this Test, more mites were found on the glass surface covered with permethrin deposits than on the control surface. As mentioned in 'Materials and Methods', these deposits had

previously caused 100% mortality under continuous exposure for 24 h. Signs of poisoning were apparent throughout this Test, and 2 of the 8 females died during the 24 h of observation.

Similar results were obtained by Cartwright (1979) in 'split arena' tests with the insect predator Anthocoris nemorum. It stayed longer in the half of a plywood arena treated with the lethal dosage of 100 mg permethrin/litre than on the control half. In additional tests measuring the activity of the predator, Cartwright found that permethrin residues reduced its locomotion, thus explaining the longer stay on the treated surface. Reduced activity may also be the cause of the longer stay of T. pyri on the permethrin side of the arena in this Test. A reduction in activity of the mites while on the deposits was noted but no specific test was set up to measure it.

6.4.3 Tests 3 and 4

In conclusion, T. pyri (a) did not avoid glass surfaces sprayed with sub-lethal concentrations of azinphos-methyl or carbaryl, (b) showed a marked avoidance reaction to sub-lethal residues of permethrin both on glass and leaf surfaces, and (c) laid consistently fewer eggs on permethrin-treated than on untreated leaf surfaces.

Similar results have been reported by several authors showing that residues of permethrin and other pyrethroids, but not azinphos-methyl or carbaryl, elicited avoidance reactions in phytoseiids as well as in phytophagous mites. In mortality tests of Hoy, Flaherty, Peacock and Culver

(1979) permethrin residues had an irritant effect on Typhlodromus occidentalis, on the Pacific spider mite, Tetranychus pacificus McGregor, and on the tydeid mite Promenatus anconai Baker, causing 'run off' from sprayed leaf disks. Permethrin residues also induced 'run off' in T. pyri during tests reported in sections 4 and 5 in this Thesis. Hall (1979) found that T. urticae fed less and laid fewer eggs on halves of leaf disks treated with fenvalerate than on the untreated halves. Penman, Chapman and Jesson (1981) reported that fenvalerate residues (a) were avoided by T. occidentalis in 'split arena' tests on glass at dosages as low as 0.05 mg/litre, (b) had an antifeedant and irritant effect on A. fallacis fed on sprayed T. urticae eggs, causing 'run off' from leaf disks, and (c) were irritant to T. urticae also causing 'run off' from sprayed leaf disks. In later tests of Penman and Chapman (1983) fenvalerate induced 'spin down' in T. urticae when sprayed on infested bean plants and prevented colonisation of pre-treated leaves. In glasshouse experiments of Aerts (1978) deltamethrin had a strong repellent effect on T. urticae and kept fully grown tomato plants practically free of spider mite infestation.

Regarding azinphos-methyl and carbaryl, no avoidance reactions were observed in residue tests with T. pyri reported in sections 4 and 5 of this Thesis, or in tests of Penman, Chapman and Jesson (1981) working with T. urticae. The same authors reported that the commercial formulation of azinphos-methyl which they tested had no antifeedant effects on A. fallacis and did not elicit any avoidance

reactions in T. occidentalis at various concentrations. Also Penman and Chapman (1983) found that azinphos-methyl did not affect the distribution of T. urticae on bean plants.

These last two reports appear to conflict, as regards results on azinphos-methyl, with earlier observations (no detailed data were reported) from New Zealand: Collyer (1976) noted that OP-resistant T. pyri showed an avoidance reaction to azinphos-methyl deposits in the laboratory, and Walker and Penman (1978), and also Penman, Wearing, Collyer and Thomas (1979), attributed localised bronzing of leaves in apple trees (caused by P. ulmi) to distributional imbalances in T. pyri caused by azinphos-methyl sprays.

Likewise, Hislop, Auditore, Weeks and Prokopy (1981) found that deposits from a field concentration of a commercial azinphos-methyl wettable powder were repellent to A. fallacis females on leaf disks for 7 days; they report reduced consumption of T. urticae eggs, a lower frequency of A. fallacis presence, and a lower number of eggs laid on the treated halves of disks than on the untreated. This result may have been influenced by the formulation ingredients in the wettable powder used.

6.4.4 Discussion on all Tests

The results obtained in this study, and also by the authors cited above, indicate that the behavioural reactions of T. pyri to permethrin deposits are caused by irritation after contact with the deposits, resulting in decreased or increased undirected activity and biased

distribution. In Tests 3 and 4 the sub-lethal deposits caused increased activity resulting in avoidance of the deposits. In Test 2, however, the higher deposits (well within the lethal range for T. pyri) reduced activity resulting in a higher number of mites being on the permethrin-sprayed surface. These results and those of Cartwright (1979) (see 6.4.2 'Test 2' above) indicate the importance of dosage when testing the behavioural responses of arthropods to pesticides. Also, when Rogoff (1952) tested various insecticides at many different concentrations for their repellency to the greenhouse thrips, Heliothrips haemorrhoidalis, he concluded that although some insecticides elicited an avoidance reaction at certain concentrations, a further increase in concentration resulted "..... in a greater number of thrips being found on the treated surface, an apparent lowering of repellency being indicated". He assumed that toxicity obscured the avoidance reaction at the higher dosages. Similarly, Mial and Turner (1982) testing permethrin, deltamethrin, cypermethrin and fenvalerate on the German cockroach, Blattella germanica, found that irritancy leading to avoidance was only apparent when pick up of the insecticide was greatly reduced, whereas with lethal deposits any irritant effects became unimportant. Apparently this conclusion would apply only to 'excito-repellents' (Busvine, 1971 - p.58) with rapid toxic action such as pyrethroids, and not to olfactory repellents. Test 1 showed that T. pyri was unable to detect and avoid permethrin deposits at the high dosage of 1000 mg/litre.

In other tests carried out at the end of the present study (but not completed) T. pyri was unable to detect deposits of permethrin at 10 mg/litre on T. urticae eggs and preyed on equal numbers of sprayed and unsprayed eggs. As in similar tests of Penman, Chapman and Jesson (1981) testing A. fallacis on fenvalerate-treated eggs of T. urticae, this resulted in undirected excitation and trapping of the predators in the barriers of the test arenas. Other reports on pyrethroids [Tan (1981) on permethrin and cypermethrin; Rice, Gibson and Stribley (1982) on deltamethrin] also indicated that the repellent effects of pyrethroids are the result of irritation rather than an evasive reaction.

Responses to pyrethroid deposits appear to be very similar to those reported for DDT, including (a) irritation causing undirected increased activity (Dethier, 1956), (b) a contact being necessary before the insect avoided the deposit (Rogoff, 1952) and (c) hyperactivity persisting for some time after the treated surface was abandoned (Baranyovits, 1951).

6.4.5 Implications for Integrated Pest Management

High predator mortality is undoubtedly the major cause of spider mite resurgence following field application of synthetic pyrethroids (see sections 1-4). Stimulation of spider mite reproduction by these chemicals has also been claimed as a contributing factor (see 5.1 'Introduction'). Evidence presented in sections 5 and 6 shows that sub-lethal effects of permethrin, i.e. reduction

of reproductive rate, and restriction of searching capacity due to avoidance of spray residues, may delay the recovery of the small proportion of the phytoseiid population that may survive a field spray of this pyrethroid.

The avoidance reaction of predators to pyrethroid residues may be beneficial in the short term if they are able to avoid spray deposits before they pick up a lethal dose. But in the longer term, avoidance of residues may delay the development of resistance because of (a) reduced exposure and (b) lower reproductive rates due to reduced feeding, as a result of restricted searching capacity.

SECTION 7

SUMMARY OF RESULTS AND CONCLUSIONS

AND SUGGESTIONS FOR FURTHER WORK

7.1 Summary of results and conclusions

1. Using T. pyri stocks from isolated unsprayed orchards, bioassays were carried out to establish levels of base-line susceptibility to azinphos-methyl, parathion, carbaryl and permethrin. The level for azinphos-methyl was similar to that found by Hoyt (1972) in New Zealand.

2. Stocks of T. pyri taken from English orchards with a spray history of organophosphates (OPs) and carbaryl showed low resistance to azinphos-methyl (4- to 6-fold) and high resistance to parathion (c 50-fold) and carbaryl (>20-fold). Similar levels were found for an orchard population derived from T. pyri imported from New Zealand in 1977.

3. The results show that native and New Zealand T. pyri have developed resistance to carbaryl to such a high level that it is virtually innocuous. Contrary to the view of Croft (1982) the species is not inherently very tolerant of carbaryl, although it is more tolerant than certain other phytoseiid species (A. fallacis, T. occidentalis, A. potentillae).

4. All stocks from sprayed orchards exhibited resistance to both azinphos-methyl and carbaryl. In contrast, certain other phytoseiid species (A. fallacis, T. occidentalis) became highly resistant to azinphos-methyl, which did not per se confer cross-resistance to carbaryl. For T. pyri the results accord with an hypothesis of cross-resistance between OPs and carbaryl, but they do not prove it, since both chemical groups have been used widely in commercial orchards from the 1950s.

5. The results suggest that low-level resistance to azinphos-methyl and high-level resistance to carbaryl are stable under relaxation of selection pressure in the field.

6. Evidence was obtained from a field trial that these levels of resistance were sufficient for T. pyri to survive the normal usage of these insecticides under English conditions. In the same trial T. pyri was not affected by diflubenzuron but was almost eliminated by cypermethrin.

7. There was no evidence of developed resistance to permethrin, but none of the orchards sampled had a spray history of permethrin or of other synthetic pyrethroids (SPs). Comparing OP-resistant and OP-susceptible stocks there was no cross-resistance to permethrin, positive or negative.

8. Comparing mortality bioassay techniques, T. pyri females exposed to spray residues on glass and apple leaves were killed by lower concentrations than in taped-slide tests (LC_{50} for taped-slide > apple leaf > glass); but for all three bioassay techniques the resistance factors to azinphos-methyl and carbaryl, comparing two stocks, were very similar.

9. In two field trials, permethrin was sprayed high-volume using the lowest recommended rate. This was highly lethal to T. pyri whether used pre-blossom, at 'bud-burst' or 'mouse-ear', or after blossom in June. Observations on the emergence of T. pyri from hibernating sites (crevices in the bark) in relation to the use of a 'bud-burst' spray showed that the lethal effect of the spray was only

delayed; no marked selectivity was obtained by this early timing. Bioassays showed that the toxic effect of permethrin residues on bark or on leaves was less persistent (c 3 wk) than were the chemical residues determined by G.C. analyses (detectable for 3 months or longer). However, numbers of T. pyri were so reduced that effective predation of spider mites, P. ulmi, was disrupted for a long period, and was only fully re-established after 3-4 months. A. schlechtendali increased when T. pyri was reduced by spraying. Tydeid mites were killed by permethrin.

10. The effects of pesticide residues on the fecundity and egg hatch of T. pyri were studied in bioassays. Dosages of azinphos-methyl and carbaryl that caused no mortality during the test period had no appreciable effect on egg production. In contrast, survivors on permethrin residues suffered a severe reduction in egg production, especially for the first week. None of the pesticides affected egg hatch.

11. The ability of T. pyri to avoid pesticide residues was also tested. The mites did not avoid residues of azinphos-methyl or carbaryl on glass, but they showed a marked avoidance of residues from a spray of 0.5 mg permethrin/litre on glass or on apple leaves, and they laid fewer eggs on the treated halves of 'choice chambers' on leaves. In other tests female mites seemed unable to detect discrete droplets of permethrin on glass, at the concentration of 1000 mg a.i./litre. These tests on the

behaviour of T. pyri suggest that the avoidance of permethrin residues results from an irritation effect rather than olfactory repellency.

12. Over a period of forty days, a susceptible stock of T. pyri produced a total of 1.5 times as many eggs as did a resistant stock. Although at first the susceptible stock produced fewer eggs/day, egg laying continued for a longer time than with the resistant stock.

7.2 Suggestions for further work

1. In relation to the scope for practical mite management in commercial orchards, the occurrence of populations of T. pyri resistant to OPs and carbaryl needs to be surveyed. The taped-slide technique would produce results comparable with existing data.

2. The cross-resistance spectrum for the various OPs in common use in commercial orchards merits study. Information on resistance factors, and comparisons with field dosages, would complement results from field trials and establish the relative safety of various OPs for T. pyri.

3. Analysis is needed of the genetic and biochemical background of resistance to OPs and carbaryl in T. pyri, to determine whether cross-resistance exists between these two chemical groups.

4. Survey of the levels of tolerance to permethrin and other synthetic pyrethroids, sampling commercial orchards which have appropriate spray histories, is required.

Since SPs have already been used extensively for six years or more, such a survey would be the obvious first stage in seeking resistant stocks. Low numbers of T. pyri could be built up on plate cultures, with broad bean pollen as food, to the numbers required for bioassays. If indications of increased tolerance are found, a selection programme on mass cultures might elevate the level.

5. It would be of interest to pursue further the studies on the effects of pesticides, especially SPs, on reproduction and behaviour of T. pyri. This work could be extended to P. ulmi and involve both laboratory and field work.

Following compilation of these 'suggestions for further work' in 1982, some of them were partially materialised and reported:

Suggestions nos 1 and 4	:	in Flegg (1983) (see list of references), and in M.M. Heisig (1983). A survey of Typhlodromus pyri in apple orchards of Kent and an investigation into factors affecting their abundance, M.Sc. Thesis, University of Reading, 149 pp.
Suggestion no. 2	:	in Cranham, Kapetanakis and Fisher (1983) (see list of references).
Suggestion no. 3	:	in Overmeer and van Zon (1983) (see list of references).

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REFERENCES

ABDEL-SALAM, F.A. & NASR, F.A. (1968). The effect of some insecticides on the stages of the Egyptian cotton leaf-worm, Prodenia litura Fab. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz **75**, 687-689.

AERTS, J. (1978). Repellent-werking van decamethrin tegen kasspint bij tomaten. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent **48** (2) 655-660.

AHMADI, A. (1983). Demographic toxicology as a method of studying the dicofol two-spotted spider mite (Acari: Tetranychidae) system. Journal of Economic Entomology **76**, 239-242.

ALINIAZEE, M.T. & CRANHAM, J.E. (1980). Effects of four synthetic pyrethroids on a predatory mite, Typhlodromus pyri, and its prey, Panonychus ulmi, on apples in south-east England. Environmental Entomology **9**, 436-439.

ALMEIDA, A.A. (1967). Reactions of larvae of Plodia interpunctella (Hb.) (Lep., Pyralidae) to insecticidal droplets. Bulletin of Entomological Research **57**, 221-226.

ANONYMOUS (1980). Revised method for spider mites and their eggs. F.A.O. method No. 10a. In: J.R. Busvine (Ed.). Recommended methods for measurement of pest resistance to pesticides, F.A.O. Plant Production and Protection Paper No. 21, F.A.O. Rome, 132 pp.

ASCHER, K.B.S. (1960). A review on 'resistance-induced enhanced susceptibility' in insects, with some notes on similar phenomena (especially 'collateral sensitivity') in microorganisms. Drug Research (Arzneimittel-Forschung) **10**, 450-461.

ATTALAH, Y.H. & NEWSON, L.D. (1966). The effect of DDT, toxaphene and endrin on the reproductive and survival potentials of Coleomegilla maculata. Journal of Economic Entomology **59**, 1181-1187.

AVERY, D.J. (1964). Carbon dioxide exchange by plum and apple leaves damaged by fruit tree red spider mite. Report of East Malling Research Station for 1963, pp. 94-97.

BALL, H.J. & SU, P.P. (1979). Effect of sublethal dosages of carbofuran and carbaryl on fecundity and longevity of the female western corn rootworm. Journal of Economic Entomology **72**, 873-876.

BARANYOVITS, F. (1951). Fly reactions to insecticidal deposits. Nature **168**, 960-961.

BARTLETT, B.R. (1965). The repellent effects of some pesticides to hymenopterous parasites and coccinellid predators. Journal of Economic Entomology **58**, 294-296.

BARTLETT, B.R. (1968). Outbreaks of two-spotted spider mites and cotton aphids following pesticide treatment. I. Pest stimulation vs. natural enemy destruction as the cause of outbreaks. Journal of Economic Entomology **61**, 297-303.

BESSON, J., JOLY, E. & TOUZEAU. J. (1978). Les actions secondaires des antiparasitaires agricoles. Phytoma - Défense des cultures - Mars 1978, pp. 18-26.

BLAIR, C.A. (1951). Damage to apple leaves by the fruit tree red spider mite, Metatetranychus ulmi (Koch). Report of East Malling Research Station for 1950, pp. 152-154.

BOWER, C.C. (1980). An evaluation of the potential of diflubenzuron for integrated pest control in apples. General and Applied Entomology **12**, 41-48.

BOWER, C.C. & KALDOR, J. (1980). Selectivity of five insecticides for codling moth control: Effects on the two-spotted spider mite and its predators. Environmental Entomology **9**, 128-132.

BOYKIN, L.S. & CAMPBELL, W.V. (1982). Rate of population increase of the two-spotted spider mite (Acari: Tetranychidae) on peanut leaves treated with pesticides. Journal of Economic Entomology **75**, 966-971.

BRAVENBOER, L. & DOSSE, G. (1962). Phytoseiulus riegeli Dosse as a predator of red spider mites of the Tetranychus urticae group. Entomologia experimentalis et applicata **5**, 291-304.

BROWN, T.M., JONSON, D.R., HOPKINS, A., DURANT, J. & MONTEFIORI, D.C. (1982). Interactions of pyrethroid insecticides and toxaphene in cotton. Journal of Agricultural and Food Chemistry **30**, 542-545.

BUSVINE, J.R. (1971). A critical review of techniques for testing insecticides. Commonwealth Agricultural Bureau, London, 345 pp.

BUTT, D.J. & JEGER, M.J. (1982). Decision based management of orchard pathogens and pests in the United Kingdom. Pp. 167-189 in R.B. Austin (Ed.) Decision Making in the Practice of Crop Protection. Monograph No. 25. British Crop Protection Council, Croydon, U.K., 238 pp.

CARTWRIGHT, J. (1979). A study of the factors affecting recolonization of beneficial insects into apple orchards after treatment with permethrim. MSc Thesis, University of London, 73 pp.

CHABOUSSOU, F. (1962). Efficacité de divers produits utilisés en traitement sur vigne contre Panonychus ulmi Koch. Répercussions sur les populations des oeufs d'hiver. Revue de Zoologie Agricole et Appliquée (Quatrieme trimestre, 1961), No. 10-12, 18 pp.

CHAMBERLAIN, W.F. (1959). The behaviour of agricultural insects toward olfactory repellents in the olfactometer and in split arena tests. Journal of Economic Entomology **52**, 286-289.

CHAPMAN, R.A. & HARRIS, C.R. (1978). Extraction and liquid-solid chromatography clean-up procedures for the direct analysis of four pyrethroid insecticides in crops by gas-liquid chromatography. Journal of Chromatography **166**, 513-518.

CHAPMAN, R.A. & SIMMONS, H.S. (1977). Gas-liquid chromatography of picogram quantities of pyrethroid insecticides. Journal of the Association of Official Analytical Chemists **60**, 977-978.

CHAPMAN, R.B. & PENMAN, D.R. (1979). Negatively correlated cross-resistance to a synthetic pyrethroid in organophosphorus-resistant Tetranychus urticae. Nature **281**, 298-299.

CHELLIAH, S., FABELLAR, L.T. & HEINRICHS, E.A. (1980). Effect of sub-lethal doses of three insecticides on the reproductive rate of the brown plant-hopper, Nilaparvata lugens, on rice. Environmental Entomology **9**, 778-780.

CHIBA, M. (1970). An analytical chemist's contribution to Applied Entomology. Proceedings of the Entomological Society of Ontario **101**, 24-27.

COLLYER, E. (1976). Integrated control of apple pests in New Zealand. 6. Incidence of European red mite, Panonychus ulmi (Koch), and its predators. New Zealand Journal of Zoology **3**, 39-50.

COLLYER, E. & KIRBY, A.H.M. (1959). Further studies on the influence of fungicide sprays on the balance of phytophagous and predacious mites on apple in South-East England. The Journal of Horticultural Science **34**, 39-50.

COLLYER, E. & VAN GELDERMALSEN, M. (1975). Integrated control of apple pests in New Zealand. 1. Outline of experiment and general results. New Zealand Journal of Zoology **2**, 101-134.

CRANHAM, J.E. (1978). Control of codling moth with diflubenzuron. Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft, Berlin-Dahlem, Heft 180, 108-110.

CRANHAM, J.E. (1979). Managing spider mites on fruit trees. SPAN **15**, 19-22.

CRANHAM, J.E. (1982). Pome fruit pest management in Northern Europe. Scientific Horticulture **33**, 100-112.

CRANHAM, J.E., KAPETANAKIS, E.G. & FISHER, A.J. (1983). Resistance to insecticides in the predatory mite Typhlodromus pyri and its spider mite prey. Proceedings, 10th International Congress of Plant Protection, Brighton, England, November 20-25, 1983, p. 638.

CRANHAM, J.E. & SOLOMON, M.G. (1981). Mite management in commercial apple orchards. Report of East Malling Research Station for 1980, pp. 171-172.

CRANHAM, J.E., TARDIVEL, G.M. & KAPETANAKIS, E.G. (1984). Orchard trials to assess the effects of pesticides on Typhlodromus pyri and its prey, Panonychus ulmi. Proceedings of the VIth International Congress of Acarology, Edinburgh, 1982, Vol. 2, in press.

CROFT, B.A. (1975a). Integrated control of apple mites. Extension Bulletin E-825, Michigan State University, 12 pp.

CROFT, B.A. (1975b). Tree fruit pest management. Pp. 471-507 in R.L. Metcalf and W.H. Luckman (Eds.). Introduction to insect pest management. John Wiley and Sons, New York 587 pp.

CROFT, B.A. (1977). Susceptibility surveillance to pesticides among arthropod natural enemies: Modes of uptake and basic responses. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz **84**, 140-157.

CROFT, B.A. (1981). Use of crop protection chemicals for integrated pest management. Philosophical Transactions of the Royal Society, London, U.K. **B-295**, 125-141.

CROFT, B.A. (1982). Arthropod resistance to insecticides: A key to pest control failures and successes in North American apple orchards. Entomologia experimentalis et applicata **31**, 88-110.

CROFT, B.A. (1983). Status and management of pyrethroid resistance in the predatory mite, Amblyseius fallacis (Acarina: Phytoseiidae). The Great Lakes Entomologist **16**, 17-32.

CROFT, B.A. & ALINIAZEE, M.T. (1983). Differential resistance to insecticides in Typhlodromus arboreus Chant and associate phytoseiid mites of apple in the Willamette Valley, Oregon. Environmental Entomology **12**, 1420-1423.

CROFT, B.A. & BROWN, A.W.A. (1975). Responses of arthropod natural enemies to insecticides. Annual Review of Entomology **20**, 285-335.

CROFT, B.A., BROWN, A.W.A. & HOYING, S.A. (1976). Organophosphorus-resistance and its inheritance in the predacious mite Amblyseius fallacis. Journal of Economic Entomology **69**, 64-68.

CROFT, B.A. & HOYING, S.A. (1975). Carbaryl resistance in native and released populations of Amblyseius fallacis). Environmental Entomology **4**, 895-898.

CROFT, B.A. & HOYING, S.A. (1977). Competitive displacement of Panonychus ulmi (Acarina: Tetranychidae) by Aculus schlechtendali (Acarina: Eriophyidae) in apple orchards. The Canadian Entomologist **109**, 1025-1034.

CROFT, B.A. & HOYT, S.C. (1978). Considerations for the use of pyrethroid insecticides for deciduous fruit pest control in the U.S.A. Environmental Entomology **7**, 627-630.

CROFT, B.A. & JEPPSON, L.R. (1970). Comparative studies of four strains of Typhlodromus occidentalis. II. Laboratory toxicity of ten compounds common to apple pest control. Journal of Economic Entomology **63**, 1528-1531.

CROFT, B.A. & MEYER, R.H. (1973). Carbamate and organophosphorus resistance patterns in populations of Amblyseius fallacis. Environmental Entomology **2**, 691-695.

