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THE ROLE OF LEAF SURFACE CHARACTERISTICS IN THE MEDIATION OF PESTICIDE AVAILABILITY TO INVERTEBRATES

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Dedicated to my parents especially to my late father who dedicated his past for our future, to my uncle Dr. Shamsul Alam who gave all the important turn of my success, to my elder brother Mr. Salim Uddin Chowdhury and my beloved wife, daughter and son whose smile keep my hope all the time alive.

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ABSTRACT

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THE ROLE OF LEAF SURFACE CHARACTERISTICS IN THE MEDIATION OF PESTICIDE AVAILABILITY TO INVERTEBRATES

by A. B. M. Nasir Uddin Chowdhury

The role of leaf surface characteristics in mediating the toxicity of the pesticides deltamethrin and dimethoate to the springtail *F. candida* and a parasitoid *A. colemani* has been studied. This study is important for the interpretation of the behaviour of foliar applied pesticides on various plant surfaces and the subsequent transfer of toxicant to the target invertebrates. There have been few quantitative assessments of the fate of pesticide deposits and their action in various crops.

Bioassays on residues showed significant differences in mortality of *F. candida* on different leaf types. On deltamethrin treated surfaces LD_{50} values ranged from 6.36 (g AI ha^{-1}) to 77.14 (g AI ha^{-1}). Significant differences in *F. candida* mortality on three species of cereal crops suggest that it may necessary to recommend different application rates for different crops, instead of adhering to a conventional single application rate. On dimethoate treated surfaces the range of LD_{50} values was 1.35 to 8.69 g AI ha^{-1}. *F. candida* was found to survive on some leaf surfaces that had been sprayed with deltamethrin at a rate of 16 times greater than the field rate. On dimethoate treated surfaces mortality was observed on some leaf surfaces sprayed with over 100 times less pesticide than used in the field.

The amount of wax on various leaf surfaces was positively correlated with the residual toxicity of deltamethrin to *F. candida* and *A. colemani*. The lipophilicity of deltamethrin may be an important factor in this correlation. No such relationship between the epicuticular wax content of various leaf types and residual toxicity of dimethoate to *F. candida* was observed despite significant differences in mortality on different leaf surfaces. This highlights the importance of the nature of the active ingredient, the formulation of pesticide, and other leaf surface characteristics in mediating toxicity.

The wettability of leaf surfaces was found to be negatively correlated with mortality of *F. candida* exposed to deltamethrin. Wettability may, however, be important in increasing spray efficacy and the behaviour of deposits on the substrate, but it is not necessarily positively correlated with increased mortality of target invertebrates.

F. candida is highly suitable for laboratory bioassays, with no control mortality and stable end-points in toxicity measurements. The susceptibility trend for *A. colemani* on different leaf types treated with deltamethrin are similar to those using *F. candida* and suggest that results may be extrapolated between related species

The results are discussed in terms of the physico-chemical properties of leaf surfaces and the ways in which they can modify the pesticide availability and can be used to predict the ultimate mortality of exposed invertebrates. These predictions may be used to aid the production of effective formulations leading to lower application rates for various crops. The experimental framework developed in this study may be adapted to evaluate pesticide side-effects on other beneficial invertebrates in various crops.

CHAPTER 1

Introduction and literature review

1.1 General introduction

The toxicity of foliar-applied pesticides depends, to a large extent, on the 'bioavailability' of the active ingredient (a.i.) to exposed organisms. Bioavailability is the term used to define the level of exposure of components of the biological system to the toxicant in its active form.

Besides species-dependent factors such as activity patterns, behaviour and the rate of contact with insecticides (Jepson *et al.* 1990; Wiles & Jepson, 1993), and a variety of chemical properties, the bioavailability of insecticides applied to plants is affected by a number of plant-surface factors. These factors, alone or in combination, influence both the physical and chemical states of the pesticide resulting in a modification of its toxic action.

The behaviour of a pesticide deposit after impaction and the subsequent transfer to the target insect is greatly influenced by the affinity of a deposit for the plant surface (Ford & Salt, 1987). Surface tension and the force of adhesion are the two main factors which determine the affinity of a deposit and thereby, wettability. A physical property that defines the relationship between the deposit droplet and the solid surface body, commonly known as contact angle is used to quantify surface tension (Holloway, 1970). Variation in contact angle represents variation in the rate of adhesion of droplets to polar and hydrophobic surfaces. Although no plant surface is either completely hydrophobic or completely wettable, however, wettability is governed by the degree of hydrophobicity. Hydrophobicity to a great extent results from the presence of plant cuticular waxes and the degree of hydrophobicity depends upon the chemical composition and orientation of the wax molecules. Chemical groups containing only carbon and hydrogen form the largest contact angles. Increases in the number of functional groups by the introduction of oxygen, prevent close packing and

introduce hydrogen bond potential which results in lowering the contact angle with the water droplet (Holloway, 1970). The highest contact angle occurs in *Brassica oleracea* whose cuticular waxes are mainly composed of normal alkanes.

Following impaction, the wettability of the leaf surface is defined by the spread of the deposit, which is calculated as a 'spread factor' r_2^2/r_1^2 ; where r_1 is the radius of the in-flight drop and r_2 is the radius of the dried deposit (Baker *et al.* 1983). High adhesion (low contact angle) causes greater spread factors whereas on the hydrophobic surfaces of plants such as carnation, clover and strawberry, which have high contact angles, there is poor adhesion and low spread. On the smooth and wax-covered surfaces of some crops such as sugar beet, dwarf bean and lemon, spread factor ranges from 3.2-4.0, which ceases with the rapid drying of the deposit (Ford & Salt, 1987). In general, the spread of polar deposits is restricted by non-polar waxy surfaces, whereas non-polar deposits show the opposite trend.

The interaction between the plant cuticle and spray droplets was investigated by Baker *et al.* (1983). Early studies (Holloway, 1970) focused mainly upon aqueous solution where it has been shown that leaf surface topography (caused by underlying venation, wax molecular structures, arrangement of trichomes etc) decreases the area of contact between the impacting droplet and the leaf surface, and the hydrophobic nature of cuticular waxes restricts overall wettability. Baker *et al.* (1983) also investigated the impact of oil-based emulsions and wettable powder formulation on leaf surfaces. Oil-based deposits spread rapidly through layers of crystalline wax and the aqueous formulations distribute more readily over smooth leaf surfaces. It is clear from the above result that cuticular waxes, their composition and some cases their distribution on the leaf surface (Crease *et al.* 1985) play an important role in leaf wettability.

A comprehensive study of the plant cuticle was also carried out by Farnendes, *et al.* (1964). To understand all aspects of

foliar retention and uptake of pesticides, from landing, impaction and retention to the final transfer of active ingredient to the target insect, other factors must also be considered. These include leaf topography. Variation in deposit redistribution between smooth and glaucous or hairy leaves is not only a result of wax thickness but also the presence of pronounced venation, or other cuticular features such as asperites, papillae, secretory glands and trichomes (Baker *et al.* 1983). Increasing in surface roughness tend to increase the contact angles greater than 90^0, decreases contact angle if it is less than 90^0 (Hartley & Bryce, 1980). Studies on artificial surfaces with paraffin waxes demonstrate that it is possible to increase contact angle from a normal 110^0 to 158^0 by roughening (Dettre & Johnson, 1964). Wenzel (1936) described an equation for non-composite roughness to relate the roughness and contact angle (Q) of a given system in terms of a roughness co-efficient (r);
$r = \cos Q_2 / \cos Q_1$, where r is the ratio of the true surface area to the apparent area of the rough solid, Q_2 is the contact angle on the rough surface (apparent contact angle) and Q_1 is the contact angle on a smooth surface (absolute contact angle). The roughness will decrease the value of the apparent contact angle when Q_1 is less than 90^0 and the reverse will happen when Q_1 is greater than 90^0. When the roughened surface is composite (having solid/liquid and air/liquid interfaces), a different equation applies by Cassie and Baxter (1944), as $\mathrm{Cos}Q_2 = f_1 \mathrm{Cos}Q_1 - f_2$, where f_1 is the area of liquid/solid contact, f_2 is the area of liquid/air contact per unit area , and Q_1 is the absolute and Q_2 the apparent contact angle.

Microscopic roughness resulting from leaf venation and from epidermal cell boundaries can produce composite surfaces which can remain water repellent even after chloroform washing of waxes. Similarly, trichomes play an important role in determining the composite nature of leaf surfaces which eventually affect wettability. The factor involved is the close association of trichomes which produce composite surfaces that allow air to become trapped between the drop

and the leaf surfaces, resulting in water repellency, and trichome with open patterns enhance wetting by capillarity (Challen, 1960).

The aim of the present study is to examine the differences in the surface-mediated toxicity of various insecticides and to determine the factors responsible for the differences in pesticide toxicity on different leaf surfaces.

This review is divided into three parts. Part one deals with the different leaf characteristics and their interactions with deposit droplets and the insect. Part two is aimed at describing the studies of leaf surface features using scanning electron microscope and finally part three is concerned with the bioassay procedures.

Part I

1.2 Epicuticular waxes

The leaf surface of almost all higher plants is covered by non-cellular and non-living cuticles which are heterogeneous in chemical composition and which contain epicuticular waxes on their surfaces. Although the amounts vary greatly with species, surface, plant part, and age, wax may comprise up to 4 percent of the fresh weight of the leaf (Eglinton & Hamilton, 1967). Variation occurs not only in amount but also in size, shape and constituents. Generally, waxes are complex mixtures of various classes of aliphatic compounds, each class comprising homologues with predominantly odd or even carbon numbers (Kreger, 1948; Eglinton & Hamilton, 1967). The nature and number of classes and the chain length of homologues differ among plant species (Holloway, 1970). Even numbered classes are commonly esters, primary alcohols and fatty acids and sometimes aldehydes, α-ω- diols and ω- - hydroxy acids and unsaturated fatty acids. Odd numbered classes mainly comprise alkanes and secondary alcohols, less frequently ketones and β-diketones and infrequently ketols, alkanes, and 2-methyl and 3-methyl branched alkanes. Overall chain length varies, from C_{10} to C_{70} (Holloway, 1969a).

In addition to species variation, cuticular waxes also vary according to leaf and plant age, size, surface (adaxial & abaxial) and even with the growth conditions. For example, immature dwarf bean leaves are coated with a thin film of amorphous wax, but plate waxes are formed during expansion on the adaxial surface. Field-grown plants exhibit larger amounts of wax than glass-house-grown plants (Baker & Hunt, 1981). There is no relationship between leaf size and the amount of wax. Environmental conditions such as radiant energy, humidity and temperature affect wax production. An increase in radiant energy or a decrease in humidity or temperature, induces the largest deposits of wax (Baker, 1974).

Differences in wax morphology are believed to result from chemical composition, however, wax ultra-structure may also be influenced by environmental conditions (Jeffree *et al.*, 1976). For example, pea plants grown in darkness develop little wax and have smooth surfaces. When these plants are exposed to strong light, young leaves rapidly produce prominent wax structures (Juniper, 1960b). Similarly, on wheat leaves, the occurrence of rodlet and platelet structures is related to light intensity and temperature (Throughton & Hall, 1967). Changes resulting from light and temperature regime have also been observed on *Brassica napus* (Whitecross & Armstrong, 1962). Sometimes, chemical and structural modification occurs during development and ageing (Rentschler, 1971; Bain & McBean, 1967; Majliak & Pommier-Miard, 1963; Skene, 1963; Fernandes *et al.* 1964; Schuck, 1969; Schutt & Schuck, 1973). Hallam (1967) and Hallam and Chamber (1970) detected a correlation between tubular wax morphology and a predominance of β-diketones in a large number of *Eucalyptus* species. This was later supported by von Wettstein-Knowles (1972) who reported similar findings with various mutant varieties of barley.

The appearance of wax on leaf surfaces also varies from species to species and even variety to variety. Because of their waxy appearance, leaves are sometimes classified as

glaucous, sub-glaucous and non-glaucous. According to Netting and von Wettestein-Knoweles (1973), increasing glacuousness is correlated with an increasing proportion of β-diketones and hydroxy-β-diketones in the wax. Glacuousness in barley (Lundqvist *et al.* 1968; von Wettstein-Knowles, 1972), *Poa colensoi* and *Eucalyptus urnigera* (Hall *et al.* 1965) is also associated with wax tubes. It may also however, be associated with other wax structures including the "loofah-like" structures of *Brassica* spp. (Juniper & Bradley, 1958), which have waxes rich in hydrocarbons, ketones and secondary alcohols (Purdy & Truter, 1963; Macey & Barber, 1970).

The relationship between the chemical constituents of wax and the form of its thin, long, tube structures in *Eucalyptus* has also been confirmed by the work of Horn *et al.* (1968); Hallam, (1967); Hallam & Chambers, 1970; in barley by von Wettstein-Knowles (1972, 1974a) and in wheat by Tulloch (1973). More recently, it has been observed that in wheat (*Triticum* spp.), the glaucousness or waxy bloom on the surface of the leaves and other plant parts and the quantity of epicuticular waxes is associated with water loss through the cuticle, wettability by pesticide sprays and disease susceptibility (Clarke, *et al.* 1994).

1.2.1 The importance of epicuticular waxes

The importance of the presence of epicuticular waxes on the leaf surfaces as a barrier to the transcuticular movement of many substances is well-established (Pfeiffer, *et. al.* 1959; Norris & Bukovac, 1972, Schönherr, 1976; Juniper & Bradley, 1958; Throughton & Hall, 1967; Holloway, 1969a). One major role of waxes on epicuticle is to prevent wetting of leaves by water repellency (Eglinton & Hamilton, 1967; Fogg, 1947, '48) whilst waxes embedded in cuticle have a water resistant action (Scheiferstein & Loomis, 1956; Grncarevic & Radler, 1967; Hall & Jones, 1961; Horrocks, 1964, Denna, 1970). Although hydrophobicity is the most important physicochemical property of the superficial wax, if this barrier can overcome, then the superficial wax may play an important role in facilitating the passage of lipophilic substances into the

cuticle by a process of solution (Holloway, 1970).

From a pest management perspective, the importance of waxes has recently received wide attention. The physicochemical environment on the leaf surface, as it affects wetting, spreading, coverage, retention and penetration plays an important role in determining the effectiveness of sprayed agro-chemicals. Physicochemical factors are governed by leaf surface characteristics such as epicuticular and cuticular waxes and their ultrastructure, and both the macro- and micro-roughness (which will be discussed later) of the leaf surface. Although the cuticle provides the initial site for spray deposition, its subsequent role in penetration is poorly understood (Baker & Hunt, 1981).

Following the impaction or landing of spray droplets, with initial bouncing and drop off, the important factors to be considered are retention, spreading and coverage, wetting and penetration or the uptake processes. The latter is more important for systemic chemicals whereas other factors are important for contact toxicants.

1.2.2 Spreading and distribution

Wide variation has been observed in spreading factors between pesticide formulations and between leaves of different plant species, which subsequently affect spray performance. The factors affected include coverage, contact and hence the residue concentration gradient within the cuticle. Baker *et al.* (1983) found that the greatest amount of spread occurred on maize leaves sprayed with oil-based chemicals. These moved readily across the glaucous surfaces, with redistribution proceeding primarily in a lateral direction. In strawberry, oil-based formulations moved preferentially along venation, suggesting that the surface wax was thicker in this region. Probably as a result of same process, different spread factors are observed on maize leaves of varying age. Seedling and immature leaves contain more waxes, with a greater density of crystalline platelets, than mature leaves.

When aqueous solutions are applied to smooth leaf surfaces, high spreading occurs, although the formulations dry rapidly. Redistribution largely depended on drop size, velocity and solution concentration at impaction (Baker *et al.* 1983). Baker *et al.* (1983) also found that at low concentrations (0.5-1.0%) of organic solvent, the properties of the central aqueous zone principally determined the deposit area. The organic phase was restricted to a narrow annulus and partitioned only marginally into the wax layers. This annulus became thicker and lipophilic constituents moved extensively in a lateral direction. In the vicinity of the annulus epicuticular waxes are badly disrupted, but the crystalline structure at the centre of the droplet is largely unchanged. The property of water repellency seems most to affect deposition, distribution and retention of chemicals applied to the foliage as suspensions or aqueous solutions (Crafts & Foy, 1962).

Wax may also play an important role in droplet reflection, which may occur from either a dry or a uniformly wetted leaf surface and is probably a result of the air film trapped between the droplet and the surface (Hartley, 1967). On dry surfaces, the causes of reflection are more preferentially attributed to the presence of micro-roughness, usually wax crystals or a dense covering of trichomes (Holloway, 1994). The droplet reflection phenomenon was examined in detail by Brunskill (1956) and Hartley and Brunskill (1958) on the waxy and highly water-repellent leaf surfaces of pea (*Pisum sativum*). However, the processes that control foliar distribution of sprayed chemicals are not fully understood and involve complex interactions between the nature and formulation of the sprayed chemicals (even application methods) and the physicochemical nature of the leaf surface. Simple relationships have not been established between foliar spread and the equilibrium surface tension of the spray solution. Abbot *et al.* (1990) carried out an extensive study with a wide range of organic liquids and adjuvants (many of which are in emulsifiable concentrate (EC) formulation) but he was unable to quantify spread in relation to a particular

physical parameter, such as equilibrium surface tension, contact angle or critical micelle concentration (CMC). It is generally assumed that EC formulations can spread more effectively on leaves that possess high amounts of crystalline waxes such as on Johnson grass (McWhorter & Barrentine, 1988), thus supporting the early work of Baker *et al.* (1983).

1.2.3 Retention

Retention of spray droplets is also influenced greatly by waxes and their ultrastructure, in combination with other aspects of macro- and micro-structure of leaf surfaces (Wattanabi & Yamaguchi, 1991). The retention of applied spray has been extensively covered in the literature (Stock & Davies, 1994). Differential retention by crop and weed species has been invoked as an important mechanism of selectivity for some herbicides. (Davies *et al.* 1967; Blackman *et al.* 1958; Holly, 1976; Hibbitt, 1969). Stock and Davies (1994) investigated the importance of formulation on retention.

The major focus of recent studies (Anderson & Hull, 1989; de Ruiter *et al.* 1990; Grayson *et al.* 1991) has been the dynamic surface tension of the spray solutions. However, it is important not to overlook the fact that in order to understand the factors that make formulations more effective, we must understand leaf surface characteristics and their role. It has been observed that EC formulations are retained more effectively than WPs, although WPs contain fairly high proportions of surfactants to promote wetting and agents for dispersion of particles in the spray tank. Wetting/dispensing agents for AIs are generally not the most appropriate materials for leaf wetting.

Studies under controlled conditions have demonstrated the importance of dynamic surface tension in spray retention phenomena for surfactant-containing solutions. Reductions in this parameter are not a guarantee of good retention however, because much depends upon the wettability of the leaf surface

(Stock & Davies, 1994), which in turn depends largely on wax content and other physicochemical properties of the leaf surface itself.

Some apparent conflicts exist between retention and coverage (Furmidge, 1962; Holloway 1993). The organosilicone 'Silwet L77', which is known to reduce dynamic surface tension, caused a significant reduction in retention on the leaves of sugar beet compared with a conventional organic surfactant or even water. If the coverage of a leaf surface is effective, (characterised by a low advancing contact angle, which again largely depends on wax ultra-structures and leaf macro- and micro-roughness), mean retention per unit area can be considerably reduced. The importance of foliar coverage versus retention depends upon the mode and site of the active ingredient (Stock & Davies, 1994).

1.2.4 Wetting

Morphological and chemical characteristics of the leaf surface have a considerable influence on overall wetting (Fogg, 1947; Baker & Bukovac, 1971). Research into the role of surface chemistry on the wettability, retention and penetration of applied chemicals (Zisman, 1964; Possingham *et al.* 1967; Holloway 1969a) has focused upon the nature of the surface waxes and their orientation (Holloway 1969a). The quantity of surface waxes is not critical, provided the entire surface is covered, and thus a monomolecular layer of wax can sufficiently reduce wetting (Baker & Bukovac, 1971).

Wettability of leaf surfaces is generally governed by the same physicochemical factors that control the wetting of any solid surface and is largely determined by the nature of chemical groups exposed on the surface (Adams & Jessop 1925). Despite an extensive knowledge of wax chemistry, the precise relationship between composition and wetting properties has been under explored. The external surfaces of plants show considerable differences in their wettability, ranging from completely wettable to highly water repellent (Holloway, 1969a). However, differences in leaf wettability are not

categorised wholly by the differences that occur in the chemical and hydrophobic properties of their isolated surface waxes (Holloway, 1969a).

Waxes with large amounts of alkanes are the least wettable (Holloway 1969b). It is not possible to correlate directly the quantity of wax with the wettability of the surface. Some wettable leaves such as *Rhododendron ponticum* are more waxy than other highly water repellent leaves, e.g. those of *Brassica oleracea*. The orientation of wax molecules in the solid state is also important. Orientation may be investigated using X-ray and electron diffraction (Kreger, 1941; Piper *et al.* 1931; Kreger & Schamhart 1956). Aliphatic chains are arranged in several monomolecular layers with the chain placed perpendicularly to certain places in the crystalline structure. This results in the exposure of methyl groups on the surface of the solid. Wax constituents containing primary alcohol and fatty acid (functional group) also expose methyl groups by forming dimers consisting of two condensed monolayers orientated with polar groups sandwiched between the aliphatic chain (Holloway, 1970). According to Adam (1963), pure hydrocarbons are the second most hydrophobic materials and maximum hydrophobicity occurs when the methyl groups are arranged in the closest possible packing. Fluorinated hydrocarbons exhibit the most hydrophobic surfaces known (Hare *et al.* 1954).

Differences in wettability may result from the differential packing of groups at the surface. Normally, functional groups in the chain prevent close packing and consequently close arrangement of the methyl groups at the surface (Holloway, 1970).

Wettability of leaf surfaces devoid of superficial waxes will be governed by the chemical groups available. The non-waxy cuticle components (cutin, pectin and cellulose) are more polar than waxes and consequently more hydrophillic (Fog, 1948; Bartell & Ray 1952; van Overbeek, 1956). When washed with chloroform, a reduction in contact angle has however

been observed, indicating a role for waxy component in wettability (Holloway, 1970). In a very recent work by Schreiber (1996), it was found that pH dependency of wetting sometimes influenced by the epiphyllic micro-organisms rather than cuticular wax composition.

1.3 Contact angle, wax content and surface roughness

Contact angle is a widely accepted technique for measuring the wettability of any solid by a liquid (Adam, 1941). It gives an inverse measure of the adhesion between a solid and a liquid (Holloway, 1971). Wetting is normally restricted because of complex structure of the leaf surface which results from underlying venation, projecting wax plates and dense arrangements of trichome which decrease the area of contact between the impacting droplet and the leaf surface. The hydrophobic properties of cuticular components impose further limitations (Adam, 1963). On dry leaf surfaces, the occurrence of droplet reflection is believed to be due to the presence of micro-roughness, usually due to wax crystals and a dense covering of trichomes.

Crystalline surface waxes in some cases exhibit a bloom, producing large contact angles (>120°) and make the leaf surface highly hydrophobic. Surface waxes without "bloom" form flattened deposits, which become more wettable and normally produce a contact angle between 80 and 100 degrees.

Leaf macro-structures, including trichomes, can cause water repellency (Challen, 1962). Similarly, venation systems in parallel ridges on many grasses and cereals also produce high contact angles and less wettability, because air-films are trapped. This also occurs with the papillose surfaces of sycamore and the finely corrugated surfaces of clover and laburnum leaves produced by small convex-surfaced epidermal cells. There was little or no change in contact angle after chloroform washing, indicating that in such cases, although wax may be present, they do not contribute significantly to wetting phenomena (Holloway, 1969a). Contact angles below 90° suggest that wax is not a prominent feature of the cuticular

surface, whereas angles above 90° usually signify a major role of wax in wettability (Holloway, 1970). Contact angles between 90 and 110° indicate that leaf surfaces may be covered by a smooth layer of superficial wax.

Similarity between contact angles for leaf surfaces and the contact angles on their isolated waxes is occasionally observed. For example in *Saponaria officinelis*, additional factors such as surface roughness are capable of modifying the hydrophobic properties of the surfaces and became more prominent where the contact angle exceeded 110°.

Although large reductions of the contact angle after chloroform washing are encountered on these surfaces due to removal of wax, the contact angles of smooth films of the isolated waxes only occurred for 50-60% of the contact angles measured on the leaf surface. These results also revealed the importance of additional factors (e.g. wax ultrastructure) that reside within the wax itself (Holloway, 1970). Leaf surface topography is affected by the shape, size and arrangement of the cells, which may be flat, convex or papillose. The cuticle itself possesses minute surface ornamentation, of granular, grooved or ridged appearance (Stace, 1965). Contact angles on wheat are always greater than 130°. These high contact angles are believed to be a result of the wax structures present (Throughton & Hall, 1967). It follows that differences in the magnitude of these high contact angles may result from changes in the density, distribution and morphology of the wax structures (Netting & von Wettstein-Knowles, 1973). However, observations on the adaxial (with both hairs and ridges) and abaxial (without hairs and ridges) of wheat leaves which show no marked differences in contact angle between the two surfaces, contradicts the findings of Hall and Throughton (1967) and Holloway (1970). According to these findings, wax morphology appears is important in determining contact angles on the leaves of wheat than do the other structural modifications. In a very recent study, Schreiber (1996) showed changes in contact angle may not always result from changes in leaf

surface chemistry and/or the fine structure of leaf surface waxes, but may also be a result of increased amounts of epiphyllic micro-organisms.

1.3.1 Sorption and penetration

The routes of uptake of any sprayed chemical on foliage include diffusion through the cuticle, absorption through trichomes or other surface macro- and micro-structures or entry through the stomata, either by vapour movement or liquid infiltration (Crafts & Foy, 1962; Sands & Bachelard, 1973a). Although the importance of wax as a water barrier is well established, there is no simple correlation between the thickness of the wax layer and the penetration rate (Baker & Hunt, 1981). Variation in penetration rates between adaxial and abaxial surfaces has been attributed to the density and orientation of wax deposits (Bukovac & Norris, 1966; Norris & Bukovac, 1972). Wide differences observed in sorption between species may be attributed to varying leaf age, environmental conditions during spraying and the formulation of the sprayed chemical (Hull, 1970). However, variability in patterns of NAA (Naphthalenacetic acid) uptake provide conflicting evidence on the role of waxes on the leaf surfaces. Rates of entry into the heavily waxed surfaces of *Eucalyptus globulus* are low at all stages of growth, whereas in dwarf bean and grape vine leaves this rate decreases with development and corresponds with an increase of wax content on these leaf surfaces (Baker & Hunt, 1981). During development, these two species, especially dwarf bean, synthesise waxes to cover the whole surface, although they are very thin, preventing any sites being given access to the applied chemical (Hoch, 1979).

It has also been observed that NAA penetrates more readily through abaxial than adaxial surfaces, although the latter yield smaller quantities of wax for example in mature leaves of sugar beet, cherry and *M. hupehensis* (Baker & Hunt, 1981). Sands and Bachelard (1973b) suggested that, in such cases, stomata play a part in the infiltration process but this can be generally accepted only in the case of aqueous solutions

where the surface tension is reduced below 30 mNm^{-1} (Schönherr & Bukovac, 1972). It has not yet been proved that stomatal penetration is the predominant mechanism of transport into plant tissues (Still *et al.* 1970). This becomes more complicated by the continuous layer enclosing the substomatal chamber (Norris & Bukovac, 1968) and remain as a prime barrier to penetration (Still *et al.* 1970). Bukovac and Norris (1967) showed that removal of wax from isolated tomato fruit cuticle greatly enhances the permeability to 2,4-D, but this ceased after further removal of cuticular wax that is occluded within the membrane. Removal of waxes from pear leaf cuticle increases sorption of NAA and plating back the wax onto dewaxed cuticle reduces sorption and penetration (Norris & Bukovac, 1972).

Sorption of 2,4-D by the cuticular membrane is inversely related to the amount of cuticular wax (Baker & Bukovac, 1971). It is well established that the macro-structure of the leaf surface and ultrastructure of the epicuticular wax influences the retention of chemicals applied in aqueous media (Fogg, 1947; Linskens, 1950; Juniper, 1959; Challen, 1960; Brian & Cattlin, 1968; Holloway, 1969a, b) and evidence is increasing that wax is the chief barrier to the penetration of water soluble materials (Juniper, 1959; Bukovac & Norris, 1966; Throughton & Hall, 1967; Norris & Bukovac, 1969). Baker and Bukovac (1971) examined different leaf species which varied as much as 100% in their wax content, but were unable to find any relationship between the quantity of wax and susceptibility to 2,4-D. The orientation of cuticular waxes may be more important than the quantity of wax in regulating sorption and penetration (Norris & Bukovac, 1969).

Many insecticides are fat soluble and likely to pass into surface waxy layers. Further penetration may occur with the loss of availability as surface contact. Gamma-BHC is known to pass readily into leaf tissue and also DDT does so to a limited extent (Martin & Batt, 1958). If it is retained in the cuticle, a solution of the insecticide in the waxy layer

may be as toxic to a pest and more resistant to weathering than crystal deposits on the waxy surface (Martin & Batt, 1958).

There are few publications that deal with foliar penetration of different types of formulation. Most early work dealt with the penetration properties of active ingredients (AI) in ideal conditions, including the use of aqueous acetone for whole plant applications (Stock *et al.* 1993; Urvoy & Gauvrit, 1991) and aqueous systems on isolated cuticle surfaces (Schrieber & Schönherr, 1992).

A few studies have been undertaken on uptake using commercial products (Holloway *et al.* 1992; Writh *et al.* 1991). In a recent study of penetration using herbicide quizalofop-ethyl (Manthey *et al.* 1992), it was shown that foliar penetration can be enhanced by improvising the activity of lipid additives. This confirmed the findings of Gauvrit and Dufour (1990) and Schott *et al.* (1991) on the penetration of diclofop-methyl into grass species. By using suitable surfactants/adjuvants, penetration can be increased even on water repellent surfaces (Stock *et al.* 1993). The prevention of recrystallisation during droplet drying has been invoked as a reason for efficient penetration (Maclssac *et al.* 1991; Nalewaja *et al.* 1992).

The penetration of water soluble compounds *via* open stomata could be enhanced by using an adjuvant. Organosilicone surfactants are at present the only agents capable of facilitating such entry (Field & Bishop, 1988; Stevens *et al.* 1991). However, the importance of the stomatal route of penetration in the presence of silicone-based surfactants is currently being challenged (Roggenbuck *et al.* 1993).

1.4 Availability of insecticide on the plant surfaces and uptake by insects

To utilise the full potential of an insecticide, the mechanisms which result in increased availability of active ingredient to the target insect must be explored and the

interactions between these mechanisms and the substrate must be understood.

Hydrophobicity determines the extent of adhesion of a material on a particular surface. For example, when oil-based formulations of pirimicarb are sprayed onto the polar surfaces of broad bean leaves (*Vicia fabae*), it is observed that the droplets remain available above the leaf surface without spreading, whereas when black bean aphids (*Aphis fabae*), come into contact with a similar droplet, a thin film is transferred to the insect which spreads, pulling the legs and antenna into close proximity with the body (Hart, 1979). Although the spread of droplets on plant surfaces increases the chance of encounter with a moving insect by increasing the deposit area, it also reduces the chance of the deposit being transferred to the exposed insect because the material becomes more intimately associated with the relatively more polar underlying surface (Ford and Salt, 1987).

Lipophilic insecticides spread into a thin film on the waxy surfaces of both waxy leaves and the insect cuticle but remain as discrete droplets proud of the surface, when placed on a hydrophilic surface, such as the leaves of broad bean (Hart, 1979).

The efficacy of a contact insecticide depends largely upon its availability on the plant surface. The accumulation of the active ingredient by the exposed insect will be reduced by transfer of a.i. into the underlying surface of the leaves, which is more desirable for systemic insecticides. It can be predicted that non-polar insecticides have a better chance of being retained on hydrophobic surfaces.

1.4.1 Transfer of insecticide to the insect body

The ultimate efficacy of an insecticide depends largely on successful transfer to the exposed insect body and subsequently to the site of action. This process requires firstly, sufficient pesticide availability on the plant surfaces, and secondly, transfer to the site of action *via*

the insect body or body parts. The extent of the transfer process is controlled by the probability of an insect encountering the a.i. deposit and the amount of active material that adheres to insect cuticle. Once attached to the cuticle, further penetration is influenced by the nature of the cuticle and the a.i. reaching the site of action.

The encounter probability of larvae of *Spodoptera littoralis* with oil-based ULV deposits of permethrin has been described and modelled by Salt and Ford (1984). The encountering process was also studied by Jepson *et al.* (1990a). According to these authors, the quantity of insecticide encountered can be determined by the encounter rate (which is a function of the proportion of the body making contact with the treated surface and behavioural activity) and the intrinsic susceptibility of an insect to a particular insecticide. Ford and Salt (1987) suggested that the extent of insecticide transfer to the insect from a treated surface will be determined by the adhesion forces acting between the deposit, plant (intermediate) and the insect (target) surfaces. Competition therefore exists between plant and insect surfaces, which results in a steady primary accumulation of a.i. by the insect until a steady state is reached at which the rate of pick up by the insect equals the rate of detachment (Salt and Ford, 1984). Some insect epicuticles contain a substantial amount of wax. Armstrong *et al.* (1951) recovered 20µg cm^{-1} of epicuticular wax from the grain weevil *Calandra granaria*. Hartley and Graham-Bryce (1980) also reported that a number of insect species, including phytophagous pests, have similar quantities of epicuticular wax. The average thickness of epicuticular waxes ranged from 0.05 to 0.4µm, which is similar to, or even greater than, those found on many leaf surfaces (ranges 0.1 to 0.2µm). In both cases waxes have a similar type of chemical composition (Hadley, 1981; Blomquist & Jackson, 1979), and should have similar affinities for insecticide (Ford and Salt, 1987). An increase in wax cover over insect cuticle relative to the wax on plant surface will increase the probability of transfer of insecticide particles from the plant surface to insect body.

Some plant surfaces with low wax may make insecticide particles less available to the insect body because the insecticide penetrates further inside the plant surface. Thus waxy surfaces of plant parts are required for retention of the insecticide deposit on the surface in order make it more available to exposed organisms.

A further route of uptake which has not been considered previously concerns the direct pick up of plant waxes contaminated with insecticide, by the insects. The plant wax and associated insecticide particles may slowly release or transfer insecticide to the insect cuticle. As an insect exerts a moving pressure, it has a greater chance of picking up the waxes from the static plant surface rather than the converse.

The precise sites of uptake vary according to species and the stage of the insect. In many cases, the predominant sites of uptake of particles are tarsi and pretarsi. Pulvilli can also be important sites of contamination when species of Diptera are exposed to deposits. Lewis and Hughes (1957) studied the contamination of Bowflies, *Phormia (Protophormia) terraenovae* exposed to lipophilic and lipophobic particles. They also tested the pick up rate of these particles from waxy and fibrous surfaces, and concluded that lipophilic particles are more likely to be picked up and retained by the waxy cuticle of the insect. However, this pick up rate is reduced when the plant surface is lipophilic, suggesting that the existence of competition between insect and plant surfaces for retaining lipophilic particles. It has also been observed that the primary and predominant site of particle attachment was the tip of the ventral tarsal setae. These numerous setae, which cover the tarsal segments, have clearly-defined patterns (Lewis 1954a) and exert greater pressure on the substrate than any other tarsal part. During cleaning movements the ventral side of the tarsi, with their numerous spines, act as instruments of distribution of particles from contaminated ventral tarsal setae to apparently uncontaminated areas such as the microtrichia of

the main tarsal cuticle, to the basal parts of setae and to the head, abdomen and leading edges of wings. As this cleaning movement takes place frequently, a proportion of particles is being constantly transferred to different parts of the body (Lewis and Hughes 1957).

The pick-up of oily crystalline deposit of DDT deposits is likely to be more toxic than the pick-up of dry deposit (Barlow & Hadaway, 1952b). This is probably because of the increased rates of penetration of DDT through the insect cuticle in the presence of oil. The increase in toxicity, is not, therefore, a result of greater availability but a consequence of the successful penetration of the toxic ingredient in the presence of oil. Although, DDT can be taken up by a typical plant wax, this effect is unlikely to influence the toxicity of the deposit unless the DDT is able to penetrate further than the wax layer. Even in conditions where plant waxes exceed insect waxes (which according to many authors, decreases the probability of adhesion of insecticide particles to the insect body by increasing the deposits chance of adhesion to the plant surface) the retention of insecticide on the plant surface, which is static, increases the probability of uptake to the insect body (especially when the insect is in contact with that surface for a considerable period), because of the insect's physical activity on the surface. The adhesion theory could be more effective for short-exposed and apparently inactive organisms, where the process of steady transfer to attain a steady rate can take place because the surfaces are in steady state relative to each other.

Part II

1.5 Scanning electron microscopy

The above review shows that the chemical and physical nature of leaf surfaces has profound effects on pesticide availability to target organisms. To study the physical characteristics of leaf surfaces, scanning electron microscopy offers a great advantage. It yields in a single operation the maximum amount of information on leaf surfaces,

for example roughness, and is highly recommended for routine examination of plant surfaces (Holloway, 1970).

