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A BIOLOGICAL STUDY OF THE SPREAD OF PESTICIDES FROM SMALL DROPLETS

by

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ABSTRACT

The spread of insecticide from ULV droplets across leaf surfaces was studied by determining the distribution of mortality of whitefly larvae around individual droplets. Thus the effects of varying such factors as droplet size, concentration of active ingredient (a.i.), formulation and plant species were examined.

Monosize droplets (59 - 117 μm) of oil-based formulations of permethrin and pirimiphos-methyl were sprayed on leaf pieces (2 x 2 cm) of tobacco (Nicotiana tabacum), tomato (Lycopersicon esculentum) and French bean (Phaseolus vulgaris) infested with settled first instar whitefly larvae (Trialeurodes vaporariorum (Westwood)). Death of larvae was found to be related to distance from droplets. The distribution of mortality around the droplet showed a very regular pattern. Most mortality occurred within a limited area around the droplet which was influenced by concentration of a.i., droplet size, formulation of carrier solution and species of plant. Concentration of a.i. and droplet size had much less effect than might be anticipated, indicating possibilities of considerable economy in use. Increasing the concentration of a.i. from 0.5 to 20% increased the area of influence of a single droplet on tobacco from 1.25 to 2.15 mm^2; an increase of only 72% although the concentration increased forty times. Estimated LD_{50} values were 0.26 and 5.18 $\mu g/cm^2$ respectively, showing that a spray concentration of 0.5% was about twenty times more economical than that of 20%. The areas of influence for 59 and 114 μm diameter droplets of 10% permethrin on tobacco were 1.07 and 1.63 mm^2 respectively. Estimated LD_{50}s were 1.01 and 4.64, demonstrating that the smaller droplet was 4.6 times more efficient than the larger. The

formulation of the carrier solution influenced the spread of the insecticide and the efficiency of the spray, thus 10% permethrin formulated in JF 8130, JF 9079, VK1 and JF 8133 on tobacco affected 0.99, 1.40, 1.90 and 1.92 mm^2 respectively. Estimated LD_{50} values were 7.63, 4.62, 3.67 and 3.43 $\mu g/cm^2$.

The species of plant modified the spread of insecticide and effectiveness of spray droplets. Areas of influence of droplets of 10% permethrin on tobacco, tomato and French bean were 1.90, 3.44 and 4.39 mm^2. Estimated LD_{50} values were 3.67, 1.81 and 1.42 $\mu g/cm^2$ respectively.

Scanning electron microscope studies of the physical form and spread of droplets of permethrin on leaves and the bioassay results showed that the effectiveness of the spray deposit is related to the length of the perimeter of the droplet, such that:

$$LS_{50} = 1.47 \text{ droplet Perimeter}$$

where LS_{50} is the median lethal spacing of droplets (in the same units).

Droplets of 10% pirimiphos-methyl in still air gave an area of influence of 402 mm^2 and an estimated LD_{50} value of 0.014 $\mu g/cm^2$ on tobacco, compared with 1.40 mm^2 and 4.62 $\mu g/cm^2$ respectively for 10% permethrin. Simple bioassay techniques were devised showing that the effect of pirimiphos-methyl over such a large area was due to its vapour pressure properties.

The spatial distribution of chemical around the droplet was deduced by mathematical means and theories of diffusion were considered. The results are discussed in relation to the practical problems of pest control and the economic and ecological implications.

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1. INTRODUCTION

Development of spraying methods dates back to the end of the last century when equipment was made to apply Bordeaux mixture in French vineyards (Lodeman, 1896). Most pesticide formulations are diluted in water and applied as medium or high volume sprays using hydraulic nozzles. In some crops high volumes are even applied to 'run-off' in an attempt to completely wet the whole surface of plants. Hydraulic nozzles produce a wide range of droplet sizes, the smaller are subject to drift, while the wasteful larger droplets are poorly retained. Sometimes over 99% of the pesticide applied to foliage fails to reach the intended target. The need to combat the escalating chemical and operational costs of conventional spraying and the desire to reduce off-target contamination have stimulated interest in alternative methods of application. The ultra-low volume (ULV) method, which was developed after its success in controlling the desert locust in East Africa shortly after the Second World War, proved a valuable tool in areas where water is scarce, and its logistic advantages are useful especially for aerial application. The ULV concept has recently been extended where greater emphasis has been placed on developing equipment with greater control of droplet size. Controlled droplet application (CDA) systems are now available which enable better deposition on the target, thus offering a great potential for reducing dose rates and wastage. These developments in application technology have been backed up with relatively little research in relation to the precise requirements of the biological target.

Only a small proportion of target organisms is hit directly by a ULV spray on foliage, especially when the target pest is small and sessile (Munthali and Scopes, 1982). The effectiveness of such a spray is, therefore, largely dependent on the spread of pesticide from initial deposits. The present work was undertaken to study the spread of pesticides from spray droplets on leaf surfaces, by determining the distribution of whitefly larval mortality on leaves of tobacco, tomato and French bean around individual droplets of oil-based formulations of permethrin, a widely used contact insecticide and pirimiphos-methyl, an insecticide with fumigant and contact action. The aim was to gain a better understanding of the role of such factors as concentration of active ingredient, droplet size, formulation of carrier solution and plant species to optimize application systems and formulations and achieve effective pest control with minimum wastage and less harmful side effects.

2. LITERATURE REVIEW

1) ULTRA-LOW VOLUME AND CONTROLLED DROPLET APPLICATION

The ultra-low volume (ULV) application technique originated in the control of the desert locust Schistocerca gregaria Forsk in East Africa shortly after the Second World War (Sayer, 1959). ULV application acquired a variety of definitions. The USDA definition quoted by Courshee et al. (1971) restricts the use of the term to volume application rates of less than 0.5 US gallon/acre, while EPPO (1972) has adopted 5 litres/ha as an arbitrary maximum. Joyce et al. (1968) defined it as waterless spraying at very low volume. Other definitions were fine spray (drift spray) and concentrate spray. None of these definitions is universally acceptable. ULV sprays have been applied at rates greater than 5 litres/ha (Morgan, 1974) with large droplets (Taylor & Merritt, 1975) and diluted with water (Moulam et al., 1975). 'The application of the minimum volume per unit area compatible with economic control' (WHO, 1971) is possibly the most suitable definition.

Since its successful use against the desert locust, ULV application methods have been used against a variety of agricultural, medical and veterinary pests, diseases and weeds worldwide. The various technical aspects of the method and its merits have been the subject of much investigation and discussion (Bals, 1969, 1973 & 1982; Courshee, 1971; Coutts & Furmidge, 1971; Johnstone, 1974; Joyce, 1974; Lofgren, 1970; Maas, 1971; Matthews, 1971; Owen & Bennett, 1977; Skoog et al., 1965).

It has been established that the method has logistic, economic and biological advantages. The main attraction is that handling small volumes results in more economic and speedier application as only lightweight equipment and containers are required enabling cheaper and faster vehicles to be used. This allows for improved timing of application especially when spraying is part of a pest management system. The method is especially suited for regions where water is scarce and transportation of large volumes of spray liquid is a limitation. The main advantage for aerial application is that it makes possible the treatment of considerably increased areas per tankload and hence per flight.

A narrow spectrum of correct droplet size for a specific target needs to be applied to optimise use of the minimum volume to achieve effective control. Hence the need for controlled droplet application (CDA) rather than merely reducing the volume application rate (Matthews, 1977a). The term was first coined by J. Fryer in 1975 during discussions on herbicide application (Matthews, 1977a). Nevertheless, the need to use sprays with a narrower spectrum of droplet size was recognized many years earlier (Fraser, 1957; Bals, 1969). In practice, commercial hydraulic sprayers produce wide droplet size spectra (15-300 µm diameter or even a maximum droplet size (D-max) of 950 µm) with vmd/nmd* ratios of 5-15. In such sprays, droplets of the optimum size form less

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*FOOTNOTE:

Droplet size is described in terms of volume, number or mass as the volume median diameter (vmd), the number median diameter (nmd) or the mass median diameter (mmd), respectively. The uniformity of droplet size in any particular spray is described by the vmd/nmd ratio. A value of one indicates completely monosize droplets, while higher values indicate correspondingly higher droplet size spectra.

than 2% of the spray volume (Himel, 1969a). Centrifugal energy nozzles (spinning discs) are capable of producing narrow droplet size spectra with vmds ranging from 30-250 μm depending on disc speed and flow rate (Heijne, 1980). The hand-held herbicide sprayer 'Herbi'(R) (Bals, 1975) is capable of producing droplets having vmd of 250 μm with vmd/nmd ratio of about 1.2 when rotating at about 2000 rpm while the mini ULVA(R) produces 75 μm vmd droplets when rotating at 7000 rpm with vmd/nmd ratio of about 1.5 upwards depending on the condition of the disc and formulation. Tractor mounted spinning discs are in use for applying pesticides to field crops.

One of the major recent advances in CDA technology is the development of electrostatic spraying. 'Electrostatic spraying opens up the prospect of improving deposition on the intended target and matching drop specifications more closely to the biological requirements without attendant problems of wastage or of drift where the requirement is for small droplets' (Graham-Bryce, 1981). The electrodynamic sprayer 'Electrodyn'(R) described by Coffee (1979, 1981) atomises purely by electrostatics with very low power requirement, has no moving parts, and produces very narrow droplet size spectra. Electrostatic charging is also applied to spinning discs to improve droplet deposition (Arnold & Pye, 1980). In laboratory tests with small droplets (< 30 μm) Law (1980) obtained up to a sevenfold increase in deposition with charged sprays. Morton (1982) reported 2.5 times increase in recovery between charged and discharge sprays applied at low rates on cotton in the field. Morton (1981) also reported average

increases of 34% in cotton yield and 47% net profit obtained by farmers in Paraguay with the 'Electrodyn' sprayer compared with knapsack sprayers applying cypermethrin and dimethoate. One problem with electrostatic sprayers is that penetration may be poor especially in dense canopies as the surface nearest to the nozzle will collect most of the spray when the charge to mass ratio is high. Efforts are continuing to improve penetration of charged sprays into plant canopies (Coffee, 1981; Durand et al., 1983; Hislop, 1983; Hislop et al., 1983; Pye, 1983; Sherman & Sullivan, 1983).

2) SPRAY COVER

The degree of pesticide cover which proves effective when pesticides are applied to foliage is determined by the behaviour and mobility of the pest and the mode of action of the pesticide. Thus with non-systemic pesticides the more static the pest, the more complete the spray cover required at the site of attack. For example, whitefly larvae are sedentary on the lower surface of the leaf so they are less exposed to insecticide sprays than jassid nymphs which move actively over both upper and lower leaf surfaces. Many major pests are exposed to spray deposits on the surfaces of plants for only a short period. Larvae of stem or fruit borers may be able to enter the plant at the site of oviposition so the period when a lethal deposit of insecticide can be picked up is minimal. The distance from egg to entry site of newly hatched larvae of the Oriental fruit moth Grapholitha molesta (Busck) on peach may be as short as 10-15 cm (3-4 minutes). Therefore, the

density of deposit on sprayed leaves must be high enough to ensure a lethal accumulation in a short exposure period. Fisher & Menzies (1976) concluded that a minimum deposit of 35 droplets/cm^2 (0.175 $\mu g/cm^2$) of carbaryl 50% WP will be required for the control of the larvae of the Oriental fruit moth on peach. 'Complete' coverage with contact fungicides is generally considered essential to ensure protection against penetration of fungal hyphae at the sight of spore germination. However, redistribution of initial deposit by rain or leaf wetness sometimes assists the active material to reach inaccessible sites, or to wash spores and fungicide to the same site thus improving disease control (Courshee, 1980; Hislop, 1969, 1970). Local movement of vapour could modify the 'biocidal' range of pesticide droplets. Hislop (1967) demonstrated that phenyl mercury chloride, maneb, mancozeb, dichlo- fluanid and oxythioquinox protect areas of Vicia faba leaf against Botrytis fabae beyond the limits of the fungicide deposit due to the release of fungicidal vapour.

Hartley & Graham-Bryce (1980) discussed the physics of pesticide cover and spread. Good target coverage with low volume application rates can be achieved in part by the spreading of a suitably formulated liquid and in part, by dispersing the spray in droplets as small as other factors allow. Spread from a pattern of discrete droplets is not likely to provide a continuous film of spray deposit. Therefore, to improve coverage in the sense of leaving the minimum distance between spray residues, it is generally considered necessary to reduce the droplet size.

Hartley & Graham-Bryce (1980) compiled a table showing the purely geometrical relationships between spray volume, coverage, mean size and distance between droplets.

Courshee et al. (1954) have drawn attention to the fact that lime sulphur sprays have a range of fungicidal influence around the deposit of a droplet; they termed the diameter of this 'biocidal' range 'd'. If the area covered by the spray residue of droplet on drying is called 'S', they postulated that the ratio between the effective fungicidal cover and the actual cover by the deposit of the fungicide is $(S + d)^2/S^2$ and is a maximum for a minimum drop size.

Munthali (1981) calculated the biocidal area (BA) per active ingredient (a.i.) for dicofol in VK1 (a carrier solution containing methylene chloride and risella oil) against immature stages of the red spider mite Tetranychus urticae. He defined the biocidal area, which he used as a measure of effective pesticide coverage, as half the treated area divided by the LN_{50} which he defined as the LD_{50} expressed as the number of droplets per cm^2 - i.e.:

$$BA = \frac{0.5}{LN_{50}}$$

Biocidal area per a.i. increased with decreasing active ingredient per droplet. Thus the smaller the droplet the more efficient is the spray. Highest biocidal area per a.i. was obtained with lowest initial a.i. per deposit at the optimum dicofol concentration of 1%.

Fluorescent dyes and pigments like Fluorescent Yellow, Lumogen and Saturn Yellow have been used as tracers to observe

distribution and assess deposits on foliage and other targets (Dean et al., 1961; Pereira, 1967; Sharp, 1973; Staniland, 1959). Samples of the target, often leaves, are collected and sorted out in various arbitrary categories depending on the amount of cover achieved. Courshee & Ireson (1961) combined subjective assessment of a large number of samples from the field with the chemical measurement of deposits on small sub-samples taken from each class of spray deposit in the subjective assessment. This technique was used to assess spray deposits on cotton (Matthews & Johnstone, 1968) and was further developed by Uk & Courshee (1982) so that deposit distribution probability in conjunction with laboratory dose mortality measurements could be used to predict the likely field effectiveness of sprays. Menzies & co-workers (1979, 1980) established a relationship between visual coverage ratings of spray deposits using fluorescent dye and the mortality of first instar larvae of the Oriental fruit moth Grapholitha molesta with phosmet. Himel (1969b) described a fluorescent tracer particle method that enables detection of spray droplets by size and number on insects, foliage and other substrates.

Spray coverage has also been assessed by measuring the emission of visible light with a fluorimeter (Yates & Akesson, 1963; Stafford et al., 1970) and by autoradiography (Pickering, 1966; Philips et al., 1978). The scanning electron microscope (SEM) coupled with cathodoluminescence and X-ray analysis has been used to study distribution patterns of spray deposits and spread of pesticides on leaves (Hart, 1979; Hess et al., 1975; Leuthold et al., 1978; Ong et al., 1973).

3) LEAF-PESTICIDE INTERACTIONS

The biological activity of pesticidal sprays on foliage is a function of many factors relating to the physico-chemical interaction of toxicant formulation and plant material (Johnstone, 1973a). The effects of the nature of leaf surfaces and spray liquid characteristics on deposition, retention, wetting, spread and distribution of pesticide sprays applied to foliage have been studied and reviewed by many workers (Crafts & Foy, 1962; Fogg, 1948; Ebeling, 1939; Furmidge, 1959 and 1962; Hartley, 1966; Holloway, 1967, 1970; Martin & Juniper, 1970; Hess et al., 1974, 1981).

The wettability of leaves is influenced by the roughness of their surface and the hydrophobic properties of cuticular components and by the physical properties of spray liquid and droplets. Spray liquid factors involved are surface tension, viscosity, velocity and droplet size. Brunskill (1956) showed that spray retention on a surface that is difficult to wet could be improved by reducing the surface tension of the spray. In agricultural practice wettability of leaves is improved by lowering the contact angle of aqueous sprays by the addition of surface-active agents. The low surface tension of oils reduces the bouncing of drops from leaves and, after impaction, their low contact angles enable the droplets to spread thereby increasing the cover of the target. Boize et al. (1976) studied the effect of leaf surface roughness on the retention and spread of oil droplets. They recognized three types of roughness and concluded that the influence of each type of roughness on droplet spread would depend on droplet size. In

general, the spread of droplets of more than 20 μm is governed by microscopic roughness which is determined by leaf hairs and protruding or recessed veins underlying the cuticle, and microscopic roughness determined by epidermal cell size and arrangement, since they contain sufficient volume to be conducted along the relatively wide grooves between epidermal cells. The spread of droplets of less than 20 μm is more likely to be limited to the epicuticular grooves;where the size, shape and organisation of wax constitute an ultra-microscopic roughness.

Baker et al. (1983) studied the effects of cuticular properties on the impaction and redistribution of aqueous and oil solutions, emulsions and wettable powders. The wide variation in spread factors, noted between formulation and between different leaf species influenced spray performance significantly as regards the coverage of the spray, its contact with plant surface and hence the residue concentration gradients within the cuticle. Oil formulations spread rapidly through the layers of crystalline wax whereas aqueous sprays distributed most readily over smooth surfaces. Munthali (1981) found that an oil-based formulation of dicofol was more efficient against red spider mite eggs on French bean than on the more waxy pepper leaf surface.

Phillips et al. (1978) studied the translocation of C^{14} dieldrin from droplets applied to the upper surface of cotton leaves. They found that one fifth of the applied dose spread more than 8 mm across the leaf surface within a day, while penetration to the lower surface took place in four days. Movement from emulsion deposits was slightly greater than from those of

suspensions. Plaut (1964) drew evidence that the non-systemic acaricide dicofol can penetrate and move in leaves after application to the leaf surface.

Hess et al. (1981) demonstrated that different formulations of a single herbicide could result in different distribution patterns on the leaf. Sodium and amine salts of MCPA sprayed on sugar beet (Beta vulgaris L.) leaves appeared as discrete deposits above the anticlinal cell walls that represented the location of spray drops that adhered to the leaf, while iso-octyl esters coalesced into numerous small thick deposits. One commercial emulsifiable concentrate formulation of propanil applied to sugar beet leaves yielded many small smooth non-crystalline deposits, another gave partially crystalline while a third resulted in crystalline deposits. Field observations indicated that the formulations followed the same order in efficiency in controlling barnyardgrass (Echinchola crus-galli (L.) Beauv.), the first being the most efficient. Malathion applied in 100 µm droplets penetrated the cuticle faster and less was washed off by rain compared with deposits from 600 µm droplets (Polles & Vinson, 1969).

4) SPRAY FACTORS AND BIOLOGICAL RESPONSE

Field and laboratory studies with foliar applied pesticides have shown the importance of spray factors in affecting their biological efficiency and indicated the complex interaction between these factors (Frick, 1970; Hislop & Baines, 1980; Mckinlay, 1974; Merritt, 1980; Munthali, 1981; Palmer, Wyatt & Scopes, 1983; Phillips et al., 1980; Scopes, 1981). Methods have been

improved for measuring and quantifying the physical features of sprays by the application of significant developments in instrumentation and data handling technology. Progress has been made in separating the various factors and in examining the individual contribution of each to the overall result (Nation, 1982).

The behaviour, distribution and deposition of spray droplets is considerably affected by their size. Numerous techniques have been developed to sample and measure droplet size (Matthews, 1975, 1979). Himel (1969a) referred to the optimum droplet size as that which gave maximum control using a minimum amount of insecticide and minimum ecosystem contamination. If droplets are much larger than the optimum the amount of chemical wasted rapidly increases as a cube function of droplet diameter. Optimum droplet size will vary with different targets, but in general droplets less than 100 µm are most effectively collected by insects and foliage (Himel, 1969a; Joyce, 1975). Flying insects such as mosquitoes collect 10-20 µm vmd droplets (David, 1946; Mount & Pierce, 1972) while resting tsetse flies collect 30-40 µm droplets (Hadaway & Barlow, 1965). In the past, small droplets (< 50 um diameter) were difficult to detect, so their role was not fully understood (Himel, 1969). However, droplets collected on the underside of leaves and in the concealed habitats of bollweevils and bollworms were less than 40 µm so this author concluded that the optimum size for insecticide droplets was 20 µm. Mboob (1975) showed that 20 µm vmd droplets of resmethrin were more effective against the greenhouse whitefly than those of 78 µm on tomatoes. Owen & Bennett (1978a, 1978b) confirmed that the optimum droplet size for

whitefly control on poinsettia is in the range of 15-25 μm mmd. Reay & Ford (1977) obtained greater mortality of Spodoptera littoralis with permethrin on cabbage using 83 μm compared with 123 and 164 μm droplets applying the same mass application rate and same droplet density. Munthali (1981) found that the relationship between LD_{50} and droplet diameter for red spider mite eggs using dicofol was positive and curvilinear: at any one concentration LD_{50} was directly proportional to the square root of active ingredient per droplet. Thus the smaller the droplet, the more efficient is the spray. Penetration of herbicide sprays into the canopy of large plants may require small droplets. However, large droplets (250-300 μm) are often used with herbicides to avoid drift which damages susceptible crops growing nearby. A survey of the Ministry of Agriculture, Fisheries & Food in the year 1980/81 (quoted by Elliott & Wilson, 1983) reported 101 incidents of damage due to drift of herbicides, covering a wide range of agricultural and horticultural crops in England. The smallest droplet size used with barban applied to Avena fatua L., namely 110 μm was the most effective (Lake & Taylor, 1974), but no detailed examination of herbicides with droplets smaller than 100 μm has been considered.

The required droplet density will vary with the mobility of the pest, the characteristics of active ingredient and formulation, and redistribution of active ingredient over the target. Johnstone (1973b) postulated that as many as 100 droplets/cm^2 may be required to give a high probability of contacting any sessile scale insects, whereas 5 droplets may be more than sufficient if a caterpillar is moving quickly over a considerable area of leaf or stem. Munthali & Scopes (1982) demonstrated that the

proportion of Tetranychus urticae eggs hit by droplets of dicofol landing on the leaf was less than 10% even when up to about 150 droplets/cm^2 were applied. They concluded that the activity of ULV sprays against sedentary targets would rely on the spread of pesticide from initial deposits. Some pests like stem and fruit borers can enter plant tissue within a few minutes after hatching, so adequate droplet density is necessary to ensure that the pest accumulates a lethal dose within such a short exposure period. Fisher & Menzies (1976) suggested a minimum of 35 droplets/cm^2 (0.175 $\mu g/cm^2$) of carbaryl for controlling the larvae of the Oriental fruit moth Grapholitha molesta on peach. Johnstone (1973b) worked out the volume of spray containing uniform sized droplets required to achieve different droplet densities. As little as 30 ml/ha has been applied in tsetse fly control with 30 µm vmd droplets (Lee et al., 1975), whereas 6-20 litres/ha have been needed for herbicide sprays with 250 µm (Taylor & Merritt, 1975). Droplet density is not always improved by an increase in spray volume unless the distribution and deposition of droplets is improved. Thus, with hand-held spinning disc sprayers doubling the volume of spray applied as 80-100 µm vmd droplets gave no improvement in control of Heliothis armigera (Hb.), or yield of seed cotton; presumably because the droplets were deposited more or less in the same area of foliage (Matthews, 1973). Two and a half times more 160 µm droplets of phosmet were required to achieve the same level of control of Laspeyresia pomonella compared with 350 µm droplets, but with one fifth of the deposit (Fisher & Menzies, 1979). Phillips, Bradford & Harris (1980)

reported that the best weed control in field trials was achieved with treatments giving more droplets per unit area, either by reducing droplet size from 265 to 135 μm or increasing the volume application rate from 15 to 60 litres/ha.

The optimum concentration of active ingredient in the spray formulation will vary with the susceptibility of the pest to the toxicant, depending partly on the intrinsic toxicity of the material and partly on the developmental stage of the pest. For example, the larval mass of Pieris brassicae caterpillar increases from about 0.25 to 250 mg between early first and late fifth instar, and as an appropriate insecticide will normally have about 10 μg/g insect body weight, the required toxic dose increases commensurately (Johnstone, 1973b). Scopes (1981) and Palmer, Wyatt & Scopes (1983) have shown that the required dose increased progressively with the developmental stage of the greenhouse whitefly, thus the LD_{50}/cm^2 of permethrin for third instar larvae was about 15 times that for early first larval instar. The importance of timely spraying to combat the early stages of the pest is thus emphasised. Johnstone (1973b) calculated the concentration of spray in one droplet of different diameters required to achieve an LD_{50} dose ranging from 1-1000 ng/insect. He reported that most insects will be killed by less than 100 ng of an appropriate insecticide so 5 to 50% concentration would be required for one 70 μm droplet deposited on target. In practice, more than one droplet would be collected, especially when smaller droplets are applied, so higher concentrations are not justified (Matthews, 1977a). Graham-Bryce (1977) illustrated theoretically

how wastage is reduced if a target is hit by more than one droplet at a lower concentration than a single droplet carrying a lethal dose.

On cotton a 10-fold increase in concentration of DDT applied in 80-100 µm droplets did not improve control of Heliothis armigera except when droplet density was adequate with narrow swaths, thus 5 litres/ha of 10% spray over a 0.9 m swath was more effective than the same dose applied at 2 litres/ha of 25% spray over 4.5 m swath (Matthews, 1973). Munthali (1981) using a series of dicofol concentrations ranging from 0.05 to 4% against mite eggs found that there is an optimum concentration of about 1%, below or above which efficiency of the spray decreased. Merritt (1982a) reported that increasing the concentration of the herbicide difenzoquat applied to Avena fatua could reduce activity as a result of localized scorch which limited herbicide mobility in the plant. However, for a given dose per plant, herbicide activity was unaffected by concentration of MCPA or paraquat, but was enhanced as the concentration of glyphosate was increased (Merritt, 1982b).

The subject of formulation and selection of solvents for ULV application has been considered by Barlow & Hadaway (1974), Coutts & Parish (1967), Furmidge (1968), Maas (1971, 1972) and Wrigley (1973). The traditional carrier, water, is technically the least suitable material for ULV sprays because of its high volatility and poor wetting ability. Aerial application of an involatile formulation of fenitrothion in butyl dioxtol at 1 litre/ha gave a threefold increase in the amount of chemical recovered in dead Panolis sp. larvae and 75% more collection by pine needles compared with the use of the same dosage as an emulsifiable concentrate

diluted in water and applied at 20 litres/ha (Joyce & Beaumont, 1979). Mineral and vegetable oils, molasses and other additives have been used to reduce the effect of evaporation when applying formulations diluted in water.

Hartley & Howes (1961) reduced evaporation of spray droplets by the addition of amine stearate formulations to technical material but the commercial development of this technique has been limited. Tunstall (1968) reported improved control of bollworms on cotton when molasses were added to carbaryl sprays. Molasses presumably served as a humectant and feeding stimulant. Wodageneh & Matthews (1981) demonstrated that the addition of oil could reduce change in size of water droplets resulting from evaporation. When water without oil was sprayed evaporation caused some of the droplets to disappear completely so that the number of droplets sampled at 2 metres away from the nozzle was reduced to only 56% of the number sampled at 10 cm. When oil was added to water the proportion of droplets sampled at 2 metres increased to 85-87% of the number sampled at 10 cm.

A wide range of organic solvents and mineral oils have been used or suggested as useful ULV carriers (Wrigley, 1973). Hadaway & Barlow (1958) studied the effect of varying the solvent upon the toxicity of DDT, prolan, dieldrin, endrin and malathion to mosquitoes (Aedes aegypti L.) and houseflies (Musca domestica L.). They found that all insecticides tested were more effective in paraffinic hydrocarbons than aliphatic long-chain esters and were least effective in aromatic esters. When the solutions were applied topically the differences in activity between solvents

were greatly reduced compared with the residual application on filter paper, showing that an important influence of the solvent had been upon the pick-up of the insecticide, while the differences obtained with topical application provided evidence that the solvent is also concerned with the passage of insecticide through the insect cuticle. Scopes (1981) demonstrated that formulation could dramatically affect pesticide efficiency. LD_{50} values for second instar greenhouse whitefly larvae using 10% permethrin formulated in five carriers varied from 0.41 $\mu g/cm^2$ to 3.18 $\mu g/cm^2$ using 50 μm diameter droplets. Richardson (1977) reported that 2,4-D formulated as a lipophilic ester will penetrate plant surfaces better than with the hydrophilic salt formulation thus resulting in better weed control.

5) NOTES ON TEST INSECT AND CHEMICALS USED IN THE PRESENT STUDY

i) The Greenhouse Whitefly Trialeurodes vaporariorum

The greenhouse whitefly, T. vaporariorum (Westwood), is a common and persistent pest in greenhouses and out of doors in warmer climates. A world list of 204 host plants of the greenhouse whitefly was published by Russell (1963). Crops subjected to severe attack in greenhouses are tomatoes, cucumber, French bean, aubergine and pepper, and a number of ornamentals such as poinsettia, fuchsia and Gerbera (Anon., 1981). Detailed studies of the biology of this whitefly were made by Hargreaves (1915) and Lloyd (1922). At 21°C each female lays about 200-250 eggs at a rate of about eight per day during a life span of 3 to 6 weeks (Anon., 1981). The incubation period is about 9 days. Eggs hatch into

scale-like larvae which move for 1-2 days but then settle down on the leaf till adult emergence. The insect passes through three larval instars and a fourth stage which corresponds to a pupa. Total developmental period is about 27 days. Besides a debilitating effect on the plant due to feeding of both adults and immature stages which suck plant sap, the principal injury caused by whiteflies is the excretion of sticky honeydew onto the foliage and fruit. When excessive, the honeydew encourages the growth of the sooty mould fungus (Cladosporium sphaerospermum Penz.) and, in extreme cases, fruits have to be cleaned before marketing. Hussey et al. (1958) reported that there was no loss in tomato yield until the density of whitefly scales reached 70 per 0.75 inch (1.9 cm) leaf disc. They concluded that the density should not exceed a mean of 20 scales per 0.75 inch leaf disc on the upper leaflets throughout the life of the plant.

Biological control of the greenhouse whitefly using the chalcid parasite, Encarsia formosa Gahan, is widely used in greenhouses (Scopes, 1969; Gould et al., 1975; Parr et al., 1976). But chemical control still plays a major role in controlling this pest. Penna et al. (1983) list 24 chemicals as effective against whitefly larvae and/or adults on tomatoes (resistant strains excepted). Resistance of whitefly to a range of organochlorine and organophosphorous insecticides has been reported by Wardlow et al. (1976).

ii) Permethrin

Technical grade permethrin is a yellow-brown to brown liquid, which sometimes tends to crystallise partly at room temperature. Vapour pressure is 261 mPa at 30°C. Chemical name is 3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. Chemical formula is $C_{21}H_{20}Cl_2O_3$. Oral LD_{50} values of permethrin depend on such factors as carrier, cis/trans ratio of the sample, the test species, its sex, age and degree of fasting. Values reported sometimes differ markedly. For example, typical oral LD_{50} value for a cis/trans ratio of 40:60 for rats is 430-4000 mg/kg. Formulations for agronomic use include emulsifiable concentrate (e.c.) (100-500 g a.i./litre), solution (50 g/litre) for fogging and ULV concentrate (50 or 100 g/litre) (Worthing & Walker, 1983).

Permethrin is a contact insecticide effective against a broad range of pests. The insecticidal properties of this synthetic pyrethroid were described by Elliott et al. (1973). Excepting the low level against pupae, permethrin gave excellent control of all stages of both greenhouse whitefly populations susceptible and resistant to DDT and malathion, although the kill of the latter was consistently better (Wardlow et al., 1976).

iii) Pirimiphos-methyl

Pirimiphos-methyl is a straw-coloured liquid. Vapour pressure is c. 13 mPa at 30°C. Chemical name is O-2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate. Chemical formula is $C_{11}H_{20}N_3O_3PS$. Acute oral LD_{50} for female rats is

2050 mg/kg. Formulations for agronomic use include e.c. (80, 250 or 500 g a.i./litre) and ULV concentrate (500 g/litre) (Worthing & Walker, 1983).

Pirimiphos-methyl is a fast acting insecticide and acaricide with both contact and fumigant action, able to penetrate the leaf to give translaminar action. Its range of activity includes many crop pests and is also used against pests of stored products, in public health, domestic and amenity use. Pirimiphos-methyl was found to be more effective in controlling the greenhouse whitefly compared with other organophosphates like malathion, dimethoate and methyl-parathion (Sharaf, 1978).

3. MATERIALS AND METHODS

1) INSECT CULTURING

Settled first instar whitefly larvae (T. vaporariorum Westwood) were produced on 7-8 week-old tobacco plants (Nicotiana tabacum) cv. Brazilian Blend, 5 week-old tomato plants (Lycopersicon esculentum) cv. Moneymaker and 5 week-old French bean plants (Phaseolus vulgaris) cv. The Prince (Scopes & Biggerstaff, 1971) in the following way. Potted plants were placed in a greenhouse containing tobacco plants heavily infested with adult whiteflies for 10-14 hours to allow the adults to lay eggs on the experimental plants. The stock plants were shaken 3-4 times to ensure even distribution on the introduced plants. The experimental plants were removed after shaking off most of the adult whiteflies, and were then fumigated with dichlorvos (2,2-dichlorovinyl dimethyl phosphate) slow-release strips for 12 hours to kill any remaining adults. Plants with eggs were then placed in a glasshouse (25-27^{o}C) to allow the eggs to hatch and the larvae to develop. Eggs hatched in 8-9 days and most of the crawlers (mobile first instar whitefly larvae) settled on the leaf within 48 hours of hatching.

2) SPRAYING SOLUTIONS

Oil-based formulations of permethrin and pirimiphos-methyl were used in all experiments. The spraying solutions were made up by dissolving the pesticide (technical material with a known amount of active ingredient, a.i.) in a carrier (formulating

liquid). U.v. fluorescent dyes were added to allow detection of droplets on leaves.

Solutions of permethrin* were made up by dissolving technical permethrin in VK1 (a carrier solution for fogging pesticides in greenhouses supplied by Timmer Ltd. and made of dichloromethane, 75%, and risella oil, 25%). The fluorescent tracers used were Calcofluor White RW (American Cyanamid Corporation) and Uvitex OB (Ciba Geigy Ltd.). A stock solution of fluorescent tracer was prepared by dissolving 1.5 g Calcofluor + 0.2 g Uvitex in acetone. The solution was filtered (Whatman filter paper) and the filtrate was made up to 5 ml and was then diluted to 20 ml with VK1. Solutions of different concentrations were made up as shown in Table 1.

In addition, ready-made formulations (JF 8130, JF 8133 and JF 9079) containing 0.5% Uvitex OB, kindly supplied by the Plant Protection Division of Imperial Chemical Industries PLC, were used. Table 2 shows the constituents of these formulations as disclosed by the Company.

All solutions were stored in sealed glass tubes at 1°C.

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* Cis/trans ratio 40:60

TABLE 1: Volumes of component solutions used to produce spraying solutions

Concentration of spraying solution (a.i. %)	Technical permethrin (90.7% a.i.) (ml)	Tracer stock solution (ml)	VK1 (ml)
20	4.40	6.0	9.60
10	2.20	6.0	11.80
5	1.10	6.0	12.90
2	0.44	6.0	13.56
1	0.22	6.0	13.78
0.5	0.11	6.0	13.89

TABLE 2: Description of formulations

Formulation	Description
VK1	Very volatile chlorinated solvent (dichloromethane) + very low volatile paraffin (risella oil)
JF 8130	Very low volatile chlorinated and paraffinic solvents
JF 8133	Volatile polar solvent + low volatile paraffin + very low volatile paraffin
JF 9079	Ketonic solvent + low volatile paraffin

3) SPRAYING EQUIPMENT

A twin fluid Micro-Tip nozzle (D in Figure 1) (Uk, 1978) was used to produce monosize droplets using a rapid flow of carbon dioxide (A) to shear a slow moving column of liquid from the tip of a fine stylus (H). The liquid was fed by gravity from a small reservoir (F) through a G30 needle (G) onto a fine central stainless steel stylus (H) in the orifice of the chamber. Monosize droplets could be produced within the range of 50-130 um by adjusting the flow of carbon dioxide between 150 to 400 cm^3/minute with a needle valve. Gas flow was measured by a simple rotameter (B). Smaller droplets were produced with greater gas flows but the range of droplet size could also be altered by raising and lowering the stylus within the orifice using the adjustor (I), raising the stylus tended to produce smaller droplets. The nozzle, when not in use, was kept in acetone to ensure that fine deposits did not accumulate in the needle causing erratic spray patterns.

This nozzle assembly has recently been modified by Coggins & Baker (1983) by the introduction of knurled thimbles giving controlled adjustment of needle and stylus settings and by the provision of an offset liquid reservoir (0.1 - 2 ml), but was not available for use in this study.