CROFT, B.A. & STEWART, P.G. (1973). Toxicity of one carbamate and six organophosphorus insecticides to O-P resistant strains of Typhlodromus occidentalis and Amblyseius fallacis. Environmental Entomology **2**, 486-488.

CROFT, B.A. & STRICKLER, K. (1983). Natural enemy resistance to pesticides: documentation characterization, theory and application. Pp. 669-702 in G.P. Georghiou and T. Saito (Eds.) Pest resistance to pesticides. Plenum Pub. New York.

CROFT, B.A., WAGNER, S.W. & SCOTT, J.G. (1982). Multiple- and cross-resistance to insecticides in pyrethroid-resistant strains of the predatory mite, Amblyseius fallacis. Environmental Entomology **11**, 161-164.

DABROWSKI, Z.T. (1969). Laboratory researches on the toxicity of pesticides to Typhlodromus finlandicus (Oud.) and Phytoseiulus macropilis (Banks) (Phytoseiidae, Acarina). Rocznik Nauk rolniczych 95-A-3, 337-369. (In Polish with English summary).

DETHIER, V.G. (1956). Repellents. Annual Review of Entomology 1, 181-202.

DETHIER, V.G., BROWNE, L.B. & SMITH, C.N. (1960). The designation of chemicals in terms of the responses they elicit from insects. Journal of Economic Entomology **53**, 134-136.

DITTRICH, V., STREIBERT, P. & BATHE, P.A. (1974). An old case re-opened: Mite stimulation by insecticide residues. Environmental Entomology **3**, 534-540.

DOWNING, R.S. & MOILLIET, T.K. (1967). Relative densities of predacious and phytophagous mites on three varieties of apple trees. The Canadian Entomologist **99**, 738-741.

DOWNING, R.S. & MOILLIET, T.K. (1974). Comparisons between an exotic and an endemic phytoseiid mite predator (Acarina: Phytoseiidae) in British Columbia. The Canadian Entomologist **106**, 773-776.

EASTERBROOK, M.A. (1979). The life history of the eriophyid mite Aculus schlechtendali on apple in South-east England. Annals of Applied Biology **91**, 287-296.

EASTERBROOK, M.A. (1984). Effects of pesticides on the apple rust mite Aculus schlechtendali (Nal.) (Eriophyiidae). Journal of Horticultural Science **59**, 51-55.

EASTERBROOK, M.A., SOLOMON, M.G. & CRANHAM, J.E. (1982). Integrated pest management trials in English apple orchards. Bulletin International Organisation for Biological Control, West Palaearctic Regional Section 1982/V/2, pp. 68-71.

EASTERBROOK, M.A., SOLOMON, M.G., CRANHAM, J.E. & SOUTER, E.F. (1984). Trials of an integrated pest management programme based on selective pesticides in English apple orchards. Crop Protection, in press.

EASTERBROOK, M.A., SOUTER, E.F., SOLOMON, M.G. & CRANHAM, J.E. (1979). Trials on integrated pest management in apple orchards. Proceedings, British Crop Protection Conference - Pests and Diseases, November 19th-22nd 1979, Brighton, England, pp. 61-67.

EGGERS-SCHUMACHER, H.A. (1983). A comparison of the reproductive performance of insecticide-resistant and susceptible clones of Myzus persicae. Entomologia experimentalis et applicata **34**, 301-307.

EL-LAKWAH, F. & ABDEL SALAM, F.A. (1974). The action of some organophosphates in sublethal doses on Spodoptera littoralis (Boisd.). Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz **47**, 28-30.

ESAAC, E.G., EL-GROGARY, S., ABDEL-FATAH, M.S. & ALI, A.M. (1972). Effect of carbaryl, methyl parathion and dieldrin on egg production and percent pupation of the Egyptian cotton leafworm, Spodoptera littoralis (Boisd.) Zeitschrift für angewandte Entomologie **71**, 263-270.

FERRARI, R. (1980). Ricerche sugli effetti repellenti di alcuni piretrinoidi nei confronti di Myzodes persicae Sulz. e Aphis fabae Scop. Redia **63**, 25-36.

FISHER, R.W. & HANSELL, R.I.C. (1964). Effect of pre- and post-treatment temperatures, age of deposit, and repellency on the toxicity of Kelthane to the two-spotted mite, Tetranychus telarius (L.) (Acarina: Tetranychidae). The Canadian Entomologist **96**, 1307-1312.

FISHER, R.W. & MORGAN, N.G. (1968). The effect on the two-spotted spider mite, Tetranychus urticae of dicofol concentration and deposit distribution on the leaf surface. The Canadian Entomologist **100**, 777-781.

FLAHERTY, D.L. & HUFFAKER, C.B. (1970). Biological control of Pacific mites and Willamette mites in San Joaquin Valley vineyards. I. Role of Metaseiulus occidentalis. II. Influence of dispersion patterns of Metaseiulus occidentalis. Hilgardia **40**, 267-330.

FLEGG, J.J.M. (1979). Zoology. Report of East Malling Research Station for 1978, pp. 119-131.

FLEGG, J.J.M. (1980). Zoology. Report of East Malling Research Station for 1979, pp. 119-132.

FLEGG, J.J.M. (1981). Zoology. Report of East Malling Research Station for 1980, pp. 97-106.

FLEGG, J.J.M. (1982). Zoology. Report of East Malling Research Station for 1981, pp. 99-112.

FLEGG, J.J.M. (1983). Zoology. Report of East Malling Research Station for 1982, pp. 101-112.

FLESCHNER, C.A. & SCRIVEN, G.T. (1957). Effect of soil type and DDT on ovipositional responses of Chrysopa californica (Coq.) on lemon trees. Journal of Economic Entomology **50**, 221-222.

FORD, J.B. & GIBONEY, F.M. (1980). Video recording techniques for studying predatory mite behaviour. Bulletin, International Organization for Biological Control, 1980/III/6, p. 35.

FRANZ, J.M. (1974). Die Prüfung von Nebenwirkungen der Pflanzenschutzmittel auf Nutzarthropoden im Laboratorium-ein Sammelbericht. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz **81**, 141-174.

FUJIE, G.H. & FULLMER, O.H. (1978). Determination of FMC 33297 residues in plant, animal and soil matrices by gas chromatography. Journal of Agricultural and Food Chemistry **26**, 395-397.

GEORGHIOU, G.P. & TAYLOR, C.E. (1977a). Genetic and biological influences in the evolution of insecticide resistance. Journal of Economic Entomology **70**, 319-323.

GEORGHIOU, G.P. & TAYLOR, C.E. (1977b). Operational influences in the evolution of insecticide resistance. Journal of Economic Entomology **70**, 653-658.

GILES, D.P. & ROTHWELL, D.N. (1983). The sub-lethal activity of amidines on insects and acarids. Pesticide Science **14**, 303-312.

GLASS, E.H. (1975). Recent developments in deciduous orchard pest management in the United States. EPPO Bulletin **5** (2) 101-111.

GORDON, P.L. & McEWEN, F.L. (1984). Insecticide stimulated reproduction of Myzus persicae, the green peach aphid (Homoptera: Aphididae). The Canadian Entomologist **116**, 783-784.

GROSCH, D.S. (1975). Reproductive performance of Bracon hebetor after sublethal doses of carbaryl. Journal of Economic Entomology **68**, 659-662.

GRUYS, P. (1980). Significance and practical application of selective pesticides. Proceedings, International Symposium of IOBC/WPRS on Integrated Control in Agriculture and Forestry, Vienna, 8-12 October 1979, pp. 107-112.

GRUYS, P. (1982). Hits and misses. The ecological approach to pest control in orchards. Entomologia experimentalis et applicata **31**, 70-87.

HALL, F.R. (1979). Effects of synthetic pyrethroids on major insect and mite pests of apple. Journal of Economic Entomology **72**, 441-446.

HALSTEAD, A.J. (1970). The effect of some systemic fungicides and other pesticides on the feeding preference of Phytoseiulus persimilis Athias-Henriot (Acarina: Phytoseiidae). MSc Thesis, University of Wales, 47 pp.

HARTLEY, G.S. & GRAHAM-BRYCE, I.J. (1980). Physical principles of pesticide behaviour, Vol. 2, London, Academic Press.

HASSAN, S.A., BIGLER, F., BOGENSCHÜTZ, H., BROWN, J.U., FIRTH, S.I., HUANG, P., LEDIEU, M.S., NATON, E., OOMEN, P.A., OVERMEER, W.P.J., RIECKMANN, W., SAMSØE-PETERSEN, L., VIGGIANI, G. & VAN ZON, A.Q. (1983). Results of the second joint pesticide testing programme by the IOBC/WPRS - Working Group 'Pesticides and Beneficial Arthropods'. Zeitschrift für angewandte Entomologie **95**, 151-158.

HELLE, W. & OVERMEER, W.P.J. (1973). Variability in tetranychid mites. Annual Review of Entomology **18**, 97-120.

HENDERSON, C.F. & McBURNIE, H.V. (1943). Sampling technique for determining populations of the citrus red mite and its predators. United States Department of Agriculture Circular 671, 11 pp.

HERBERT, H.J. (1974). Notes on the biology of the apple rust mite, Aculus schlechtendali (Prostigmata: Eriophyidae) and its density on several cultivars of apple in Nova Scotia. The Canadian Entomologist **106**, 1035-1038.

HISLOP, G.R., AUDITORE, P.J., WEEKS, B.L. & PROKOPY, R.J. (1981). Repellency of pesticides to the mite predator Amblyseius fallacis. Protection Ecology **3**, 253-257.

HISLOP, G.R. & PROKOPY, R.J. (1981). Integrated management of phytophagous mites in Massachusetts (U.S.A.) apple orchards. 2. Influence of pesticides on the predator Amblyseius fallacis (Acarina:Phytoseiidae) under laboratory and field conditions. Protection Ecology **3**, 157-172.

HOBART, J., FORD, J., KIRKBY, R. & JACKSON, G.J. (1970). Report of the work carried out on Phytoseiulus, 1968/69. Spider Mite Newsletter **4**, 10-12.

HOCART, C.W. (1978). A comparative study of some biological characteristics of two strains of Typhlodromus pyri (Scheuten) (Acarina:Phytoseiidae). MSc. Thesis, University of London, 69 pp.

HOY, M.A., FLAHERTY, D., PEACOCK, W. & CULVER, D. (1979). Vineyard and laboratory evaluations of methomyl, dimethoate and permethrin for a grape pest management program in the San Joaquin Valley of California. Journal of Economic Entomology **72**, 250-255.

HOY, M.A., KNOP, N.F. & JOOS, J.L. (1980). Pyrethroid resistance persists in spider mite predators. California Agriculture **32** (11&12) 11-12.

HOY, M.A., ROSS, N.W. & ROUGH, D. (1978). Impact of NOW insecticides on mites in Northern California almonds. California Agriculture **32** (5) 10-12.

HOY, M.A., ROUSH, R.T., SMITH, K.B. & BARCLAY, L.H. (1979). Spider mites and predators in San Joaquin Valley almond orchards. California Agriculture **33** (10) 11-13.

HOY, M.A., WESTIGARD, P.H. & HOYT, S.C. (1983). Release and evaluation of a laboratory-selected, pyrethroid-resistant strain of the predacious mite Metaseiulus occidentalis (Acari:Phytoseiidae) in Southern Oregon pear orchards and a Washington apple orchard. Journal of Economic Entomology **76**, 383-388.

HOYT, S.C. (1969). Population studies of five mite species on apple in Washington. Proceedings of the 2nd International Congress of Acarology, 1967, pp. 117-133.

HOYT, S.C. (1972). Resistance to azinphosmethyl of Typhlodromus pyri (Acarina:Phytoseiidae) from New Zealand. New Zealand Journal of Science **15**, 16-21.

HOYT, S.C. & BURTS, E.C. (1974). Integrated control of fruit pests. Annual Review of Entomology **19**, 231-252.

HOYT, S.C. & CALTAGIRONE, L.E. (1971). The developing programs of integrated control of pests of apples in Washington and peaches in California. Pp. 395-421 in C.B. Huffaker (Ed.) Biological Control. Plenum Press, New York, 511 pp.

HOYT, S.C., WESTIGARD, P.H. & BURTS, E.C. (1978). Effects of two synthetic pyrethroids on the codling moth, pear psylla and various mite species in Northwest apple and pear orchards. Journal of Economic Entomology **71**, 431-434.

HUDSON, J.P. (1976). Fruit crops: a rather special case. Pp. 81-91 in D.L. Gunn and J.G.R. Stevens (Eds.) Pesticides and Human Welfare. Oxford University Press, Oxford, 278 pp.

HUFFAKER, C.B. & KENNETT, C.E. (1953). Differential tolerance to parathion in two Typhlodromus predatory on cyclamen mite. Journal of Economic Entomology **46**, 707-708.

HUSSEY, N.W. & HUFFAKER, C.B. (Co-ordinators) (1976). 7. Spider mites. Pp. 179-228 in V.L. Delucchi (Ed.) Studies in biological control. Cambridge University Press, Cambridge.

INOUE, K. (1981). The fitness of resistant populations of citrus red mite to acaricides in Japan. Proceedings of the International Society of Citriculture, 1982, Vol. 2, 663-666.

JACKSON, G.J. & FORD, J.B. (1973). The feeding behaviour of Phytoseiulus persimilis (Acarina: Phytoseiidae), particularly as affected by certain pesticides. Annals of Applied Biology **75**, 165-171.

JEPPSON, L.R., KEIFER, H.H. & BAKER, E.W. (1975). Mites injurious to economic plants. University of California Press, Berkeley, 614 pp.

JOLLANDS, P. (1979). An investigation into the factors determining the effectiveness of cypermethrin and pirimiphos-methyl at ultra-low volume. MSc Thesis, University of London, 81 pp.

KAPETANAKIS, E.G. & CRANHAM, J.E. (1983). Laboratory evaluation of resistance to pesticides in the phytoseiid predator Typhlodromus pyri from English apple orchards. Annals of Applied Biology **103**, 389-400.

KEIDING, J. (1967). Persistence of resistant populations after the relaxation of the selection pressure. World Review of Pest Control **6**, 115-130.

KENNEDY, J.S. (1947). The excitant and repellent effects on mosquitoes of sublethal contacts with DDT. Bulletin of Entomological Research **37**, 593-607.

KENNEDY, J.S. (1977). Behaviourally discriminating assays of attractants and repellents. Pp. 215-229 in H.H. Shorey and J.J. McKelvey, Jr. (Eds.) Chemical control of insect behaviour. Wiley-Interscience, New York, 414 pp.

KIRBY, A.H.M. (1973). Progress in the control of orchard pests by integrated methods. Horticultural Abstracts **43**, 1-16 & 57-65.

LEES, A.D. (1953). Environmental factors controlling the evocation and termination of diapause in the fruit tree red spider mite, Metatetranychus ulmi Koch (Acarina, Tetranychidae). Annals of Applied Biology **40**, 449-486.

LEIGH, T.F. & WYNHOLDS, P.F. (1980). Insecticides enhance spider mite reproduction. California Agriculture **34** (10), 14-15.

LORRIMAN, F. & LLEWELLYN, M. (1983). The growth and reproduction of hop aphid (Phorodon humuli) biotypes resistant and susceptible to insecticides. Acta entomologica bohemoslovaka **80**, 87-95.

MADSEN, H.F. & CARTY, B.E. (1977). Pest management: Four years experience in a commercial apple orchard. Journal of the Entomological Society of British Columbia **74**, 3-6.

MAGGI, V.L. & LEIGH, T.F. (1983). Fecundity response of the two-spotted spider mite to cotton treated with methyl parathion or phosphoric acid. Journal of Economic Entomology **76**, 20-25.

MANSOUR, M.H. (1978). Inhibitory and stimulatory effects of low doses of insecticides on growth and reproductivity of the cotton leaf-worm, Spodoptera littoralis Boisd. Zeitschrift fur Pflanzenkrankeiten und Pflanzenschutz **85**, 570-575.

McENROE, W.D. & NAEGELE, J.A. (1968). The coadaptive process in an organophosphorus resistant strain of the two-spotted spider mite, Tetranychus urticae. Annals of the Entomological Society of America **61**, 1055-1059.

McMURTRY, J.A., HUFFAKER, C.B. & VRIE, M. VAN DE (1970). Ecology of tetranychid mites and their natural enemies: A review. I. Tetranychid enemies: Their biological characters and the impact of spray practices. Hilgardia **40**, 331-390.

McMURTRY, J.A., MAHR, D.L. & JOHNSON, H.G. (1976). Geographic races in the predacious mite, Amblyseius potentillae (Acari:Phytoseiidae). International Journal of Acarology **2**, 23-28.

McMURTRY, J.A. & SCRIVEN, G.T. (1965). Insectary production of phytoseiid mites. Journal of Economic Entomology **58**, 282-284.

MIAL, S. & TURNER, I.F. (1982). The irritancy of insecticidal deposits to insects: residual pyrethroids vs Blattella germancia. Abstracts of papers in meeting 'Uptake of Pesticides', Association of Applied Biologists and Society of Chemical Industry, 16th February 1982, Natural History Museum, London, p. 5.

MOORE, R.F. (1980). Behavioural and biological effects of NRDC-161 as factors in control of the boll weevil. Journal of Economic Entomology **73**, 265-267.

MORGAN, C.V.G., CHANT, D.A., ANDERSON, N.H. & AYRE, G.L. (1955). Methods of estimating orchard mite populations, especially with the mite brushing machine. The Canadian Entomologist **87**, 189-200.

MORIARTY, F. (1969). The sublethal effects of synthetic insecticides on insects. Biological Reviews **44**, 321-357.

MORSE, J.G. & CROFT, B.A. (1981). Developed resistance to azinphosmethyl in a predator-prey mite system in greenhouse experiments. Entomophaga **26**, 191-202.

MOTOYAMA, N., ROCK, G.C. & DAUTERMAN, W.C. (1970). Organophosphorus resistance in an apple orchard population of Typhlodromus (Amblyseius) fallacis. Journal of Economic Entomology **63**, 1439-1442.

MUIR, R.C. (1965). The effect of sprays on the fauna of apple trees. II. Some aspects of the interaction between populations of Blepharidopterous angulatus (Fall.) (Heteroptera:Miridae) and its prey, Panonychus ulmi (Koch) (Acarina:Tetranychidae). Journal of Applied Ecology **2**, 43-57.

NAKASHIMA, M.J. & CROFT, B.A. (1974). Toxicity of benomyl to the life stages of Amblyseius fallacis. Journal of Economic Entomology **67**, 675-677.

NEVILLE, A. (1979). Some effects of sublethal doses of pyrethroid insecticides on the rice brown planthopper Nilaparvata lugens (Stahl). MSc. Thesis, University of London, 49 pp.

NIEMCZYK, E. (1964). The distribution of Typhlodromus pyri Scheut. (Acarina, Phytoseiidae) on apple trees in spring. Report of East Malling Research Station for 1963, pp. 180-183.

OATMAN, E.R. (1966). Studies on integrated control of apple pests. Journal of Economic Entomology **59**, 368-373.

OVERMEER, W.P.J. (1981). Notes on breeding phytoseiid mites from orchards in the laboratory. Mededelingen van del Faculteit Landbouwwetenschappen Rijksuniversiteit Gent **46** (2), 503-509.

OVERMEER, W.P.J., DOODEMAN, M. & VAN ZON, A.Q. (1982). Copulation and egg production of Amblyseius potentillae and Typhlodromus pyri (Acari, Phytoseiidae). Zeitschrift fur angewandte Entomologie **93**, 1-11.

OVERMEER, W.P.J. & VAN ZON, A.Q. (1981). A comparative study of the effect of some pesticides on three predacious mite species: Typhlodromus pyri, Amblyseius potentillae and A. bibens (Acarina:Phytoseiidae). Entomophaga **26**, 3-9.

OVERMEER, W.P.J. & VAN ZON, A.Q. (1982). A standardised method for testing the side effects of pesticides on the predacious mite, Amblyseius potentillae (Acarina: Phytoseiidae). Entomophaga **27**, 357-364.

OVERMEER, W.P.J. & VAN ZON, A.Q. (1983). Resistance to parathion in the predacious mite Typhlodromus pyri Scheuten (Acarina: Phytoseiidae). Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent **48** (2) 247-251.

PARKER, B.L., SOOK MING, N.G., SZE PENG, T. & SINGH, G. (1976). The effect of malathion on fecundity, longevity and geotropism of Menochilus sexmaculatus. Environmental Entomology **5**, 495-501.

PARRY, W.H. & FORD, J.B. (1971). The artificial feeding of phosphamidon to Myzus persicae. III. Effects of phosphamidon on the longevity, fecundity and liquid uptake. Entomologia experimentalis et applicata **14**, 389-398.

PENMAN, D.R. & CHAPMAN, R.B. (1983). Fenvalerate-induced distributional imbalances of two-spotted spider mite on bean plants. Entomologia experimentalis et applicata **33**, 71-78.

PENMAN, D.R., CHAPMAN, R.B. & JESSON, K.E. (1981). Effects of fenvalerate and azinphos methyl on two-spotted spider mite and phytoseiid mites. Entomologia experimentalis et applicata **30**, 91-97.

PENMAN, D.R., FERRO, D.N. & WEARING, C.H. (1976). Integrated control of apple pests in New Zealand. VII. Azinphosmethyl resistance in strains of Typhlodromus pyri from Nelson. New Zealand Journal of Experimental Agriculture **4**, 377-380.

PENMAN, D.R., WEARING, C.H., COLLYER, E. & THOMAS, W.P. (1979). The role of insecticide resistant phytoseiids in integrated mite control in New Zealand. Pp. 59-69 in J.G. Rodriguez (Ed.) Recent Advances in Acarology, Vol. I. Academic Press, London, 631 pp.

PERRY, A.S. & AGOSIN, M. (1974). Physiology of insecticide resistance. In: M. Rockstein (Ed.) The Physiology of Insecta, 2nd Edition, Vol. VI. Academic Press, New York.

PLUTHERO, F.G. & SINGH, R.S. (1984). Insect behavioural responses to toxins: practical and evolutionary considerations. The Canadian Entomologist **116**, 57-68.

PUTMAN, W.L. (1959). Hibernation sites of phytoseiids (Acarina: Phytoseiidae) in Ontario peach orchards. The Canadian Entomologist **91**, 735-741.

QUINLAN, J.D. (1981). Recent developments in the chemical control of tree growth. Acta Horticulturae **114**, 144-151.

REISSIG, W.H., STANLEY, B.H., VALLA, M.E., SEEM, R.C. & BOURKE, J.B. (1983). Effects of surface residues of azinphosmethyl on apple maggot behaviour, oviposition and mortality. Environmental Entomology **12**, 815-822.

RICE, A.D., GIBSON, R.W. & STRIBLEY, M.F. (1983). Effects of deltamethrin on walking, flight and potato virus Y-transmission by pyrethroid resistant Myzus persicae. Annals of Applied Biology **102**, 229-236.

RIEDL, H. & HOYING, S.A. (1980). Impact of fenvalerate and diflubenzuron on target and nontarget arthropod species on Bartlett pears in Northern California. Journal of Economic Entomology **73**, 117-122.

ROCK, G.C. (1972). Integrated control of mites. Agricultural Experimental Service, Fruit Insect Note, A-1, North Carolina State University Publication, 18 pp.

ROCK, G.C. (1979). Relative toxicity of two synthetic pyrethroids to a predatory mite Amblyseius fallacis and its prey, Tetranychus urticae. Journal of Economic Entomology **72**, 293-294.

ROCK, G.C. & YEARGAN, D.R. (1971). Relative toxicity of pesticides to organophosphorus-resistant orchard populations of Neoseiulus fallacis and its prey. Journal of Economic Entomology **64**, 350-352.

ROGOFF, W.M. (1952). The repellency of chlordane, DDT, and other residual insecticides to greenhouse thrips. Journal of Economic Entomology **45**, 1065-1071.

ROUSH, R.T. & HOY, M.A. (1978). Relative toxicity of permethrin to a predator, Metaseiulus occidentalis, and its prey, Tetranychus urticae. Environmental Entomology **7**, 287-288.

ROUSH, R.T. & HOY, M.A. (1980). Selection improves Sevin resistance in spider mite predators. California Agriculture **34** (1) 11-13.