Since the commercial introduction of the scanning electron microscope (SEM) its popularity in scientific research is ever increasing. Presently, SEM has been widely used in biological sciences. By means of SEM, it is easy to study the complex surface topography of biological materials at a great depth of focus (around 300x higher than the normal optics) and magnification which may range from X10 to a maximum of X100,000 or even more. Moreover, a nearly three dimensional view is also possible (Jeffree & Read 1991). In the last two decades, techniques, protocols and comparisons between different SEM techniques have been studied and reviewed extensively (Echlin & Kaye 1979; Beckett & Read, 1986; Read & Jeffree, 1991; Heslop-Harrison, 1970; Davies 1971; Baker & Parsons 1971; Parson *et al.* 1974; Baum & Hadland 1975; Sargent, 1983; Pearce & Beckett 1985; Read & Jeffree 1991). Different techniques varying from the observation of uncoated fresh specimens to specimens prepared with procedures involving chemical fixation, dehydration, drying and coating have been elaborated.

Specimen preparation techniques to study the surface topography of botanical specimens (Echlin, 1968; Heywood, 1969) are important, because a true representation of the original specimen can only be obtained from material which can withstand a high vacuum (10^{-5} torr; 13 mN/m^2) and the specimen must also have sufficient electrical conductivity to prevent the accumulation of surface charge during examination (Parson *et al.* 1974). Some specimens have inherent conductivity and can be examined for 10-15 minutes at low accelerating voltages (2-5 Kv) without pre-treatment (Heslop-Harrison, 1970). Others may need to be vacuum coated with a conducting layer. The problem of observing fresh material without coating is that accelerating voltage must be kept low, limiting the resolution and allowing magnification up to X2000. Tissue also progressively desiccates in the vacuum, and cells collapse with the loss of structural water.

Investigation of leaf surface micro- and ultrastructure (including cell patterns and surface wax morphology) requires high magnification and resolution, for which accelerating voltages of 10 kV (even higher) and magnification well above X2000 are needed. This is possible only when fresh material is coated with an electrically conducting layer before scanning. Again the vacuum used may cause collapse of epidermal cells, although in some cases, wax crystals may remain unaffected (Amelunxen *et al.* 1967; Still *et al.* 1970; Hanover & Reicosky, 1971; Baker & Holloway, 1971; Baker & Parsons, 1971). Other preparative techniques are used for removing surface water. Normally such drying of specimens is carried out by either air-drying (AD), freeze-drying (FD), critical point drying (CPD) and recently cryo-preservation.

1.5.1 Air drying

Air drying is the simplest and most rapid method of drying specimens. The movement of the water/air interface through the specimen during drying causes distortion of the epidermal cells by surface tension forces. Boyde and Wood (1969) and Fujita *et al.* (1971) have used this technique successfully, however, Boyde *et al.* (1972) and Polliack *et al.* (1973) have favoured the more sophisticated techniques of freeze and critical point drying.

1.5.2 Freeze drying

Although this method produces better results than air drying, because it avoids the artifacts produced at the water/air interface (Boyde and Franc, 1981), the movement of solid/liquid and solid/vapour phase boundaries may still cause some distortion.

1.5.3 Critical point drying

This technique has now widely been accepted as a method of specimen drying and is practised by many biologists. In this technique the artifacts caused by the movement of phase boundaries can be avoided (Parsons *et al.* 1974). Specimen are immersed in liquid, pressurized and heated. At a certain temperature and pressure (the critical point) the liquid

changes into a gas. Water possesses high critical point value (T= 374°C and P= 22 mN/m²) and therefore it is more easy to replace water within the specimen by a liquid which has more easily attainable constants. Anderson (1951, '56) used liquid carbon-dioxide whereas Koller and Bernhard (1964) used nitrous oxide.

1.5.4 Cryopreservation

One further method has now been used for drying specimens, commonly known as cryopreservation. This is believed to be superior to the three techniques reviewed above (Sargent, 1983). This technique was not used in the present investigation.

Recently, with the introduction of Low temperature scanning electron microscope (LTSEM), it is possible to overcome many of difficulties that arise regarding specimen drying for ambient temperature SEM. Different aspects of LTSEM was reviewed extensively in recent literature (Echlin, 1978; Wilson & Robards, 1984; Robards & Sleytr, 1985; Beckett & Read, 1986; Bastacky *et al.* 1987b, 1988; Marshall, 1987; Sargent, 1988; Read, 1990; Jeffree & Read, 1991).

Part III

1.6 Bioassay procedures

Individual insect species have unique tolerance distributions to particular pesticides. This tolerance distribution is a function of several factors including the physiology of the species, individual body weight and activity patterns, the chemistry of the pesticide, its formulation and application methods.

The response of individual insect species to pesticides also differs on the basis of their exposure levels and the nature of the treated surface. With residual deposits, toxic effects following indirect exposure will be a function of the level and duration of contact. Thus residual susceptibility is a combined function of several factors including the characteristics of the treated surface. In the early stages

of pesticide bioassay development, emphasis was laid on the intrinsic susceptibility factor of the organism/toxicant and its behaviour pattern (Jepson *et al.* 1990,), and little or no attention was given to the substrate mediating toxicity (Wiles & Jepson, 1993), especially when the substrate was the sprayed leaf itself.

For a given pesticide and each dose rate a specific effect will exist on the exposed insect. In order to determine LD_{50} values for a particular insect/pesticide/substrate combination, it is necessary to carry out a dose response assay. Residual exposure bioassays were developed to derive estimates of the median lethal dose rates obtained from analysis of dose-response data for the insect species on treated substrates (Finney, 1971; Busvine, 1971). A classical approach was taken in the present investigation, using these approaches to investigate the differing toxicities of pesticides to a standard organism on a wide variety of substrates. Although these techniques have been used in thousands of investigations, they still have excellent potential for exploring basic questions of pesticide toxicology and environmental fate.

1.7 Deltamethrin and dimethoate as test substances

1.7.1 The advent of synthetic pyrethroids and their importance

Synthetic pyrethroids were developed during the 1970's and are used widely to control a wide range of arthropod pests (Elliot, 1989). Pyrethroids are the derivatives of natural pyrethrins, a group of esters that occur in the flowers of a number of *Crysanthemum* species (Asteraceae) (Leahey, 1985). Natural pyrethrins have low mammalian toxicity coupled with high toxicity to invertebrates but their photostability is very poor. The need for a more photostable insecticide product lead to the development of synthetic pyrethroids. Following the development of permethrin (Elliot, *et al.* 1973a, b), in the late 1970's many more synthetic pyrethroids have been developed internationally. Their broad spectrum activity results in high levels of control against many

important pests in different insect orders. By the late 1980's they comprised approximately one quarter of all foliar-applied insecticides worldwide, and were sprayed over 100 million hectares (Cox, 1990). Properties such as broad spectrum activity, high toxicity to insects, low mammalian toxicity and lack of persistence in the environment are the major contributory factors to their popularity (Hirano, 1989). Pyrethroid insecticides are highly lipophilic and low in volatility, they have proved most effective as both direct and residual deposit or contact, insecticides.

1.7.2 Mode of action of synthetic pyrethroid

The precise mode of action and toxicity level varies between different organisms and between compounds and formulations. Activity is modified by species-dependent differences in the site of primary action and also by the detoxifying capabilities of the organism, in particular, variation in esterase activity (Soderland & Bloomquist, 1989). The primary site of action is considered to be the sodium-ion channel. Progression from primary irritation and hyperactivity to knockdown and mortality is an indication of sequential poisoning that progresses from the peripheral, sensory system to the central nervous system. Many authors have investigated the biochemical (Ruight, 1985; Leahey, 1985; Soderland & Bloomquist, 1989) and kinetic (Ford *et al.* 1981; Greenwood *et al.* 1990) processes involved in the toxic action of pyrethroids.

The behaviour of pyrethroid residues on foliage and in soil is related to factors such as temperature, soil type and moisture content. In some pyrethroids, toxicity normally increases as the temperature decreases (Sparks *et al.* 1983; Ruight, 1985; Heimbach & Baloch 1994).

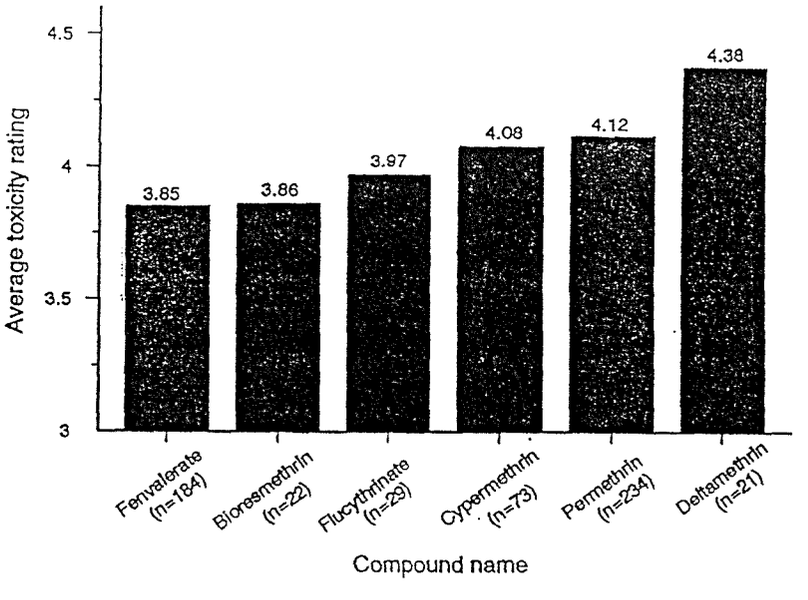
1.7.3 Effects on non-target organisms

The effects of synthetic pyrethroids on non-target invertebrates are now widely accepted and established (Croft & Whalon, 1982; Hill, 1985; Smith & Straton 1986; Ingesfield 1989; Croft, 1990a). The SELCTV database of Theiling (1987)

and Theiling and Croft (1988) suggests that pyrethroids are probably the most toxic class of organic insecticides to non-target invertebrates. Despite this, Croft (1990a) reported that several pyrethroids exhibit physiological selectivity to certain species of parasitoid and predator e.g. *Venturia canescens* (L) and *Chrysopa carnea* (Stephens). This is probably a result of the variable detoxifying capability of some non-target species (Raja Kulendran & Plapp, 1982; Croft & Mullin, 1984)

1.7.4 Deltamethrin compared with other synthetic pyrethroids

Deltamethrin was selected for the present experiment. It is known to be one of the most toxic pyrethroid compounds (Fig. 1.1) and is the most widely used pyrethroid worldwide, with an approximate 30% share of pyrethroid market. It is registered in 100 countries around the world (AGROW, 1991b). There is already a relatively large base of information available on its effects and behaviour in the ecosystem.



(Figure 1.1 Toxicity rating of six synthetic pyrethroids insecticide to beneficial organism from the SELCTV database (adapted from Croft, 1990a). Toxicity rating based on a scale of 1 to 5:1= no effect on beneficials, 2= <10% effect, 3= 10 to 30% effect, 4+ 31 to 90% effect, n= number of record.)

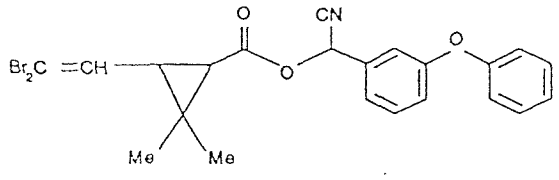


Figure 1.2. Chemical structure of deltamethrin

The chemical structure is given in Figure 1.2 and and a summary of the chemical properties is given below.
Chemical properties of deltamethrin (From pesticide manual 1994)

Common name - Deltamethrin
Code names - NRDC 161 (Licensed to Russel Uclaf)
CODEX 135
OMS 1998
Chemical name - (S)-α-cyano-3-phenoxybenzyl (1R, 3R)-3-(2,2-
(IUPAC) dibromovinyl)-2,2-dimethylcyclopropan-1-
carboxylate.
Empirical formula - $C_{22}H_{19}Br_2NO_3$
Properties - Technical grade (Russel Uclaf ≥98-5% deltamethrin m/m; colourless crystalline powder; melting point 98-101°C; vapor pressure 2μPa at 25°C; solubility at 20°C, < 2μg l^{-1} in water, 500g l^{-1} in acetone. Stable to air, more stable in acid than alkaline media.
Formulated deltamethrin 2.5%EC (Decis, 25g l^{-1}, Hoechst, UK. Ltd.) obtained from a commercial supplier was used in all experiments in this study contain xylene, tolunes, ethyl and propyl benzenes.

1.7.5 Dimethoate

1.7.5.1 Advent of dimethoate and its importance

Dimethoate was developed much earlier than deltamethrin, and represents another group of widely-used pesticides, the organophosphates. These were in use long before the advent of synthetic pyrethroids as a commercially formulated pesticides often with high mammalian toxicity. First introduced as commercial insecticide by American Cyanamid Co,

BASF, Boehringer Sohn (now Cynamid Argor) and Montecatini S. p. A (now Isagro Srl), dimethoate is now widely used around the world to control a number of pests including Aphididae, Coccidae, Coleoptera, Collembola, Diptera and Lepidoptera. It is also used as an acaricide.

1.7.5.2 Mode of action of dimethoate

Dimethoate is a systemic insecticide with contact and stomach activity. Its intoxication pathway is as a cholinesterase inhibitor and it has high mammalian toxicity. One of the most important contributing factors to its toxicity is that metabolism in plants is the same as in mammals. Hydrolysis to *o,o*-dimethylated phophorodithioate,-phosphorothioate and phosphate occurs and it is also oxidised to the phosphorothioate. This oxidation gives the corresponding oxone, which is highly toxic, acts as a strong cholinesterase inhibitor, and appears to be more persistent than the original parent dimethoate. The ester group is demethylated and methylamine group is hydrolytically cleaved.

1.7.5.3 Side-effects and environmental fate of dimethoate in comparison to deltamethrin

Although some authors have found similar intrinsic toxicities to predatory Coleoptera in the laboratory (Jepson *et al*, 1995), deltamethrin and dimethoate have a wide range of differences in their properties (Table 1.) that affect environmental fate. Moreover, field application rates are widely different, for example, rates of 6.25g a.i. ha^{-1} for deltamethrin and 340 g a.i. ha^{-1} for dimethoate as applied in winter wheat in the UK.

Table 1. Physicochemical properties of dimethoate and deltamethrin (adapted from Jepson *et al.* 1995).

Property	Dimethoate	Deltamethrin
Octanol:water partition coefficient	5	270,000
Water solubility	25g/l @ 21°C	0.0002 mg/l @ 20°C
Vapour pressure	0.29mPA @ 20°C	0.002 mPA @ 25°C

Considering dimethoate and deltamethrin have at least similar toxicities to a number of species, then some differences in risk might be expected to arise as a result of their widely different field application rates (Jepson *et al.* 1995).

LD50 values for dimethoate have been found to be lower when expressed per arthropod and are generally similar when expressed per body weight, although *Demetrias atricapillus* (Coleoptera:Carabidae) was found to be highly tolerant to deltamethrin (LD_{50} of 66.17 µg a.i. g^{-1}, Wiles & Jepson, 1992). Cilgi *et al.* (1994) showed similar toxicities for both dimethoate and deltamethrin to *Bembidion lampros* on glass (0.72g a.i. ha^{-1}), equivalent to 0.21% of the field application rate of dimethoate (340 g a.i. ha^{-1}) and 11.52% of the field rate of deltamethrin (6.25 g a.i. ha^{-1}). When both these pesticides were applied at full field-rate in *in-situ* bioassays on treated foliage, have produced similar toxicities after 24h confinement of predatory Coleoptera (Unal & Jepson, 1991). Such similarities, despite 54-fold differences in application rate, could arise through trade-offs in rate of uptake and elimination of the two pesticides. Such trade-offs are of critical importance in field condition, where the interaction between the products with substrate and environment are more likely to affect the overall process of toxicity mediation.

In the short-term, adsorption to leaf and soil and volatilization from both of these substrates will determine the fate and bioavailability of the two pesticides (Arnold and Briggs (1990). From Table 1, it can be predicted that the higher vapour-pressure, higher water solubility and lower octanol:water partition coefficient of dimethoate, will make it more readily volatile from leaf and soil, and also more readily taken up systemically by the plant, and less subject to adsorption into soil organic matter or leaf epicuticular waxes. On the other hand, due to high lipophilicity, deltamethrin has a greater likelihood of adsorption by plant epicuticular waxes but is not systemically transmissible to inner plant parts. Jepson *et al.* (1995) reported that the

ratios of relative toxicity of deltamethrin and dimethoate declined most rapidly in the first 24h after spray application, reaching a more stable rate of decline between 24 and 96h after treatment. Following an initial 54-fold difference in chemical availability at the time of spraying, there was a rapid decline in dimethoate availability relative to deltamethrin, even hours after treatment, which tend to result in a convergence in toxic effects.

Boehncke *et al.* (1990), investigated the rapid loss of another organophosphate, mevinphos, (mevinphos is more water soluble and volatile than dimethoate) in comparison with deltamethrin. The overall evaporative loss of mevinphos in the crop environment after 24h was 94%, but only 49% of deltamethrin was lost. In the short-term i.e. after 1, 3, 6 and 24h, the mean percentage loss of mevinphos from a range of crop types was much greater than deltamethrin. As a result of high lipophilic properties, deltamethrin has a greater chance of binding with plant waxes than dimethoate positively making it more persistent as a surface deposit. Jepson *et al.* (1995) explained that despite high application rates, dimethoate showed similar toxicities to deltamethrin against arthropods, as a result of those environmental trade-offs. However, in the laboratory, they can only show these similarities when both are applied at similar dose rates. The high application rate of dimethoate in the field implies higher toxic effects on soil and foliage, including higher toxicity to predatory fauna. This prediction is supported by work of Vickerman *et al.* (1987a, 1987b). These two contrasting insecticides were selected for the present study.

1.8 *Folsomia candida* as a test species

1.8.1 *Folsomia candida* in field and its uses in pesticide side-effect predictions

Collembola are widespread over the world and many species play an important role in the decomposition of plant residues as well as the mineralization of nutrients (Kiss & Bukony, 1992).

Long-term field investigations of pesticide ecotoxicity, including the Boxworth project (Vickerman, 1992), suggest that the contrasting spatial and temporal occurrences of invertebrate species determines their relative exposures and vulnerabilities to pesticides. This includes the soil fauna, and surface-active species of macroinvertebrates, including Collembola. Collembolan species are of considerable interest as important prey items for polyphagous predators including Carabidae and Linyphiidae. Overwintering populations of predators may be present at a stage in the year before pests invade and Collembola may represent an important alternative food source at this time (Tania *et al.* 1997).

Recently, several authors have suggested using collembolan species, including *F. candida*, as indicator species to study the side-effects of pesticide in arable farmland (Frampton, 1994, Wiles & Frampton 1996). The selection criteria for indicator species have been reviewed by Cilgi (1994). Axelsen *et al.* (1997) used a collembolan species *F.fimetaria* to develop a mathematical simulation model investigating the predator-prey interactions in a two species ecotoxicological test system. Krough *et al.* (1997) used *F. candida* to study the adverse effects of industrial residues including synthetic organic pesticides, both in the field and laboratory. In 1995, the same authors studied the influence of the insecticide dimethoate on the reproduction of *F. candida* and *F. fimetaria*. Short-term effects of the insecticide dimethoate on activity and spatial distribution of the *F. fimetaria* were explained under laboratory conditions by Fabian *et al.* (1994) who observed the adverse effects of the pesticide on activity patterns.

A variety of authors have investigated the bioavailability and toxicity of cadmium, lead and zinc in soil ecosystems (both in the field and laboratory), exploiting *F. candida* (Crommentujin, *et al.* 1997; van Gestel *et al.* 1997a, 1997b; Sandifer, *et al.* 1996; Smit *et al.* 1996). Haux *et al.* (1996) used *F. candida* to test for acute toxicity, in terrestrial hazard assessment for pesticides. Similarly, Sorensen *et al.*

(1995) studied the effects of sub-lethal exposure to dimethoate on the locomotor behaviour of *F. candida*.

F. candida has recently been used for a number of environmental and toxicological studies. New test systems are being suggested for this species as a candidate indicator for pesticide side-effects, both *in-situ* and *in-vitro* (Wiles & Frampton, 1994). More studies are required to explore the overall potential of this species as an indicator organism. Most of the work so far done has been concerned with protection of soil ecosystems. In the present study *F. candida* was selected to examine chemical bioavailability on leaf surfaces.

Several collembolan species have been tried as test animals for laboratory experiments (Scops & Lichtenstein, 1967; Sanocka & Woolszyn, 1970; Thompson & Gore, 1972; Thompson, 1973; Eijsackens, 1978; Ulber, 1978, '79; Subagja & Snider, 1981; Mela *et al.* 1987). *Folsomia candida* Willem, is a white eyeless soil invertebrate of 1.5 to 3mm long with a powerful tail. It is very easy to culture, an important criterion for a potential standard laboratory test organism. A relatively large amount of information exists about collembolan biology in particular for *F. candida* (Kiss & Bakonyi, 1992). Under laboratory conditions, *F. candida* multiplies parthenogenetically (Torne, 1964, '66; Goto, 1960; Goto & Oegel, 1961; Marshall & Kevan, 1962; Green, 1964a; Husson & Palevody, 1966).

Until quite recently, the use of Collembola in toxicological testing has escaped the attention of entomologists and ecotoxicologists. The greater part of the existing literature is mainly concerned with taxonomy and more comprehensive work on the biology of the group, (Gisin, 1944, '60; Maynard 1951; Salmon 1951, '56; and Stach 1947, '60). Paclt (1956) reviewed the biology and Salmon (1951, '56) given an extensive bibliography. However, there are few published papers concerning their ecology (Holdaway, 1927; Ripper, 1930; Davidson, 1931-33; Maclagan, 1960). Work on their rearing and

feeding habits has been carried out by Rhode (1956); Hubber (1958) and Goto (1961). The species was excluded from the candidate lists made for the pesticide side-effects data base of Croft (1990). Thompson & Gore (1972) used this insect to test the toxicity of twenty-nine insecticides.

Numerous investigators have studied the biology of collembolan species. For example, the influence of temperature on development, mortality and fecundity (Sharma & Kevan, 1963a, b; Hale, 1965a, b), of moisture and various soil factors on egg laying (Davidson, 1931, '32), of temperature and humidity on embryonic and post embryonic development (Thibued, 1968a, b, c), and of crowding on population growth (Green, 1964b, Usher, 1971). For *F. candida* 21°C is an optimal temperature for hatching with 94.7% of eggs hatching (Snider, 1973).

A mixture of plaster of Paris and charcoal is now widely accepted as a suitable substrate for rearing, with a moist environment of near 100% humidity with yeast as food (Snider, 1973). Snider and Butcher (1973) maintained a continuous population of *F. candida* in their laboratory for eight years.

1.9 Hymenopteran parsitoids

1.9.1 Parasitoids as natural enemies of crop pests

In addition to polyphagous and pest specific predators and entomopathogenic fungi, a wide range of dipteran and hymenopteran parasitoids attack insects pests, especially aphids (Wratten & Powell, 1991). For example, aphid primary parasitoids belonging to the family Aphidiinae alone, comprise more than 400 species of this family parasitising only aphids (Stary, 1970).

Three important aphid pests of cereal crops, the English Grain Aphid *Sitobion avenae*, (F), Rose-Grain Aphid *Metopolophium dirhodum* (Wlk) and Bird-Chery-Oat Aphid, *Rhopalosiphum padi* (L) (Homoptera:Aphididae) (Dixon, 1987) are attacked by at least seven different genera of primary parasitoids, which are themselves attacked by at least five

different genera of hyperparasitoids (Powell, 1982). These primary parasitoids have their major effects at the early stages of aphid population growth, at densities as low as 0.1 aphids/shoot (Chambers, *et al.* 1986). They appear to be less effective during later stages of aphid population development (Vorley & Wratten, 1985).

More broadly, twenty three species of parasitoids in the family Aphidiinae have been included in classical biological control in the field and they have become established in 32 out of 55 attempts (Greathead, 1989).

1.9.2 Effects of pesticide on parasitoids

It has been well-documented that pesticides reduce natural enemy populations (Vickerman & Sunderland, 1977,; Basedow *et al.* 1985,; Fischer & Chambon, 1987,; Vickerman *et al.* 1987a, 1987b). In some cases this is even lead to local extinction (Burn, 1992). Many registered pesticide application rates lie beyond the upper asymptote of the residual dose-response curves of pests, to ensure complete eradication (van Emden, 1989). For this reason, they are often toxic to natural enemies as well. The possibility of achieving differences in the primary toxic effects on target and non-target organisms by reducing these dose rates below their maxima is being increasingly explored (Poehling, 1989). The range of insecticide doses spanning low to high kills of herbivores is generally larger than that for carnivores (van Emden, 1989). The probable explanation is that herbivores carry a wider range of detoxifying enzymes than carnivores, some with the ability to detoxify pesticides. Herbivores exploit these enzymes to detoxify secondary compounds they encounter in host plants. It may therefore be postulated that a decrease in the dose rates of pesticides could reduce natural enemy mortality much faster than the rate of pest mortality.

Waage (1989) gave five possible explanations for decreases in natural enemy densities following pesticide application. These were, 1) direct mortality by the toxicant, 2) sub-lethal effects, 3) pest resistance causing increases in

populations, which in turn allow more pests to escape control by natural enemies, showing inverse density-dependent functional responses, 4) changes in prey distribution, which force natural enemies to emigrate temporarily or which result in reduced foraging efficiency and finally 5) synchronisation of pest populations which may cause critical shortages in host or prey availability.

The SELCTV database (Theiling & Croft, 1988) indicates that parasitoids are generally more susceptible to pesticides than predators (Croft & Morse, 1979). Among different life stages, egg and adult stages show the most susceptibility and synthetic pyrethroids show the highest proportion of toxic effects (Theiling & Croft, 1988).

1.9.3 Mode of pesticide uptake by parasitoids

Three main routes of exposure to pesticide toxicity are 1) direct contact, either through interception of pesticide droplets or by vapor inhalation, 2) residual contact with pesticide on a substrate, such as the plant or soil surface and 3) transfer of the toxicant through the food chain that already contaminated with pesticide spray.

1.9.4 Assessment of pesticide side-effects on parasitoids

Various organisations such as the IOBC (International Organisation of Biological Control), BART (Beneficial Arthropod Regulation Testing Group) develop testing methods and strategies for evaluating the effects of pesticides on non-target arthropods for regulatory and IPM purposes. Normally, side-effect quantification requires a combination of tests that include laboratory, semi-field and field experiments (Hassan, 1989). Jepson *et al.* (1989) defined these sequences as falling within the "micro" (=laboratory) scale of test methodology. At this level, the extent of initial uptake and the individual toxicity responses of organisms in the crop during and just after spray application may be determined. The "meso" (=semi-field) scale includes approaches that determine within-year effects on populations within treated plots. Finally, the macro (=field) scale

incorporates those methods that determine effects on populations between fields and between growing seasons.

According to IOBC, WPRS (West Palaearctic Regional Section) testing procedures, if a pesticide is found to be "harmless" to a particular beneficial arthropod in initial laboratory tests then no further experiments are required. On longer spatial or temporal scale it is assumed that the pesticide will remain "harmless" in the field at similar rate application. However, if a pesticide is found to be "harmful" in laboratory screening, further experimentation is triggered to determine whether effects are found under normal environmental conditions in the "real world".

Hassan (1989) suggested a number of methods for development of laboratory bioassays:
1)For exposed life stages (e.g. adult parasitoids) the design parameters for the bioassay should include a) exposure to freshly dried pesticide deposits, b) use of recommended concentrations of pesticide, c) application on glass plates, leaf or sand, d) creation of even films of pesticide, in standard amounts of (1-2) mg fluid cm^{-2} on glass or leaf or 6mg fluid cm^{-2} on sand, e) use of laboratory-reared organisms, uniform in age, f) an adequate exposure period before evaluation, g) adequate ventilation, h) water-treated controls, j) measurements of reduction in beneficial capacity as well as mortality, k) use of four evaluation categories: 1= "harmless" (<50%), 2= "slightly harmful" (50-79%), 3= "moderately harmful" (80-99%), 4= "harmful" (>99%).

Hassan (1992) suggested an adjustment of the "harmless" trigger value to less than 30% and established "slightly harmful" as 30-79% mortality or effect 2) For "protected" or "less exposed" life stages (e.g. parasitoids within their hosts) design parameter for bioassays should include: a) direct sprays onto hosts, b) use of recommended concentrations of pesticide, c) adequate ventilation, d) use of laboratory reared organisms uniform in age; e) water-treated controls; f) measurements of reduction in beneficial

capacity as well as mortality, g) four evaluation categories, as in test 1.

1.10 Aims of the study

This study was undertaken to investigate the role of leaf characteristics in the mediation of toxicity to exposed invertebrates. By understanding the importance of different surface factors, it may be possible to predict the susceptibilities of exposed organisms on a range of leaf treated with a range of different pesticides. This may permit us to improve prediction criteria for beneficial species, and improve predictions of efficacy against certain pests.

Folsomia candida was selected as a standard test organism for two reasons: 1) these types of experiment require large numbers of laboratory-reared test individuals, and it is uneconomic to use rare or expensive test organisms. *F. candida* is also parthenogenetic and easy to rear. Large cultures can be maintained in the laboratory for a long periods 2) if *F. candida* could be exploited successfully for this type of experiment, it could extend the range of organisms that may be exploited as standard laboratory test species in risk assessment.

The primary objective of the present study was to focus on the effects of leaf micro- and macro- structures (e.g. wax content, wax ultrastructure etc.) on the transfer of pesticide to exposed organisms. It seemed wise to explore these questions with a species that was readily available, in large numbers, easy to rear and maintain in uniform age groups. After collecting the relevant information it is then possible to verify findings with economically important test species and to develop predictive approaches for a wide range of taxonomic groups.

In addition to taking an interest in leaf surface characters, especially wax content and wettability, two contrasting pesticides were selected from two chemically diversified classes of insecticide to increase the generality of the

findings.

In an attempt to expand the validity of preliminary findings to beneficial species, a short experiment was also carried out with the parasitoid, *Aphidius colemani*.

1.11 Experimental framework and goals of the project

Laboratory bioassays were used to determine the residual toxicity of deltamethrin and dimethoate to *F. candida* and a parasitoid *A. colemani*, and to establish the susceptibility spectra exhibited on a wide taxonomic range of leaf substrates. Emphasis was also given to developing a clear understanding of different substrates and different insecticide behaviours on the mediation of toxicity to the exposed organisms.

The goals of the study were to establish susceptibility rankings of test organisms to two different insecticides sprayed on sixteen different leaf substrates, and to predict the possible role of leaf characteristics. The gained knowledge can be used in insecticide formulation and field applications for different crop types and habitats (both pest and natural enemies).

The experiment was also extended to a study on the potential of *F. candida* as a standard laboratory test species for studies on insecticide side-effects.

CHAPTER 2

Development of a general bioassay methodology for *Folsomia candida* Willem (Collembola:Isotomidae) on leaf surfaces

2.1 Introduction

Laboratory bioassays confine, and therefore expose, the test organism to pesticides under controlled laboratory conditions. Although experimental conditions differ from the real world of the field, control of factors such as age, temperature, humidity and light, which are important in providing reliable comparisons between test organisms or substrates give laboratory bioassays an important role in basic toxicology. Bioassays provide detailed information concerning the toxicity of compounds to given species on a variety of substrates and can aid understanding of insecticide-substrate-invertebrate interactions, including chemical bioavailability in specified conditions.

Folsomia candida (Collembola:Isotomidae) Willem, which is predominantly a soil inhabitant was chosen for the present study. It has potential for use as a standard laboratory test species (Thompson & Gore 1972; Thompson 1973; Mela *et al.* 1987; Fabian *et al.* 1994; Wiles & Frampton 1994; Krogh, 1995; Krogh & Pederson, 1997) and is discussed in detail in chapter 1.8. However, to undertake successful laboratory bioassays it is important to develop reliable test methodologies. This is of particular importance to the design of the present experiment. The substrates under evaluation were leaf surfaces, which differ completely from the natural substrate of *F. candida*. The advantages of taking this approach however were as follows:

i) use of a standardised organism is cost-effective; for example, only a small number of experimental units are required, which may be sprayed with a laboratory sprayer such as Potter Laboratory Spray Tower (Potter, 1952). Small bioassay chambers may be inexpensive and simple to construct and therefore more practical for the design of replicated experiments. Standardised test organisms, including *F. candida*, can also be cultured in large numbers at low

expense, and can be made readily available for experiments where large numbers of test organisms are required.
ii) Standardised experimental systems also enable the design of methodologies that may answer more specific questions about different compounds and substrates.
iii) These methods also enable effective comparisons of the toxicities of different compounds and application rates.
iv) Finally, by establishing a test methodology that works effectively with many compounds and substrates, these methods may be used to generate data on efficacy and side-effects that can be extrapolated to a wide range of other invertebrates. These species may be expensive and difficult to rear or buy, and it is problematic for laboratories to collect them in large numbers from the field on a regular basis.

During the development of a new bioassay technique, careful consideration must always given to factors relating to the aim of the study, such as biological and operational factors. If *F. candida* is to be developed as a test organism, as intended in the present investigation, then attention must be given to moisture, relative humidity, temperature, light and ventilation, all of which might affect survival. Survival in test conditions must be of prime concern, especially in experiments which are framed to evaluate pesticide availability and toxic responses on different substrates. The test organism should come into intimate contact with the pesticide to ensure reliable and repeatable comparisons between substrates and to evaluate their role in mediating toxicity to exposed invertebrates. Test invertebrates should therefore be confined to the treated surface and unable to rest on unsprayed surfaces and, must also be active enough to ensure exposure to the toxicant.

The bioassay described here was developed specifically for *F. candida* on different leaf substrates. This chapter provides details of bioassay chamber construction and experimental procedures in the laboratory.

2.2 Materials and methods

2.2.1 Modified Petri dishes

Petri dishes were used to confine *F. candida* on the test leaf surfaces and accommodated 10-20 test individuals without control mortality for a period exceeding 96 h.

The bioassay apparatus comprised two chambers. An outer chamber, (Chamber 1, Fig 2.1) was made from a plastic Petri dish (5.5cm diameter) covered with a lid (2.5cm deep). The cover was perforated by a needle to provide aeration. A commercially available covered cotton pad was attached to the underside of the lid by double-sided adhesive tape. Before setting up each experiment, this cotton pad was soaked with 5ml of distilled water. Moist air was provided inside each Petri dish by means of aquarium pump feeding a rubber tube which connected to the chamber via microlance sterile needles. The second chamber, (Chamber 2, Fig 2.1) was 3.0cm diameter and 1.5cm high. The lid had a 1.5cm diameter opening and was covered with a net of fine mesh. Test insects were exposed to the treated surface in this chamber, which was placed carefully within the first chamber.

Leaf discs (3cm diameter) of cabbage, tomato, dwarf bean, orange and pear were attached to filter paper discs of the same size using strips of double-sided adhesive tape. Barley, wheat, maize and sugar cane leaves were attached to the filter paper using strips of double sided adhesive tape and were placed in parallel, base to tip, on each paper with their adaxial surfaces exposed. The prepared samples were then cut into 3.0cm diameter circular discs. Each leaf surface sample was placed on the Petri dish base in the second, inner chamber. The test substrate was sprayed under a Potter Laboratory Spray Tower (Potter, 1952), calibrated to deliver a spray volume equivalent to 200 litres ha^{-1}. The tower was thoroughly cleaned and flushed with alcohol and water between treatments. Before spraying, a dilution series of deltamethrin was prepared from formulated "Decis" (deltamethrin 25 g l^{-1} EC, Hoechst UK Ltd). Similarly, dilution series for dimethoate were prepared from "Croptex

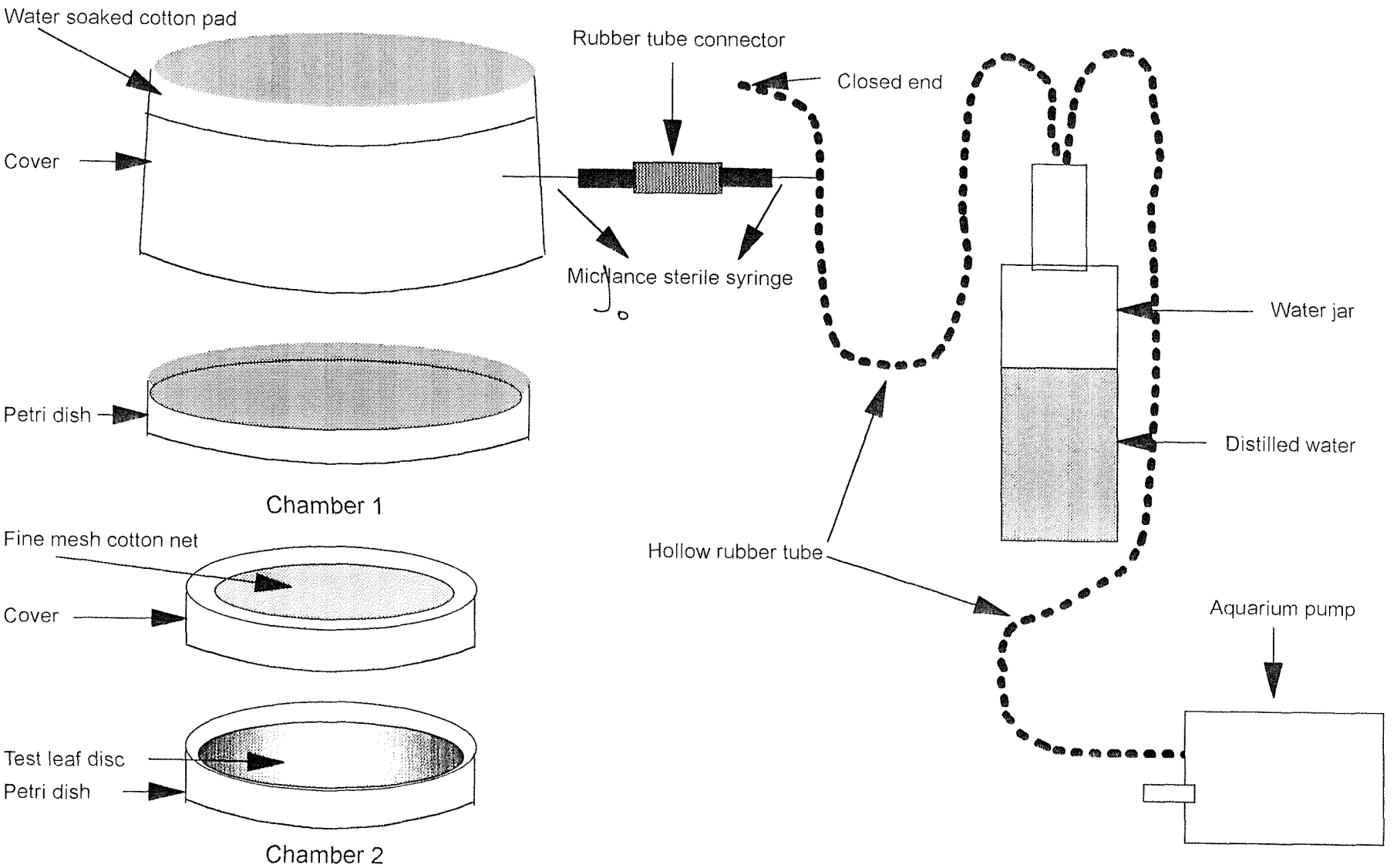


Figure 2.1. Bioassay chamber and technique

dimethoate" (dimethoate 400 g l^{-1} EC, Hortichem Ltd. UK).