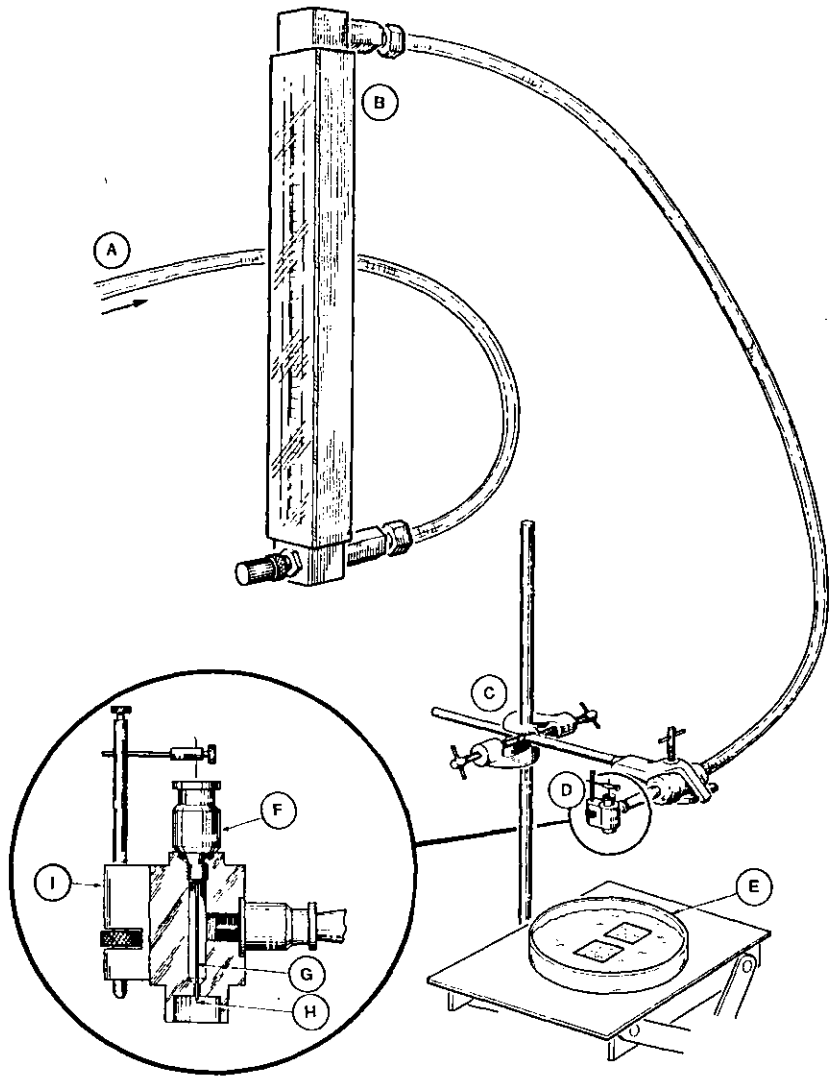


Figure 1.

Spraying equipment.

A. CO_2 from gas cylinder
B. Rotameter
C. Retort stand
D. Micro-tip nozzle
E. Petri-dish with 2 infested leaf pieces
F. Pesticide reservoir
G. G30 needle
H. Fine stainless steel stylus
I. Stylus adjustor

4) PREPARING INSECTS FOR SPRAYING

Square leaf pieces (4 cm^2) without main veins were cut from infested leaf pieces when most of the crawlers (mobile first instar whitefly larvae) had settled. Leaf pieces were placed inverted on wet cotton wool in 9 cm polystyrene Petri dishes. Any remaining mobile larvae were carefully removed from the leaf pieces using the tip of a needle. Since it usually took over eight hours to complete this process, all leaf pieces were finally checked within an hour before spraying and any first instar larvae which were close to moulting were removed. Thus all test insects were of very uniform age and development.

5) BIOASSAY TECHNIQUE

i) Dosing Procedure

A known number of droplets (12 droplets/2 cm) was sprayed along the middle of each of ten infested leaf pieces for each treatment. Five leaf pieces were sprayed with the blank formulation (carrier solution) alone as control. Samples of droplets were collected on magnesium oxide coated slides (May, 1950) immediately before treating the first piece and after each experimental spraying. Droplet size was determined by measuring 50 droplets on each of the two slides under a microscope (at 200X magnification) using an eye-piece graticule. Variation in droplet size was small ($< \pm$ 2 µm), so the sprays were assumed to contain only monosize droplets. Figure 2 shows the frequency distribution of droplet size of one treatment.

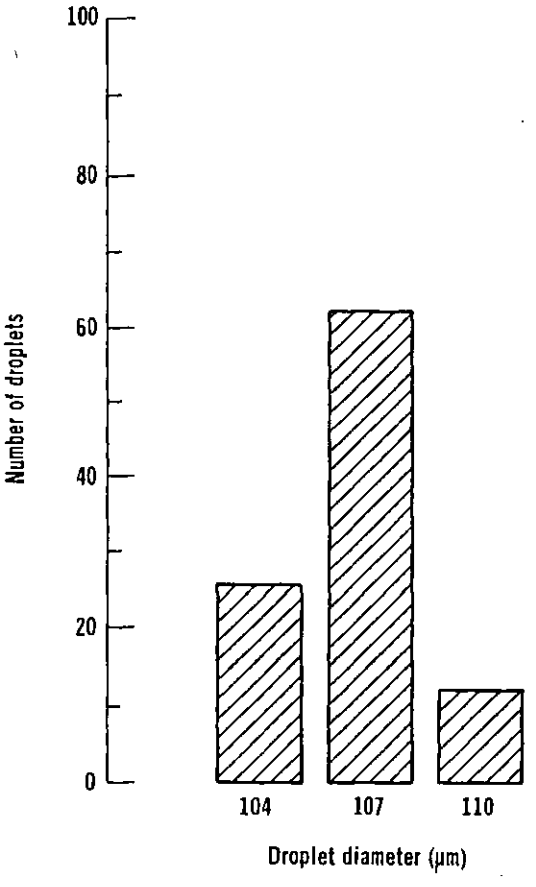


Figure 2.
Frequency distribution of droplet size of one treatment of 10% permethrin in VK1.

ii) Measuring Distances of Larvae from Droplets

In the method initially developed, the distance of each larva from the nearest spray droplet was measured immediately after spraying using an eye-piece graticule in a dissecting stereomicroscope under u.v. and subdued white light. Measurements were made from the centre of the droplet to the nearest edge of the larva. Maps of leaf pieces showing the positions of larvae in relation to droplets were made to be used for recording mortality four days later. This method proved to be very tedious and time consuming and allowed only a limited number of leaf pieces to be treated, with a long time lag between recording the first and last pieces, during which some spread of the droplets might have taken place as suggested by the fluorescent tracer.

A photographic technique was accordingly developed. The leaf pieces were photographed immediately after spraying under u.v. and subdued white light using a photomacrographic zoom system (Tessovar, Carl Zeiss) (A in Plate 1) and 35 mm camera attachment (B). A yellow gelatin filter (Wratten 1A) was placed above the nosepiece (E) of the zoom system to absorb any excess u.v. light that might fog the film, and a second yellow filter (Wratten 12) (F) was placed in front of the white light source (C) to cut down the amount of light and improve contrast. The white light source was angled at about 25° to the horizontal to give depth and shadow to the insects. Each treated leaf piece was photographed at an exposure period of 30 seconds using a medium speed film. The negatives were printed on resin-coated paper, having the advantage of staying stable in size through processing, to give a 10X magnification of the leaf pieces.

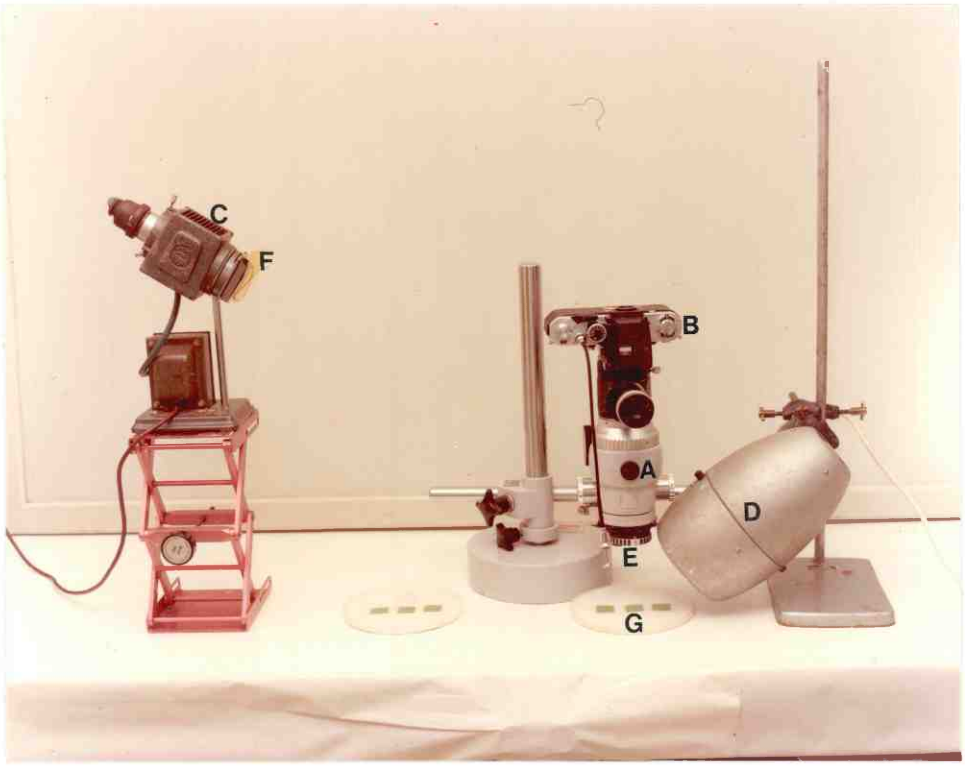


PLATE 1: Apparatus used for photographing leaf pieces:

A. Photographic zoom system
B. 35 mm camera attachment
C. White light source
D. U.v. lamp
E. Nosepiece
F. Filter (Wratten 12)
G. Petri dish with 3 treated leaf pieces

iii) Assessing the Mortality of Whitefly Larvae

Treated leaf pieces were kept on wet cotton wool in 14 cm polystyrene Petri dishes. Ten holes (1.5 cm diameter) were drilled in each Petri dish lid to allow for ventilation. The dishes were placed inverted on rack shelves at 22 ± 3°C. Mortality was recorded four days after spraying. Larvae which failed to moult were considered dead and were marked on the prints (Plate 2). Death was recorded in relation to distance from droplets on the leaf pieces.

6) STUDY OF THE DISTRIBUTION OF ACTIVE INGREDIENT AROUND SINGLE DROPLETS OF PERMETHRIN ON TOBACCO LEAVES

The distribution of active ingredient around single droplets of permethrin on tobacco leaves was studied using X-ray micro-analysis by the detection of the specific X-ray photons from the chlorine atoms present in the insecticide (Falk et al., 1975). This was carried out at Rothamsted Experimental Station.

Monosize droplets of 10% permethrin in VK1 were sprayed on the under surfaces of tobacco leaves. Leaf pieces (1 x 1 cm) were cut from treated leaves 48 hours after spraying and affixed to aluminium specimen stubs with colloidal silver adhesive. The specimens were then examined with a scanning electron microscope (SEM) (Cambridge Stereoscan 250) equipped to detect X-ray emissions. Detected X-rays were processed by a computer-based energy dispersive analyser. An element map was generated during the scan in which the X-ray analyser modulated the SEM cathode ray tube at specimen locations corresponding to the locations of detected chlorine in the insecticide.

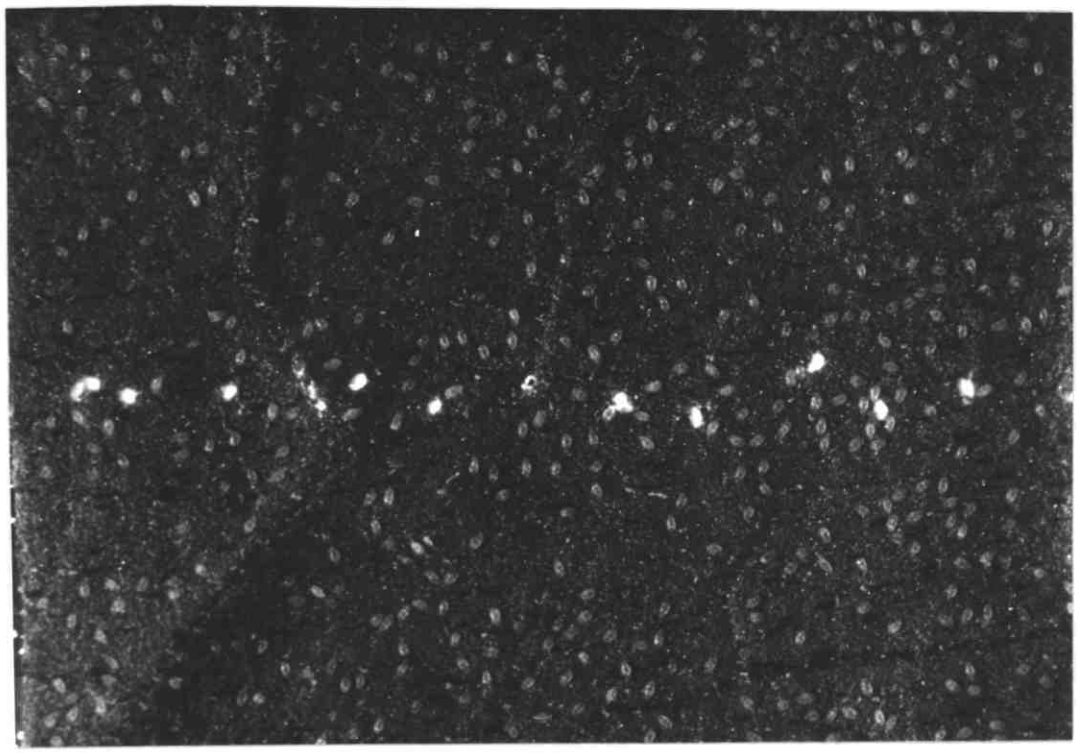


PLATE 2: Tobacco leaf piece with settled first instar whitefly larvae (A) and line of spray droplets (B).
(C) Dead larvae (recorded 4 days after spraying).

(X 7)

7) DIPPING TESTS OF WHITEFLY LARVAE

Solutions of permethrin in 1% acetone were made up by diluting a stock solution of permethrin in acetone with distilled water containing 0.2% manoxol (wetting agent). A stock solution (A) of 0.2% permethrin in acetone was made up by dissolving 2.2 ml technical permethrin (90.7% a.i.) into 90 ml acetone, and diluting 10 ml of the resultant solution with 90 ml acetone. A stock solution (B) of 1% permethrin in acetone was made up by dissolving 50 ml of solution (A) into 50 ml acetone. A series of concentrations (20, 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16 ppm) of permethrin in 1% acetone were prepared by diluting stock solution (A) or (B) with distilled water, or distilled water and acetone (Appendix Table 1).

Ten tobacco leaf discs (1 cm^2) infested with settled first instar whitefly larvae were each immersed for 1 second in the dipping solution for each treatment. Fifteen leaf discs were similarly dipped in 1% acetone as a control. Ten discs were also kept as undipped controls. All leaf discs were kept as in previous tests. Mortality was assessed four days later.

8) STUDY OF THE PHYSICAL FORM AND SPREAD OF DROPLETS OF PERMETHRIN ON LEAVES USING A SCANNING ELECTRON MICROSCOPE

A method described by Atkey, Abdalla & Pegler (1983) was used to study the physical form and spread of droplets on leaf surfaces. Effects of formulation and plant species on the physical form and spread of droplets were examined.

Monosize droplets of 10% permethrin formulated in JF 8130, VK1

and JF 8133 were sprayed on the under surfaces of tobacco leaves. Droplets of 10% permethrin were similarly sprayed on leaves of tomato and French bean. Leaf pieces (1 x 1 cm) were cut from treated leaves and glued to SEM specimen stubs with a small amount of fast drying electroconductive silver paint and inserted into a transfer stage of a sputter-cryo device. The pressure in the transfer stage was reduced to ca. 0.2 mm Hg to remove free water vapour and the specimen was rapidly transferred, under vacuum, to a shielded freezing stage at -150°C where it was held for 2 minutes to freeze while the pressure was further reduced to 0.05 mm Hg. The pressure was raised to 0.1 mm Hg with cooled dry argon, and a coating of gold was sputtered onto the specimen which was then transferred, frozen and under vacuum, to a freezing stage on a Jeol T20 SEM for examination. Leaf pieces were cut from treated leaves and immediately examined at 0.5, 24, 48 and 96 hour intervals from spraying for each treatment. Five micrographs (at X24 magnification) were taken for each treatment and recorded on films at a frame speed of 60 seconds.

It was attempted to use a Quantimet image analyser to measure areas and perimeters of droplets on 4 x 6 cm prints of the micrographs. This method did not prove successful because there was not enough contrast between the images of droplets, and epidermal cells and leaf structures. The magnification on the screen of the device was not high enough to enable accurate tracing of the outlines of droplets from the prints of micrographs taken at the convenient microscope magnification of X24. A paper tracing method was finally used. A slide was prepared of each micrograph and was projected at constant magnification on a paper sheet using a slide

projector. The outlines of droplet images were traced on the paper sheets. Perimeters of droplet images were measured with a map measurer. The mean perimeter of droplet on the leaf was calculated by measuring samples of more than 20 droplets for each treatment. Areas of droplets were determined by cutting paper tracings of droplet images, weighing and converting into areas from the known weight per unit area of the paper sheets used. The mean area of droplet on the leaf was calculated by measuring samples of more than 20 droplets for each treatment. The theoretical mean radius of droplet assuming the droplets were circular was derived from the mean area of droplet and was used to calculate the mean theoretical perimeter of droplet for each treatment. The mean maximum length of droplet (a) and the mean maximum width at right angles to this (b) were measured. These two dimensions were used to calculate the mean area ($ab\,\pi/4$) of the enclosing ellipse and of its perimeter ($\pi\sqrt{\frac{a^2 + b^2}{2}}$).

9) COMPARISON BETWEEN THE EFFECTS OF DROPLETS OF PERMETHRIN AND PIRIMIPHOS-METHYL

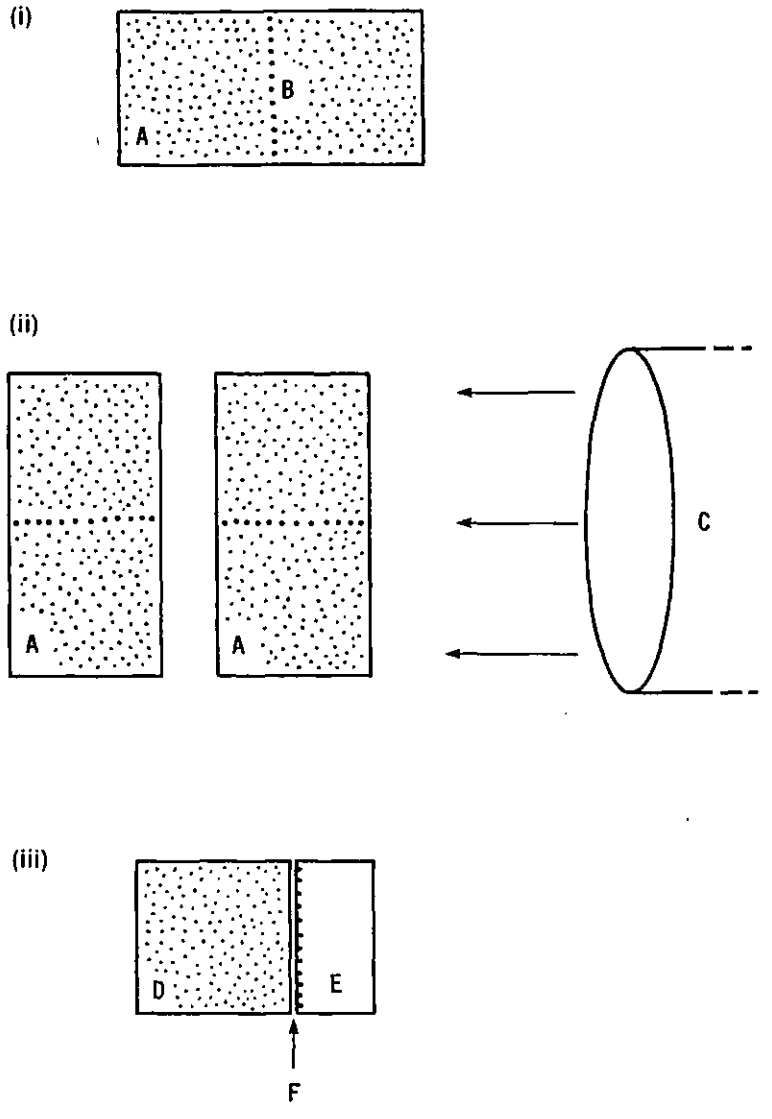
A line of droplets (12 droplets/2 cm) of 10% permethrin formulated in JF 9079 was sprayed across the middle of each of ten tobacco leaf pieces (2 x 2 cm) infested with settled first instar whitefly larvae. Pirimiphos-methyl was found to produce a considerable kill even at the margin of a 2 x 2 cm leaf, so larger pieces were used. Therefore six leaf pieces (4 x 2 cm) were similarly treated with 10% pirimiphos-methyl in the same formulation giving ca. 2 cm of leaf on either side of the droplet

line. Six leaf pieces were treated with the blank formulation as control. All treated leaf pieces were kept as in previous tests and mortality was assessed four days after spraying.

10) ASSESSING FUMIGANT AND CONTACT EFFECTS OF PIRIMIPHOS-METHYL

Simple bioassay techniques were devised to assess the relative importance of fumigant and contact effects of pirimiphos-methyl on its activity. Twelve tobacco leaf pieces (4 x 2 cm) infested with settled first instar whitefly larvae were each treated with a single line (12 droplets/2 cm) of droplets of 10% pirimiphos-methyl in JF 9079 (Figures 3i and ii). Six of the treated leaf pieces were kept as in previous tests. A continuous air stream (5 litres per second) was generated over the remaining six treated leaf pieces, using a centrifugal fan (Figure 3ii), to prevent build-up of vapour at the leaf surface. The direction of the air stream was along the line of droplets, thus preventing an asymmetrical drift.

A single line of droplets was similarly sprayed along the edge of each of twelve 2 cm long tobacco leaf strips, each of which was placed alongside an infested 2 x 2 cm leaf piece with a gap of about 0.2 mm (Figure 3 iii) to eliminate contact and assess the effect due to vapour. These and six infested leaf pieces treated with the blank formulation alone as control were kept like the test leaves of the first treatment. Mortality was assessed four days after spraying.



A Infested tobacco leaf piece (4 x 2cm)
B Line of droplets
C Centrifugal fan
D Infested tobacco leaf piece (2 x 2cm)
E Tobacco leaf strip
F Gap (*c.* 0.2mm)

Figure 3.
Schematic diagrams of pirimiphos-methyl experiment.

4. RESULTS AND DISCUSSION

4a. RESULTS

1) DEFINITION OF TERMS

Ld_{50} - Median lethal distance is defined as the distance from the centre of an isolated droplet at which 50% of the target population is killed.

LS_{50} - Median lethal spacing is defined as the theoretical distance between uniformly spaced droplets which would produce 50% mortality of the total target population.

Area of Influence -

The hexagonal area around each droplet when uniformly spaced droplets cause 50% mortality of the target population. It is the area of a hexagon with a least diameter equal to the LS_{50}.

LN_{50} - Median lethal number of droplets is defined as the number of uniformly spaced droplets per unit area of leaf which will produce 50% mortality.

LD_{50} - Median lethal dose is defined as the amount of active ingredient per unit area that give 50% mortality of the target population.

2) DISTRIBUTION OF MORTALITY OF WHITEFLY LARVAE AROUND DROPLETS OF PERMETHRIN

Results of mortality of whitefly larvae in relation to distance from droplets for the different treatments were recorded as in the example data sheets (Appendix 2, Table 3).

Response curves of mortality against distance from droplets were fitted using a logit:log log transformation expressed by the equation:

$$\text{Logit } p = a + b \log_{10} \log_{10} (100\ d)$$

where p is corrected mortality expressed as a proportion and d is the distance of the nearest edge of a larva from the centre of the nearest droplet in mm. This is explained in detail in Section 4b.

Comparisons of the effects of concentration, droplet size, formulating liquid and plant species on the distribution of mortality around droplets of permethrin were made.

i) Effect of Concentration of active ingredient

The effects of three concentrations (20, 10 and 5%) on the distribution of mortality of settled first instar whitefly larvae around 107 to 117 µm diameter droplets of permethrin in VK1 on on tobacco leaves were first studied (Figure 4, Appendix Table 3). The logit:log log regression lines gave slopes of -15.54, -13.87 and -17.45; and intercepts of 3.45, 2.79 and 3.45 for 20, 10 and 5% concentrations respectively. There were no significant differences between the slopes and intercepts of the three treatments (Table 4a). Calculated median lethal distance (Ld_{50}) values were 0.466, 0.388 and 0.327 mm, the value for 20% differing

TABLE 3: Example data sheet showing numbers, observed and corrected mortalities of whitefly larvae treated with droplets of 5% permethrin in VK1

Substance: 5% permethrin in VK1 Plant: Tobacco

Droplet size: 117 ± 2 µm Data sheet:

Target: Settled 1st instar whitefly larvae Date:

Distance from centre of droplet	No. of insects	No. of insects dead	Observed mortality (%)		*Corrected mortality (%)
0.0–0.1 mm	-	-	-		-
0.1–0.2	12	12	100		100
0.2–0.3	36	28	77.78		77.42
0.3–0.4	67	31	46.27		45.41
0.4–0.5	66	11	16.67		15.33
0.5–0.6	77	5	6.49		4.99
0.6–0.7	63	6	9.52		8.07
0.7–0.8	84	4	4.76		3.23
0.8–0.9	86	6	6.98		5.49
0.9–1.0	74	2	2.70		1.14
1.0–1.1	82	3	3.66	2.44	0.87
1.1–1.2	82	1	1.22		
1.2–1.3	94	3	3.19		
1.3–1.4	60	0	0		0.21
1.4–1.5	72	1	1.39	1.79	
1.5–1.6	59	0	0		
1.6–1.7	51	2	3.92		
1.7–1.8	77	0	0		
1.8–1.9	66	3	4.56		
1.9–2.0	56	0	0		
2.0–2.1	65	1	1.54		
2.1–2.2	70	0	0		
2.2–2.3	72	2	2.78	1.67	0.09
2.3–2.4	63	1	1.59		
2.4–2.5	56	2	3.57		
2.5–2.6	55	0	0		
2.6–2.7	60	0	0		
2.7–2.8	53	1	1.89		
2.8–2.9	49	3	6.12		
2.9–3.0	37	0	0		

* See Section 4b.2)

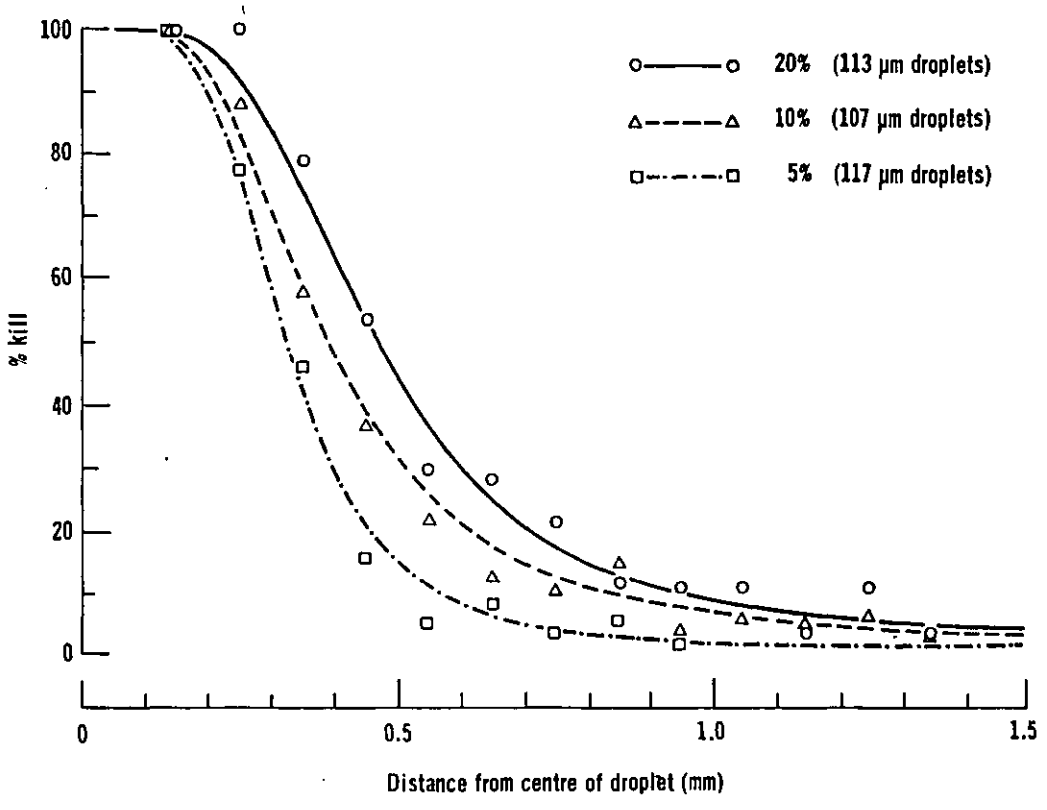


Figure 4.
Effect of concentration on the distribution of mortality of settled first instar whitefly larvae around droplets of permethrin in VK1 on tobacco leaves. (3 - treatment experiment).

significantly ($P<0.001$) from that for 5%.

A second experiment using a wider range of concentrations (20, 10, 5, 2, 1 and 0.5%) with droplets of permethrin in VK1 of 101 to 110 µm diameters was conducted to extend the findings (Figure 5, Appendix Table 4). The slopes were -12.36, -14.27, -18.78, -17.06, -14.34 and -13.58. The intercepts were 2.63, 2.89, 3.7, 3.25, 2.83 and 2.51. Ld_{50} values obtained were 0.430, 0.393, 0.377, 0.355, 0.376 and 0.339 mm respectively. Only Ld_{50} values for 2% ($P<0.01$), 1% ($P<0.05$) and 0.5% ($P<0.01$) differed significantly from that for 20% (Table 4a).

ii) Effect of Droplet Size

It was not possible to produce uniformly sized droplets below 50 µm with the nozzles available. Therefore, a comparison between two droplet sizes only was made. These were 59 and 114 µm diameter droplets of 10% permethrin in JF 8133 sprayed on tobacco leaves against settled first instar whitefly larvae (Figure 6, Appendix Table 5). The logit:log log regression lines had slopes of -14.69 and -18.93; intercepts of 2.61 and 3.97 for 59 µm and 114 µm droplets respectively. Calculated median lethal distance (Ld_{50}) value for 59 µm droplet (0.320 mm) differed significantly ($P<0.001$) from that for 114 µm droplet (0.417 mm) (Table 4b).

Due to the ease of control in placing droplets at low gas flow rates, which tended to give large droplets, sizes of about 100 µm were used in all other experiments.

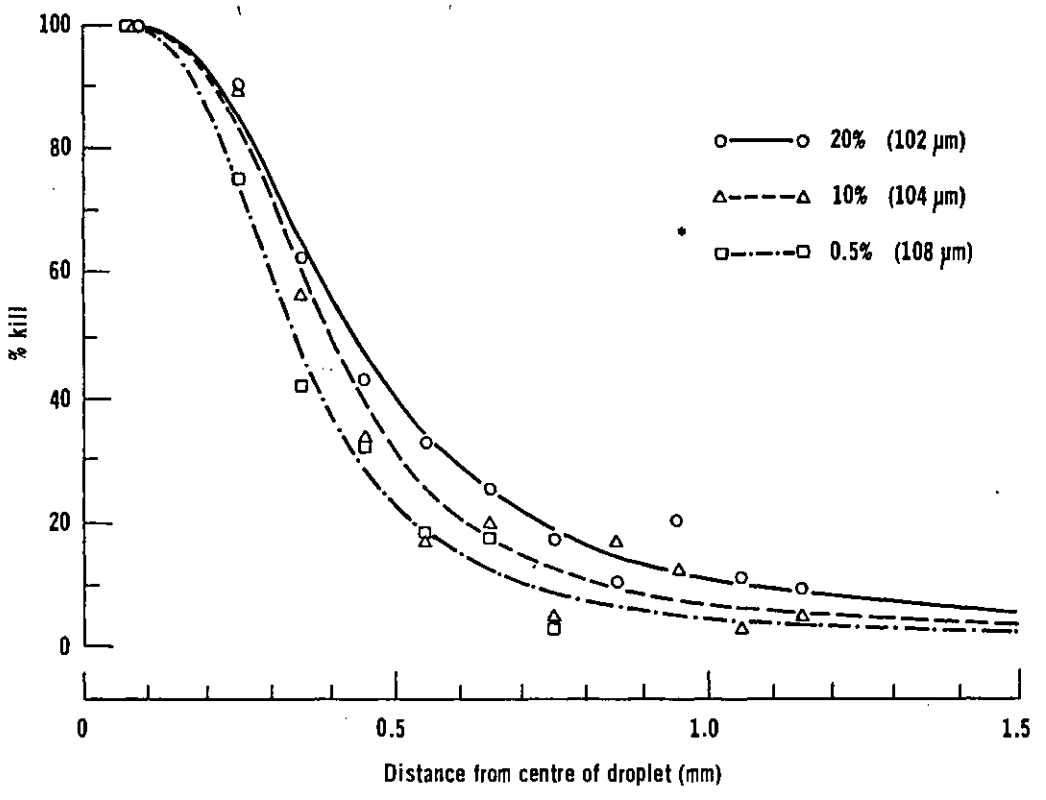


Figure 5.

Effect of concentration on the distribution of mortality of settled first instar whitefly larvae around droplets of permethrin in VK1 on tobacco leaves. (6 - treatment experiment).

* Lines of 5, 2 and 1% concentrations are omitted for clarity.

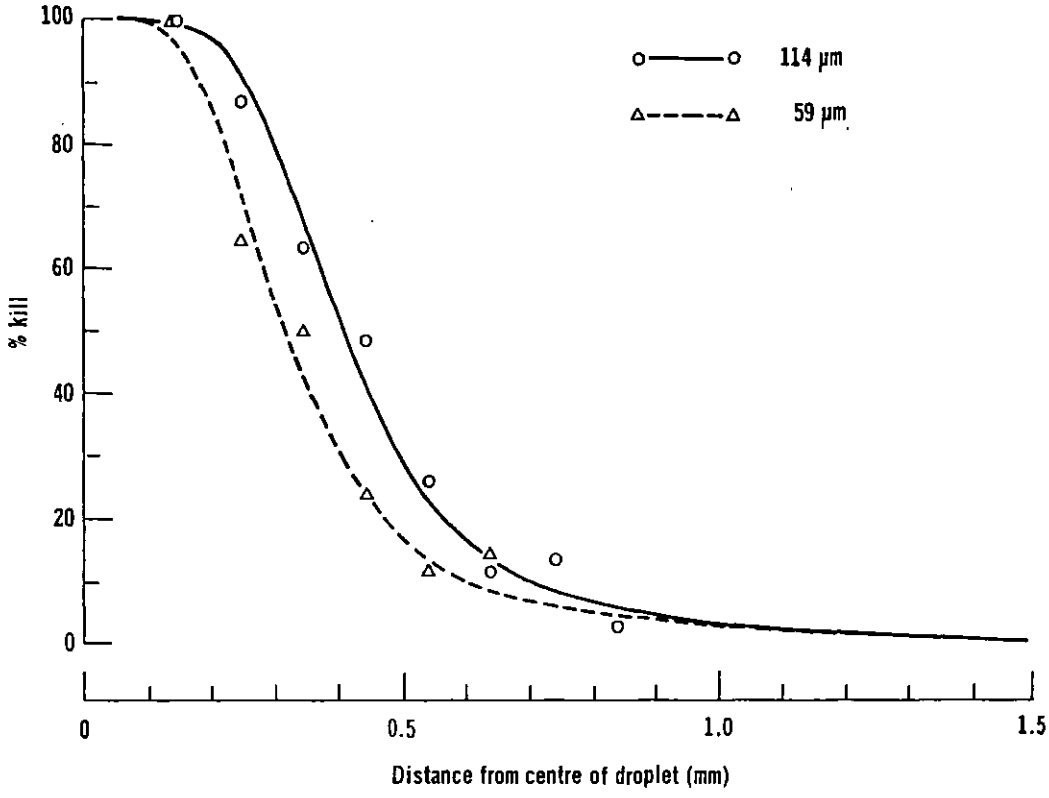


Figure 6.
Effect of droplet size on the distribution of mortality of settled first instar whitefly larvae around droplets of 10% permethrin in JF 8133 on tobacao leaves.

iii) Effect of Formulation

Comparisons were made between the effects of three formulating liquids (JF 8130, VK1 and JF 8133 described in Table 2) on the distribution of mortality of settled first instar whitefly larvae around 108 to 113 µm diameter droplets of 10% permethrin. The slopes were -14.80, -19.40 and -11.49 for droplets formulated in VK1, JF 8133 and JF 8130. The slope for JF 8130 was significantly different ($P<0.05$) from that for JF 8133. The intercepts were 3.14, 4.22 and 1.90 respectively. There were no significant differences between intercepts for VK1 and JF 8133, but the intercept for JF 8130 was significantly different from that for VK1 ($P<0.05$) and JF 8133 ($P<0.001$). Calculated Ld_{50} values were 0.427, 0.447 and 0.290 mm respectively; showing no significant differences between formulations VK1 and JF 8133, but the value for JF 8130 was significantly different from both formulation VK1 and JF 8133 ($P<0.001$) (Table 4c).

iv) Effect of Plant Species

The effects of three species of plant (tobacco, tomato and French bean) on the distribution of mortality of settled first instar whitefly larvae around 106 to 110 µm droplets of 10% permethrin in VK1 were compared (Figure 8, Appendix 7). The slopes were -14.80, -12.09 and -13.13 on tobacco, tomato and French bean respectively. The intercepts were 3.14, 2.88 and 3.32. There were no significant differences between the slopes and intercepts on the three plant species. Calculated Ld_{50} values were 0.426, 0.537 and 0.618 mm respectively. Ld_{50}

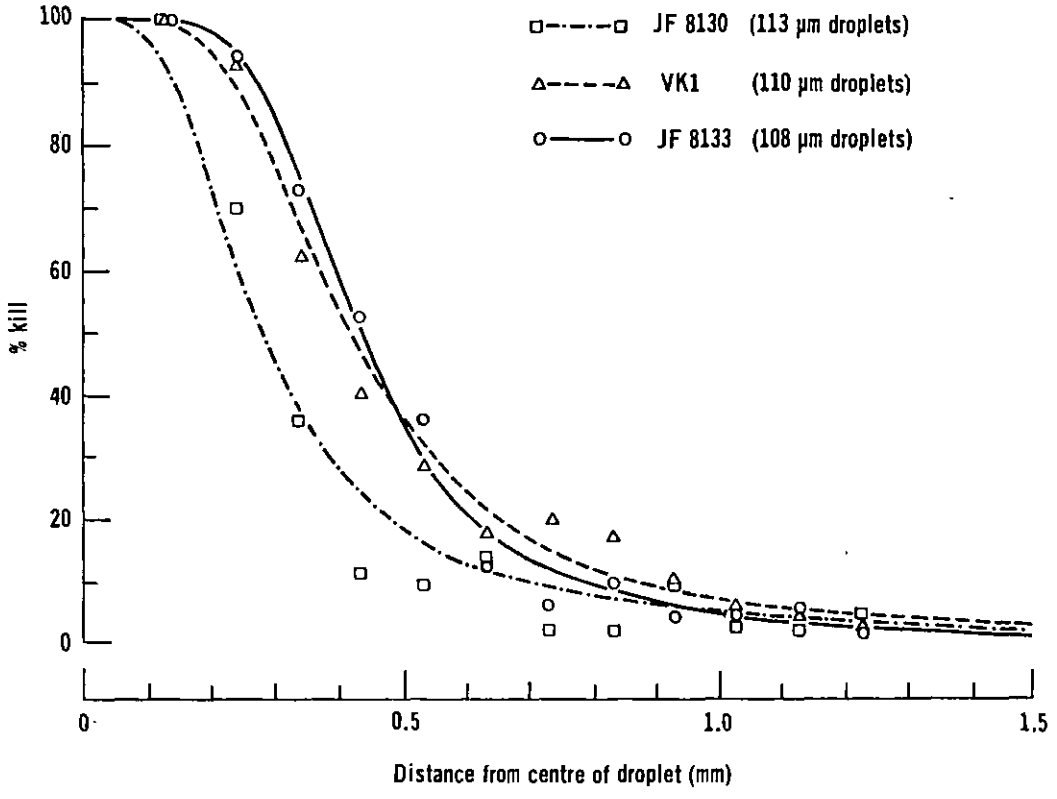


Figure 7.
Effect of formulation on the distribution of mortality of settled first instar whitefly larvae around droplets of 10% permethrin on tobacco leaves.