RUSCOE, C.N.E. (1977). The new NRDC pyrethroids as agricultural insecticides. Pesticide Science **8**, 236-242.

SABELIS, M.W. (1981). Biological control of two-spotted spider mites using phytoseiid predators. Part I. Modelling the predator-prey interaction at the individual level. PUDOC, Wageningen, 242 pp.

SAMSØE-PETERSEN, L. (1983). Laboratory method for testing side effects of pesticides on juvenile stages of the predatory mite, Phytoseiulus persimilis (Acarina: Phytoseiidae) based on detached bean leaves. Entomophaga **28**, 167-178.

SANTOS, M.A. (1976). Evaluation of Zetzellia mali as a predator of Panonychus ulmi and Aculus schlechtendali. Environmental Entomology **5**, 187-191.

SAWICKI, R.M. & RICE, A.D. (1978). Response of susceptible and resistant peach-potato aphids Myzus persicae (Sulz.) to insecticides in leaf-dip bioassays. Pesticide Science **9**, 513-516.

SOLOMON, M.G. (1975). The colonization of an apple orchard by predators of the fruit tree red spider mite. Annals of Applied Biology **80**, 119-122.

SOLOMON, M.G. (1982). Phytophagous mites and their predators in apple orchards. Annals of Applied Biology **101**, 201-203.

SOUTHWOOD, T.R.E. (1978). Ecological Methods, (2nd edition). Chapman and Hall, London, 524 pp.

STRICKLER, K. & CROFT, B.A. (1981). Variations in permethrin and azinphos-methyl resistance in populations of Amblyseius fallacis (Acarina: Phytoseiidae). Environmental Entomology **10**, 223-236.

SWIFT, F.C. (1970). Predation of Typhlodromus (A.) fallacis on the European red mite as measured by the insecticidal check method. Journal of Economic Entomology **63**, 1617-1618.

SWIRSKI, E., AMITAI, S. & DORZIA, N. (1967). Field and laboratory trials on the toxicity of some pesticides to predacious mites (Acarina: Phytoseiidae). The Israel Journal of Agricultural Research **17**, 149-159.

TAN, K.-H. (1981). Antifeeding effect of cypermethrin and permethrin at sub-lethal levels against Pieris brassicae larvae. Pesticide Science **12**, 619-626.

VAN ZON, A.Q. & VAN DER GEEST, L.P.S. (1980). Effect of pesticides on predacious mites. Pp. 227-230 in E.K. Minks and P. Gruys (Eds.) Integrated control of insect pests in The Netherlands. PUDOC, Wageningen, 304 pp.

VAN ZON, A.Q. & WYSOKI, M. (1978). The effects of some fungicides on Phytoseiulus persimilis (Acarina: Phytoseiidae). Entomophaga **23**, 371-378.

VIRGONA, C.T., HOLAN, G. & SHIPP, E. (1983). Repellency of insecticides to resistant strains of housefly. Entomologia experimentalis et applicata **34**, 287-290.

VOSS, G. (1961). Ein neues Akarizid-Austestungsverfahren für Spinnmilben. Anzeiger fur Schädlingskunde **34** (5) 76-77.

VRIE, M. VAN DE (1962). The influence of spray chemicals on predatory and phytophagous mites on apple trees in laboratory and field trials in The Netherlands. Entomophaga **7**, 243-250.

VRIE, M. VAN DE, McMURTRY, J.A. & HUFFAKER, C.B. (1972). Ecology of tetranychid mites and their natural enemies: A review. III. Biology, ecology and pest status, and host-plant relations of tetranychids. Hilgardia **41**, 343-432.

WALKER, J.T.S. & PENMAN, D.R. (1978). Integrated control of apple pests in New Zealand. 11. The influence of field application of azinphos-methyl on predation of European red mite by Typhlodromus pyri. Proceedings of the 31st New Zealand Weed and Pest Control Conference, pp. 208-213.

WATVE, C.M. & LIENK, S.E. (1975). Responses of two phytoseiid mites to pesticides used in New York apple orchards. Environmental Entomology **4**, 797-800.

WATVE, C.M. & LIENK, S.E. (1976). Toxicity of carbaryl and six organophosphorus insecticides to Amblyseius fallacis and Typhlodromus pyri from New York apple orchards. Environmental Entomology **5**, 368-370.

WAY, M.J. (1949). Laboratory experiments on the effect of DDT and BHC on certain aphidophagous insects and their hosts. Bulletin of Entomological Research **40**, 279-297.

WAY, M.J. (1978). Integrated pest control with special reference to orchard problems. Report of East Malling Research Station for 1977, pp. 187-204.

WEARING, C.H. & ASHLEY, E. (1978). A recommended spray programme for integrated control of European red mite on apples. The Orchardist of New Zealand **51**, 355-356.

WEARING, C.H. & THOMAS, W.P. (1978). Integrated control of apple pests in New Zealand. 13. Selective insect control using diflubenzuron and Bacillus thuringiensis. Proceedings of the 31st New Zealand Weed and Pest Control Conference, pp. 221-228.

WEARING, C.H., WALKER, J.T.S., COLLYER, E. & THOMAS, W.P. (1978). Integrated control of apple pests in New Zealand. 8. Commercial assessment of an integrated control programme against European red mite using an insecticide-resistant predator. New Zealand Journal of Zoology **5**, 823-837.

WHALON, M.E., CROFT, B.A. & MOWRY, T.M. (1982). Introduction and survival of susceptible and pyrethroid-resistant strains of Amblyseius fallacis (Acari: Phytoseiidae) in a Michigan apple orchard. Environmental Entomology **12**, 1096-1099.

WICKENDEN, M.F. (1982). Meteorological records 1980-1981. Report of East Malling Research Station for 1981, pp. 51-55.

WILLIAMS, I.H. (1976). Determination of permethrin by gas-liquid chromatography with electron capture detection. Pesticide Science **7**, 336-338.

WONG, S.W. & CHAPMAN, R.B. (1979). Toxicity of synthetic pyrethroid insecticides to predacious phytoseiid mites and their prey. Australian Journal of Agricultural Research **30**, 497-501.

WORTHING, C.R. (Ed.) (1983). The pesticide manual (7th edition). The British Crop Protection Council, Croydon, UK, 695 pp.

WYATT, I.J. & WHITE, P.F. (1977). Simple estimations of intrinsic increase rates for aphids and tetranychid mites. Journal of applied Ecology **14**, 757-766.

APPENDICES

Appendix 2.1

Detailed effects of carbaryl and diflubenzuron sprayed on 22 April on numbers of *Typhlodromus pyri* and *Panonychus ulmi*. For sample sizes see text 2.2.2.

Treatment and Plot	Mite numbers: Date of sampling 17 April	19 May	9 June
	a. *T. pyri*		
carbaryl			
plot no. 3	31	3	9
" " 4	50	6	7
" " 5	57	6	5
" " 8	36	9	10
total	174	24	31
diflubenzuron			
plot no. 1	4	0	4
" " 2	42	2	2
" " 6	15	3	5
" " 7	17	3	2
total	78	8	13
	b. *P. ulmi*, all stages		
carbaryl			
plot no. 3	-	0	33
" " 4	-	3	2
" " 5	-	9	27
" " 8	-	5	2
total	-	17	64
diflubenzuron			
plot no. 1	-	2	4
" " 2	-	1	0
" " 6	-	1	9
" " 7	-	1	0
total	-	5	13

Appendix 2.2

Detailed effects on *Typhlodromus pyri* of carbaryl and diflubenzuron sprayed on 23 June and 16 July; mite numbers per 48 leaves/plot (96 leaves/variety sub-total).

Treatment and Plot	Numbers of *T. pyri*				
	Date of sampling				
	9 June*	30 June	15 July	4 Aug.	9 Sept.
carbaryl					
plot no. 2	4	3	11	16	35
" " 4	14	19	8	17	35
" " 5	10	24	13	43	48
" " 6	10	10	2	41	31
total	38	56	34	117	149
sub-totals : Cox	18	26	13	50	65
Laxton	20	30	21	67	84
diflubenzuron					
plot no. 1	8	1	1	10	56
" " 3	18	2	10	15	40
" " 7	4	10	8	13	44
" " 8	20	16	9	35	28
total	50	29	28	73	168
sub-totals : Cox	16	16	17	41	93
Laxton	34	13	11	32	75

* Originally 24 leaves per plot were examined. Mite numbers of this sample were doubled to correspond to 48 leaves

Appendix 2.3

Detailed effects on *Panonychus ulmi* of carbaryl and diflubenzuron sprayed on 23 June and 16 July; mite numbers per 48 leaves/plot (96 leaves/variety sub-total)

Treatment and Plot	Numbers of *P. ulmi*, all stages				
	Date of sampling				
	9 June*	30 June	15 July	4 Aug.	9 Sept.
carbaryl					
plot no. 2	0	3	1	38	27
" " 4	4	63	1	159	18
" " 5	54	9	26	86	54
" " 6	18	1	9	52	105
total	76	76	37	335	204
sub-totals : Cox	26	12	3	101	43
Laxton	50	64	34	234	161
diflubenzuron					
plot no. 1	8	4	8	148	225
" " 3	66	27	43	29	41
" " 7	0	0	1	79	83
" " 8	4	0	2	28	2
total	78	31	54	284	351
sub-totals : Cox	4	3	28	115	248
Laxton	74	28	26	169	103

* Originally 24 leaves per plot were examined. Mite numbers of this sample were doubled to correspond to 48 leaves

Appendix 2.4

Detailed effects on *Typhlodromus pyri* and *Panonychus ulmi* of azinphos-methyl and cypermethrin sprayed on 16 July; mite numbers per 33 leaves/plot.

Treatment and Plot	Mite numbers		
	Date of sampling		
	15 July	4 August	9 September
a. *T. pyri*			
azinphos-methyl			
plot AZI	5	12	62
" AZII	3	8	45
" AZIII	4	3	34
total	12	23	141
cypermethrin			
plot RI	4	0	5
" RII	8	0	0
" RIII	7	0	0
total	19	0	5
b. *P. ulmi*, all stages			
azinphos-methyl			
plot AZI	8	91	154
" AZII	21	58	314
" AZIII	3	215	279
total	32	364	747
cypermethrin			
plot RI	4	78	492
" RII	3	8	45
" RIII	0	39	297
total	7	125	834

Appendix 2.5

Effects on tydeid mites of carbaryl and diflubenzuron sprayed on 23 June and 16 July; mite numbers per 192 leaves/treatment (96 leaves/variety sub-total).

Treatment and Variety sub-total	Tydeid numbers			
	Date of sampling			
	30 June	15 July	4 August	9 September
carbaryl				
sub-total Cox	0	7	6	24
sub-total Laxton	0	0	0	3
treatment total	0	7	6	27
diflubenzuron				
sub-total Cox	4	1	27	81
sub-total Laxton	0	2	3	9
treatment total	4	3	30	90

Appendix 3.1

1981 trial: detailed data on the effects of a single spray of 25 mg permethrin/litre on numbers of *Typhlodromus pyri* up to blossom

Date of spray (growth stage of apple)	Replicates and total	Numbers of *T. pyri* per plot* Date of sampling 12 March	30 March	6 April**	19 April	11 May
17 March (bud-burst)	I	42	0	10	1	0
	II	5	2	0	1	0
	III	11	1	3	1	3
	IV	39	82	16	1	20
	total	97	85	29	4	23
6 April (mouse-ear)	I	14	19	2	0	0
	II	23	17	1	0	0
	III	18	28	28	0	4
	IV	25	26	44	0	0
	total	80	90	75	0	4
Unsprayed	I	8	19	8	18	16
	II	66	4	6	7	8
	III	2	71	64	14	33
	IV	25	26	42	12	51
	total	101	120	120	51	108

* For sample sizes see Table 3.2

** Sample taken before application of April spray

Appendix 3.2

1981 trial: detailed data on the effects of a single spray of 25 mg permethrin/litre on numbers of *Typhlodromus pyri* in the summer

Date of spray (growth stage of apple)	Replicates and total	Numbers of *T. pyri* per plot (50 leaves) Date of sampling: 1 June	23 June*	2 July	22 July	6 Aug.	25 Aug.
17 March (bud-burst)	I	2	5	5	13	29	23
	II	0	2	5	13	15	6
	III	6	6	2	6	5	6
	IV	11	6	14	6	4	25
	total	19	19	26	38	53	60
6 April (mouse-ear)	I	3	0	0	9	25	37
	II	0	1	1	7	20	6
	III	2	3	3	5	4	9
	IV	3	10	8	8	10	11
	total	8	14	12	29	59	63
23 June (1 month after petal-fall)	I	9	26	1	0	4	15
	II	7	11	0	1	2	0
	III	4	6	1	1	2	3
	IV	32	7	0	0	0	0
	total	52	50	2	2	8	18
Unsprayed	I	9	5	13	9	14	16
	II	5	25	8	13	8	11
	III	17	6	9	5	1	5
	IV	12	15	13	6	15	8
	total	43	51	43	33	38	40

* Sample taken before application of June spray

Appendix 3.3

1981 trial: detailed data on the effects of a single spray of 25 mg permethrin/litre on numbers of *Panonychus ulmi* in the summer

Date of spray (growth stage of apple)	Replicates total and sub-totals	Numbers of *P. ulmi*, all stages per plot (50 leaves) Date of sampling 1 June	23 June*	2 July	22 July	6 Aug.	25 Aug.
	I	0	7	2	16	6	46
	II	238	290	182	436	166	150
17 March	III	1444	3280	1620	1318	372	334
(bud-burst)	IV	2128	1486	630	248	126	116
	total	3810	5063	2434	2018	670	646
sub-total :	eggs	3154	4751	2094	1968	361	510
	active stages	656	312	340	50	309	136
	I	1	25	2	16	14	34
	II	86	284	162	510	56	154
6 April	III	102	212	152	51	14	30
(mouse-ear)	IV	4200	5188	2072	1184	404	212
	total	4389	5709	2388	1761	488	430
sub-total :	eggs	3883	5442	2132	1705	327	350
	active stages	506	267	256	56	161	80
	I	0	14	1	17	17	30
23 June	II	3	33	12	74	142	452
(1 month after	III	306	266	49	660	340	840
petal-fall)	IV	34	68	4	130	52	544
	total	343	381	66	881	551	1866
sub-total :	eggs	286	345	42	846	303	1448
	active stages	57	36	24	35	248	418
	I	51	13	13	3	4	0
	II	5	55	6	23	10	74
Unsprayed	III	38	9	1	7	0	4
	IV	210	49	14	152	30	8
	total	304	126	34	185	44	86
sub-total :	eggs	252	117	18	177	21	72
	active stages	52	9	16	8	23	14

* Sample taken before application of June spray

Appendix 3.4

1981 trial: persistence of field-weathered residues of permethrin sprays on apple bark and leaves.

Results of chemical analysis by gas chromatography, and bioassay with young Amblyseius potentillae

Date of sampling	Time interval from spray	Chemical analysis: Total (cis+trans) permethrin µg/cm²	Bioassay: % mortality of young A. potentillae
	Days	Means per plot	Means per plot
Bark sprayed 17 March			
17 March*	0	0.185	100
27 March	10	0.087	35.0
6 April	20	0.118	22.5
30 April	44	0.058	10.0
29 May	73	0.042	- **
23 June	98	0.018	- **
28 September	195	0.016	- **
Bark sprayed 6 April			
6 April*	0	0.150	70.0
17 April	11	0.063	50.0
30 April	24	0.055	20.0
11 May	35	0.052	17.5
29 May	53	0.047	- **
23 June	78	0.035	- **
28 September	175	0.007	- **
Leaves sprayed 23 June			
23 June*	0	0.130	87.5
30 June	7	- **	55.0
7 July	14	0.055	37.5
14 July	21	- **	20.0
21 July	28	0.041	10.0
4 August	42	0.035	7.5
24 August	62	0.025	5.0
28 September	97	0.020	- **

* Sample taken immediately after spray application

** Not tested

Appendix 4.1 Results for taped-slide bioassays of organophosphates on Typhlodromus pyri: detailed data from probit analyses (LC_{50}s and LC_{90}s in mg/litre)

Insecticide / Mite stock	24 h assessment		48 h assessment		Common slope[2] ± standard error	Total heterogeneity[2] (chi sq./degrees of freedom/significance[4])	Position difference[3] of the 2 lines (24 h and 48 h)
	LC_{50} (95% FL[1])	LC_{90} (95% FL)	LC_{50} (95% FL)	LC_{90} (95%FL)			
A. Azinphos-methyl							
Amsterdam	218 (196-242)	399 (347-480)	114 (101-128)	208 (182-249)	4.87 ± 0.47	1.48/ 5/n.s.	***
Amsterdam (fed on T. urticae)	226 (191-267)	456 (373-607)	118 (98-140)	238 (196-311)	4.21 ± 0.52	4.38/ 6/n.s.	***
Bangor	122 (112-134)	198 (177-228)	96 (87-106)	155 (137-180)	6.13 ± 0.56	3.35/ 4/n.s.	***
Bristol	191 (161-227)	369 (297-528)	100 (81-121)	194 (158-264)	4.48 ± 0.70	1.72/ 4/n.s.	***
Evesham	270 (244-300)	478 (418-567)	174 (155-194)	308 (270-362)	5.16 ± 0.46	8.83/ 6/n.s.	***
MP4	357 (312-409)	785 (654-1010)	243 (209-280)	534 (447-680)	3.75 ± 0.39	3.25/ 5/n.s.	***
MP9	714 (618-832)	1240 (1030-1630)	543 (470-641)	941 (755-1270)	5.38 ± 0.75	3.80/ 4/n.s.	*
Lynsted	460 (410-515)	860 (752-1020)	357 (316-402)	666 (577-798)	4.72 ± 0.41	8.82/ 8/n.s.	**
Norton	558 (486-639)	1170 (992-1450)	374 (326-427)	787 (671-963)	3.97 ± 0.35	11.99/10/n.s.	***
WF (Hadlow)	739 (627-886)	1410 (1130-2020)	578 (490-705)	1100 (869-1640)	4.56 ± 0.68	2.84/ 4/n.s.	n.s.
B. Parathion							
Bangor	36.8 (30.6-44.2)	77.7 (61.9-111)	26.8 (21.9-32.2)	56.5 (45.4-78.8)	3.95 ± 0.56	1.84/ 5/n.s.	*
MP9	2250 (1910-2660)	4180 (3420-5700)	1820 (1550-2190)	3390 (2720-4800)	4.76 ± 0.67	0.44/ 4/n.s.	n.s.
Norton	1790 (1520-2090)	3110 (2580-4270)	1350 (1110-1590)	2360 (1970-3110)	5.32 ± 0.88	1.07/ 4/n.s.	n.s.

1. FL ≡ Fiducial limits

2. The data of this Table were derived from 'adjusted parallel lines', as the two lines, for 24 h and 48 h, were parallel (P=0.05) for each one of the mite stocks

3. Significance of chi sq.: n.s. ≡ not significant; * ≡ P=0.05; ** ≡ P=0.01; *** ≡ P=0.001

Appendix 4.2 Results for taped-slide bioassays of carbaryl on Typhlodromus pyri:

detailed data from probit analyses (LC_{50}s and LC_{90}s in mg/litre)

Mite stock	24 h assessment				48 h assessment				Parallelism[2]	Position[3] difference of the 2 lines (24 h and 48 h)
	LC_{50} (95% FL[1])	LC_{90} (95% FL)	Slope ± standard error	Heterogeneity (chi sq./degrees of freedom/ significance)	LC_{50} (95% FL)	LC_{90} (95% FL)	Slope ± standard error	Heterogeneity (chi sq./degrees of freedom/ significance)		
Amsterdam	2910 (2180-4220)	18 800 (10 600-49 800)	1.58 ± 0.22	2.380/4/n.s.	795 (641-976)	2510 (1910-3770)	2.56 ± 0.31	8.340/4/n.s.	**	***
Bangor	990 (722-1370)	7630 (4230-23 500)	1.44 ± 0.25	0.445/3/n.s.	323 (258-395)	953 (716-1530)	2.73 ± 0.41	0.765/2/n.s.	**	***
Bristol	3110 (2410-4310)	6100 (4380-21 500)	4.38 ± 1.39	2.263/2/n.s.	1710 (1080-2370)	4990 (3260-19 200)	2.75 ±0.80	1.296/1/n.s.	n.s.	**
Evesham	4750 (3310-8100)	>20 000 (>20 000)	1.41 ± 0.22	4.730/5/n.s.	420 (318-532)	1630 (1170-2770)	2.18 ± 0.32	0.637/3/n.s.	*	***
MP4	>20 000 (n.e.)	>20 000 (n.e.)	5.23 ± 3.25[4]	0.336/3/n.s.	13,400 (9790-17 300)	>20 000 (>20 000)	2.65 ± 0.83	0.618/1/n.s.	n.s.	***
MP9	>20 000 (n.e.)	>20 000 (n.e.)	n.e.	n.e.	>20 000 (n.e.)	>20 000 (n.e.)	9.76 ± 5.84[4]	0.268/1/n.s.	n.e.	n.e.
Lynsted	>20 000 (n.e.)	>20 000 (n.e.)	n.e.	n.e.	>20 000 (n.e.)	>20 000 (n.e.)	3.35 ± 3.34[4]	0.219/3/n.s.	n.e.	n.e.
Norton	>20 000 (n.e.)	>20 000 (n.e.)	n.e.	n.e.	>20 000 (n.e.)	>20 000 (n.e.)	0.76 ± 0.92[4]	1.876/1/n.s.	n.e.	n.e.
WF (Hadlow)	9900 (7140-13 400)	>20 000 (>20 000)	2.46 ± 0.59	4.171/2/n.s.	7100 (5100-10 500)	>20 000 (>20 000)	2.20 ± 0.50	0.349/2/n.s.	n.s.	n.s.

1. FL ≡ Fiducial limits

2. n.s. ≡ chi sq. for parallelism is not significant, i.e. the 2 lines (for 24 h and 48 h) are parallel; *, ** ≡ chi sq. significant for $P=0.05$ and $P=0.01$ respectively, i.e. lines are not parallel; n.e. ≡ not estimable

3. n.s. ≡ the two lines, for 24 h and 48 h, do not differ in position; **, *** ≡ the two lines differ for $P=0.01$ and $P=0.001$ respectively

4. Not significantly different from zero

Appendix 4.3 Results for taped-slide bioassays of insecticides on Typhlodromus pyri and Amblyseius potentillae: detailed data from probit analyses (LC_{50}s and LC_{90}s in mg/litre)

MITE SPECIES / Insecticide / Mite stock	24 h assessment		48 h assessment		Common slope[2] ± standard error	Total heterogeneity[2] (chi sq./degrees of freedom/significance[3])	Position difference[3] of the 2 lines (24 h and 48 h)
	LC_{50} (95% FL[1])	LC_{90} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)			
A. T. PYRI							
permethrin							
Bangor	20.4 (16.8-24.5)	37.7 (30.6-51.9)	19.8 (16.1-24.0)	36.5 (29.5-50.4)	4.81 ± 0.74	5.306/8/n.s.	n.s.
Bristol	20.1 (15.1-26.0)	57.6 (41.0-108)	20.8 (15.8-27.4)	59.6 (41.5-117)	2.80 ± 0.51	1.420/7/n.s.	n.s.
Evesham	25.0 (20.1-33.8)	52.3 (37.5-110)	20.9 (16.6-26.9)	43.6 (32.3-83.8)	4.01 ± 0.87	4.157/4/n.s.	n.s.
MP9	30.7 (23.5-44.3)	115 (69.5-337)	25.8 (19.7-35.4)	96.6 (60.8-258)	2.24 ± 0.45	1.448/6/n.s.	n.s.
Lynsted	15.2 (11.9-18.4)	31.1 (25.2-42.6)	14.9 (11.7-18.2)	30.5 (24.5-42.3)	4.11 ± 0.65	5.380/7/n.s.	n.s.
Norton	18.3 (14.8-22.2)	39.5 (31.5-55.7)	17.7 (14.3-21.7)	38.2 (30.0-55.0)	3.84 ± 0.56	3.022/7/n.s.	n.s.
WF (Hadlow)	18.1 (14.7-22.2)	50.8 (38.6-77.1)	16.9 (13.4-20.8)	47.1 (36.0-70.7)	2.87 ± 0.38	7.142/8/n.s.	n.s.
B. A. POTENTILLAE							
azinphos-methyl	55.8 (45.8-65.1)	97.3 (82.3-125)	45.6 (38.6-53.9)	79.4 (65.2-110)	5.31 ± 0.87	3.015/3/n.s.	n.s.
carbaryl	413 (300-553)	1350 (950-2280)	261 (185-355)	852 (601-1430)	2.50 ± 0.36	2.032/5/n.s.	*
permethrin	7.88 (6.18-10.0)	27.0 (20.0-40.1)	6.38 (4.98-8.11)	21.8 (16.3-32.0)	2.40 ± 0.24	4.922/8/n.s.	n.s.