Initially a series of range-finding doses were applied to small numbers of insects. From these, definitive dose-ranges of between five and seven doses were selected. After each treatment, the treated substrate was allowed to dry for approximately 30 min. Freshly collected *Folsomia* (10 to 20) were then placed in each replicate dish. Each study was replicated 3 to 4 times. Immediately after the introduction of live insects, the Petri dishes were covered with their respective lids. Mortality response data were taken at 24h intervals for 3 to 4 days. The bioassay data were then analyzed statistically.

2.3 Results

Examples of bioassay results are given in Figures 2.2, 2.3, 2.4 and 2.5 for *F. candida* on barley and cabbage leaf surfaces, sprayed with deltamethrin and dimethoate.

Fig 2.2 and 2.3 give the mortality trends for *F. candida* on two different leaf surfaces during continuous exposure to deltamethrin residues using the modified Petri dish chamber.

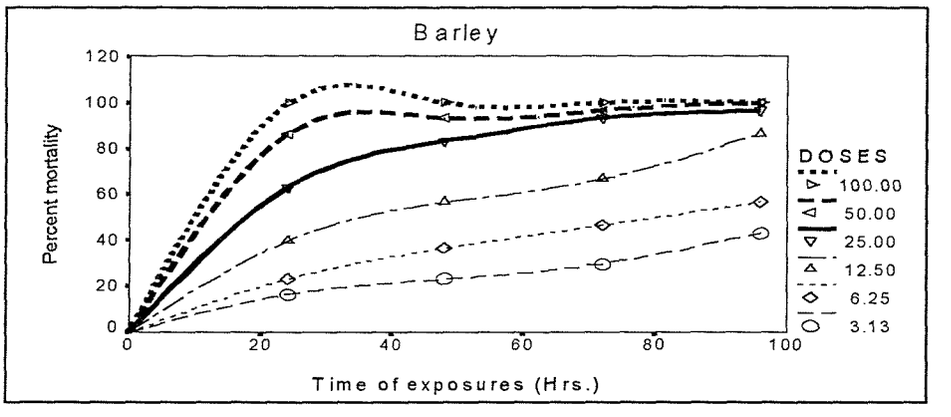


Figure 2.2. Individual dose responses of *F. candida* to deltamethrin 2.5EC on barley leaf surfaces(g a.i. ha^{-1}).

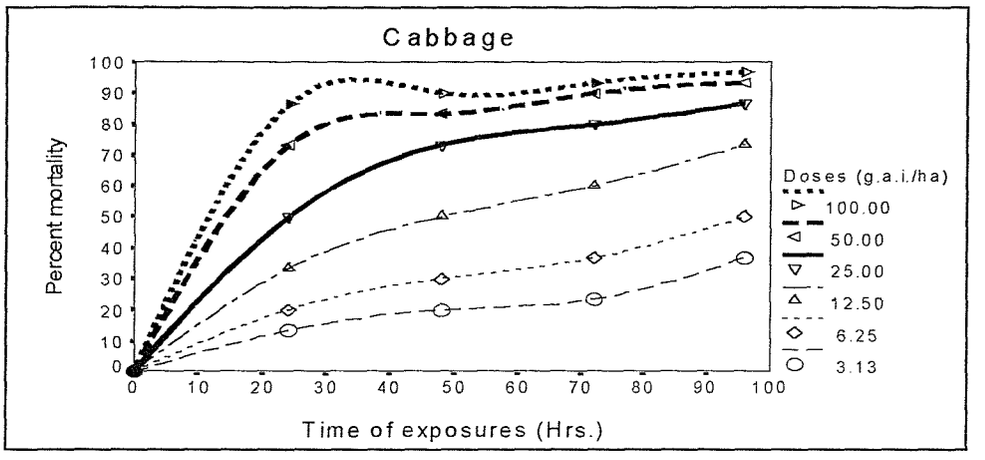


Figure 2.3. Individual dose responses of *F. candida* to deltamethrin 2.5EC on cabbage leaf surfaces(g a.i. ha^{-1})

Data were taken at 24h intervals. No control mortality was observed. Both figures reveal differences in mortality at different dose rates. The end points that are evident at different dose-rates define the equilibrium that is reached between deposit availability, uptake rates and metabolism/elimination rates. The shallow incremental trend indicates that a certain amount of residual toxicity remained throughout the exposure period.

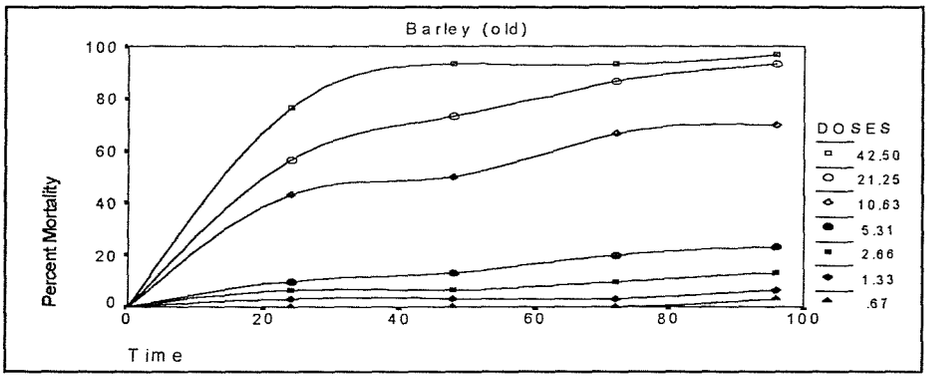


Figure 2.4 Individual dose responses of *F. candida* to dimethoate 40EC on barley leaf surfaces (g a.i. ha^{-1})

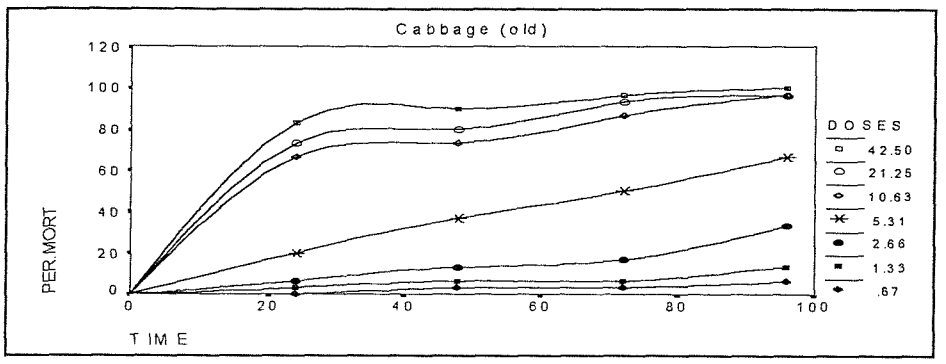


Figure 2.5 Individual dose responses of *F. candida* to dimethoate 40EC on cabbage leaf surfaces (g a.i. ha^{-1})

Figures 2.4 and 2.5 show similar trends for dimethoate. However there are clear separations of response between the three high and four lower dose rates. This indicates a steep dose-response curve over the range 5-10 g a.i. ha^{-1}.

2.4 Discussion

Laboratory bioassays have a range of advantages, especially for basic research which is conducted to answer specific questions centred upon relatively unexplored areas. In susceptibility studies with invertebrates, many factors should be carefully considered which might influence pesticide toxicity. These include the characteristics of the organism tested, including life stage, age, size and sex, characteristics of substrate, the nature of the test pesticide, i.e. particularly active ingredient, its formulation and application method (Jepson 1989; Croft, 1990a) and environmental conditions including temperature, light and humidity. For reliable comparison, (i.e. in this case the susceptibility of *F. candida* on different leaf surfaces) these factors must be controlled as far as possible.

These types of bioassay system provide a highly cost-effective tool for quantifying the toxicity of a pesticide and the susceptibility of test organisms on different leaf

surfaces. This requires large numbers of replicated range-finding experiments on a number of test substrates and with more than one pesticide. A large number of test individuals are needed. *F. candida* was considered to meet these needs, but in order to undertake such experiments with a soil invertebrate, reliable bioassay techniques are essential.

Differences in substrate-mediated toxicity may separate taxonomic groups of plants which induce higher relative toxicities when treated with specific pesticides. Data of this form may encourage more selective spraying and even improved formulations of pesticides for targeting on specific groups of plants surfaces and invertebrates. Great care must, however, be taken in generalization and in extrapolation to the field. This requires more extensive experiments to determine stable and widely-accepted indices of toxicity that are considered applicable to natural environments.

CHAPTER 3

The Susceptibility of *Folsomia candida* (Collembola:Isotomidae) to deltamethrin and dimethoate on different leaf types.

3.1 Introduction

The possible interaction between a sprayed agrochemical and the substrate has received much attention (Ford and Salt, 1987). Leaf characteristics such as surface tension (Holloway, 1970), surface roughness (Jeffree *et al.* 1976, Holloway, 1970, Baker *et al.* 1983), wax content and wax structures (Clarke, *et al.* 1994) have definitive roles in wettability, retention, sorption and penetration of sprayed chemicals. These physical processes determine the process of transfer and uptake of the toxicant by the exposed organism. Although the final toxicity responses to a particular chemical are modified further by the detoxifying ability of the exposed organism, the rate at which the active ingredient reaches the site of action is quite clearly dependent upon the nature of the substrate and the chemical concerned. Until now there has been relatively little research into the interactions between these factors and the ultimate toxic responses of exposed organisms. In order to understand the role of leaf characteristics in the transfer of toxicant to exposed organisms, it is necessary to determine what differences in toxicity occur when particular organisms are exposed to pesticides sprayed on different substrates.

The effects of a specific pesticide in residual deposits will be a function of the level and duration of contact. Residual susceptibility is a combined function of several factors, including the characteristics of the treated surface. In early studies emphasis was mainly given to the intrinsic factors associated with the organism and toxicant and to the behavioural patterns of the invertebrates, as they affected contact with the deposit (Jepson *et al.* 1990). Little attention was given on the substrate as a mediator of toxicity (Wiles and Jepson, 1993), especially when the substrate is the leaf itself.

The most commonly used index of susceptibility is the LD_{50}, an estimate of the median lethal dose, normally obtained from analysis of dose-response data (Finney, 1971; Busvine, 1971; Robertson and Preisler, 1992). For residual deposits LD_{50}'s are expressed in terms of dose per arthropod. LD_{50} may also be expressed in dose-per unit body weight, which gives a measure of intrinsic susceptibility of the treated population to the toxicant. Many factors can affect the ultimate toxic response of the organism to the toxicant. These include the physical and chemical characteristics of the exposed organism (e.g. size, weight, sex, age, life stage, nature and the availability of detoxifying enzymes) and also the nature of the pesticide e.g. active ingredient, method of application, formulation etc. (Jepson, 1989; Croft, 1990).

It is important to minimize sources of variability to achieve acceptable comparability of results. To satisfy this requirement, it is necessary to undertake controlled dose treatments under controlled laboratory conditions and to use laboratory-cultured and therefore uniform test individuals, identical in age and physiological condition. *F. candida* was cultured in the laboratory and used in this experiment.

By comparing the susceptibility of *F. candida* with deltamethrin and dimethoate on a wide range of leaf types this study aimed to address the questions:
1) Does susceptibility vary significantly with different leaf types, varieties and age
2) Does this variation follow the same trend for different classes of pesticide and thus provide a basis for further studies of the role of leaf characteristics in mediating toxicity to exposed organisms.

3.2 Materials and methods

3.2.1 Test invertebrates

Folsomia candida was selected as a test species because it has a well described biology and experimental usage in toxicology (BSI standard 1992). This species is

distributed over a wide geographical range and is abundant in almost every terrestrial habitat. Most importantly, as a primary test organism, it is very easy to maintain and inexpensive. The parthenogenetic life cycle makes culturing very straight-forward and able to provide a constant supply of new generation (Crommentuijin *et al.* 1993).

3.2.2 Culturing of test species

A mixture of Plaster of Paris and charcoal was used to make the rearing substrate for the test species. Moderately large (29 X 29 X 13 cm) plastic boxes ("Giant Storer" mnf. by Stewart, UK) with transparent plastic lids were used as containers. To prepare the substrate, a tablespoonful of charcoal was added to 250ml of distilled water in a 1000ml beaker with continuous stirring until the mixture became homogenous. Plaster of Paris was then added slowly, with constant stirring, until the whole mixture became a semi-solid paste. This was then poured into the plastic box to make a base of approximately one centimetre thickness. It was allowed to dry for several hours, but water was added as a spray to prevent sudden cracking due to excessive dryness. The dried substrate was saturated with distilled water before inoculating the Collembola collected from a parent stock in the Biology Department at Southampton University. Commercially available dried active baking yeast (Allinson, Westmill foods Ltd. Berks, UK.) was used as food. The food was given three times a week. Each time a small quantity of yeast was placed on moist filter paper (4.25cm) which was then placed carefully at the middle of the substrate. At each change, the old filter paper with or without food was thrown away and replaced.

3.2.3 Test leaf types

Plants were mainly selected from crops of economic importance and included examples of leaves having glaucous, sub-glaucous and glossy surfaces. They included orange, cabbage, barley, wheat, sugar cane, pear, maize, tomato, rape and dwarf bean. To determine any differences between varieties and age classes, rape leaves were collected from three different

varieties, including young and old leaves. The maize leaves tested also included young and old leaves whereas barley leaves were collected from seedlings. Barley, orange, cabbage, sugar cane, maize, tomato, rape and dwarf bean were grown in glass houses. Wheat leaves were collected from a field at Manydown, Hampshire, UK, whereas pear leaves were collected from plants grown on the campus of the University of Southampton, Hampshire, UK.

3.2.4 Bioassay chamber and technique

The test species, *F. candida*, selected for the present investigation was regarded as a challenge in the sense that it is a soil inhibitant. To use this as a laboratory test species on a completely different substrate i.e. on leaf surfaces, required the development of an appropriate bioassay technique. The method used for the present experiment was described in Chapter 2.

3.2.5 Statistical analysis

Probit analysis was carried out on the 24h, 48h, 72h and 96h dose-response data to obtain dose response statistics (Finney 1971). Only dead insects were included in the calculation. The slopes of the probit lines were compared for *F. candida* on different leaf surfaces using a parallelism test from a computer package of statistics (SPSS). Linear regression analyses were carried out to determine overall susceptibility.

3.3 Results

3.3.1 Determination of the end-point of toxic effects

The 24, 48, 72 and 96h LD_{50} values given by probit analysis were plotted against time for all leaf species to determine the end-point of the toxic effect. Figure 3.1 shows plots for *F. candida* exposed on sixteen different type of leaf surface treated with deltamethrin. Figure 3.2 shows the same data for the pesticide dimethoate. Although the curves indicate different rates of poisoning, the plots showed a similar trend of decline in LD_{50} over time with all sixteen leaf types. In most cases, the LD_{50} values approached a stable end

Table-3.1. 72-h Probit statistics of responses to Deltamethrin 2.5 EC for *F. candida* . and different leaf surfaces

Leaf species	Probit slope (SE ±)	LD50 (95%cl) detransformed (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	2.11 (.31)	6.36 (4.58-8.25)	1.69 (4) ns
Cabbage (*Brassica oleracea*) v. Prixie	1.62 (.24)	8.96 (6.18-12.11)	0.744 (4) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	1.66 (0.23)	16.87 (12.48-22.67)	0.744 (4) ns
Pear (*Pyrus communis*)	1.32 (.21)	14.43 (9.82-20.50)	0.798 (4) ns
Sugarcane (*Saccarum officinerum*)	1.15 (.20)	20.94 (13.98-32.40)	1.427 (4) ns
Wheat (*Triticum aestivum*) v. Hereward	1.08 (.20)	24.86 (16.38-41.00)	0.326 (4) ns
Orange (*Citrus spp.*)	1.15 (.21)	40.79 (27.15-73.70)	1.057 (4) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	1.20 (.17)	77.14 (54.45-119.26)	3.208 (4) ns
Rape (*Brassica napus*) v. Tanto (old)	1.78 (.25)	8.23 (5.82-10.92)	0.187 (4) ns
Rape (*B. napus*) v. Tanto (Young)	2.16 (.30)	7.91 (5.92-10.12)	1.267 (4) ns
Rape (*B. napus*) v. Lirawell (old)	1.77 (.25)	9.42 (6.77-12.46)	0.046 (4) ns
Rape (*B. napus*) v. Lirawell (Young)	2.26 (.30)	8.61 (6.58-10.92)	1.481 (4) ns
Rape (*B. napus*) v. Starlight (old)	1.91 (.26)	9.80 (7.25-12.76)	0.779 (4) ns
Rape (*B. napus*) v. Starlight (Young)	2.02 (.28)	8.24 (6.07-10.66)	0.919 (4) ns
Maize (*Zea mays*) v. Marcia (old)	1.27 (.23)	66.65 (43.44-134.60)	0.954 (4) ns
Maize (Zea mays) v. Marcia (young)	1.27 (.22)	37.53 (25.94-61.89)	1.254 (4) ns

a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

Table-3.2. 72-h Probit statistics of responses to Dimethoate 40 EC for *F. candida* and different leaf surfaces

Leaf species	Probit slope (SE ±)	LD50 (95%cl) detransformed (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	2.53 (0.29)	8.69 (6.99-10.86)	3.150 (5) ns
Cabbage (*Brassica oleracea*) v. Prixie	2.45 (0.27)	5.20 (4.16-6.49)	4.079 (5) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	1.95 (0.23)	2.80 (2.13-3.60)	1.564 (5) ns
Pear (*Pyrus communis*)	1.86 (0.25)	1.76 (1.26-2.31)	2.232 (5) ns
Sugarcane (*Saccarum officinerum*)	1.27 (0.17)	4.19 (2.92-5.91)	1.722 (5) ns
Wheat (*Triticum aestivum*) v. Hereward	2.29 (0.27)	2.77 (2.17-3.49)	4.087 (5) ns
Orange (*Citrus spp.*)	1.55 (0.22)	1.62 (1.08-2.23)	1.274 (5) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	2.32 (0.28)	2.36 (1.83-2.97)	3.296 (5) ns
Rape (*Brassica napus*) v. Tanto (old)	3.01 (0.39)	1.95 (1.58-2.39)	2.416 (5) ns
Rape (*B. napus*) V. Tanto (Young)	2.44 (0.33)	1.57 (1.20-1.97)	1.309 (5) ns
Rape (*B. napus*) v. Lirawell(old)	3.04 (0.39)	1.91 (1.55-2.34)	3.040 (5) ns
Rape (*B. napus*) v. Lirawell (young0	2.37 (0.33)	1.56 (1.18-1.97)	2.268 (5) ns
Rape (*B. napus*) v. Starlight (old)	3.14 (0.41)	1.91 (1.55-2.32)	3.913 (5) ns
Rape (*B. napus*) v. Starlight (Young)	2.52 (0.37)	1.35 (1.02-1.70)	1.200 (5) ns
Maize (Zea mays) v. Marcia (old)	2.01 (0.23)	4.17 (3.25-5.36)	4.525 (5) ns
Maize (*Zea mays*) v. Marcia (Young)	2.09 (0.23)	5.95 (4.67-7.60)	0.906 (5) ns

a+ Significance level, ns= not significant, P<0.05, d.f.= degrees of freedom

Figure 3.1 Variation of residual LD50 of *F. candida* on different leaf surfaces with time after treatment with deltamethrin 2.5 EC (bar indicates 95% fiducial limit)

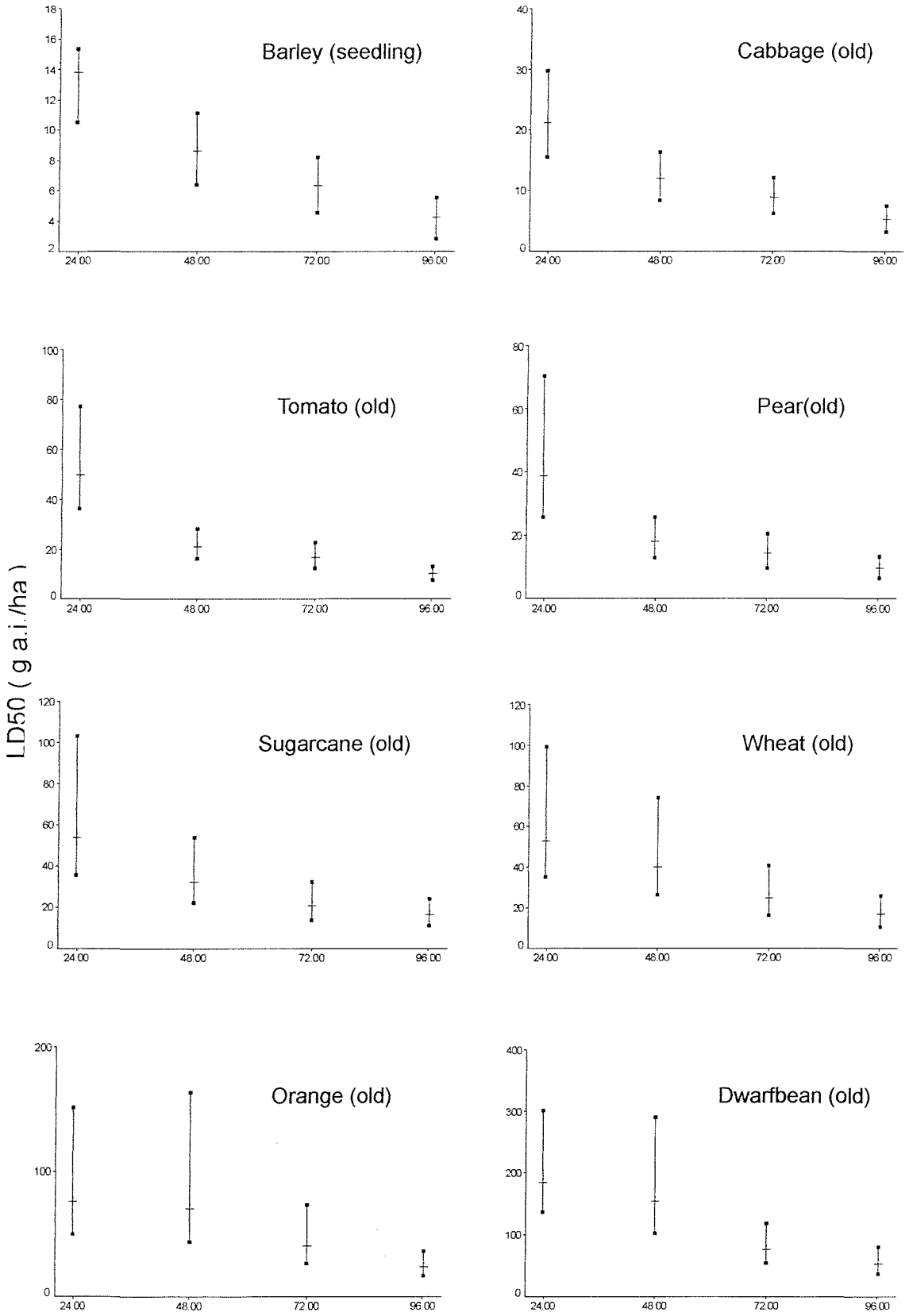


Figure 3.1 (cont.) Variation of residual LD50 of *F. candida* on different leaf surfaces with time after treatment with deltamethrin 2.5 EC (bar indicates 95% fiducial limit)

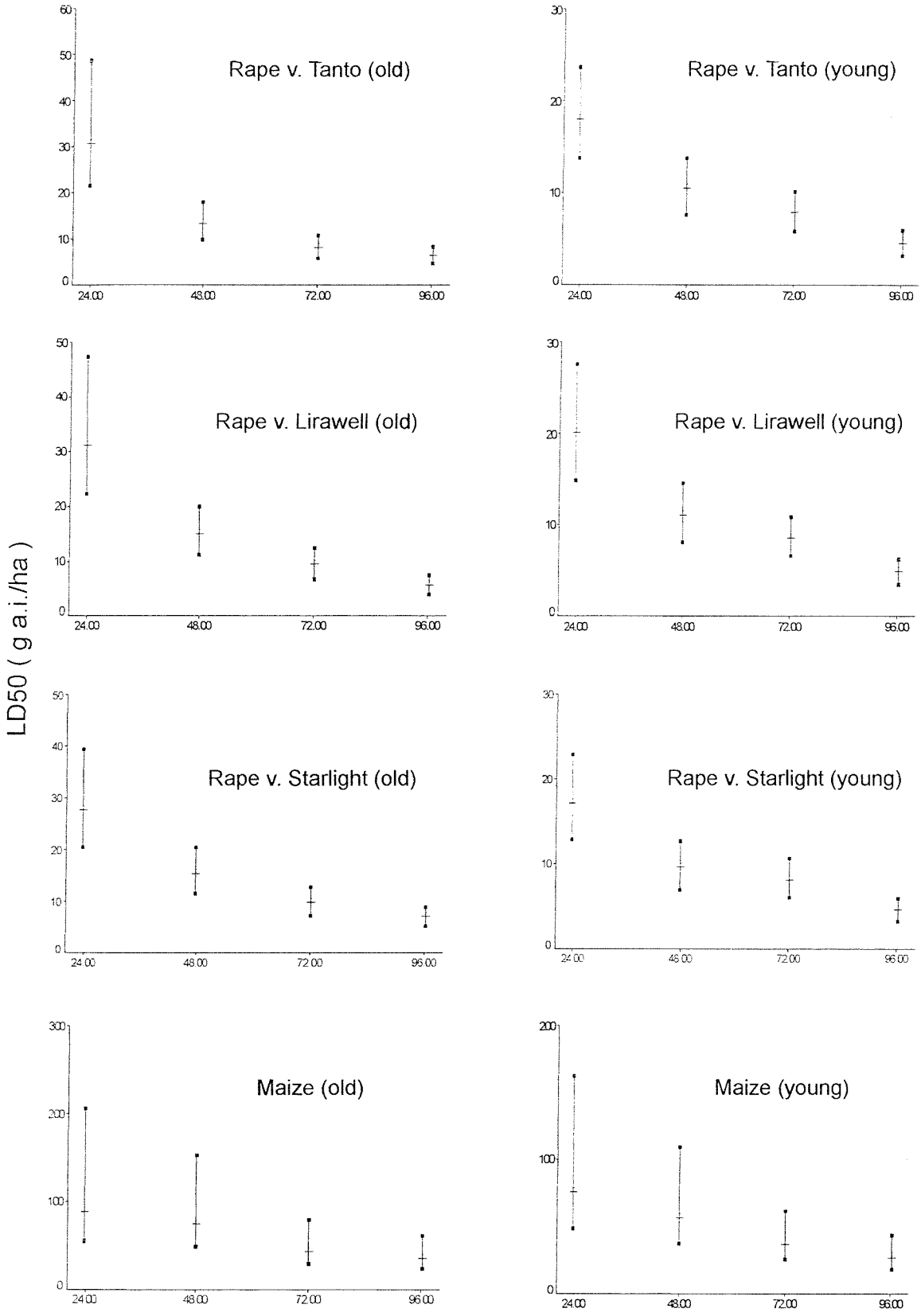


Figure 3.2 . Variation in residual LD50 of *F. candida* on different leaf surfaces with time after treatment with Dimethoate 40EC (bar indicates 95%fiducial limit)

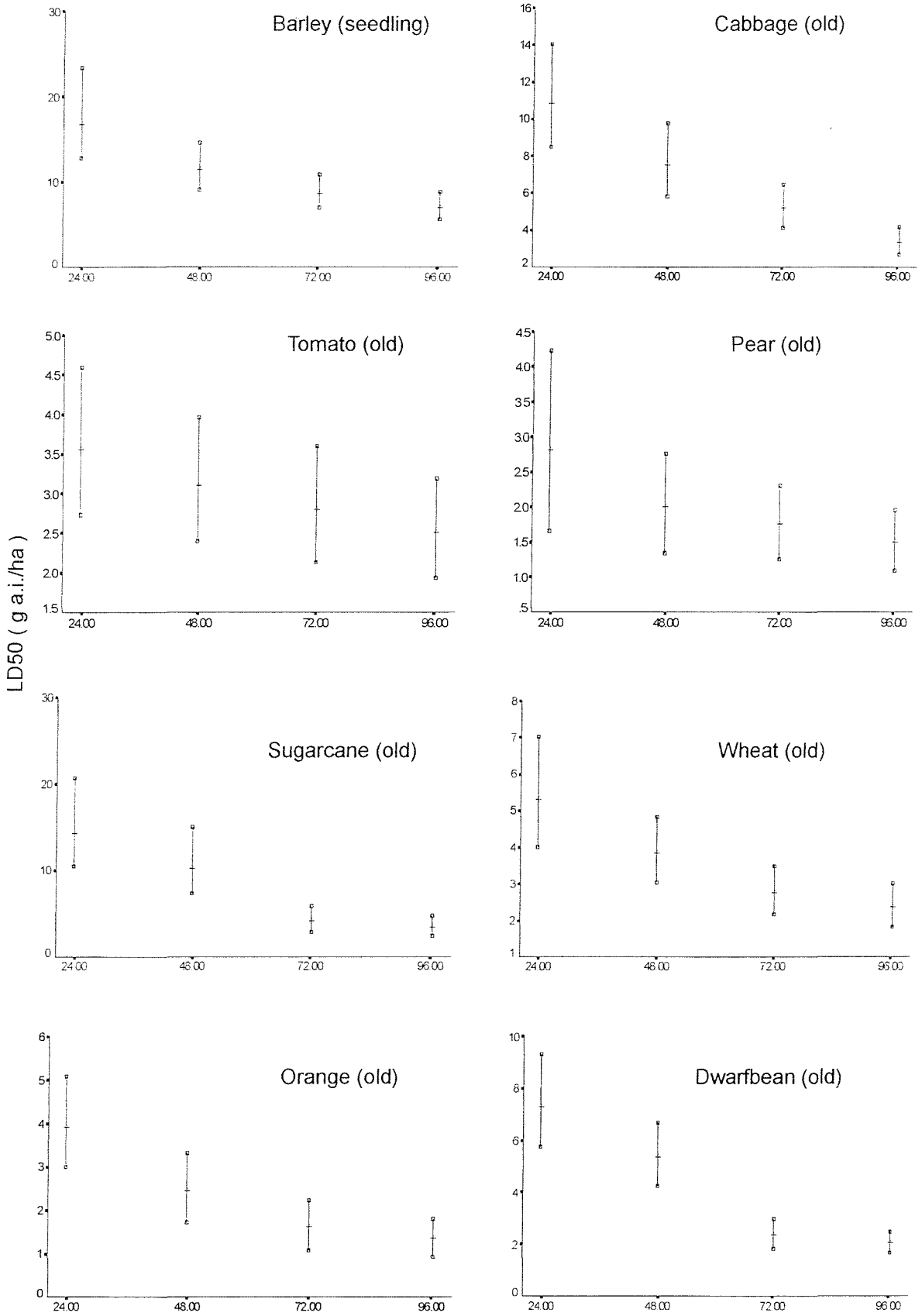
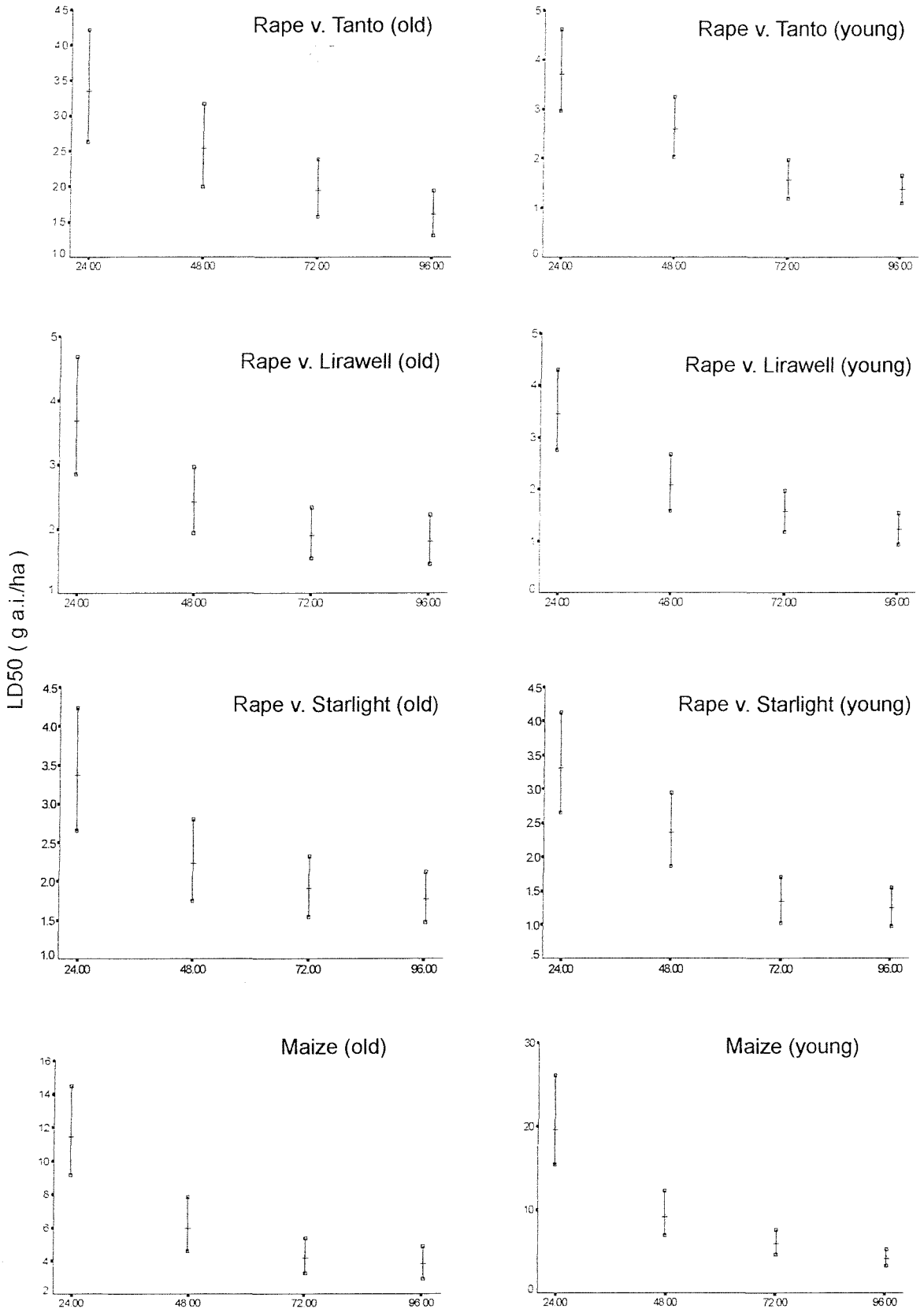


Figure 3.2 (cont.). Variation in residual LD50 of *F. candida* on different leaf surfaces with time after treatment with Dimethoate 40EC (bar indicates 95%fiducial limit)



point, but with an indication of further low level mortality which may have extended for a longer period. The 72-h assessment data were selected for comparison of susceptibilities.

3.3.2 Analysis of the dose-response relationship

The toxicological statistics from probit analysis are given in Table 3.1 for deltamethrin and Table 3.2 for dimethoate. In almost all cases, χ^2 statistics indicated non-significant heterogeneity.