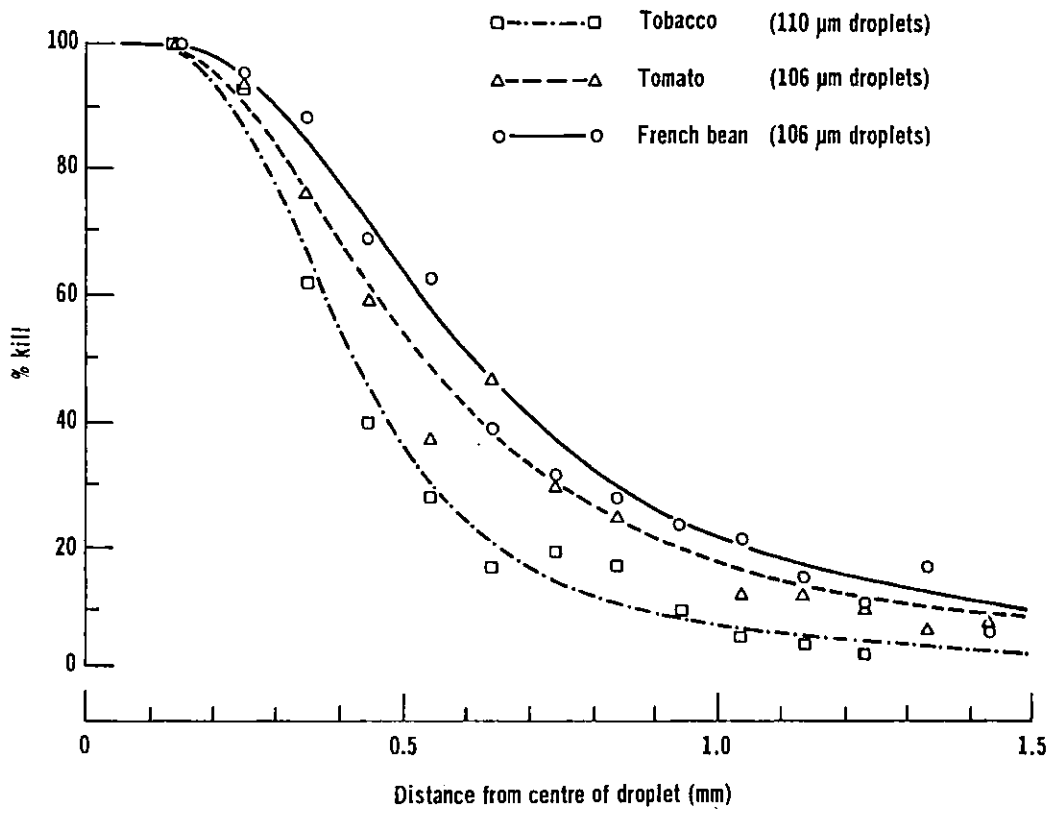


Figure 8.
Effect of plant species on the distribution of mortality of settled first instar whitefly larvae around droplets of 10% permethrin in VK 1.

TABLE 4: Tests of significance for intercepts, slopes and Ld_{50}s for treatments of all bioassay droplet experiments (d values)

a) Effect of concentration

1) First experiment

		10%	20%
5%	a	0.22	0.77
	b	1.17	0.47
	Ld_{50}	2.32*	4.35***
10%	a		1.16
	b		0.76
	Ld_{50}		2.32*

/Continued ...

TABLE 4 (Continued)

a) Effect of concentration

2) Second experiment

		10%	5%	2%	1%	0.5%
20%	a	0.14	1.30	0.89	0.46	0.03
	b	0.43	1.95*	2.08*	1.15	0.89
	Ld_{50}	1.02	1.59	2.97**	1.96*	2.79**
10%	a		0.91	0.50	0.24	0.10
	b		1.52	1.01	0.50	0.31
	Ld_{50}		0.25	1.27	0.71	1.46
5%	a			0.64	0.77	1.16
	b			0.39	0.75	0.96
	Ld_{50}			1.37	0.60	1.51
2%	a				0.27	1.03
	b				0.52	0.22
	Ld_{50}				1.01	0.69
1%	a					0.39
	b					0.22
	Ld_{50}					0.53

/Continued ...

TABLE 4 (Continued)

b) Effect of droplet size

	114 µm	
59 µm	a	1.52
	b	0.70
	Ld_{50}	3.54***

c) Effect of formulation

		VK1	8130
JF 8133	a	1.62	3.31***
	b	1.93	2.02*
	Ld_{50}	0.03	5.26***
VK1	a		2.07*
	b		0.49
	Ld_{50}		5.25***

/Continued ...

TABLE 4 (Continued)

d) Effect of plant species

		Tomato	Tobacco
French bean	a	1.77	0.96
	b	1.18	0.88
	Ld_{50}	3.12**	7.82***
Tomato	a		0.68
	b		1.78
	Ld_{50}		4.01***

e) Comparison between effects of permethrin and pirimiphos-methyl

		Pirimiphos-methyl (still air)	Pirimiphos-methyl (moving air)
Permethrin	a	11.93***	1.77
	b	3.80***	1.44
	Ld_{50}	37.95***	13.35***

/Continued ...

TABLE 4 (Continued)

f) Pirimiphos-methyl experiment

		Still air (separate strips)	Moving air
Still air (leaf pieces)	a	0.46	12.52***
	b	0.44	8.29***
	Ld_{50}	0.39	35.19***
Still air (separate strips)	a		14.14***
	b		8.97***
	Ld_{50}		35.71***

a Intercept

b Slope

Values with no asterisk are not significant

* Significantly different ($P<0.05$)

** Significantly different ($P<0.01$)

*** Significantly different ($P<0.001$)

value on tobacco differed significantly from that on tomato ($P<0.01$) and from that on French bean ($P<0.001$) (Table 4d).

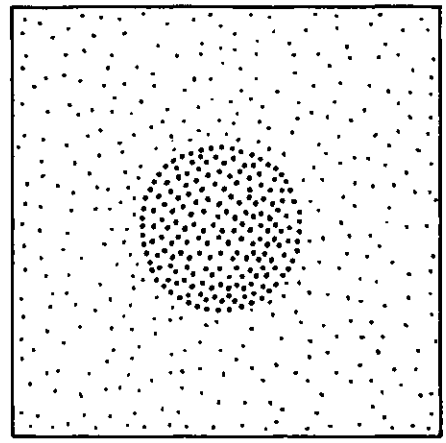
3) DISTRIBUTION OF PERMETHRIN (A.I.) AROUND DROPLETS ON TOBACCO LEAVES

X-ray microanalysis was attempted to quantify and study the distribution of active ingredient around individual droplets of permethrin on tobacco leaves. Distribution images of chlorine (Figure 9a) showed dense circles on a fairly uniform lighter background, indicating the positions of droplets and probably background chlorine in and on the leaf. Line scans (Figure 9b), revealed wide flat-topped curves falling very abruptly at both ends. It was concluded that the method was only able to detect chlorine in the droplet but was not sensitive enough to cope with the small amounts around the droplet. The minimum detectable chlorine level using this method is 500 ppm.

4) DIPPING TESTS OF WHITEFLY LARVAE IN PERMETHRIN IN 1% ACETONE

Bioassay dipping tests were carried out to provide an alternative indirect method for assessing the distribution of active ingredient around droplets on the leaf. The mortality response of whitefly larvae to a known concentration range of permethrin was assessed and thus the concentration was determined on an arbitrary scale. It was also intended to confirm that the mortality response followed the expected linear transformation of a probit or logit curve. Probit analysis of mortality data of

(A) Distribution image of Cl



(B) Line scan

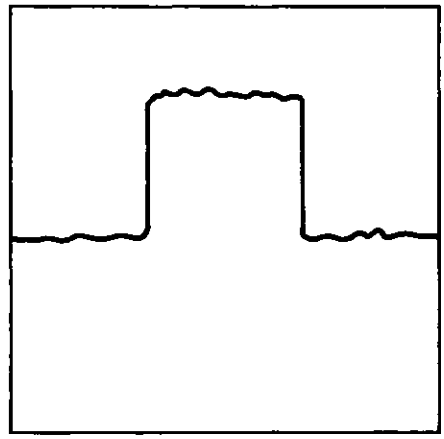


Figure 9.
Element map of permethrin on tobacco leaf 48 hours after spraying.

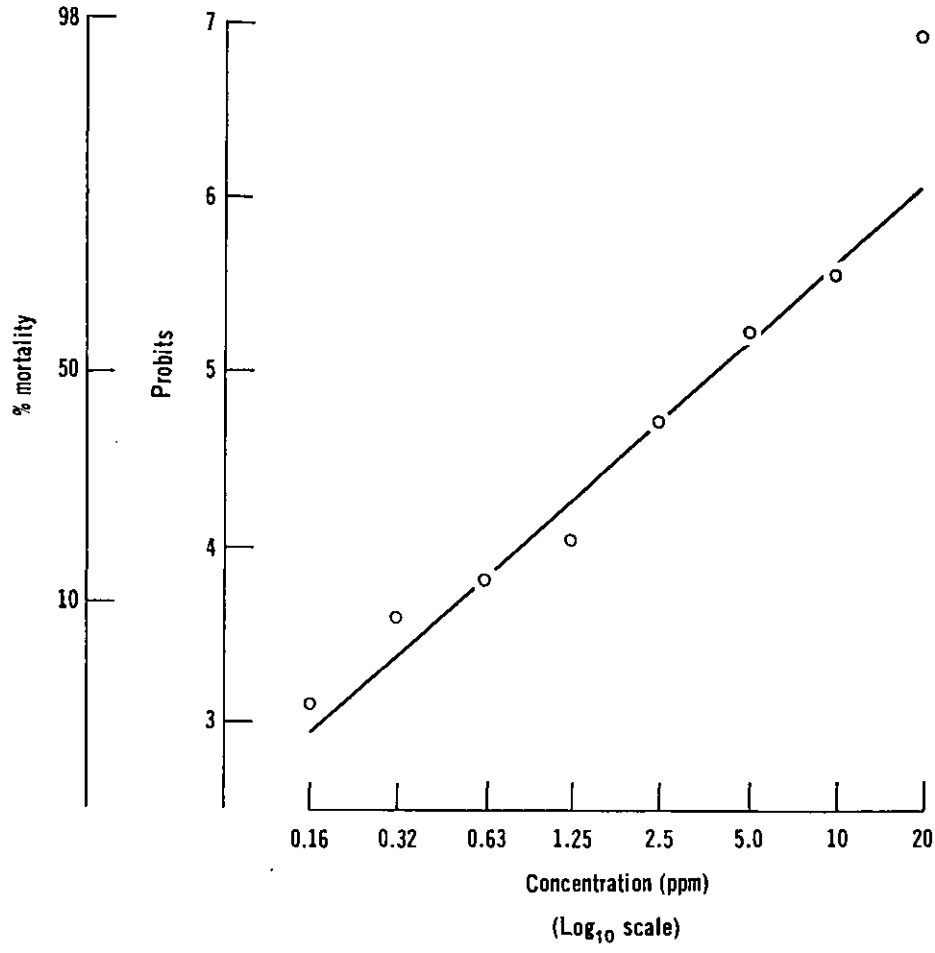


Figure 10.
Bioassay of settled first instar whitefly larvae dipped in solutions of permethrin in 1% acetone.

settled first instar larvae dipped in a series of concentrations (0.16 to 20 ppm) of permethrin in 1% acetone (Figure 10) gave the following equation:

Probit mortality = $4.136 + 1.489 \log_{10}$ Conc.

(Concentration in ppm)

This produced an LD_{50} of 3.81 ppm.

Weighted logit analysis gave the equation:

Logit mortality = $- 0.747 + 1.288 \log_{10}$ Conc.

(Concentration in ppm)

This gave an LD_{50} of 3.80 ppm.

Anomalies in these results and possible alternative interpretations will be discussed later.

5) PHYSICAL FORM AND SPREAD OF DROPLETS OF PERMETHRIN: SEM RESULTS

The physical form and spread of 76 to 80 µm droplets of 10% permethrin formulated in VK1, JF 8133 and JF 8130 on tobacco, and in VK1 on tobacco, tomato and French bean leaves were studied.

i) Effect of Formulation

The effects of three formulations (JF 8130, VK1 and JF 8133) on the physical form and spread of droplets of 10% permethrin were compared. Formulation JF 8130 formed dome shaped droplets with

very smooth circumferences (Plates 3 and 4). Droplets of formulations VK1 (Plates 5 and 6) and JF 8133 (Plate 7) were much flatter and with relatively irregular margins. The top surface of many droplets of formulation JF 8130 contained discrete crystal-like structures (Plate 4), while scum of material was visible on droplets of JF 8133 (Plate 7) and VK1 (Plate 6). Spread of droplets of JF 8130 measured by the mean droplet area after 48 hours from spraying was very limited (0.0196 mm^2) and differed significantly ($P<0.001$) from that of VK1 (0.0285 mm^2) and JF 8133 (0.0313 mm^2). The mean droplet perimeter for JF 8130 (0.541 mm) also differed significantly ($P<0.001$) from that for VK1 (0.719 mm) and JF 8133 (0.788 mm) (Table 5a).

ii) Effect of Plant Species

Comparisons were made between the effects of three species of plant (tobacco, tomato and French bean) on the physical form and spread of droplets of 10% permethrin in VK1. On tomato, the droplets had less regular shape (Plate 8) than on tobacco (Plate 6). On French bean appreciable proportions of the droplet content ran between the epidermal cells forming irregular indentations (Plate 10) which were more prominent in the vicinity of veins (Plate 9). The mean droplet perimeter on tobacco (0.783 mm) differed significantly ($P<0.05$) from that on tomato (0.917 mm) and on French bean (0.997 mm). The mean droplet areas on the surfaces of the three plants, however, did not differ appreciably, being 0.0285, 0.0303 and 0.0295 mm^2 on tobacco, tomato and French bean, respectively (Table 6a).

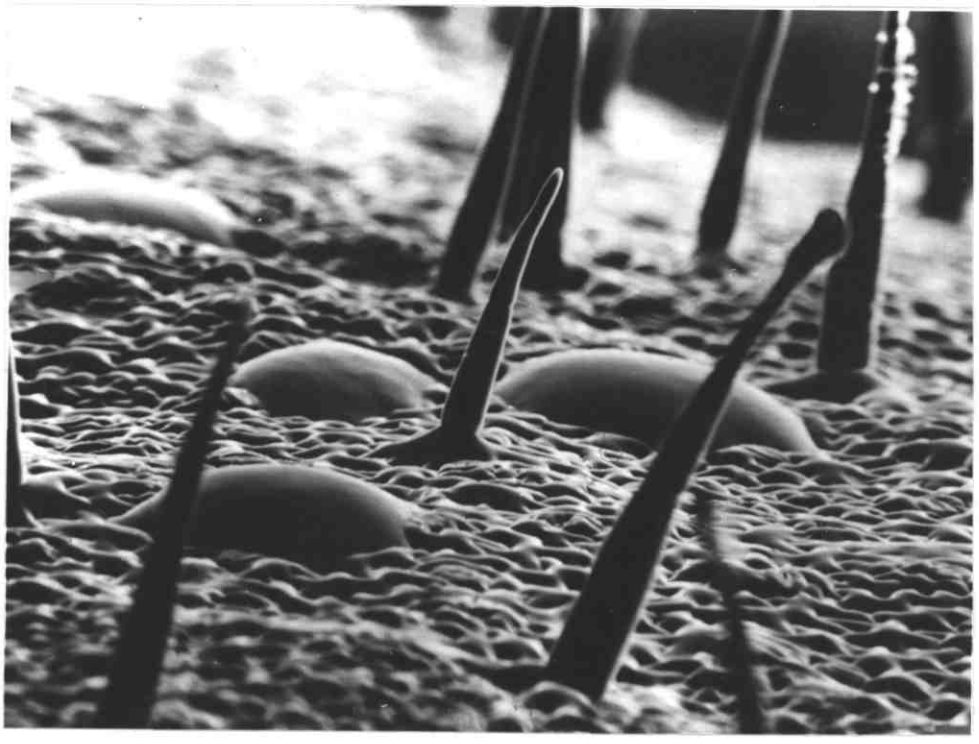


PLATE 3: Scanning electron micrograph showing droplets of 10% permethrin in JF 8130 on tobacco leaf 48 hours after spraying (tilt angle 80°) (X 150)

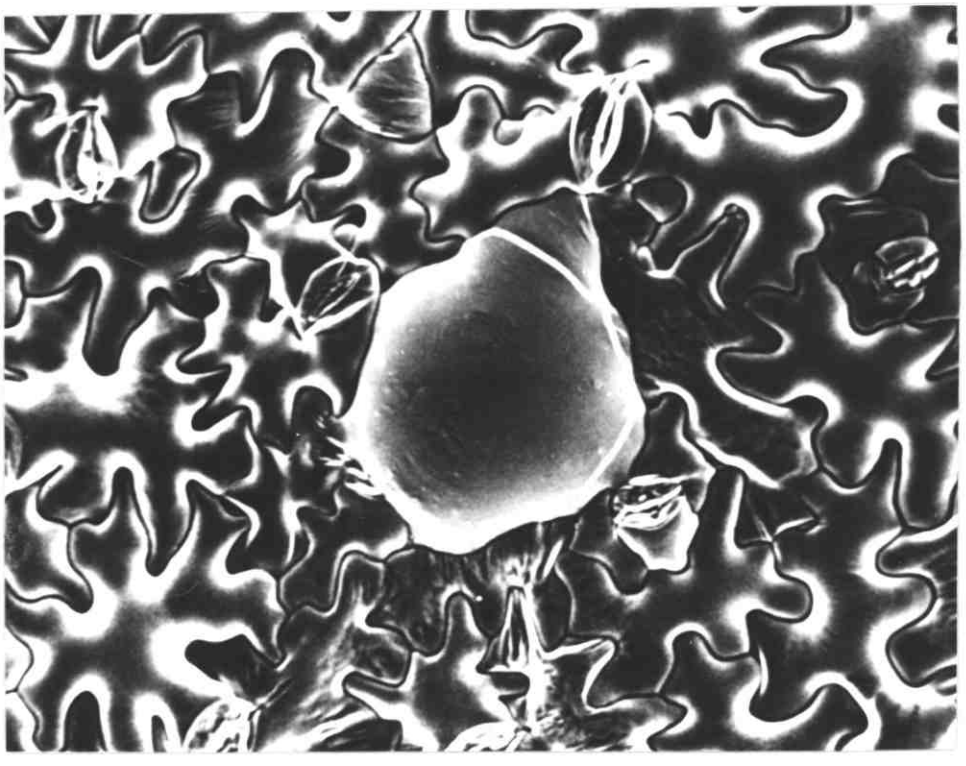


PLATE 4: Scanning electron micrograph showing a droplet of 10% permethrin in JF 8130 on tobacco (no tilt) (X 300)

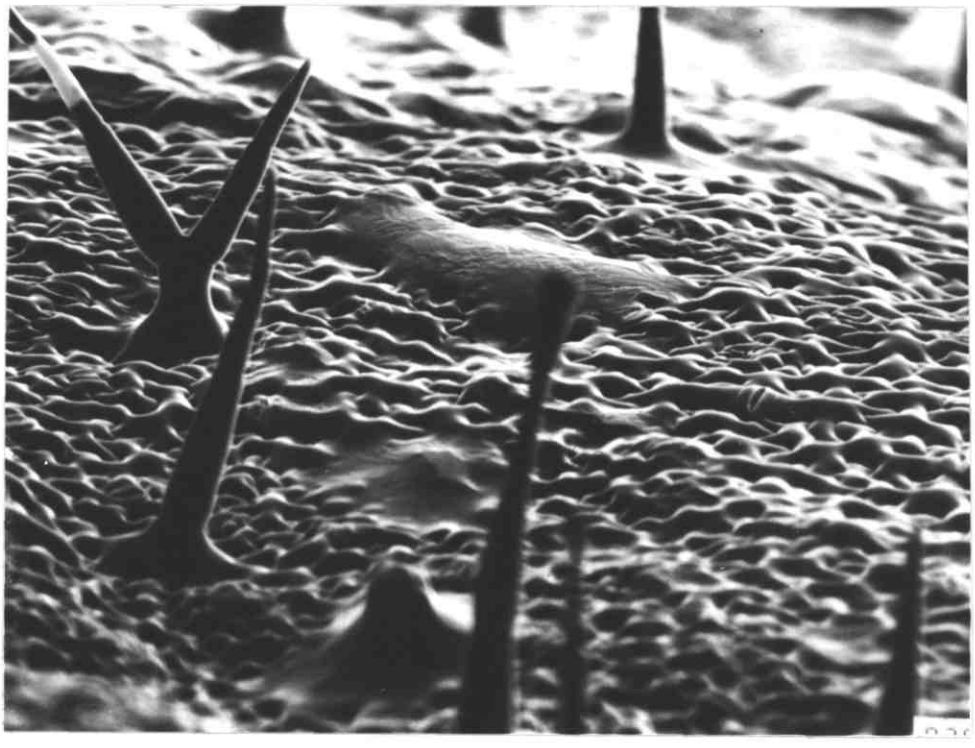


PLATE 5: Scanning electron micrograph showing droplets of 10% permethrin in VK1 on tobacco leaf 48 hours after spraying (tilt angle 80°) (X 150)

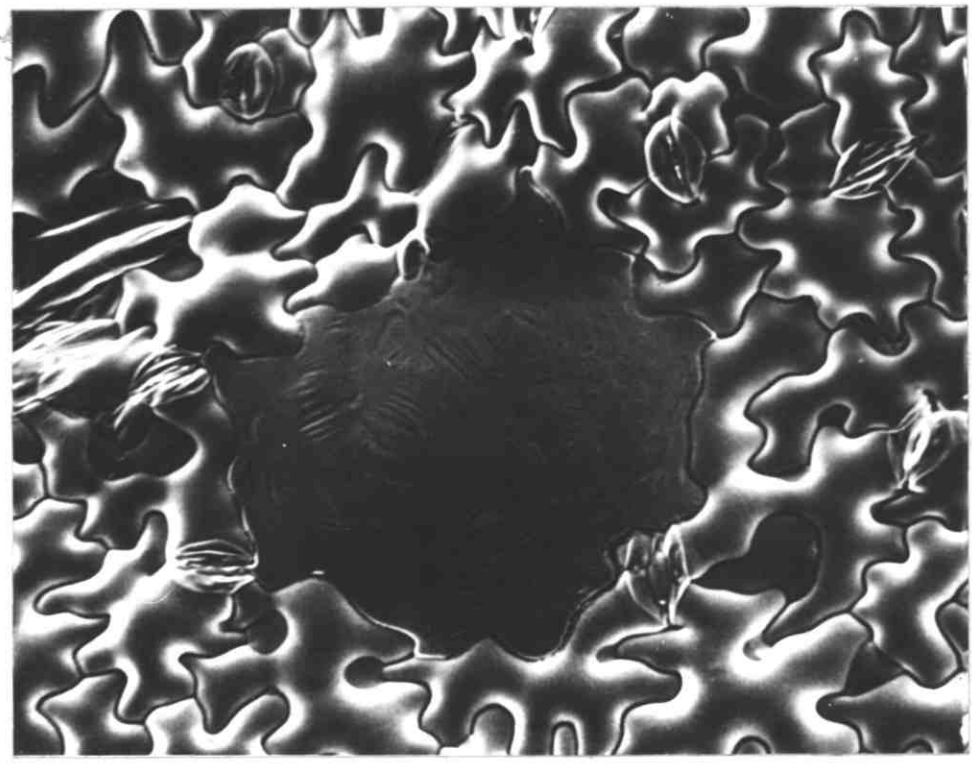


PLATE 6: Scanning electron micrograph showing a droplet of 10% permethrin in VK1 on tobacco leaf (no tilt) (X 300)

TABLE 5: Effect of formulation on the physical spread of permethrin droplets

a) Mean areas, theoretical mean radii and mean perimeters of droplets of 10% permethrin in three formulations on tobacco leaves

Droplet age (hours)	Formulation ...	JF 8130	VK1	JF 8133
	Droplet size (µm) ...	76	80	78
0.5	Mean droplet area (mm^2)	0.0193	0.0251	0.0214
	Theoretical mean radius (mm)	0.0784	0.0894	0.0825
24	Mean area	0.0205	0.0273	0.0270
	Theoretical mean radius	0.0808	0.0932	0.0921
48	Mean area	0.0196	0.0285	0.0313
	Standard error (S.E.)	3.43×10^{-3}	4.84×10^{-3}	6.09×10^{-3}
	Theoretical mean radius	0.0790	0.0952	0.0998
	Mean perimeter (mm)	0.541	0.719	0.788
	S.E.	0.0082	0.0131	0.0165

/Continued ...

TABLE 5 (Continued)

b) Mean maximum lengths and widths of droplets, mean diameters, mean areas and mean perimeters of ellipses enclosing droplets of 10% permethrin in three formulations on tobacco leaves

Formulation	Droplet		Mean maximum droplet		Mean dia. $\frac{1}{2}(a+b)$ (mm)	Mean ellipse area (mm^2)	Mean ellipse perimeter (mm)
	Size (µm)	Age (h)	Length (a) (mm)	Width (b) (mm)			
JF 8130	76	48	0.179	0.145	0.162	0.0205	0.512
VK1	80	48	0.229	0.180	0.205	0.0324	0.647
JF 8133	78	48	0.251	0.194	0.223	0.0384	0.704

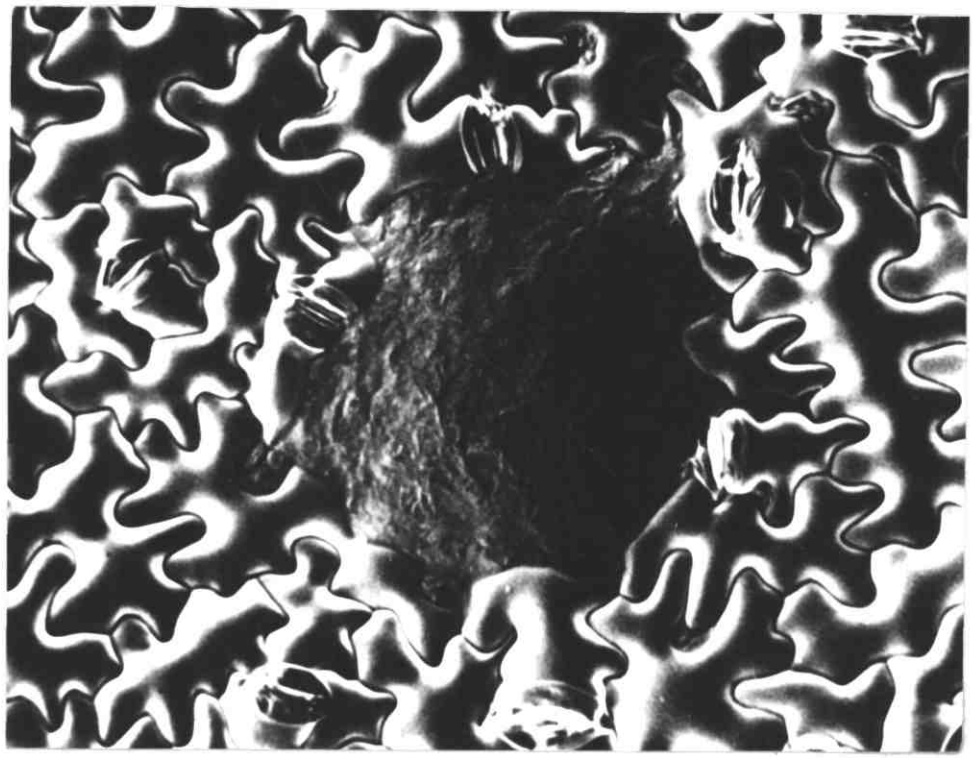


PLATE 7: Scanning electron micrograph showing a droplet of 10% permethrin in JF 8133 on tobacco leaf 48 hours after spraying (X 300)

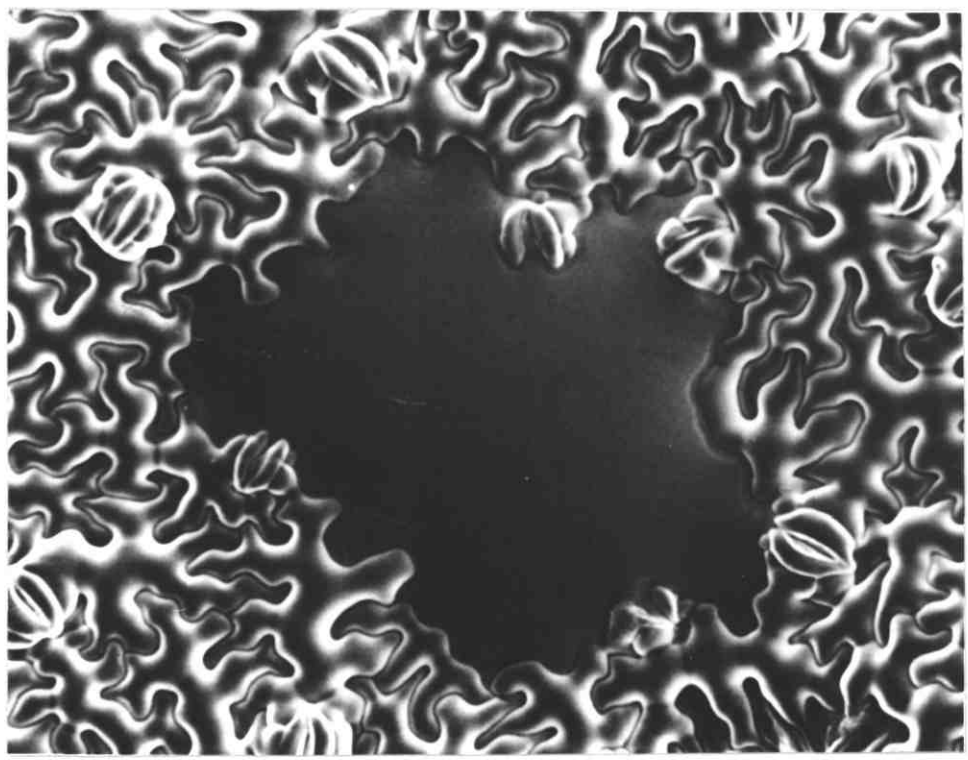


PLATE 8: Scanning electron micrograph showing a droplet of 10% permethrin in VK1 on tomato leaf 48 hours after spraying (X 300)

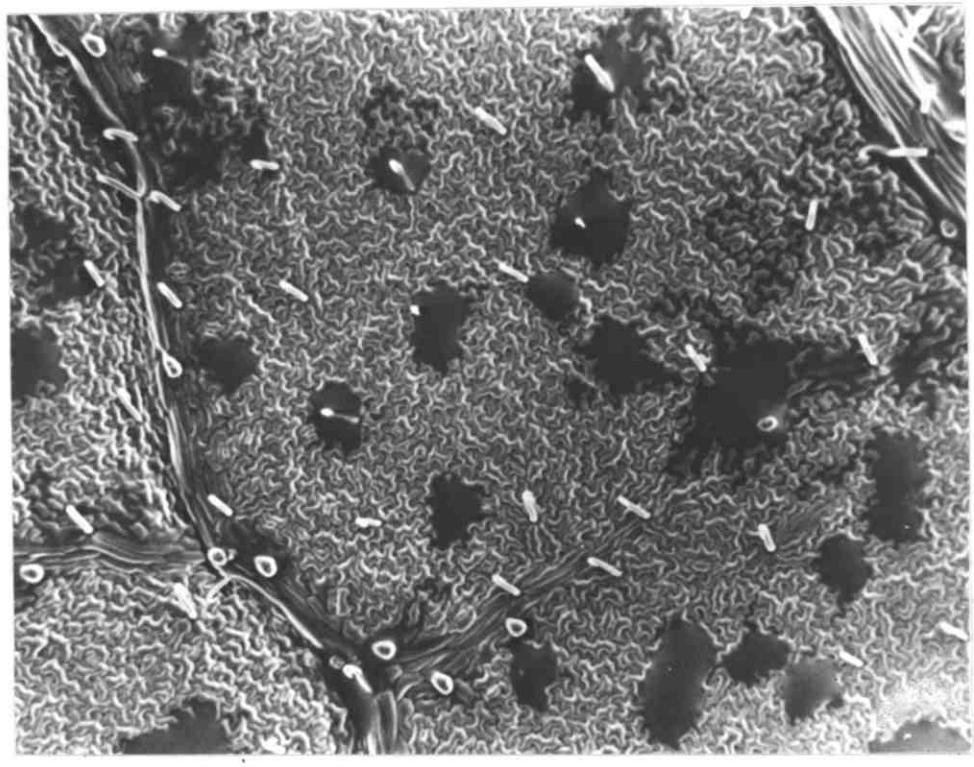


PLATE 9: Scanning electron micrograph showing droplets of 10% permethrin in VK1 on French bean leaf 48 hours after spraying (X 50)

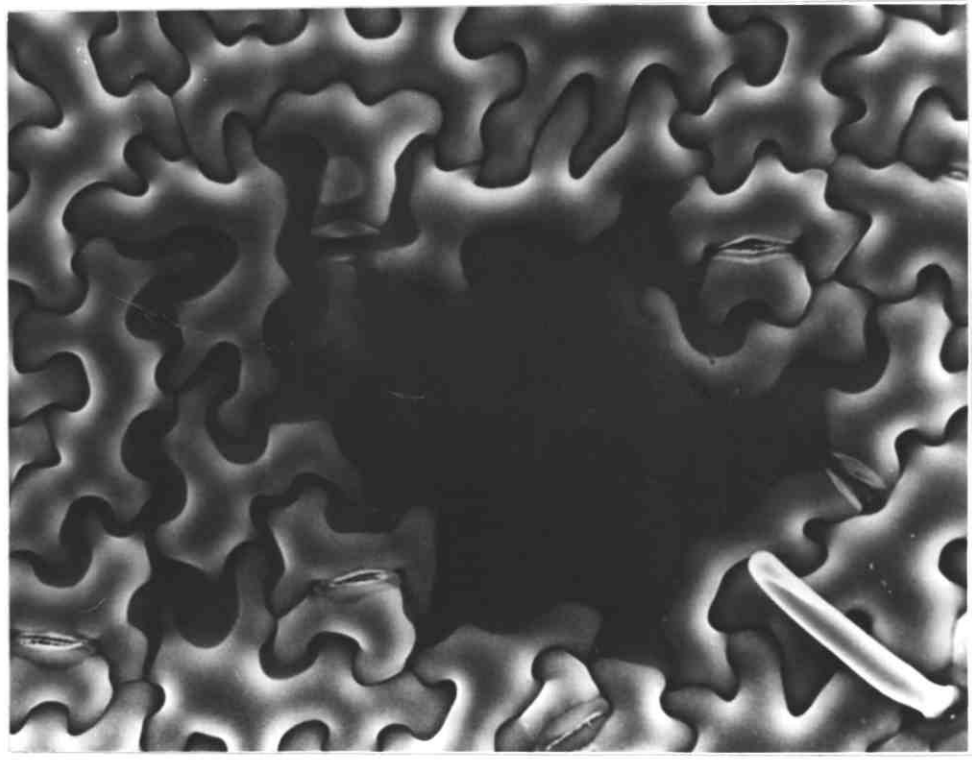


PLATE 10: Scanning electron micrograph showing a droplet of 10% permethrin in VK1 on French bean leaf (X 400)

TABLE 6: Effect of plant species on the physical spread of permethrin droplets

a) Mean areas and theoretical mean radii of droplets of 10% permethrin in VK1 on three species of plant

Droplet size = 80 µm

Droplet age (hours)	Plant species ...	Tobacco	Tomato	French bean
0.5	Mean droplet area (mm^2)	0.0251	0.0261	0.0253
	Theoretical mean radius (mm)	0.0894	0.0911	0.0897
24	Mean area	0.0273	0.0307	0.0287
	Theoretical mean radius	0.0932	0.0989	0.0956
48	Mean area	0.0285	0.0303	0.0295
	S.E.	4.84×10^{-3}	5.65×10^{-3}	5.08×10^{-3}
	Theoretical mean radius	0.0952	0.0982	0.0969
	Mean droplet perimeter (mm)	0.783	0.917	0.997
	S.E.	0.0131	0.0253	0.0395

/Continued ...