1. FL = Fiducial limits

2. The data of this Table were derived from 'adjusted parallel lines', as the two lines, for 24 h and 48 h, were parallel ($P=0.05$) for each one of the mite stock-insecticide combinations shown

3. Significance of chi sq.: n.s. = not significant; * = $P=0.05$

Appendix 4.4 Results for residue bioassays of three insecticides on Typhlodromus pyri: detailed data from probit analyses (LC_{50}s and LC_{90}s in mg/litre)

MITE STOCK Insecticide Technique	24 h assessment LC_{50} (95% FL[1])	24 h assessment LC_{90} (95% FL)	48 h assessment LC_{50} (95% FL)	48 h assessment LC_{90} (95% FL)	Common slope[2] ± standard error	Total heterogeneity[2] (chi sq./degrees of freedom/significance[4])	Position difference[3] of the 2 lines (24 h and 48 h)
A. AMSTERDAM							
azinphos-methyl							
leaf/residue	67.0 (52.0-86.5)	117 (89.5-245)	56.5 (42.9-80.9)	94.4 (71.6-236)	5.32 ± 1.48	0.277/2/n.s.	n.s.
glass/residue	4.82 (2.82-8.76)	18.0 (9.68-64.0)	2.05 (1.15-3.43)	7.69 (4.41-22.4)	2.24 ± 0.48	1.435/3/n.s.	*
carbaryl							
leaf/residue	923 (575-1610)	3550 (1930-14,200)	554 (319-887)	2130 (1250-6730)	2.19 ± 0.50	0.484/4/n.s.	n.s.
glass/residue	468 (247-1090)	4120 (1580-34,500)	140 (69.8-259)	1230 (567-6440)	1.36 ± 0.29	2.485/7/n.s.	**
permethrin							
leaf/residue	4.27 (3.50-5.25)	7.97 (6.31-11.6)	4.34 (3.45-5.42)	8.10 (6.34-11.6)	4.73 ± 0.74	0.730/2/n.s.	n.s.
glass/residue	0.644 (0.500-0.991)	2.49 (1.47-7.27)	0.401(0.278-0.584)	1.55 (0.956-4.06)	2.18 ± 0.43	0.271/3/n.s.	n.s.
B. MP4							
azinphos-methyl							
leaf/residue	145 (115-183)	281 (217-437)	132 (101-176)	255 (189-425)	4.46 ± 0.80	3.207/6/n.s.	n.s.
glass/residue	14.3 (11.0-17.9)	26.0 (20.3-42.7)	10.8 (7.95-13.7)	19.6 (15.2-31.4)	4.92 ± 1.09	0.964/3/n.s.	n.s.
carbaryl							
leaf/residue	16 600 (7580-57 900)	>20 000 (>20 000)	7240 (3030-17 700)	>20 000 (>20 000)	1.27 ± 0.33	1.267/4/n.s.	n.s.
glass/residue	5110 (not estimable)	>20 000 (not estimable)	not estimable	not estimable	3.35 ± 3.34[4]	(0.280/1/n.s.)	(not estimable)

1. FL = Fiducial limits

2. The data of this Table were derived from 'adjusted parallel lines', as the two lines, for 24 h and 48 h, were parallel (P=0.05) for each one of the mite stock-insecticide combinations shown with the exception of the MP4-carbaryl-glass/residue; for that case the data shown refer to the 24 h assessment

3. Significance of chi sq.: n.s. = not significant; * = P=0.05; ** = P=0.01

4. Not significantly different from zero

Appendix 5.1 Life table with data from a laboratory experiment to compare the fecundity of Typhlodromus pyri adult females reared on broad bean pollen vs females reared on Tetranychus urticae

Symbols used in the table:

a = numbers of T. pyri adult females alive at the beginning and end of the 2-day period
b = number of eggs laid in the 2-day period
x = pivotal age of each age group
l_x = number of adult females of pivotal age x alive, as a fraction of an initial population of 1
m_x = age specific fecundity per female, i.e. the number of eggs laid per female during the age interval with pivotal age x
$l_x m_x$ = fecundity rate for age x
$Re = \Sigma l_x m_x$ = Net Rate of Egg Production

Day of test	a	b	x	l_x	m_x	$l_x m_x$	$Re = \Sigma l_x m_x$
A. T. pyri reared on pollen							
Replicate I							
2	5-5	0	1	1	0	0	
4	5-5	0	3	1	0	0	
6	5-5	2	5	1	0.40	0.40	
8	5-5	7	7	1	1.40	1.40	
10	5-5	3	9	1	0.60	0.60	
12	5-5	4	11	1	0.80	0.80	
							Re = 3.20
Replicate II							
2	5-5	0	1	1	0	0	
4	5-5	2	3	1	0.40	0.40	
6	5-5	3	5	1	0.60	0.60	
8	5-5	4	7	1	0.80	0.80	
10	5-5	3	9	1	0.60	0.60	
12	5-5	5	11	1	1.00	1.00	
							Re = 3.40
B. T. pyri reared on T. urticae							
Replicate I							
2	5-5	6	1	1	1.20	1.20	
4	5-5	7	3	1	1.40	1.40	
6	5-5	8	5	1	1.60	1.60	
8	5-4	8	7	0.9	1.78	1.60	
10	4-4	8	9	0.8	2.00	1.60	
12	4-4	6	11	0.8	1.50	1.20	
							Re = 8.60
Replicate II							
2	5-5	7	1	1	1.40	1.40	
4	5-5	6	3	1	1.20	1.20	
6	5-5	12	5	1	2.40	2.40	
8	5-5	12	7	1	2.40	2.40	
10	5-5	9	9	1	1.80	1.80	
12	5-5	10	11	1	2.00	2.00	
							Re = 11.20

Appendix 6.1. Distribution of *Typhlodromus pyri* adult females between the permethrin-treated (2 mg/litre) and the control surface of a glass arena

Serial number of count	Time from start of test	Number of mites counted: permethrin	Number of mites counted: control
A. First series of counts			
1	1 min	7	1
2	2	5	3
3	3	4	4
4	4	5	3
5	5	3	5
6	6	6	2
7	7	5	3
8	8	6	2
9	9	5	3
10	10	4	4
11	11	5	3
12	12	3	5
13	13	7	1
14	14	8	0
15	15	3	5
16	16	4	4
17	17	6	2
18	18	4	4
19	19	2	6
20	20	7	1
Total 20		99	61
B. Second series of counts*			
1	2h	1	6
2	2h 25min	3	4
3	2 50	4	3
4	3 15	4	3
5	3 40	4	3
6	4 05	5	2
7	4 30	5	2
8	4 55	5	2
9	5 20	5	2
10	5 45	5	2
Total 10		41	29
C. Third series of counts**			
1	22h	5	1
2	22h 30min	4	2
3	23h	3	3
Total 3		12	6

* one mite was dead in the wet barrier when these counts started
** one mite was dead at edge of wet barrier when these counts started

Appendix 6.2. Distribution of *Typhlodromus pyri* adult females between the permethrin-sprayed (0.5 mg/litre) and the control halves of glass arenas

A. First series of counts

Serial number of count	Time from start of test	Number of mites counted					
		Replicate I		Replicate II		Total	
		permethrin	control	permethrin	control	permethrin	control
1	10min	4	6	8	2	12	8
2	20	4	6	6	4	10	10
3	30	2	8	4	6	6	14
4	40	1	9	2	8	3	17
5	50	1	9	3	7	4	16
6	1h	1	9	2	8	3	17
7	1h 10min	1	9	2	8	3	17
8	1 20	1	9	3	7	4	16
9	1 30	2	8	3	7	5	15
10	1 40	1	9	3	7	4	16
11	1 50	1	9	2	8	3	17
12	2h	0	10	0	10	0	20
13	2h 10min	0	10	0	10	0	20
14	2 20	2	8	0	10	2	18
15	2 30	1	9	1	9	2	18
16	2 40	0	10	1	9	1	19
17	2 50	1	9	2	8	3	17
18	3h	1	9	3	7	4	16
19	3h 10min	2	8	2	8	4	16
20	3 20	0	10	1	9	1	19
Total 20		26	174	48	152	74	326

(continued)

Appendix 6.2 (continued)

B. Second series of counts

Serial number of count	Time from start of test	Number of mites counted					
		Replicate I		Replicate II		Total	
		permethrin	control	permethrin	control	permethrin	control
1	17h 40min	4	6	2	8	6	14
2	17 50	6	4	2	8	8	12
3	18h	4	6	4	6	8	12
4	18h 10min	3	7	3	7	6	14
5	18 20	4	6	4	6	8	12
6	18 30	5	5	3	7	8	12
7	18 40	4	6	3	7	7	13
8	18 50	4	6	4	6	8	12
9	19h	4	6	3	7	7	13
10	19h 10min	4	6	3	7	7	13
Total 10		42	58	31	69	73	127

Appendix 6.3. Distribution of *Typhlodromus pyri* adult females between the azinphos-methyl-sprayed and the control halves of glass arenas

Serial number of count	Time from start of test	Number of mites counted					
		Replicate I		Replicate II		Total	
		azinphos-m.	control	azinphos-m.	control	azinphos-m.	control
1	10min	5	5	5	5	10	10
2	20	6	4	4	6	10	10
3	30	4	6	2	8	6	14
4	40	6	4	4	6	10	10
5	50	4	6	5	5	9	11
6	1h	3	7	4	6	7	13
7	1h 10min	3	7	6	4	9	11
8	1 20	5	5	9	1	14	6
9	1 30	9	1	6	4	15	5
10	1 40	7	3	7	3	14	6
11	1 50	4	6	8	2	12	8
12	2h	6	4	8	2	14	6
13	2h 10min	7	3	7	3	14	6
14	2 20	5	5	7	3	12	8
15	2 30	5	5	4	6	9	11
16	2 40	4	6	5	5	9	11
17	2 50	5	5	6	4	11	9
18	3h	6	4	6	4	12	8
19	3h 10min	6	4	5	5	11	9
20	3 20	4	6	5	5	9	11
Total 20		104	96	113	87	217	183

Appendix 6.4. Distribution of *Typhlodromus pyri* adult females between the carbaryl-sprayed and the control halves of glass arenas

Serial number of count	Time from start of test	Number of mites counted					
		Replicate I		Replicate II		Total	
		carbaryl	control	carbaryl	control	carbaryl	control
1	10min	6	4	7	3	13	7
2	20	3	7	2	8	5	15
3	30	1	9	3	7	4	16
4	40	5	5	6	4	11	9
5	50	0	10	3	7	3	17
6	1h	4	6	6	4	10	10
7	1h 10min	4	6	6	4	10	10
8	20	6	4	8	2	14	6
9	30	2	8	6	4	8	12
10	40	2	8	9	1	11	9
11	50	2	8	6	4	8	12
12	2h	7	3	6	4	13	7
13	2h 10min	5	5	6	4	11	9
14	2 20	3	7	7	3	10	10
15	2 30	5	5	8	2	13	7
16	2 40	3	7	7	3	10	10
17	2 50	2	8	4	6	6	14
18	3h	6	4	5	5	11	9
19	3h 10min	7	3	5	5	12	8
20	3 20	8	2	2	8	10	10
Total 20		81	119	112	88	193	207

Appendix 6.5. Distribution of *Typhlodromus pyri* adult females between the permethrin-sprayed and the control halves of apple leaf arenas

p = permethrin c = control

A. First series of counts

Serial number of count	Time from start of test	Number of mites counted									
		Rep.I		Rep.II		Rep.III		Rep.IV		Total	
		p	c	p	c	p	c	p	c	p	c
1	10min	2	8	4	6	7	3	3	7	16	24
2	20	1	9	3	7	5	5	4	6	13	27
3	30	2	8	3	7	5	5	2	8	12	28
4	40	3	7	5	5	4	6	2	8	14	26
5	50	0	10	3	7	3	7	4	6	10	30
6	1h	2	8	6	4	3	7	2	8	13	27
7	1h 10min	1	9	6	4	1	9	3	7	11	29
8	1 20	2	8	5	5	3	7	5	5	15	25
9	1 30	1	9	4	6	2	8	5	5	12	28
10	1 40	3	7	5	5	3	7	3	7	14	26
11	1 50	1	9	5	5	2	8	2	8	10	30
12	2h	0	10	5	5	5	5	3	7	13	27
13	2h 10min	2	8	4	6	5	5	2	8	13	27
14	2 20	0	10	4	6	5	5	2	8	11	29
15	2 30	1	9	3	7	5	5	3	7	12	28
16	2 40	0	10	5	5	3	7	3	7	11	29
17	2 50	0	10	4	6	2	8	1	9	7	33
18	3h	0	10	3	7	3	7	1	9	7	33
19	3h10min	0	10	4	6	4	6	2	8	10	30
20	3 20	0	10	3	7	4	6	0	10	7	33
21	3 30	0	10	5	5	5	5	0	10	10	30
22	3 40	0	10	5	5	3	7	1	9	9	31
23	3 50	2	8	6	4	3	7	0	10	11	29
Total 23		23	207	100	130	85	145	53	177	261	659

(continued)

Appendix 6.5. (continued)

B. Second series of counts

Serial number of count	Days from start of test	Number of mites counted									
		Rep.I		Rep.II		Rep.III		Rep.IV		Total	
		p	c	p	c	p	c	p	c	p	c
1	1	0	10	5	5	4	6	3	7	12	28
2	2	0	10	4	6	5	5	3	7	12	28
3	3	0	10	4	6	1	9	0	10	5	35
4	4	0	9*	3	7	3	7	2	8	8	31
5	5	0	9	2	8	7	3	2	8	11	28
6	6	0	9	6	4	3	7	3	7	12	27
7	7	1	8	3	7	4	6	4	6	12	27
Total 7		1	65	27	43	27	43	17	53	72	204

* one mite found dead in control half of arena

Appendix 6.6. Distribution of eggs laid by *Typhlodromus pyri* adult females in the permethrin-sprayed and the control halves of apple leaf arenas

p = permethrin c = control

Serial number of count	Days from start of test	Number of eggs laid									
		Rep.I		Rep.II		Rep.III		Rep.IV		Total	
		p	c	p	c	p	c	p	c	p	c
1	1	0	2	0	3	0	1	0	0	0	6
2	2	0	2	2	1	0	1	0	2	2	6
3	3	3	4	1	6	1	6	0	2	5	18
4	4	0	8	2	7	1	3	0	7	3	25
5	5	0	5*	7	2	1	6	0	3	8	16
6	6	0	5*	4	5	2	6	0	2	6	18
7	7	0	6*	5	6	0	5	0	3	5	20
Total 7		3	32	21	30	5	28	0	19	29	109

* Laid by 9 females; one female was found dead in Rep.I on Day 4

PUBLICATION OF WORK RELEVANT TO THIS THESIS

1. E.G. KAPETANAKIS. Laboratory bioassays for checking the persistent toxicity of permethrin to phytoseiid mites on apple trees.
   Paper presented in the Fifth Meeting of the Working Group WPRS/IOBC 'Pesticides and Beneficial Arthropods', Rothamsted Experimental Station, Harpenden, Herts. England, 27-30 October 1981.
   (Related to bioassays reported in section 3)

2. J.E. CRANHAM, G.M. TARDIVEL and E.G. KAPETANAKIS. Orchard trials to assess the effects of pesticides on Typhlodromus pyri and its prey Panonychus ulmi.
   Paper presented by the senior author in the 'VI International Congress of Acarology' Edinburgh, Scotland, 5-11 September 1982 (proceedings in press).
   A photocopy of the paper as submitted (8 pp. + 1 Table and 2 Figs) is enclosed in the pocket inside the back cover of this Thesis.)
   (Includes results of field trial reported in section 2)

3. J.E. CRANHAM, E.G. KAPETANAKIS and A.J. FISHER. Resistance to insecticides in the predatory mite Typhlodromus pyri and its spider mite prey.
   Paper presented by the senior author in the '10th International Congress of Plant Protection 1983', Brighton, England, 20-25 November 1983.
   A photocopy of the paper (Proceedings of the Congress, Vol. 2, p. 638), together with a graph presented in a

poster session during the Congress, are enclosed in the pocket inside the back cover of this Thesis.
(Includes results of bioassays reported in section 4)

4. E.G. KAPETANAKIS and J.E. CRANHAM. Laboratory evaluation of resistance to pesticides in the phytoseiid predator Typhlodromus pyri from English apple orchards.
Paper published in the Annals of Applied Biology (1983), **103**, 389-400.
A photocopy is enclosed in the pocket inside the back cover of this Thesis.
(Includes results of bioassays reported in section 4)

5. J.E. CRANHAM, M.A. EASTERBROOK and E.G. KAPETANAKIS. Effects of pyrethroids on apple orchard mites.
Paper presented by the senior author in the Symposium 'Pyrethroid Insecticides in the Environment', Southampton University, Southampton, England, 12 and 13 April 1984. Abstracts of papers will be published in Pesticide Science.
(Includes results from tests reported in section 6)

6. E.G. KAPETANAKIS, T.M. WARMAN and J.E. CRANHAM. Effects of permethrin sprays on the mite fauna of apple orchards. Paper submitted to the Annals of Applied Biology in May 1984. A photocopy of the paper as submitted (19 pp. + 2 Tables and 4 Figs.) is enclosed in the pocket inside the back cover of this Thesis.
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Laboratory evaluation of resistance to pesticides in the phytoseiid predator *Typhlodromus pyri* from English apple orchards

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SUMMARY

Typhlodromus pyri, reared on plate cultures and fed on pollen of *Vicia faba*, were bioassayed using a taped-slide technique. Mite stocks from isolated unsprayed orchards were used to establish base-line susceptibility to azinphos-methyl, parathion, carbaryl and permethrin. Stocks from English orchards with a spray history of organophosphates and carbaryl showed resistance of 4 to 6× to azinphos-methyl, *c* 50× to parathion, >20× to carbaryl, and no resistance to permethrin. An orchard population derived from *T. pyri* imported from New Zealand in 1977 responded similarly. In other tests, mites exposed to spray residues on glass and on apple leaves were killed by lower concentrations than in taped-slide tests (glass < apple leaf < taped-slide technique); but for all three assay techniques the resistance factors to azinophos-methyl and carbaryl, comparing two stocks, were similar. All stocks from sprayed orchards were resistant to both azinphos-methyl and carbaryl, suggesting cross-resistance; and resistance to both these pesticides appeared to be stable in the field when selection pressure was relaxed. The results are discussed in relation to earlier work on *T. pyri* and two other resistant orchard-inhabiting phytoseiid species.

INTRODUCTION

In England and in the Netherlands recent work on integrated pest management on apple has shown that the predatory phytoseiid mite *Typhlodromus pyri* Scheuten is able to regulate fruit tree red spider mite (*Panonychus ulmi* Koch) at very low, non-damaging, numbers (Easterbrook, Souter, Solomon & Cranham, 1979; Gruys, 1982). In New Zealand *T. pyri* has developed marked resistance to azinphos-methyl (Hoyt, 1972; Penman, Ferro & Wearing, 1976), permitting the development of 'integrated mite control' programmes in which azinphos-methyl is used to control orchard tortricids (Wearing, Walker, Collyer & Thomas, 1978). Resistance of *T. pyri* to various organophosphates (OPs) was also reported from New York State (Watve & Lienk, 1975, 1976). In England, the accumulated field evidence from 1977 indicates that OP resistance has become common in commercial sprayed orchards (Cranham, Tardivel & Kapetanakis, 1983; Flegg, 1983) and that *T. pyri* usually tolerates high rates of carbaryl. Similar evidence for high tolerance to carbaryl in this species was reported from the Netherlands (Gruys, 1980), New Zealand (Wearing & Ashley, 1978) and New York State, USA (Watve & Lienk, 1976). Resistant strains of two other phytoseiid species, *Amblyseius fallacis* and *Typhlodromus occidentalis*, have been widely exploited in integrated mite control programmes in N. America and Australasia (Croft, 1981). When resistance to OPs developed in these two species it did not *per se* confer cross-resistance to carbaryl (Croft, 1982); it was desirable therefore to study this aspect for *T. pyri*.

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An OP-resistant strain of *T. pyri* was imported to England from New Zealand in January, 1977 (courtesy of Dr H. Wearing, D.S.I.R., Auckland); it was mass-reared on potted plum trees under glass and released in August, 1977 in Middle Park orchard, East Malling Research Station (EMRS). In the same year, *T. pyri* in a commercial apple orchard at Hadlow, Kent (12 km south of EMRS) were observed to survive repeated OP sprays; mass releases of this population were made in Weston's Field orchard, Rocks Farm, EMRS in autumn 1977 and spring 1978. With the apparent development of resistance in native *T. pyri* to several commonly used effective pesticides (certain OPs, carbaryl, HCH) (Solomon & Cranham *in* Flegg, 1982 pp. 100–101) and the commercial use of several non-acaricidal fungicides, 'integrated mite management' was demonstrably practical in commercial orchards (Cranham & Solomon, 1981). However, a major threat to this development stems from the current popularity of synthetic pyrethroid (SP) insecticides, of which permethrin was the first to be marketed; all SPs sold for orchard use proved persistently toxic to *T. pyri* (AliNiazee & Cranham, 1980; J. E. Cranham *in* Flegg, 1982, p. 101).

The aims of the bioassay studies reported in this paper were (1) using stocks from isolated, unsprayed orchards, to establish levels of 'base-line' susceptibility in *T. pyri* to azinphos-methyl, parathion, carbaryl and (2) using stocks from orchards with various spray histories of OPs and/or carbaryl, to quantify the levels of resistance occurring. Three different bioassay techniques were compared. In addition, it was hoped that these studies would shed light on the relation between resistance to OPs and to carbaryl in this species, the stability of such resistance and whether the acquisition of resistance to OPs/carbaryl influenced the response to permethrin. To add to available data (reviewed by Croft, 1982) on the comparative toxicological responses of important orchard phytoseiid species, assays were also run on *Amblyseius potentillae* Garman, a species considered important in the Netherlands.

MATERIALS AND METHODS

Provenance of stocks of T. pyri

Table 1 lists details of the stocks of *T. pyri* reared in the laboratory and used in bioassays. These four 'unselected' stocks either had no known history of pesticide exposure, or were known to have been exposed to very few spray applications. Other phytoseiid species were present in these collections, and particular care was taken to start cultures with *T. pyri* only. The remainder of the stocks were collected from mature apple orchards with varying histories of spraying over many years; in these collections, *T. pyri* was the only phytoseiid species present.

OP-selected stocks

The OP-resistant *T. pyri* imported from New Zealand (see above) were released in Middle Park (MP) orchard, Home Farm, EMRS on 10 August 1977, shortly after two sprays of azinphos-methyl, intended to eliminate susceptible native *T. pyri*, were applied on 27 July and 4 August. The whole orchard received one spray of azinphos-methyl in 1978.

In a mite-management trial started in 1979 in a commercial orchard (Fant Farm), Blocks A and B, of 2 ha each, were separated by a third 2 ha block on which pyrethroids kept *T. pyri* at very low numbers. Survival of *T. pyri* on Block B in 1979 showed that they were already resistant to OPs when the trial was started.

Carbaryl-selected stocks

Commercial orchards at Lynsted and Norton had received 2–3 sprays of carbaryl per year for many years, and in certain years a single pre-blossom spray of demeton-S-methyl for aphid control. There appeared to be no commercial orchards that had received only carbaryl and no OPs whatever.