The probit responses of *F. candida* to deltamethrin on different leaf surfaces are shown in Figure 3.3 and to dimethoate in Figure 3.4. The susceptibility ranking, in terms of g AI ha^{-1} showed a range of variation between different leaf surfaces for both deltamethrin and dimethoate. For deltamethrin-treated surfaces, *F. candida* showed high susceptibility on barley (seedling) leaves (0.80 g AI ha^{-1}) followed by rape v. Tanto (young), rape v. Starlight (young), rape v. Tanto (old), rape v. Lirawell (young), cabbage (old), rape v. Lirawell (old), rape v. Starlight (old), pear (old), tomato (old), sugarcane (old), wheat (old), maize (young), orange (old), maize (old) and dwarf bean (old). *F. candida* showed least susceptibility on dwarf bean leaves. The dose-range used for all the leaf types, except dwarf bean ranged from 3.125 g AI ha^{-1} to 100 g AI ha^{-1}. Low mortality responses on dwarf bean required the addition of two more concentrations (150 g AI ha^{-1} and 200 g AI ha^{-1}). A number of significant separations were also evident in the parallelism tests of probit lines (Table 3.3).

A linear regression model was fitted to the probit slope against log 72-h LD_{50} (g AI ha^{-1}) for *F. candida* on the different leaf surfaces sprayed with deltamethrin (Figure 3.7). This gave a significantly negative correlation (r^2 = 0.67 ; F = 28.67; d.f. = 1,14 ; $p < 0.001$). The *F. candida* tolerance distribution was narrowest i.e. highest regression slope on rape v. Lirawell (young) and lowest on wheat (old). All three varieties of rape including both old and young,

Figure.-3.3 Probit transformed responses of *F. candida* on different leaf typesafter 72 hrs of treatment with deltamethrin 2.5EC

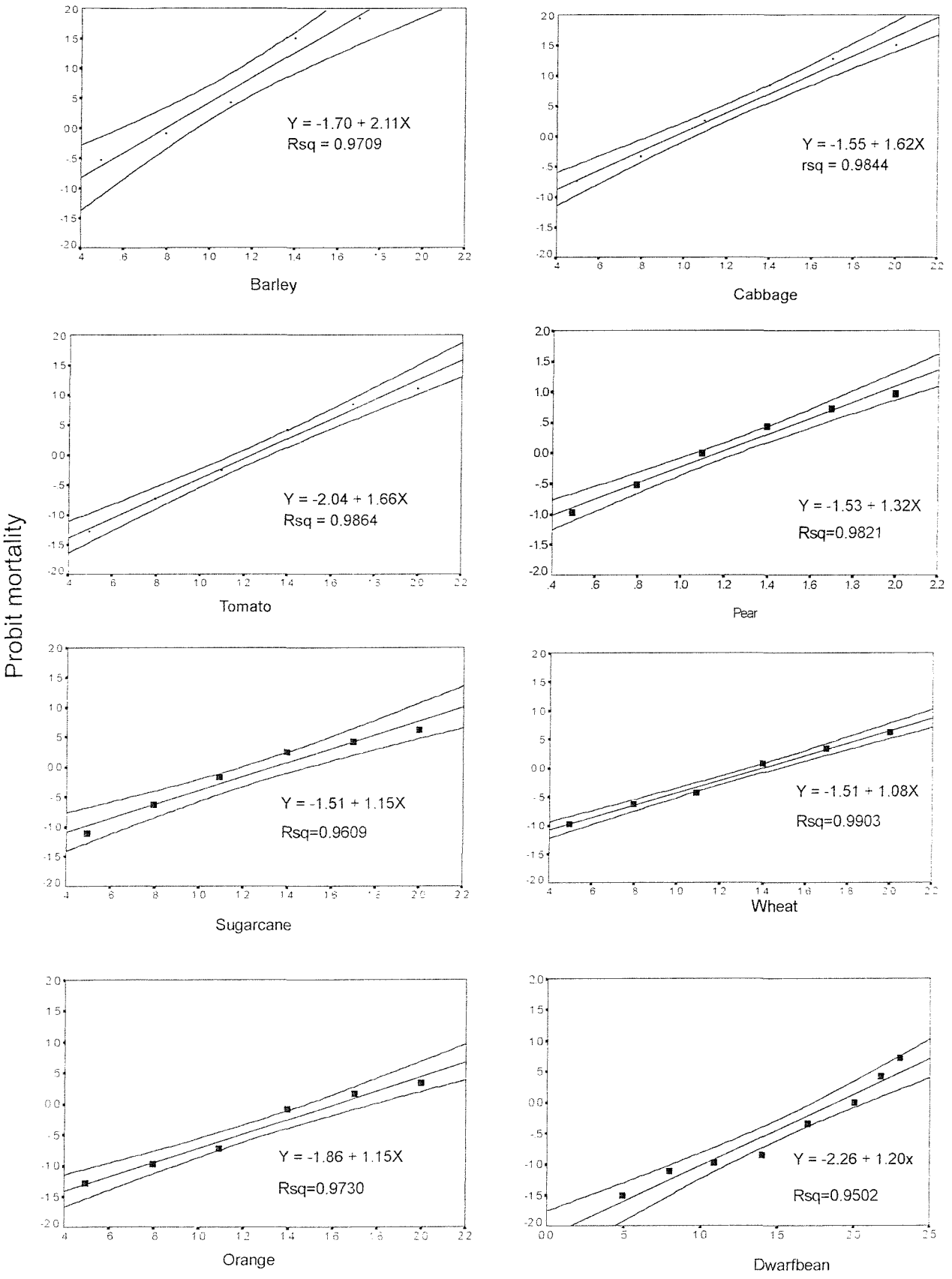
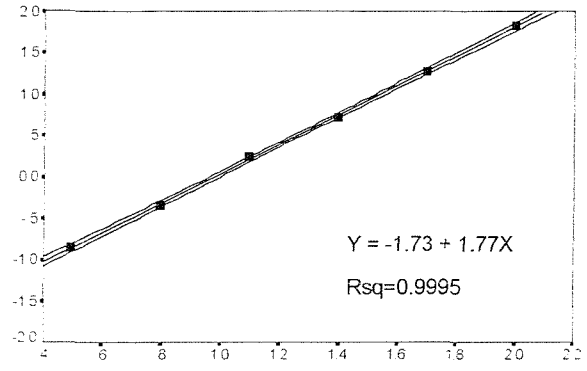
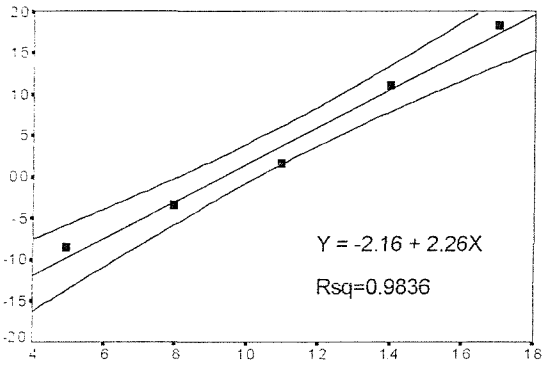


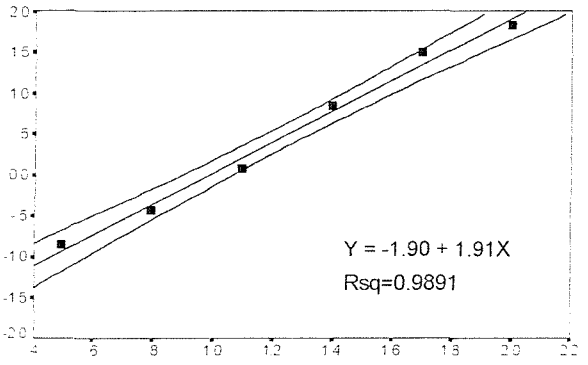
Figure-3.3(cont.). Probit transformed responses of *F. candida* on different leaf types after 72 hrs of treatment with deltamethrin 2.5EC



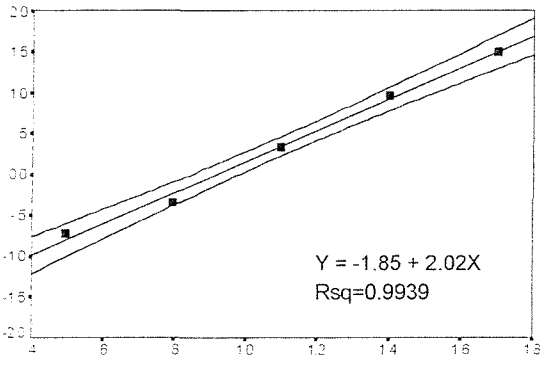
Rape v. lirawell (mature)



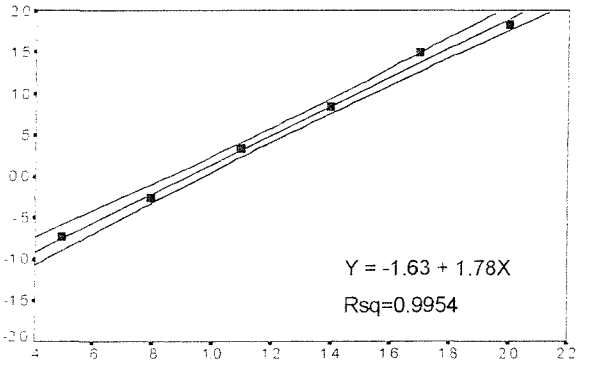
Rape v. lirawell (young)



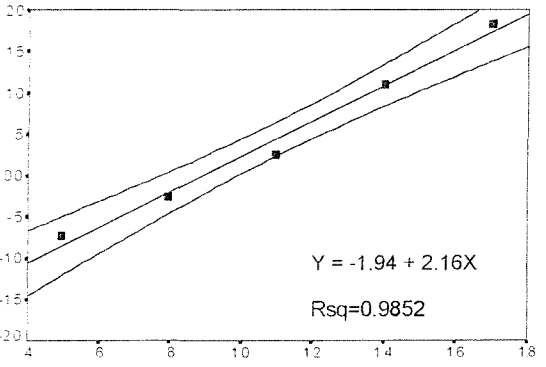
Rape v. starlight (mature)



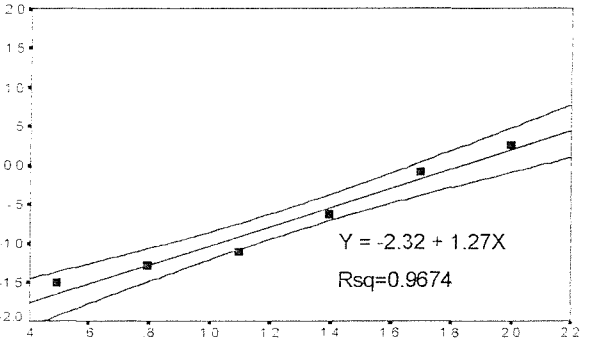
Rape v. starlight (young)



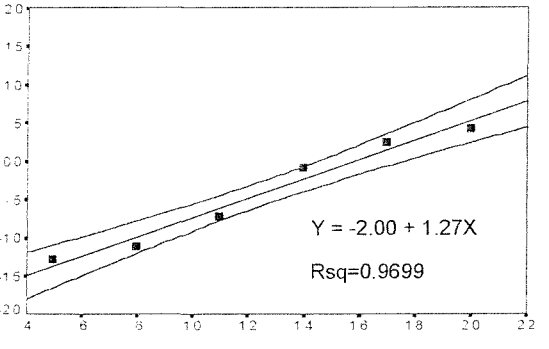
Rape v. tanto (mature)



Rape v. tanto (young)



Maize (mature)



Maize (young)

Probit mortality

Log of doses (g a.i./ha)

Figure 3.4 . Transformed probit responses of *F. candida* on different leaf types after 72-hrs of Treatment with dimethoate 40 EC

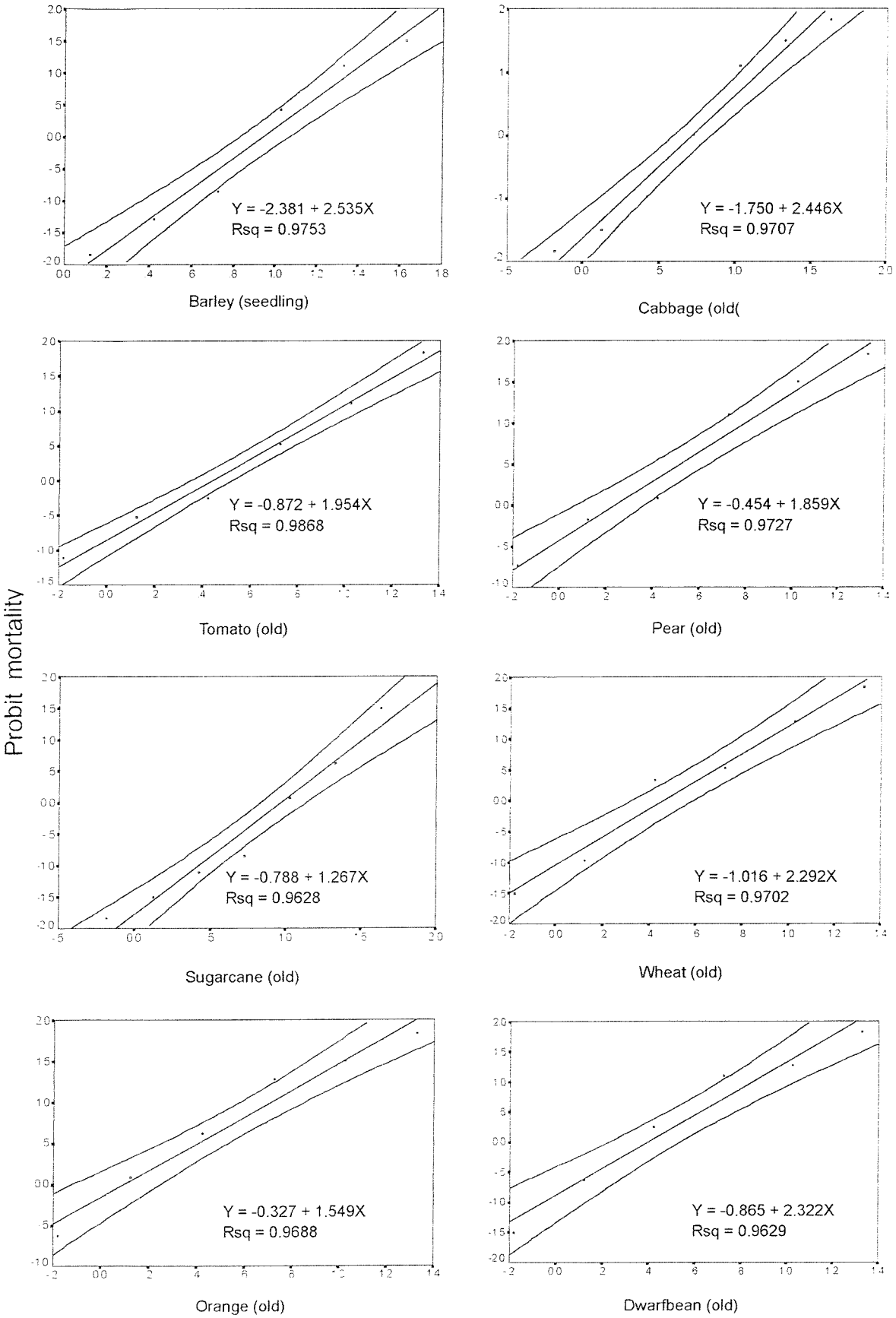
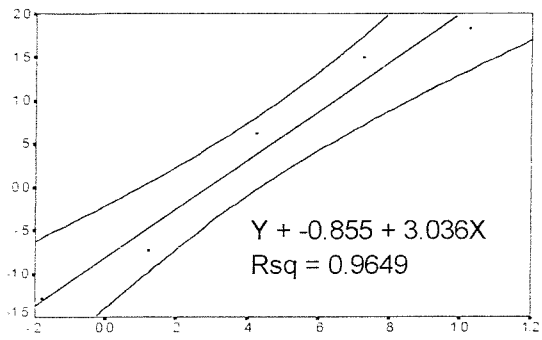
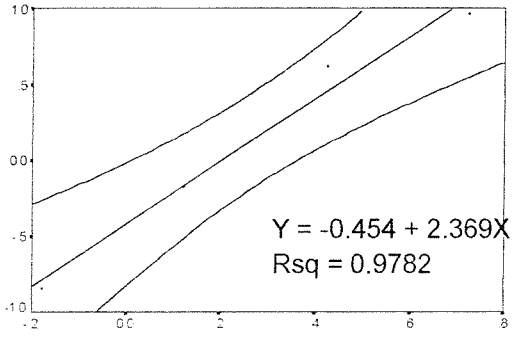


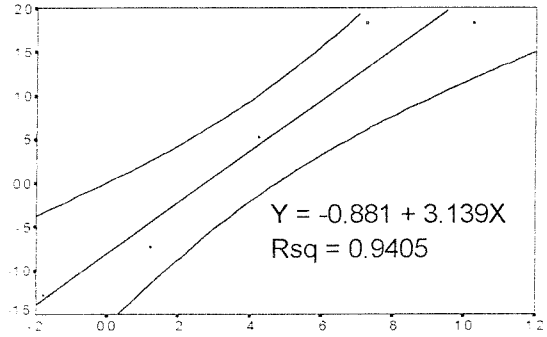
Figure 3.4 (cont.) . Transformed probit responses of *F. candida* on different leaf surfaces after 72-hrs of Treatment with dimethoate 40 EC



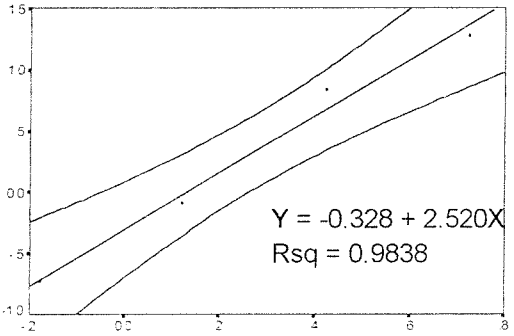
Rape v. Lirawell (old)



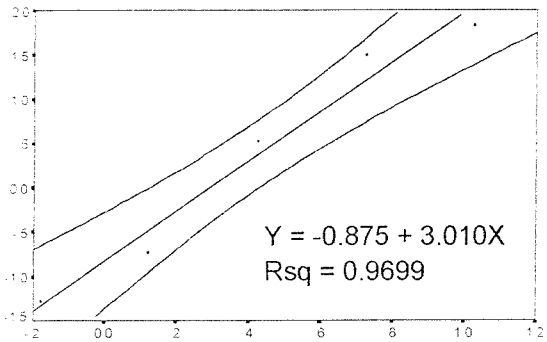
Rape v. Lirawell (young)



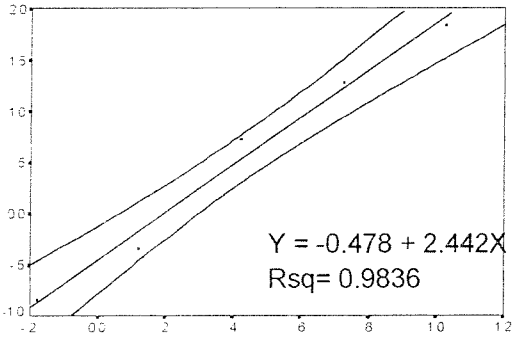
Rape v. Starlight (old)



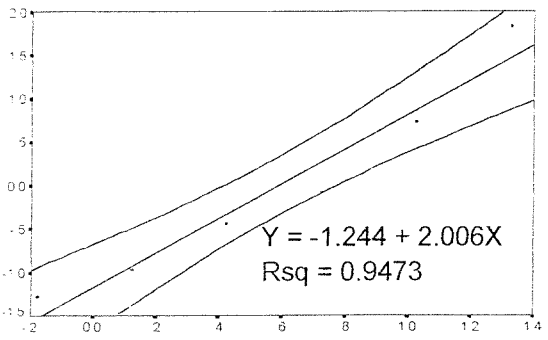
Rape v. Starlight (young)



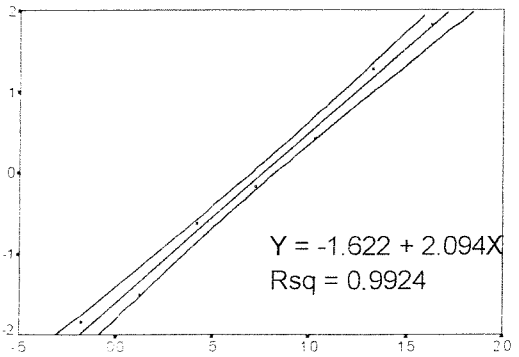
Rape v. Tanto (old)



Rape v. Tanto (young)



Maize (old)



Maize (young)

Probit mortality

Log of doses (g a.i./ha)

Table 3.3. Matrix of $\chi 2$ Statistics from Pairwise Maximum Likelihood Analysis for Differences in Parallelism of 72-h Probit Lines of the Test Leaf Species (Deltamethrin g a.i./ha)

Leaf spp.	Cabbage (o)	Dbean (o)	Maize (o)	Maize (y)	Orange (o)	Pear (o)	R.v.L (o)	R.v.L (y)	Sugarcane (o)	Tomato (o)	Wheat (o)	R.v.S (o)	R.v.S (y)	R.v.T (o)	R.v.T (y)
Barley (s)	1.551 ns	7.262 **	5.146 *	4.844 *	6.298 *	4.228 *	0.530 ns	0.122 ns	6.625 *	1.232 ns	7.473 **	0.170 ns	0.051 ns	0.524 ns	0.012 ns
R.v.T (y)	1.977 ns	8.555 **	6.100 **	5.712 *	7.316 **	5.015 *	0.749 ns	0.059 ns	7.672 **	1.597 ns	8.618 **	0.296 ns	0.120 ns	0.743 ns	
R.v.T (o)	0.278 ns	4.391 *	2.652 ns	2.544 ns	3.785 ns	2.079 *	1.000 ns	1.278 ns	4.075 *	0.155 ns	4.184 *	0.120 ns	0.270 ns		
R.v.S (y)	1.148 ns	6.911 **	4.665 *	4.394 *	5.887 *	3.777 ns	0.276 ns	0.360 ns	6.228 *	0.865 ns	7.109 **	0.032 ns			
R.v.S (o)	0.807 ns	6.345 *	4.074 *	3.890 *	5.384 *	3.303 ns	0.130 ns	0.660 ns	5.730 *	0.580 ns	6.615 *				
Wheat (o)	3.070 ns	0.139 ns	0.360 ns	0.407 ns	0.059 ns	0.670 ns	4.798 *	10.339**	0.033 ns	3.658 ns					
Tomato (o)	0.020 ns	3.177 ns	1.691 ns	1.620 ns	2.718 ns	1.232 ns	0.142 ns	2.375 ns	2.976 ns						
Sugarcane (o)	2.458 ns	0.026 ns	0.174 ns	0.205 ns	0.003 ns	0.397 ns	4.051 *	9.291 **							
R.v.L (y)	2.855 ns	10.471**	7.673**	7.148**	8.909**	6.351*	1.280 ns								
R.v.L (o)	0.260 ns	4.379 *	2.622 ns	2.515 ns	3.761 ns	2.049 ns									
Pear(o)	0.919 ns	0.296 ns	0.038 ns	0.030 ns	0.316 ns										
Orange (o)	2.230 ns	0.004 ns	0.124 ns	0.150 ns											
Maize (y)	1.255 ns	0.117 ns	1.000 ns												
Maize (o)	1.313 ns	0.094 ns													
Dbean (o)	2.561 ns														

Key to test leaf species: (s), Seedlings; (o),Old Leaf; (y),Young Leaf; Dbean, Dwarfbean; R.v.T, Rape var. Tanto; R.v.S, Rape var. Starlight; R.v.L, Rape var. Lirawell.Values in boxes give $\chi 2$ statistics (d.f.1) and level of significance: ns = not significant; *, $P < 0.05$; **, $P < 0.01$.

Table 3.4 . Matrix of $\chi 2$ Statistics from Pairwise Maximum Likelihood Analysis for Differences in Parallelism of 72-h Probit Lines of the Test Leaf Species (Dimethoate 40EC g a.i./ha)

Leaf spp.	Cabbage (o)	Dbean (o)	Maize (o)	Maize (y)	Orange (o)	Pear (o)	R.v.L (o)	R.v.L (y)	Sugarcane (o)	Tomato (o)	Wheat (o)	R.v.S (o)	R.v.S (y)	R.v.T (o)	R.v.T (y)
Barley (s)	0.180 ns	0.566 ns	2.973 ns	1.309 ns	7.800 **	7.098 **	1.154 ns	0.265 ns	15.94 ***	2.47 ns	0.341 ns	1.200 ns	0.003 ns	1.124 ns	0.020 ns
R.v.T (y)	1.000E-08 ns	0.223 ns	1.805 ns	0.716 ns	5.373 *	4.535 *	1.209 ns	0.089 ns	11.01 ***	1.522 ns	0.137 ns	1.213 ns	1.000E-08 ns	1.188 ns	
R.v.T (o)	2.567 ns	3.218 ns	6.587 *	4.110 *	11.76 ***	11.91 ***	1.000E-08 ns	1.962 ns	19.94 ***	5.619 *	2.319 ns	1.000e-08 ns	0.874 ns		
R.v.S (y)	0.091 ns	0.326 ns	1.802 ns	0.708 ns	4.990 *	4.340 *	0.884 ns	10.017 **	1.463 ns	0.163 ns	0.163	0.855 ns			
R.v.S (o)	2.957 ns	3.583 ns	7.001 **	4.330 *	12.23 ***	12.72 ***	1.000E-08 ns	2.047 ns	20.61 ***	5.883 *	2.430 ns				
Wheat (o)	1.000E-08 ns	1.000E-08 ns	1.105 ns	0.259 ns	4.622 *	3.712 ns	2.368 ns	1.000E-08 ns	10.750 **	0.895 ns					
Tomato (o)	1.552 ns	0.693 ns	1.000E-08 ns	0.221 ns	1.586 ns	0.798 ns	5.720 *	0.626 ns	5.633 *						
Sugarcane (o)	14.95 ***	10.55 **	5.808*	8.582 **	0.942 ns	2.302 ns	20.12 ***	8.313 **							
R.v.L (y)	0.017 ns	0.018 ns	0.895 ns	0.117 ns	3.608 ns	2.988 ns	1.998 ns								
R.v.L (o)	2.657 ns	3.308 ns	6.707 **	4.194 *	11.91 ***	12.10 ***									
Pear(o)	5.818 *	3.53 ns	0.833 ns	2.031 ns	0.163 ns										
Orange (o)	6.657 **	4.394 *	1.502 ns	3.056 ns											
Maize (y)	0.560 ns	0.096 ns	0.243 ns												
Maize (o)	1.986 ns	0.950 ns													
Dbean (o)	0.127 ns														

Key to test leaf species: (s), Seedlings; (o),Old Leaf; (y),Young Leaf; Dbean, Dwarfbean; R.v.T, Rape var. Tanto; R.v.S, Rape var. Starlight; R.v.L, Rape var. Lirawell.Values in boxes give $\chi 2$ statistics (d.f.1) and level of significance: ns = not significant; *, $P < 0.05$; **, $P < 0.01$ ***.

barley (seedling), cabbage (old) and tomato (old) showed relatively steeper slopes than wheat (old), sugarcane (old), orange (old), dwarf bean (old), maize (old and young), and pear (old).

The susceptibility of *F. candida* to dimethoate on different leaf surfaces was significantly different from deltamethrin. The 72-h dose-response data showed *F. candida* to be highly susceptible to dimethoate on rape v. Starlight (young) (1.35 g AI ha^{-1}). The ranking sequence of susceptibility on other leaf surfaces are: rape v. Lirawell (young) > rape v. Tanto (young) > orange (old) > pear (old) > rape v. Starlight (old) > rape v. Lirawell (old) > rape v. Tanto (old) > dwarf bean (old) > wheat (old) > tomato (old) > maize (old), sugarcane(old) > cabbage (old) > maize (y) > barley (seedling). A number of significant separations were also evident from the analysis of parallelism of probit lines (Table 3.4). This indicates significant variation in the form of the tolerance distribution on different leaves which may reflect differences in exposure caused by leaf surface properties.

A linear regression model was fitted to data for probit slopes against log 72-h LD_{50} for *F. candida* on the different leaf surfaces for dimethoate (Fig. 3.8). No correlation was this time observed between slope and LD_{50} indicating an irregular pattern of tolerance distribution for *F. candida* as a function of susceptibility.

From these results it can be concluded that there are significant differences in the toxicity responses of *F. candida* on the different leaf substrates. Variation in susceptibility as a result of varietal differences was less than that arising from age differences. For example, the susceptibility ranking of *F. candida* exposed to three varieties of rape showed smaller differences between varieties than susceptibilities on the old and young leaves of those varieties (Tables 3.1 and 3.2).

Figure3.5 .Individual dose responses of *F. candida* to Deltamethrin 2.5EC on different leaf types at 24 hrs intervals.

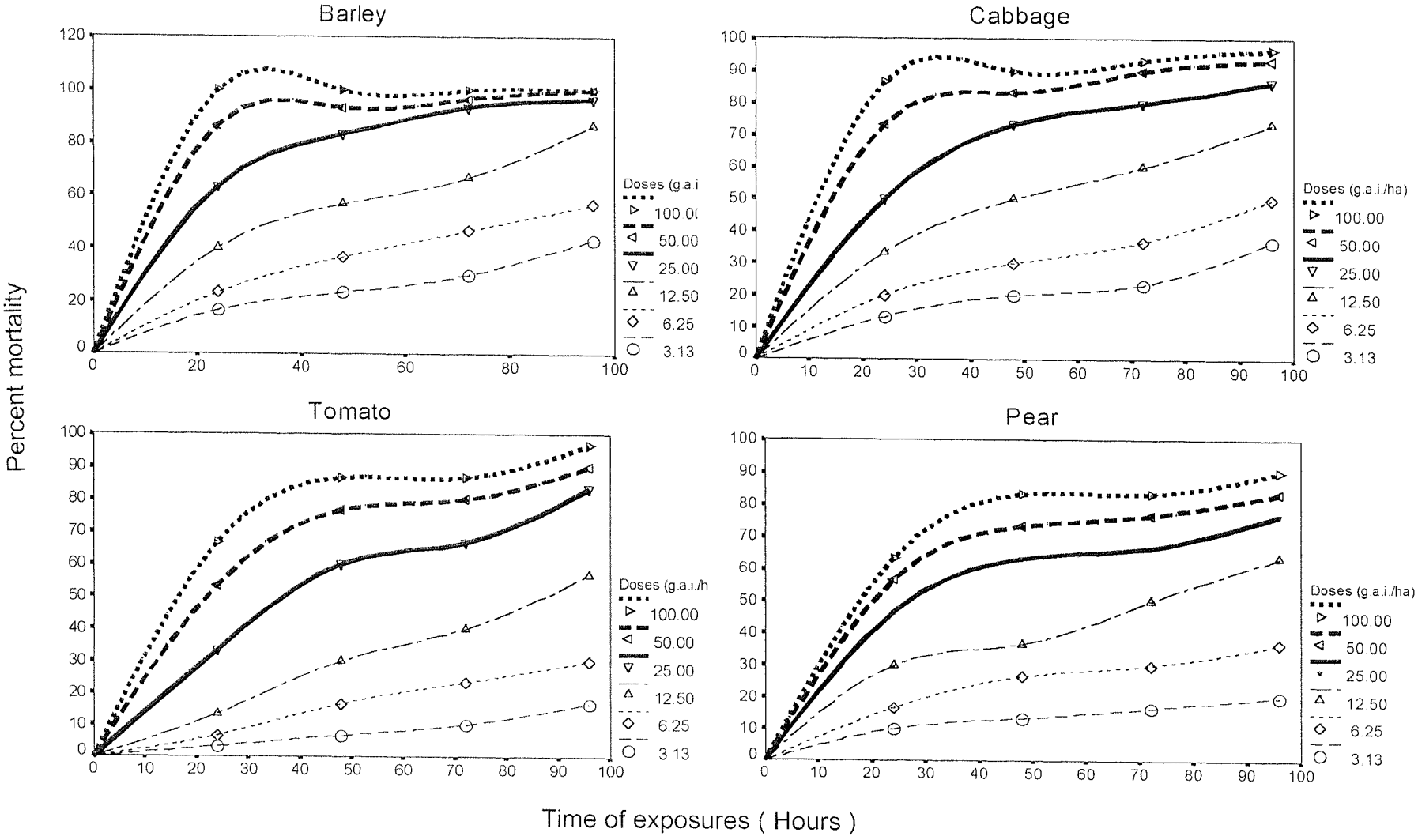


Figure3.5 (cont.). Individual dose responses of *F. candida* to Deltamethrin 2.5EC on different leaf types at 24 hrs intervals.

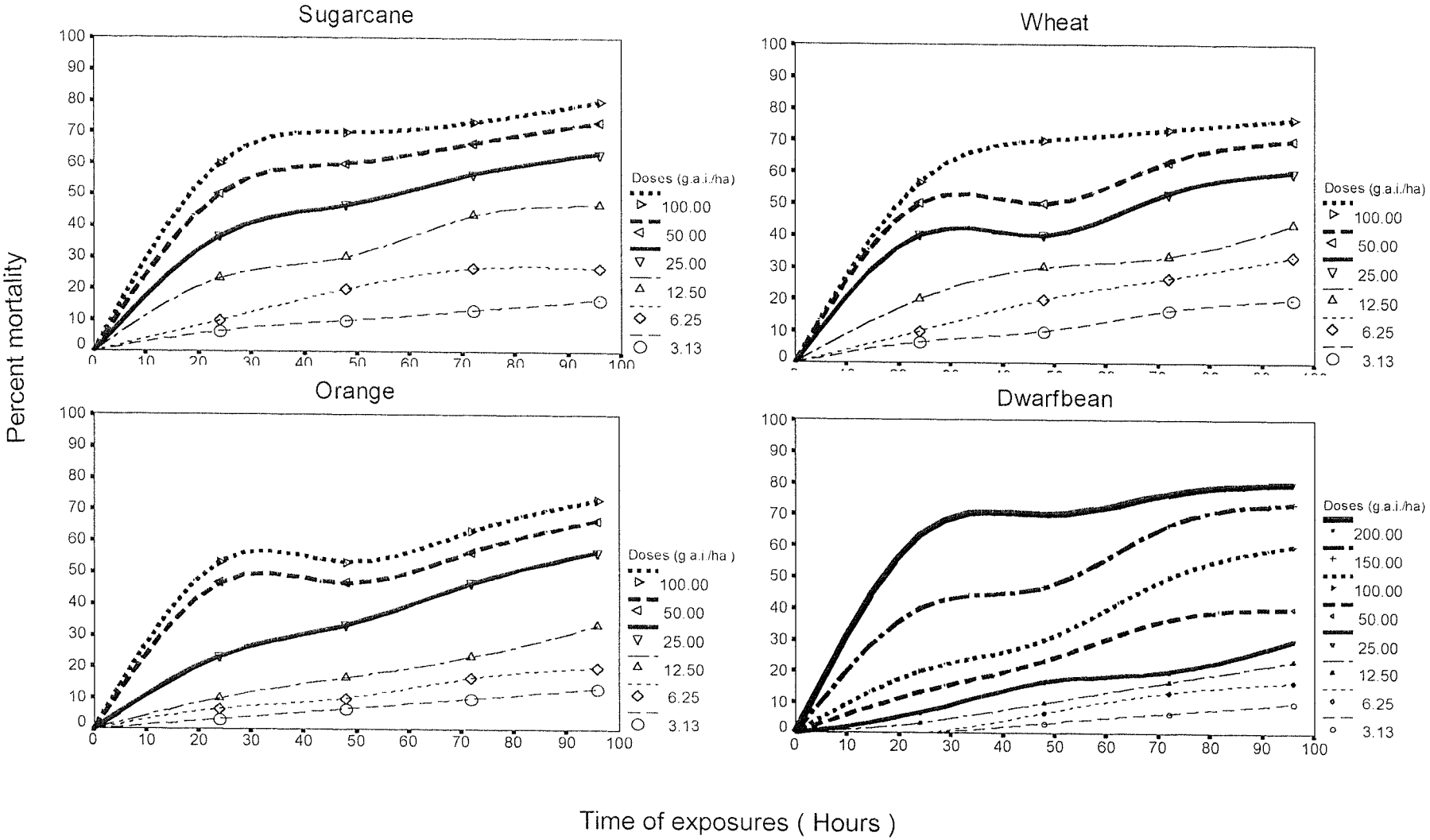


Figure3.5 (cont.). Individual dose responses of *F. candida* to Deltamethrin 2.5EC on different leaf types at 24 hrs intervals.