TABLE 6 (Continued)

b) Mean maximum lengths and widths of droplets, mean diameters, mean areas and mean perimeters of ellipses enclosing droplets of 10% permethrin in VK1 on three species of plant

Droplet size = 80 µm

Plant species	Droplet age (hours)	Mean maximum droplet		Mean dia. (mm)	Mean ellipse area (mm^2)	Mean ellipse perimeter (mm)
		Length (mm)	Width (mm)			
Tobacco	48	0.229	0.180	0.205	0.0324	0.647
Tomato	48	0.236	0.175	0.206	0.0325	0.656
French bean	48	0.239	0.179	0.209	0.0335	0.663

6) COMPARISON BETWEEN THE DISTRIBUTION OF WHITEFLY LARVAL MORTALITY AROUND DROPLETS OF 10% PERMETHRIN AND 10% PIRIMIPHOS-METHYL

A comparison was made between the distribution of mortality of settled first instar whitefly larvae around 102 to 107 µm droplets of permethrin and pirimiphos-methyl, both at 10%, and formulated in JF 9079 (Figure 11, Appendix 8). Permethrin droplets produced a response slope of -15.66, an intercept of 3.14 and Ld_{50} of 0.390 mm. The shape of the response curve of pirimiphos-methyl conformed very closely with the logit:log log transformation ($p<0.001$, based on the correlation coefficient). The slope was -22.43, differing significantly ($P<0.01$) from that of permethrin and the intercept was 9.98 compared with 3.14, also differing significantly ($P<0.001$). The Ld_{50} was 6.10 mm also differing significantly ($P<0.001$) from the value for permethrin (Table 4e).

7) EFFECTS OF FUMIGANT AND CONTACT ACTION OF PIRIMIPHOS-METHYL ON THE DISTRIBUTION OF MORTALITY AROUND DROPLETS

The distribution of mortality around droplets of pirimiphos-methyl was studied in more detail to assess the relative importance of fumigant effect in relation to surface mobility of the toxicant. Mortalities around 104 µm droplets of 10% pirimiphos-methyl in JF 9079 sprayed on test leaves were compared with those around droplets sprayed on separate leaf strips placed adjacent to test leaves. The two treatments gave a very similar response (Figure 12, Appendix 9). The slopes were -22.22 and -22.58. The

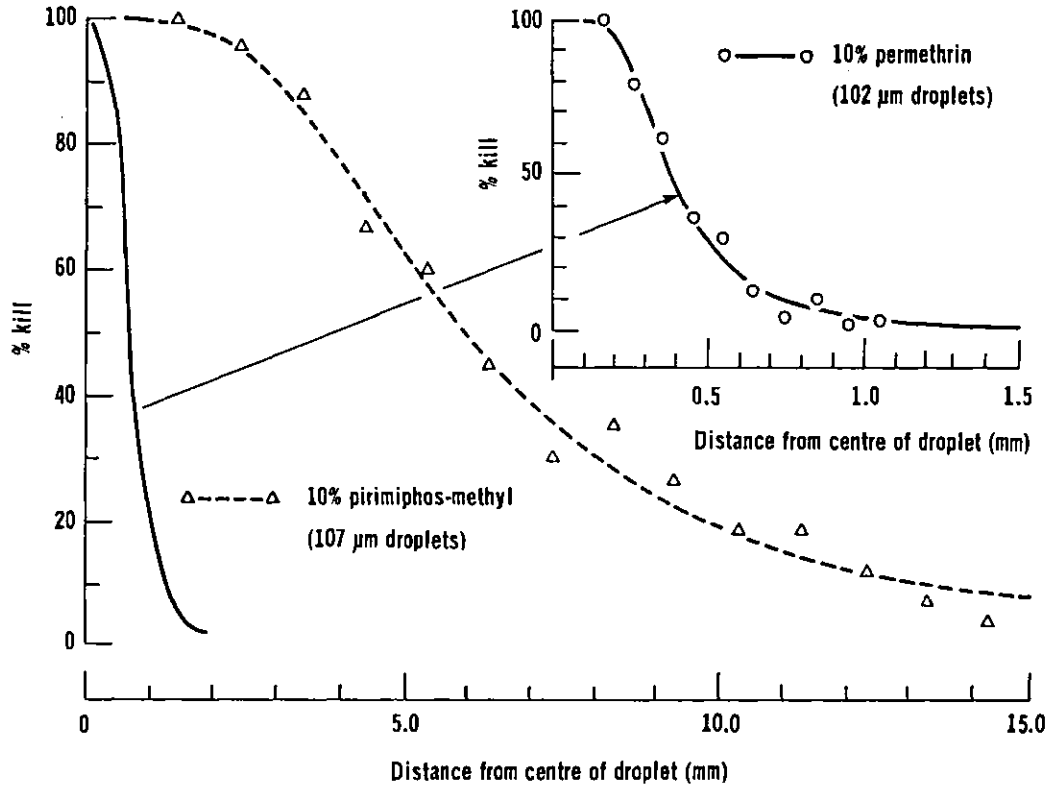


Figure 11.
The distribution of mortality of settled first instar whitefly larvae around droplets of 10% permethrin and 10% pirimiphos - methyl in JF 9079 on tobacco leaves.

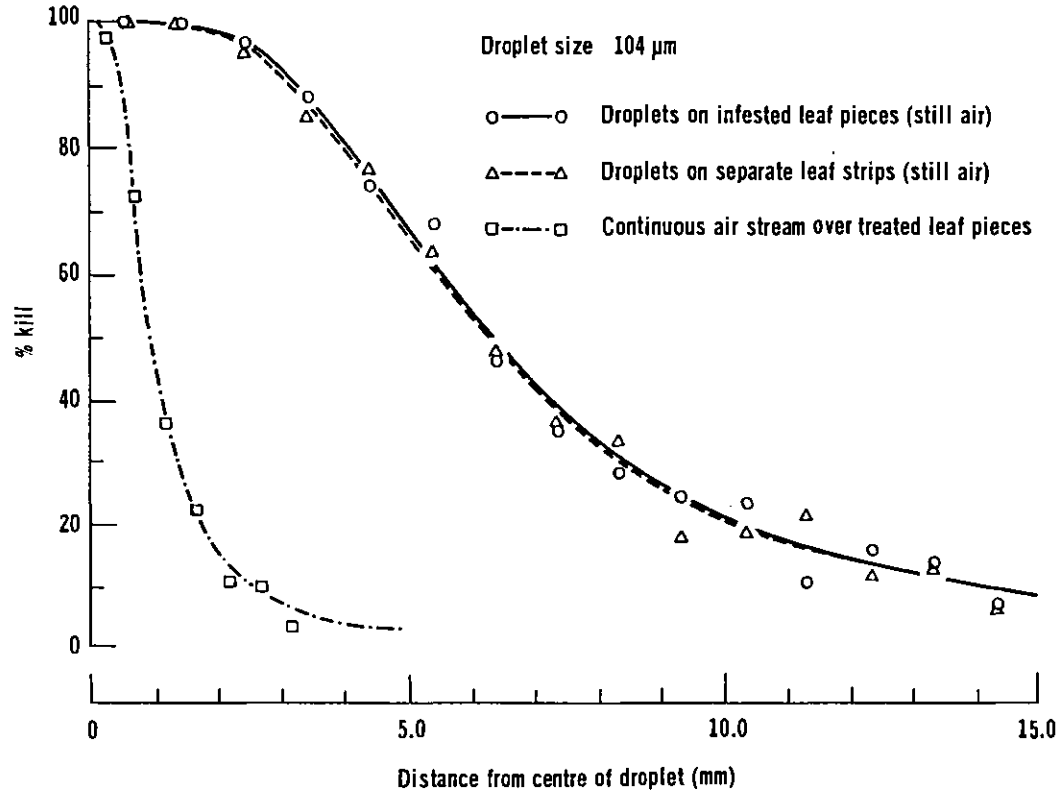


Figure 12.
Effect of fumigant and contact action on the distribution of mortality of settled first instar whitefly larvae around droplets of 10% pirimiphos - methyl in JF 9079 on tobacco leaves.

intercepts were 9.95 and 10.1. Ld_{50} values were 6.34 and 6.42 mm. When a gentle air flow was passed over a set of treated leaf pieces a different response was obtained. This gave a slope of -14.40, an intercept of 4.33 and an Ld_{50} of 1.01 mm. All slope, intercept and Ld_{50} showed significant differences ($P<0.001$) compared with the first two treatments (Table 4f).

4b. DISCUSSION

1) EMPIRICAL MODEL

Numerous attempts were made, in the early stages of interpretation, to find a mathematical expression which would both describe and explain the results obtained. The principle most likely to be involved was diffusion with disposition of chemical around a droplet resulting from random molecular movements, i.e. basically a normal distribution. Examination of the laws of diffusion showed that the mathematics of diffusion from a circular source are exceedingly complex and research is still in progress elsewhere to devise a model on this basis. This study will be discussed later.

The measurements taken in these experiments were of percentage mortality, to be used as a bioassay to determine the fall-off of insecticide at distances progressively further from the droplet. It was felt necessary to find an equation which would adequately describe this fall-off, in order to summarize the results and make valid comparisons. Initial plotting of mortality against distance revealed a distinct sigmoid form, being 100% close to the droplet

then falling fairly abruptly to about 20% but then falling gradually over a considerable distance until a steady low level was reached, explained by the amount of natural mortality. The curve was reminiscent of the response curve of mortality to concentration, namely that the probit of mortality is proportional to the logarithm of the concentration. Attempts to plot the probit or the logit of mortality against the log of the distance however resulted in a curved response, still with a long 'tail' even when natural mortality was taken into account. It was therefore decided to log the distance twice to achieve further forshortening of the extreme distances. This had the desired effect of producing remarkably straight lines in all sets of data examined.

The relationship between mortality (as a proportion, p) and distance from the centre of the droplet (d in mm) can therefore be expressed by the equation:

$$\text{logit } p = a + b \log_{10} \log_{10} (100\ d)$$

The value of the logit was used throughout in preference to the probit because of the ease of programming on a desk calculator and because it gave a somewhat better fit in most cases. 'The logit transformation does not differ appreciably from the probit and statistical analyses based on the two transformations will always lead to essentially the same result' (Hewlett & Plackett, 1979). Thus:

$$\text{Logit } p = \frac{1}{2} \log_e \frac{p}{1 - p}$$

2) NATURAL MORTALITY

The amount of control mortality in each set of experiments was determined by spraying infested leaves with the carrier solvent alone and assessing mortality between the droplets. When this value was used to correct mortalities in the treatments, the resulting values expressed the effect of insecticide alone, whereas the value usually required was for the insecticide as formulated. Further, it was found, in some instances, that the mortality in the 'tail' of the distribution was lower than the assessed control mortality, causing difficulties in logit fitting. So natural mortality was assessed by another method, namely by determining the asymptote to which the 'tail' tended. Since deaths in the tail were sparse, they were combined into convenient groups to present a progressively declining series.

A calculator was programmed to store the data of a set and, being given an estimate of natural mortality, to correct by Abbott's formula, regress the logit against log log distance and calculate the correlation coefficient. After several iterations the natural mortality giving the best correlation could be assessed. This usually gave a value near or somewhat below the control mortality, but was always positive (Appendix Tables 3 to 9). No weighting was used in the regression at this stage, so there was a strong bias to the numerous low values in the tail, giving reasonable confidence in the estimate of the asymptote, namely the natural mortality. It had very little effect on the fit of the region of greatest slope, however, where much of the interest in interpretation lies.

As an example, in this and the later sections on discussion, the 5% and 20% concentrations of permethrin in VK1 in the 3-treatment experiment (Figure 4, Appendix Table 3) will be considered. In the 5% treatment (Table 3), mortality was 100% from the droplet centre to 0.15 mm, then dropped rapidly from 78% at 0.25 to about 3% at 1 mm. From there to 3 mm there were only scattered deaths accounting for only 1.7% of the total. The observed control mortality was higher at 2.26%, however, and it was therefore not possible to correct this outer region or use it for logistic fitting. The natural mortality clearly lay at or below 1.7% and after a few iterations, regressed values indicated the best fit, as measured by a correlation coefficient of 0.960, when the natural mortality was 1.58%. In the 20% treatment, 100% mortality extended to 0.25 mm: the only permethrin treatment where this was observed. It then fell from 79% at 0.35 mm rapidly at first and then more gradually to 1.2 mm. From there to 1.9 mm the mortality averaged 3.1% and for the remaining 1.1 mm of the tail averaged only 1.2%. Observed control mortality was again 2.26% but natural mortality on the treated leaf was clearly well below this and iterated regressions indicated a value of 0.616% with a correlation coefficient of 0.977.

3) FITTING RESPONSE CURVES

Having obtained acceptable estimates of the proportion dying from natural causes (Pc), the observed proportion dying (Po) could be corrected by Abbott's formula to give an estimate of the

proportion killed by the insecticide formulation (p).

$$p = \frac{P_o - P_c}{1 - P_c}$$

The logit could then be calculated from the equation:

$$\text{logit } p = \frac{1}{2} \log_e \frac{p}{1 - p}$$

or directly from the observed mortality by substituting Abbott's formula into this:

$$\log p = \frac{1}{2} \log_e \frac{Po - Pc}{1 - P_o}$$

The logit could then be regressed against the $\log_{10} \log_{10} (100\ d)$, but this time weighting each value by $p(1 - p)$ to give greatest weight to values near the middle of the sigmoid and least to those near the upper and lower asymptotes, where variability was greatest. It was also possible to give added weighting according to the number of scales observed at each distance, $n\ p(1 - p)$, but this was found to give little advantage since the numbers, particularly in the centre of the range, did not vary greatly. The practice was therefore not continued.

In this way equations were obtained, relating mortality to distance, of the form:

$$\text{logit } p = a + b \log_{10} \log_{10} (100\ d)$$

In an analogous process to probit analysis, a median lethal distance (Ld_{50}) can then be derived. Since the logit of 50% is 0 then:

$$\log_{10} \log_{10} (100\ Ld_{50}) = -\frac{a}{b}$$

or

$$Ld_{50} = 10^{-\frac{a}{b}}/100$$

This value indicates the distance from the centre of a droplet at which 50% of the scales are killed. It corresponds roughly with the concept of the biocidal radius as defined by Munthali (1981), who assumed that, on a uniformly sprayed leaf on which 50% of the targets were killed, the area circumscribed by these radii would occupy half the total area of the leaf. These concepts will be discussed further in a later section.

Returning to the examples, correction for such a low natural mortality made little difference to the estimated mortalities except on the 'tails' of the responses, where mortality at the extremities was estimated at only 0.09 and 0.22% in the 5% and 20% concentrations respectively. Weighted logistic regression gave intercepts (a) of 3.14 and 3.45 and slopes (b) of -17.45 and -15.54 respectively. The fit of the resulting curves was remarkably good at both concentrations, as it was in all the other experiments. The best comparison of the two concentrations is made on the Ld_{50} which were 0.326 and 0.466 mm respectively. Thus the areas within which 50% or more scales were killed were

0.334 mm^2 for 5% and 0.682 mm^2 for 20%. On this basis, four times the concentration of insecticide achieved only twice the effectiveness of kill.

4) RELATING MORTALITY TO CONCENTRATION

The main objective of assessing mortality was to investigate the distribution of the insecticide around the droplet. No method could be devised of direct chemical analysis of the leaf on such a small scale around a droplet, in order to calibrate the effects. It was therefore accepted that concentration was related to mortality by the log concentration:probit relationship which normally holds in such cases (Finney, 1952). The dipping tests were therefore carried out and the results subjected to probit analysis in the normal way. The resulting equation was:

$$\text{Probit kill} = 4.136 + 1.489 \log_{10}\text{Conc.}$$

(Concentration in ppm)

This indicated an LD_{50} of 3.81 ppm.

Since logits are more easy to calculate than probits in the ensuing calculations a weighted logit fit was attempted. The second equation was:

$$\text{Logit kill} = 0.747 + 1.288 \log_{10} \text{Conc.}$$

(Concentration in ppm)

The resultant LD_{50} was 3.80 ppm, very close to the probit estimate.

In the dipping tests the entire leaf piece was immersed in the formulation and therefore the whitefly scale was wetted by the insecticide on all its surfaces. In the droplet tests, however, the insecticide reached the scale apparently only by diffusion across the leaf surface and would have contacted only the underside of the scale. There are, therefore, fundamental differences in the mode of action in the two cases. It is not possible to obtain an absolute measure of insecticidal effect by this method and the responses were assumed to be proportional. Although the above logit equation is used throughout in the later calculations, it must be accepted that the concentration values are only in arbitrary units and that even the slope of 1.288 may not necessarily be correct.

5) DISTRIBUTION OF INSECTICIDE AROUND THE DROPLET

This logit equation can now be used to translate any mortality value into concentration units. Performing this transformation on the smoothed mortalities in the 5% treatment shows that the amount of insecticide at 0.25 mm was 11.1 units; at 0.35 mm, 2.9 units; and at successive distances, 1.1, 0.57, 0.33, 0.21, 0.14, 0.10 until at 2.95 mm it had fallen, in theory, to 0.005 arbitrary units (Appendix Table 3). For the 20% concentration there were an estimated 31.9 units at 0.25 mm and successively 9.6, 4.2, 2.3, 1.4, 0.93, 0.66 and 0.49, reaching 0.03 at 2.95 mm. Thus the concentration appears to have fallen very rapidly in the vicinity of the droplet but changed to a much slower decline further out. This form of curve is very reminiscent of an inverse square relationship and comparisons which have been made so far between

estimated concentration and an inverse square law indicate a very close fit (see Section 4b. 12) i)).

A point of considerable interest in these concentration estimates is that the values for the 20% concentration are between 3 and 4 times those for 5%. When the total insecticide between the 0.2 and 3.0 mm radii was estimated, allowing for the increasing areas of concentric circles, it was found that the mean concentration from 20% was 0.509 units and, from 5%, 0.144 units. Thus 4 times the concentration has released 3.53 times the amount of insecticide into the surrounding area, which is therefore not greatly different from a proportional release. Calculations for the 10% treatment confirm this by giving an intermediate value of 0.300 units. Comparable calculations for mortality gave mean percentage deaths of 1.67, 3.86 and 4.79 for 5, 10 and 20%. Thus 4 times the concentration gave only 2.87 times the kill, the difference clearly being due to the non-proportional response.

It should perhaps be pointed out at this stage that the proportionality of insecticide release was not confirmed in the second experiment, using six concentrations, which will be considered later.

6) OVERLAP OF DROPLET INFLUENCE IN ONE DIMENSION

In order to relate these findings to those of Munthali (1981) and also to deduce any practical implications, it is necessary to consider what might happen if droplets were dispersed across the whole surface of a leaf. It has been assumed that, with the fairly wide spacing of the droplets in these experiments, they

did not interfere with each other in the direction of measurement and that the mortalities represent those of single droplets. With closely spaced droplets on a leaf, the diffusing insecticide from one will clearly interact, to some extent, with that of its neighbour. The indications are that the dispersal of insecticide across the leaf surface is entirely by random diffusion of the molecules (to be discussed later). If this contention is accepted, the overlap of effect between one droplet and its neighbour will be additive.

In Munthali's research he assessed the density of droplets on a leaf which would result in 50% mortality of his target organisms (mite eggs). This he described as the LN_{50} or median lethal number of droplets per cm^2. For comparison, it is therefore necessary to express the present findings in similar terms. Consider firstly the overlap of effect between two droplets, on the line between the two. For 20% permethrin the assessed concentration falls from 31.95 units at 0.25 mm to 0.17 at 1.45 mm. If droplets are spaced at 1.7 mm apart (Table 7, Figure 13), a point at 0.25 mm distance from one droplet will receive 31.95 units of insecticide from one droplet and 0.17 from the other making 32.12 in all. It can then be estimated that this will kill 91.60%, compared with 91.55% without overlap: scarcely any difference. At the mid-point 0.85 mm, however, 0.66 units are received from each droplet giving 1.32 in all and raising the mortality from 12.3 to 23.4%. To estimate the total mortality around a droplet successive observations must be taken to represent successive concentric circles, whose areas increase in the ratios of 1:2:3 etc. The assessed mortalities

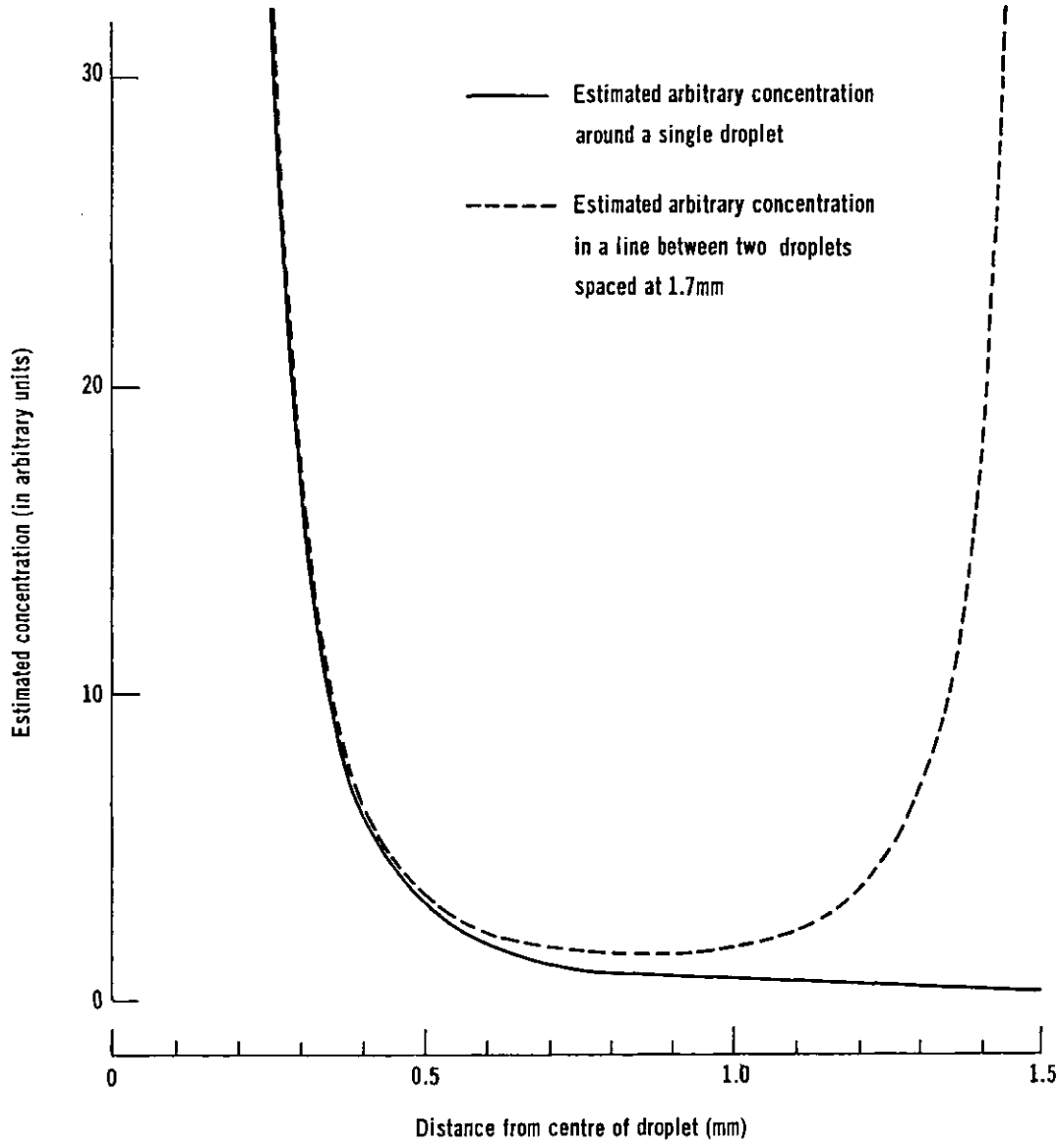


Figure 13.
Estimated arbitrary concentration around a single droplet and between two droplets of 20% permethrin in VK1 spaced 1.7 mm apart on tobacco leaf (one dimensional overlap of droplet influence).

TABLE 7: Calculation of median lethal spacing (LS_{50}) for droplets of 20% permethrin in VK1 on tobacco leaf

Distance	Smoothed % kill	Est. conc.	1.7 mm spacing (x_2) Conc. from next drop	Total conc.	Resultant % kill	Relative area	Mortality product	1.6 mm spacing (x_1) Conc. from next drop	Total conc.	Resultant % kill	Relative area	Mortality product
0.05	100.00		0.12		100	1	100	0.14		100	1	100
0.15	99.11	256.64	0.14	256.78	99.11	2	198.22	0.17	256.81	99.11	2	198.22
0.25	91.55	31.95	0.17	32.12	91.60	3	274.80	0.20	32.15	91.61	3	274.83
0.35	73.92	9.64	0.20	9.84	74.35	4	297.40	0.24	9.88	74.45	4	297.80
0.45	53.00	4.23	0.24	4.47	54.53	5	272.65	0.30	4.53	54.88	5	274.40
0.55	36.05	2.28	0.30	2.58	39.27	6	235.62	0.37	2.65	40.07	6	240.42
0.65	24.52	1.39	0.37	1.76	29.79	7	208.53	0.49	1.88	31.25	7	218.75
0.75	17.09	0.93	0.49	1.42	24.86	8	198.88	0.66	1.59	27.31	8	218.48
0.85	12.29	0.66	0.66	1.32	23.34	4.5	105.03					
0.95	9.13	0.49					1891.13					1822.90
1.05	6.97	0.37				40.5	40.5				36	36
1.15	5.45	0.30	Estimated mean mortality (y_2) = 46.69					Estimated mean mortality (y_1) = 50.64				
1.25	4.36	0.24	Median lethal spacing $(LS_{50}) = x_1 + (x_2 - x_1)\frac{y_1 - 50}{y_1 - y_2}$									
1.35	3.55	0.20										
1.45	2.94	0.17	$= 1.6 + (1.7 - 1.6)\frac{50.64 - 50}{50.64 - 46.69} = \underline{1.616 \text{ mm}}$									
1.55	2.47	0.14										
1.65	2.10	0.12										
1.75	1.80	0.11										

must therefore be multiplied by the appropriate area, summed and divided by the total area. Thus, for 20% permethrin droplets at 1.7 mm spacing, the mean mortality can be obtained by dividing the summed mortality by the units of area dividing the central region between two droplets, i.e.:

$$\frac{1891}{40.5} = 46.7\%$$

Clearly to obtain 50% mortality the droplets would need to be somewhat nearer. Recalculation at 1.6 mm gives a mortality of 50.6% and, by proportion it can be estimated that the distance giving 50% kill would be 1.61 mm (Table 7).

Similar calculations with 5% permethrin show that the estimated 'median lethal spacing' (LS_{50}) would be 1.09 mm. Assuming a perfect equilaterial triangle spacing of droplets on a surface (the closest possible packing), each droplet would influence a hexagonal area with the least width equal to the spacing distance. The area of the hexagon would then be $6(LS_{50}/2)^2 \tan 30^\circ = 0.866\ LS_{50}^2$ mm^2. The areas of influence of the 5% and 20% droplets can then be calculated as 1.03 and 2.24 mm^2 respectively. The median lethal number of droplets per unit area of leaf (LN_{50}/cm^2) can then be calculated by dividing 100 mm^2 by these figures, giving 97.2 and 44.5 respectively. Thus, although 5% droplets contain a quarter of the insecticide of 20% droplets, only twice as many are needed to achieve the same effect.

7) OVERLAP OF DROPLET INFLUENCE IN TWO DIMENSIONS

The above calculations considered only two droplets, ignoring those in surrounding positions and the mortality in the spaces between the direct lines. Examples of spread in two dimensions were therefore considered to test the validity of the one-dimensional method. Again, considering droplets on a triangular grid pattern, it is convenient to divide the area between into hexagonal areas, each 0.1 mm across (Figure 14). For 20% permethrin at 1.6 mm spacing, each droplet is enclosed in its own hexagon with 15 hexagons between it and its nearest neighbours, and further hexagons filling all spaces between. The area of influence of one droplet then comprises 256 hexagons which can be conveniently divided into 12 congruent triangles (Figure 14a). It is then possible, for each of the 30 hexagons wholly or partly within the triangle, to measure or calculate its distance from neighbouring droplets. The four nearest were found to be sufficient. The insecticide contribution from each droplet could then be calculated and summed and, hence, the mortality in each hexagon could be estimated (Figures 14b and c). The mean mortality for the whole area of influence can then be calculated by summing the mortality in one triangle, multiplying by 12 and dividing by 256, the total number of hexagons. The resulting value is found to be 49.56% which compares very favourably with 50.6% assessed in one dimension.

Similar calculations were also performed for 5% permethrin in VK1 at 1.1 mm spacing and 10% permethrin in JF 8133 at 1.4 mm spacing. The resulting mortality estimates were 47.0 and 46.3

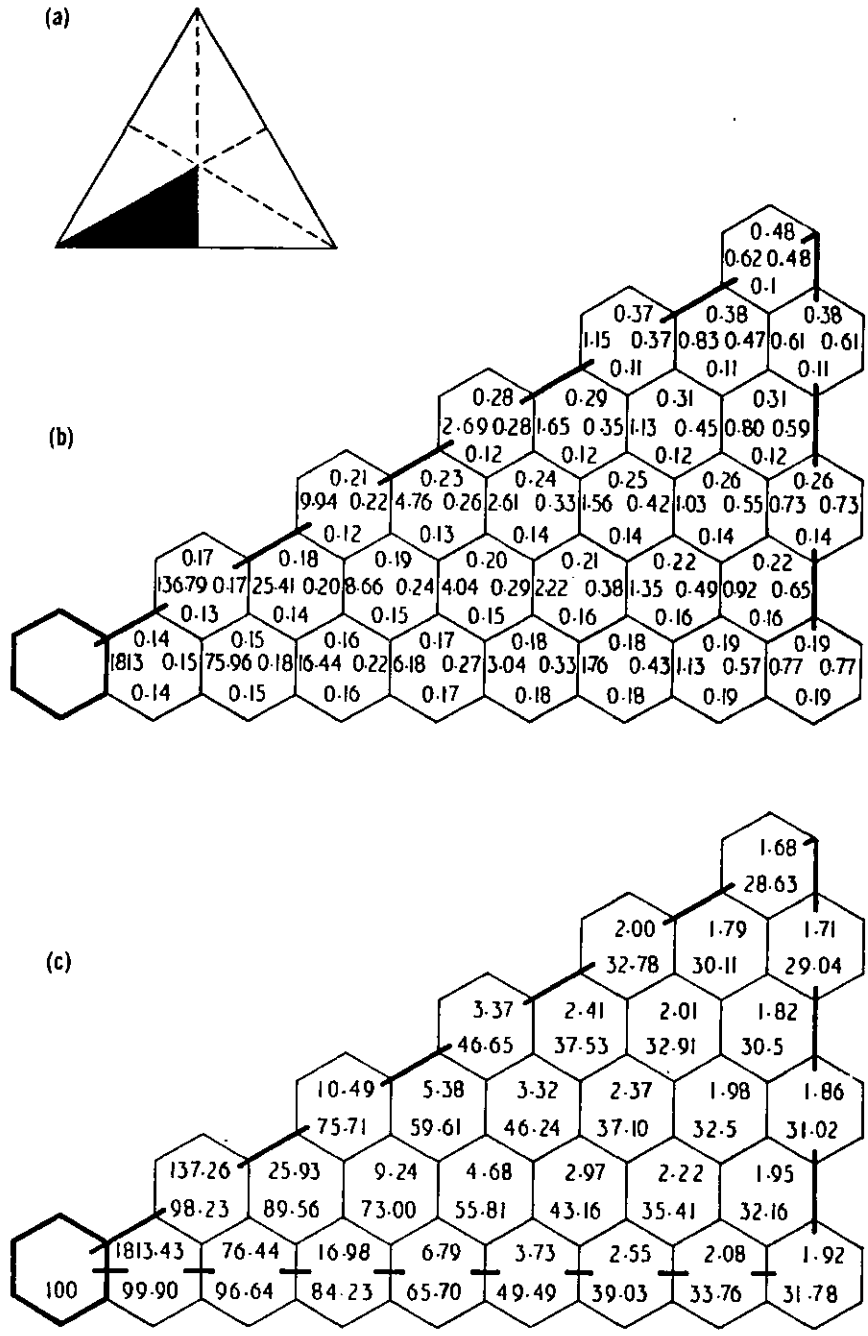


Figure 14.

Two dimensional overlap considering four droplets of 20% permethrin in VK1 spaced 1.6mm apart in a triangular grid on tobacco leaf.

(a) Equilateral triangle enclosing half the area of influence between four droplets.

(b) Insecticide contribution (in arbitrary units of concentration) from four droplets (shaded triangle).

(c) Summed estimated concentrations (top) and estimated mortalities (bottom) for four droplets.

compared with 49.6 and 49.4 respectively on a one-dimensional basis. It was concluded that the simpler one-dimensional method was adequate for all practical purposes, especially since the two dimensional method could give a slight underestimate due to considering only four droplets.

It was also possible, by this hexagon method, to deduce relatively how much insecticide had diffused onto the leaf surface from each droplet. For 20% permethrin (in VK1) this amounted to 13,305 units; for 10% (in JF 8133), 29,851 and for 5% (in VK1), 7,071. These values must, however, be accepted with caution since they are largely determined by the very high concentration in the immediate vicinity of the droplet, the estimation of which is subject to very considerable error.

The hexagonal design can also be extended to illustrate the distribution of mortality over the entire area. If five equally spaced droplets are depicted with mortalities for all hexagons between, it is possible to plot contours at 10% intervals (Figure 15). It then becomes clear that there is a plateau of very high (>90%) mortality, immediately around the droplet. There are then close concentric circles as mortality falls rapidly to 40%. This may be envisaged as a steep truncated cone of mortality. The entire area between the droplets has a fairly uniform mortality between 29 and 40%. This would appear to be the pattern of mortality in all cases where 50% of the target organisms are killed, regardless of the spacing of the droplets.

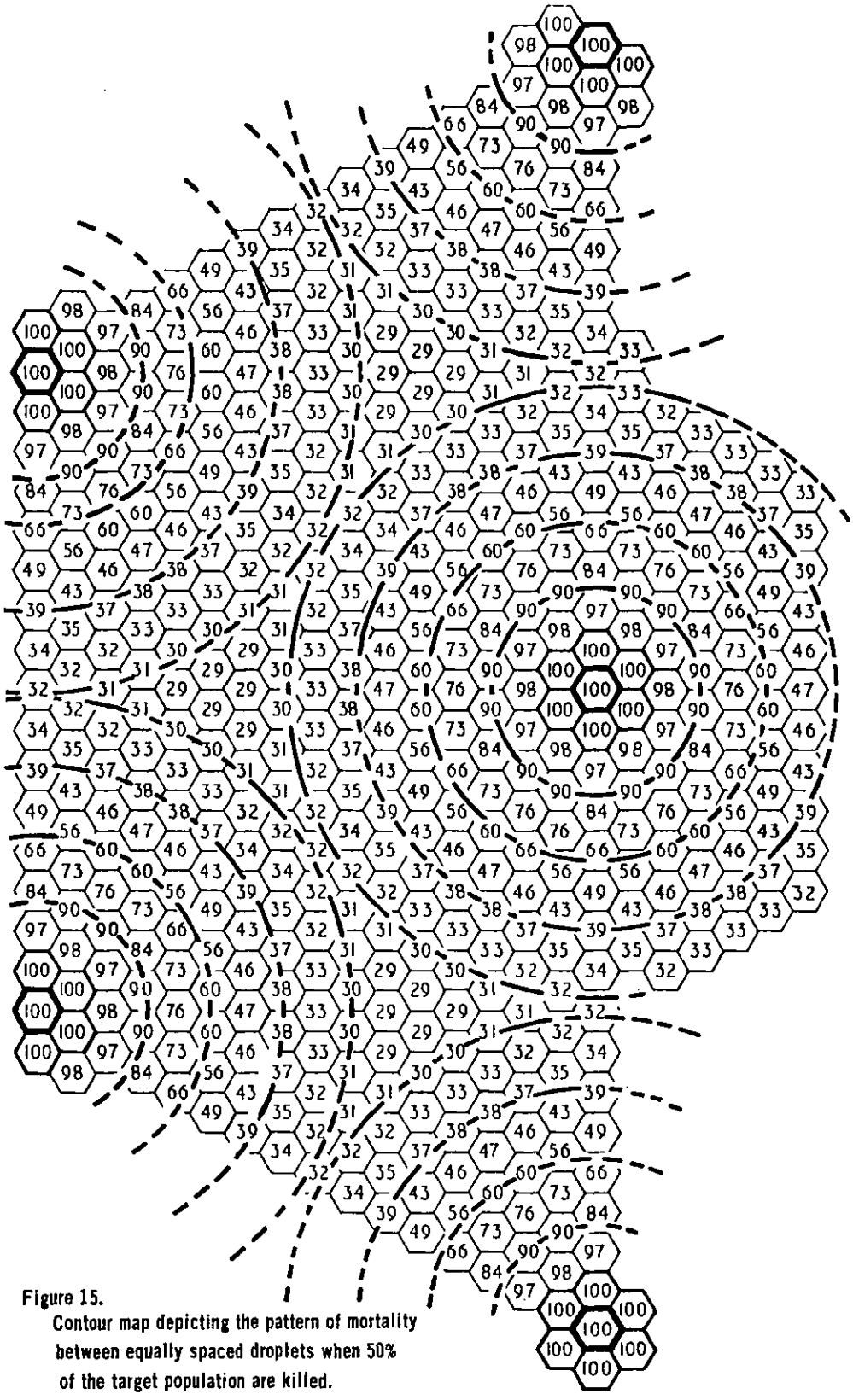


Figure 15.
Contour map depicting the pattern of mortality between equally spaced droplets when 50% of the target population are killed.