Table 1. *Laboratory stocks of* Typhlodromus pyri *used in bioassays*

Code name	Site of collection; spray history	Date of collection	Initial no. of females
'Unselected' stocks			
Amsterdam	Lab. of Experimental Entomology, University of Amsterdam, Netherlands; unsprayed apples	March 1981	20
Bangor	near Bangor, N. Wales; unsprayed apple trees	October 1981	6
Bristol	Bristol, Avon; ADAS apple orchard established *c.* 1968 minimal use of insecticides *c.* 1970, unsprayed since.	July 1982	35
Evesham	Near Evesham, Worcs.; plum orchard; one DDT spray/ year for 20 yr, no OPs or carbaryl recorded	October 1981	50
OP-selected stocks			
Fant B	Commercial orchard near Maidstone, Kent; 16 OP sprays 1979–82, no carbaryl.	September 1982	50
MP/4	Middle Park Orchard, EMRS; New Zealand stock established 1977, azinphos-methyl four sprays 1979–80, no carbaryl.	June 1981	50 50
MP/9	Different section of same orchard; azinphos-methyl nine sprays 1979–81, no carbaryl.	June 1982	50
Carbaryl-selected stocks			
Lynsted	Commercial orchard near Faversham, Kent; mainly carbaryl, occasional demeton-S-methyl for many yr	October 1981	50
Norton	As Lynsted	October 1981	50
Selection reduced			
Fant A	As Fant B, but separate 2 ha block; no OPs or carbaryl 1979–1981.	September 1982	50
WF (Hadlow)	Weston's Field orchard, EMRS; Few OP and carbaryl sprays 1978–82	June 1982	50

Reduced selection

The OP-resistant Hadlow stock were released in Weston's Field orchard, Rocks Farm, EMRS in 1977–78 (see above). The use of OPs and carbaryl was much lighter than average for commercial orchards: OPs (azinphos-methyl and demeton-S-methyl) were sprayed once pre-blossom in 1979 and 1982, and carbaryl was sprayed three times in 1980 on half the orchard, and once on the whole orchard in 1981. Stock Fant A came from a 2 ha block on which OP selection pressure was totally relaxed for 3½ yr (1979–82); only diflubenzuron and pirimicarb were used as insecticides, but after blossom in 1982 two OP sprays (azinphos-methyl, fenitrothion) were applied.

Amblyseius potentillae *Garman*

A susceptible stock of Dutch origin was supplied by Dr W. P. J. Overmeer, University of Amsterdam; it had been maintained as a standard laboratory stock for some years.

Rearing

Both species of phytoseiid were reared in a constant environment room at 25 °C, 50–60% r.h. and 17-h daily photophase, on plate cultures without plant leaves; the method was modified from McMurtry & Scriven (1965). They were fed with either *Tetranychus urticae* every second day or with pollen of dwarf broad bean (*Vicia faba*) once per week. *T. urticae* was reared on broad bean. The use of pollen as the only food for *A. potentillae* was standard practice at the University of Amsterdam (Overmeer, 1981) and tests showed that *T. pyri* would also reproduce at a satisfactory rate on fresh pollen. Pollen remained in acceptable condition when the r.h. of the room was 50–60%; below this humidity the pollen dried out too quickly and at over 70% r.h. it

'solidified' and became unsuitable. The use of pollen was less time-consuming, individual culture plates could be maintained for longer and mites recovered more easily for bioassays. The bioassay results reported here are for mites fed on pollen, except when otherwise noted.

The rectangular plastic plates, 20 × 10 cm, had a black, matt, non-refractive surface which aided observations under a microscope. Each clear plastic box, 25 × 15 × 10 cm, contained distilled water to a depth of 3–4 cm. A thoroughly soaked plastic foam sponge, 17 × 8 × 5 cm, rested in the water with a plate on top. Paper tissues folded twice and thoroughly wetted were draped from all four sides of the plate to cover a border strip 2–3 cm wide and to act as a wick, thus providing a water barrier and a source of drinking water. Because some mites occasionally walked on to the water barrier a complete ring of 'Tangle-Trap' non-drying glue (The Tanglefoot Co., Grand Rapids, Mich. 49504, USA) was syringed on top of the wet tissues as a 'fail-safe' barrier. Each culture plate was provided with a few small pieces of clear gelatin sheet folded to form inverted V-shaped 'roofs'; these provided shelter and acceptable oviposition sites.

Bioassay techniques

All bioassays were on adult female mites and mortality was assessed after 24 and 48 h. Most of the tests employed the taped-slide technique (Voss, 1961) which has been standardised and widely used for spider mites and phytoseiids (Anon., 1980), but slightly modified in that slides were sprayed with serial concentrations of each toxicant under a Potter tower. Of each serial concentration of toxicant, 3 ml were sprayed at 48 kPa (7 p.s.i.) to give a standard deposit close to 4 mg wet spray/cm^2. After spraying, slides were allowed to dry for up to 1 h and then stored at 25 °C over wet capillary matting in boxes to maintain a high humidity. Mortality was assessed after 24 h and 48 h by prodding each mite with a fine brush.

Certain *T. pyri* stocks were also assayed using pesticide residues on apple leaf and glass surfaces. Plastic cartons, 112 ml capacity with lids, were used as water reservoirs, with filter paper on the carton lid kept moist by paper wicks. Leaf discs, 25 mm diameter, were sprayed in the Potter tower (deposit *c.* 4 mg/cm), allowed to dry and placed on the carton lids. A filter paper with an 18 mm diameter hole was fitted over each leaf disc, in contact with the wick, to provide both a barrier and drinking water. As with the rearing method, an annulus of 'Tangle-Trap' was syringed on top of the wet paper as an additional 'fail-safe' barrier. Five mites were moved to each arena and a little pollen was added as food. The technique with glass (microscope slides) was similar except that a 5 × 8 mm gelatin roof was also added to each arena.

Tests with each toxicant and mite stock were repeated two or three times and results were pooled. Assay data were subjected to probit analysis.

Commercial formulations of pesticides were used: (1) azinphos-methyl 22% e.c. ('Gusathion'), (2) carbaryl 85% w.p. ('Sevin'), (3) permethrin 25% e.c. ('Ambush') and (4) parathion 20% e.c. (from I.C.I. Ltd but no longer marketed in UK).

RESULTS

Responses of T. pyri *to OPs and carbaryl in taped-slide tests*

The site of collection of the Bangor stock (North Wales) was the most remote from sprayed orchards; sites for Bristol and Evesham stocks were isolated but closer to commercial fruit-growing areas. Even the collection site for Amsterdam in the Netherlands was not so remote from sprayed orchards as was Bangor. The latter was the most susceptible stock to both azinphos-methyl and carbaryl (Table 2); at 48 h Bristol and Amsterdam also gave LC_{50} s close to 100 mg azinphos-methyl/litre but they were rather more tolerant of carbaryl than was Bangor.

Amsterdam stock fed on (a) pollen and (b) *Tetranychus urticae* gave very similar responses to azinphos-methyl (Table 2), providing evidence that a diet confined to pollen did not affect the level of response to this pesticide.

Table 2. *Results for 'taped-slide' bioassays of OPs and carbaryl on* T. pyri: LC_{50}*s (mg/litre) and slope values from probit analyses*

	Azinphos-methyl			Carbaryl			
	LC_{50}			LC_{50}		Slope	
	24 h	48 h	Common slope	24 h	48 h	24 h	48 h
Amsterdam	220	110	4·87	2 910	800	1·58	2·56
Amsterdam (fed *T. urticae*)	230	120	4·21	–	–	–	–
Bangor	120	100	6·13	990	320	1·44	2·73
Bristol	190	100	4·48	3 110	1 710	4·38	2·75
Evesham	270	170	5·16	4 750	420	1·41	2·18
Fant B	640	510	5·99	–	–	–	
MP/4	360	240	3·75	>20 000	13 400	*	2·65
MP/9	710	540	5·38	>20 000	>20 000	*	
Lynsted	460	360	4·72	>20 000	>20 000	*	
Norton	560	370	3·97	>20 000	>20 000	*	
Fant A	630	530	7·17	>20 000	>20 000	*	
WF (Hadlow)	740	580	4·56	10 000	7 000	2·46	2·20

	Parathion		
	LC_{50}		
	24 h	48 h	Common slope
Bangor	37	27	3·95
MP/9	2 250	1 820	4·76
Norton	1 790	1 350	5·31

1. The 95% confidence limits of LC_{50}s for azinphos-methyl and parathion were in the range of 9–20% of the values; those for carbaryl, when estimable, in the range 23–70% of the values.

2. S.E.S of slopes for azinphos-methyl and parathion were in the range 9–17% of the values and those for carbaryl in the range 12–32% (* = not estimable; – = not tested).

With Bangor as a measure of base-line susceptibility, MP/9 (of New Zealand origin) and native stocks (Lynsted, Norton, WF, Fant A and B) exhibited a 4–6 fold resistance to azinphos-methyl and a >20-fold resistance to carbaryl, except for a slightly lower level in WF (Hadlow). Evidence that the rather low level of increased tolerance to azinphos-methyl represents a development of resistance to OPs was seen in the responses to parathion (Table 2) i.e. a 48- and 61-fold resistance in Norton and MP/9 respectively as compared with Bangor.

In spite of the very limited use of OPs in the WF (Hadlow) and Fant A orchards these stocks gave LC_{50} s for azinphos-methyl as high as those obtained for MP/9 (nine sprays, 1979–81) and Fant B (16 sprays 1979–82). However, MP/4 (four sprays, 1979–80) was appreciably less tolerant of azinphos-methyl than MP/9; this accords with the effects of azinphos-methyl sprays recorded in the orchard in 1978–80 (J. E. Cranham, unpublished) and it is possible that MP/4 was not an homogenous resistant stock. Purity was not checked in the laboratory.

With azinphos-methyl, kills at 48 h were always higher than at 24 h, although a common slope for the log-concentration (lc–p) lines could always be fitted (Table 2). With assays of carbaryl on the four 'unselected' stocks, the increased kill at 48 h was more marked than it was for azinphos-methyl and the slopes of the lc–p lines increased, except that for Bristol. For stocks from sprayed orchards, however, carbaryl was virtually innocuous; it was not practicable to test concentrations above 2% carbaryl, which except with WF (Hadlow) gave much less than 50% kill. The parameters for lc–p lines could not be estimated.

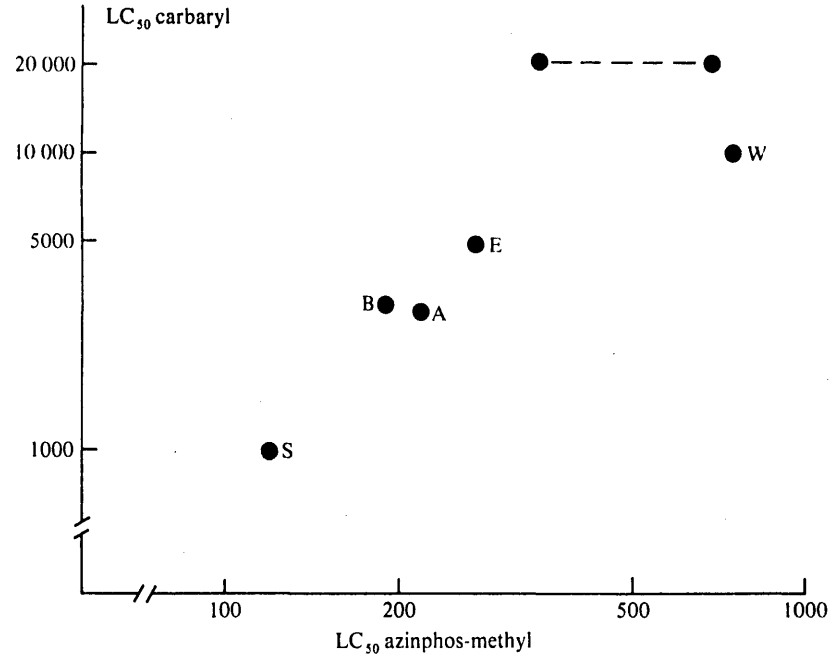


Fig. 1. Relation between LC_{50} s (24 h, mg/litre, log scale) for carbaryl and azinphos-methyl: S, Bangor; B, Bristol; A, Amsterdam; E, Evesham; W, WF (Hadlow); ●——●, range of values for other stocks of *Typhlodromus pyri*.

The same very high resistance to carbaryl was found with MP/4, MP/9 and Fant A, which received no sprays of carbaryl in 1977–82, as for the Lynsted and Norton stocks which received two to three sprays/year for a longer period; thus, resistance to carbaryl, once developed, appears to be stable when selection is relaxed.

Fig. 1 shows the relationship between the 24 h LC_{50} s for azinphos-methyl and carbaryl for the four 'unselected' stocks, WF (Hadlow) and the range of values obtained for the other resistant stocks. Since for the latter, LC_{50} s for carbaryl could be quantified only as >20 000 mg/litre there are too few data for a correlation coefficient to be meaningful. However, the data suggest a positive correlation between the resistance to both pesticides. No stock was susceptible to azinphos-methyl and highly resistant to carbaryl, or *vice-versa*.

Responses of T. pyri *to permethrin in taped-slide tests*

None of the stocks had a spray history involving synthetic pyrethroids and the main purpose of the tests was to establish the level of 'base-line' susceptibility to permethrin, and to look for any influence of resistance to OPs and carbaryl on the response. MP was slightly more tolerant to permethrin than OP-susceptible or native OP-resistant stocks which gave LC_{50} s in the small range 15–25 mg/litre (Table 3). The New Zealand mites from which MP was developed were received in 1977 i.e. almost certainly before *T. pyri* in New Zealand could have received any exposure to synthetic pyrethroids. For the range of samples tested there is no indication that resistance to OPs and carbaryl affects the level of response to permethrin.

Responses of T. pyri *in residue bioassays*

The Amsterdam and MP/4 stocks of *T. pyri* which, in taped-slide tests, showed a small difference in response to azinphos-methyl and a large difference with carbaryl, were assayed by the alternative techniques described under 'Methods', involving residues on (1) leaf surfaces and

Table 3. *Results (24 h) for 'taped-slide' bioassays of permethrin on* T. pyri: *LC_{50}s (mg/litre) and slope values*

Mite stock	Azinphos-methyl LC_{50}	Permethrin LC_{50}*	Permethrin Slope*
Bangor	120	20	4·81
Bristol	190	20	2·80
Evesham	270	25	4·01
MP	710	31	2·24
Lynsted	460	15	4·11
Norton	560	18	3·84
WF (Hadlow)	740	18	2·87

* 95% confidence limits of LC_{50}s were in the range of 15–42% of values; S.E.s of slopes in the range of 13–22% of values.

Table 4. *Comparison of three techniques of bioassay on two stocks of* Typhlodromus pyri: *24 h LC_{50}s (mg/litre) and slope values*

Pesticide: technique	Amsterdam stock LC_{50}	(R1)	Slope	MP/4 stock LC_{50}	(R1)	Slope	(R2)
azinphos-methyl:							
taped-slide	218	(45)	4·87	357	(26)	3·75	(1·6)
leaf/residue	67	(14)	5·32	145	(10)	4·46	(2·2)
glass/residue	4·8	(1)	2·24	14	(1)	4·92	(2·9)
carbaryl:							
taped-slide	2 910	(6·2)	1·58	>20 000	(4)	n.s.	(>10)
leaf/residue	923	(2·0)	2·19	16 600	(3·2)	1·27	(18)
glass/residue	468	(1)	1·36	5 110	(1)	0·87	(11)
permethrin:							
taped-slide	37	(58)	1·95	not tested			–
leaf/residue	4·3	(6·7)	4·73	not tested			–
glass/residue	0·64	(1)	2·18	not tested			–

R1: Ratio of LC_{50} to that obtained by the glass/residue technique with the same insecticide and mite shock.
R2: Ratio of LC_{50} MP/LC_{50} Amsterdam for the same insecticide and bioassay technique ('tolerance factor').
n.s. = Not significant.

(2) glass. Table 4 compares the LC_{50} s and slope values (24-h kills) obtained by probit analyses. Comparison of the 48 h values gave much the same picture. With all three pesticides, mites were killed by lower concentrations in leaf residue assays, and by much lower concentrations in glass residue assays, than were required in taped-slide tests: in Table 4 the ratio R1 compares the three techniques for each stock/pesticide. The ratio R2 compares LC_{50} s for the two stocks of *T. pyri* obtained by each technique and the results show a rather small variation in these estimated 'resistance factors'.

Responses of Ambylyseius potentillae *to three pesticides*

In taped-slide assays, this species proved to be more susceptible than the Bangor stock of *T. pyri* to azinphos-methyl and carbaryl and considerably more susceptible to permethrin than all stocks of *T. pyri* (Table 5).

Table 5. *'Taped-slide' tests on* Amblyseius potentillae: *data from probit analyses*

Pesticide	LC_{50} (24 h)	95% confidence limits)	LC_{50} (48 h)	Slope ± S.E.
azinphos-methyl	56	(46–65)	46	5·31 ± 0·87
carbaryl	413	(300–553)	261	2·50 ± 0·36
permethrin	8	(6–10)	6	2·40 ± 0·24

DISCUSSION

Several authors used the taped-slide bioassay technique to assess tolerance levels in phytoseiid mites; usually the mites were dipped, not sprayed as in the present study, in serial concentrations of the pesticides. This probably makes little difference to the mortality obtained for any given concentration, since in both spraying and dipping the mites are thoroughly wetted. The time between treatment and assessment – 24 or 48 h – affected the response recorded, as shown in the present study.

Information from the literature concerning bioassays on *T. pyri* has been collated in Table 6; LC_{50} values are for 24 h at a temperature of 25–27 °C. Only the present study and that of Hoyt (1972) report tests on OP-susceptible (OP-S) stocks of *T. pyri*, and LC_{50} values were of the same order. Estimations of the resistance factor for azinphos-methyl by Penman *et al.* (1976) and by Wong & Chapman (1979) in New Zealand, and by Watve & Lienk (1976) in New York State, were based on the LC_{50} value (72 mg/litre) obtained by Hoyt (1972) for an OP-S stock; this has therefore been adopted for uniform comparisons in Table 6. This evidence points to similar levels for both 'base-line' susceptibility and for resistance in New Zealand in 1970–71 (Hoyt, 1972) and from the present study in England in 1981–82. This relatively low level of 10-fold or less was insufficient for *T. pyri* to survive the eight or more sprays of azinphos-methyl common in certain apple-growing districts of New Zealand. However, by 1976 Penman *et al.* (1976) confirmed that levels as high as 40-fold had developed after 10–15 yr of intensive use. This

Table 6. *Comparison of LC_{50}s (mg/litre) from the literature on the responses of* Typhlodromus pyri *to azinphos-methyl and carbaryl; all were obtained by taped-slide technique, 24 h assessment*

Authors: country	Azinphos-methyl: OP susceptible	Azinphos-methyl: OP resistant	Resistance factor
Hoyt (1972): N. Zealand	72	700	10
Penman *et al.* (1976): N Zealand	–	800–3 000	11–42
Wong & Chapman (1979): N. Zealand	–	990	14
Watve & Lienk (1976): New York State, USA	–	840, 1150, 1404	12–20
Kapetanakis & Cranham (this paper): UK			
MP (New Zealand origin)	–	359–714	5–10
English stocks	122	460–739	6–10
	Carbaryl		
Watve & Lienk (1976): USA	–	>18 000	–
Kapetanakis & Cranham (this paper): UK	990	>20 000	20

permitted the development of an integrated mite control system in commercial orchards at least for certain important districts where such high levels were found (Wearing *et al.*, 1978).

Recent trial results in England show that 5–10 fold resistance to azinphos-methyl will allow *T. pyri* to survive up to four sprays per season (two pre- and two post-blossom) without disrupting the effective predation of *P. ulmi*. More intensive insecticide spraying is very seldom required in England; average commercial use is three to five insecticide sprays per season.

The results obtained by Watve & Lienk (1976) show a variation in the level of resistance through the season. Mites in reproductive diapause, collected October 1974, showed 20-fold resistance to azinphos-methyl; after emergence in the spring (collected March, 1975, tested when out of diapause) the factor was 16-fold. The same population collected in August 1975 showed 12-fold resistance. Pesticide usage in New York State is more intensive than in England, reflecting higher summer temperatures and more species of 'key' pests which can cause direct damage to fruits (Glass, 1975) but usage is probably less intensive than in New Zealand. The levels of resistance found appear to reflect the intensity of use.

In the present study, it is notable that the MP/9 stock (of New Zealand origin) and native stocks had similar levels of resistance. The MP stock (imported in 1977) was taken from a district in New Zealand where high levels of resistance had been recorded but the level of resistance for the sample was not assessed either before despatch or after arrival in England. It is, however, known that 2 yr after the releases in 1977 the population on Middle Park orchard had a relatively low resistance; AliNiazee & Cranham (1980) recorded 80% reduction in numbers in 1979 from a single pre-blossom spray of azinphos-methyl + demeton-S-methyl sulphone. In the post-blossom period it was evident (J. E. Cranham *in* Flegg, 1980) that two to three sprays of azinphos-methyl distrupted predation of *P. ulmi*. It seems probable that the released mites had hybridised with susceptible native *T. pyri* and/or that, under relatively low selection pressure, the level of resistance had declined. Numerous genetic studies of OP-resistance in spider mites, reviewed by Helle & Overmeer (1973) and similar studies on the phytoseiid *A. fallacis* (Croft, Brown & Hoying, 1976), showed that resistance to OPs was controlled by a single major gene, dominant or semi-dominant in expression. Also, in spider mites, the level of resistance to individual OPs can usually be increased, under specific selection, by minor gene effects. A reasonable hypothesis, therefore, for the genetic background to resistance in *T. pyri* is that a single major gene confers low-level resistance to azinphos-methyl and a high level to parathion, but that the level to azinphos-methyl can be increased by intensive selection with that pesticide. If selection is relaxed, such high levels may not be 'stable', but the low level due to the major gene probably is. The results obtained for the WF and Fant A stocks suggest such stability.

Comparisons with other phytoseiid species

The comparisons developed for *T. pyri* in Table 6 have been extended to other species in Table 7. As Croft (1982) noted: 'interspecific differences within the family (Phytoseiidae) are as variable as those of their tetranychid prey'.

With *T. occidentalis*, Croft & Jeppson (1970) compared two stocks from unsprayed and two from sprayed orchards (*ex* Washington, Utah and California); the two unselected strains susceptible to azinphos-methyl (Az-S) and the two strains resistant to azinphos-methyl (Az-R) were equally tolerant to parathion, and susceptible to carbaryl. This species appears to be inherently tolerant to parathion (Croft, 1982), as observed by Huffaker & Kennett (1953) working on strawberry in California, when the use of parathion and other OPs first began. The data of Croft & Stewart (1973) (Table 7) also confirm that high resistance to azinphos-methyl does not *per se* confer resistance to carbaryl. Roush & Hoy (1980) obtained a stock of this species with increased resistance to carbaryl by laboratory selection; the resistance level is not easily quantified from their results but was probably less than 10-fold.

Table 7. *Comparison of LC_{50}s (mg/litre) from the literature on the responses of three species of phytoseiids to two OPs and carbaryl in bioassays using the taped-slide technique, 24 h assessment, except when noted otherwise*

Species: authors	Resistance status	Azinphos-methyl	Parathion	Carbaryl
Typhlodromus occidentalis:				
Croft & Jeppson (1970) (48 h)	Az-S	20, 30	5100, 5400	310, 350
	Az-R	1760, 2031	5400, 5800	210, 520
Croft & Stewart (1973) (leaf discs, 48 h)	Az-R	10 920	–	78
Amblyseius fallacis:				
Croft & Hoying (1975)	OP-R	–	–	54–68
	OP-R	–	–	295*
Rock & Yeargan (1971)	OP-S	42	48	–
	OP-R	1205, 3495	2658, 2766	–
Croft *et al.* (1976)	OP-S	11	4	–
	OP-R	810	670	–
A. potentillae:				
Kapetanakis & Cranham (this paper)	OP-S	56	–	413

* From an orchard with a history of heavy carbaryl spraying.