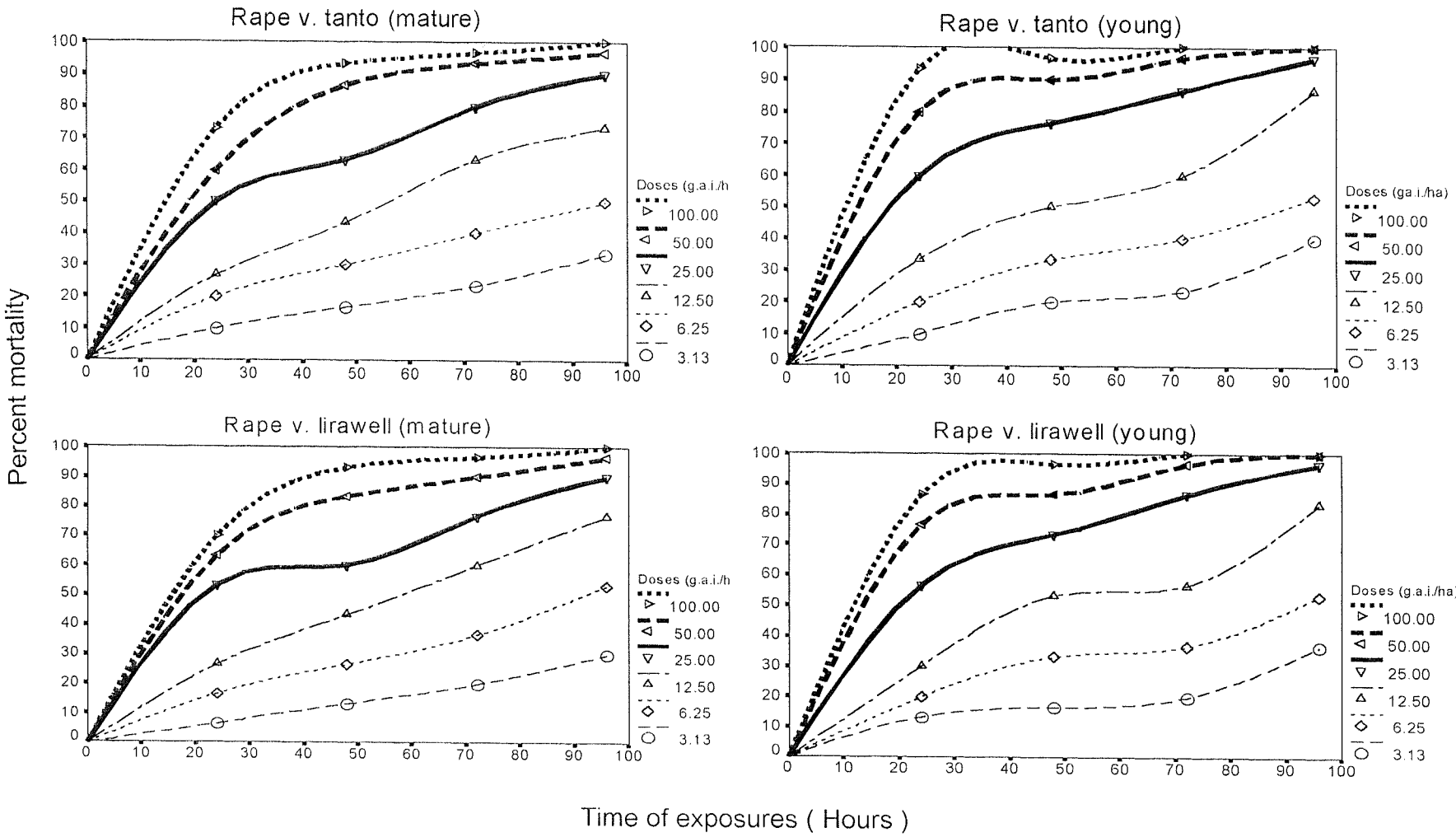


Figure3.5 (cont.). Individual dose responses of *F. candida* to Deltamethrin 2.5EC on different leaf types at 24 hrs intervals.

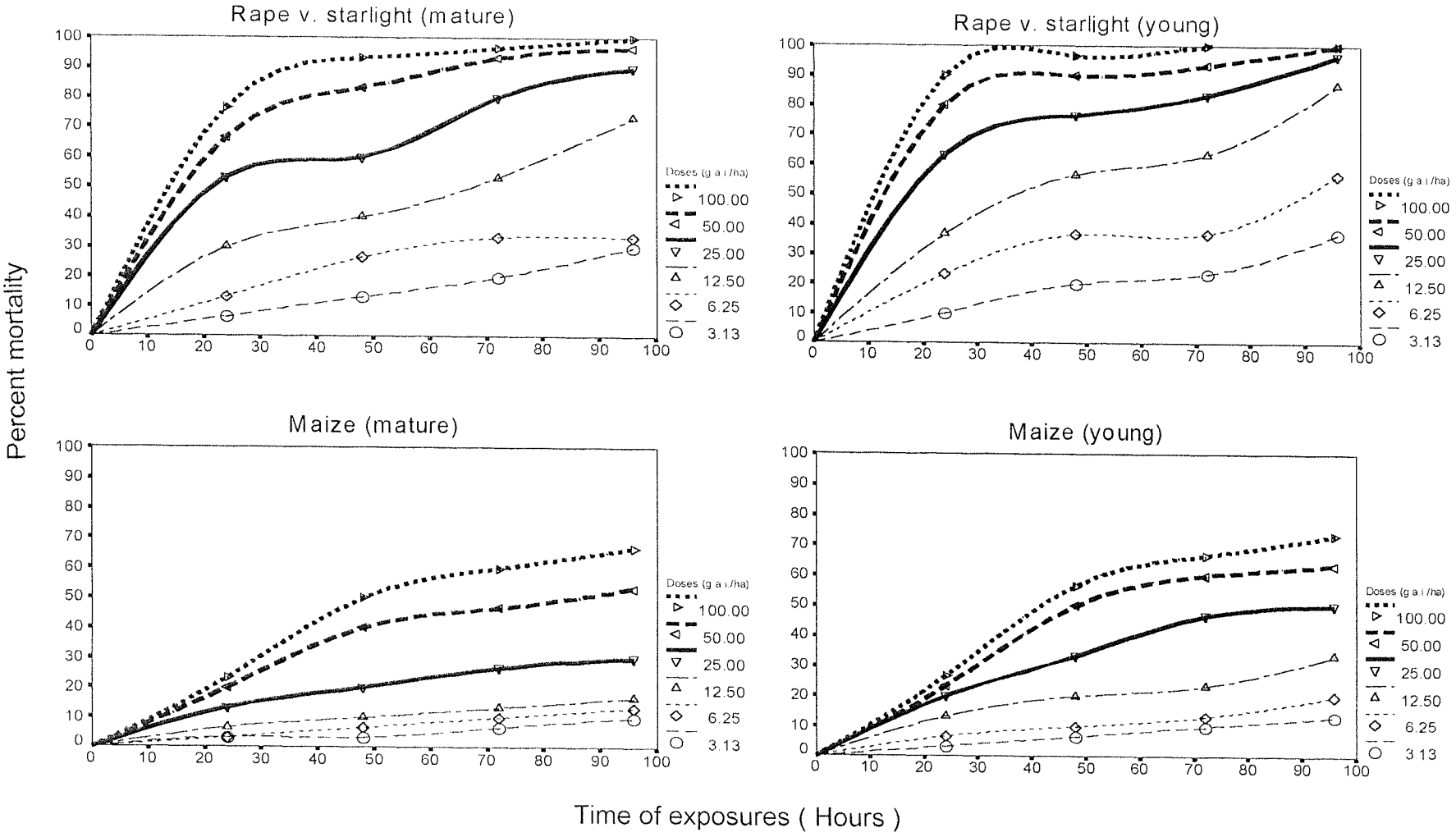


Figure 3.6. Individual dose response of *F. candida* to dimethoate 40 EC on different leaf surfaces at 24 hrs intervals.

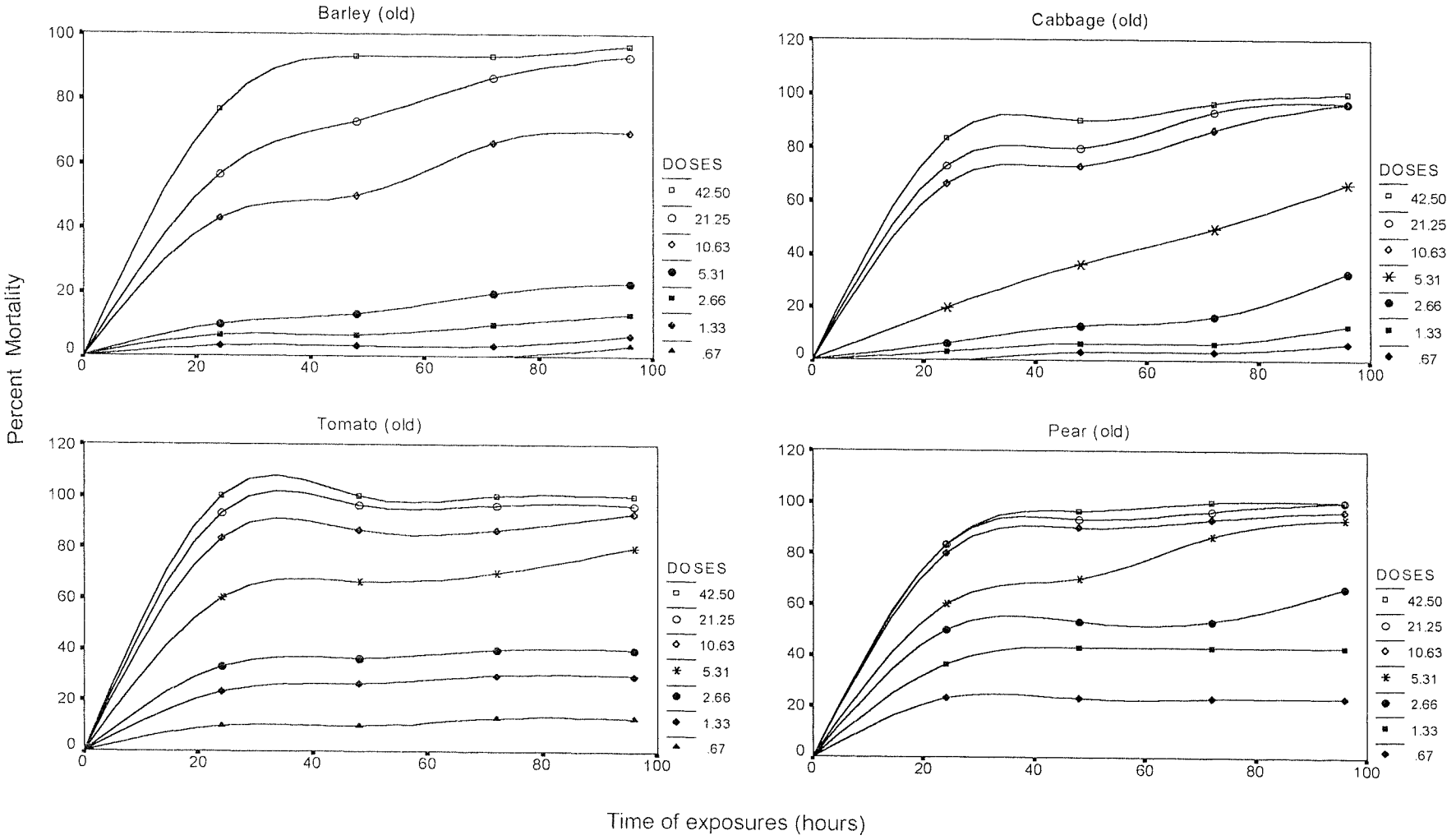


Figure 3.6 (cont.).Individual dose response of *F. candida* to dimethoate 40 EC on different leaf types at 24 hrs intervals.

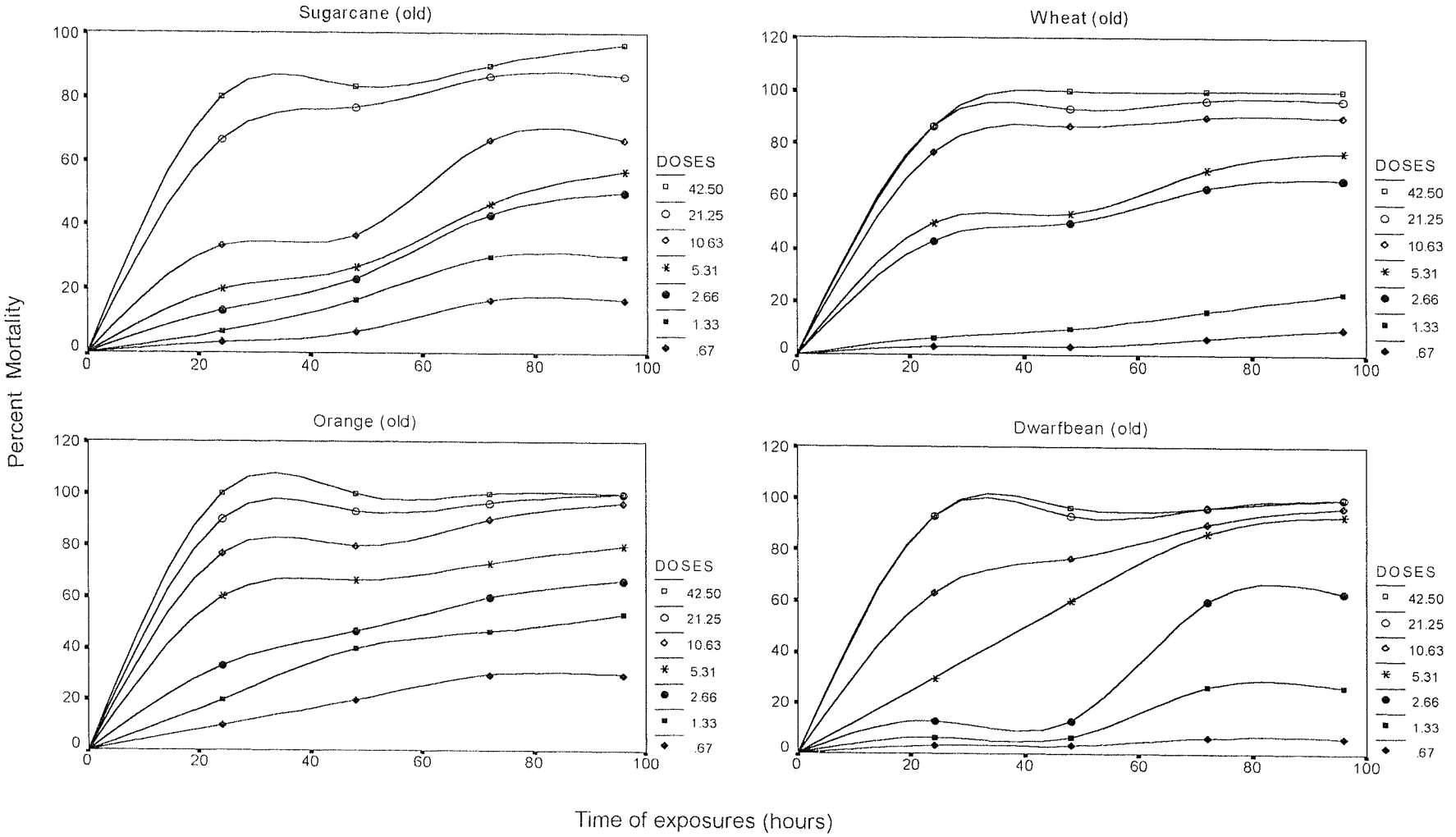


Figure 3.6 (cont.). Individual dose response of *F. candida* to dimethoate 40 EC on different leaf types at 24 hrs intervals.

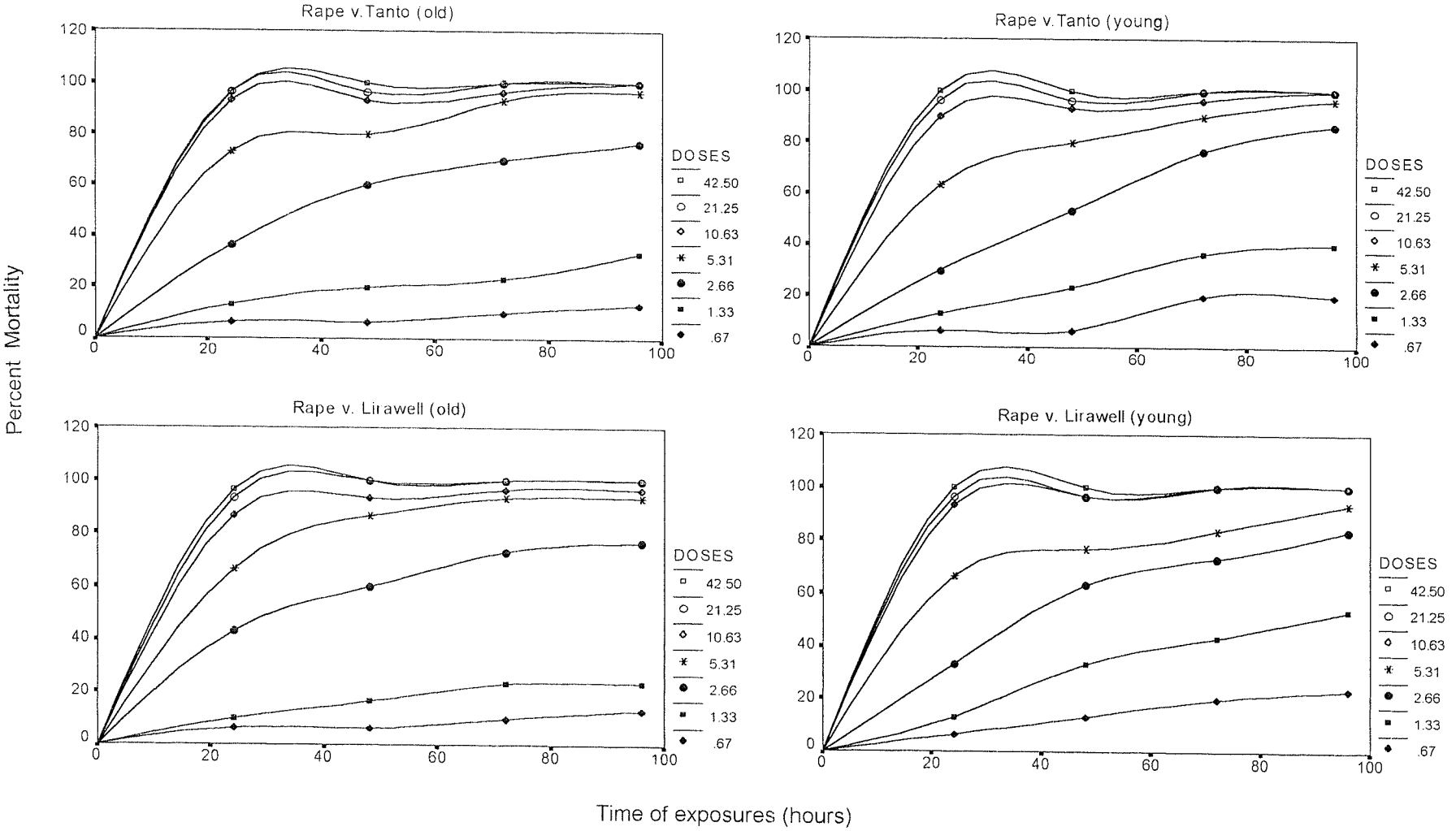
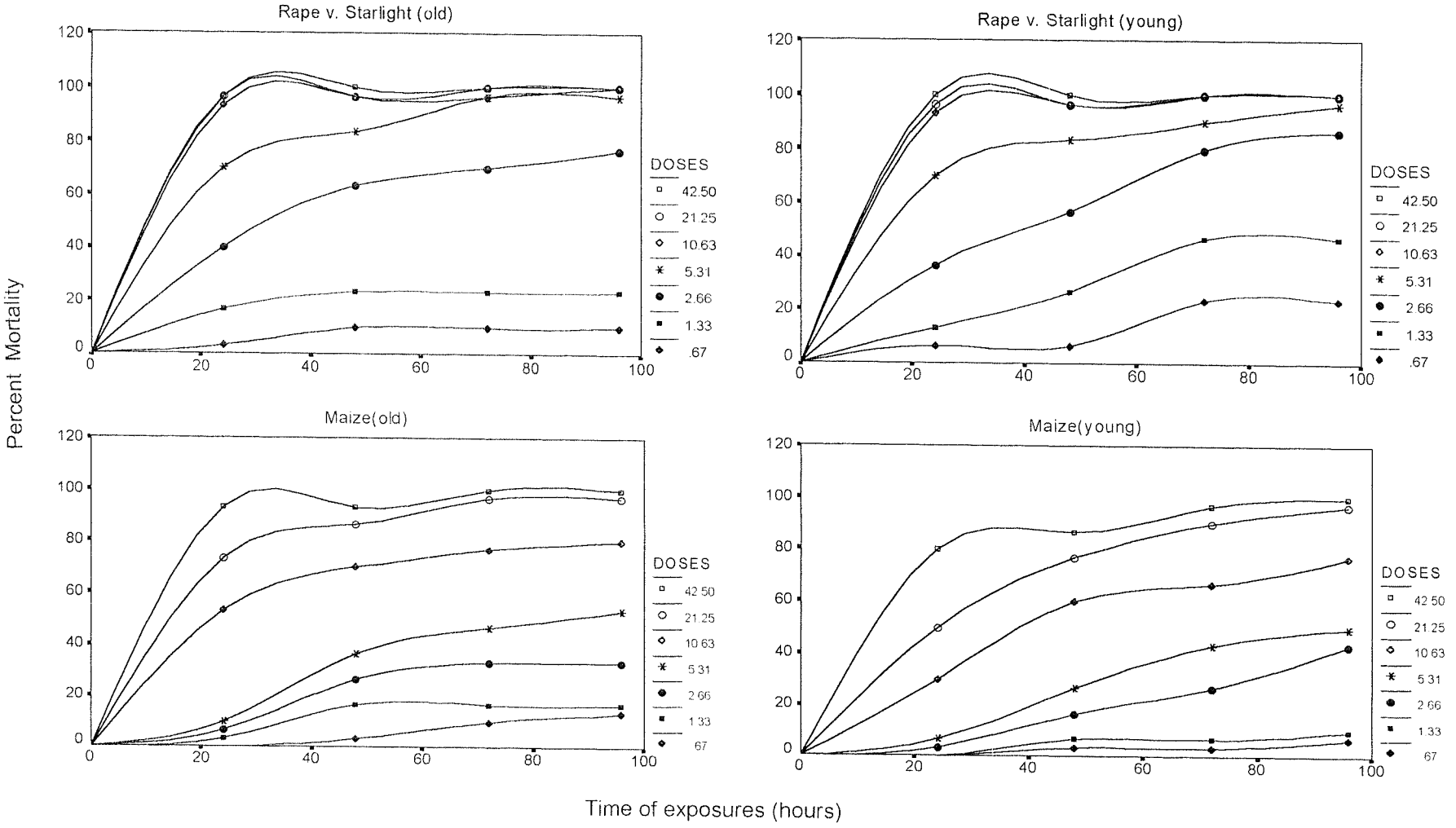


Figure 3.6 (cont.). Individual dose response of *F. candida* to dimethoate 40 EC on different leaf types at 24 hrs intervals.



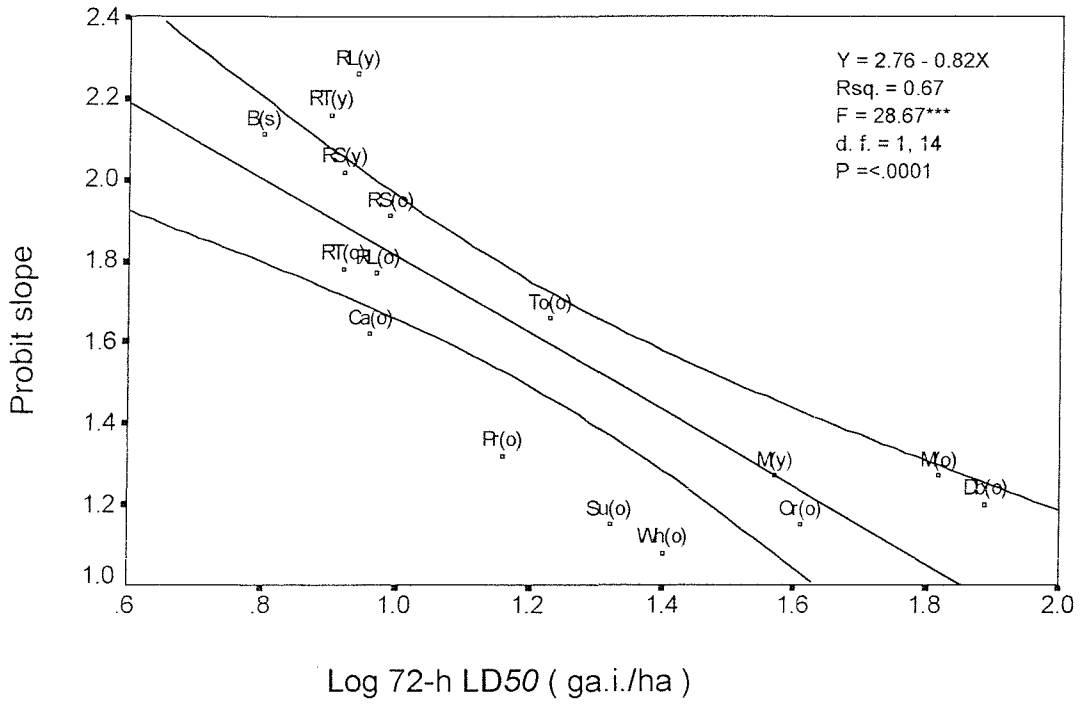


Figure3.7. Correlation between probit slope and Log 72-h LD50 (g a.i./ha) for different leaf types sprayed with deltamethrin 2.5 EC. (Curved lines indicate 95% confidence intervals.) Key to test types: B, Barley; Ca, Cabbage, RT, Rape var. Tanto; Rl, Rape var.Lirawell; RS, Rape var.Starligh t;Db, Dwarfbean; To, Tomato; Pr, Pear; Su, Sugarcane; Wh, Whaet; Or, Orange; M, Maize; (o), Old leaves; (y), Yoyng leaves; (s), Seedlings

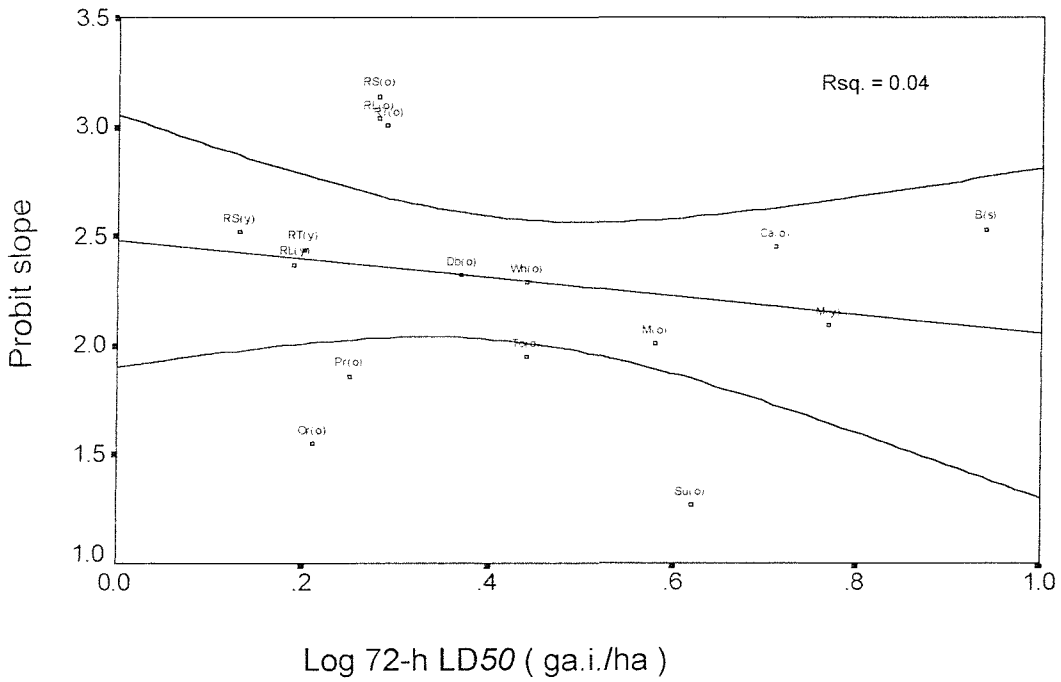


Figure 3.8. Correlation between probit slope and Log 72-h LD50 (g a.i./ha) for different leaf types sprayed with ddimethoate 40 EC. (Curved lines indicate 95% confidence intervals.) Key as figure 3.7.

3.3.3 Insecticide toxicity

Dimethoate was more toxic than deltamethrin in the present investigation - even in comparison with their respective recommended field application rates. On deltamethrin- treated leaves, the test insect *F. candida* was alive at spray rates up to 16 times the field application rate (6.25 g AI ha^{-1}). On dimethoate-treated surfaces, 100% mortality was observed at spray rates 8 times lower than the field rate (340 g AI ha^{-1}); mortality was even observed on the surfaces treated at a deposition rate of 144 times lower than the field rate. With deltamethrin- treated surfaces mortality was first observed at half the field rate.

Both insecticides showed similar LD_{50}'s on barley and cabbage leaves (Tables 3.1 and 3.2). In other cases, the differences in LD_{50} values for the two insecticides were much wider. The lowest LD_{50} value observed on barley seedlings with deltamethrin 6.36 (g AI ha^{-1}). The lowest value observed for dimethoate was 1.35 (g AI ha^{-1}), found on the leaf surface of rape v. Starlight (young). For deltamethrin, the LD_{50} values on different leaf surfaces ranged from 6.36 (g AI ha^{-1}) to 77.14 (g AI ha^{-1}). The range for dimethoate-treated surfaces ranges from 1.35 (g AI ha^{-1}) to 8.69 (g AI ha^{-1}). The lowest value for dimethoate was very close to the highest value for deltamethrin. In general, the two species from the *Brassica* family, rape and cabbage, and the three species of *Graminae* (wheat, maize and sugar cane) showed close patterns in terms of LD_{50} values. Barley showed differences from three other species in the same family, probably a result of physiological differences associated with different growth stages. These differences were not detected on the leaf surfaces treated with dimethoate.

Figures 3.5 and 3.6 display the time course of poisoning as a function of dose rate on each leaf surface treated with deltamethrin and dimethoate respectively. The end-points at different dose rates indicate the equilibrium points between deposit availability, uptake and metabolism/elimination rates. The shallow incremental trend, in most cases following

the plateau shows that a degree of residual toxicity remained throughout the exposure period.

3.4 Discussion

3.4.1 Susceptibility trends on different leaf surfaces

The results of the present experiments showed the existence of differences in both apparent susceptibility and tolerance distributions of *F. candida* on different leaf surfaces. On deltamethrin treated surfaces, the sixteen leaf types used in the study can be grouped into two broad categories on the basis of high and low apparent susceptibility, and also on the basis of tolerance distribution. Given the level of experimental control of physical and biological conditions, these differences can reflect only differences in the form and rate of exposure to the toxicants. The fact that these differences are statistically significant indicates that leaf surface characteristics do play an important role in the overall impact of applied pesticides.

There are several factors that could have contributed to the variation in residual effects of the test insecticides. Following spray application, the successful residual transfer of a toxicant to the site of action in the target invertebrate depends upon substrate characteristics and pesticide properties, providing other environmental factors are more or less similar. The factors to consider in defining the physical and chemical interactions between pesticide, substrate and invertebrate include the affinity of the deposit for the substrate (Ford and Salt 1987), spreading and coverage (Baker *et al.* 1983), deposit size (Spillmann, 1984), retention (Linskens *et al.* 1965; Stock and Davies, 1994) and penetration (Ford and Salt, 1987).

The accumulation of insecticide deposits by an exposed invertebrate will be reduced by the transfer of active ingredient into the plant cuticle. Thus for good contact action, it is desirable to retain the deposit on the surface by restricting foliar penetration (Hartley and Graham-Bryce, 1980; Wilson *et al.* 1983). It is possible that certain leaf

surfaces retain a high insecticide deposit on their surfaces by restricting foliar penetration which in turn makes it more readily available to invertebrates. This might include Brassicae treated with deltamethrin in the present study.

The surfaces on which *F. candida* showed lowest apparent susceptibility had lower amounts of wax deposit. For example, dwarf bean and maize leaves have wax deposits of 1-2 and 10-15 ug cm^{-2} (Baker *et al.* 1983). The morphological characters of these surfaces also place them within different categories. These include amorphous (dwarf bean), glossy (orange), semi-glaucous (maize, wheat and sugar cane) and glaucous (rape). It was observed during spraying that the spray droplets on barley (seedling) leaves, upon which the *F. candida* were highly susceptible, were in a fine continuous pattern, with tiny individual droplets that stayed until the droplets apparently dried. On the surfaces like dwarf bean and orange, the fine droplets joined soon after the spray application to form large, isolated drops of irregular shape. This behaviour of the deposit might leave areas without significant accumulation of insecticide.

Drops with such high affinity may show significant gravitational effects, which must have an adverse effect on the coverage and retention of the insecticide on the leaf surface, especially in field conditions. *Baker et al.* (1983) found a high spread factor following application of an aqueous solution (0.31) of 'Utivex 2B', on dwarf bean and lemon leaves (3.7 and 4.0 respectively) in comparison to rape leaves (0.1).

Other leaf surface characteristics such as the arrangement of basal cells and cell boundaries, the presence of 'open' or 'closed' pattern trichomes, and the micro-structures of epicuticular wax deposits can also contribute to the process of substrate-deposit-invertebrate interactions. For example, tomato leaf surfaces have prominent trichomes. Droplet behaviour on such surfaces is quite different from amorphous or glossy surfaces, like dwarf bean or orange leaves. The

susceptibility ranking of *F. candida* on tomato leaves was intermediate. Although tomato leaves have a low wax content (Chapter 6), the retention of pesticide deposits might be increased by the physical nature of the surface. For example, following spray application, a substantial number of fine droplets are likely to be trapped by trichome tips and spread towards their bases by capillary action. This may enable droplets to be retained on the surface until drying. Since these droplets may not be in direct contact with the leaf surface just after impaction, they avoid immediate foliar penetration and may be more readily available to the invertebrates. Trichome structures may also effectively increase the total contact area with, *F. candida*. Normally, the primary and most vulnerable site of contact for *F. candida* on any relatively flat and smooth surface will be the legs and tail tip. It is possible however, that on leaf surfaces like tomato, the sides of the body may come into contact with dense trichomes and accumulate additional amounts of insecticide and increase the total contact area (Jepson, 1990).

The susceptibility trends for *F. candida* on different leaf surfaces sprayed with dimethoate is more difficult to interpret. Although there were differences in the apparent susceptibility and tolerance distributions on different leaf surfaces, patterns were less evident within this overall variability. The mortality responses of *F. candida* on dimethoate-treated surfaces increased rapidly at high doses, while on deltamethrin-treated surfaces, these increases followed a more gradual trend. On the deltamethrin-treated surfaces of sugar cane, maize, wheat, orange and dwarf bean, mortality did not reach 100% during the period of observation, whereas, in the case of dimethoate-treated surfaces, 100% mortality was observed on most surfaces at some point during the observation period. This will reflect differences in basic physiological susceptibility, combined with differences in the mode and rate of exposure to the individual pesticides.

Dimethoate is more hydrophilic than deltamethrin and also has systemic properties. Although *F. candida* was again highly susceptible to dimethoate on rape varieties, it also exhibited high susceptibility on leaf surfaces like pear and orange. *F. candida* was least susceptible on barley leaves and on other species of *Graminae*, such as maize (both old and young), sugarcane and wheat.

Unlike deltamethrin, dimethoate is recommended as a control measure for collembolan species (Pesticide Manual, 1994). This may indicate that it has highly toxic properties to Collembola and is less detoxifiable by the collembolan enzyme system. Deltamethrin however, is bound to soil organic matter and is not effective as a soil insecticide.

The mode of action of dimethoate is complex. Metabolism in plants is similar to processes in animals (Pesticide Manual, 1994). In addition to hydrolysis, it is also oxidized to the phosphorothioate and the corresponding oxone, which is highly toxic and a stronger cholinesterase inhibitor with greater persistence than dimethoate itself. It is possible that the metabolism of dimethoate on different leaf surfaces produces variable levels of oxone and variable rates of persistence which influence the toxicity to invertebrates. High foliar penetration and persistence within leaf tissues will make the toxicant less available to surface active invertebrates. Persistence on outer leaf surfaces will, however, make the chemical more available as a contact poison to exposed invertebrates.

Foliar penetration results in the loss of availability to surface contact. For example, Gamma BHC is known to pass readily into leaf tissue as does DDT to a limited extent (Martin and Batt, 1958). However, if retained in the cuticle without further penetration, a solution of the insecticide in the waxy layer may be as toxic to an exposed invertebrate and more resistant to natural weathering than the crystal deposition on the surface (Martin and Batt 1958).

The steeper increase in the dose response of *F. candida* to dimethoate may be a result of rapid evaporative loss and volatilization. The short-term adsorption and volatilization from substrates like leaf surfaces will determine the fate and bio-availability of the pesticide (Arnold and Briggs, 1990). The rapid decline in dimethoate availability relative to deltamethrin is also supported by Jepson *et al.* (1990). Higher vapor-pressure, water solubility and the lower octanol-water partition co-efficient for dimethoate make it more volatile on leaves. In addition, the fact that it is systemically taken up by the plant surface makes it less subject to adsorption into leaf epicuticular waxes. The role of wax deposits on leaf surfaces may therefore be less evident than it was for deltamethrin.