8) EFFECTS ON THE BIOLOGICAL EFFICIENCY OF PESTICIDE DROPLETS

i) Effect of Concentration of active ingredient

Two experiments were carried out on the effects of concentration on effectiveness. The first experiment having three concentrations, 20, 10 and 5% permethrin in VK1, has already been considered. The second, with six concentrations 20, 10, 5, 2, 1 and 0.5%, was to confirm and extend the findings. Taking the area of influence as a convenient comparative measure of effectiveness, there was good agreement between the two experiments at 20% (2.25 and 2.15 mm^2) and at 10% (1.72 and 1.65 mm^2) (Table 8). However, the area of influence for 5% in the first experiment (1.03 mm^2) was considerably lower than that in the second (1.43 mm^2) and was, in fact, much lower than 2, 1 and 0.5% (1.26, 1.50 and 1.25 mm^2 respectively). The value for 1% in the second experiment is rather high when plotting area of influence against concentration (Figure 16). It is clear from the graph that there is a strong linear relationship and even including the anomalous 5 and 1% values in a regression analysis, a good correlation was obtained ($P<0.001$). The resulting equation was:

$$\text{Area of influence} = 1.18 + 0.487\ (\text{Concentration})$$

The remarkable conclusion from this equation is that, in theory, if concentration was reduced to 0% the area of influence of the droplet would still be 1.2 mm^2. The actual area of spread of the oil, as observed under the scanning electron microscope, would have been only about 0.039 mm^2 for a 100 µm droplet and even

TABLE 8: Summary table showing regression coefficients, Ld_{50} values, areas of influence and relative amounts of released chemicals ($\propto$) for all droplet experiments of permethrin and pirimiphos-methyl

Experiment	Treatment	Droplet size (µm)	Intercept	Slope	Adjusted intercept*	Mean co-ordinates $\bar{x}$	Mean co-ordinates $\bar{y}$	Ld_{50} (mm)	Area of influence (mm^2)	$\propto$ (arbitrary units)**
Concentration (1)	20%	113	3.45	-15.54	3.27	0.267	-1.38	0.466	2.24	1307
(permethrin in VK1 on	10%	107	2.79	-13.87	3.03	0.252	-1.40	0.388	1.72	858
tobacco)	5%	117	3.14	-17.45	2.59	0.212	-1.12	0.326	1.03	385
Concentration (2)	20%	102	2.63	-12.36	3.30	0.269	-1.37	0.430	2.15	1389
(permethrin in VK1 on	10%	104	2.89	-14.27	3.03	0.249	-1.33	0.393	1.65	858
tobacco)	5%	105	3.70	-18.73	2.84	0.223	-0.96	0.377	1.43	602
	2%	101	3.25	-17.06	2.75	0.226	-1.19	0.355	1.26	522
	1%	110	2.83	-14.34	2.95	0.233	-1.03	0.376	1.50	739
	0.5%	108	2.51	-13.58	2.80	0.235	-1.37	0.339	1.25	570
Formulation (10%	JF 8130	113	1.90	-11.49	2.71	0.242	-1.77	0.290	0.99	483
permethrin on tobacco)	VK1	110	3.14	-14.80	3.15	0.256	-1.29	0.426	1.90	1054
	JF 8133	108	4.22	-19.40	3.11	0.243	-0.99	0.447	1.92	987
Droplet size (10%	59	59	2.61	-14.65	2.65	0.223	-1.33	0.321	1.07	433
permethrin in JF 8133)	114	114	3.97	-18.93	3.02	0.231	-0.82	0.417	1.67	840

/Continued ...

TABLE 8 (Continued)

Experiment	Treatment	Droplet size (μm)	Intercept	Slope	Adjusted intercept*	Mean co-ordinates $\bar{x}$	Mean co-ordinates $\bar{y}$	Ld_{50} (mm)	Area of influence (mm^2)	α (arbitrary units)**
Plant species	Tobacco	110	3.14	-14.80	3.15	0.256	-1.29	0.426	1.90	1054
(10% permethrin	Tomato	106	2.88	-12.09	3.67	0.286	-1.15	0.537	3.44	2660
in VK1)	French bean	106	3.32	-13.13	3.85	0.295	-1.10	0.618	4.39	3553
Insecticides	Permethrin	102	3.14	-15.66	2.95	0.233	-1.00	0.386	1.40	742
(10% in JF 9709)	Pirimiphos-methyl	107	9.98	-22.43	-	0.455	-0.46	6.099	401.50	-
Pirimiphos-methyl	Still air	104	10.12	-22.58	-	0.458	-0.45	6.424	444.45	-
(10% in JF 9709)	Still air (cut)	104	9.95	-22.22	-	0.457	-0.43	6.345	436.95	-
	Moving air	104	4.33	-14.37	-	0.325	-0.67	1.007	12.10	11576

* See Section 4b. 12) ii)

** See Section 4b. 12) iii)

TABLE 9: Effect of concentration on the biological efficiency of droplets of permethrin in VK1

Concentration (%)	20	10	5	2	1	0.5
Droplet size (μm)	102	104	105	101	110	108
Ld_{50} (mm)	0.430	0.393	0.377	0.355	0.376	0.339
LS_{50} (mm)	1.57	1.38	1.29	1.20	1.32	1.20
Area of influence (mm^2)	2.15	1.65	1.43	1.26	1.50	1.25
LN_{50} (droplets cm^{-2})	46.6	60.5	69.8	69.7	66.5	79.9
LD_{50} (μg cm^{-2})	5.18	3.56	2.12	0.752	0.463	0.264

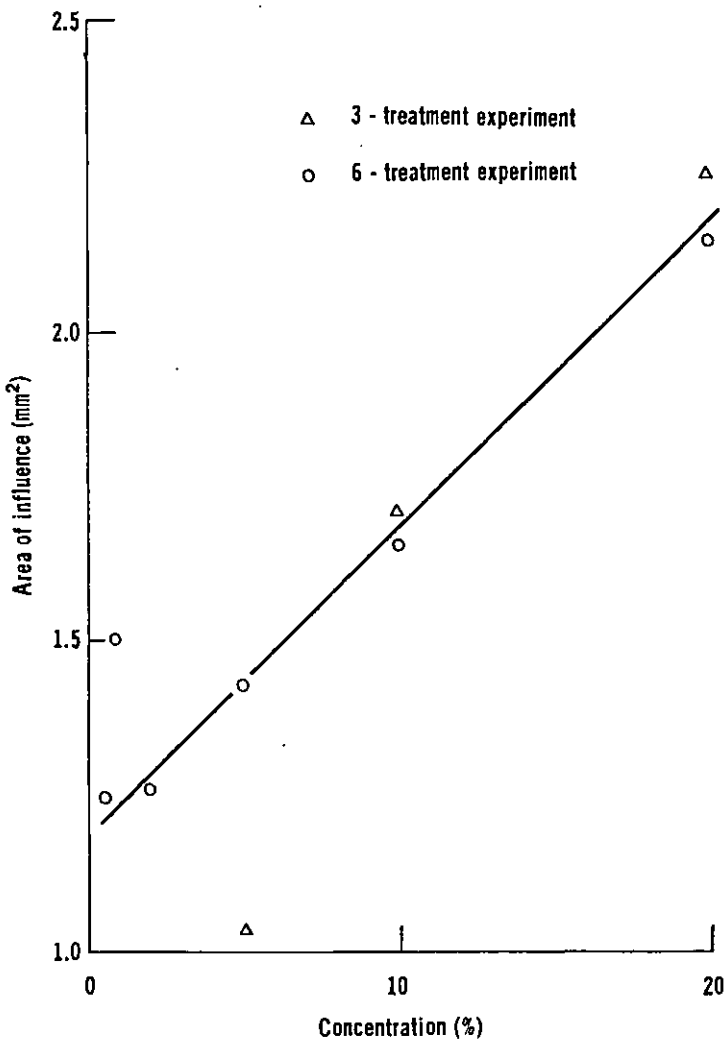


Figure 16.
Relationship between area of influence and concentration of active ingredient.

mortalities observed in the vicinity of droplets of the blank formulation were far too low to account for such an effect. It must be concluded that the effectiveness of droplets only fell off appreciably at concentrations below 0.5%.

The graph also shows that from 0.5% to 20% the area of influence only increases from 1.2 to 2.2 mm^2 although the concentration has increased 40 times. This indicates a great loss of efficiency with increasing toxicant concentration. The best comparison to make is on the estimated LD_{50}: the amount of insecticide per unit area of leaf required to give 50% mortality (in $\mu g/cm^2$). The derived figures (Table 9) are 0.264 and 5.18 ug/cm^2 respectively, showing that 0.5% concentration is 19.6 times more economical than 20%.

ii) Effect of Droplet Size

A comparison between two droplet sizes was made. These were 59 and 114 μm droplets of 10% permethrin in JF 8133. Logit-log log regression gave median lethal distance (Ld_{50}) values of 0.32 and 0.42 mm for the 59 and 114 μm diameter droplets respectively. Estimated areas of influence were 1.07 and 1.63 mm^2 (Table 10). Thus, although the larger droplet contained 7.2 times as much insecticide as the smaller, it was able to affect only 52% more area. Looked at from the point of view of estimated LD_{50}s, the smaller droplets would require 1.01 $\mu g/cm^2$ to give 50% mortality, whereas the larger would need 4.64 $\mu g/cm^2$. Therefore halving the size of droplets can increase the efficiency and economy of spraying by at least four times.

TABLE 10: Effect of droplet size on the biological efficiency of droplets of 10% permethrin in JF 8133

Droplet size (µm)	59	114
Ld_{50} (mm)	0.321	0.417
LS_{50} (mm)	1.11	1.39
Area of influence (mm^2)	1.07	1.63
LN_{50} (droplets cm^{-2})	93.7	59.8
LD_{50} (µg cm^{-2})	1.01	4.64

iii) Effect of Formulation

The effects of three formulations (JF 8130, VK1 and JF 8133) were studied in a direct comparison. Permethrin was dissolved in each of these to give a 10% solution and droplets of closely-similar size were sprayed. In a separate experiment 10% permethrin was also used in JF 9079. Some difference in slope was evident between VK1 and JF 8133 but the behaviour of the two was remarkably similar, giving Ld_{50}s of 0.43 and 0.45 mm and areas of influence of 1.90 and 1.92 mm^2 respectively (Table 11). JF 8130, on the other hand, gave an Ld_{50} of 0.29 mm and an area of influence of 0.99 mm^2. Thus to achieve the same kill, twice as much permethrin would need to be used when formulated in JF 8130.

Although not strictly comparable, as it was tested at a different time, JF 9079 gave an Ld_{50} of 0.39 mm and an area of influence of 1.40 mm^2, and was apparently intermediate in its effect.

iv) Effect of Plant Species

Comparisons were made between the effectiveness of permethrin (10% in VK1 with 106 to 110 µm droplets) on the leaves of three species of plant: tobacco, tomato and French bean. Calculated median lethal distances (Ld_{50}) were found to be 0.43, 0.54 and 0.62 mm respectively and the areas of influence were 1.90, 3.44 and 4.39 mm^2 (Table 12). Thus the insecticide was assessed to be most effective on bean, and that 2.3 times as much would be required to attain the same kill on tobacco and 1.3 times as much on tomato.

TABLE 11: Effect of formulation on the biological efficiency of droplets of 10% permethrin

Formulation	JF 8130	JF 9079	VK 1	JF 8133
Droplet size (μm)	113	107	110	108
Ld_{50} (mm)	0.290	0.386	0.426	0.447
LS_{50} (mm)	1.07	1.27	1.48	1.49
Area of influence (mm^2)	0.99	1.40	1.90	1.92
LN_{50} (droplets cm^{-2})	101.0	72.1	52.7	52.0
LD_{50} ($\mu g\ cm^{-2}$)	7.63	4.62	3.67	3.43

TABLE 12: Effect of plant species on the biological efficiency of droplets of 10% permethrin in VK1

Plant	Tobacco	Tomato	French bean
Droplet size (μm)	110	106	106
Ld_{50} (mm)	0.426	0.537	0.618
LS_{50} (mm)	1.48	1.99	2.25
Area of influence (mm^2)	1.90	3.44	4.39
LN_{50} (droplets cm^{-2})	52.7	29.1	22.8
LD_{50} ($\mu g\ cm^{-2}$)	3.67	1.81	1.42

Munthali (1981) similarly found that twice the amount of dicofol was needed on pepper as on French bean, to kill mite eggs. So, although direct comparisons cannot be made, in both cases pesticide was considerably more effective on bean than on the other test plants.

v) Comparison Between the Effectiveness of Permethrin and Pirimiphos-Methyl

A comparison was made between the effects of permethrin and pirimiphos-methyl, at 10% in a suitable solvent, JF 9079. Permethrin gave an Ld_{50} of 0.39 mm and an area of influence of 1.40 mm^2, as already noted. The behaviour of pirimiphos-methyl however was fundamentally different. Although the shape of the response curve conformed very closely with the logit:log log model the slope and intercept differed significantly from those of permethrin. The Ld_{50} was calculated as 6.10 mm and the area of influence 402 mm^2 (Table 13). Thus only one droplet would be needed for every 4 cm^2. Therefore 290 times as much permethrin would be required to achieve the same result! This level of activity cannot be explained by diffusion of chemical through the leaf cuticle (see Section 4b. 12)) and may be explained by the known vapour pressure properties of pirimiphos-methyl and theories of gaseous diffusion.

TABLE 13: A comparison between the biological efficiencies of droplets of 10% pirimiphos-methyl and 10% permethrin in JF 9079

Chemical	Pirimiphos-methyl	Permethrin
Droplet size (μm)	102	107
Ld_{50} (mm)	6.10	0.386
LS_{50} (mm)	21.6	1.27
Area of influence (mm^2)	402	1.40
LN_{50} (droplets cm^{-2})	0.249	72.1
LD_{50} ($\mu g\ cm^{-2}$)	0.0138	4.62

vi) Fumigant and Contact Effects of Pirimiphos-Methyl

The effect of pirimiphos-methyl over a larger area was examined in more detail to assess the relative importance of fumigant effects in contrast to diffusion of the spray liquid over the surface of leaf. A line of droplets was sprayed along the edge of each separate leaf strip which was placed each alongside a test leaf piece with a gap of about 0.2 mm, cutting the recorded scales completely from the source of insecticide. Those and test leaves on which the droplets were directly applied were kept in still air and mortalities were recorded. The responses of the two treatments were virtually identical: The slopes, -22.5 and -22.6; The intercepts, 9.95 and 10.1; Ld_{50}s, 6.34 and 6.42 and the areas of influence 437 and 444 mm^2 (Table 14). The main activity of pirimiphos-methyl was undoubtedly by fumigant action bridging the leaf pieces.

When a gentle air flow was passed over treated leaf pieces the response was quite different. The slope of the response line became -14.4, typical of the values for permethrin. The intercept became 4.33, a little higher than permethrin but less than a half of the value for pirimiphos-methyl in still air. The Ld_{50} was 1.01 mm, a sixth of that in still air, and the area of influence was 12.1 mm^2 compared with 1.4 for permethrin and 444 for pirimiphos-methyl in still air. The behaviour of pirimiphos-methyl in moving air was very similar to that of permethrin, so that activity was mainly by diffusion across the leaf surface. However, pirimiphos-methyl was more toxic than permethrin under similar conditions. Most or all toxicity due to vapour pressure effects was removed by the air flow.

TABLE 14: Effects of fumigant and contact action on the effectiveness of droplets of 10% pirimiphos-methyl in JF 9079

	Still air (droplets on .. Test leaves)	Still air (droplets on .. Separate leaf strips)	Air flow over test leaves
Droplet size (µm)	104	104	104
Ld_{50} (mm)	6.42	6.35	1.01
LS_{50} (mm)	22.6	22.4	3.74
Area of influence (mm^2)	444	437	12.1
LN_{50} (droplets cm^{-2})	0.225	0.229	8.27
LD_{50} (µg cm^{-2})	0.0133	0.0135	0.487

9) INTERACTION OF DROPLETS

The interaction between neighbouring droplets was investigated to confirm whether theories proposed earlier were valid. Droplets of 10% permethrin in VK1 were sprayed on infested tobacco leaves in equilateral triangles (as far as practicable) spaced either 3.0 or 1.1 mm apart, on average.

At 3 mm spacing interference between droplets is negligible in this area since mortality was no higher than that expected from single droplets at that distance. The distribution of mortality around the droplets, in fact, was very similar to that previously found with single droplets of 10% permethrin in VK1. It was therefore concluded that no measurable interference had occurred at a distance of 3 mm. It was decided that this treatment could act as a better 'control' for comparison with 1.1 mm spacing treatment than other tests at about the same time.

Assuming that the droplets were behaving as if they were isolated, the effect that might be expected if the droplets were brought within 1.1 mm of each other was estimated. Fitting a logit:log log equation in the usual way the following relationship was found; giving a good fit to the data (Figure 17a):

$$\text{Logit } p = 3.707 - 18.60 \log_{10} \log_{10} (100\ d)$$

Estimated concentrations were calculated from the smoothed mortality values (Table 15, Figure 17b). By adding these in two directions with a 1.1 mm spacing and including small estimated contributions from the third droplet, an U-shaped curve (Figure 17c) was obtained. This, in turn, was translated back into

TABLE 15: Calculations of estimated concentrations and expected mortalities between droplets spaced 1.1 mm apart using mortality data of droplets of 10% permethrin in VK1 spaced 3.0 mm apart in a triangular pattern and mean mortalities between three droplets at 3.0 mm and 1.1 mm spacings

Distance (mm)	Observed		Smoothed		Contributions from 2nd & 3rd droplets			Total conc.	Logit mort.	Expected % mort.	Relative area	Mort. product
	Mortality	Conc. (arbitr. units)	Mortality	Concentration								
0.05	100		100							100	1	100
0.15	100		99.24	294.44	+0.202	+0.162	=	294.804	2.835	99.24	2	198.48
0.25	91.67	32.380	89.65	26.137	+0.286	+0.176	=	26.599	1.089	89.83	3	269.49
0.35	70.59	8.310	64.55	6.489	+0.427	+0.189	=	7.105	0.351	66.83	4	267.32
0.45	28.57	1.675	38.41	2.492	+0.684	+0.197	=	3.373	-0.067	46.68	5	233.40
0.55	18.18	0.991	21.79	1.213	+1.213	+0.202	=	2.628	-0.206	39.83	3	119.49
0.65	8.70	0.465	12.80	0.684						Summation	18	1188.18
0.75	11.11	0.593	7.97	0.427								
0.85	7.41	0.398	5.24	0.286								
0.95	6.67	0.359	3.62	0.202								
1.05			2.60	0.149								
1.15			1.93	0.114								
1.25												
1.35	0.89	0.094										
1.45												
1.55												
1.65												

Estimated mean mortality between 3 droplets spaced at 3 mm = $\frac{1465.04}{98} = 14.95\%$

Expected mean mortality between 3 droplets spaced at 1.1 mm = $\frac{1188.18}{18} = 66.01\%$

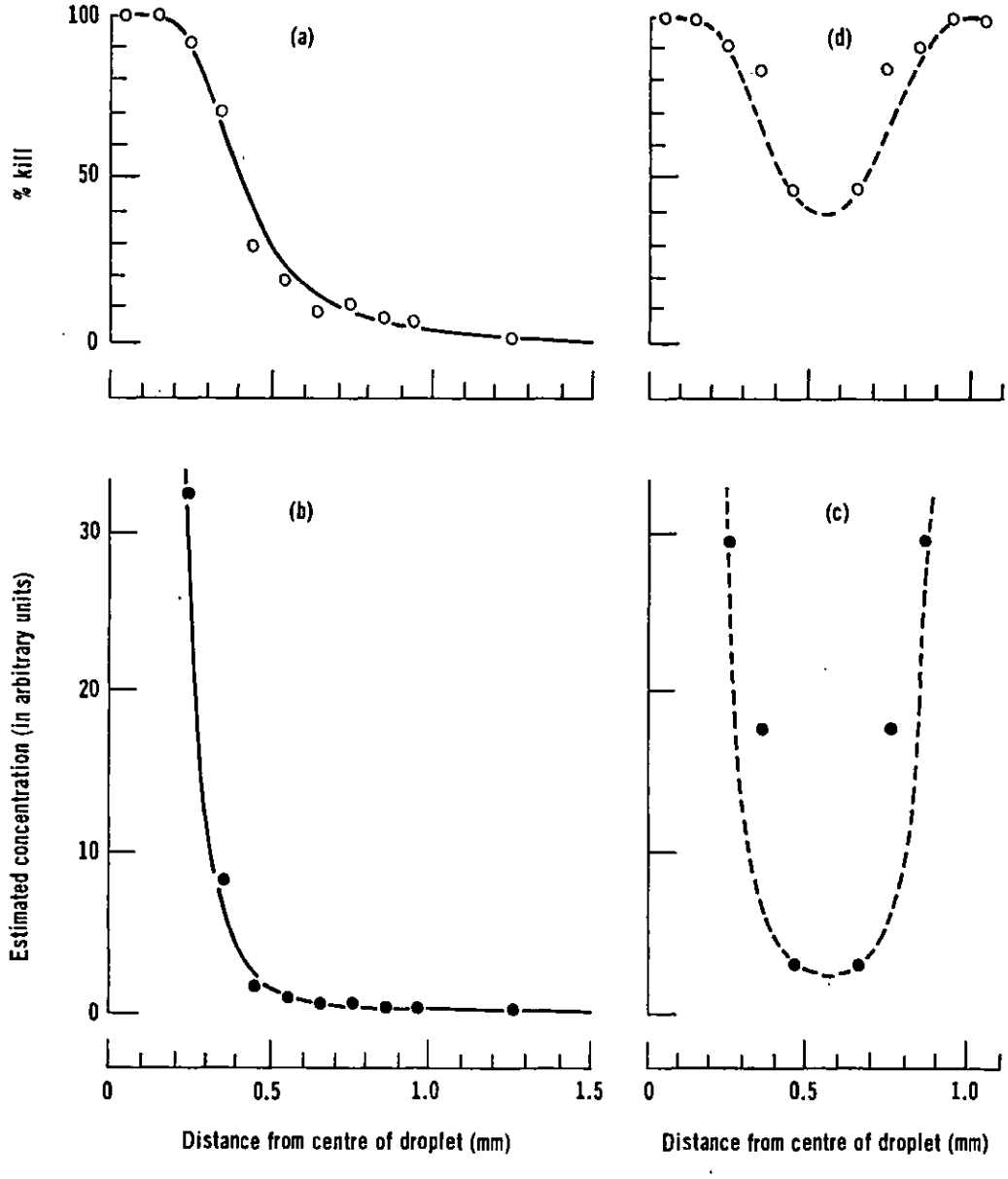


Figure 17.

Interaction of droplets of 10% permethrin in VK 1 at 3.0 and 1.1 mm spacings in a triangular pattern on tobacco leaves.

(a) Mortality between three droplets spaced 3.0 mm apart.

(b) Estimated concentration between three droplets spaced 3.0 mm apart.

(c) Estimated concentration between three droplets spaced 1.1 mm apart calculated by the overlap method.

(d) Theoretical regression line of mortality between three droplets spaced at 1.1 mm apart, and points of corrected mortality of the 1.1 mm spacing treatment.

mortalities which might be expected when three droplets were spaced at 1.1 mm intervals (Figure 17d). This curve was then compared with the observed mortalities obtained from the 1.1 mm spacing experiment. The agreement between fact and theory was excellent. Only one point lay at any distance from the line and this, in any case, was inconsistent with the neighbouring points. The likelihood of error, in fact, was very great, for the displacement of this point could have been brought about by the death of only four additional scales out of forty one. The mortality equidistant from two droplets was found to be about 40%, or about double that at the same distance from isolated droplets. At this point mortality would appear to be additive, but this certainly was not the case at other positions.

These calculations were used to assess the overall mortality between droplets spaced at 1.1 mm. Making allowance for increasing areas of concentric circles, the mean mortality was calculated as 66%. A similar calculation for 3 mm spacing revealed a mean mortality of about 15%. These values would also apply in theory with a uniform triangular spacing over the entire leaf surface.

10) THEORETICAL MORTALITY RESPONSE CURVES

The droplet spacing which would, in theory, produce 50% mortality of whitefly scales was determined. Using the same method of calculating mortality with droplets at different distances, mortality was estimated over a wide range of spacings between droplets and therefore over a range of dosages per unit area. In this way an expected mortality response curve could be obtained.

The response curve of mortality against dose or $\log_{10}$ dose obtained using 20% permethrin in VK1 (Appendix Table 4) showed a smooth sigmoid curve (Figure 18a). It follows that, in theory, if a sedentary target is subjected to a range of spray droplet densities, a sigmoid (closely resembling a probit-log response) can be expected. This conclusion was made quite independently of the normal explanation of a probit response based on probability theory.

For comparison, the same process was carried out for the 5% permethrin treatment. Although a shallower curve was obtained when plotting mortality against dose, very similar sigmoid curves were obtained when plotting mortality against $\log_{10}$ dose (Figure 18a). the only obvious difference being a lateral displacement due to differences in LD_{50}.

When the logit (or probit) transformations of the percentage kill were plotted against $\log_{10}$ dose a gently rising curve rather than a straight line was obtained (Figure 18b). However, it was sufficiently close to a straight line, particularly in the central region, to consider fitting a weighted logit line. The resulting equation for 20% permethrin was:

$$\text{Logit kill} = -0.696 + 1.412 \log_{10} \text{dose}$$

It is constructive to compare this with the logit equation obtained from the dipping experiment:

$$\text{Logit kill} = -0.747 + 1.289 \log_{10} \text{dose}$$

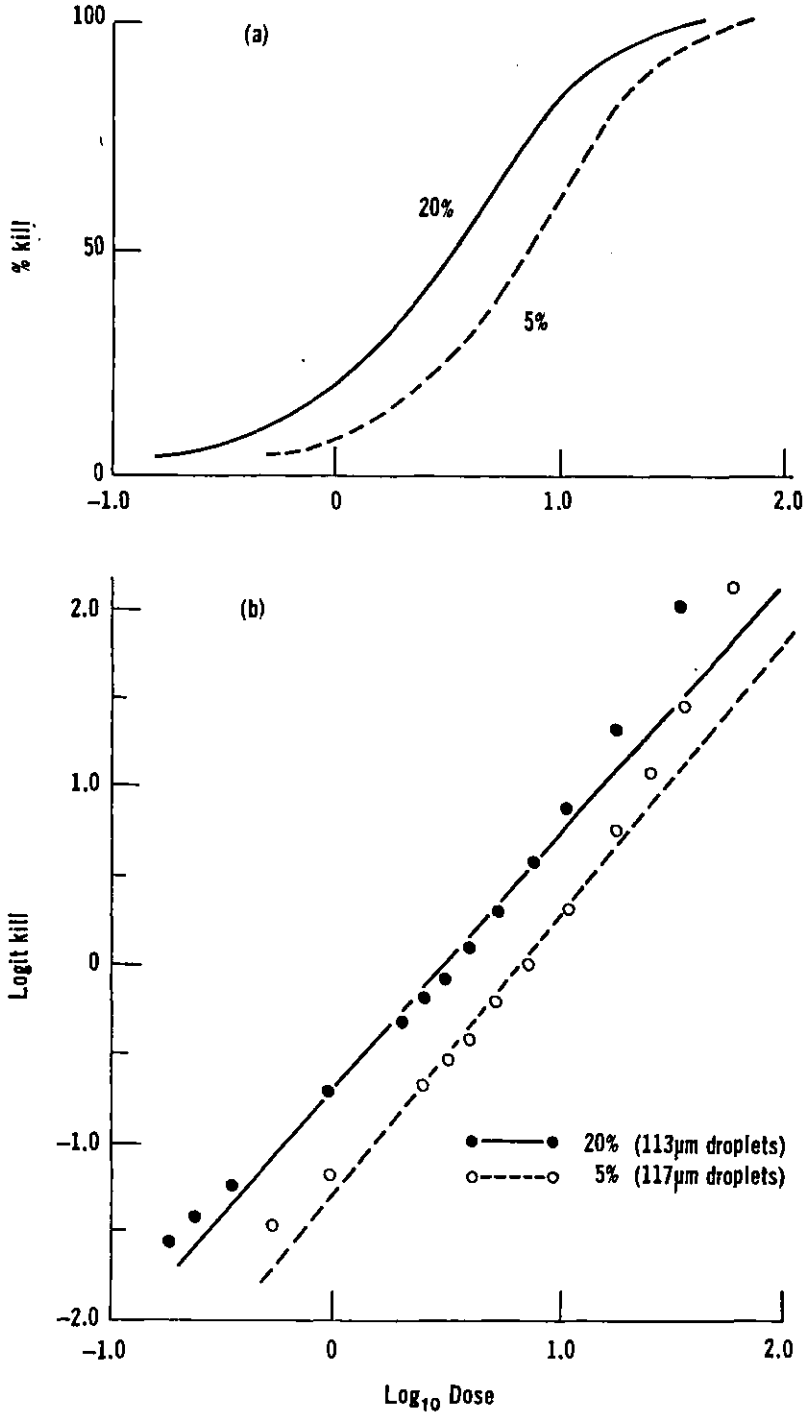


Figure 18.

Theoretical response curves of mortalities of whitefly settled first instar larvae treated with droplets of 20% and 5% permethrin in VK1 on tobacco leaves.

(a) Percentage kill against $\log_{10}$ dose.

(b) Logit kill against $\log_{10}$ dose.

Thus, although the differences are considerable between dipping and a theory based on spraying, the slopes of the response lines are very similar. The similarity of the intercept is only coincidental since the doses from dipping can only be regarded as arbitrary.

Calculations for the 5% permethrin treatment also gave a gently rising curve. Fitting a weighted regression gave a closely parallel line with the following equation:

$$\text{Logit kill} = -1.294 + 1.552 \log_{10} \text{dose}$$

The line was again displaced by the difference in LD_{50}s. The calculation of LD_{50}s from these equations is less accurate than the previous method due to the curvature of the true line.

Hypothetical transformations and other assumptions have been made to arrive at the above response curves which cast doubt on the validity of the results. The mortality around a single droplet has been used to predict the mortality over the whole leaf surface from scattered droplets.

How well does this prediction conform with fact? It is necessary to find comparable data from sprayed surfaces to test that. Only one set of data appears to be available. Palmer (personal communication) sprayed first instar whitefly scales on tomato leaves with varying densities of 107 µm droplets of 10% permethrin in VK1. Thus the results should be directly comparable with the one tomato treatment in the present series of experiments. Weighted logit analysis of the data gave an estimate for the median lethal number of droplets per cm^2 (LN_{50}) of 32.1. This compares very favourably with the estimate for tomato by the present

method, of 29.1 and contrasts markedly with the values for tobacco of 59.8, 52.7, 58.1 and 60.5. This agreement was only for the density of droplets causing 50% mortality and could well have occurred by chance. Both methods were also used to determine densities killing other percentages, as expressed by the slope of the logit lines. Here the agreement was even closer. The slope from the spray experiment was 1.245 and that derived from the single droplet experiment was 1.247. The full extent of the agreement between the two methods is shown by the regression equation which, for the spray experiment was:

$$\text{Logit kill} = -1.876 + 1.245 \log_{10} N$$

and from the single droplet experiment:

$$\text{Logit kill} = -1.803 + 1.247 \log_{10} N$$

A further point of comparison is the upward curvature of the logit response. In the spray experiment of Palmer, the mortality at the higher droplet densities was much higher than would be expected for either a logit or a probit response (Figure 19) giving a poor fit despite ample data in this area. This increased mortality at high droplet densities is reflected in the curvature of the theoretical points derived from single droplets. Thus there was better agreement between the observed and theoretical points than between the observed points and the fitted logit line.

Another fact seeming to confirm this curvature was the result of the dipping tests, which also showed a greater response from

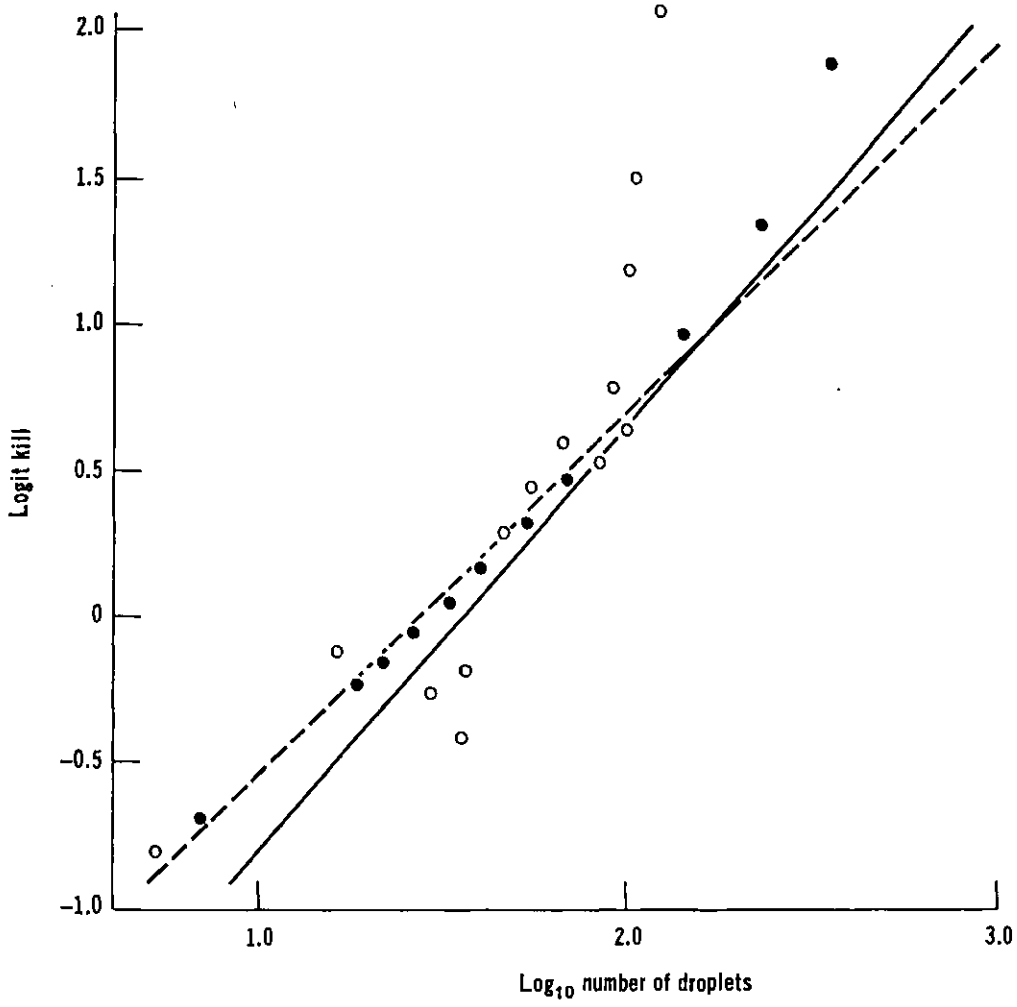


Figure 19.

Comparison between mortality of first instar whitefly larvae sprayed with different droplet densities of 10% permethrin in VK1 on tomato plants and estimated mortality from single droplets.

○ Spray experiment, ● Single droplet experiment.

the highest concentration than would be expected from a probit relationship (Figure 10). In this case, however, the slope of the response curve cannot be attributed to the spacing of droplets, as in the previous two instances, since the insecticidal film was assumed to be continuous and mortality was probably due to direct contact of the formulation with all the test insects. If the reason for the curvature was the same in all cases (apart from by chance), it must reflect an unusual response of whitefly scales to permethrin which does not conform with normal probit-log response found with other insects. It might be associated with the sedentary habit of the insect, but is not necessarily a result of the spacing of droplets as appeared at first sight.

11) FURTHER THEORETICAL CONSIDERATIONS

i) Relationship Between Median Lethal Spacing (LS_{50}) and Median Lethal Distance (Ld_{50})

Since the 'median lethal spacing' (LS_{50}) of droplets is complicated to calculate using the overlapping method, it would be more convenient to find a simple relationship between this and some more readily-measured criteria. The median lethal distance (Ld_{50}), as previously defined, has little practical meaning but is readily calculated and suffers from little inaccuracy, since it is on the steepest part of the response curve. It could therefore provide a useful measure if it can be related to the median lethal spacing (LS_{50}). Plotting the calculated values of LS_{50} for all permethrin treatments against corresponding Ld_{50}s shows little divergence from a straight line (Figure 20a). Calculation of the

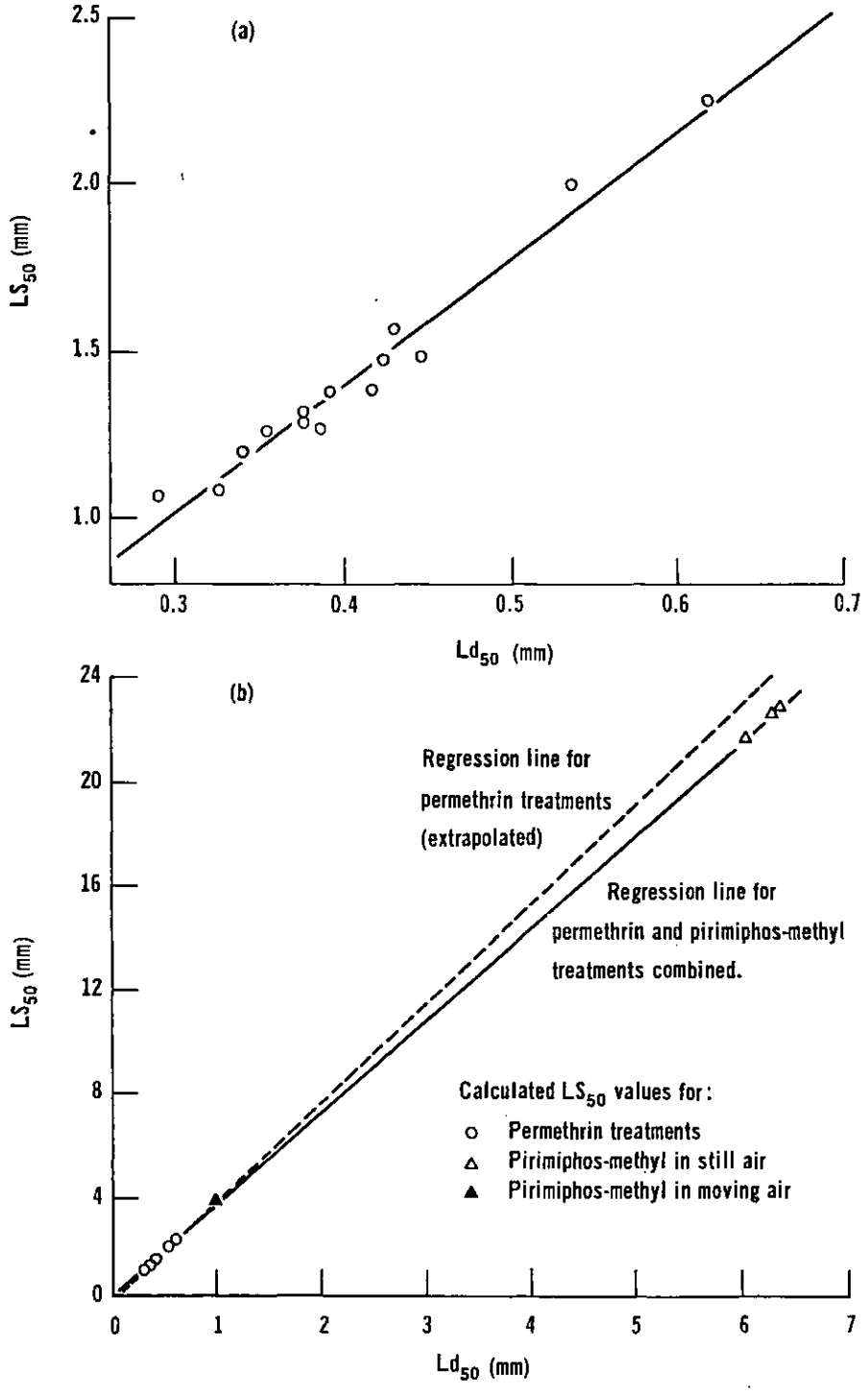


Figure 20.
Relationship between median lethal spacing (LS_{50}) and median lethal distance (Ld_{50})
(a) for permethrin treatments
(b) for treatments of permethrin and pirimiphos - methyl combined.

regression equation gives:

$$LS_{50} = -0.1221 + 3.803\ Ld_{50}$$

with a correlation coefficient of 0.985, showing a very precise linearity and an intercept close to the origin.