With *A. fallacis*, the data of Rock & Yeargan (1971) and that of Croft *et al.* (1976) show that this species is not inherently tolerant of parathion and the development of OP resistance in the field resulted in a high level to both azinphos-methyl and parathion. Resistance to OPs did not confer cross-resistance to carbaryl, but where there was intensive use moderate resistance developed to this pesticide (Croft, 1982). Croft & Hoying (1975) tested several OP-resistant stocks from orchards in Michigan and were able to develop a moderate level of resistance after prolonged selection.

No developed resistance has been recorded for *A. potentillae* and tests in the present study confirmed the stock was susceptible to azinphos-methyl and to carbaryl.

No other reports were found which compared stocks of *T. pyri* susceptible and resistant to carbaryl. The data of Watve & Lienk (1976) for an OP-resistant stock show an extremely high resistance to carbaryl (Table 6). A review by Croft (1982) states that carbaryl 'is almost non-toxic to *T. pyri*'. If the result obtained with the Bangor stock represents 'base-line' susceptibility, *T. pyri* is indeed rather more tolerant than the above three species but it has been shown (J. E. Cranham, unpublished) that the level of tolerance in native OP-susceptible *T. pyri* did not prevent some reduction of a field population after a carbaryl spray.

It would appear that *T. pyri* has easily developed very high levels of resistance to carbaryl, and that this has occurred in England, New York State, New Zealand (Wearing & Ashley, 1978) and the Netherlands (Gruys, 1980). Croft (1982) further states that 'there is no documented cross-resistance between carbaryl and azinphos-methyl in any phytoseiid species'. The results reported here for *T. pyri* accord with a hypothesis of cross-resistance between OPs and carbaryl; however, they do not prove it, since both chemical groups have been widely used in English commercial orchards for *c.* 25 yr, as they have been more widely in Europe, N. America and Australasia. Further investigation of this topic might require genetic and/or biochemical studies.

Responses to permethrin

In the bioassays with permethrin in the present study the LC_{50} values for OP-resistant and OP-susceptible stocks of *T. pyri* were of a similar order i.e. there was no clear indication of cross-resistance, positive or negative.

Some English orchards have been sprayed with synthetic pyrethroids for 4–5 yr; on these plots very few *T. pyri*, often none, can be found (M. G. Solomon *in* Flegg, 1983). Hence, field observations so far do not suggest the development of resistance to SPs in England.

Comparison of three bioassay techniques

Comparing results with the three bioassay techniques (Table 4) there were large differences between the LC_{50} s, in the same order for all three pesticides: taped-slide > leaf/residue > glass/residue. Experimental work to seek explanation for these differences was not within the scope of this study. Hartley & Graham-Bryce (1980) have reviewed the topic of pick-up of pesticides from residues on different surfaces. It seems probable that on glass virtually all the pesticide would remain 'available' for pick-up by mites walking over the surface. On a leaf surface, some of the pesticide would be 'unavailable' in micro-crevices of the surface structure and some absorbed in the waxy components of the leaf cuticle. It is more difficult to suggest possible explanations for the higher concentrations required in the taped-slide tests. The rate of penetration of the pesticide to the site of toxic action may be reduced if mites are relatively inactive i.e. held on their backs as in the taped-slide tests; alternatively, penetration through the tarsi may be an exceptionally efficient route of entry which is minimised in this test. Experiments could be devised to investigate such aspects.

Possibly the leaf/residue technique approximates to field conditions more closely than either the taped-slide or glass/residue techniques; however, no bioassay technique is fully adequate for predicting field results and the information must be extrapolated with considerable caution and experience. In the field, weather conditions, the complex of life stages present, and behavioural aspects, may all affect the result. The purpose of bioassays was to compare the responses of different stocks to pesticides and it is notable that this comparison (R2, Table 4) was of the same order for all three techniques.

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REFERENCES

ALINIAZEE, M. T. & CRANHAM, J. E. (1980). Effect of four synthetic pyrethroids on a predatory mite, *Typhlodromus pyri*, and its prey, *Panonychus ulmi*, on apples in south-east England. *Environmental Entomology* **9**, 436–439.

ANON. (1980). Revised method for spider mites and their eggs. F.A.O. method No. 10a. In *Recommended methods for measurement of pest resistance to pesticides, F.A.O. Plant Production and Protection Paper No. 21*, F.A.O. Rome, by J. R. Busvine, 132 pp.

CRANHAM, J. E. & SOLOMON, M. G. (1981). Mite management in commercial apple orchards. *Report of East Malling Research Station for 1980*, pp. 171–172.

CRANHAM, J. E., TARDIVEL, G. M. & KAPETANAKIS, E. G. (1983). Orchard trials to assess the effects of pesticides on *Typhlodromus pyri* and its prey, *Panonychus ulmi*. *Proceedings of the 6th International Congress of Acarology, Edinburgh, 1982*, in press.

CROFT, B. A. (1981). Use of crop protection chemicals for integrated pest control. *Philosophical Transactions of the Royal Society, London* **B295**, 125–141.

CROFT, B. A. (1982). Arthropod resistance to insecticides: a key to pest control failures and successes in North American apple orchards. *Entomologia experimentalis et applicata* **31**, 88–110.

CROFT, B. A., BROWN, A. W. A. & HOYING, S. A. (1976). Organophosphorus-resistance and its inheritance in the predacious mite *Amblyseius fallacis*. *Journal of Economic Entomology* **69**, 64–68.

CROFT, B. A. & HOYING, S. A. (1975). Carbaryl resistance in native and released populations of *Amblyseius fallacis*. *Environmental Entomology* **4**, 895–898.

CROFT, B. A. & JEPPSON, L. R. (1970). Comparative studies on four strains of *Typhlodromus occidentalis*. II. Laboratory toxicity of ten compounds common to apple pest control. *Journal of Economic Entomology* **63**, 1528–1531.

CROFT, B. A. & STEWART, P. G. (1973). Toxicity of one carbamate and six organophosphorous insecticides to O-P resistant strains of *Typhlodromus occidentalis* and *Amblyseius fallacis*. *Environmental Entomology* **2**, 486–488.

EASTERBROOK, M. A., SOUTER, E. F., SOLOMON, M. G. & CRANHAM, J. E. (1979). Trials on integrated pest management in English apple orchards. *Proceedings 1979 British Crop Protection Conference – Pests and Diseases* **1**, 61–67.

FLEGG, J. J. M. (1980). Zoology. *Report of East Malling Research Station for 1979*, pp. 119–132.

FLEGG, J. J. M. (1982). Zoology. *Report of East Malling Research Station for 1981*, pp. 99–112.

FLEGG, J. J. M. (1983). Zoology. *Report of East Malling Research Station for 1982*, pp. 101–112.

GLASS, E. H. (1975). Recent developments in deciduous orchard pest management in the United States. *EPPO Bulletin* **5**(2), 101–111.

GRUYS, P. (1980). Significance and practical application of selective pesticides. *Proceedings of the International Symposium of IOBC/WPRS on Integrated Control in Agriculture and Forestry, Vienna, 8–12 October, 1979*, pp. 107–112.

GRUYS, P. (1982). Hits and misses. The ecological approach to pest control in orchards. *Entomologia experimentalis et applicata* **31**, 70–87.

HARTLEY, G. S. & GRAHAM-BRYCE, I. J. (1980). *Physical principles of pesticide behaviour*. Vol. 2, London: Academic Press.

HELLE, W. & OVERMEER, W. P. J. (1973). Variability in tetranychid mites. *Annual Review of Entomology* **18**, 97–120.

HOYT, S. C. (1972). Resistance to azinphos-methyl of *Typhlodromus pyri* (Acarina: Phytoseiidae) from New Zealand. *New Zealand Journal of Science* **15**, 16–21.

HUFFAKER, C. B. & KENNETT, C. E. (1953). Differential tolerance to parathion in two *Typhlodromus* predatory on cyclamen mite. *Journal of Economic Entomology* **46**, 707–708.

MCMURTRY, J. A. & SCRIVEN, G. T. (1975). Population increase of *Phytoseiulus persimilis* on different insectary feeding systems. *Journal of Economic Entomology* **68**, 319–321.

OVERMEER, W. P. J. (1981). Notes on breeding phytoseiid mites from orchards in the laboratory. *Mededelingen van de Faculteit landbouwwetenschappen Rijksuniversiteit Gent* **46**(2), 503–509.

PENMAN, D. R., FERRO, D. N. & WEARING, C. H. (1976). Integrated control of apple pests in New Zealand. VII. Azinphos-methyl resistance in strains of *Typhlodromus pyri* from Nelson. *New Zealand Journal of Experimental Agriculture* **4**, 377–380.

ROCK, G. C. & YEARGAN, D. R. (1971). Relative toxicity of pesticides to organophosphorus-resistant orchard populations of *Neoseiulus fallacis* and its prey. *Journal of Economic Entomology* **64**, 350–352.

ROUSH, R. T. & HOY, M. A. (1980). Selection improves Sevin resistance in spider mite predator. *Californian Agriculture* **34**, 11–14.

VOSS, G. (1961). Ein neues Akarizidaustestungsverfahren für Spinnmilben. *Anzeiger für Schädlingskunde* **34**(5) 76–77.

WATVE, C. M. & LIENK, S. E. (1975). Responses of two phytoseiid mites to pesticides used in New York apple orchards. *Environmental Entomology* **4**, 797–800.

WATVE, C. M. & LIENK, S. E. (1976). Toxicity of carbaryl and six organophosphorous insecticides to *Amblyseius fallacis* and *Typhlodromus pyri* from New York apple orchards. *Environmental Entomology* **5**, 368–370.

WEARING, C. H. & ASHLEY, E. (1978). A recommended spray programme for integrated control of European red mite on apples. *The Orchardist of New Zealand* **51**, 355–357.

WEARING, C. H., WALKER, J. T. S., COLLYER, E. & THOMAS, W. P. (1978). Integrated control of apple pests in New Zealand. 8. Commercial assessment of an integrated control programme against European red mite using an insecticide-resistant predator. *New Zealand Journal of Zoology* **5**, 823–837.

WONG, S. W. & CHAPMAN, R. B. (1979). Toxicity of synthetic pyrethroid insecticides to predacious phytoseiid mites and their prey. *Australian Journal of Agricultural Research* **30**, 497–501.

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RESISTANCE TO INSECTICIDES IN THE PREDATORY MITE TYPHLODROMUS PYRI AND ITS SPIDER MITE PREY

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Background and objectives

Resistance to organophosphates (OPs) in Panonychus ulmi developed from c. 1960, reaching high levels (Cranham, 1982); the continued use of OPs for insect control has resulted some 20 years later in development of resistance in the predatory phytoseiid mite Typhlodromus pyri. Results are reported of bioassays and field trials to evaluate resistance of the predator to a range of insecticides, and the effects on the balance of predator and prey in orchards.

Materials and methods

T. pyri stocks collected from sprayed and unsprayed orchards were reared separately on plate cultures with pollen of Vicia faba as food, and bioassayed using a taped slide technique (Kapetanakis & Cranham 1983). Insecticides were also evaluated in well-replicated field trials of randomised block design, on young apple trees sprayed to 'point of drip', both mite species being present. Numbers of mites on leaf samples were assessed, using a brushing machine, at successive 3-week intervals after spraying, and when possible before spraying.

Results and conclusions

In bioassays, T. pyri collected from isolated apple trees in North Wales provided standards of 'base-line' susceptibility for carbaryl and nine OPs in common use, as follows. T. pyri was moderately tolerant to carbaryl (LC_{50} 1000 mg/l). With the OPs there was a large (> 200 fold) range of LC_{50}s in the order phosalone (500 mg/l), fenitrothion, azinphos-methyl, pirimiphos-methyl, chlorpyrifos, parathion, demeton-S-methyl, vamidothion and dimethoate (2.6 mg/l).

Stocks of T. pyri with a long history of exposure to OPs and carbaryl (three native stocks, one from New Zealand) were resistant to these insecticides. The cross-resistance spectrum was similar for all four test stocks: azinphos-methyl, pirimiphos-methyl and dimethoate had low (< 10) resistance factors whilst the other OPs gave factors in the range 35-76, and carbaryl > 20. The range of LC_{50}s was again very large, and suggested that carbaryl and certain OPs were probably harmless in practice to resistant T. pyri, whilst others might be harmful. Results of field trials on resistant populations were in agreement; phosalone, fenitrothion, chlorpyrifos, azinphos-methyl and demeton-S-methyl were harmless at normal field rates, and dimethoate and pirimiphos-methyl were very harmful.

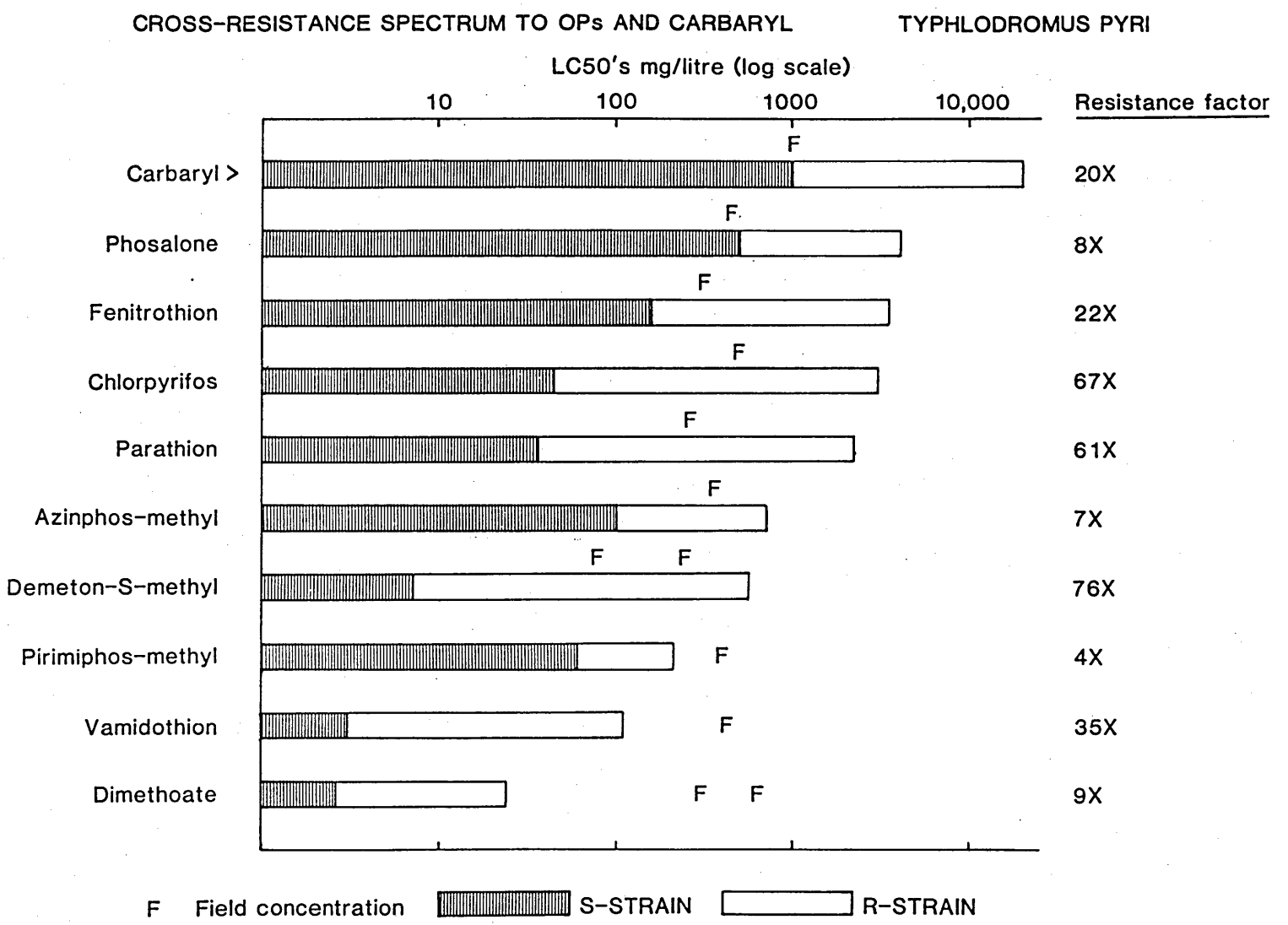
Each phytoseiid species that has developed OP resistance exhibits a specific cross-resistance spectrum with a wide range of responses to different OPs; the differences between species in the responses to certain OPs are very pronounced (Croft, 1982). In England, T. pyri provides excellent stable regulation of P. ulmi at non-injurious numbers. A range of OPs, and carbaryl, can now be used as selective agents for insect pest control, without harm to T. pyri. Several non-acaricidal fungicides are available. By a suitable choice of pesticides, resistance in the predator can be exploited, in order to minimise use of acaricides and so prevent further development of resistance in P. ulmi.

References

Cranham, J. E. (1982) Resistance to organophosphates, and the genetic background, in fruit tree red spider mite, Panonychus ulmi, from English apple orchards. Annals of Applied Biology 100, 11-23.

Croft, B. A. (1982) Arthropod resistance to insecticides: a key to pest control failures and successes in North American apple orchards. Entomologia experimentalis et applicata 31, 88-110.

Kapetanakis, E. G.; Cranham, J. E. (1983) Laboratory evaluation of resistance to organophosphates and carbaryl in the phytoseiid predator Typhlodromus pyri from English apples. Annals of Applied Biology, in press.


CROSS-RESISTANCE SPECTRUM TO OPs AND CARBARYL
TYPHLODROMUS PYRI
LC50's mg/litre (log scale)
10
100
1000
10,000
Resistance factor
Carbaryl >
Phosalone
Fenitrothion
Chlorpyrifos
Parathion
Azinphos-methyl
Demeton-S-methyl
Pirimiphos-methyl
Vamidothion
Dimethoate
F
20X
8X
22X
67X
61X
7X
76X
4X
35X
9X
F Field concentration
S-STRAIN
R-STRAIN

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Orchard trials to assess the effects of pesticides on *Typhlodromus pyri* and its prey, *Panonychus ulmi*

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INTRODUCTION

The capacity of the predatory phytoseiid *Typhlodromus pyri* Scheuten to regulate the numbers of its main prey, the spider mite *Panonychus ulmi* (Koch), at very low, non-injurious levels has been evident from recent trials of integrated pest management (Easterbrook, *et al.*, 1979, Gruys, 1982). Cranham and Solomon (1981) concluded that the possibilities for integrating biological control of *P. ulmi* with chemical control of fungal diseases and insect pests in England are now better than ever before; the major contributing factors are the current commercial acceptance of non-acaricidal fungicides, and the development of resistance to many organophosphate (OP) insecticides and to carbaryl in some native strains of *T. pyri*. In this paper, evidence from recent orchard trials on the effects of various pesticides on the balance of *T. pyri* and its prey is reviewed, and a general framework for the results is discussed.

METHODS

Three strains of *T. pyri* of differing provenance, two of them resistant to OPs, were mass-reared in separate glasshouses on potted Myrobalan B plum rootstocks, with *P. ulmi*

and Tetranychus urticae (Koch) as prey, and were released in various orchards from 1977/78.

1. 'N.Z.' (OP-R), imported from New Zealand in January 1977, kindly supplied by Dr. H. Wearing, DSIR, Auckland.
2. 'Hadlow' (OP-R), collected from a commercial apple orchard at Hadlow, Kent in August 1977.
3. 'S', susceptible to OPs, collected from South Park orchard, EMRS, in 1977/78. There had been only a very limited use of OPs in this orchard since 1950.

Mite numbers were assessed on foliage samples (72-120 leaves per treatment) taken at c. 3 weekly intervals after spraying and whenever possible before spraying. Using a mite-brushing machine, mites were brushed on to discs of white card which had been varnished and dried and then coated very thinly before use with a non-drying adhesive of the type used in pheremone traps. Mites were counted immediately after brushing.

'NC21314' is a code number of FBC Ltd. for a novel acaricide under development (Bryan et al., 1981).

RESULTS

Fant Farm trial, 1979-82. Three insecticide programmes were compared on plots of 1.6 ha in this commercial orchard. Indigenous T. pyri were abundant from the first season of the trial on plot A (diflubenzuron, pirimicarb) and on plot B (azinphos-methyl, demeton-S-methyl) and on both plots they regulated spider mite numbers for the 3 years 1979-81 without need for acaricides. On plot C, permethrin kept

T. pyri at very low numbers and cyhexatin was needed annually for spider mite control.

Westons Field, 1980 (EMRS). The Hadlow strain of T. pyri had been released and established in this 2 ha orchard in 1977/78. Tetradifon was applied at petal fall in 1978 to reduce P. ulmi and thereafter no further acaricides were needed in 1978/81. In a 1980 trial the treatments were carbaryl (2 kg/ha) and diflubenzuron (0.6 kg/ha) airblast-sprayed once pre-bloom and twice post-bloom in a 2 x 2 design duplicated on plots of 0.2 ha. Neither pesticide caused disruption and T. pyri kept P. ulmi at very low numbers throughout the season. Fig.1 shows the mite numbers from 16th July, when azinphos-methyl and cypermethrin were applied to other, separate, plots. T. pyri tolerated azinphos very well but were nearly eliminated by cypermethrin, resulting in an upsurge of P. ulmi.

Trials of 6-12 weeks duration, West Egham, 1978-81

These trials were in a 0.8 ha orchard of semi-dwarf trees (Cox, Worcester on M.9 rootstocks) for the purpose of comparing the effects of individual pesticides on different strains of T. pyri in the same orchard. Treatments were replicated on six single-tree plots. The S, Hadlow and N.Z. strains of T. pyri were released in discrete blocks separated by at least 30 m. In time, aerial dispersal of T. pyri caused problems of cross-infestation between blocks. In 1980 T. pyri were intentionally eliminated by pre-bloom sprays of cypermethrin and dicofol and the S and N.Z. stocks were re-established by post-bloom releases. From 1981 only

only the N.Z. stock was used, after sprays of azinphos-methyl to eliminate S mites.

A detailed description of the trial results is beyond the scope of this short paper. The N.Z. and Hadlow strains tolerated azinphos-methyl, chlorpyrifos and demeton-S-methyl, whilst the S strain was reduced by these OPs but not by phosalone. In 1978 all three strains were reduced by carbaryl although laboratory bioassays indicated that the OP-R strains were more tolerant than the S strain to this pesticide. However, in 1980, neither N.Z. or S (nor Hadlow in the Westons Field trial, see above) were reduced by carbaryl.

No difference was detected between OP-R and S strains in response to other classes of pesticides: (a) pyrethroids, which were highly disruptive, (b) acaricidal fungicides such as binapacryl, dinocap and wettable sulphur which reduce both predator and prey about equally, (c) non-acaricidal fungicides including bupirimate, fenarimol and triadimefon and (d) non-acaricidal chemical fruit thinning agents such as ephephon.

For the N.Z. strain in 1980 Fig.2 shows that over a 6-week period the ratio of prey/predator declined from c. 100:1 to 10:1 on unsprayed and azinphos-sprayed plots alike. In contrast, pirimiphos-methyl prevented an increase of T. pyri and the ratio remained at 100:1 after 6 weeks. A new selective acaricide (NC21314) greatly reduced P. ulmi without affecting T. pyri, so that after 6 weeks the prey: predator ratio was about 1:1.

In a 1981 trial deltamethrin and fenpropathrin applied

pre-bloom reduced T. pyri by 99% after 5 weeks, whilst permethrin, cypermethrin and fluvalinate caused c. 90% reduction. In a nearby pre-bloom trial in the same orchard, five OPs (azinphos-methyl, demeton-S-methyl, fenitrothion, malathion and vamidothion) caused no significant reductions of the N.Z. strain.

DISCUSSION

An OP-R strain was imported from New Zealand and released in August 1977 in Middle Park orchard, EMRS. In the same year, an OP-R strain was collected from a commercial orchard at Hadlow, 12 km south of EMRS. The occurrence of other OP-R orchard populations was evident at Fant Farm, Barming (9 km south-east of EMRS) in 1979, and at Norton, Faversham (38 km east of EMRS) in 1980. In 1981, the Agricultural Development and Advisory Service found clear evidence of OP-R T. pyri in several orchards in the Evesham (Worchestershire) district, 200 km north-west of EMRS (R.A. Vernon, personal communication). Thus, although T. pyri can spread by aerial dispersal, and indeed such spread has been noted on EMRS, there appears to be unequivocal evidence that resistance to OPs has developed in some indigenous strains after selection for 25 years or more. It is not yet known how commonly such strains occur in commercial orchards; at the present time the evidence for widespread resistance to carbaryl is stronger than the evidence for widespread resistance to OPs.