CHAPTER 4

Susceptibility of *Aphidius colemani* (Hymenoptera:Aphidiinae:) Viereck to deltamethrin residues on three different leaf substrates

4.1 Introduction

Hymenopteran parasitoids play an important role in controlling cereal aphids (Wratten & Powell 1991). Modern agriculture has however come to rely extensively on synthetic chemical pesticides. Most of these synthetic pesticides are broad spectrum and therefore pose a potential threat to non-target invertebrates. Accompanied by high lipid solubility, these compounds may be biomagnified, resulting in direct toxicity in higher trophic levels, penetrating even the top of the food chain (Carson 1962). Over use of pesticides, especially in under developed countries, causes the development of resistance and resurgence (Metcalf, 1986). To solve these problem and ensure a high demand for agricultural products, it has not been possible to depend upon biological control as an alternative to pesticides. Therefore, a combination of several control methods is considered to be the only alternative approach to minimizing the high use of synthetic pest control chemicals which led to the development of the IPM (Integrated Pest Management) concept.

Pesticide applications in cereals have been shown to reduce natural enemy population densities (Vickerman *et al.* 1987a, 1987b). Deltamethrin is one of the most widely-used pesticides to control cereal pests. Considerable research has been undertaken to quantify the side-effects of pesticides over a wide range of species (Elzen 1989). However, little work has been undertaken on the effects of pesticides on one of the most important aphid parasitoids, *Aphidius colemani*.

The parasitoid *A. colemani* has recently received worldwide attention. It has been introduced to the Kingdom of Tonga, from Australia, as a potential control agent for Banana aphid, *Pentalonia nigronervosa* (Wellings *et al.* 1994). In an evaluation of four aphidiine parasitoids species both in the laboratory and glasshouse (Vansteents, 1995), for controlling

Aphis gossypii Glover, *A. colemani* was found to be the most effective aphid parasitoid, parasitizing 72-80% of the test aphid population.

The reasons for inclusion of a parasitoid in the present experiments were twofold 1) to verify results obtained from previously conducted experiments with *F. candida* with an economically important biological control agent , which has a greater chance of coming into contact with pesticide residues on leaf surfaces in field conditions, and 2) to develop a more reliable test system of laboratory bioassay for a parasitoid. Several test methods have been reported for evaluating the side-effects of pesticides on parasitic Hymenoptera (Mead-Briggs, 1992; Polgar, 1988; Hassan, 1988; Oomen, 1985). In those works substantial differences have been found in the range of chemicals tested and methodologies used. Currently, laboratory pesticide testing methods with parasitoids can not be used to extrapolate to field conditions. Nevertheless such studies still provide important information concerning pesticide side-effects on parasitoids. This chapter aimed to address the following questions:
i) Are there any differences in the toxic responses of a parasitoid to deltamethrin residues that result from differences in leaf substrates ?
ii) If these exist, do they follow the similar trends as for *F. candida* ?
iii) To what extent does the residual toxicity of deltamethrin to *A. colemani* relate to that of *F. candida*?

4.2 Methods and materials

The parasitoids used in the present experiment were supplied by 'Kopert UK Ltd'. A large number of parasitoids were used to develop suitable methods and to determine the definitive dose-range. The number of leaf types tested were therefore restricted and three leaf species defined as 'low', 'medium' and 'high' on the basis of their wax content and having glaucous, semi-glaucous and amorphous surface morphology were selected. These included barley seedling (high wax content and semi-glaucous), cabbage (medium wax content and glaucous) and dwarf bean (low in wax content and amorphous) (for wax

content see Chapter 6). Three bioassay methodologies were considered.

4.2.1 Experimental method one

In this method, the exposure chamber (modified from Mead-Briggs (1992) and Longley (1994) was used. This consisted of two glass plates (12cm X 12cm) fitted to a section of plastic drain-pipe (10.5cm diameter and 2.5cm thin) with five holes (10mm diameter) drilled through the side walls for ventilation. The holes were covered on the inside with fine gauze, attached via non-toxic glue (UHU). One hole was left uncovered, and closed with a cotton wool plug. This was soaked in a 50:50 honey-water solution as a food source, prior to starting each experiment.

The upper surface of the lower glass plate was covered with freshly collected, visibly uninjured and untreated leaves from glasshouse grown plants by double-sided adhesive tape. The leaves were attached adaxial surface facing upwards. A dilution series of 4, 6.25, 7.81, 9.76 and 12.20 g AI ha^{-1} of formulated deltamethrin was prepared before setting up each experiment. A series of range-finding experiments had been undertaken to determine the dose range using smaller numbers of test parasitoids.

The glass plates, with attached leaves, were sprayed under a Potter Laboratory Spray Tower (Potter, 1952), calibrated to deliver a spray volume of 200l ha^{-1}. The tower was thoroughly cleaned and flushed with alcohol and distilled water between treatments. The upper glass plate and the drain-pipe section were kept unsprayed. The treated surfaces were then allowed to dry for approximately 30 minutes in the spray room. The treated surfaces, with their respective chambers were placed in a cold room, where the test parasitoids were kept for 10-20 minutes to render them partially paralysed. This enabled handling with low risk of escape and parasitoids soon regained their normal levels of activity. Ten individual parasitoids were carefully introduced onto the treated surface with a fine point brush. The whole unit was held together firmly with a rubber band and returned to the

insectary. In order to minimise the build-up of pesticide vapor, each exposure chamber was ventilated with humidified air, using a small aquarium pump connected to one of the holes in the plastic drain-pipe by rubber tubing. The whole experiment was conducted in a controlled environment of 18-21^{0}C with a relative humidity of 50-80%. Light was provided as continuous illumination above the chambers. To restrict parasitoid activity around the edge of untreated drain-pipe, the top plate was covered by placing a 2cm wide strip of masking tape around the glass plate edge. The test parasitoids remained active in the illuminated area. Mortality data was taken at 24 h intervals for 96 h.

4.2.2 Experimental method two

To minimize the problems associated with a relatively large chamber area of 12cm X 12cm, and to permit a similar bioassay procedure to that used for *F. candida* (see chapter 2), a Petri dish chamber of 5.5cm diameter and 2.5cm height was used. Two holes (10mm diameter) were drilled through the side walls of the upper portion (top lid) of the Petri dish. A fine hole was made to insert a microlance syringe, in order to provide moist air by the same procedure as mentioned in method one. One hole was covered with fine gauze and another was fitted with a cotton plug, soaked in 50:50 honey:water solution as food. All other procedures were same as in method one, except the lowest dose rate of 4 g AI ha^{-1} was excluded and a higher dose rate of 15.25 g AI ha^{-1} was added. The observation periods were also changed from 24h, 48h, 72h and 96h to 2h, 8h, 16h and 24h respectively.

4.2.3 Experimental method three

This was a modification of method one, and involved some aspects of method two. The chamber was much smaller than that used in method one. The glass plates used were 7.5 X 7.5cm in diameter and the plastic drain-pipe used was 5.5 cm in diameter. The other design features of the chamber, and most of the techniques used were similar to those used in method one. The most significant changes made were that both glass plates were covered with leaves and sprayed. The doses used and data observation periods were same as in method two. The

plastic drain pipes used were transparent and brushed with 'fluon' in order to make it difficult for parasitoids to grasp the wall and rest upon them.

During each observation, the chambers were returned to the cold room and left for period of 10 to 15 minutes. This is because, with the covering of both chamber surfaces, it was difficult to collect data without opening the whole chamber. Keeping the chamber in the cold room for a short period made the parasitoids immobile and reduced the risk of escape during data collection.

4.3 Results

Figures 4.1 and 4.2 show the dose response curve of *A. colemani* on cabbage and dwarf bean leaf surfaces respectively at 24h intervals with continuous exposure to deltamethrin residues, observed in test method one. The final dose-ranges used in the present experiment were multiples of the recommended field rate of deltamethrin (6.25 g AI^{-1}). Both the figures shown inconsistent increases in mortality of *A. colemani* on both leaf surfaces after 24h of treatment with deltamethrin. Experiments with barley leaf surfaces were abandoned due to the problems associated with this method. At 48h, 72h and 96h of treatment, the curves were more sigmoid in trend. It is also evident from these curves that the mortality of *A. colemani* was higher on cabbage leaf surfaces than on dwarf bean.

Figure 4.3 indicates stable end-point toxicities in response to time of exposure. However, the time that the test parasitoid actually spent on the treated substrate was in doubt in this method and it was difficult to reach acceptable conclusions concerning residual toxicity from the above figure. For close comparisons with the two other test method used in this study, only the 24h probit statistics are given in Table 4.1 (for 48h, 72h and 96h data see appendices).

Figures 4.4 and 4.5 show the variation in residual LD_{50} of *A. colemani* on different leaf surfaces, with time after treatment with deltamethrin, for methods 2 and 3

Figure 4.1 Dose response curve of *A. colemani* on Cabbage leaf surface treated with deltamethrin at 24 h intervals of exposure in test method 1.

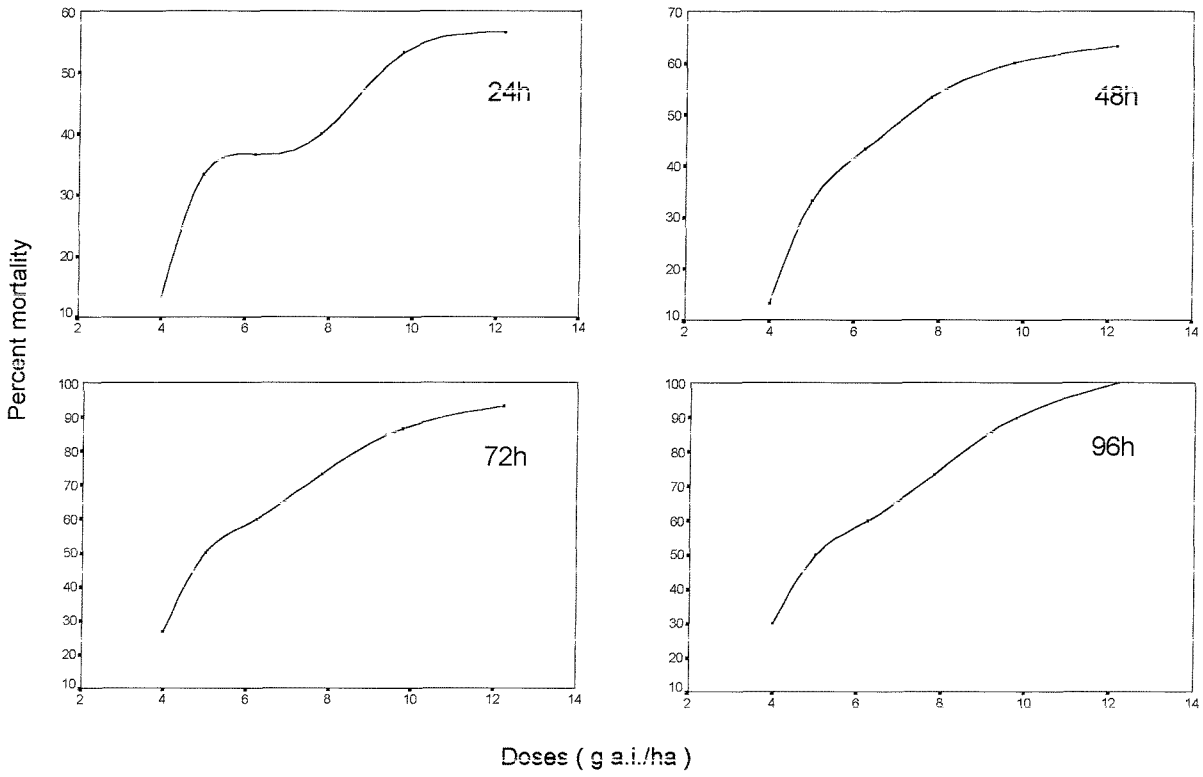


Figure 4.2 Dose response curve of *A. colemani* on Dwarfbean leaf surface treated with deltamethrin at 24 h intervals of exposure in test method 1.

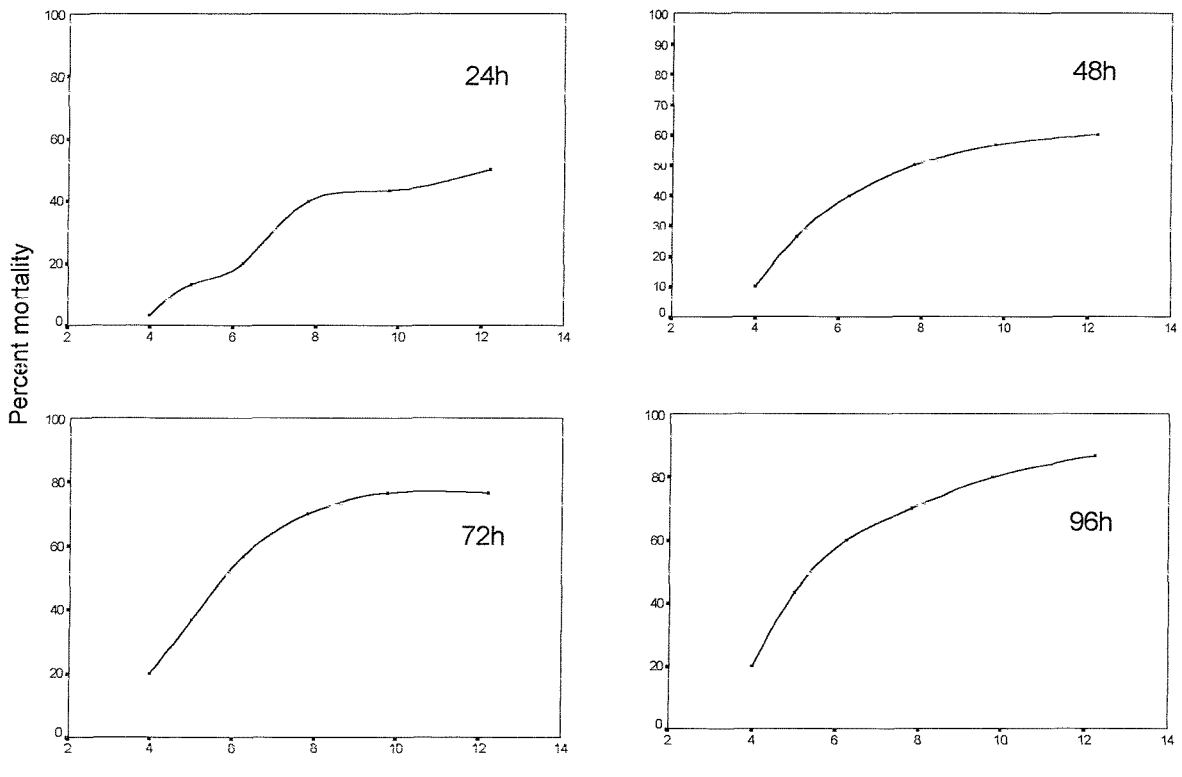


Figure 4.3 Variation of residual LD50 of *A. colemani* with time after treatment with deltamethrin 2.5EC (bar indicates 95% fiducial limit) in test method 1.

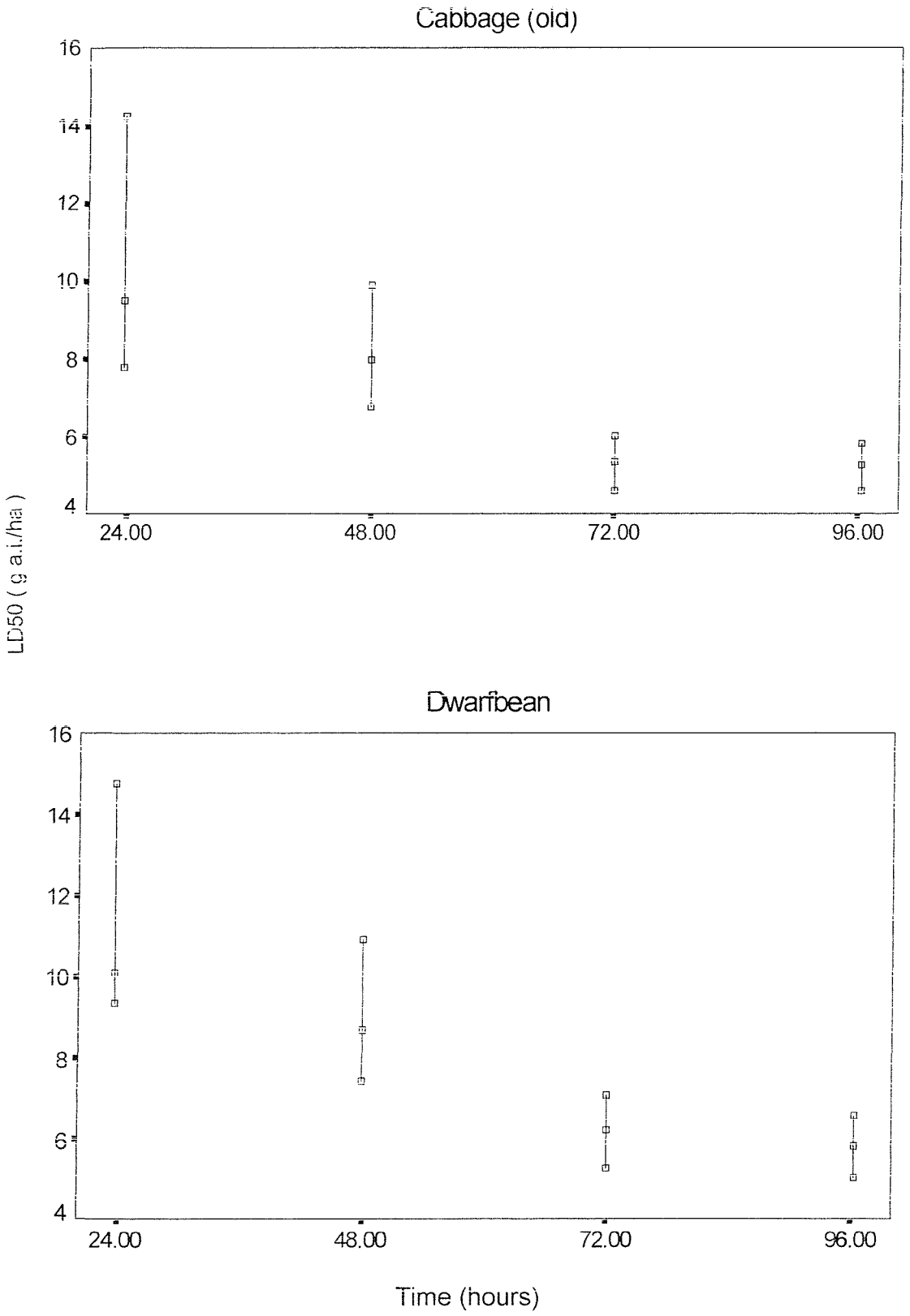


Figure 4.4 Variation of residual LD50 of *A. colemani* with time aftetreatment with deltamethrin 2.5EC (bar indicates 95% fiducial limit) in test method 2.

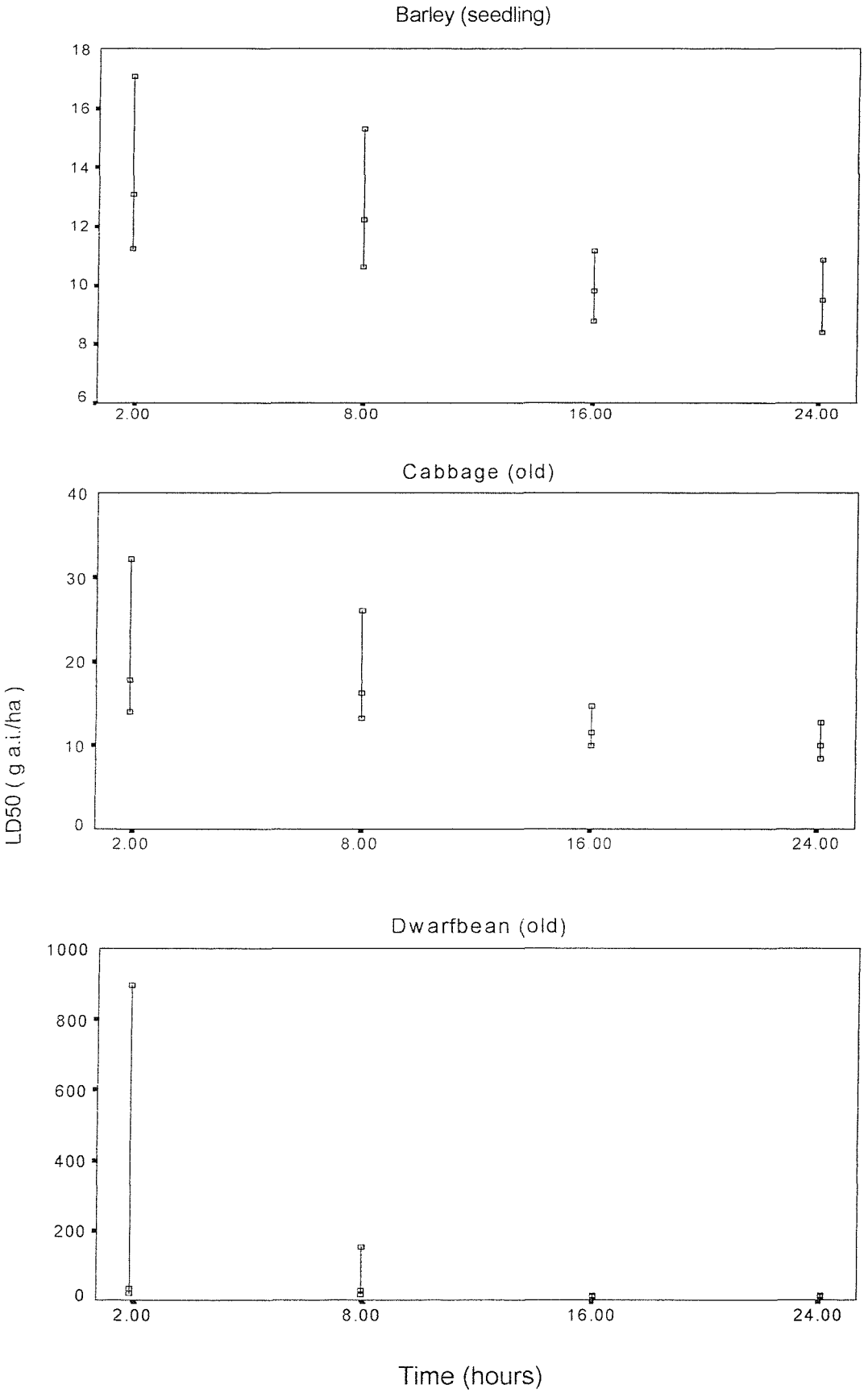
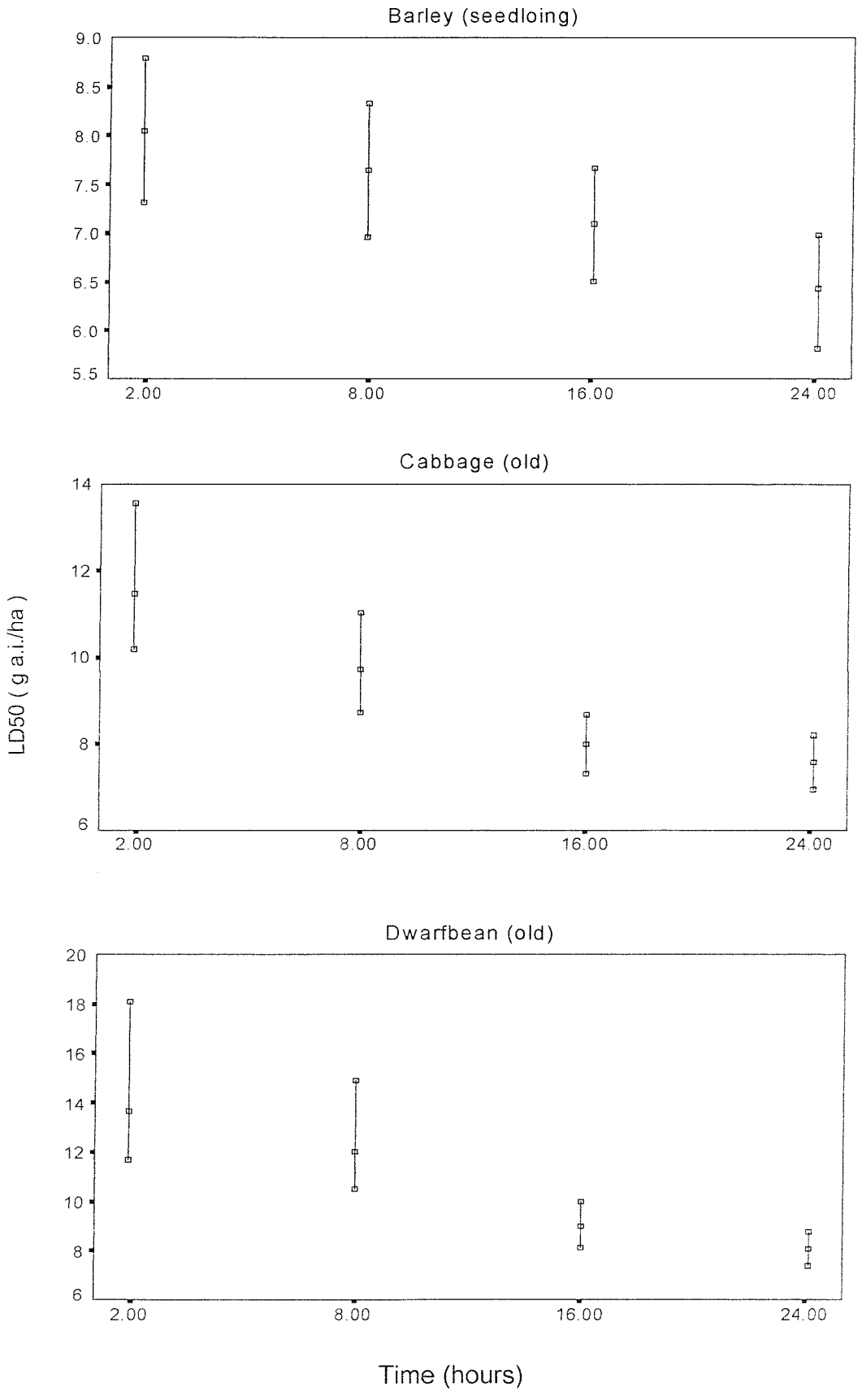


Figure 4.5 Variation of residual LD50 of *A. colemani* with time aftetreatment with deltamethrin 2.5EC (bar indicates 95% fiducial limit) in test method 3.



respectively. Although the curves indicate different rates of poisoning, the plots showed a similar trend of decline in LD_{50} over time on all three leaf surfaces. In most cases, these values reached a stable end-point with an indication of further low level mortality which may have continued for a longer period. However, as a result of changes in leaf morphology, especially drying and other related phenomena that take place in such closed chambers and artificial arenas, further mortality may not simply be an indicator of direct poisoning effects. 24h assessment data were therefore chosen for a comparison of the susceptibilities of *A. colemani* on test leaf surfaces.

Tables 4.1, 4.2 and 4.3 show the probit statistics on the responses to deltamethrin on different leaf surfaces using methods 1, 2 and 3 respectively. In almost all cases, χ^2 statistics indicated non-significant heterogeneity. The probit responses of *A. colemani* to deltamethrin on different leaf surfaces, assayed from the three methods tested, are shown in Figures 4.6, 4.7 and 4.8. Figures 4.12 and 4.13 shows the dose response curve of *A. colemani* on the three test leaf surfaces assayed using methods 2 and 3 respectively.

The ranking sequence of susceptibilities for *A. colemani* on three different leaf surfaces in methods 2 and 3 were as, barley > cabbage > dwarf bean and that in method 1 was cabbage > dwarf bean. The trends were similar to that of *F. candida* (Chapter 3). The LD_{50} values observed on cabbage and dwarf bean in methods 1 and 2 were close for each leaf type. These values found using method 3 were lower than for the two other methods. These results indicate differences in exposure level for *A. colemani* in the three methods.

In method 1, the leaf surface area used in each chamber was larger (7.5cm diameter) than that used in method 2 (5.5cm diameter). The unsprayed inner side-wall of drain-pipe used in each chamber of method 1, was 2.5cm high and 7.5cm in diameter and comprised a larger unsprayed area than in the other methods. This wall was also darker because of the side

Table 4.1. 24-h Probit statistics for the responses to deltamethrin 2.5 EC *of A. colemani* on three different leaf types (test method 1)

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) detransformed (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Cabbage (*Brassica oleracea*) v. Prixie	2.26 (0.60)	9.51 (7.80-14.27)	1.86 (4) ns
Dwarfbean *(Phaseolus vulgaris*) v. Sutton	3.38 (0.69)	10.10 (9.37-14.76)	2.27 (4) ns

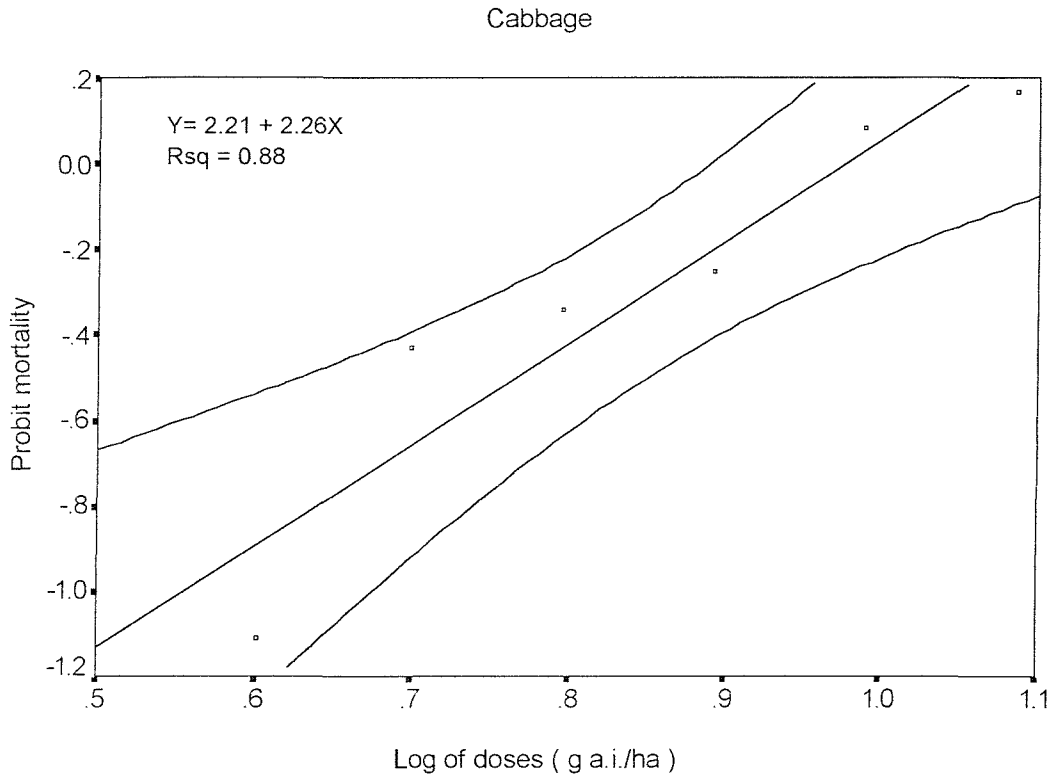
Table 4.2 24-h Probit statistics for the responses to Deltamethrin 2.5 EC of *A. colemani* on three different leaf types (test method 2)

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) detransformed (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	3.76 (0.64)	9.48 (8.39-10.87)	0.378 (4) ns
Cabbage (*Brassica oleracea*) v. Prixie	2.52 (0.60)	10.04 (8.43-12.81)	0.379 (4) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	2.91 (0.63)	12.13 (10.32-16.09)	0.951 (4) ns

Table 4.3 24-h Probit statistics for the responses to Deltamethrin 2.5 EC of *A. colemani* on three different leaf types(test method 3)

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) detransformed (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	6.71 (0.97)	6.43 (5.81-6.98)	1.052 (4) ns
Cabbage (*Brassica oleracea*) v. Prixie	6.62 (0.85)	7.58 (6.96 - 8.20)	3.493 (4) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	5.90 (0.77)	8.07 (7.38-8.79)	1.247 (4) ns

Figure 4.6. Transformed probit responses of *A. colemani* on two different leaf surfaces after 24h of treatment with deltamethrin in test method 1.



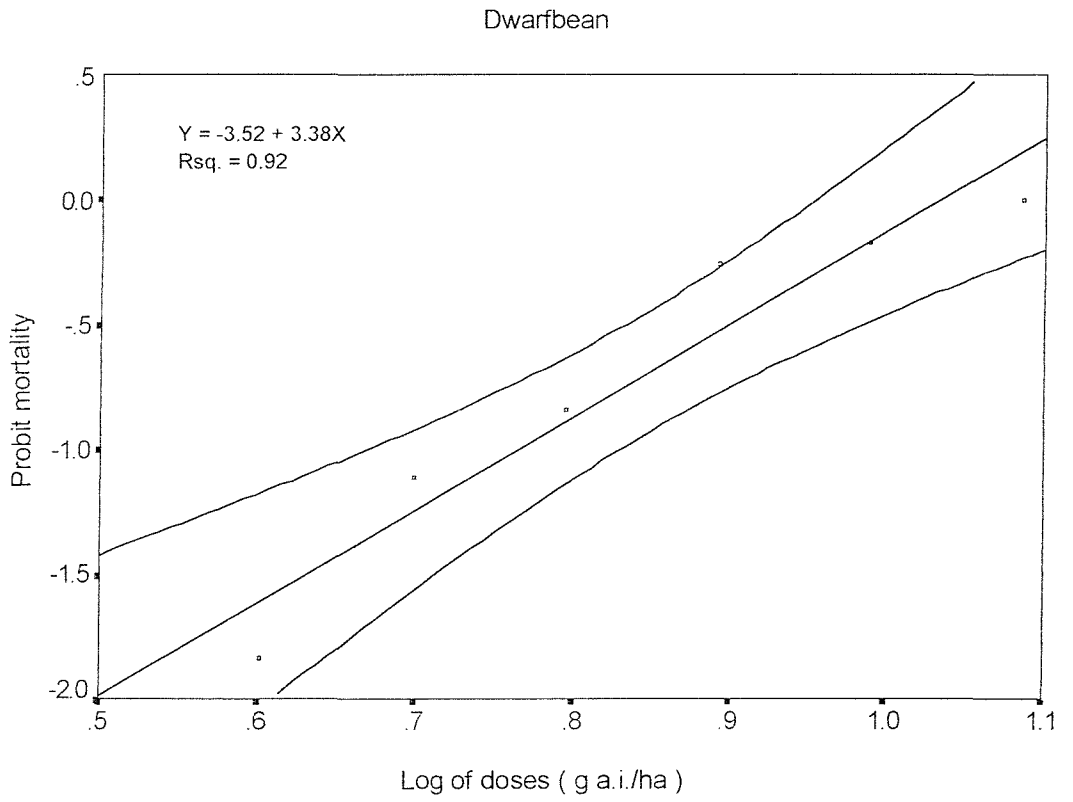
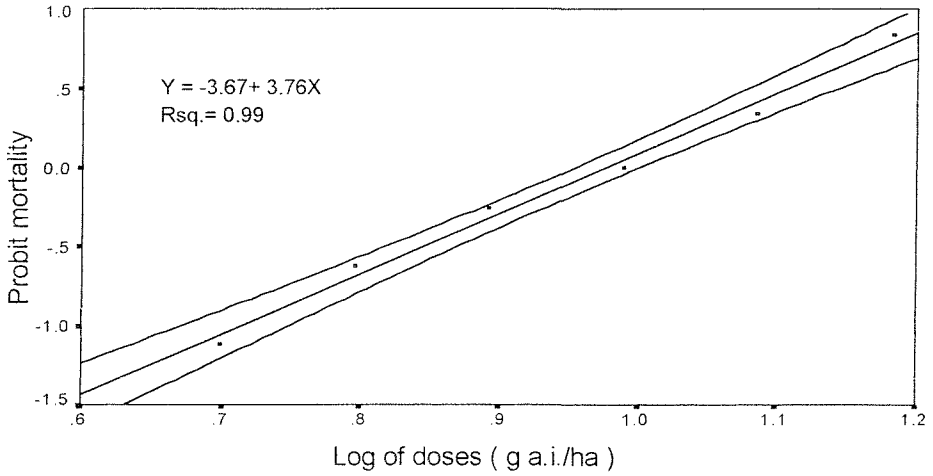
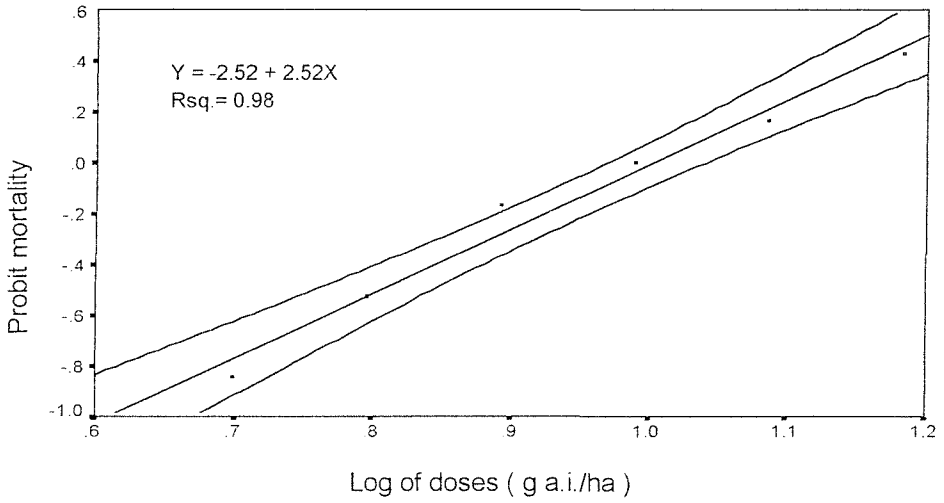


Figure 4.7. Transformed probit responses of *A. colemani* on two different leaf surfaces after 24h of treatment with deltamethrin in test method 2.