Extrapolating to an Ld_{50} of 6.3, the mean for pirimiphos-methyl in still air, gives an estimate of 23.8 for the LS_{50} compared with an observed value of 22.2 (Figure 20b). Similarly the estimate for pirimiphos-methyl in moving air is 3.71 compared with an observed value of 3.73. It would appear, therefore, that even extreme cases conform to the same law as for permethrin. Including these extra four points in the regression gives the equation:

$$LS_{50} = -0.00373 + 3.534\ Ld_{50}$$

with a correlation coefficient of 0.999. The intercept is so close to zero that it may be assumed that the line passes through the origin and that LS_{50} is precisely proportional to Ld_{50} so that:

$$\left[LS_{50} = 3.53\ Ld_{50} \right]$$

Since the area of influence (AoI) equals $0.866\ LS_{50}^2$, then it will also equal $0.866\ (3.53\ Ld_{50})^2$ or $10.8\ Ld_{50}^2$. Then since the median lethal number of droplets (LN_{50}) per cm^2 is 100/AoI it will also equal $9.26/Ld_{50}^2$. Further, a 100 um droplet of 10% permethrin contains 0.05236 ug of active ingredient, so the median lethal dose, LD_{50}/cm^2, will be $0.485/Ld_{50}^2\ \mu g/cm^2$. Equivalent values

for other droplet sizes and concentrations can readily be calculated from this simple formulae by proportion, remembering that the amount of active ingredient per droplet is proportional to the cube of the diameter.

ii) Relationship Between Slope and Intercept

The slopes of the logit:log log lines for permethrin vary from -11.5 to -18.9 (Table 8). It is not immediately clear whether this variation is due to natural causes, to an inability to determine the slopes with accuracy, or whether the slope varies with extent of spread of the insecticide, as measured, for instance, by the Ld_{50}. Slopes were plotted against Ld_{50} values to test this (Figure 21a). The resulting scatter of points is such that there is no indication of any relationship; nor is there any sign that concentration has any effect in the two concentration experiments.

Whether the slope can be regarded as constant for all permethrin treatments must be considered. A mean slope of -14.85 was calculated and, assuming this was constant, new values for intercepts were estimated by determining the line passing through the means and the log log co-ordinates (Table 8). Response curves which do not conform with observed data would invalidate the assumption that the slope is constant. To test this, two treatments (5% and 20%) having extreme slopes, namely -18.73 and -12.36 respectively (Table 8) were compared.

Using newly calculated intercepts of 5.670 and 6.605 with the mean slope, new response curves were plotted (Figures 21b and c), but these differed only slightly from the original curves so

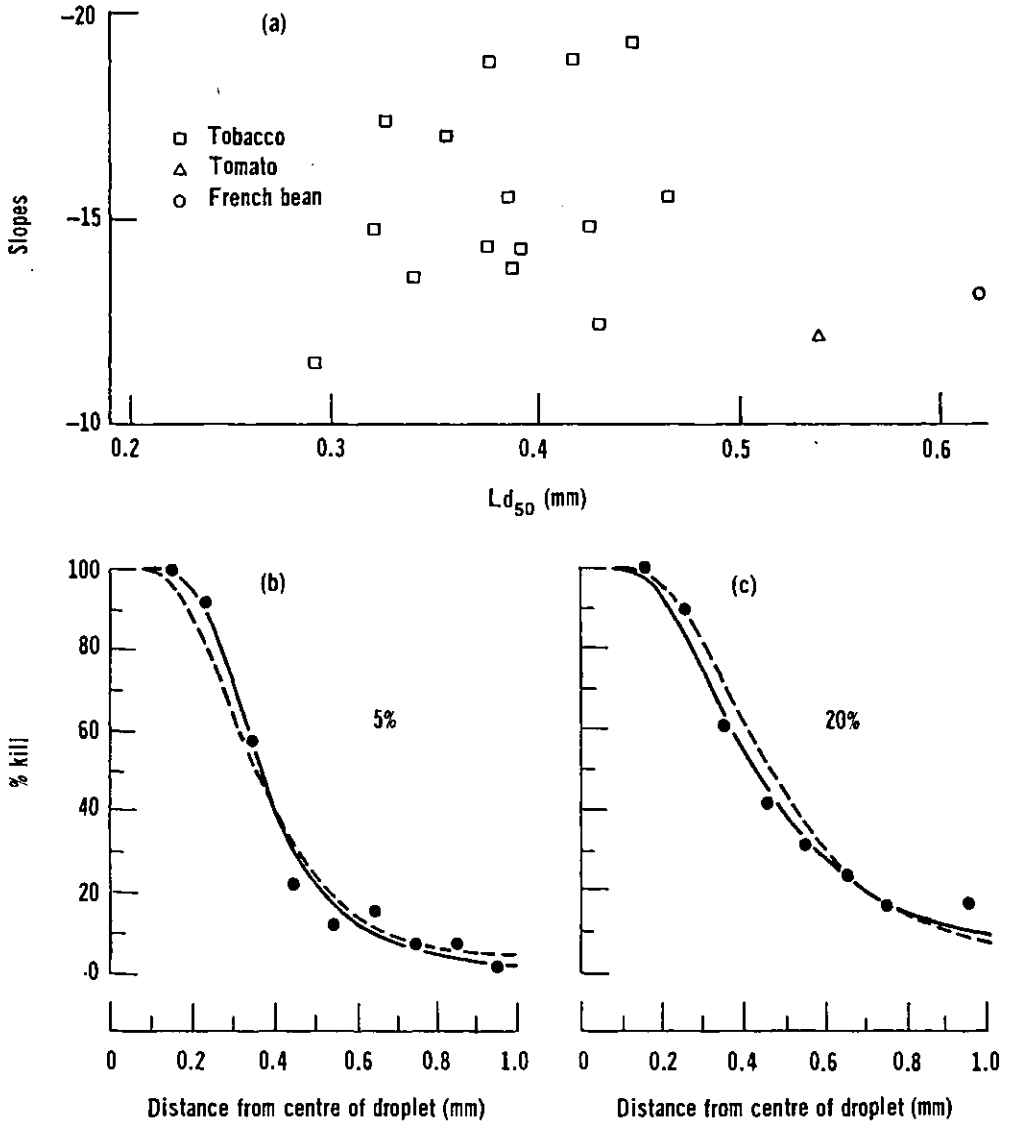


Figure 21.

(a) Slope against Ld_{50} for permethrin treatments.

Comparison between regression lines of mortality using original calculated slope (——) and fixed slope (– – –) of:

(b) 5% permethrin in VK1,

(c) 20% permethrin in VK1.

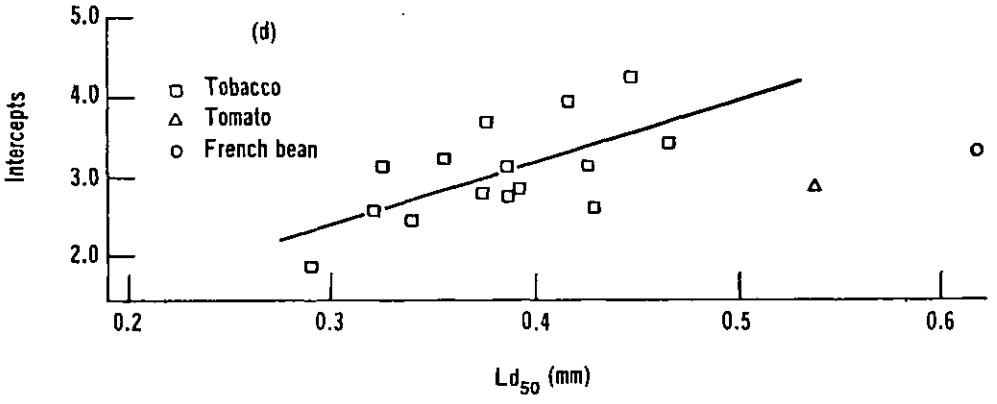


Figure 21.

(d) relationship between intercept and Ld_{50} for permethrin treatments. (The regression line is for treatments on tobacco only).

showed no marked inconsistencies with the observed data.

Since the two alternative lines cross near the centre of their range (at the weighted mean of the co-ordinates), the difference in slope makes little difference to the estimates of Ld_{50}s (0.36 and 0.46 compared with 0.38 and 0.43 originally). Nor is there any change in the general interpretation of the experiments.

The slopes calculated for pirimiphos-methyl in still air also appeared to be constant at about -22.4 but consistently different from slopes for permethrin. This suggests that the slope may be a value akin to the diffusion constant, having one value for diffusion within wax and another for diffusion through air. This supposition is reinforced by the observation that, when air is moved over the pirimiphos-methyl deposit, the slope reverts to 14.37, close to the mean for permethrin, suggesting that diffusion is only taking place through the leaf surface and that the fumigant effect of the toxicant has been removed.

It might equally be contended that the intercept remains a constant while the slope varies. However, plotting the intercept against the Ld_{50} shows a fairly clear relationship between the two (Figure 21d). Regression analysis for droplets of permethrin treatments on tobacco leaves gives an equation:

$$\text{Intercept} = 0.149 + 7.65\ Ld_{50}$$

with a correlation coefficient of 0.641 ($P<0.1$). The intercept, therefore, appears to be dependent upon the spread of the insecticide, rather than the slope. Once it is assumed that the

slope is constant, the intercept becomes directly related to the spread of insecticide as indicated by the Ld_{50}.

iii) Application of the Constant Slope Relationship

Calculating the relative total amount of chemical diffusing from a droplet on to theleaf surface from the weighted regression line of logit on log log results in considerable distortion at points close to the droplet due to differences in the estimates for the value of the slopes. When a constant value for slope is used, this problem no longer exists; thus with a constant mean slope of -14.85 the regression equation becomes:

$$\text{Logit kill} = a - 14.85 \log_{10} \log_{10} (100\ d)$$

Also, from the dipping tests:

$$\text{Logit kill} = -0.747 + 1.289 \log_{10} \text{Conc.}$$

It follows that the concentration, 'C' in arbitrary units, at any distance, 'd' in mm, from the centre of the droplet may be estimated by:

$$\text{Log}_{10}\ C = 0.58 + 0.776a - 11.52 \log_{10} \log_{10} (100\ d)$$

Converting this to a power equation by taking antilogs,

$$C = 10^{0.58 + 0.776a} (\log_{10} 100\ d)^{-11.52}$$

Thus, at any distance from the centre of droplet, the concentration is proportional to the value antilog_{10} (0.58 + 0.776a). This

value is a constant for any treatment and may be termed α. Since the concentration at all distances is proportional to α, it follows that the summation of all concentrations, and therefore the total amount of toxicant on the leaf surface will be proportional to α. Thus α may be used as a comparative value expressing the amount of toxicant leaving the droplets in any one treatment. Values of α have therefore been calculated for all permethrin treatments (Table 8) and will be used for comparison later.

The value of α is also of use in estimating the concentration at any distance from a droplet or preparing distribution curves. The concentration can simply be estimated from the equation:

$$C = \alpha (\log_{10} 100\ d)^{-11.52}$$

When d = 0.1 mm, then $(\log_{10} 100\ d)^{-11.52}$ equals unity and the concentration equals α. Thus α always represents the expected concentration at 0.1 mm from the droplet centre.

The value of α is calculated from the adjusted intercept, 'a', assuming the constant slope. The value of 'a' may be calculated without recourse to regression analysis from the mean values of $\bar{x}$ and $\bar{y}$ co-ordinates, where $\bar{x}$ is the mean of $\log_{10} \log_{10} (100\ d)$ values and $\bar{y}$ is the mean of the logit kill values, provided these means are estimated near the middle of the response range. The value of 'a' can then be determined by:

$$a = \bar{y} + 14.85\ \bar{x}$$

Similarly, the median lethal distance (Ld_{50}) can be calculated with equal simplicity since the logit kill will be zero:

$$Log_{10} \log_{10} (100\ d) = \frac{\bar{y}}{14.85} + \bar{x} = \frac{-a}{14.85}$$

12) ALTERNATIVE MODELS

i) Normal Distribution of Toxicant From a Point Source

It was originally anticipated that the distribution of insecticide around a minute droplet would follow a normal curve with distance based on the laws of diffusion from a point source (Crank, 1956; equation 3.4). If this were so, the expected mortality around a droplet could be calculated from the probit-log dose relationship. The resulting curve (Figure 22) is flat topped over a considerable distance, is broadly convex for much of the declining region and has an abrupt 'tail'. This is quite unlike any of the mortality curves found in the present study which have a short plateau, fall abruptly into a concave curve and then extend into a long tail. No modification of constants could achieve a better agreement between the two basic curves.

It was therefore concluded that either the insecticide around the droplet was not normally distributed or that the mortality response of whitefly scales to the insecticide was not of the probit form. It was partly for this reason that the dipping tests were carried out. However, the results seemed to indicate a probit response apart from one anomalous point (Figure 10) where mortality was very high and therefore errors were greater and weighting was

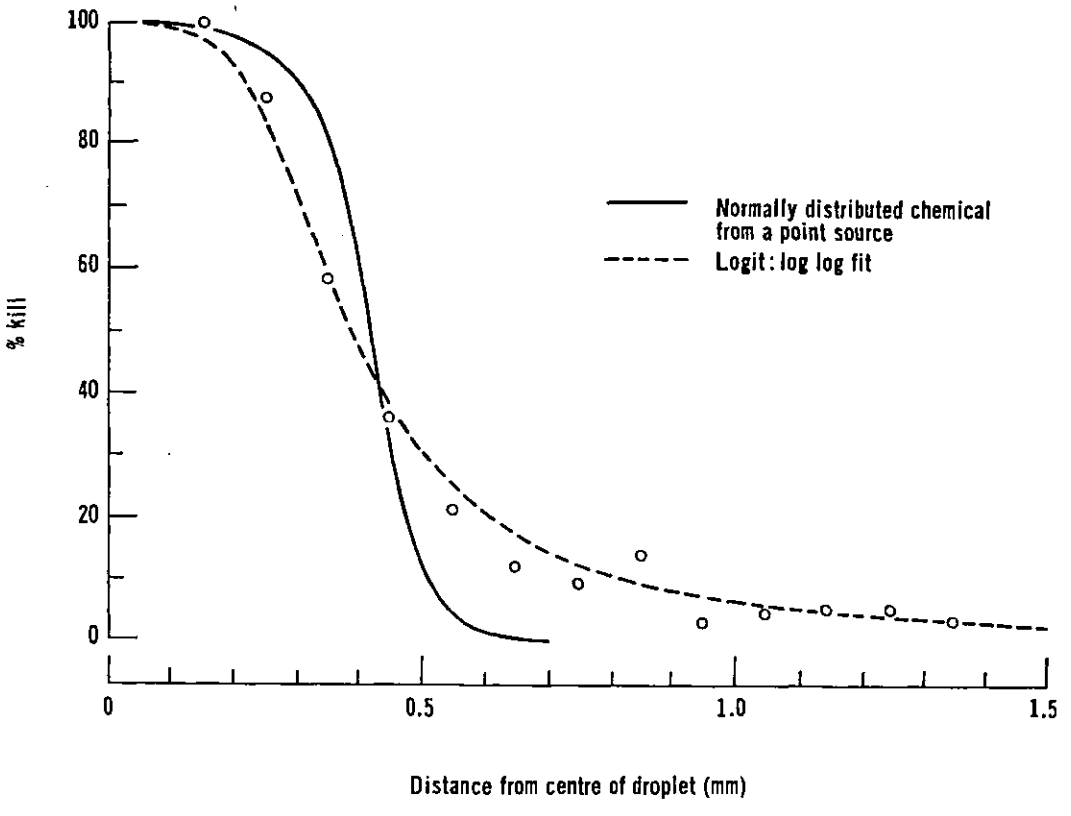


Figure 22.

Comparison between expected mortality for a normally distributed chemical from a point source and logit: log log fit for droplets of 10% permethrin in VK1 on tobacco.

very low. It was therefore decided to proceed on the assumption that the response was of a probit form and accept that it was likely that the distribution of insecticide was not entirely determined by random diffusion.

Further evidence from the results of Palmer discussed above indicates that the mortality response of whitefly scales to permethrin is not of a probit-log dose form. The possibility of an alternative interpretation was therefore considered. A mathematical expression which would adequately describe the mortality response in the dipping experiment was sought. The simplest suitable equation was:

$$\% \text{ kill} = 23 \sqrt{\text{Conc.}}$$

Using this equation, concentrations, on an arbitrary scale, for any observed kill can be calculated. To test this further, the results of five 10% permethrin treatments were combined to reduce variability. Mean mortalities were calculated for each distance from the droplet giving 88.3% at 0.25 mm and then, successively, 61.8, 38.1, 24.6, 15.6, 9.8, 13.0, 6.7% etc. Corresponding estimated concentrations were 14.74, 7.21, 2.75, 1.15, 0.46, 0.18, 0.32, 0.09 units. When plotted against distance, these points follow a descending curve resembling the lower half of a normal curve. A normal curve can, in fact, be fitted closely to the first few points. The curve shown (Figure 23a) is derived from the equation:

$$\text{Conc.} = 32\, e^{-12.25\, d^2}$$

and has its apex at the centre of the droplet (d = 0). This indicates that the 'theoretical' concentration at this point is 32 arbitrary units. The mortality equation above indicates a concentration of 18.9 with 100% mortality, any higher concentrations also clearly giving total kill. This point is reached on the normal curve (Figure 23a) at 0.2 mm from the centre of droplet, which is in conformity with the observation that at 0.15 mm, total kill was always observed.

The diffusion equation given by Crank (1956) for a point source on an infinite plane is:

$$C = \frac{M}{4\pi Dt} e^{-r^2/4Dt}$$

The value of 12.25 in the previous equation clearly corresponds with 1/4 Dt in this equation, 'D' being the diffusion constant and 't' the time in seconds. Taking the time at observed mortality, namely 4 days or 345600 seconds, it is possible to estimate the value of the diffusion constant as 6×10^{-8}, which is the order of value expected for diffusion through a solid. This therefore confirms the impression that the mortality is due to the diffusion of toxicant through the surface wax of the leaf or perhaps through the cuticle or epidermis.

One anomaly of this model is that the normal curve indicates that the concentration falls quite rapidly to infinitesimal levels reaching 0.00015 arbitrary units and mortality of only 0.28% at 1 mm for the five combined treatments considered above. The observed mortality at this distance was about 5% and continued to fall, not

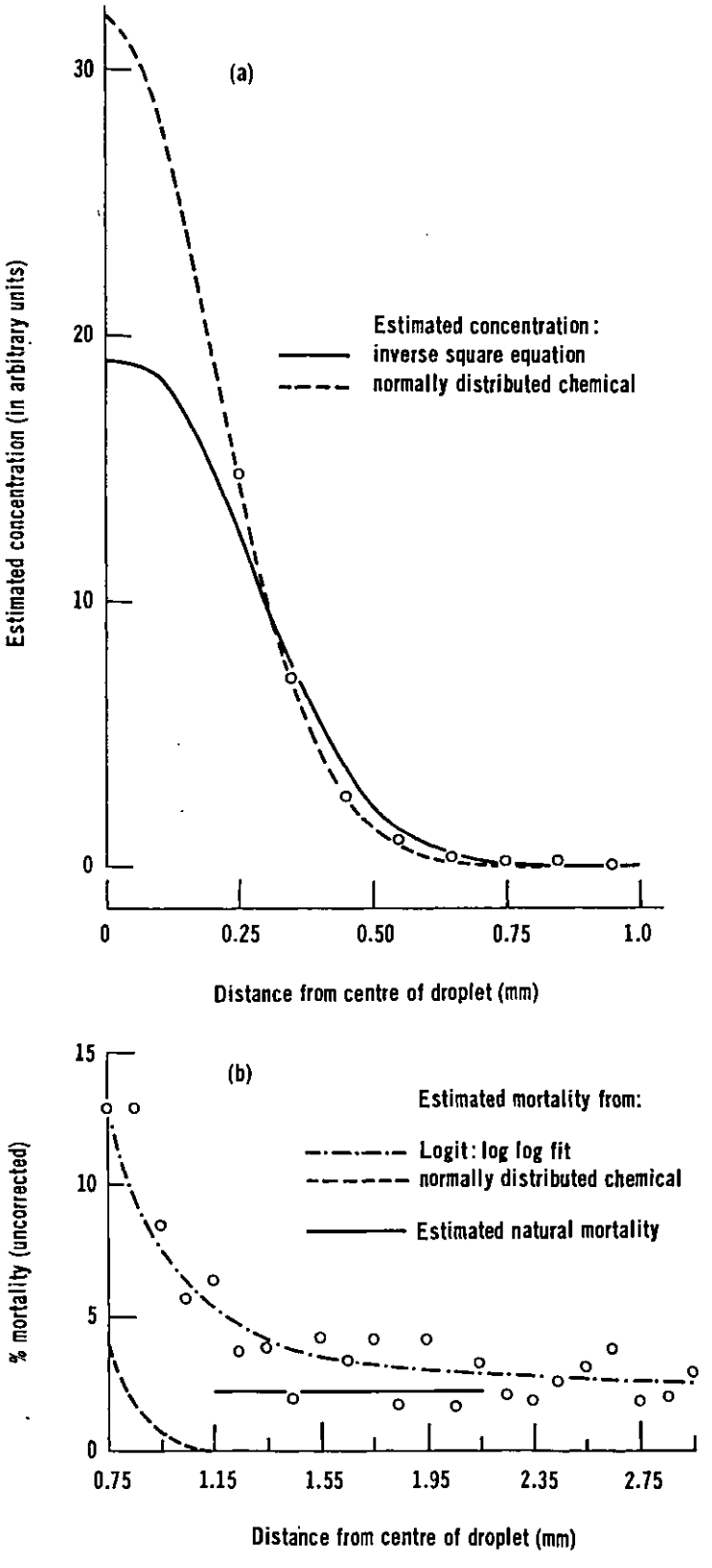


Figure 23.

(a) Estimated concentration for five combined treatments of permethrin calculated by the inverse square equation and estimated concentration of a normally distributed chemical.

(b) Comparison between expected mortalities from logit: log log fit and from a normally distributed chemical for five combined treatments of droplets of 10% permethrin.

approaching the estimated control mortality of 2.2% until beyond 2 mm. In confirmation of this there is a significant regression ($P<0.01$) of mortality on distance between 1 mm and 2.5 mm.

If the observed mean mortalities (without correction) are plotted against distance on an exagerated scale (Figure 23b) and the expected mortality, based on a normal distribution of permethrin, is plotted against it, there is clearly no agreement, the mortality falling to the natural level long before the observed mortality ceases to fall. On the other hand, if a curve is fitted using the logit:log log transformation, a gradual decline is indicated over the entire experimental distance and fitting closely throughout to the observed points.

Several other expressions have been investigated to relate mortality to concentration in the dipping experiment. In some, the fit was better than the square root relationship used above but it became more difficult to fit normal curves. In all cases, however, a fitted normal curve gave an abrupt end to the resultant mortality curve and did not conform to the observed distribution of mortality. The most obvious conclusion is that the distribution of toxicant around the droplet does not follow a normal curve near the outer limits of chemical and that there is some mechanism moving small lethal quantities to a greater distance than would be expected. Some doubt is therefore cast on the validity of models based on diffusion, although in many respects, the concept is logical and gives reasonably good fits in the vicinity of the droplet. Considerably more attention needs to be given both to the determination of the dose response curve and to its application in these experiments.

ii) Distribution of Pirimiphos-Methyl

In experiments using pirimiphos-methyl the level of mortality was so great that the source of insecticide can probably be regarded, for practical purposes, as a straight line. The proximity of the droplets was such that complete overlap of effects would be expected within a few mm and certainly within the 6 mm distance of the Ld_{50}. In the treatment where there was a physical gap between the row of droplets and test leaf it is most unlikely that effects of individual droplets would be experienced beyond the gap, since gaseous diffusion is implied. Since there were no perceptible differences between this and the treatment where droplets were sprayed on the test leaves, it appears that droplets cannot have acted individually in this case either.

The equation applicable when diffusion occurs from a line source (Crank, 1956; equation 3.7) is:

$$C = \frac{M}{4\pi Dt} e^{-r^2/4Dt}$$

This is the same expression as for a point source except that 'M' is now defined as the amount of substance per unit length. Since the amounts of chemical considered have not been quantified and are, therefore, arbitrary, this definition makes no difference to our calculations. The distribution of chemical about the line will thus be expected to be normal, as for a point source.

The diffusion constant, 'D', for permethrin was estimated as 6×10^{-8} mm^2 S^{-1}. To a general approximation, since the area of influence of a pirimiphos-methyl droplet was about 300 times that

of permethrin, the diffusion constant can be estimated as 2×10^{-5} mm^2 s^{-1}, a value close to that to be expected for gaseous diffusion.

iii) Computer Model of Diffusion Laws

The diffusion law considered so far has applied to the diffusion of a substance from a point source on an infinite plane surface. The equation involved is relatively simple, expressing a normal distribution around the source. However, experimentation has revealed that the distance of diffusion does not exceed a few mm, and therefore the size of the droplet itself may become involved in the shape of the distribution curve. The mathematical aspects of this have been explained by Crank (1956) and the application of his equations to the present studies have been explored by Dr Rose of the Glasshouse Crops Research Institute and by Dr Salt, Mrs Sharkey and Dr Ford of Portsmouth Polytechnic.

The law most probably involved in this droplet study is that concerning a disc source on an infinite plane surface, since the droplet spreads to a disc-like shape on the surface of the leaf and releases toxicant from its perimeter into the surface layers of the leaf which, in view of the small diffusion distances involved, can be regarded as infinite. The equation given by Crank (1956; equation 3.10) calculates the concentration, 'C', at a distance, 'r', from the centre of a droplet of radius, 'a', as:

$$C = \frac{C_o}{2Dt} e^{-r^2/4Dt} \int_o^a e^{-r'^2/4Dt} I_o \left(\frac{rr'}{2Dt}\right) r' \, dr'$$

where 'I_o' is the Bessel function of the second kind of order zero, 'C_o' is the initial concentration within the droplet, 't' is time in seconds and 'D' is the diffusion constant. The integral in the equation has to be evaluated numerically except in the centre of the droplet.

A further complication in the calculations is that the distribution varies with time (Figure 24). At any one point, away from the perimeter of the droplet, the concentration will initially increase (consider the point at 1.5 on Figure 24) and will subsequently decline as chemical disperses still further. Thus the toxic stress to which a target is subjected is in reality a concentration time product, summing a complex sequence of changes over the total exposure time. The response of whitefly scales to concentration time products is entirely unknown and can only be assumed to be the usual probit form of response. If the mortality response is to a summation of a series of curves as illustrated in Figure 24, there is likely to be a long 'tail' of concentration extending from the droplet, especially as time proceeds. This may explain why mortality continues to fall over a much wider distance than is explained by a normal distribution. Thus the deaths occurring at the greater distances would be the latest ones within the exposure period.

Salt, Sharkey & Ford (personal communication) devised a

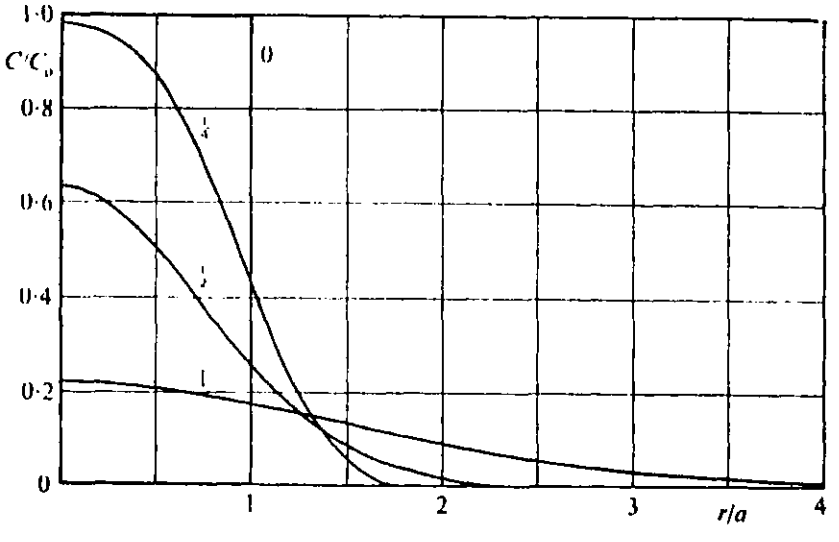


Figure 24.

Concentration distributions for a circular disc source. Numbers on curves are values of $(Dt/a^2)^{1/2}$.

(From Crank, 1956)

computer model to simulate the effects of diffusion from a disc-like droplet. They assumed that mortality was determined by the highest concentration reached at any point, and that a probit:log concentration response was involved. Then by varying parameters such as concentration and droplet diameter they were able to predict the mortality responses with distance for any combination of parameters. The shape of the response curve was very close to the observed shape and could be fitted by iterative methods, varying the relevant parameters (Figure 25). The fit to the observed data was excellent so that it was concluded that the results were entirely consistent with random diffusion.

Using the computer model, it was possible to investigate the effect of changing the concentration, for example from 5% to 10 or 20% (Figure 26a). Increasing the concentration four times in this way clearly doubles the median lethal distance (Ld_{50}) and therefore increases the affected area by four times. Thus the increase in area is proportional to the increase in concentration. Comparing this with the experimental results in the first concentration experiment (Figure 4, Table 8) increasing the concentration four times, from 5% to 20% increased the Ld_{50} from 0.466 mm, an increase of 43% and an increase in the area affected by 104%. Thus the area of influence was only doubled as opposed to a four times increase in theory. In the second experiment (Figure 5; Table 8) increase from 5% to 20% raised the Ld_{50} from 0.377 to 0.430 mm, an increase of 14% or, on area basis, of only 30%. It would appear therefore that there is a considerable discrepancy between observed results and mathematical theory.

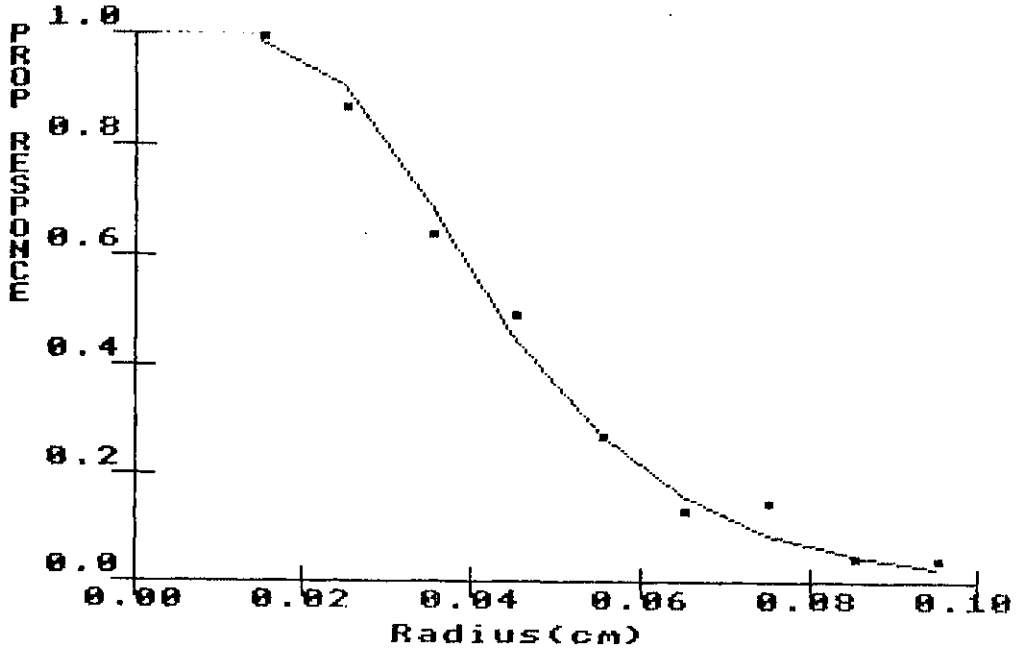
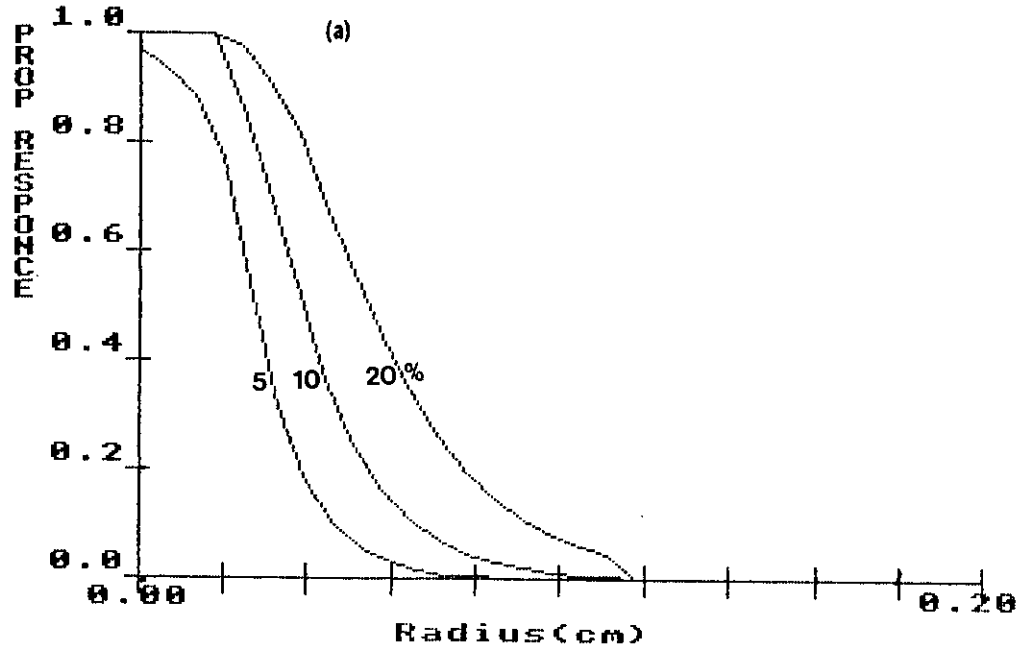


Figure 25.

Computer model fit (based on diffusion of toxicant from a disc source) of the distribution of mortality of settled first instar whitefly larvae around droplets of 10% permethrin in JF 8133 on tobacco leaves.



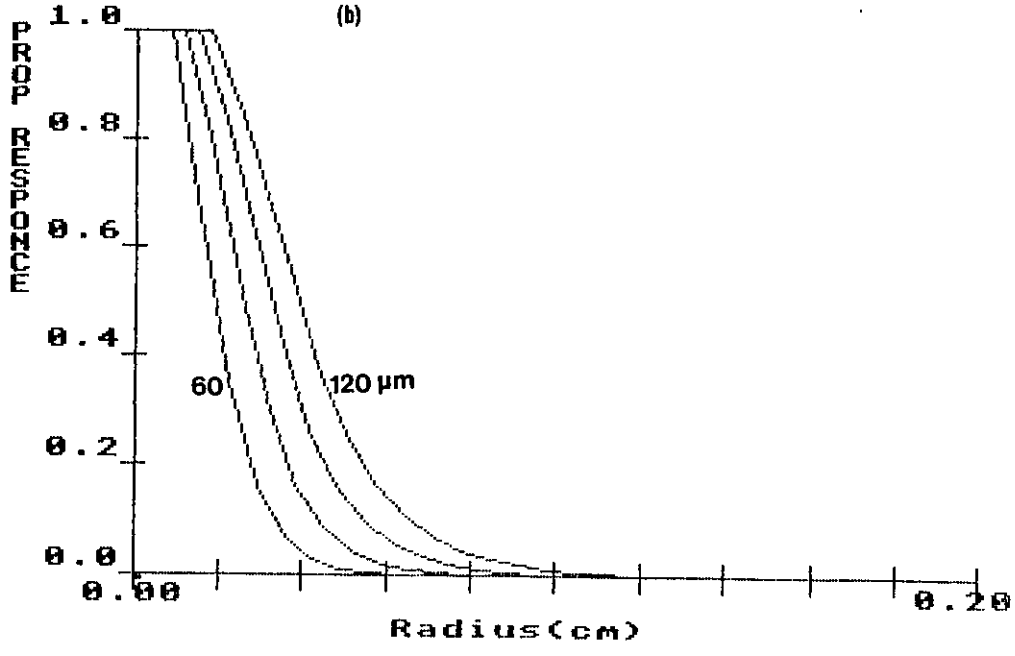


Figure 26.

Computer model predictions of the effects of change in concentration of active ingredient and droplet size on whitefly larval mortality at radial distances from droplets of permethrin.

(a) Effect of concentration.

(b) Effect of droplet size.