In orchards where T. pyri is well established it is the relative toxicity of a pesticide to both the predator

and its prey which determines the likely 'numerical response' of P. ulmi after application, i.e. whether the effect will be (1) 'disruptive', leading to increase of spider mites, (2) 'suppressive' to the numbers of both species, (3) neutral or non-disruptive or (4) 'corrective', reducing spider mites with little or no effect on the predators. Table 1 categorises various pesticides used in English apple orchards in this regard.

The pyrethroids currently most used commercially give little or no control of P. ulmi, whilst they are probably the most persistently toxic compounds for T. pyri. In all trial studies, pyrethroids led to resurgence of spider mites, often requiring the subsequent use of acaricides. Pirimiphos-methyl, used for control of apple rust mite, Aculus schlechtendali, differed from other OPs tested in being highly toxic to the N.Z. strain.

Single applications of acaricides (2A) highly toxic to both species will suppress spider mites effectively; but, since the predator never has the chance to be effective, repeated use is required and this entails the danger of resistance developing. Several fungicides (2B) are mild acaricides exhibiting about equal toxicity to both species. Full programmes of these fungicides suppress T. pyri almost completely; but their occasional use, sometimes advantageous for disease control, is not incompatible with practical 'mite-management'.

In the neutral or non-disruptive category are those pesticides with little or no toxicity to either species.

Several OPs have become neutral due to resistance development in both species, although historically they were at first suppressive, and rapidly became disruptive as OP resistance developed in P. ulmi.

In the remaining category are those selective acaricides with no toxicity (4A) or low toxicity (4B) to the predator. The corrective action is well illustrated by the response obtained to NC21314 (Fig.2). Tetradifon was also useful in this way in the above trials, and in others (Easterbrook et al.,1979), but will obviously be less effective in orchards where a substantial proportion of P. ulmi remain resistant to it. Cyhexatin is appreciably toxic to T. pyri, especially immature stages, but it is more toxic to P. ulmi; experience in England has shown that low rates (e.g. 0.2 kg/ha) provide a good corrective action.

Dosages, even of selective acaricides, which almost eliminate P. ulmi ('overkill') will result in starvation of the predators, unless there are sufficient alternative prey, such as Aculus schlechtendali, present. Interactions between these three species, and the effects of pesticides and resistance development on the whole mite fauna, deserve further study.

To ensure effective biological control of P. ulmi by T. pyri the choice of pesticides seems to be the paramount factor for success.

SUMMARY

The effects of various pesticides in common use in English apple orchards on the predatory phytoseiid

Typhlodromus pyri, and on its main prey Panonychus ulmi, were studied in orchard trials, 1978-81. Three strains of T. pyri were involved: an OP-resistant strain from New Zealand, a native OP-R strain, and a native strain more susceptible to organophosphate insecticides. The disruptive effect of individual pesticides depended on the responses of both T. pyri and its prey. Trial results are described and their practical implications discussed. Commercial acceptance of non-acaricidal fungicides for mildew control, and the development of resistance in native strains to several organophosphates and to carbaryl, now enhance the potential for biological control of P. ulmi by T. pyri.

REFERENCES

BRYAN, K.M.G., GEERING, Q.A. & REID, J. (1981). Proceedings 1981 British Crop Protection Conference - Pests and diseases, 67-74.

CRANHAM, J.E. & SOLOMON, M.G. (1981). Report East Malling Research Station for 1980, 171-172.

EASTERBROOK, M.A., SOUTER, E.F., SOLOMON, M.G. & CRANHAM, J.E. (1979). Proceedings British Crop Protection Conference - Pests and diseases, 61-67.

GRUYS, P. (1982). Entomologia experimentalis et applicata 31, 70-87.

Table 1. Some orchard pesticides categorised with regard to their relative toxicity to OP-resistant Typhlodromus pyri and Panonychus ulmi: x, low; xx, moderate; XXX, high toxicity; O, no toxicity

Category	Toxicity to T. pyri	Toxicity to P. ulmi	Pesticides
1. "disruptive"	xxx	0 or x	permethrin, cypermethrin deltamethrin, pirimiphos-methyl
2A "suppressive"	xxx	xxx	fenpropathrin, dicofol, amitraz
2B "suppressive"	xx	xx	binapacryl, dinocap, wettable sulphur
3A "neutral"	0	0	bupirimate, fenarimol, triadimefon, captan, dodine, dithianon, diflubenzuron, pirimicarb
3B "neutral"	0 or x	0 or x	most OP insecticides, carbaryl
4A "corrective"	0	xxx	tetradifon, NC 21314
4B "corrective"	x	xxx	cyhexatin

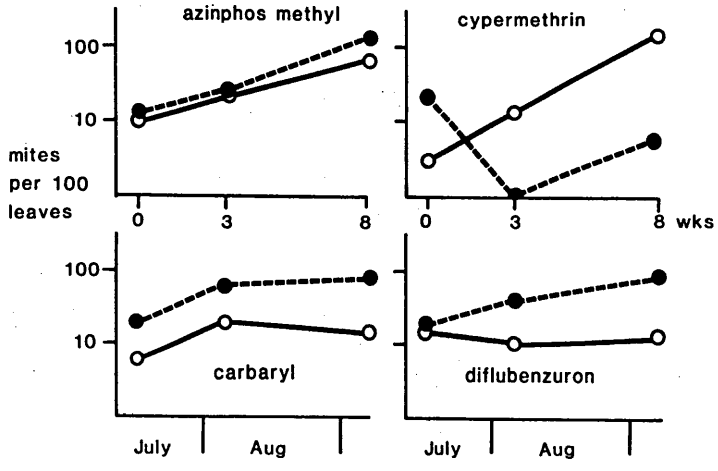


Fig. 1. The disruptive effect of a cypermethrin spray on the relative numbers (log scale) of Panonychus ulmi (o——o) and OP-resistant Typhlodromus pyri (● ---●) compared with the 'neutral' effects from sprays of azinphos-methyl, carbaryl and diflubenzuron.

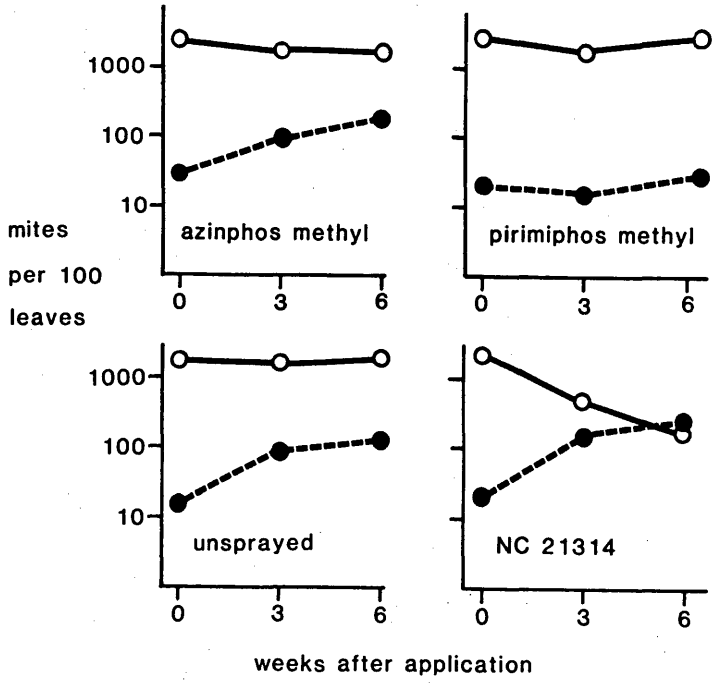


Fig. 2. The effects of three pesticides on the relative numbers (log scale) of P. ulmi (o——o) and OP-resistant T. pyri (● --- ●): azinphos-methyl (neutral); pirimiphos-methyl (disruptive); NC 21314 (corrective); unsprayed comparison.

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Effects of Permethrin Sprays on the Mite Fauna of Apple Orchards

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(Accepted _____)

SUMMARY

In two apple orchard trials single high volume sprays of 25-50 mg permethrin/litre greatly reduced numbers of the phytoseiid predatory mite Typhlodromus pyri whether the spray was timed pre-bloom at growth stages from 'bud-burst' to 'late green cluster', or post-bloom in June. At 'bud-burst' T. pyri were partially protected in hibernating sites on the bark, but the lethal effect of the spray was only delayed; no appreciable selectivity could be obtained by this early timing. Bioassays showed that the persistent toxicity of permethrin residues on bark or on leaves declined to a low level within 20 days, and was related to the decline in residue levels determined by G.C. analyses. Residues were detectable for three months or longer. Resurgence of spider mites, Panonychus ulmi, occured within two months after

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all spray timings, and effective predation by T. pyri was only fully re-established after several months. In one trial, apple rust mite, Aculus schlechtendali also increased when T. pyri was reduced by spraying. Tydeid mites were killed by permethrin.

INTRODUCTION

Synthetic pyrethroids (SPs) now in common use in English apple orchards show a high persistent toxicity to most natural enemies, including the key phytoseiid predator Typhlordomus pyri Scheuten, their use often resulting in resurgence of the spider mite Panonychus ulmi (Koch) and apple rust mite, Aculus schlechtendali (Nalepa). AliNiazee and Cranham (1980) showed that sprays of four SPs sprayed at 'pink bud' stage were highly injurious to T. pyri. Gruys (1980) reported that permethrin was damaging but less so at 'bud-burst' than during the summer, which suggests a degree of selectivity by judicious timing. This paper reports the results of field trials in which the effects of pre-bloom and post-bloom sprays of permethrin were tested and compared. The early-season emergence and behaviour of T. pyri were studied in relation to the effects of sprays. The persistent toxicity of field-weathered residues of permethrin was assessed in bioassays and related to the levels of residues determined by chemical analyses.

MATERIALS AND METHODS

1980 trial. A single spray of 50 mg permethrin/litre (as 'Ambush', I.C.I. Ltd; 25% a.i.) was applied high-volume to 'point of drip' on (a) 25 March at 'late-dormant' stage and (b) 5 May at 'late green cluster' stage, and compared with (c) unsprayed control trees. Plots were single apple trees of cv. Worcester Pearmain replicated in six randomised blocks on the perimeter rows of a large orchard. No other insecticides were applied to these trees during 1980. Samples were taken at a height of 1.6-1.9 m from each tree as follows:

- 13 March, late dormant stage, 'pre-spray' samples, spur shoots 15 cm long (total 18/treatment)
- 20 May, full bloom, leaf and blossom clusters (total 18)
- 2 and 16 June, 28 July, mature leaves (total 120).

These were stored in polythene bags at 8°C and examined within 24 h.

1981 trial. The effects were compared of single sprays of 25 mg permethrin/litre applied (a) 17 March at 'bud-burst' stage, (b) 6 April at 'mouse-ear' stage, and (c) 23 June, c. 1 month after the 'petal-fall' stage, when the first spray for control of codling moth is usually applied. Comparison was made with unsprayed check plots. Plots were of 8-12 trees replicated four times in a randomized block design. Sprays were applied by hand-lance to 'point of drip'. The 'bud-burst' spray was originally applied on 12 March but heavy rainfall (c. 8 mm) started before spraying was completed and before deposits had dried. It was considered that much of the deposit would have been removed by the rain

and therefore these plots were resprayed on 17 March. Subsequently, residue measurements suggested that between 12 and 17 March 30% or more of the initial deposit (12 March) was removed by weathering and other processes. For the control of apple sucker, Psylla mali Schm., it was necessary to apply gamma HCH (as 'Gamma-Col', Imperial Chemical Industries; 80% a.i.) over all plots on 13 April; this insecticide had not reduced numbers of T. pyri in another trial (Flegg, 1981). No other insecticides were used in 1981; apple scab and mildew were controlled by routine sprays of captan and bupirimate, fungicides known to be harmless to T. pyri (Easterbrook, Souter, Solomon and Cranham, 1979; Gruys, 1980; Flegg, 1983).

Sampling for mite numbers and distribution. Samples were taken at a height of 1.6-1.9 m in the foliage canopy of two trees per plot:

- on 12 and 30 March, 6 April ('late-dormant' to 'mouse-ear' stages), twigs 20 cm long bearing leaf and flower buds (total 16).
- 19 April and 11 May ('pink bud' to 'petal fall'), flower clusters each with c. 5 cm of wood (total 16)
- 1 June to 25 August, fully expanded leaves (total 200).

Hibernating female T. pyri are quickly aroused by warmth and leave their shelters (Putman, 1959). In order to obtain valid data on their emergence and distribution, all plant material was sealed in polythene bags and carried in a cooler box at 6-10°C to a cold store at 7-8°C. On collection dates up to and including 11 May, samples were sub-divided in the field into (a) wood and (b) buds, leaf and flower clusters, and these were sealed in separate bags. Twig and cluster

samples were then examined by dissection under the microscope within 24 h of collection. Each bag from cold store was emptied into a small cooler box at 5°C, and as each part was removed from the box it was quickly examined under cool lighting.

Leaf samples, from 1 June, were also bagged and cold-stored, and assessed within 24 h by brushing (Henderson & McBurnie, 1943; Morgan, Chant, Anderson & Ayre, 1955); a brushing machine made by Leedom Engineering, California, U.S.A. was used. Mites were brushed on to paper cards with a counting grid covered with a very thin layer of 'Tangle Trap', a non-drying adhesive (ex The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.). Cards were scanned within 1 h and the numbers of T. pyri, P. ulmi, Zetzellia mali (Ewing), tydeid and tarsonemid mites were counted. Mite counts were subjected to analysis of variance for each sampling date using the transformation Log(1+X). Counts on pre-spray samples were used as covariates. Numbers of A. schlechtendali were counted on a 1/16th sector of the counting grid on each card and categorized as 0-50, 50-200 or >200.

Sampling to estimate persistence. After the pre-bloom sprays T. pyri were active on the bark surface, whilst after the spray on 23 June their activity was largely confined to leaves. Therefore, after the pre-bloom sprays samples of 4-yr old wood (15-20 mm diameter) and, after the June spray fully expanded leaves, were taken at intervals. The sample from each plot was divided for use in bioassays and chemical analysis.

Chemical analysis. Four 10 cm shoots (with side shoots

and buds removed) or 20 leaves (with petioles removed) were soaked overnight in 100 ml of 1:1 acetone/petroleum spirit (60°-80°C boiling range). The extract was decanted into 175 ml distilled water and the liquid from rinsing the sample with three washes of 5 ml petroleum spirit and 5 ml acetone was added to it. After the addition of 2g sodium sulphate the whole volume was shaken for 3 minutes and when separation was complete the aqueous layer was discarded. The petroleum spirit layer was washed with distilled water (2 x 175 ml) and then dried over anhydrous sodium sulphate. This extraction procedure is based on those described by Williams (1976) and Fujie and Fullmer (1978).

A 1 ml aliquot of the extract was cleaned up by column chromatography using a procedure based on that described by Chapman & Harris (1978). For very low residues, a larger aliquot was taken and reduced to 1 ml by evaporation under nitrogen. The column consisted of a pasteur pipette plugged with cotton wool on to which a 5:1 mixture of 'Florisil' (deactivated with 3% water) and activated charcoal (Nuchar C 190 N) was added to give a 6 cm layer of adsorbent. The loaded column was eluted with 3 ml petroleum spirit and 1 ml 4:1 benzene/hexane mixture and the permethrin was then eluted with a further 2.5 ml of the benzene/hexane mixture. The eluate was evaporated to dryness and taken up in 0.5 ml hexane.

Determination of permethrin was by gas chromatography (Pye 104) using a 1.5 m x 4 mm glass column packed with 2% QF-1 on 80-100 mesh Gas Chrom Q. Column temperature was 205°C and gas flow (nitrogen) was 1.94 litres/h. The electron

capture detector was maintained at 270°C. Cis- and trans-isomers of permethrin were resolved under the conditions used (compare work by Chapman & Simmons 1977) with retention times 4.6 min (cis) and 5.4 min (trans), and the concentrations of each were determined by comparison of peak heights with those of standards.

Concentrations in solution down to 0.005 $\mu g\ ml^{-1}$ of cis- or trans-permethrin could be detected and 0.01 $\mu g\ ml^{-1}$ determined, leading to a limit of determination (total permethrin) of 0.006 $\mu g\ cm^{-2}$ of sample surface. Where concentration was carried out prior to clean-up, down to 0.0006 $\mu g\ cm^{-2}$ could be determined on bark (10-fold concentration) and 0.001 $\mu g\ cm^{-2}$ on leaves (6-fold concentration). Results were corrected for the recovery efficiency of the method. Recovery of permethrin added to unsprayed twigs at levels of 0.01-0.1 $\mu g\ cm^{-2}$ averaged 86.7%. The corresponding recovery for foliage was 81.9%.

Bioassays of residues on bark. A disk of bark, 27 mm diameter, was cut from each sample shoot and held flat on the lid of a rigid plastic box (125 x 80 x 22 mm) by double-sided sticky tape. The construction of the type of cell devised to confine mites on a bark surface is shown in cross-section in Fig.1. Wet filter paper above the double-sided tape acted as a barrier and an essential supply of drinking water. A hole, 16 mm diameter, was cut in both tape and filter paper (before wetting) to form a circular arena. A ring of 'Tangle-trap' non-drying adhesive (ex The Tanglefoot Co., Grand Rapids, Michigan, U.S.A.) was syringed on top of the

wet filter paper as an additional barrier. It was necessary to prevent water creeping over the bark surface and this was achieved with an annulus of 'Sellotape', with the non-sticky side uppermost, placed below and projecting 1.5 mm within the filter paper walls of the arena.

The phytoseiid Amblyseius potentillae (Garman) was reared on 'plate' cultures (Kapetanakis & Cranham, 1983) with pollen of Vicia faba as food. To produce immature mites of known age, adult female A.potentillae were placed on a clean culture plate (Day 1) to lay eggs for 24 h and were removed (Day 2). On Day 4 any unhatched eggs were removed from the plate. On Day 5, five young mites (larvae and protonymphs) were transferred to each test arena on bark, and a trace of pollen added as food. There were eight replicates per treatment/occasion. Tests were maintained at 20°C and mortality was assessed after 24 h and 48 h; mortality was virtually complete in 24 h. Mites within each test arena, or in the edge of the water barrier, which did not show signs of movement when prodded with a fine brush were recorded as dead.

Bioassays of residues on leaves. Disks, 16 mm diameter, were cut and placed with their abaxial surfaces uppermost on wet filter paper on the lids of 113 ml plastic cartons (D.G. Containers Ltd, Liverpool) which acted as water reservoirs. The subsequent procedure and replication was the same as for the assays on bark.

RESULTS

1980 trial. Observations showed that on 13 March adult female

T. pyri were still hibernating in crevices on the bark; none were found in swelling buds. On 9 April c. 25% of the T. pyri were found in buds and the rest in bark crevices. On 12 May nearly all adult females were found in the leaf and blossom clusters and had started to produce eggs. Plots sprayed on (a) 25 March, (b) 5 May and (c) unsprayed had pre-spray counts on 13 March of 56, 3 and 48 T. pyri adult female/sample respectively, and post-spray counts on 20 May of 1, 0 and 11 T. pyri/sample. Counts on leaves are given in Table 1; T. pyri was virtually eliminated by permethrin at either spray timing. This led by July to a marked increase of P. ulmi on certain replicates; however, the differences were non-significant due to high variation between replicates.

1981 trial. Emergence and distribution of mites in the spring. Fig. 2 summarises data on the distribution of T. pyri between overwintering shelters on the bark and the developing leaf and flower clusters, from March to June on unsprayed trees. On 12 March, overwintering females of T. pyri were found only in shelters on the bark of spurs, branches and trunk. Cankers, localised areas of rotting wood, scars of removed shoots, loose bark and algae were the most common sites. Most of the mites were found in groups of 3-10 individuals; but larger groups, up to 20 or more, were found, causing a large variation between the counts for replicate plots early in the season.

Tydeid mites (species undetermined) were found in the same shelters with T. pyri, most of them a dark grey colour, a few yellowish green. About 4% T. pyri females had dark

coloured guts, an indication that they had been feeding on tydeids. The stigmaeid mite, Zetzellia mali, and tarsonemids were also found occupying similar, and quite often the same, shelters as T. pyri.

Active T. pyri were first found on 30 March ('late bud-burst') under bud scales, or in protected sites among hairs at the bases of emerging flower buds.

Most looked well fed and their gut colour, light to dark brown, suggested they were feeding on apple rust mite, Aculus schlechtendali, numbers of which had moved already to the new buds. About 20% of the tydeids of a pale green colour had moved to the new growth but most Z. mali and all tarsonemids were still found in bark crevices.

On 6 April, c. 50% of T. pyri were still in shelters on the bark, although many of these appeared well fed, an indication of movement from the shelters in search of prey and back again for protection against spring frosts (Fig.2). Z. mali and c. 75% of tydeids were still in hibernating sites on the bark, whereas a large proportion of tarsonemids were active on the new growth.

On 19 April only c. 30% T. pyri, but over 60% of tydeids, were found in shelters. Most of the rust mites were on leaves and flower sepals in the clusters. Very few Z. mali and tarsonemids were found on this occasion. A few larvae of P. ulmi were recorded.

On 11 May most T. pyri were on the new growth in protected sites including young unfolded leaves or the bases of young fruit; many had fed on immature P. ulmi. Many T. pyri eggs were counted. Tydeids, over 95% were on the young leaves.

On 1 June no T. pyri were found on bark and only trace numbers on young fruitlets; their main niche for the rest of the season was the abaxial surfaces of leaves.

Effects of sprays on T. pyri and P. ulmi.

Pre-bloom sprays. The full effect of the March spray was delayed; reductions in T. pyri numbers, compared to unsprayed plots, were non-significant on 30 March and 6 April, but were large and significant ($P = 0.001$) on 19 April and 11 May (Table 2). This delay probably resulted because mites were partially protected within shelters. In samples taken 30 March from plots sprayed on the 17th, 94% of the T. pyri found were in shelters in contrast to 41% in samples from unsprayed plots; on 6 April the proportions were 69% and 48% respectively. It may be that mites on the sprayed plots were temporarily deterred by the spray residues from leaving shelters and/or that most of those mites that had left shelters were killed.

The spray on 6 April eliminated T. pyri quickly; none were found on 19 April, when the numbers on March-sprayed plots were also very low ($P = 0.001$) compared to unsprayed (Table 2). By 11 May, and in the leaf samples of 1 June, T. pyri had begun to increase again but they were still fewer ($P. = 0.01$) than on unsprayed plots (Fig.3). From 23 June until 25 August the differences in T. pyri numbers between the pre-bloom sprayed and unsprayed plots were non-significant. Fig.3 also shows that compared to unsprayed plots on 1 June the pre-bloom sprayed plots had c. 10 x more P. ulmi and the ratio of P. ulmi to T. pyri was 35:1 and 63:1 compared

to 1.1 on unsprayed. Nevertheless from this stage onward P. ulmi numbers declined, T. pyri gradually increased, and the ratio was restored to c. 5:1 or less from 22 July to 25 August.

June spray. This virtually eliminated T. pyri and numbers did not begin to recover for six weeks; even by 25 August they were still lower ($P = 0.05$) than on plots sprayed pre-bloom and unsprayed. This resulted in a marked upward trend in P. ulmi numbers to thirty times those on the unsprayed plots by 25 August; on that occasion the ratio of P. ulmi:T. pyri was 23:1, and subsequently spider mite numbers continued to rise. In the leaf samples P. ulmi numbers were on average, through the summer, four times higher on Egremont Russet than on James Grieve; but no varietal preference was apparent for T. pyri or for other mite species found.