Barley



Cabbage



Dwarfbean

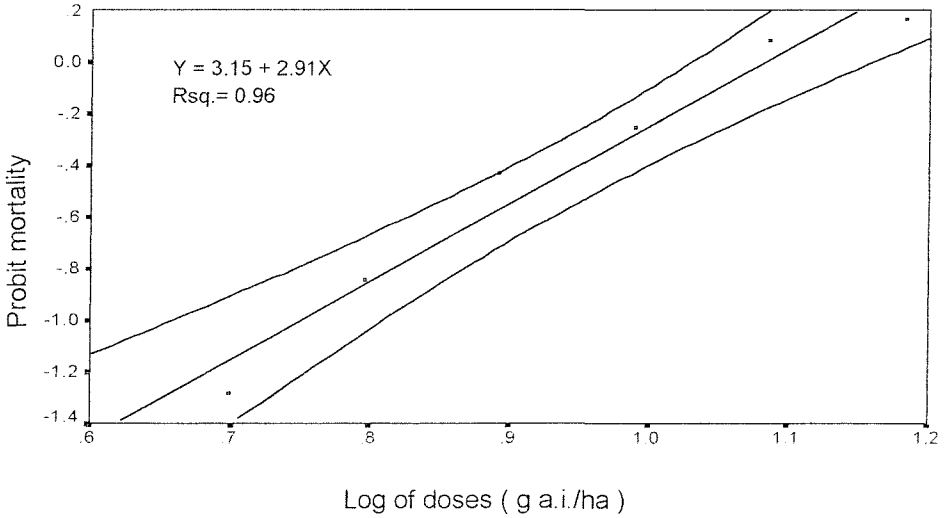
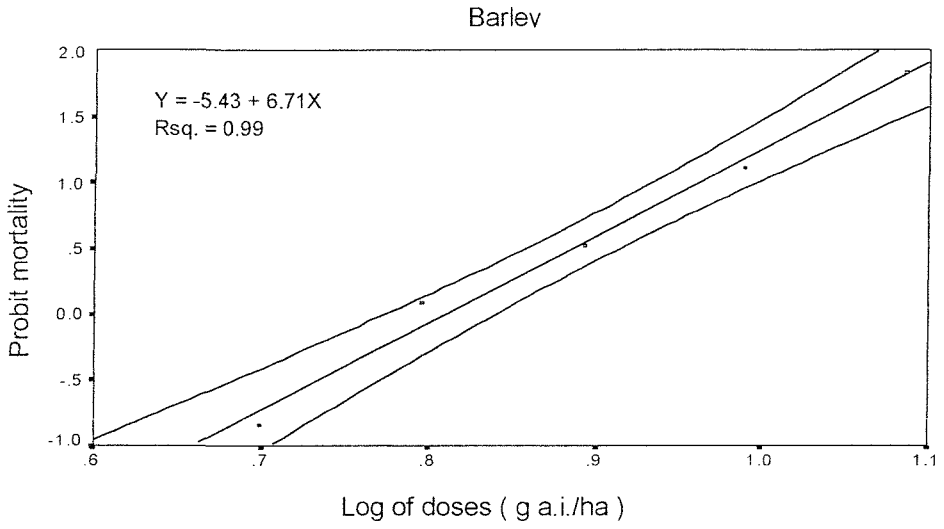
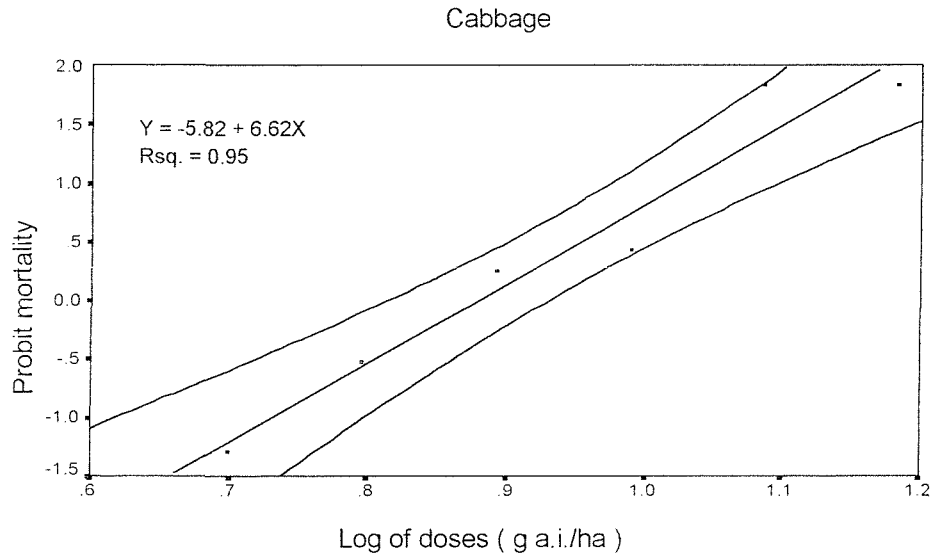


Figure 4.8. Transformed probit responses of *A. colemani* on two different leaf surfaces after 24h of treatment with deltamethrin in test method 3.





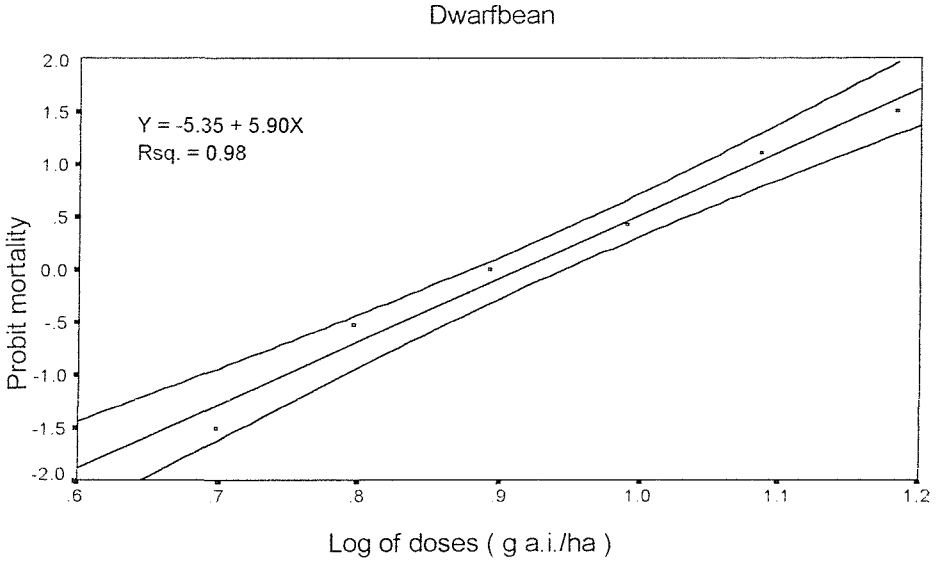
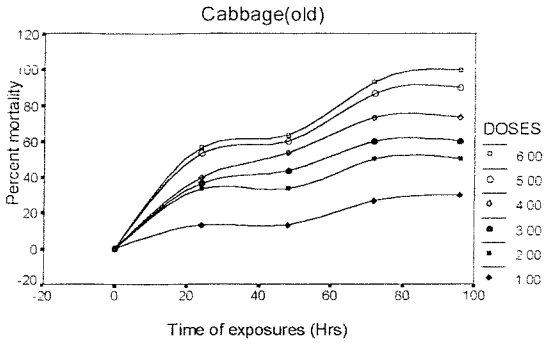


Figure 4.9 Individual dose responses of *A. colemani* to deltamethrin 2.5EC on different leaf types (test method 1)



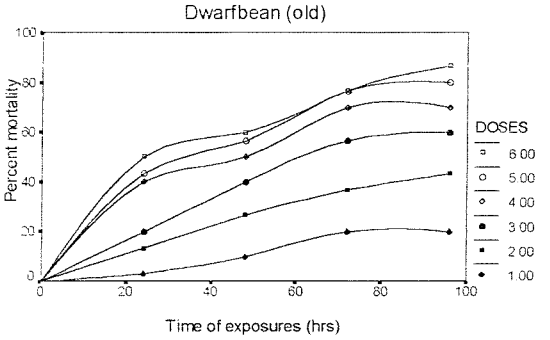
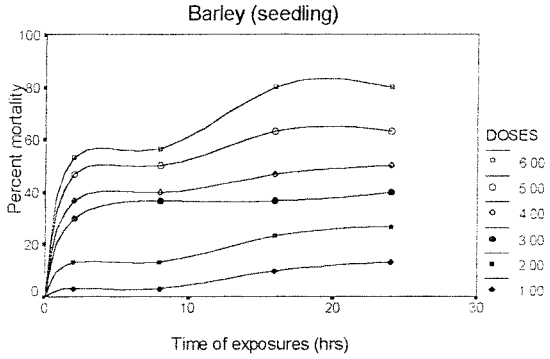
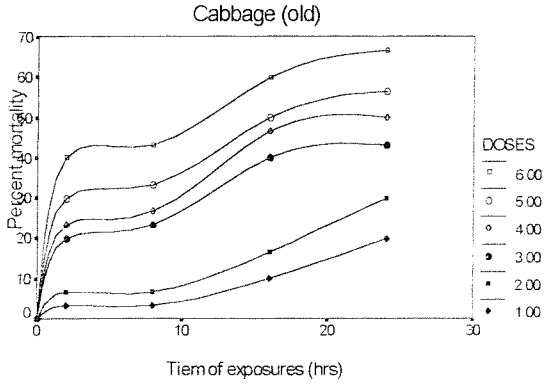


Figure 4.10 Individual dose responses of *A. colemani* to deltamethrin 2.5EC on different leaf types (test method 2)





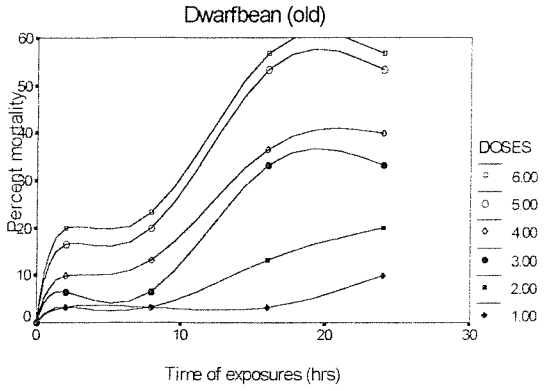
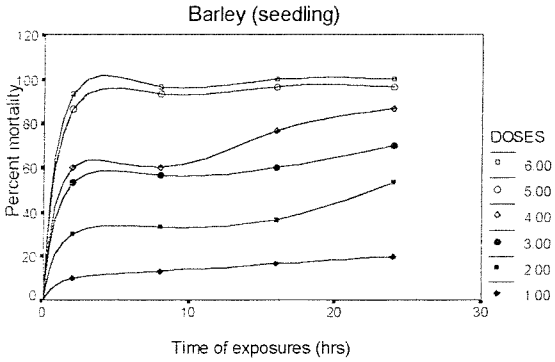
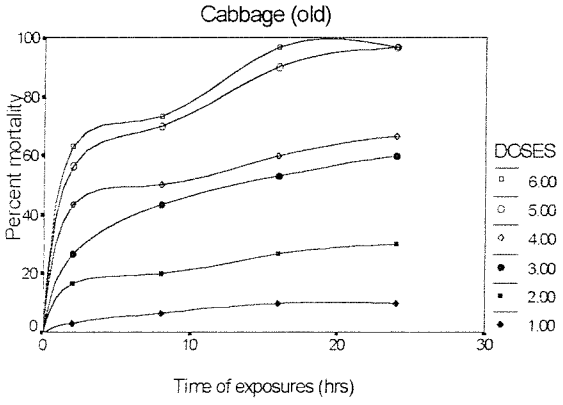


Figure 4.11 Individual dose responses of *A. colemani* to deltamethrin 2.5EC on different leaf types (test method 3)





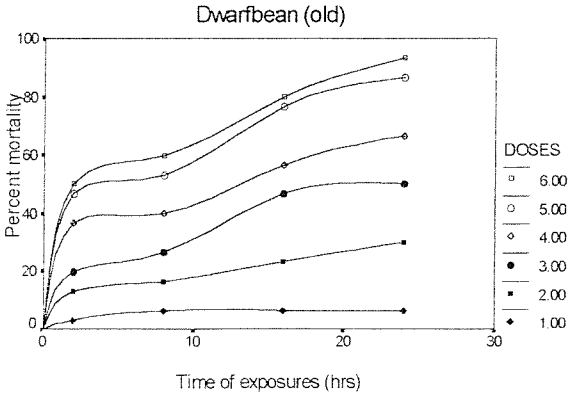


Figure 4.12 Dose response curve of *A. colemani* on three different leaf surfaces after 24hrs of treatment with deltamethrin (test method 2)

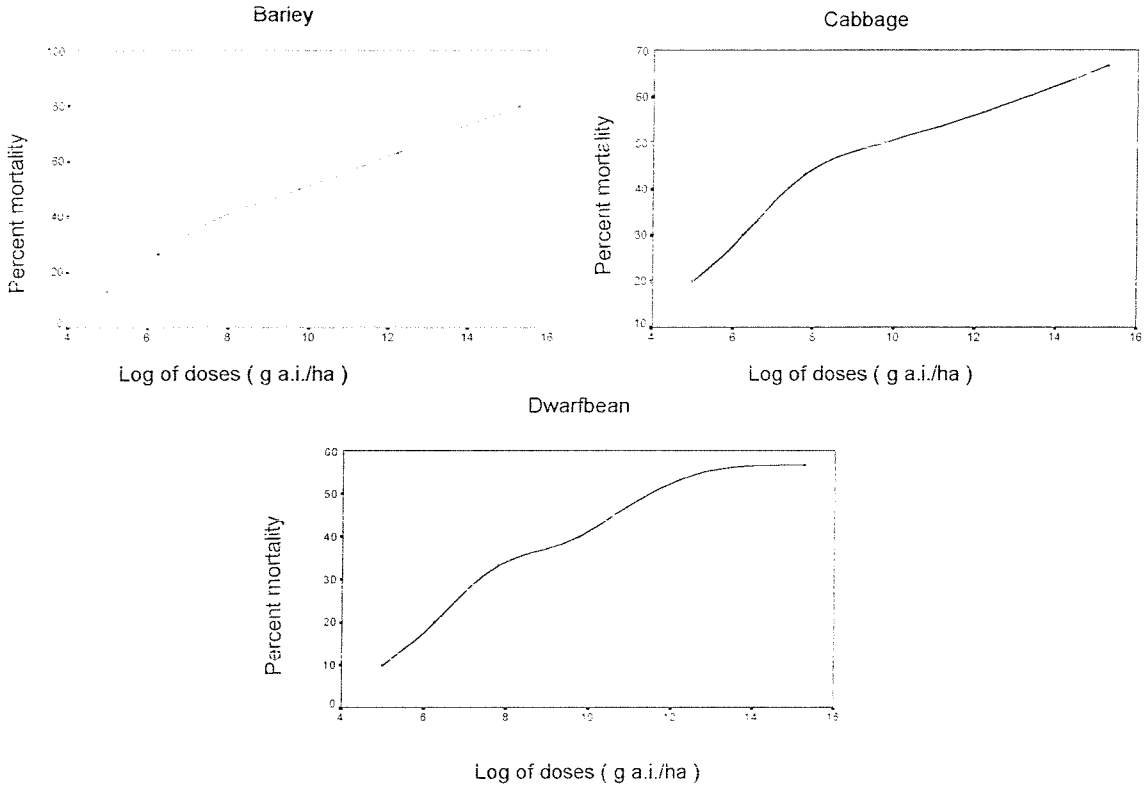
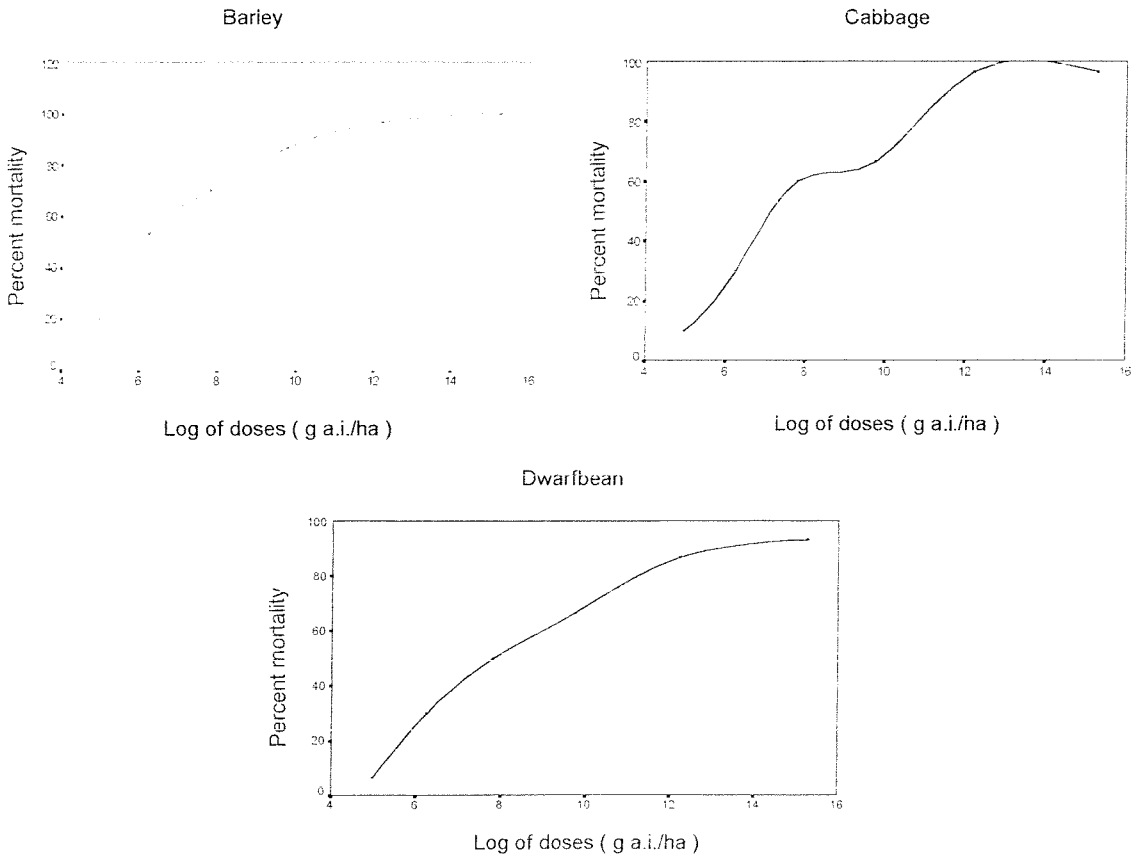


Figure 4.13 Dose response curve of *A. colemani* on three different leaf surfaces after 24hrs of treatment with deltamethrin (test method 3)



covering, which was designed to restrict parasitoid movement in this area. In method 2, the side wall of the Petri dish was 3.0cm high and 5.5cm in diameter and transparent, which encouraged the parasitoid to be more active on this unsprayed area. It is possible that in method 1, the encounter rate of the test parasitoids with treated surfaces was higher than in method 2, which led to higher apparent susceptibilities in method 1. The high susceptibility of *A. colemani* on the three leaf surfaces in method 3 was probably a result of higher encounter rates, because both the glass surfaces were covered with leaves and sprayed. The test parasitoid in this method had little option of avoiding contact with residues.

Figures 4.9, 4.10 and 4.11 show the trends in poisoning for *A. colemani* by deltamethrin on the different leaf surfaces for methods 1, 2 and 3 respectively. The shallow incremental trends in percentage mortality in most of the cases shows that some residual toxicity remained throughout the exposure period. For methods 2 and 3, the mortality rates of *A. colemani* on barley leaves showed stable responses at mid and upper doses. In method 1, the percentage mortality at the highest dose rates reached above 80% on barley leaves, whereas on cabbage and dwarf bean leaves, the percentage mortality of *A. colemani* remained below 70% and 60% at the highest dose rate. In method three, 100% mortality was observed at the highest doses on barley and cabbage leaves and became stable, whereas on dwarf bean leaves, the mortality at the highest doses reached above 90% at the end of exposure period, and the curve indicated further increases in mortality. In method 2, on dwarf bean leaves, the curve at the highest dose rate approached a stable mortality (below 60%) and no indication of further mortality was evident. It may be possible that in method 2, after preliminary encounter to the treated surface, parasitoids tended to remain upon unsprayed areas.

4.4 Discussion

4.4.1 Evaluation of the three test methods:

It is very important to develop robust bioassay methodologies for laboratory-based toxicological studies. The data can only

be considered reliable if the method used is sound and appropriate. The higher the resolution of test methodology, the more the analysis will represent the true conditions of the experiment. The advantages and disadvantages of the three methods used in the present study are outlined below.

4.4.1.1 Test method 1:

Advantages : i) Mortality data can be taken at different intervals in the exposure period by looking through the uncovered glass surface of the bioassay chamber, without having to open it. This minimizes the risks of escape of live test individuals during data taking and avoids disturbance. ii) Sufficient light can pass through the transparent glass plate into the chamber and make the parasitoid active.

Disadvantages : i) Covering a surface of 12cm X 12cm with small leaves, such as those of barley seedlings, is difficult. Small leaves, of irregular shape, such as dwarf bean, also present problems during attachment on the surface. It is not possible to cover the whole surface with one leaf. Several leaves are therefore, required and these have to be joined together to cover the surface. Such joining is difficult, especially when it aims to be edge to edge. Parasitoids can be trapped or seek refuge under the top edges of two adjacent leaves. Leaf surfaces, such as the older leaves of sugar cane and maize, have a considerable shrinkage tendency near the edges of two adjacent leaves because the leaves begin to desiccate after a few hours. This exposes the unsprayed adhesive tape beneath the leaf surfaces and causes a number of test individuals to become trapped on the sticky surface.

ii) For longer exposure periods, calculations of the actual time of contact with the treated surface are unreliable. For example, a 24h residual exposure does not ensure that the test individual spent a high proportion of this exposure time on the treated surface. The natural behaviour pattern of *A. colemani* is to spend a considerable proportion of time on the untreated upper glass surface, with occasional encounters with the treated leaf surface. The actual duration of contact with treated surface therefore remains unknown.

4.4.1.2 Test method 2

Advantages : i) This method solves, to a certain extent, the problem of leaf attachment on the lower part of the Petri dish because of its smaller arena area. For the relatively broad leaves of cabbage, or even dwarf bean, the surface can covered by one leaf. This is very helpful because there is no chance of exposing the adhesive tape from beneath of leaf surface as a result of shrinkage. Parallel sticking of smaller barley leaves, tip to base, is also much easier.
ii) Mortality data can taken easily by looking through transparent top cover and the side walls of the Petri dish.

Disadvantages : i) A wide area of the chamber remains unsprayed, therefore restricting the test parasitoid from maximum contact with the treated surface. The actual contact with the treated surface is also difficult to predict.

4.4.1.3 Test method 3

Advantages : i) This method solves the problem of leaf attachment on the glass surface again by using a smaller arena. The major difficulties of ensuring maximum exposure on treated surfaces, found with the two other methods, can also be minimized considerably. Here both glass surfaces are covered with leaves and sprayed. A very small area remains unsprayed (the side wall of plastic drain-pipe) This restricts the choice of the test individual which is more likely to be exposed to treated surfaces. Although the side walls of the drain-pipe are unsprayed, the area is much reduced and, according to the nature of parasitoid activity, it is less likely that the test individual will spend much time resting on vertical wall of the drain-pipe. The pipe was also brushed with 'fluon' in order to present a smoother surface that prevented the parasitoid from grasping the wall. Although forceful confinement of test parasitoids to treated surfaces is unrealistic relative to encounters with surfaces in the field, this form of experimental design will at least present more dependable data for laboratory analysis. Even in field conditions, when a large area is sprayed with pesticide, although the parasitoid population of that field may not remain on single treated leaves continuously, most of

the population will be unable to escape from the treated area, and they are likely to come into contact regularly and continuously with other treated plant or soil surfaces.
ii) Taking data is easy and reliable, because close observation is possible by opening the whole chamber. It is possible for the observer to distinguish between apparently knocked down and dead parasitoids. Any adults that hide under the leaf edges can also be monitored.

Disadvantages : i) Lack of light makes the chamber more artificial. However, there is compensation from using a transparent drain-pipe and keeping the chamber close to light source, which allows at least some defused light to enter the system.
ii) Keeping the chambers in a cold room prior to data collection might affect live parasitoids. However, neither any disruption in the activity of live adults nor any mortality in control treatments was observed after the chambers were brought to normal temperatures in the insectary.
From the three methods tested, method 3 seems to be the most appropriate for studies of the effects of leaf surface factors on the mediation of pesticide toxicity. In this study the major focus of investigation is the substrate and most consideration should be given to encounters with treated surfaces with low control mortality.

4.4.2 Susceptibility trends

Differences in the LD_{50} values for *A. colemani* on barley, cabbage and dwarf bean leaf surfaces were evident in all the methods used. Considering method 3 as the most appropriate among the three method tested, *A. colemani* was most apparently susceptible on the barley leaf surface, followed by cabbage and dwarf bean. The sequence of susceptibility on these three leaf surfaces was similar to that of *F. candida*. The LD_{50} values for *F. candida* after 24h of exposure on barley, cabbage and dwarf bean were 13.82, 21.30 and 186.31(g AI ha^{-1}) and were wider than those for *A. colemani*. However, the span of LD_{50} values for *F. candida* after 72h, at the end point of toxicity, was much closer to that for *A. colemani* on

barley and cabbage leaf surfaces. It is not possible to present concrete conclusions concerning extrapolation of the results of *F. candida* to those for *A. colemani*. The primary indication shows some similarities in the trends of toxicity responses. For this purpose, however, further experiments on a wide taxonomic range of parasitoids and predators should be conducted, on a range of plant species.

CHAPTER 5

Scanning electron microscopy: a study of leaf micro and macro structures

5.1 Introduction

Detailed studies of the composition and structure of the external surfaces of plant are vital to an interpretation of the responses of topically applied substances. The advent of the scanning electron microscope (SEM) offered opportunities of directly viewing leaf surface structures such as wax particles, cell structure and arrangement, stomata, trichomes etc. Prior to the development of SEM, replica techniques were used to view the leaf surfaces and certain leaf characters were damaged by temperature and the electron beam of the transmission electron microscope (TEM) (Juniper & Bradley 1958; William & Juniper 1968). The resolution of such micrographs was limited. The use of SEM in current biological studies is of great advantage in many respects. Further advances in the design of SEMs have greatly improved their resolving power, and the development of the low temperature scanning electron microscope (LTSEM) enhances routine examination of leaf surface features. This yields in a single operation the maximum information on leaf surface roughness and is therefore highly recommended for such studies (Holloway, 1970). Their immense capacity to image complex surface topographies with great depth of field make SEMs extremely valuable for many biological observations.

With the adoption of cathodoluminescence techniques, the SEM can also be used to identify spray deposits on leaf surfaces (Hart, 1979). This a valuable facility for toxicological studies. Workers have used the SEM to study the leaf surface morphology of plants from different taxonomic groups with different aims in mind. For example, the SEM has been used to investigate the leaf wettability and wax morphology of wheat (Netting, 1975); wax ultrastructure and recrystallization of barley, brussels sprouts and other plant species (Jeffree *et al.* 1975); origin of plant epicuticular waxes (Jeffree *et al.* 1978); chemical and physical characteristics of leaf surfaces

(Holloway 1971); leaf wettability (Holloway, 1970); the water proofing function of plant and animal (Hadley 1982); surface roughness and spreading of oil spray drops (Boize *et al.* 1976) and plant cuticle and spray droplet interactions (Baker *et al.* 1983).

In the complex interactions that are initiated after the impaction of pesticidal spray drops on target leaf surface (Bukovac, 1976), leaf characteristics such as surface roughness, leaf geometry, surface chemistry and the micro climate can all interact with the drop during the drying process. Some of these characteristics can be closely observed by SEM with a large depth of field (up to 1mm), coupled with high resolution (less than 10nm) with some species.

Although Heslop-Harrison (1970) managed to view leaf specimens of *Pinguicula grandifora* directly under the traditional ambient temperature SEM, it has been commonly found that the delicate structure of many biological specimens, especially leaf surfaces, suffer distortion due to exposure to the electron beam and subsequent temperature rises. It is possible to observe uncoated specimens for a short period at low pressure (10^{-5} torr) and (Heslop-Harrison 1970) found no evidence of serious charging effects with an accelerating voltage of 5Kv. With the development of the LTSEM, it can now possible to observe plant and fungal morphology in the hydrated state (Read *et al.* 1990).

Observations under traditional ambient temperature SEM still require specimen dehydration and coating. Techniques such as freeze-drying and critical-point drying were developed to dry the specimen (Parson, *et al.* 1973). Later on cryopreservation was introduced (Sargent, 1982) to overcome shrinkage and solubilization of certain leaf structures during freeze-drying and critical-point drying and fixation of specimens prior to critical-point drying. Coating of the dried specimen with metal prevents charging and makes it possible to obtain high resolution images of the epicuticular wax and other

structures. Coated materials can be preserved in a desiccator for long periods and used later.

This chapter concerns the wax structures of leaves and their arrangement, cell shape and boundaries, and trichomes to facilitate the further interpretation of the pesticide-substrate-invertebrate interaction. Leaf species used were the same as described in chapter 3.

A possible close agreement of this study with the studies on apparent susceptibilities of *F. candida* on different leaf surfaces, wax content and wettability will help better understanding of interactions between leaf surface and pesticide deposits and toxicity.

5.2 Materials and methods

5.2.1 Specimen Preparation

5.2.1.1 Specimen drying: Specimens which were fresh, unsprayed, and physically healthy without any visible injury were collected from greenhouses, orchards and the field. Immediately after picking from the live plant they were preserved in small vials containing absolute ethanol. Most of the specimens were dried in a critical-point dryer (CPD) (BALZERS, Balzers Union Aktiengesellschaft, Liechtenstein). Biological specimens are normally washed in a physiological salt solution and chemically fixed with a suitable medium. The fixing agent was then washed out with a suitable buffer and the specimen dehydrated with acetone or ethanol. In the present experiment no chemical fixation was carried out. Specimens were dehydrated directly with absolute ethanol or acetone. Chemical fixation was avoided for the reason that it may cause damage to fine delicate wax structures. The dehydrated specimen was transferred from absolute ethanol into a specimen holder immersed in the same solvent in a Petri dish. This was done to make sure that the specimen did not dry up at any stage of final transfer to the specimen pressure chamber of CPD, which was filled with liquid CO_2. The specimen holder with the specimen was then inserted into

the specimen pressure chamber. The specimen pressure chamber was precooled to the preselected temperature (10°C at a pressure of 50 bar or 20°C at a pressure of 40 bar). The shut-off valve of the pressurized gas container was opened and the transition liquid was allowed to fill the specimen chamber until the level of liquid rose to the upper edge of the front sight glass. The inlet valve was then closed. The media mixture was drained out until the specimen was still covered by the liquid and the outlet valve was closed. This process of introducing the transition liquid into the specimen chamber and draining out the media mixture was repeated several times to ensure that the media mixture brought into the pressure chamber during the transfer of the specimen was completely exchanged for the transition liquid (normally six to eight times).

After the last media exchange, the specimen chamber with the transition liquid was filled to a level just below the upper edge of the front sight glass and the inlet valve was closed. The specimen pressure chamber was then heated to raise the critical temperature and the critical pressure of transition liquid (for CO_2 is 31° and 73.8 bar). The liquid was then volatilized and the specimen dried. In practice, before releasing the gas from the specimen chamber it was heated to several degrees above the critical temperature (for CO_2 40°C) and the pressure raised to 80-85 bar. After releasing gas from the specimen pressure chamber by carefully opening the manually operated gas-dosing valve, the heating was stopped. After a reduction of the pressure in the specimen pressure chamber, the dried specimen was removed with the specimen holder.

A few specimens were prepared by the freeze-drying. Freeze-drying was carried out by quenching the fresh leaf samples (already attached to the specimen stubs) in liquid nitrogen for several hours. The sample was then placed on the specimen stage of an Edward-Pearce dryer which had been precooled to -60° C. After a few hours the specimen was removed and stored in a desiccator.

5.2.1.2 Mounting and Coating: All the specimens were mounted, either before or after drying, on 0.5" aluminium pin stubs (Agar, Agar Scientific Ltd. Essex, UK.) by means of double-sided adhesive tape. The stubs with the specimen were placed in the specimen chamber of a sputter coater (EMscope, London, UK.) and closed. The argon pressure was set to 2 lb per square inch and the time needle was set at 5. A glow discharge is then initiated between an anode and cathode plate in an argon atmosphere at a relatively high pressure of 200 millitorr. The gas ions accelerate towards the metal target (cathode) and the impact causes metallic ions to be released from the target. Due to the high gas pressure and large number of collisions, the metal atoms reach the specimen moving in all directions. This cloud of metal ion is then condensed onto the surrounding cold surface, including the specimens were evenly coated. As soon as a blue glow was visible in the chamber, the lower meter of the sputter coater was set to 25mA, if necessary, using the argon needle valve. At the end of the process, the chamber was vented and the stub with the specimen was removed.

5.2.2 Examination under the Scanning Electron Microscope (SEM): The specimen chamber of the SEM was vented by pressing the air button. After 30 seconds the specimen chamber was pulled out and the specimen stub inserted into the specimen stage and the grubscrew tightened gently. The specimen stage was then returned into the specimen chamber, the door was closed firmly and the chamber was evacuated by pressing the EVAC button for a few minutes until the high vacuum light came back on.

The specimen was then observed under a Hitachi-S-450 SEM. The view brightness control was kept at mid-position and set the condense-lens to 3. Accelerating voltage was increased stepwise and set at 10 KV. Several authors have suggested the use of 2.3 Kv for specimens which were not fixed for 1-2h in osmium tetroxide vapour (Parson, *et al.* 1974). However, using a 2-3 Kv accelerating voltage, a high resolution images was not possible to achieve and only magnifications up to 2000

were practicable (Parson *et al.* 1974). Images were focused by saturating the filament carefully and releasing the focus monitor button. Images were observed at various magnifications and depths. The emission current was set at a minimum of 20 and a maximum of 50.

5.3 Results

Figures 1-3 (plate 5.1) show the adaxial surfaces of barley seedling leaves at different magnifications showing characteristic plate-like epicuticular waxes. Figures 4-7 (Plate 5.1, 5.2) show the adaxial surfaces of Cabbage leaves. In Figure 4 (Plate 5.1) a network of wax deposits is conspicuous at 3.3k magnification. At lower magnification, wax ornamentation on the guard cells and accessory cells is often less conspicuous (Figures 5 and 6, plate 5.1). In Figure 7 (Plate 5.2) a characteristic wax bloom is shown.

The adaxial surfaces of tomato leaves are shown in Figures 8-11 (Plate 5.2). Tiny granular wax particles are present on the surface. The stomatal guard cell has smaller amounts of waxes then the basal cells (Figure 9, plate 5.2). Leaf stomata are apertures generally formed by two guard cells which are modified epidermal cells. Figure 11 (Plate 5.2) shows the waxes at a magnification of 12k. This permits comparative analysis of the thickness of wax granules. For example, on the leaf surface of cabbage a wax bloom can be observed at 3.3k magnification, whereas on the surface of a tomato leaf, the relative sizes of wax granules are still less, even after viewing at a magnification of 12k. Figure 8 (Plate 5.2) shows cell grooving with interlocking edges and a raised middle. This can affect the wetting by liquids after impaction, which may accumulate more in groves allowing the raised centres to dry out rapidly.

Figures 12-14 (Plates 5.2 and 5.3) show the adaxial surfaces of sugar cane leaves. The guard cells and the accessory cells seem to be almost wax-free (Figures 15 and 16, plate 5.3). At high magnifications, stomatal openings are seen with heavy wax deposits (Figures 17 and 18, plate 5.3). The waxes are

more or less needle-shaped with pointed tips. Trichomes are present at a low density (Figures 15 and 16, plate 5.3). The adaxial surfaces of orange leaves are shown in Figures 19-22 (Plate 5.4.). The stomatal openings are round in shape and devoid of prominent wax deposition. Tiny wax particles are found as a thin layer at some points (Figures 21 and 22, plate 5.4), however, the wax structures on cell surfaces are less frequent when viewed at a magnification of 1.2K (Figures 20, plate 5.4).