The computer model can similarly be used to determine the expected effect of doubling the droplet size (Figure 26b). By increasing the droplet size from 60 μm to 120 um, the volume and therefore the amount of active ingredient is increased eight times. The model shows that the Ld_{50} is doubled and the enclosed area is therefore increased four times. In the experiment on droplet size, however, increasing the diameter from 59 to 114 μm, a seven-fold increase in volume, increased the Ld_{50} from 0.321 to 0.417 or 30% or, on an area basis, an improvement of only 69%. Thus again the improvement in practice was much less than would be expected in theory.

Clearly, changes in characteristics of the droplet are not followed by changes in toxicity which would be expected from the mathematical laws of diffusion. Possible reasons for these discrepancies should therefore be sought.

iv) A Functional Theory of Insecticide Dispersion from Droplets

The analyses of data for all experiments have shown that the distribution of mortality, and presumably of the chemical itself, follows a very regular pattern around a pesticide droplet. This resembles a normal distribution of chemical (although probably not precisely normal) suggesting that the main mechanism for dispersal of the chemical is random diffusion of molecules through the surface layers of the leaf. Because of the known solubility of most insecticides in waxes, it seems probable that diffusion takes place in the surface wax of the leaf or in the cuticle. This was

confirmed by the estimated diffusion constant which implies that the solvent medium is in a semi-solid state.

When the diffusion laws were applied more precisely, however, as in the computer model, it was found that, although the correct shape of curve was obtained, much greater differences between treatments were predicted than were actually obtained. This applies both when changing droplet size and when altering concentration. Possible explanations for this discrepancy should therefore be sought.

(1) Change in droplet size

One factor apparently ignored in the diffusion laws used in the model is that there is a discontinuity in the solvent medium between the droplet and the leaf surface. The insecticide must pass across the interface between the formulating oil and the leaf wax. In the diffusion formulae it is assumed that the medium of the disc source is the same as the surrounding surface.

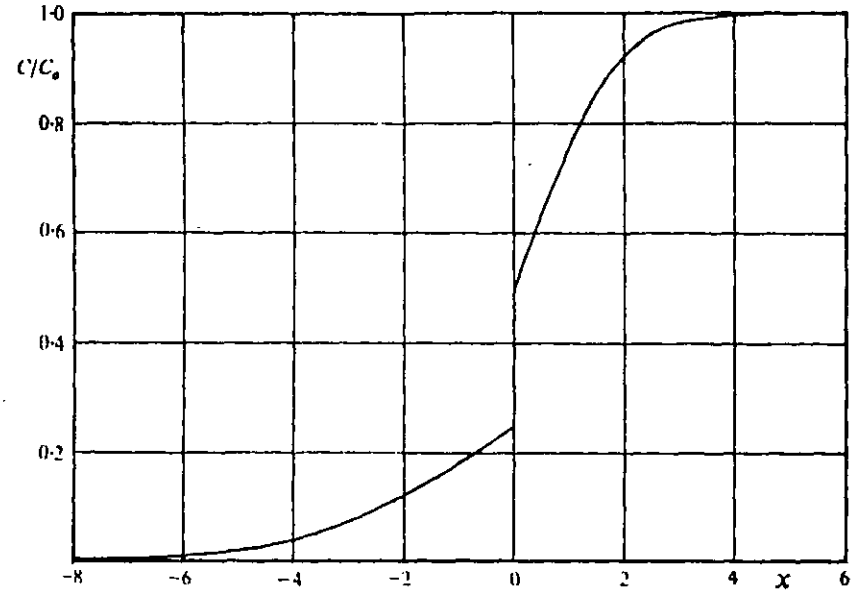
In practice still more complications may be involved at the interface. The formulating solvent could, for instance, dissolve the wax in the immediate vicinity of the droplet margin, leaving a narrow band around the droplet where diffusion is restricted. Also, on impact, the droplet may spread beyond its final bounds, dissolving or disrupting the wax over an even wider area. When the droplet again contracts under surface tension it will leave a relatively impenetrable band around it. In circumstances like this, when the passage of diffusing chemical is impeded by a barrier (of absent wax) it is logical to conclude that the amount of chemical escaping from the droplet will be proportional to the

length of the impeding barrier. In the droplet size experiment, assuming droplets spread in proportion to their in flight diameter, the larger droplets (114 μm) would have had a perimeter 93% more than the smaller droplets (59 μm). Assuming the slopes of the logit:log log response curves were constant the estimated amounts of insecticide moving onto the leaf surface should have been in the ratio 840 to 433, namely a 94% increase with the larger droplet. Thus the estimated amount of diffusing chemical was exactly in proportion to the perimeter of the droplet. It must be admitted that this was based on only a single comparison, but the above theory gives a better explanation of the observed results than the computer model based on laws of diffusion.

Crank (1956) does briefly consider diffusion across an interface between two media and also where there is a resistance at the interface, but his examples are not applicable to the present situation since they relate to two infinite media. The equations are of great complexity with too many unknowns to attempt to adjust them to this study. However, the accompanying figures (Figures 27a and b) provide information concerning the type of changes which might be expected across an interface. Also the concentration at any one point should be directly proportional to the original concentration within the droplet.

The implications of this theory, relating release of insecticide from the droplet to the length of the perimeter, has a distinct bearing on the interpretation of two other experiments. In the formulation experiment both VK1 and JF 8133 behaved similarly. They are both similar formulations containing a large

(a)



(b)

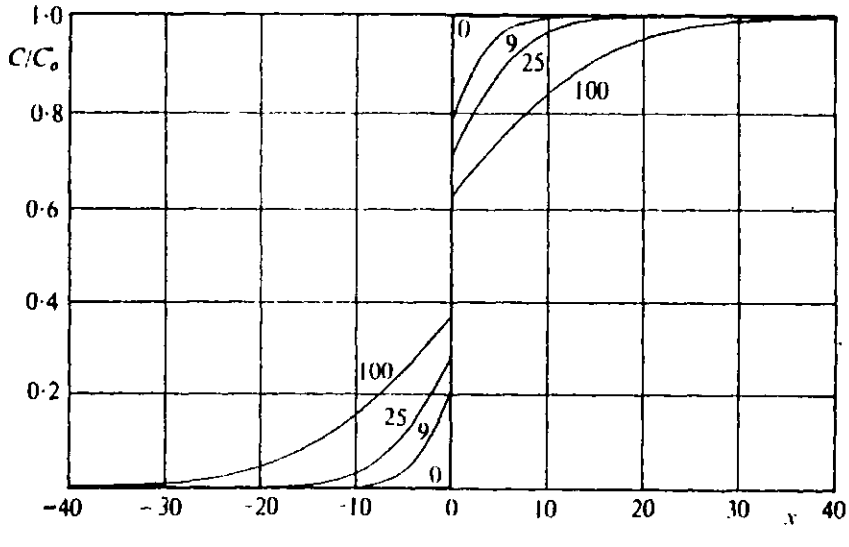


Figure 27.

(a) Concentration distribution in a composite medium.
$D_1 t = 1$, $D_2 = 4D_1$, $k = \frac{1}{2}$

(b) Concentration distribution in a composite medium with a resistance at the interface. Numbers on curves are values of $D_1 t$. $D_1 = D_2$, $k = 1/10\, D_1$

(From Crank, 1956)

proportion of volatile solvent and when they are deposited on a tobacco leaf they spread out to a certain extent. The JF 8130 formulation, on the other hand, contained little volatile component and, on a leaf, contracted to a dome-shaped droplet. Thus, although the remaining volume of oil was more than that in the droplets of the other two formulations, the perimeter of the droplet was considerably less. It is, of course, a matter for speculation whether the poor insecticidal properties of the droplets were a direct result of this poor initial spread. However, further confirmation comes from the pirimiphos-methyl experiment where, for comparison permethrin was formulated in JF 9079. These droplets tended to form discrete domes (light microscope observation) and, accordingly, their toxic activity was intermediate between VK1 and JF 8133 on the one hand and JF 8130 on the other.

Similar interpretations can be applied in the plant species comparisons. On tobacco, the plant on which most tests were done, the droplets formed fairly regular circular pools (Plate 6). On tomato the droplets spread into irregular areas (Plate 8) and on bean the oil appeared to flow out in long channels between the elongate epidermal cells (Plates 9 and 10). In these three cases, however, the areas covered by the droplets did not differ appreciably (Table 6). Permethrin was least effective on tobacco and most on bean. Whether differences in the physical form of droplets were due to the waxiness or to the surface topography of the leaf is not clear, but both in this and in the formulation experiment, the effectiveness of the toxicant appears to be related to the final perimeter of the droplet on the leaf.

The SEM studies of droplets were therefore examined in greater detail to determine what aspects of the droplet conformation were responsible for the changes in effectiveness. The perimeters of droplets of 10% permethrin were measured for three formulations (JF 8130, VK1 and JF 8133) and for one formulation (VK1) on three plant species (tobacco, tomato and French bean), and the areas of droplets were determined by weighing cut-out tracings of the SEM photographs. The mean radius of droplet assuming the droplets were circular was derived from mean area of droplet for each treatment (Tables 5 & 6). Indentations around some droplets, particularly on bean, were so deep (Plate 9) that it was difficult to imagine these areas adding to the total outward diffusion. The concentration in these areas might be assumed to be saturated. To measure the maximum extent of the droplet and a rounded shape enclosing the droplet and eliminating the indentations, the mean of the maximum length of the droplet and the maximum width at right angles to this were measured to calculate the area of the enclosing ellipse and its perimeter. The values were then corrected by proportion to the figures to be expected for sizes of droplets used in the mortality experiments.

These values were compared with various assessments of effectiveness, for instance, the median lethal distance (Ld_{50}), the median lethal spacing (LS_{50}), the area of influence (AoI), the median lethal dose (LD_{50}), or the index of diffused amount of insecticide (α). All of these were compared in turn, by graphing, with all of the aspects of conformation mentioned above. In all

the comparisons involving the actual droplet area and its derivatives, or of the ellipse dimensions, there was no semblance of a correlation between effectiveness and that dimension. As typical examples the LS_{50} was plotted against droplet area and the LD_{50} was plotted against mean ellipse diameter in Figures 28a and b. However, when measures of effectiveness were plotted against total perimeter, relationships were always clearly visible. When the Ld_{50}, AoI or α were plotted against perimeter, upward trending curves resulted. This was surprising for α (Figure 29a) since it was anticipated that the amount of diffusing insecticide (represented by α) would have been proportional to the perimeter, as argued above, and that a straight line through the origin would have resulted. The relationship is more complex, yet, nevertheless the amount of diffusion is determined by the length of the droplet margin and by no other parameter which has been examined. The upward curvature of the graph (Figure 29a) implies that as the droplet margin becomes more convoluted, the effectiveness increases out of proportion to the margin's length. This is in direct contradiction of a supposition made earlier that the indentations might not contribute appreciably to the outward diffusion. On the other hand, the two points giving the semblance of curvature are from the tomato and bean tests and could possibly involve a different mechanism due to the nature of the leaf surface or wax.

When LD_{50} was plotted against perimeter, a negatively sloping relationship was revealed, since LD_{50} is inversely related to effectiveness (Figure 29b). There is one outlying point, however: the 59 µm droplet test. This point become anomalous since the

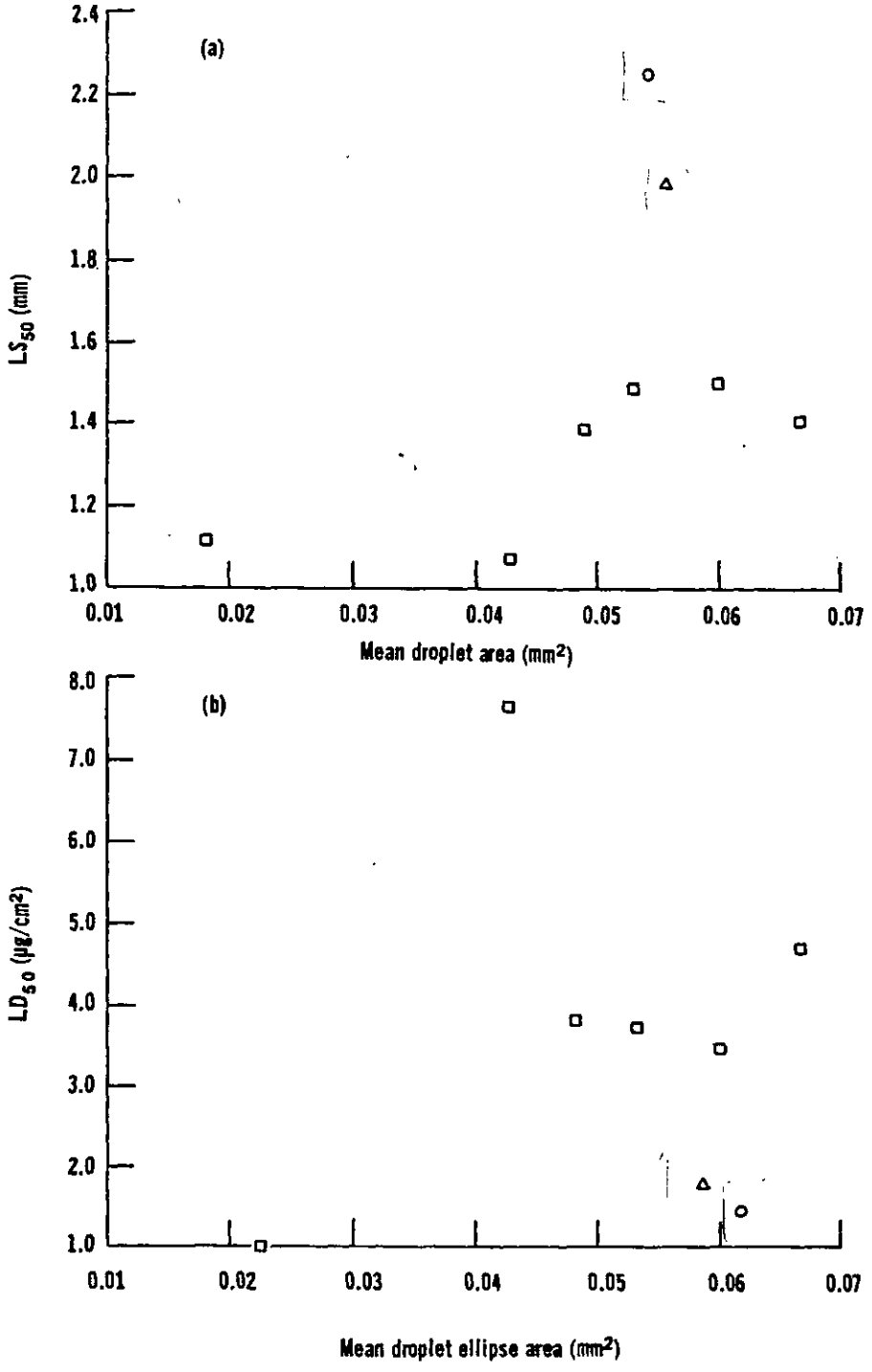


Figure 28.

(a) Median lethal spacing against mean droplet area

(b) LD_{50} against mean droplet ellipse area

□ Tobacco, △ Tomato, ○ French bean

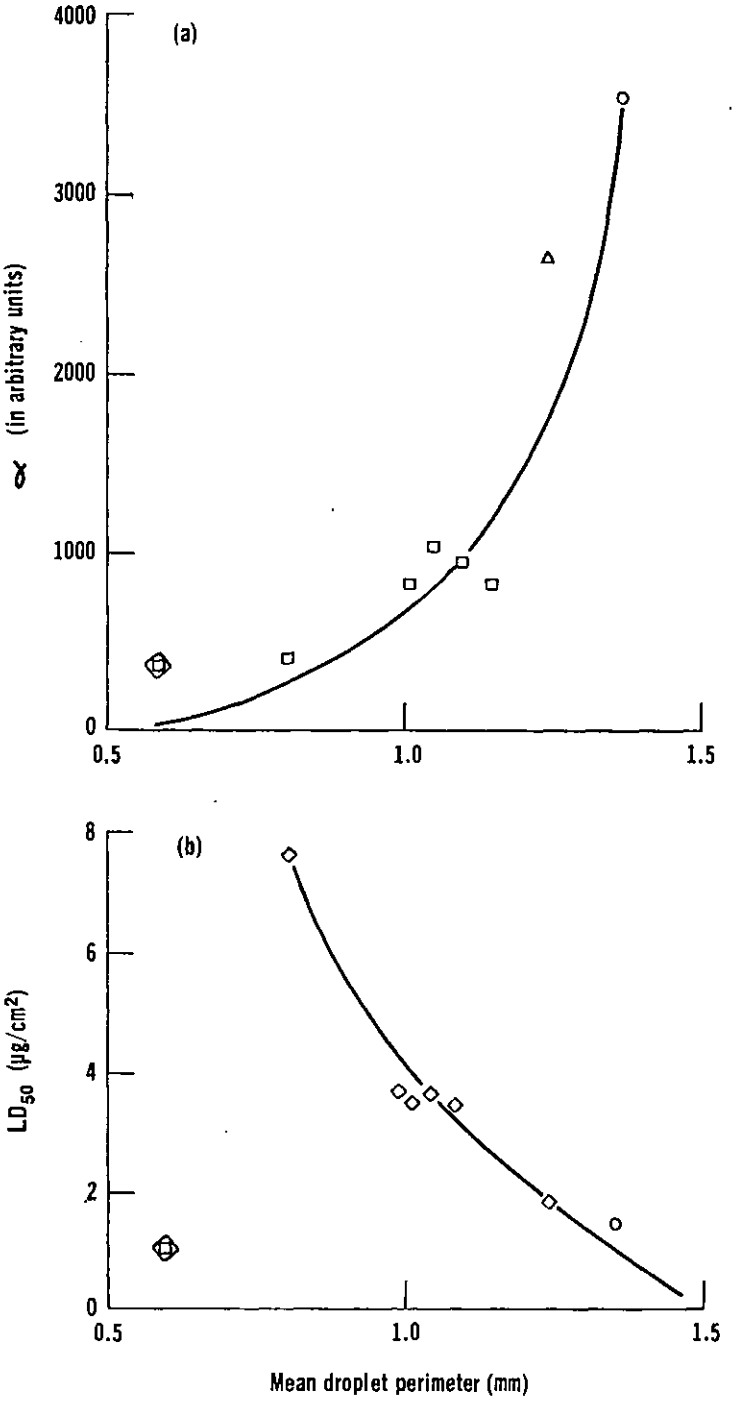


Figure 29

(a) Relationship between the relative amount of diffusing chemical (∝) and droplet perimeter.

(b) Relationship between LD_{50} and droplet perimeter.

□ Tobacco, △ Tomato, ○ French bean (*c.* 100 µm droplets)

◈ Tobacco (59 µm droplets)

amount of active ingredient is involved in the calculation of LD_{50}. These droplets, when produced, were only about half the diameter of the others and therefore contained only about one eighth of the amount of insecticide. The graph therefore demonstrates forcibly how a small droplet is more effective than larger droplets even when they have spread widely, and despite its lower chemical content. This fact casts some doubt on the validity of including this point in the other graphs. However, the chemical content does not appear to have affected its position in the curves, and it has already been concluded that concentration change has little effect.

Only when the median lethal spacing (LS_{50}) was plotted against perimeter was there anything resembling a straight line response (Figure 30). Some curvature remained, but a fitted linear regression line had the equation:

$$LS_{50} = 0.0383 + 1.437 \text{ Perimeter}$$

with a correlation coefficient of 0.866 ($P < 0.01$). The intercept is so close to the origin that for all practical purposes the equation can be rewritten:

$$\left[LS_{50} = 1.474 \text{ Perimeter} \right]$$

Thus, as a generalization, the spacing of 10% permethrin droplets, required to achieve 50% mortality of whitefly scales, is about one and a half times the perimeter of the droplet. For droplets that remain circular on the leaf, the spacing is about 9.3 times the

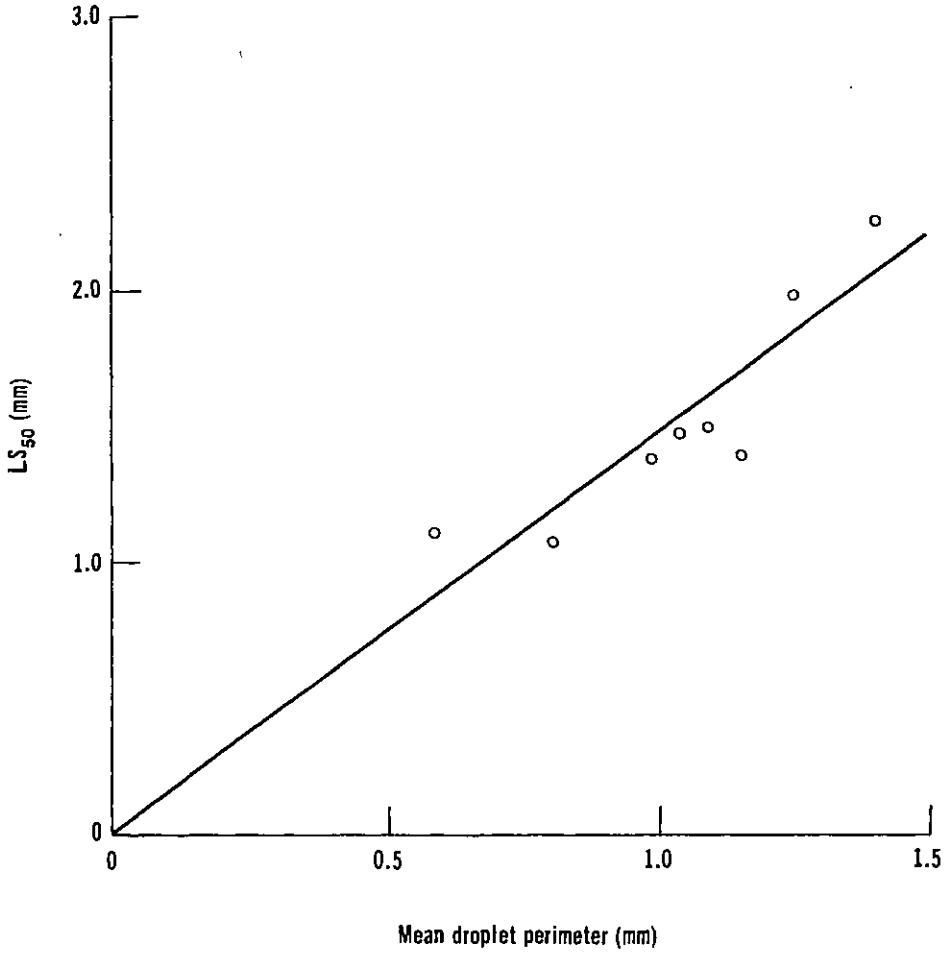


Figure 30.
Relationship between median lethal spacing of droplets and droplet perimeter.

radius. Even in this respect change of concentration has little effect on the ratio, varying from 10.5 for 20% down to 8.1 for 0.5%.

Although this simple concept relating spacing to perimeter in a sense summarizes all the permethrin findings, it is not simple to propose a mechanism to explain it.

(2) Changes in concentration of active ingredient

An explanation should now be sought for the unexpected responses to change in concentration. When estimates were made of the amounts of insecticide diffusing onto the leaf surface ($\propto$), it was found that, in the first concentration experiment, the amounts were 385, 858 and 1307: roughly in proportion to the original concentrations of 5, 10 and 20% (Table 8). It should be noted that the area of influence barely doubled between 5 and 20% (Table 8) and cannot, therefore, be taken to represent the amount of insecticide present. Yet the area of influence estimated from the computer model quadrupled between 5 and 20%. The area, and not the amount, therefore appeared to be in proportion to the concentration.

Although the amounts of chemical released from droplets seemed to be proportional to the initial concentrations in this experiment, this was not confirmed in the second experiment (Table 8). Amounts of insecticide at 20 and 10% were 1389 and 858: very similar to the first experiment. However, at 5, 2, 1 and 0.5% the estimated amounts remained relatively stable at 602, 502, 739 and 570 being quite unrelated to concentration. Thus a ten times difference in concentration had little influence on the

effectiveness of the spray droplets.

One possible explanation for this is found in the method of formulation. The carrier, VK1, used in the concentration tests, consists of a mixture of a non-volatile oil and highly volatile methylene chloride. One reason for the addition of the latter is to increase the solubility of permethrin in the oil. Very soon after spraying, and possibly while the droplet is still in the air, much of the methylene chloride will have evaporated. What is left will be a super saturated solution of permethrin in oil and permethrin will rapidly crystalize or separate out from the oil (according to ambient temperature and purity). In confirmation of this, several of the SEM micrographs appear to show crystals on or below the surfaces (Plate 4) or scum or rafts of material on the surface (Plate 7). The latter might be wax from the leaf surface but there are, nevertheless, strong indications that separated permethrin is present. If this is the case, the oil of the droplet may be a saturated solution over a wide range of original concentrations and, therefore, the partitioning of permethrin across the interface with the wax would be equal regardless of the original concentration. This suggestion could therefore explain the similarity of results observed with 5 and 0.5% permethrin.

The mechanism of action could therefore be that, around the perimeter of the droplet, the concentration of permethrin is virtually saturated and therefore constant. At the interface with the wax, according to the laws of partitioning, the wax itself would be equally saturated, but not necessarily at the same concentration. From the interface the molecules would diffuse

outwards across the leaf surface having bridged any impeding barrier. Once on the unbroken surface, the chemical would presumably obey the laws of random diffusion.

Within the droplet, the concentration at the perimeter would be continually depleted but at such a slow rate as not to reduce the saturation appreciably. Then, as the concentration within the droplet falls fractionally, more permethrin would be dissolved from the crystals. Thus the crystals would act as a continuous reservoir feeding toxicant into the system as it flowed outwards. From the point of view of immediate control, this would have the effect of 'locking up' surplus pesticide and limiting the effectiveness of spray. On the other hand it would tend to increase the persistence and to provide constant protection over a prolonged period.

In time, according to this theory, the crystals would eventually dissolve completely, the period depending on the original concentration and therefore the quantity of crystals formed. This stage would be reached when the amount of remaining pesticide was just sufficient to saturate the remaining oil. From then on the concentration would progressively diminish and the flow through the interface would decline accordingly.

There are several unknowns in this respect. VK1 contains risella oil, but this in itself is a variable mixture of oils for which no precise information is available. VK1 contains 75% methylene chloride, so a 1% solution would rapidly become 4% but some constituents of risella oil are believed to be somewhat

volatile, so the final concentration is unknown. Also because of the variable nature of both risella oil and technical permethrin it is difficult to determine solubilities with any precision. The two concentrations experiments were carried out using different samples of VK1, and it may be because of different solubilities in the two samples that somewhat contradictory results were obtained.

One observation is difficult to reconcile with this crystallization theory. Concentrations of 10%, and particularly 20%, were shown to bring increased mortality compared with the lower concentrations, whereas the theory would suggest that surplus chemical would merely crystallize within the droplets. It should be remembered however that a 20% solution would become 80% after evaporation of the methylene chloride and the droplet would therefore contain far more toxicant than oil. Under these circumstances quite different physical factors may come into play. For instance, the crystals may be brought into intimate contact with the leaf surface.

5. GENERAL DISCUSSION

The spread of insecticide from individual droplets of oil-based formulations of permethrin and pirimiphos-methyl was studied by assaying first instar whitefly larvae, T. vaporariorum (Westw.), on leaves of tobacco, tomato and French bean. Percentage mortalities were measured to determine the decline of insecticidal effect at distances progressively away from the droplet. Death was related to distance from droplets and the distribution of mortality around a droplet showed a very regular pattern. The logit:log log model (Section 4b.1)) gave an excellent fit to the data in all experiments. It was not possible to quantify the amount of active ingredient around the droplet by direct methods (Section 4a.3)), so the distribution of chemical was deduced mathematically from the dose-response relationship (Section 4b.4) and 5)). In a way analogous to probit analysis, a parameter termed the 'median lethal dose' or Ld_{50} was calculated from the logit:log log transformation. The calculations were extended to determine the 'median lethal spacing of droplets' or LS_{50} and the 'area of influence' of a droplet, and to derive the median lethal number of droplets (LN_{50}) and the median lethal dose (LD_{50}) per cm^2.

Only a small proportion of target organisms are hit directly by a ULV spray on foliage, especially when the target is small and sessile. Munthali & Scopes (1982) reported that less than 10% of Tetranychus urticae eggs were hit by droplets landing on the leaf even with a density of 150 droplets per cm^2. Mortality was, therefore, largely dependent on the spread of the pesticide from spray droplets across the leaf surface. So, the spread of

pesticides from individual droplets of oil based formulations on leaves was studied in the present investigation. The influence of spray factors and the effect of leaf surfaces of different plant species on the transfer of pesticides from droplets to the target pest were examined.

Increasing the concentration of active ingredient from 5 g/litre to 200 g/litre (the two extreme permethrin concentrations of the six tested) increased the area of influence from 1.25 to 2.15 mm^2 (Table 9); an increase of only 72% although the concentration increased forty times. Estimated LD_{50} values were 0.26 and 5.18 $\mu g/cm^2$ respectively. Thus a spray containing 5 g a.i./litre is about twenty times more economical than that of 200 g a.i./litre. As argued in Section 4b.12), the bulk of active material at the high concentration is apparently 'locked up' in the droplet and is therefore not readily transferred to the pest. Clearly lower concentrations of active ingredient are more efficient for a non-persistent effect. Such an approach is desirable to reduce costs, hazards to non-target organisms and decrease residues which may increase selection of a resistant pest population as well as pollute the environment. However, when persistence is required to control a pest over a longer period, more concentrated sprays might need to be applied to provide a reservoir of pesticide, but this needs to be administered in a carefully studied programme within an integrated pest management system. Such a reservoir of chemical from a concentrated spray is important when rain allows redistribution of a deposit, for example, fungicides are spread after deposits from dilute sprays have been depleted (Courshee, 1960; Hislop & Cox, 1970).

Palmer et al. (1983) reported a fall in efficiency of permethrin spray against first instar whitefly larvae of 7.4 times from 10 g/litre to 100 g/litre and 119 times from 10 g/litre to 500 g/litre with 50 µm droplets, and a fall of 2.1 and 30.4 times respectively for 100 µm droplets based on LD_{50} values. The present study indicated a reduction in efficiency of 7.7 times from 10 g/litre to 100 g/litre with 100 µm droplets on the basis of LD_{50} estimates (Table 9). Munthali (1981) using a series of concentrations (0.5 to 40 g/litre) of dicofol in VK1 against mite eggs, found that there was an optimum concentration of active ingredient of about 10 g/litre, below and above which the biological efficiency of the spray decreased. The fall in efficiency between 10 and 40 g/litre using 100 µm droplets was about 4.7 times. The equivalent LD_{50} values for the present experiment was 0.42 $\mu g/cm^2$ for 10 g/litre and 1.52 $\mu g/cm^2$ for 40 g/litre solution, namely a reduction in efficiency of 3.6 times: a similar effect even though a different chemical and test organism were used.

Due to difficulties with adjusting the spray nozzle, only one comparison was made on the effect of droplet size on spread and efficiency of pesticides. The areas of influence were 1.07 and 1.63 mm^2 for 59 and 114 µm diameter droplets respectively (Table 10). Thus although the larger droplet contained 7.2 times the amount of chemical as the smaller, it was able to affect only 52% more area. On the basis of estimated LD_{50} values (1.01 and 4.64 ug/cm^2) the smaller droplet is 4.6 times more efficient than the larger, needing only about one fifth the amount of toxicant to

achieve 50% mortality, but requiring 1.6 times the number of droplets/cm^2. Other workers have also demonstrated the effectiveness of small droplets applied as foliar sprays. One fifth of phosmet deposit as 150 µm droplets was required compared with 350 µm droplets to achieve the same level of control of Lyspeyresia pomonella, but with 2.5 times the number of droplets/cm^2 (Fisher & Menzies, 1979). Similarly, 50 µm droplets of permethrin in a 10 g/litre solution were 7.8 times more efficient than 100 µm against first instar whitefly larvae (Palmer et al., 1983). The relationship between LD_{50} and droplet diameter for red spider mite eggs using dicofol was positive and curvilinear: at any one concentration LD_{50} was directly proportional to the square root of active ingredient per droplet (Munthali, 1981). Thus the smaller the droplet the more efficient is the spray.

Theoretical argument and experimental evidence support the view that better target coverage and more efficient control at lower rates of application can best be effected by dispersing the spray in as many droplets as is technically possible. This emphasizes the need for the use of small, fairly uniform droplets to ensure even distribution of pesticide deposits when a low volume of spray liquid is applied. CDA equipment is now available which produces small droplets with remarkable control of droplet size spectra. Electrostatic charging improves droplet deposition and reduces the proportion of small droplets which drift. Whereas recommendations of dosages for pesticide application are still largely based on empirical observations made during field and laboratory trials relating to use of high volume, the evidence

suggests that changes in application techniques could improve efficiency of pesticide use. Existing application techniques are based largely on pesticides which had to be applied at about 1 kg/ha, but the potency of all major classes of pesticides has increased by several orders of magnitude since the beginning of this century (Graham-Bryce, 1981). CDA/ULV spraying is qualitatively and quantitatively different from conventional high volume spraying since discrete droplets can be applied offering the possibility of delivering a measured amount of chemical to the target together with better retention. There is, therefore, greater incentive to consider the optimum specifications for the size and contents of droplets in a more analytical way. This calls for a better understanding of pesticide performance in relation to the biological target. When the biological target (Matthews, 1977b) is defined and the optimum droplet size (Himel, 1969a) to reach the target is determined, droplet density, concentration of active ingredient and spray volume can be determined subsequently. Once the features of the optimum spray deposit requirements for the biological target are defined, efforts could then be made to adjust spraying procedures and formulation to achieve the desired effect. Adoption of such an approach can lead to significant reductions both in dose rates and in wastage.

The formulation of carrier solution also influenced the spread of the pesticide from droplets and the efficiency of the spray. Areas of influence were 0.99, 1.40, 1.90 and 1.92 for droplets of 100 g/litre of permethrin formulated in JF 8130, JF 9079, VK1 and JF 8133 respectively (Table 11). Estimated LD_{50} values for

settled first instar larvae on tobacco were 7.63, 4.62, 3.67 and 3.43 $\mu g/cm^2$ respectively. Estimated LN_{50} values were 101, 72, 53 and 52 droplets/cm^2 respectively. Thus twice the amount of chemical must be applied when formulated in JF 8130 compared to VK1 and JF 8133 and about one and a half times compared with JF 9079. Also twice the number of droplets must be used in the first case which means that double the volume of spray liquid has to be transported and applied. Scopes (1981) and Palmer et al. (1983) have also shown that permethrin sprays formulated in JF 8130 were less effective than those formulated in VK1 or JF 8133.

Spread of droplets of formulation JF 8130 was significantly lower than that of VK1 and JF 8133, thus reducing the perimeter of droplets as shown by SEM studies (Section 4a.5)). Mean droplet perimeters were 0.541, 0.719 and 0.788 mm for JF 8130, VK1 and JF 8133 respectively (Table 5a). The formulations followed the same order in terms of biological effectiveness. Areas of influence were 1.07, 1.48 and 1.49 mm^2 for JF 8130, VK1 and JF 8133 respectively (Table 11). Thus droplets VK1 and JF 8133 had very similar effect and were more effective than JF 8130 which had a significantly smaller droplet margin. It is worth noting that formulation JF 8130 is less volatile than both VK1 and JF 8133 (Table 2) and is more viscous.

Details of formulations are generally closely guarded by the chemical manufacturers. This has led to a lack of published research and consequently a restriction of communication among workers in the field, so that exploitation of formulation to derive maximum benefit from application changes is difficult (Merritt,

1980). If such a state of affairs is to continue the greater onus will rest on the manufacturers to consider the requirements of the biological target and to match formulation with leaf surface characteristics for improved pesticide effectiveness.

The leaf surfaces of different plant species also modified the spread and efficiency of pesticide droplets. Calculated areas of influence of droplets of 100 g/litre permethrin were 1.90, 3.44 and 4.39 on tobacco, tomato and French bean respectively (Table 12). Estimated LD_{50} values were 3.67, 1.81 and 1.42 $\mu g/cm^2$. Thus the chemical was most efficient on French bean and 2.3 times the amount was needed on tobacco and 1.3 on tomato. Similarly, twice the dose of dicofol was needed to control red spider mite eggs on pepper leaves compared with French bean (Munthali, 1981). An explanation for this is indicated in the SEM studies of droplets and bioassay results in the present investigation, namely efficiency of pesticide deposits is related to the length of droplet perimeter (Section 4b.12)). On French bean, for instance, the margin of the droplet is deeply contorted (Plates 9 and 10) resulting in a greater increase in effectiveness.

Although it is clear that the physico-chemical properties of plant surface and formulation influence the performance of foliar applied sprays (Boize, 1976; Hart, 1979; Hess et al., 1974 & 1981; Holloway, 1970; Johnstone, 1973a), the precise nature of droplet-cuticle interactions is poorly understood (Baker et al., 1983). Such basic knowledge is essential to ensure choice and development of formulations that assist efficient transfer of active material

from the site of deposition on the leaf to the intended target. Increased efficiency should be obtained by matching the surface activity of the pesticide deposit with that of the leaf. When the exact nature of the leaf surface is known and the mechanisms underlying liquid-leaf surface interactions are understood, formulation components could be adjusted for improved effectiveness. It is possible to envisage formulations specially designed or adjusted to match the surface requirements of a certain crop and pest to achieve maximum efficiency. Chemical companies could then charge accordingly for the tailor-made product, without losing profits as a result of the great reduction in the required amount of active ingredient.

It has often been reported that vapour transfer of toxicant can be useful in modifying the biocidal range of a pesticidal deposit; but, as indicated by Hislop & Baines (1980), little information on the subject is available. Hartley & Graham-Bryce (1980) have pointed out that many pesticides applied to foliage have vapour pressures sufficiently high to be transferred to the target pest via the gaseous phase. A volatile insecticide, pirimiphos-methyl (vapour pressure: c. 13 mPa at 30°C) was chosen and its effectiveness was compared with permethrin (vapour pressure: c. 261 mPa at 30°C). Median lethal distance (Ld_{50}) values were quite different for the two insecticides, namely 0.39 and 6.10 mm for droplets of permethrin and pirimiphos-methyl respectively (Table 13). The areas of influence were 1.40 and 402 mm^2, and estimated LD_{50} values were 4.62 and 0.014 $\mu g/cm^2$ respectively. The distance of response, area and level of

activity shown by pirimiphos-methyl cannot be attributable to surface mobility of toxicant on the leaf, suggesting that they are most likely to be due to vapour transfer above the leaf or possibly in air spaces within the leaf. Simple techniques were devised to test this. When the insecticidal deposit was physically separated from the test insects a response was observed identical to that obtained with continuous leaves, confirming that transfer of toxicant could be entirely attributed to vapour above the surface and not through surface mobility or movement within the leaf. When an air flow was passed over treated leaf pieces the area of influence was reduced to one sixth of that in still air. The behaviour of pirimiphos-methyl in moving air was similar to that of permethrin, but the level of activity was higher. It appears that all or most of the vapour was removed by the air flow. These results clearly demonstrate that vapour transfer from surface deposits can contribute significantly to the effectiveness of pesticides.