Residual toxicity and chemical persistence

Figs.4a-c show that after all three spray timings the percentage mortality of young A. potentillae in bioassays and the levels of permethrin residues determined by G.C. analyses, declined within 10-14 days to about half the initial values recorded on day '0'. Thereafter, mortality declined to negligible levels within 40 days, but low-level residues were detectable for considerably longer. On samples from the unsprayed plots, there were no detectable residues (< 0.002 µg total permethrin cm^{-2} on bark and < 0.001 ug cm^{-2} on leaves) and less than 5% mortality in bioassays. Fig.4d shows the relationship between the percentage kill obtained in bioassays and the residue levels determined by analyses; there were no large differences between the effects

of residues on leaves and on bark, but the results suggest that residues on leaves were on average slightly more toxic. More detailed studies would be needed to reach firm conclusions on this point.

Effects on other mite species

On all plots from March to May the numbers of *A. schlechtendali* were low. In leaf samples on 1 June they were estimated at <16/leaf. Numbers rose slowly, reaching >64/leaf in August on all sprayed plots but were markedly lower ($P = 0.05$) on unsprayed plots.

Numbers of tydeid mites were reduced ($P = 0.05$) by both pre-bloom sprays (Table 2) though, as for *T. pyri*, the full effect of the March spray was delayed. On 1 June, leaf samples showed only 15-30/100 leaves on sprayed and unsprayed plots alike, and the numbers continued to decline until August.

There was no clear evidence that permethrin was harmful to *Zetzellia mali* and to tarsonemid mites. These were fairly common on twig samples early in the season though both were very highly aggregated in distribution. Few *Z. mali* and no tarsonemids, were found in leaf samples from June to August.

DISCUSSION

In both years, *T. pyri* became active and began to move out of winter shelters at the end of March, more than a month before winter eggs of *P. ulmi* began hatching. Observations suggest that, at first, many of the mites were making foraging excursions in search of prey (including rust mites and tydeids) and returning to shelters on the bark rather than staying

in the opening buds. In the 1981 trial, there was evidence that the shelters provided temporary protection from the effects of the spray at 'bud-burst' (17 March), but residues were still present at toxic levels when the mites became more active in April (i.e. within 20 days of spraying). In the results for both trials (Tables 1 and 2, Fig.3), the disruptive effect of a permethrin spray in March at the 'late-dormant' to 'bud-burst' stages was not markedly less than that of sprays at 'mouse-ear' (6.4.81) or 'late green cluster' (5.5.80).

Residual toxicicity and chemical persistence

The toxicity of permethrin residues both on bark and on leaves declined to a low level within 20 days, when in the bioassays c. 20% of larvae and protonymphs of A. potentillae were being killed. This species was chosen for the bioassay work because T. pyri has a much lower fecundity (Overmeer, Doodeman & van Zon, 1982) and cultures would not provide the numbers of eggs required. Subsequent bioassays employing the taped slide technique (Kapetanakis & Cranham, 1983) showed that T. pyri was 2-3 times more tolerant than A. potentillae to permethrin. Immature mites are less tolerant than adults. Hence it is likely that permethrin residues in this trial ceased to be toxic to T. pyri in rather less than 20 days, especially pre-bloom when the population consisted only of adult females. However, related studies (E.G. Kapetanakis, Ph.D. thesis, in preparation), showed that T. pyri adult females tended to avoid apple leaf surfaces bearing permethrin residues at sub-lethal levels; and when confined on such surfaces egg production was reduced. Therefore it seems likely that the persistent effects of permethrin

residues to T. pyri may extend somewhat beyond the period when they cause direct mortality, as determined for example by Ruscoe (1977) for larvae of Plutella xylostella.

Solvent extraction before G.C. analysis would presumably have recovered permethrin not only from surfaces of bark and leaves where mites were exposed to it, but also from micro-crevices, leaf waxes and plant tissues where they were not. The difference between the persistent toxicity and the persistence of total chemical residues, in relation to 'biological availability', was stressed in a review paper by Chiba (1970). In a study comparing bioassay techniques on adult T. pyri (Kapetanakis & Cranham, 1983), fresh residues of permethrin on a glass surface were more toxic than on a leaf surface, so that the ratio of LC_{50}s leaf/glass was 6.6. This suggests that even with fresh residues on a leaf surface only a small fraction of the total permethrin present is available to the mites. In the current study, the correlation between percentage kill and residue levels (Fig.4d) suggests that the size of the available fraction did not change greatly with time after spraying. There is an indication from these data that the available fraction on leaves, after the June spray, was rather higher than that on bark after the March spray; though the results on bark for the April spray were not consistent with this conclusion.

Disruptive effect of sprays

After pre-bloom sprays of 50 mg permethrin/litre in 1980, T. pyri numbers had not recovered by late July and control of P. ulmi was lost for the whole season. After pre-bloom sprays of 25 mg permethrin/litre in 1981, the persistent toxicity was judged to be negligible by the end of April.

Nevertheless, it took nearly four months, until late July before the ratio of P. ulmi:T.pyri had recovered to 5:1 or less (Fig.3). With the June spray this ratio was 23:1 at the end of August; subsequently P. ulmi laid many winter eggs so that the disruptive effect was carried over to spring 1982. It is probable that on these small plots immigration of T. pyri from adjacent unsprayed trees boosted the rate at which the population recovered, so that recovery would have occurred rather faster than when whole orchards are sprayed. Weather conditions during 1981 were not conducive to a rapid build up of P. ulmi; May was one of the wettest on record, and mean temperatures in June were 2°C cooler than average. In 1981, permethrin was tested at the lowest recommended concentration. The conditions of the trial therefore tended to minimise rather than maximise the disruptive effect of permethrin. Nevertheless resurgence of P. ulmi occurred after all three spray timings.

In contrast to the report by Gruys (1980) in the Netherlands, but in agreement with the results of another pre-bloom trial of pyrethroids in England (Flegg, 1982), the results do not suggest that the disruptive effect of pre-bloom, or even of 'bud-burst', sprays of permethrin was markedly less than that of post-bloom sprays; both are unacceptable for the practice of mite management (Cranham and Solomon, 1981).

REFERENCES

ALINIAZEE, M.T. & CRANHAM, J.E. (1980). Effect of four synthetic pyrethroids on a predatory mite. Typhlodromus pyri, and its prey, Panonychus ulmi, on apples in south-east England. Environmental Entomology 9, 436-439.

CHAPMAN, R.A. & HARRIS, C.R. (1978). Extraction and liquid-solid chromatography clean-up procedures for the direct analysis of four pyrethroid insecticides in crops by gas-liquid chromatography. Journal of Chromatography 166, 513-518.

CHAPMAN, R.A. & SIMMONS, H.S. (1977). Gas-liquid chromatography of picogram quantities of pyrethroid insecticides. Journal of the Association of Official Analytical Chemists, 60, 977-978.

CHIBA, M. (1970). An analytical chemist's contribution to applied entomology. Proceedings of the Entomological Society of Ontario 101, 24-27.

CRANHAM, J.E. & SOLOMON, M.G. (1981). Mite management in commercial apple orchards. Report of East Malling Research Station for 1980, pp. 171-2.

EASTERBROOK, M.A., SOUTER, E.F., SOLOMON, M.G. & CRANHAM, J.E. (1979). Trials on integrated pest management in apple orchards. Proceedings, British Crop Protection Conference - Pests and Diseases. November 19th-22nd 1979, Brighton, England, pp 61-7.

FLEGG, J.J.M. (1981). Zoology. Report of East Malling Research Station for 1980, pp. 97-106.

FLEGG, J.J.M. (1982). Zoology. Report of East Malling Research Station for 1981, pp. 99-112.

FLEGG, J.J.M. (1983). Zoology. Report of East Malling Research Station for 1982, pp. 101-112.

FUJIE, G.H. & FULMER, O.H. (1978). Determination of FMC 33297 residues in plant, animal and soil matrices by gas chromatography. Journal of Agricultural and Food Chemistry 26, 395-397.

GRUYS, P. (1980). Significance and practical application of selective pesticides. Proceedings of the International Symposium of the IOBC/WPRS on Integrated Control in Agriculture and Forestry, Vienna, 8-12 October, 1979 pp. 107-112.

HENDERSON, C.F. & McBURNIE, H.V. (1943). Sampling technique for determining populations of the citrus red mite and its predators. United States Department of Agriculture circular 671, 11 pp.

KAPETANAKIS, E.G. & CRANHAM, J.E. (1983). Laboratory evaluation of resistance to organophosphates and carbaryl in the phytoseiid predator Typhlodromus pyri from English apple orchards. Annals of Applied Biology, 103, 389-400.

MORGAN, C.V. CHANT, D.A., ANDERSON, N.H. & AYRE, G.L. (1955). Methods for estimating orchard mite populations, especially with the mite brushing machine. The Canadian Entomologist 87, 189 - 200.

OVERMEER, W.P.J., DOODEMAN, MARGA & VAN ZON, A.Q. (1982). Copulation and egg production in Amblyseius potentillae and Typhlodromus pyri (Acari, Phytoseiidae). Zeitschrift für angewandte Entomologie 93, 1-11.

PUTMAN, W.L. (1959). Hibernation sites of phytoseiids (Acarina: Phytoseiidae) in Ontario peach orchards. The Canadian Entomologist 91, 735-741.

RUSCOE, C.N.E. (1977). The new NRDC pyrethroids as agricultural insecticides. Pesticide Science 8, 236-242.

WILLIAMS, I.H. (1976). Determination of permethrin by gas-liquid chromatography with electron capture detection. Pesticide Science 7, 336-338.

Table 1. 1980 trial : effects of a single spray of 50 mg permethrin/litre on numbers of Panonychus ulmi and Typhlodromus pyri per 100 leaves

Date of spray (growth stage of apple)	Date of sampling		
	2 June	16 June	28 July
	P.ulmi, active stages (eggs)		
25 March (late dormant)	37 (10)	38 (736)	239 (2705)
5 May (late green cluster)	315 (23)	54 (1551)	421 (4378)
Unsprayed	12 (5)	6 (193)	18 (286)
	T. pyri		
25 March	0	0	2
5 May	0	0	0
unsprayed	13	7	7

Table 2. 1981 trial : effects of a single spray of 25 mg permethrin/litre on numbers of Typhlodromus pyri and tydeid mites up to bloom

(see text for sample sizes)

Date of spray (growth stage)	Date of sampling 12 March	30 March	6 April	19 April (2)	11 May (2)
	T. pyri				
17 March (bud burst)	97⇒	85	29	4 b	23 a
6 April (mouse ear)	80	90	75⇒	0 a	4 a
unsprayed	101	120	120	51 c	108 b
	Tydeid mites				
17 March	263⇒	103	57	19 ab	10 a
6 April	162	125	115 ⇒	9 a	6 a
unsprayed	177	138	143	52 b	52 b

(1) ⇒ spray applied between sample dates

(2) values in each column followed by the same letter do not differ significantly (P = 0.05)

Legends to Figures

Fig.1. The type of cell devised for bioassays of *Amblyseius potentillae* on permethrin residues on apple bark, shown in cross-section.

Fig.2. Distribution of *Typhlodromus pyri* between overwintering shelters on bark and the developing leaf and flower clusters of apple trees, from March to May, 1981; data from unsprayed plots.

Key: ☐ on bark; ⚄ in buds; ▩ in flower clusters; ▨ at bases of floral pedicels; ▧ on flowers; ▤ on fruitlet clusters; ▥ on leaves.

Letters refer to the apple growth stages: LD, late dormant; LBB, late bud-burst; ME, mouse-ear; FB, full blossom; PF, petal fall.

Figs.3a-d. The effects of 25 mg permethrin/litre sprayed (a) 17 March, (b) 6 April, (c) 23 June, 1981, compared with (d) unsprayed trees: numbers of *Typhlodromus pyri* (●---●) and *Panonychus ulmi* (o——o) active stages per 100 leaves (log scale).

Figs.4a-c. Biological and chemical persistence of field-weathered residues of permethrin on (a) bark, sprayed 17 March, (b) bark, sprayed 6 April, and (c) leaves, sprayed 23 June: o——o % kill of young *Amblyseius potentillae* in bioassays; ●——● µg permethrin cm^{-2}, determined by G.C. analyses.

Fig.4d. Data of Figs.4a-c: relation between % kill and permethrin residue levels: Δ——Δ, spray of 17 March; ▲——▲, spray of 6 April; □——□, spray of 23 June.

Fig. 1

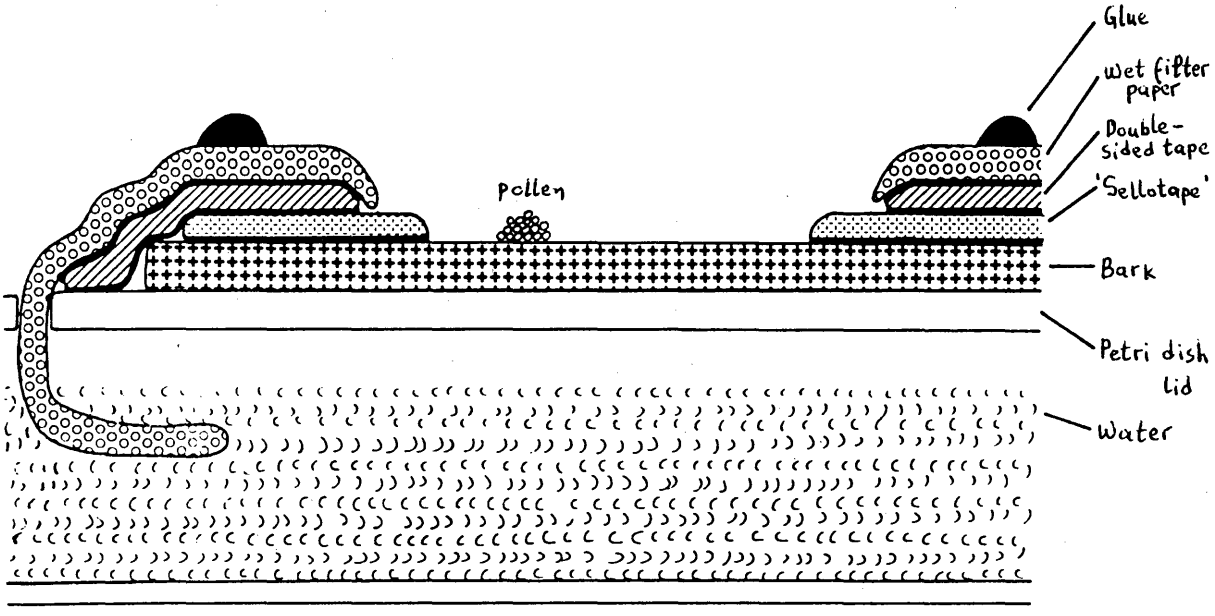

Glue
Wet filter paper
Double-sided tape
'Sellotape'
pollen
Bark
Petri dish lid
Water

Fig. 2

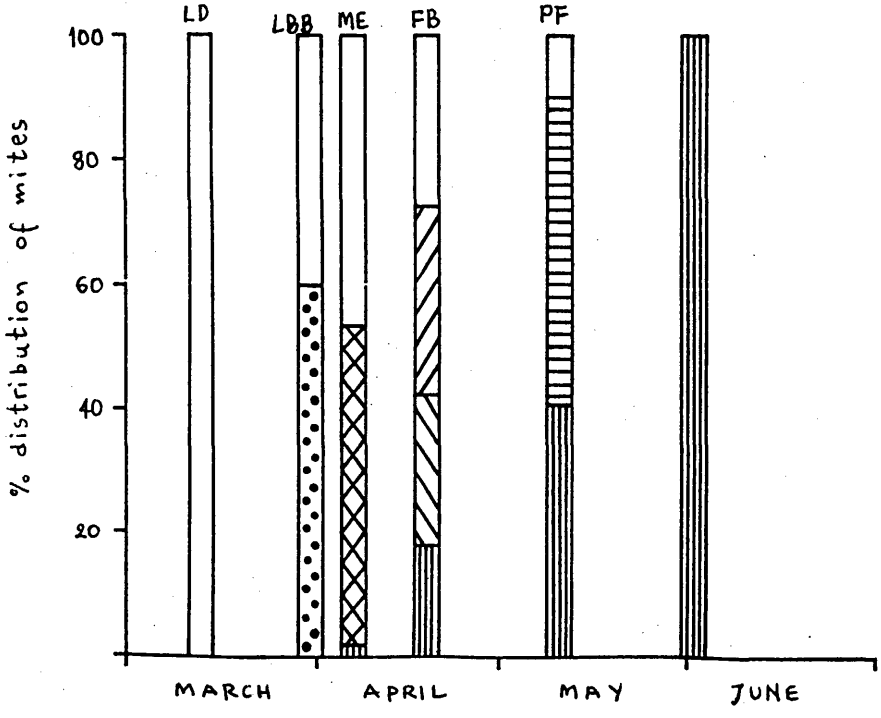

LD
LDB
ME
FB
PF
100
80
60
40
20
% distribution of mites
MARCH
APRIL
MAY
JUNE

Fig. 3

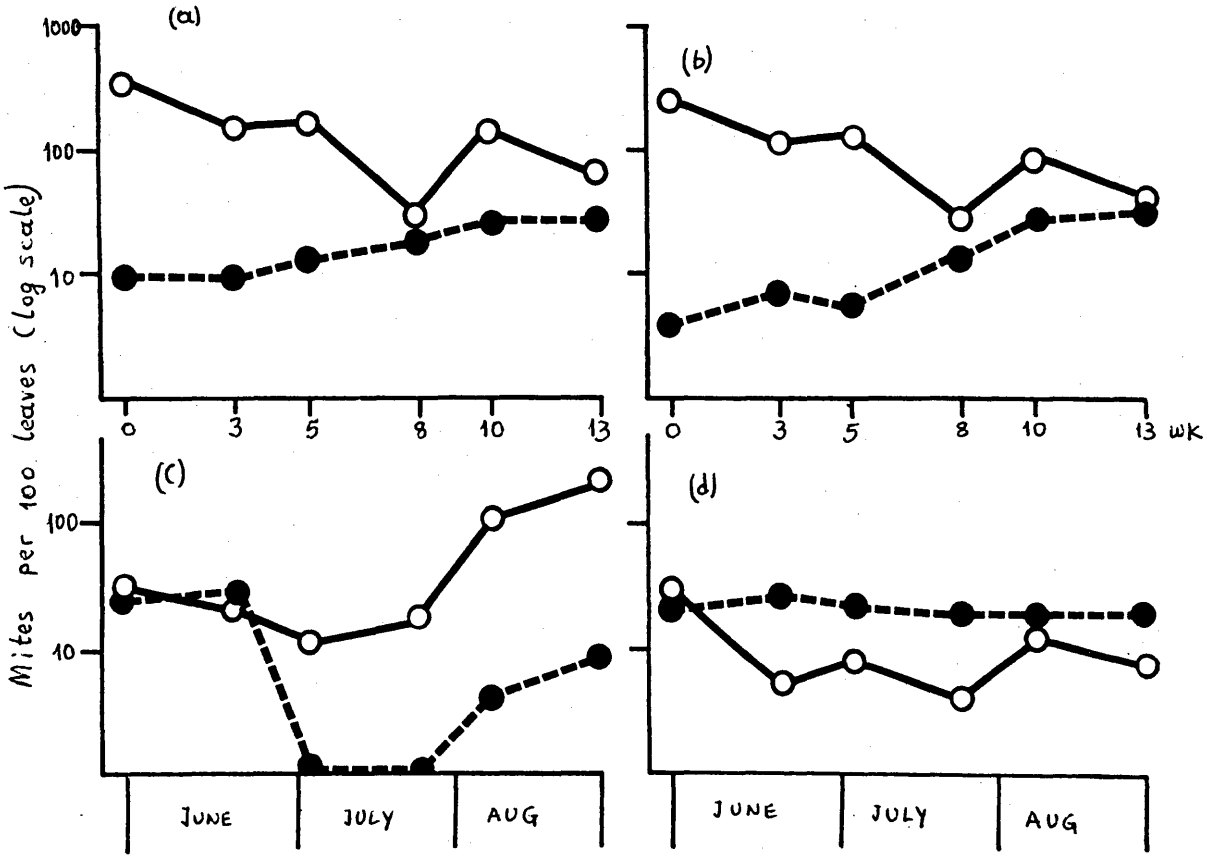
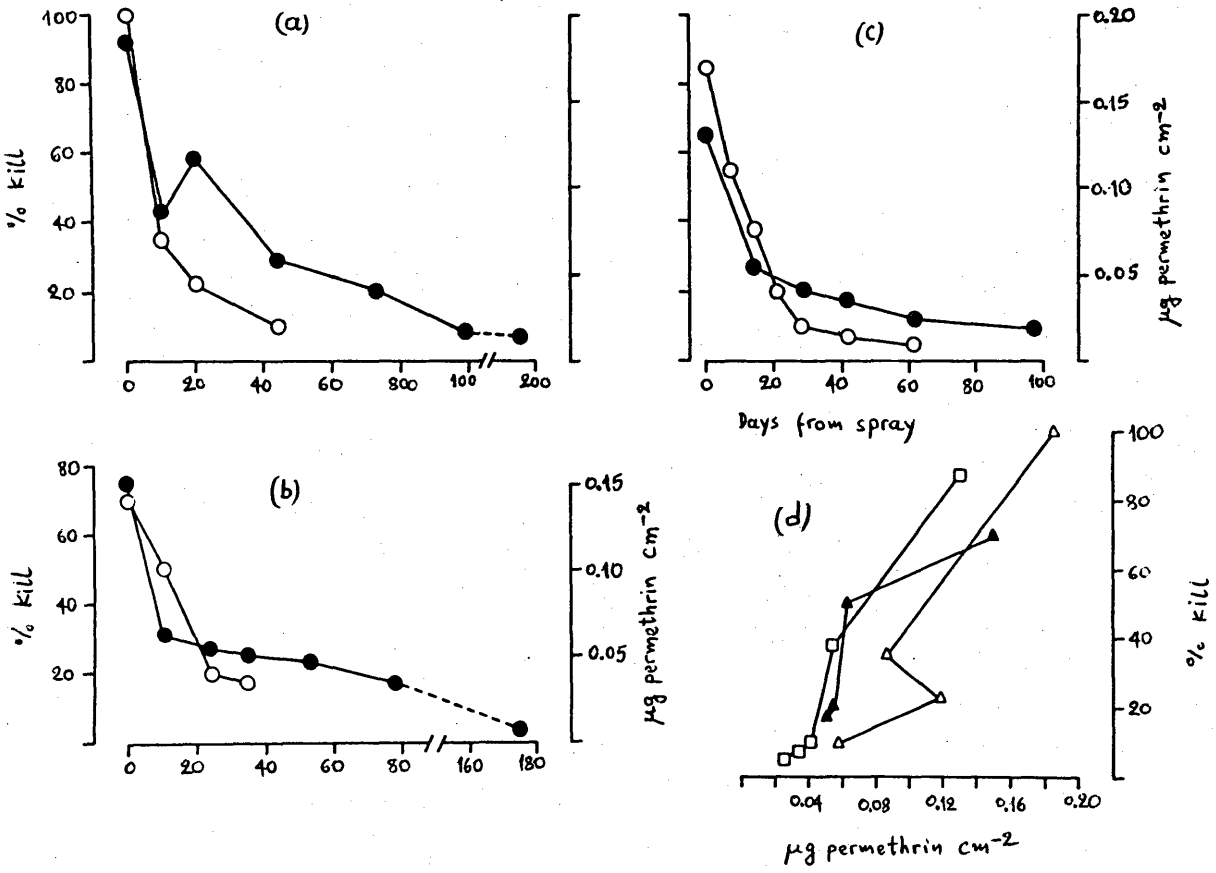

(a)
(b)
(c)
(d)
1000
100
10
Mites per 100 leaves (log scale)
0
3
5
8
10
13
wk
JUNE
JULY
AUG

Fig.4


(a)
% kill
100
80
60
40
20
0
20
40
60
800
100
200
(c)
0.20
0.15
0.10
0.05
µg permethrin cm⁻²
0
20
40
60
80
100
Days from spray
(b)
% kill
80
60
40
20
0.15
0.10
0.05
µg permethrin cm⁻²
0
20
40
60
80
160
180
(d)
100
80
60
40
20
% kill
0.04
0.08
0.12
0.16
0.20
µg permethrin cm⁻²