Bent (1967) and Hislop (1967) demonstrated that certain fungicides protected areas of leaf beyond the visible limits of the fungicide deposits due to the release of fungicidal vapour. A draught from a fan shifted the zones of inhibition to one side of the deposits, but did not reduce their areas indicating that perhaps enough volatile toxicant was retained in the boundary layer of the air at the leaf surface which was not easily moved. Bent (1967) also reported zones of protection around fungicide deposits for plants kept in an open greenhouse and he suggested that vapour phase toxicity of fungicides for the control of

powdery midlews could operate in the field. The present study, however, has shown that all or most of pirimiphos-methyl vapour was removed by the air flow indicating that conditions of still air at the leaf surface should prevail if the benefits from vapour transfer of this toxicant are to be realized in the field. This highlights the importance of the knowledge of the nature of vapour release from the source material and the effects of wind, temperature and relative humidity if practical use of vapour is to be utilized in the field.

Many pesticides are diluted in water and applied as medium or high volume sprays using hydraulic nozzles. In some crops high volumes are applied to 'run-off' in an attempt to completely wet the whole surface of plants. Hydraulic nozzles produce a wide range of droplet sizes, the smaller are subject to drift while the larger tend to be lost to the ground. There is overwhelming evidence that conventional spray applications, while giving effective control at recommended dose rates, are very inefficient in that a relatively low proportion of the spray applied reaches the target (Graham-Bryce, 1977; Matthews, 1981; Hislop, 1983). Escalating costs of chemicals, concern for environmental quality and the high operational cost of high volume have stimulated interest in search for alternative methods of application. The ultra-low volume method, which was developed after success in controlling the desert locust in East Africa shortly after the Second World War, proved a valuable tool in places where water is scarce and transportation of large amounts of liquid is a limitation and is particularly useful in aerial spraying. Ground

light-weight CDA sprayers permit faster and better timed application which is particularly important in integrated pest management programmes. Lighter sprayers and vehicles allow spraying when soil conditions make high volume equipment virtually unusable as in winter cereals in the United Kingdom. Also, and probably far more important, CDA equipment has greater control of droplet size eliminating very wasteful large spray droplets and reducing the number of small driftable droplets, resulting in increased deposition on the target. More recently, electrostatic charging has resulted in improved deposition on the target surface. Increased deposition with charged and uncharged sprays compared with conventional systems has been reported by many workers (Allen et al., 1983; Cayley et al., 1983; Jarrett et al., 1978; Morgan et al., 1983), resulting in less ground contamination and fewer hazards to non-target organisms (Endacott, 1983; Cayley et al., 1983). The Seventh Report of the UK Royal Commission on Environmental Pollution (Anon., 1979) accepted that the continued use of pesticides is essential to maintain food supplies but was concerned about the scale of their use and expressed its desire to see an official commitment to reduce pesticide usage to the minimum necessary to maintain yields and productivity. To this end the report emphasizes greater precision in spraying as a means of achieving more efficient pest control, reducing the amount of pesticide needed and minimizing environmental pollution. Criteria for evaluation of application methods should include (beside the fulfilment of their basic function of preventing reduction in yield and quality due to pests, diseases and weeds) the harmful

effects to non-target organisms and environmental quality.

Results at least equivalent to and sometimes better than those obtained with conventional application have been achieved with CDA/ULV methods at similar dose rates. In some cases CDA has enabled better control at lower dose rates and reduced costs compared with conventional methods. Allen et al. (1983) obtained a better control of pea moth Laspeyresia nigricana at half the recommended dose of permethrin applied electrostatically than conventional application at the full dose. A greater reduction of Heliothis sp. damage in cotton was obtained with one fifth the dose of permethrin applied electrodynamically compared with conventional hydraulic spraying (Sherman & Sullivan, 1983). Morton (1981) reported average increase in cotton yield of 34% and net profit of 47% obtained by farmers in Paraguay with electrodynamic compared with hydraulic nozzles applying cypermethrin.

The improved deposition obtained with CDA systems has not always however produced a better biological response from a given dose compared with conventional application or enabled the dose to be reduced with equal efficacy. Inferior control at equivalent dose with CDA/ULV systems compared with conventional methods, despite higher or at least similar deposits, have been reported by Jarrett et al. (1978) with Bacillus thuringiensis against caterpillars on chrysanthemums, Morgan et al. (1983) with fungicides against apple powdery mildew Podosphaera leucotricha, Sherman & Sullivan (1983) with fluazifop-butyl against tall grasses in soybean and cotton and Richards (1984) with a granulosis virus for control of codling moth damage Cydia pomonella, on apples.

Poor canopy penetration and inadequate spray cover were suggested as the cause for poor control at low volume application rates.

Efforts are continuing to improve penetration of charged sprays into plant canopies by using air assistance and manipulation of parameters such as droplet size, charge to mass ratio and spray volume (Durand et al., 1983; Hislop, 1983; Pye, 1983; Sherman & Sullivan, 1983). The relative importance of spray cover depends on the behaviour of the target to be controlled and the nature and mode of action of the pesticide. A thorough spray cover is generally considered essential with contact fungicides and herbicides, microbial stomach insecticides and relatively sessile pests. These are the areas where CDA, in some instances, has not proved to be as effective as conventional application.

The present study was concerned with the transfer of pesticides from droplets after they have impacted on the leaf. It illustrates how the total dose released in the environment and wastage can be reduced if spray parameters are adjusted according to a sound knowledge of the biological requirements. Effective target coverage and more efficient control should result from a careful choice of formulation which matches the surface nature of the plant and the behaviour of the pest.

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APPENDIX TABLE 1: Volumes of component solutions used to produce dipping solutions

Dipping solution concentration (ppm)	Permethrin stock solution (ml)		Acetone (ml)	Water + 0.2% manoxol (ml)
20.0	1	A*	0.0	99
10.0	1	B**	0.0	99
5.0	0.5	B†	0.5	99
2.5	0.25	B	0.75	99
1.25	0.125	B	0.875	99
0.625	0.0625	B	0.9375	99
0.313	0.0313	B	0.9687	99
0.156	0.0156	B	0.9844	99

* Stock solution A: 2% permethrin in acetone

** Stock solution B: 1% permethrin in acetone

† Dilutions of permethrin solutions in acetone were made by dissolving larger volumes of permethrin stock solution in acetone at the appropriate ratio and taking one ml of each resultant solution

APPENDIX TABLE 2: Example data sheet showing numbers of whitefly larvae on tobacco leaf pieces treated with droplets of 5% permethrin in VK1

Substance: 5% permethrin in VK1 Plant: Tobacco

Droplet size: 117 ± 2 µm Data sheet:

Target stage: Settled first instar whitefly larvae Date:

Distance (mm)	Leaf piece no. 1		2		3		4		5		6		7		8		9		10		Total no. of insects	Total no. of insects dead
	No. of insects Alive	Dead	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D		
0.0–0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.1–0.2	0	0	0	3	0	1	0	1	0	0	0	0	0	0	0	2	0	3	0	2	12	12
0.2–0.3	2	3	0	3	2	3	0	4	1	2	0	3	0	0	0	4	3	3	0	2	36	28
0.3–0.4	4	3	2	4	7	4	1	2	4	5	5	2	4	1	2	1	4	4	2	1	67	31
0.4–0.5	6	0	9	2	4	0	3	1	6	0	5	1	8	1	5	2	4	1	5	3	66	11
0.5–0.6	7	0	5	1	15	1	12	1	8	0	7	0	3	1	7	0	6	0	2	1	77	5
0.6–.07	4	1	7	1	4	0	6	1	11	0	9	0	5	0	7	0	6	0	1	1	63	6
0.7–0.8	8	0	8	0	12	1	4	3	8	0	8	0	6	1	9	0	10	1	5	0	84	4
0.8–0.9	7	0	4	0	10	1	11	0	11	1	6	1	3	0	17	0	8	0	5	0	86	6
0.9–1.0	7	0	6	0	12	1	5	1	2	0	8	0	6	1	8	0	9	1	7	0	74	2
1.0–1.1	6	1	10	0	11	0	8	0	4	0	6	0	7	1	9	0	1	1	7	0	82	3
1.1–1.2	7	0	10	0	9	0	5	0	8	0	6	1	8	0	9	0	10	0	9	0	82	1
1.2–1.3	9	0	16	0	5	0	9	0	7	0	4	1	10	1	17	0	10	1	5	0	94	3
1.3–1.4	5	0	6	0	6	0	4	0	7	0	8	0	6	0	9	0	6	0	3	0	60	0

/Continued ...

APPENDIX TABLE 2 (Continued)

Distance (mm)	Leaf piece no. 1		2		3		4		5		6		7		8		9		10		Total no. of insects	Total no. of insects dead
	No. of insects Alive	Dead	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D		
1.4-1.5	5	0	4	0	10	0	8	0	10	0	5	0	5	0	11	0	6	1	7	0	72	1
1.5-1.6	4	0	7	0	6	0	3	0	8	0	6	0	4	0	11	0	5	0	5	0	59	0
1.6-1.7	5	0	8	0	5	0	3	0	4	1	6	0	5	0	5	0	1	1	7	0	51	2
1.7-1.8	6	0	10	0	3	0	10	0	7	0	6	0	12	0	9	0	8	0	6	0	77	0
1.8-1.9	8	0	9	1	6	0	10	0	2	1	5	0	9	1	6	0	6	0	2	0	66	3
1.9-2.0	5	0	6	0	7	0	6	0	8	0	9	0	3	0	4	0	4	0	4	0	56	0
2.0-2.1	8	0	7	0	5	0	5	0	6	0	4	0	10	0	6	0	9	0	4	0	65	1
2.1-2.2	4	0	5	0	6	0	5	0	7	0	7	0	8	0	11	0	11	0	6	0	70	0
2.2-2.3	8	1	8	0	9	0	6	1	5	0	7	0	5	0	9	0	9	0	4	0	72	2
2.3-2.4	4	0	7	0	7	0	9	0	6	0	5	0	5	0	3	1	11	0	5	0	63	1
2.4-2.5	6	0	9	0	4	0	5	0	7	0	4	1	7	1	6	0	4	0	2	0	56	2
2.5-2.6	2	0	4	1	4	0	8	0	8	0	9	0	3	0	6	0	9	0	2	0	55	0
2.6-2.7	3	0	12	0	3	0	4	0	5	0	12	0	3	0	6	0	9	0	3	0	60	0
2.7-2.8	8	0	6	1	3	0	4	0	5	0	7	0	6	0	7	0	2	0	4	0	53	1
2.8-2.9	5	0	3	0	1	0	6	0	10	0	3	0	5	0	7	1	5	1	1	1	49	3
2.9-3.0	2	0	4	0	5	0	2	0	6	0	7	0	2	0	3	0	6	0	0	0	37	0

APPENDIX TABLE 3: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around droplets of permethrin in VK1 of three concentrations on tobacco leaves

Concentration ...	20%			10%			5%		
Droplet size (µm) ...	113			107			117		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
0.1-0.2	100	99.11	256.6	100	97.39	96.47	100	97.84	114.8
0.2-0.3	100	91.55	31.95	87.61	82.32	15.02	77.42	76.77	11.06
0.3-0.4	79.18	73.92	9.635	57.83	58.44	5.151	45.41	42.28	2.877
0.4-0.5	53.56	53.00	4.229	36.35	38.18	2.470	15.33	20.65	1.141
0.5-0.6	29.62	36.05	2.276	21.29	24.96	1.421	04.99	10.67	0.569
0.6-0.7	17.80	24.52	1.391	12.00	16.90	0.915	08.07	06.04	0.327
0.7-0.8	21.40	17.09	0.927	09.27	11.93	0.637	03.23	03.72	0.207
0.8-0.9	11.89	12.29	0.657	13.90	08.76	0.468	05.49	02.44	0.141
0.9-1.0	05.30	09.13	0.487	02.79	06.65	0.359	01.14	01.69	0.101
1.0-1.1	05.87	06.97	0.375	05.13	05.20	0.284	00.87	01.22	0.075
1.1-1.2	03.30	05.45	0.297	04.22	04.16	0.231		00.91	0.058
1.2-1.3	10.56	04.36	0.241	05.23	03.40	0.191		0.70	0.046
1.3-1.4	02.62	03.55	0.199	02.55	02.83	0.161		0.55	0.037
1.4-1.5		02.94	0.167	01.74	02.39	0.138	0.21	0.45	0.030
1.5-1.6	02.45	02.47	0.142	05.03	02.04	0.120		0.37	0.025
1.6-1.7		02.10	0.123		01.76	0.105		0.30	0.021

/Continued ...

APPENDIX TABLE 3 (Continued)

Concentration ...	20%			10%			5%		
Droplet size (µm) ...	113			107			117		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
1.7-1.8		01.80	0.107		01.54	0.093		0.25	0.018
1.8-1.9		01.57	0.094	0.77	01.36	0.083		0.22	0.016
1.9-2.0		01.37	0.083		01.20	0.074		0.19	0.014
2.0-2.1		01.21	0.074		01.08	0.067		0.16	0.012
2.1-2.2	01.09	01.07	0.067		00.97	0.061		0.14	0.011
2.2-2.3		00.96	0.060		00.88	0.056	0.09	0.12	0.010
2.3-2.4		0.86	0.055		0.80	0.051		0.11	0.009
2.4-2.5		0.78	0.050		0.73	0.047		0.10	0.008
2.5-2.6		0.71	0.046	0.71	0.67	0.043		0.09	0.007
2.6-2.7		0.64	0.042		0.61	0.040		0.08	0.006
2.7-2.8	0.22	0.59	0.039		0.57	0.038		0.07	0.006
2.8-2.9		0.54	0.036		0.52	0.035		0.07	0.005
2.9-3.0		0.50	0.033		0.49	0.033		0.06	0.005
Control mortality:		2.26%			2.26%			2.26%	
Estimated natural mort:		0.62%			1.18%			1.58%	
Intercept:		3.45			2.79			3.14	
Slope:		-15.54			-13.87			-17.45	
Ld50:		0.466 mm			0.388 mm			0.326 mm	

APPENDIX TABLE 4: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around droplets of permethrin in VK1 of six concentrations on tobacco leaves

Concentration...	20%			10%			5%		
Droplet size (µm) ...	102			104			10		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
0.1-0.2	100	97.15	88.83	100	97.74	110.1	100	99.15	268.4
0.2-0.3	89.95	84.20	16.94	89.22	83.56	16.24	92.17	87.60	21.79
0.3-0.4	61.86	64.71	6.531	56.90	59.71	5.400	57.28	58.36	5.137
0.4-0.5	42.57	46.84	3.393	34.86	38.86	2.534	22.73	31.58	1.904
0.5-0.6	32.97	33.57	2.073	17.33	25.16	1.435	11.71	16.68	0.903
0.6-0.7	24.59	24.67	1.401	19.36	16.85	0.913	15.12	09.34	0.499
0.7-0.8	17.05	18.57	1.014	04.04	11.77	0.628	06.29	05.62	0.306
0.8-0.9	05.37	14.37	0.771	15.93	08.56	0.458	07.12	03.61	0.202
0.9-1.0	20.27	11.41	0.608	11.76	06.45	0.348		02.44	0.141
1.0-1.1	10.24	09.25	0.494	02.30	05.00	0.274	0.30	01.77	0.106
1.1-1.2	07.83	07.65	0.410	03.66	03.98	0.221		01.27	0.078
1.2-1.3	05.11	06.43	0.347	01.91	03.23	0.182		00.96	0.062
1.3-1.4		05.48	0.298		02.67	0.152		0.74	0.048
1.4-1.5		04.73	0.260		02.24	0.130		0.59	0.039
1.5-1.6		04.13	0.229	01.54	01.91	0.112	0.07	0.47	0.032
1.6-1.7	03.48	03.63	0.203		01.64	0.098		0.39	0.027

/Continued ...

APPENDIX TABLE 4 (Continued)

Concentration ...	20%			10%			5%		
Droplet size (µm) ...	102			104			10		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
1.7-1.8		03.23	0.182		01.43	0.086		0.32	0.023
1.8-1.9		02.89	0.164		01.25	0.077		0.27	0.019
1.9-2.0		02.60	0.149		01.11	0.069		0.23	0.017
2.0-2.1		02.36	0.136		00.99	0.062		0.20	0.015
2.1-2.2		02.15	0.125		0.88	0.056		0.17	0.013
2.2-2.3		01.96	0.115		0.80	0.051		0.15	0.011
2.3-2.4		01.80	0.107		0.72	0.047		0.13	0.010
2.4-2.5	01.41	01.67	0.099	0.45	0.66	0.043	0.01	0.12	0.009
2.5-2.6		01.54	0.093		0.60	0.040		0.10	0.008
2.6-2.7		01.43	0.087		0.55	0.037		0.09	0.007
2.7-2.8		01.33	0.081		0.51	0.034		0.08	0.007
2.8-2.9		01.25	0.077		0.47	0.032		0.07	0.006
2.9-3.0		01.17	0.072		0.44	0.030		0.07	0.006
Control mortality:		1.99%			1.99%			1.99%	
Estimated natural mort:		0.54%			2.30%			1.83%	
Intercept:		2.63			2.89			3.70	
Slope:		-12.36			-14.27			-18.73	
Ld50:		0.430 mm			0.393 mm			0.377 mm	

APPENDIX TABLE 4: (Continued)

Concentration ... 2% / Droplet size (µm) ... 101				1% / 110			0.5% / 108		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
0.1-0.2	100	98.36	147.2	100	97.45	98.41	100	95.69	60.65
0.2-0.3	83.83	82.24	14.94	71.84	81.61	14.28	74.89	74.30	9.811
0.3-0.4	51.65	51.48	4.006	60.06	56.26	4.756	42.05	47.23	3.440
0.4-0.5	21.88	27.84	1.622	45.41	35.46	2.224	32.04	28.56	1.674
0.5-0.6	15.76	15.27	0.822	30.89	22.46	1.126	18.15	17.90	0.974
0.6-0.7	12.85	08.96	0.479	13.54	14.83	0.797	17.81	11.87	0.633
0.7-0.8	03.72	05.63	0.306	05.39	10.27	0.548	03.01	08.30	0.444
0.8-0.9	01.90	03.76	0.210	02.86	07.42	0.399		06.07	0.329
0.9-1.0	04.23	02.64	0.151		05.57	0.303	02.97	04.60	0.253
1.0-1.1	02.65	01.93	0.113	02.77	04.30	0.238		03.60	0.201
1.1-1.2		01.45	0.088		03.41	0.192		02.89	0.164
1.2-1.3		01.13	0.070		02.76	0.158	02.59	02.36	0.137
1.3-1.4	00.73	00.89	0.057		02.28	0.132		01.97	0.116
1.4-1.5		00.72	0.047	01.80	01.91	0.113		01.66	0.099
1.5-1.6		00.59	0.039		01.63	0.097		01.43	0.086
1.6-1.7		00.50	0.033	01.07	01.40	0.085	01.20	01.24	0.076

/Continued ...

APPENDIX TABLE 4 (Continued)

Concentration ... 2% / Droplet size (µm) ... 101				1% / 110			0.5% / 108		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
1.7-1.8		0.42	0.029		01.21	0.075		01.08	0.067
1.8-1.9		0.36	0.025		01.06	0.066		0.95	0.060
1.9-2.0	0.25	0.31	0.022		0.94	0.059	0.74	0.85	0.054
2.0-2.1		0.27	0.019		0.84	0.053		0.76	0.049
2.1-2.2		0.23	0.017		0.75	0.048		0.68	0.045
2.2-2.3		0.21	0.015		0.68	0.044		0.62	0.041
2.3-2.4		0.18	0.014	0.40	0.61	0.040		0.56	0.037
2.4-2.5		0.16	0.012		0.57	0.037		0.52	0.035
2.5-2.6		0.15	0.011		0.51	0.034	0.38	0.47	0.032
2.6-2.7	0.11	0.13	0.010		0.47	0.032		0.44	0.030
2.7-2.8		0.12	0.009		0.43	0.029		0.40	0.028
2.8-2.9		0.11	0.009		0.40	0.027		0.38	0.026
2.9-3.0		0.10	0.008		0.37	0.026		0.35	0.024
Control mortality:		1.99%			1.99%			1.99%	
Estimated natural mort:		1.08%			1.35%			0.45%	
Intercept:		3.25			2.83			2.51	
Slope:		-17.06			-14.34			-13.58	
Ld50:		0.355 mm			0.376 mm			0.339 mm	

APPENDIX TABLE 5: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around droplets of 10% permethrin in JF 8133 of two sizes on tobacco leaves

Droplet size (µm) ...	59			114		
Distance (mm)	% mortality		Estimated arbitrary concentration	% mortality		Estimated arbitrary concentration
	Corrected	Smoothed		Corrected	Smoothed	
0.1–0.2	100	95.89	63.333	100	99.49	419.37
0.2–0.3	64.64	72.12	8.881	87.22	91.86	33.127
0.3–0.4	50.10	42.20	2.868	63.91	68.77	7.690
0.4–0.5	23.50	22.44	1.319	48.86	41.73	2.820
0.5–0.6	11.62	13.74	0.736	25.85	23.54	1.326
0.6–0.7	14.43	08.65	0.463	11.89	13.59	0.728
0.7–0.8	03.48	05.81	0.315	13.64	08.29	0.444
0.8–0.9		04.11	0.228	02.84	05.35	0.291
0.9–1.0	03.40	03.04	0.172	01.02	03.63	0.203
1.0–1.1		02.32	0.134		02.56	0.147
1.1–1.2		01.82	0.108		01.88	0.111
1.2–1.3		01.47	0.088		01.42	0.086
1.3–1.4		01.20	0.074		01.10	0.068
1.4–1.5	02.79	01.00	0.063		00.87	0.055
1.5–1.6		00.85	0.054		00.70	0.045
1.6–1.7		00.74	0.047	01.07	00.57	0.038

/Continued ...

APPENDIX TABLE 5 (Continued)

Droplet size (µm) ...	59			114		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
1.7-1.8		00.63	0.041		00.47	0.032
1.8-1.9		00.55	0.036		00.40	0.027
1.9-2.0		00.48	0.032		00.34	0.024
2.0-2.1		00.43	0.029		00.29	0.020
2.1-2.2		00.38	0.026		00.25	0.018
2.2-2.3		00.34	0.024		00.22	0.016
2.3-2.4	00.09	00.31	0.022		00.19	0.014
2.4-2.5		00.28	0.020		00.17	0.013
2.5-2.6		00.26	0.018	00.02	00.15	0.011
2.6-2.7		00.24	0.017		00.13	0.010
2.7-2.8		00.22	0.016		00.12	0.009
2.8-2.9		00.20	0.015		00.11	0.008
2.9-3.0		00.19	0.014		00.10	0.008
Control mortality:		2.80%			3.90%	
Estimated natural mort:		2.22%			3.03%	
Intercept:		2.61			3.97	
Slope:		-14.65			-18.93	
Ld50:		0.321 mm			0.417 mm	

APPENDIX TABLE 6: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around droplets of 10% permethrin in three formulations on tobacco leaves

Formulation ... / Droplet size (µm) ...	JF 8130 113			VK 1 110			JF 8133 108		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
0.1-0.2	100	89.81	26.572	100	98.50	160.16	100	99.67	622.45
0.2-0.3	69.00	61.13	5.694	92.53	87.72	22.006	93.35	94.24	46.143
0.3-0.4	35.05	36.85	2.347	61.75	66.55	7.025	72.36	75.39	10.327
0.4-0.5	10.45	22.79	1.277	39.32	45.27	3.026	51.57	49.21	3.693
0.5-0.6	07.99	15.02	0.808	28.26	29.94	1.777	35.11	28.96	1.704
0.6-0.7	15.08	10.53	0.561	16.94	20.18	1.112	11.97	17.00	0.921
0.7-0.8	01.18	07.75	0.416	19.42	14.08	0.755	05.20	10.40	0.555
0.8-0.9	01.38	05.95	0.322	16.56	10.19	0.544	08.63	06.69	0.361
0.9-1.0	09.08	04.71	0.259	09.58	07.63	0.409	04.10	04.51	0.249
1.0-1.1	02.04	03.82	0.213	05.47	05.88	0.319	04.03	03.17	0.179
1.1-1.2	01.46	03.17	0.179	03.97	04.64	0.255	04.53	02.31	0.134
1.2-1.3	04.06	02.68	0.153	02.44	03.75	0.209	01.26	01.73	0.103
1.3-1.4		02.30	0.132	02.10	03.08	0.174		01.33	0.082
1.4-1.5	02.27	02.00	0.117		02.57	0.148		01.05	0.065
1.5-1.6		01.75	0.104		02.18	0.127	0.19	0.84	0.053
1.6-1.7		01.55	0.093	0.87	01.86	0.120		0.68	0.044

/Continued ...

APPENDIX TABLE 6 (Continued)

Formulation ... / Droplet size (µm) ...	JF 8130 113			VK 1 110			JF 8133 108		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
1.7-1.8		01.39	0.084		01.61	0.096		0.56	0.037
1.8-1.9	01.53	01.25	0.077		01.41	0.086		0.47	0.032
1.9-2.0		01.13	0.070		01.24	0.076		0.40	0.027
2.0-2.1		01.03	0.064		01.10	0.068		0.34	0.024
2.1-2.2		00.94	0.059		0.98	0.062		0.29	0.021
2.2-2.3		0.87	0.055		0.88	0.056		0.25	0.018
2.3-2.4		0.80	0.051	0.50	0.80	0.051	0.13	0.22	0.016
2.4-2.5	00.55	0.74	0.048		0.72	0.047		0.20	0.014
2.5-2.6		0.69	0.045		0.66	0.043		0.17	0.013
2.6-2.7		0.65	0.042		0.60	0.040		0.15	0.012
2.7-2.8		0.61	0.040		0.55	0.037		0.14	0.011
2.8-2.9		0.57	0.038		0.51	0.034		0.12	0.010
2.9-3.0		0.54	0.036		0.47	0.032		0.11	0.009
Control mortality:		3.85%			2.95%			3.42%	
Estimated natural mort:		3.21%			2.62%			3.07%	
Intercept:		1.90			3.14			4.22	
Slope:		-11.49			-14.80			-19.40	
Ld50:		0.290 mm			0.426 mm			0.447 mm	

APPENDIX TABLE 7: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around droplets of 10% permethrin in VK1 on three plant species

Plant species ...	Tobacco			Tomato			French bean		
Droplet size (µm) ...	110			106			106		
Distance (mm)	% mortality		Estimated arbitrary concentration	% mortality		Estimated arbitrary concentration	% mortality		Estimated arbitrary concentration
	Corrected	Smoothed		Corrected	Smoothed		Corrected	Smoothed	
0.1-0.2	100	98.50	160.16	100	98.29	142.11	100	99.18	275.23
0.2-0.3	92.53	87.72	22.006	92.76	90.37	28.085	95.81	94.39	47.327
0.3-0.4	61.75	66.55	7.025	76.14	76.76	11.049	88.59	84.41	17.187
0.4-0.5	39.32	45.27	3.026	58.91	61.72	5.821	69.04	71.31	8.570
0.5-0.6	28.26	29.94	1.777	37.00	48.45	3.595	62.83	58.05	5.078
0.6-0.7	16.94	20.18	1.112	46.91	37.97	2.450	38.73	46.48	3.350
0.7-0.8	19.42	14.08	0.755	30.18	30.06	1.786	31.73	37.16	2.376
0.8-0.9	16.56	10.19	0.544	24.64	24.14	1.366	28.04	29.92	1.776
0.9-1.0	09.58	07.63	0.409	29.84	19.71	1.083	23.77	24.36	1.380
1.0-1.1	05.47	05.88	0.319	12.21	16.35	0.883	21.51	20.09	1.106
1.1-1.2	03.97	04.64	0.255	11.99	13.75	0.737	20.61	16.78	0.908
1.2-1.3	02.44	03.75	0.209	10.44	11.72	0.625	10.75	14.18	0.760
1.3-1.4	02.10	03.08	0.174	06.45	10.11	0.539	17.45	12.13	0.647
1.4-1.5		02.57	0.148	0.734	08.81	0.471	06.77	10.48	0.559
1.5-1.6		02.18	0.127	11.01	07.75	0.416	13.54	09.14	0.488
1.6-1.7	0.87	01.86	0.120	04.61	06.88	0.370		08.03	0.430

/Continued ...

APPENDIX TABLE 7 (Continued)

Plant species... Droplet size (µm) ...	Tobacco 110			Tomato 106			French bean 106		
Distance (mm)	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration	% mortality Corrected	% mortality Smoothed	Estimated arbitrary concentration
1.7-1.8		01.61	0.096	08.79	06.14	0.332		07.12	0.383
1.8-1.9		01.41	0.086		05.53	0.301	06.38	06.35	0.343
1.9-2.0		01.24	0.076	04.93	05.00	0.274		05.71	0.310
2.0-2.1		01.10	0.068		04.55	0.250		05.15	0.282
2.1-2.2		0.98	0.062		04.16	0.230	06.07	04.68	0.257
2.2-2.3		0.88	0.056	02.95	03.82	0.213		04.27	0.236
2.3-2.4	0.50	0.80	0.051		03.53	0.197		03.91	0.217
2.4-2.5		0.72	0.047		03.26	0.184	03.57	03.60	0.201
2.5-2.6		0.66	0.043		03.03	0.172		03.32	0.187
2.6-2.7		0.60	0.040		02.83	0.161		03.08	0.174
2.7-2.8		0.55	0.037	02.51	02.56	0.147	02.69	02.86	0.163
2.8-2.9		0.51	0.034		02.47	0.143		02.67	0.153
2.9-3.0		0.47	0.032		02.33	0.135		02.49	0.144
Control mortality:		2.95%			2.20%			1.47%	
Estimated natural mort:		2.62%			1.33%			0.40%	
Intercept:		3.14			2.88			3.32	
Slope:		-14.80			-12.09			-13.13	
Ld50:		0.426 mm			0.537 mm			0.618 mm	

APPENDIX TABLE 8: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around droplets of 10% pirimiphos-methyl and 10% permethrin in JF 9079 on tobacco leaves

Chemical ... Pirimiphos-methyl Droplet size (µm) ... 102				Permethrin 107			
Distance (mm)	% mortality		Estimated arbitrary concentration	Distance	% mortality		Estimated arbitrary concentration
	Corrected	Smoothed			Corrected	Smoothed	
0.0–1.0	100	99.993	20710	0.0–0.1	-	-	-
1.0–2.0	100	99.19	278.79	0.1–0.2	100	93.33	144.86
2.0–3.0	95.40	94.87	51.459	0.2–0.3	79.77	84.87	17.726
3.0–4.0	87.70	85.38	18.379	0.3–0.4	62.02	59.20	5.298
4.0–5.0	66.29	72.03	08.847	0.4–0.5	36.12	36.43	2.310
5.0–6.0	59.54	57.85	05.041	0.5–0.6	29.98	22.17	1.237
6.0–7.0	44.15	45.20	03.199	0.6–0.7	13.30	14.05	0.753
7.0–8.0	29.20	35.02	02.187	0.7–0.8	04.80	09.37	0.500
8.0–9.0	33.97	27.23	01.578	0.8–0.9	10.09	06.55	0.353
9.0–10.0	24.91	21.39	01.187	0.9–1.0	02.60	04.77	0.262
10.0–11.0	17.38	17.02	0.923	1.0–1.1	03.65	03.59	0.201
11.0–12.0	17.06	13.74	0.736	1.1–1.2		02.78	0.159
12.0–13.0	10.53	11.24	0.600	1.2–1.3		02.21	0.128
13.0–14.0	05.34	09.32	0.497	1.3–1.4	01.03	01.79	0.106
14.0–15.0	02.13	07.82	0.419	1.4–1.5		01.47	0.089
15.0–16.0		06.63	0.357	1.5–1.6		01.23	0.076
16.0–17.0		05.68	0.308	1.6–1.7		01.04	0.065

/Continued ...

APPENDIX TABLE 8 (Continued)

Chemical ... Pirimiphos-methyl Droplet size (µm) ... 102				Permethrin 107			
Distance (mm)	% mortality		Estimated arbitrary concentration	Distance (mm)	% mortality		Estimated arbitrary concentration
	Corrected	Smoothed			Corrected	Smoothed	
17.0–18.0		04.91	0.269	1.7–1.8		00.90	0.057
18.0–19.0		04.28	0.236	1.8–1.9		00.77	0.050
19.0–20.0		03.75	0.209	1.9–2.0		00.68	0.044
20.0–21.0		03.32	0.187	2.0–2.1	0.28	00.60	0.039
21.0–22.0		02.95	0.167	2.1–2.2		00.53	0.035
22.0–23.0		02.64	0.151	2.2–2.3		00.47	0.032
23.0–24.0		02.37	0.137	2.3–2.4		00.40	0.028
24.0–25.0		02.14	0.125	2.4–2.5		00.38	0.026
25.0–26.0		01.94	0.114	2.5–2.6		00.35	0.024
26.0–27.0		01.77	0.105	2.6–2.7		00.31	0.022
27.0–28.0		01.62	0.096	2.7–2.8		00.29	0.020
28.0–29.0		01.48	0.089	2.8–2.9		00.26	0.019
29.0–30.0		01.36	0.083	2.9–3.0		00.24	0.018
Control mortality:		1.13%			1.13%		
Estimated natural mort:		1.13%			1.13%		
Intercept:		9.98			3.14		
Slope:		-22.42			-15.66		
Ld50:		6.10 mm			0.386 mm		

APPENDIX TABLE 9: Data comparing mortalities, estimated arbitrary concentrations and regression coefficients around 104 µm droplets of 10% pirimiphos-methyl in JF 9079 in still air and with continuous air flow over treated tobacco leaf pieces

Still air (droplets sprayed on test leaf pieces)				Still air (droplets sprayed on separate leaf strips)			Continuous air flow over treated leaf pieces			
Distance (mm)	% mortality		Estimated arbitrary concentration	% mortality		Estimated arbitrary concentration	Distance (mm)	% mortality		Estimated arbitrary concentration
	Corrected	Smoothed		Corrected	Smoothed			Corrected	Smoothed	
0.0-1.0	100	99.995	25251	100	99.994	21283	0.0-0.5	97.53	98.89	209.4
1.0-2.0	100	99.33	330.37	100	99.25	298.04	0.5-1.0	72.32	69.49	7.927
2.0-3.0	95.65	95.67	60.302	95.44	95.30	55.870	1.0-1.5	36.03	36.49	2.279
3.0-4.0	87.73	87.37	21.391	84.87	86.61	20.143	1.5-2.0	21.97	19.58	1.075
4.0-5.0	74.41	75.22	10.247	75.89	74.20	9.761	2.0-2.5	10.46	11.86	0.633
5.0-6.0	67.97	61.70	5.817	63.48	60.64	5.591	2.5-3.0	09.39	07.87	0.422
6.0-7.0	46.06	49.11	3.680	47.53	48.20	3.562	3.0-3.5		05.60	0.304
7.0-8.0	34.62	38.60	2.509	34.46	37.90	2.444	3.5-4.0	00.39	04.19	0.232
8.0-9.0	27.66	30.34	1.807	32.93	29.83	1.769	4.0-4.5		03.26	0.184
9.0-10.0	24.04	24.01	1.357	17.48	23.67	1.334	4.5-5.0		02.61	0.150
10.0-11.0	23.39	19.21	1.053	18.41	18.99	1.039	5.0-5.5		02.15	0.125
11.0-12.0	09.70	15.57	0.838	20.52	15.43	0.831	5.5-6.0		01.80	0.107
12.0-13.0	15.11	12.77	0.682	11.19	12.69	0.678	6.0-6.5		01.53	0.092
13.0-14.0	13.58	10.60	0.565	12.98	10.57	0.564	6.5-7.0		01.33	0.081
14.0-15.0	07.16	08.90	0.476	05.48	08.90	0.475	7.0-7.5		01.16	0.072
15.0-16.0		07.44	0.399		07.57	0.406	7.5-8.0		01.02	0.064
16.0-17.0		06.47	0.349		06.50	0.351	8.0-8.5		00.91	0.058

/Continued ...

APPENDIX TABLE 9 (Continued)

Still air (droplets sprayed on test leaf pieces)			Still air (droplets sprayed on separate leaf strips)			Continuous air flow over treated leaf pieces			
Distance (mm)	% mortality Corrected	Smoothed	Estimated arbitrary concentration	% mortality Corrected	Smoothed	Estimated arbitrary concentration	Distance (mm)	% mortality Corrected	Smoothed	Estimated arbitrary concentration
17.0–18.0		05.60	0.304		05.63	0.306	8.5–9.0		00.82	0.052
18.0–19.0		04.88	0.267		04.92	0.269	9.0–9.5		00.74	0.048
19.0–20.0		04.28	0.236		04.33	0.239	9.5–10.0		00.67	0.044
20.0–21.0		03.78	0.211		03.83	0.231				
21.0–22.0		03.36	0.189		03.41	0.191				
22.0–23.0		03.00	0.170		03.06	0.173				
23.0–24.0		02.70	0.154		02.75	0.157				
24.0–25.0		02.44	0.141		02.49	0.143				
25.0–26.0		02.21	0.129		02.26	0.131				
26.0–27.0		02.01	0.118		02.06	0.121				
27.0–28.0		01.84	0.109		01.88	0.111				
28.0–29.0		01.69	0.100		01.73	0.103				
29.0–30.0		01.55	0.093		01.59	0.096				
Control mortality:		1.26%			1.26%				1.26%	
Estimated natural mort:		1.26%			1.26%				1.26%	
Intercept:		10.12			9.95				4.33	
Slope:		-22.58			-22.22				-14.37	
Id50:		6.42 mm			6.35 mm				1.01 mm	