Figures 23-26 (Plates 5.4 and 5.5) show the adaxial surfaces of dwarf bean leaves at various magnifications and resolutions. The arrangement of the basal cells shows a close similarity with that of tomato leaves. Although scattered tiny waxe particles are present on the cell surfaces, no dense clusters of wax particles are observed. Figures 25 and 26 (Plate 5.5) show two stomatal openings, one with no wax deposits at all and another with a few wax deposits. The adaxial surfaces of old and young leaves of rape (varieties Tanto and Lirawell) are shown in Figures 27-30 (Plate 5.5). The surfaces of the young leaves have denser wax deposits than old leaves. Here again it can be noted that the magnifications at which wax particles are visible, are much lower than that for dwarf bean and orange leaf surfaces, which illustrates the thickness and abundance of waxes on rape leaves.

Figures 31 and 32 (Plate 5.6) show the adaxial surfaces of old and young leaves of rape (v. Tanto). The crystalline waxes are very dense on and around the stomata. It is interesting to note that wax crystals are condensed on the stomatal opening and have developed into a bloom of trichome-like structures. The basal cells have deep grooving along the edges which will mean that these areas may wet readily. At higher magnification, globular wax deposits are observed in addition to needle shape crystalline structures, (Figures 33-36, plate 5.6). Figures 33 and 34 (Plate 5.6), represent the adaxial surfaces of old and young leaves of rape (v. Lirawell) respectively. Young leaves show more wax deposition

Explanation of plates

Plate 5.1.

Fig.1. Adaxial surface of barley leaf, 3.3k.

Fig.2. Adaxial surface of barley leaf, 3.3k.

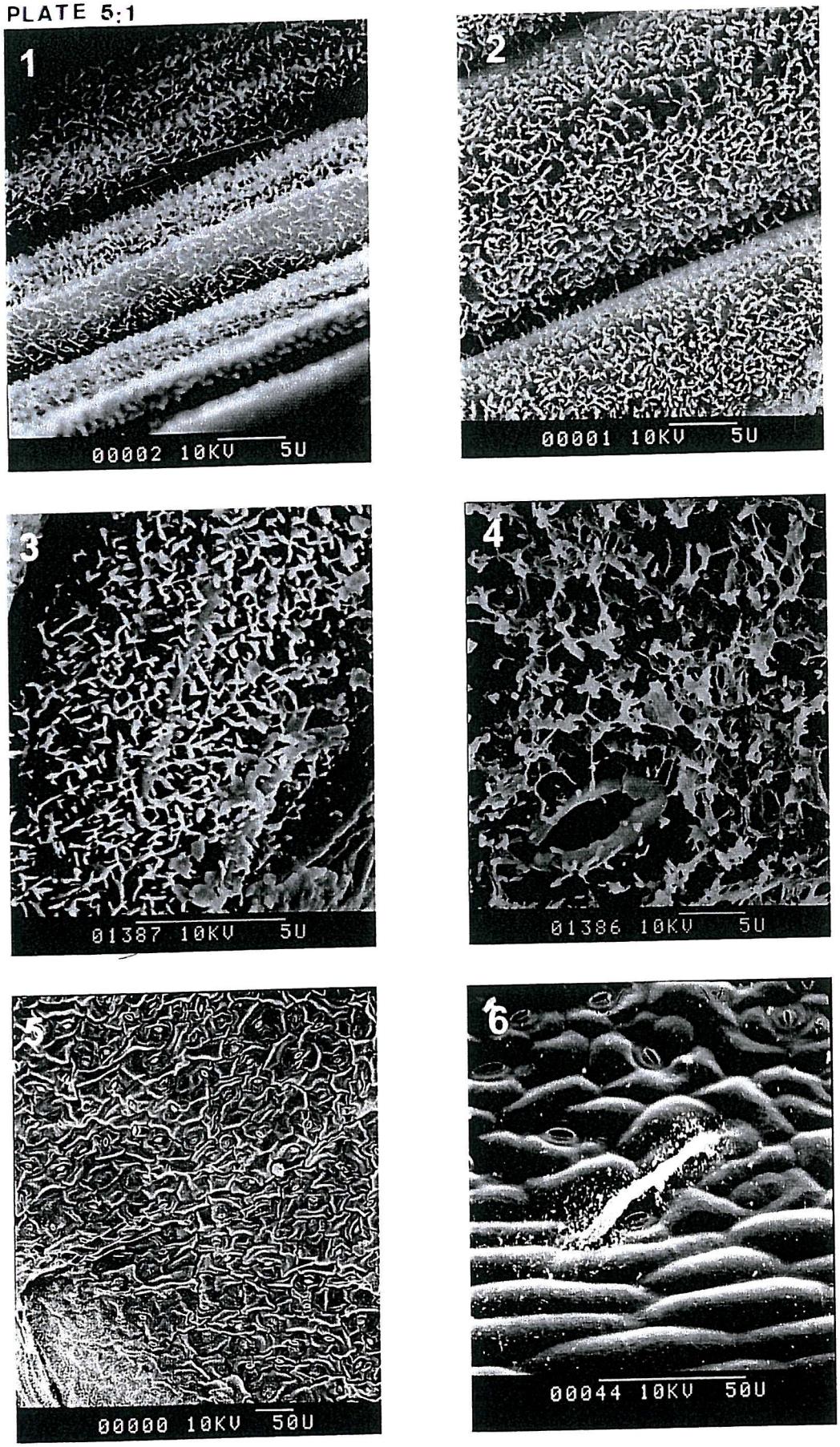
Fig.3. Adaxial surface of barley leaf, 7.4k.

Fig.4. Adaxial surface of cabbage leaf, 3.3k.

Fig.5. Adaxial surface of cabbage leaf, 180x.

Fig.6. Adaxial surface of cabbage leaf, 720x.

PLATE 5:1



Figures 1 -6 Scanning electron microscopy of different leaf surfaces

Plate 5.2.

Fig.7. Adaxial surface of cabbage leaf, 3.3k.

Fig.8. Adaxial surface of tomato leaf, 1.4k.

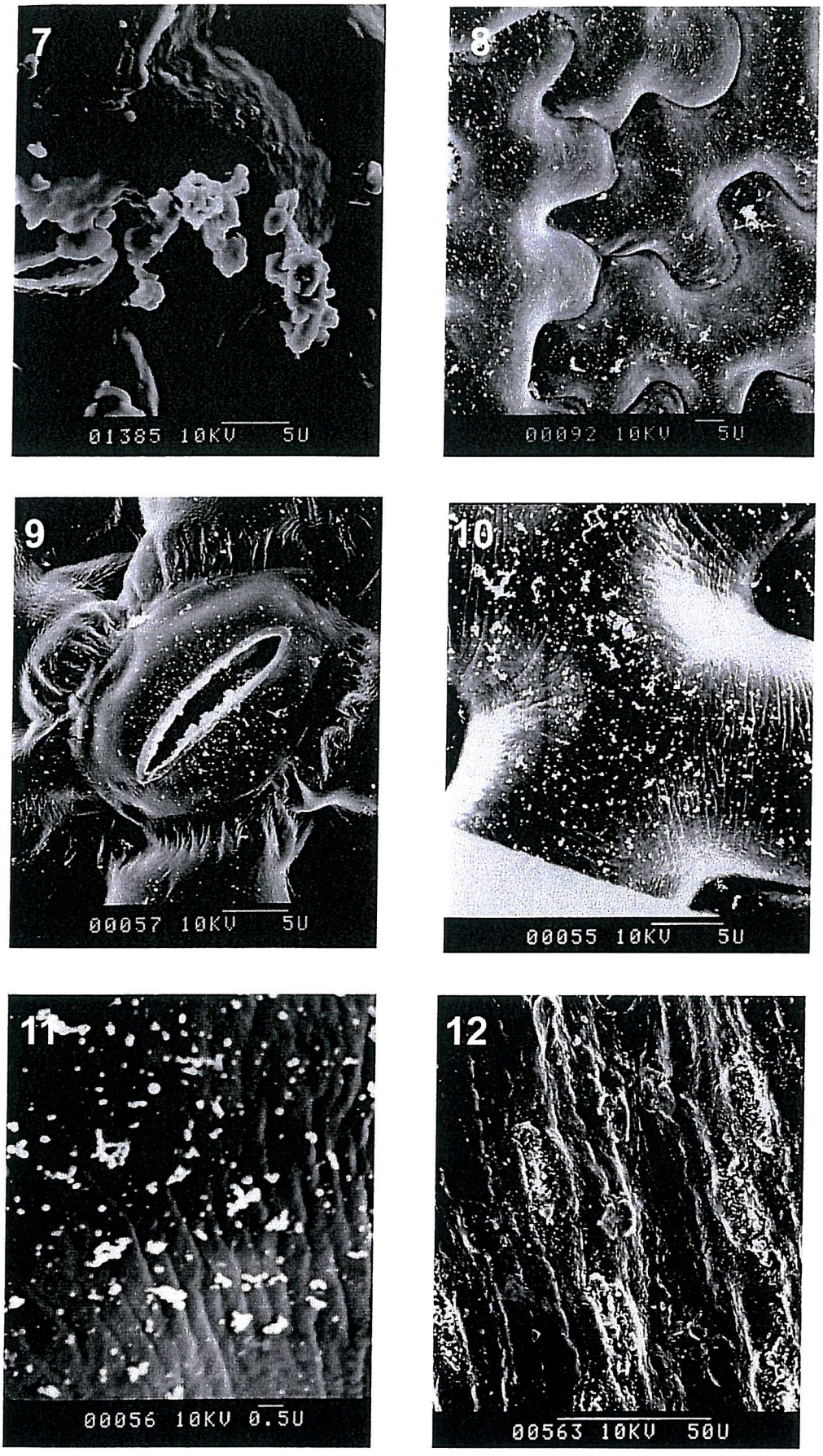
Fig.9. Adaxial surface of tomato leaf, 3.2k.

Fig.10. Adaxial surface of tomato leaf, 3.5k.

Fig.11. Adaxial surface of tomato leaf, 12k.

Fig.12. Adaxial surface of sugarcane leaf, 240x.

PLATE 5·2



Figures 7 - 12 Scanning electron microscopy of different leaf surfaces

Plate 5.3.

Fig.13. Adaxial surface of sugarcane leaf, 930x.

Fig.14. Adaxial surface of sugarcane leaf, 1.8k.

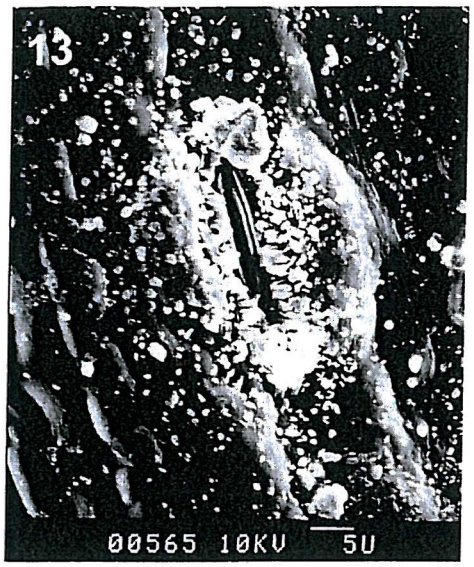
Fig.15. Adaxial surface of wheat leaf, 140x.

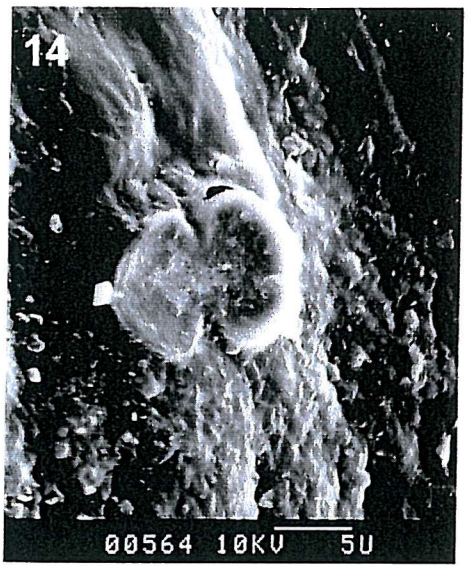
Fig.16. Adaxial surface of wheat leaf, 290x.

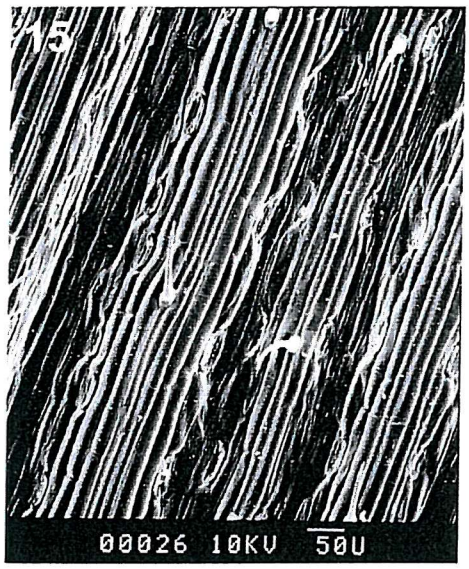
Fig.17. Adaxial surface of wheat leaf, 1.6k.

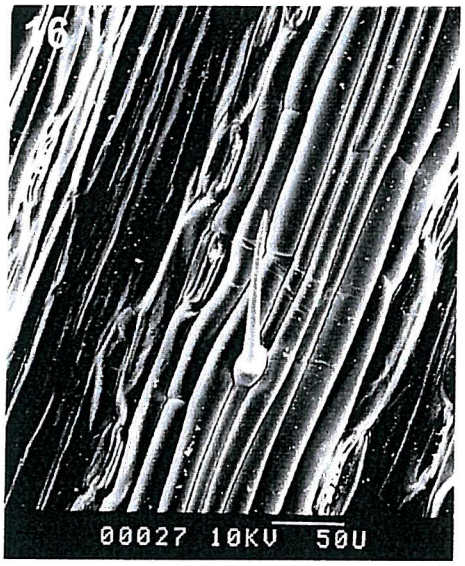
Fig.18. Adaxial surface of wheat leaf, 3.7k.

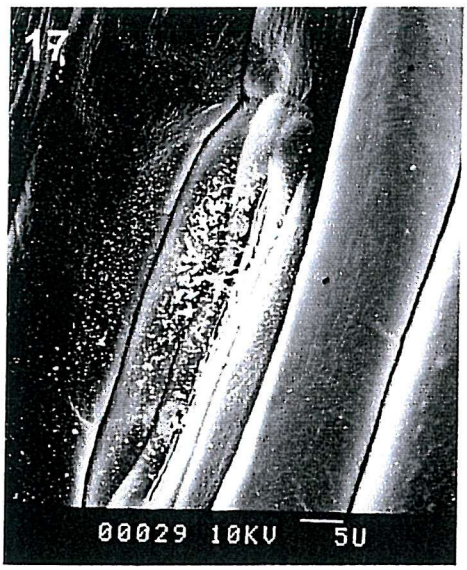
PLATE 5·3











Figures13 - 18 Scanning electron microscopy of different leaf surfaces

Plate 5.4.

Fig.19. Adaxial surface of orange leaf, 530x.

Fig.20. Adaxial surface of orange leaf, 1.2k.

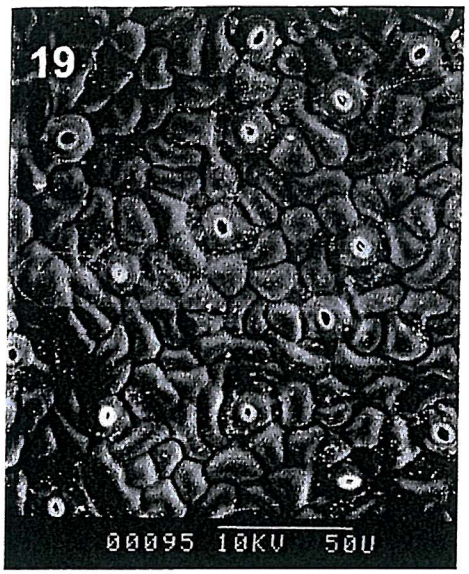
Fig.21. Adaxial surface of orange leaf, 6.3k.

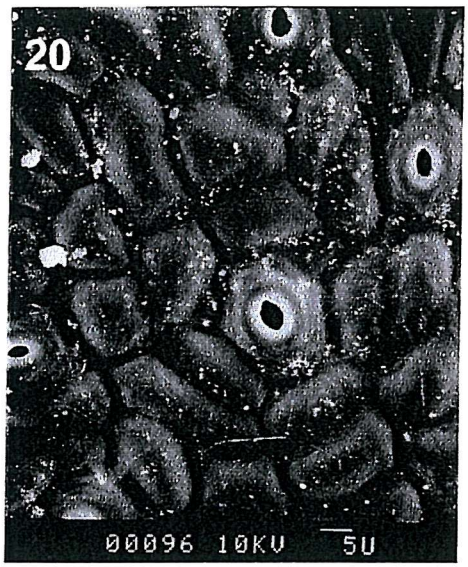
Fig.22. Adaxial surface of orange leaf, 6.3k.

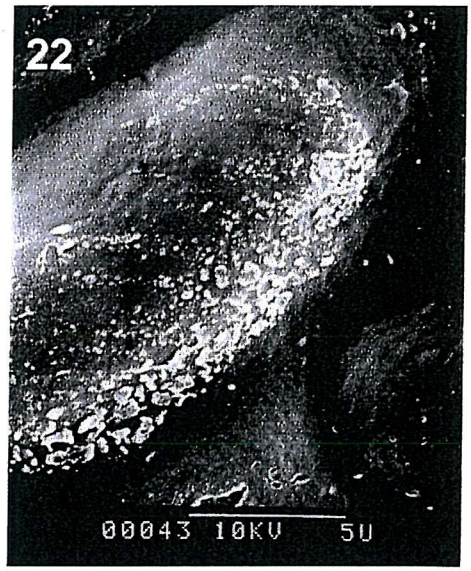
Fig.23. Adaxial surface of dwarfbean leaf, 720x.

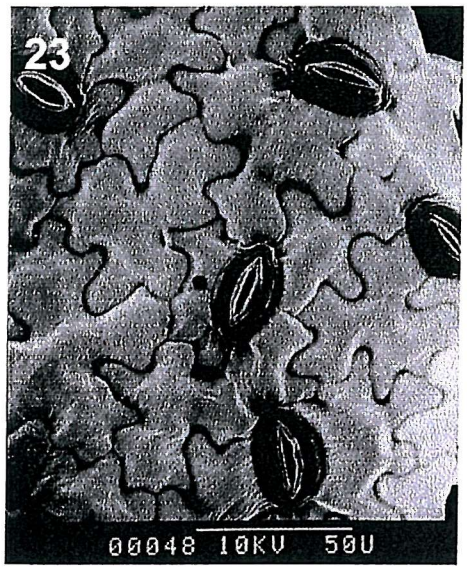
Fig.24. Adaxial surface of dwarfbean leaf, 1.6k.

PLATE 5·4









Figures19 - 24 Scanning electron microscopy of different leaf surfaces

Plate 5.5.

Fig.25. Adaxial surface of dwarfbean leaf, 2.9k.

Fig.26. Adaxial surface of dwarfbean leaf, 3.5k.

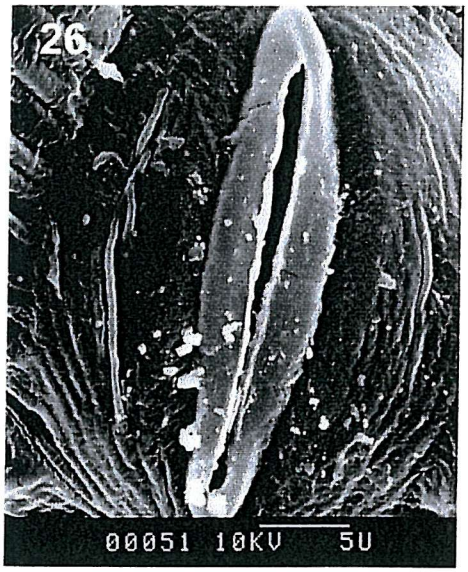
Fig.27. Adaxial surface of rape var. tanto (old) leaf, 540x.

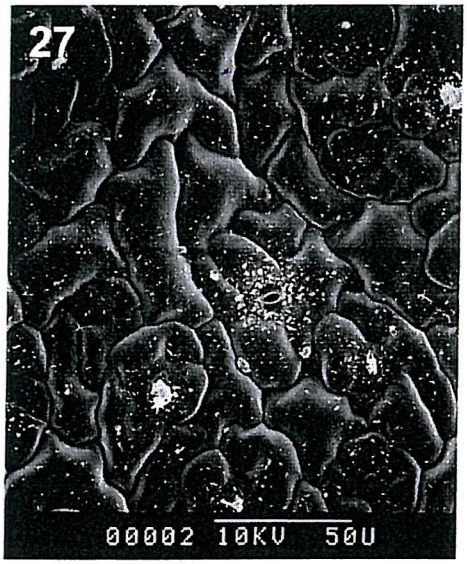
Fig.28. Adaxial surface of rape var. tanto (young) leaf, 340x.

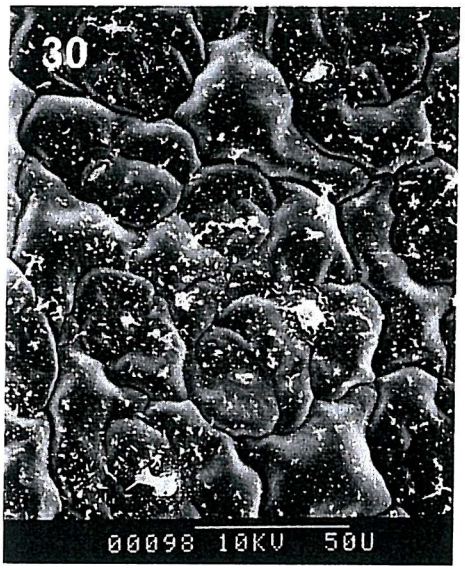
Fig.29. Adaxial surface of rape var. lirawell (old) leaf, 1.2k.

Fig.30. Adaxial surface of rape var. lirawell (young) leaf, 620x.

PLATE 5·5







Figures 25 - 30 Scanning electron microscopy of different leaf surfaces

Plate 5.6.

Fig.31. Adaxial surface of rape var. tanto (old) leaf, 1.8k.

Fig.32. Adaxial surface of rape var. tanto (young) leaf, 2.2k.

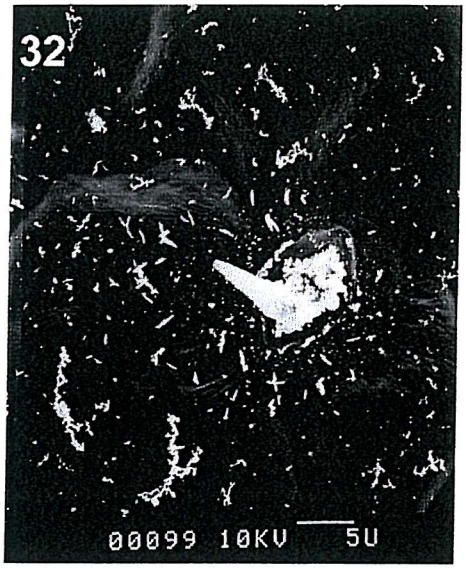
Fig.33. Adaxial surface of rape var. lirawell (old) leaf, 2.7k.

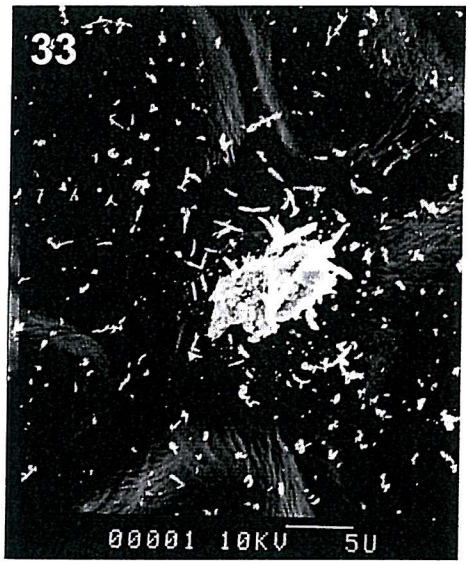
Fig.34. Adaxial surface of rape var. lirawell (young) leaf, 3.5k.

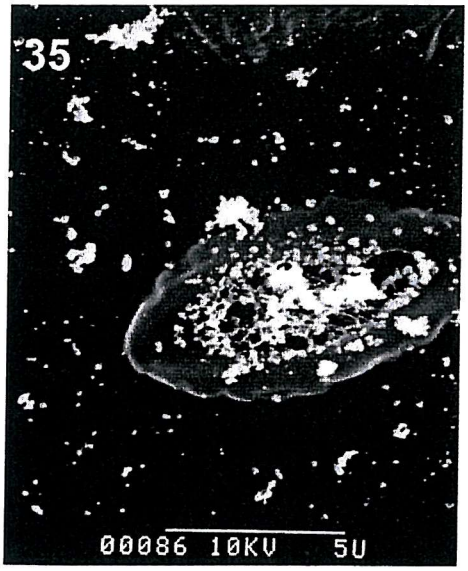
Fig.35. Adaxial surface of rape var. starlight (old) leaf, 7.1k.

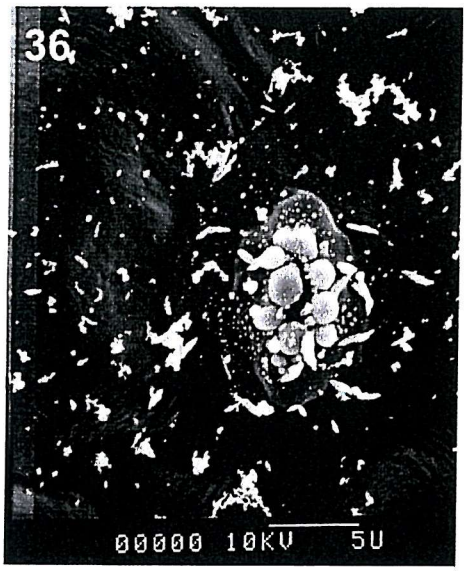
Fig.36. Adaxial surface of rape var. starlight (young) leaf, 3.5k.

PLATE 5·6









Figures 31 - 36 Scanning electron microscopy of different leaf surfaces

Plate 5.7.

Fig.37. Adaxial surface of maize (old) leaf, 680x.

Fig.38. Adaxial durface of maize (young) leaf, 1.6k.

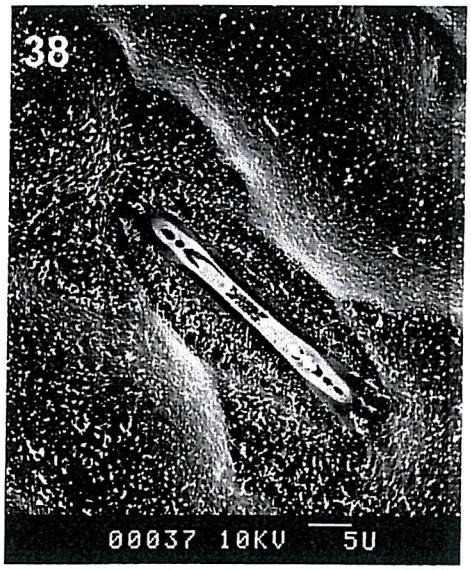
Fig.39. Adaxial durface of maize (young) leaf, 4.4k.

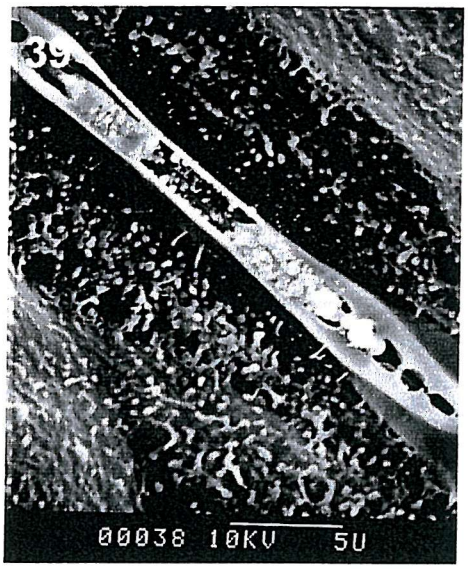
Fig.40. Adaxial surface of dewaxed barley leaf, 150x.

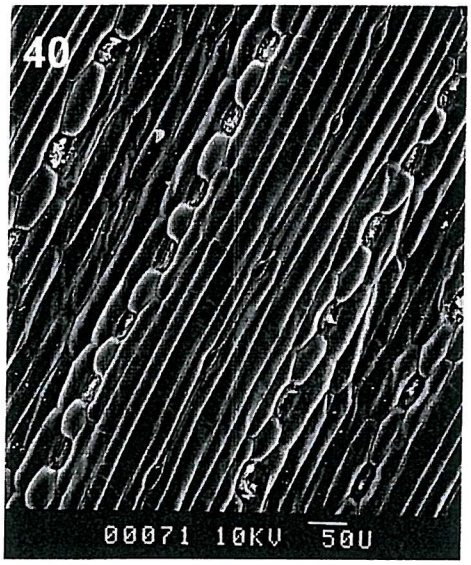
Fig.41. Adaxial surface of dewaxed tomato leaf, 110x.

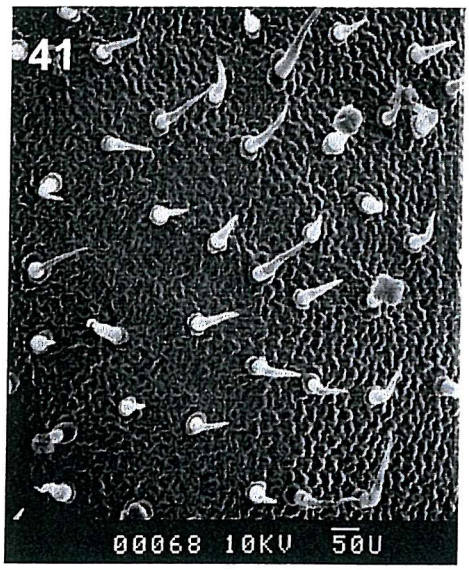
Fig.42. Adaxial surface of dewaxed tomato leaf, 240x.

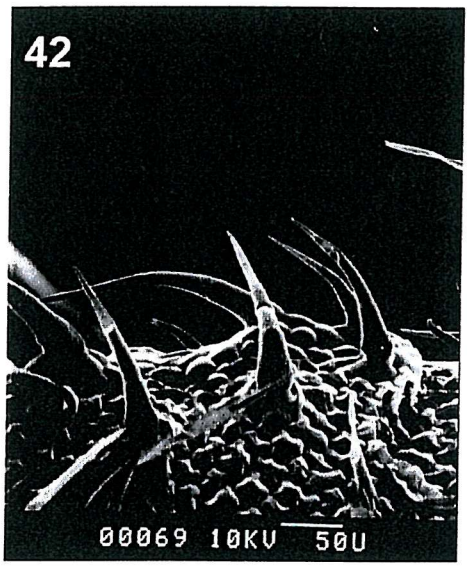
PLATE 5-7











Figures 37 - 42 Scanning electron microscopy of different leaf surfaces

Plate 5.8.

Fig.43. Adaxial surface of dewaxed tomato leaf, 310x.

Fig.44. Adaxial surface of dewaxed tomato leaf, 3.6k.

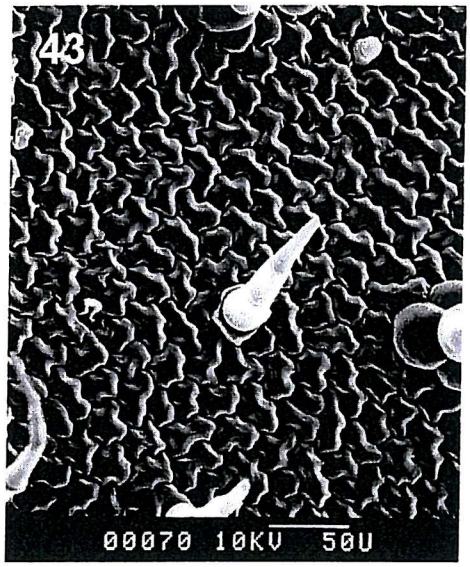
Fig.45. Adaxial surface of dewaxed sugarcane leaf, 170x.

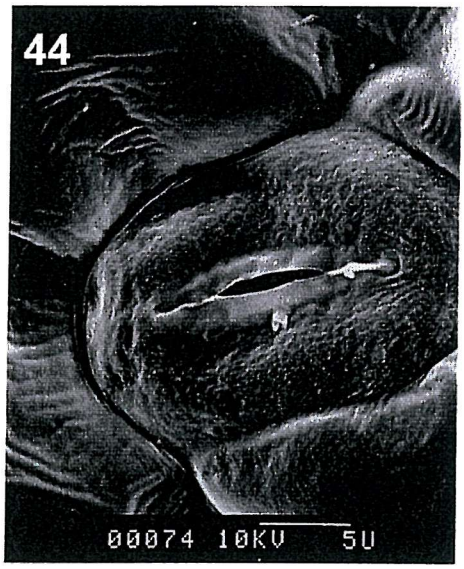
Fig.46. Adaxial surface of dewaxed sugarcane leaf, 1.7k.

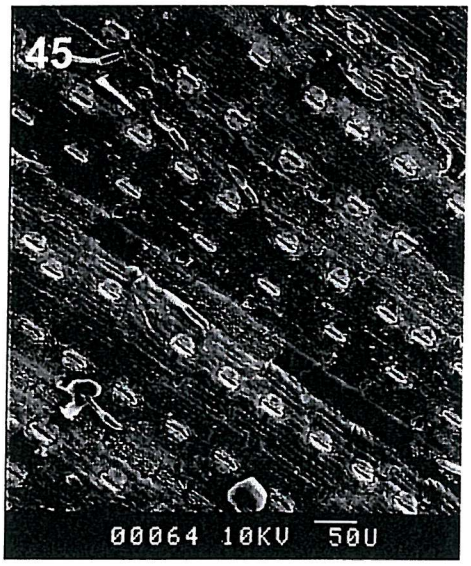
Fig.47. Adaxial surface of dewaxed maize leaf, 2.2k.

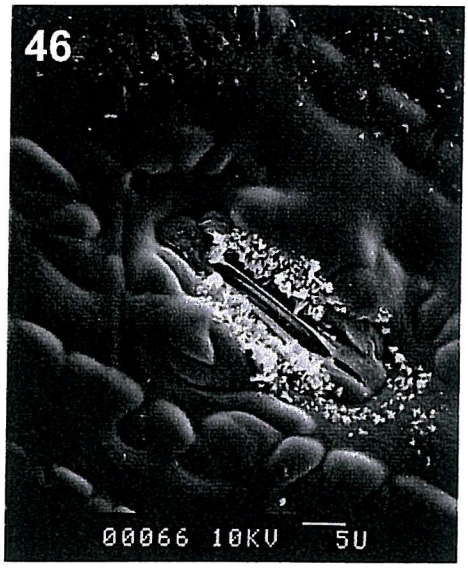
Fig.48. Adaxial surface of dewaxed maize leaf, 1.7k.

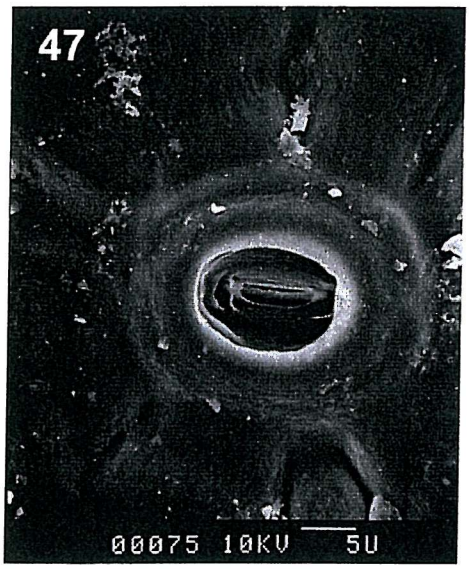
PLATE 5-2

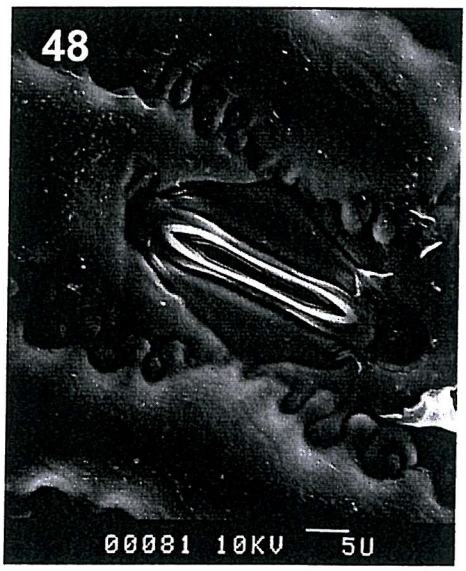












Figures 43 - 48 Scanning electron microscopy of different leaf surfaces

than the old leaves. Surprisingly, the wax depositions on the leaf surfaces of rape (v. Starlight) are different from two other varieties. On the old leaf surface the wax deposits on the stomatal opening form a net-like structure rather than a needle shaped cluster (Figure 35, plate 5.6). Although needle-shaped crystalline wax deposits are present on the surfaces of young leaf of rape (v. Starlight), the stomatal opening is full of globular shaped wax deposits (Figure 36, Plate 5.6). These differences in shape and structures of wax deposits greatly influence the micro-roughness of the whole leaf surface and will affect the overall wettability by spray droplets.

Figure 37 (Plate 5.7) shows the adaxial surface of an old leaf of maize with tiny wax particles spotted all over the cell surface. The adaxial surfaces of young leaves of maize are shown in figures 38 and 39 (Plate 5.7).

The adaxial surface of a barley leaf is shown in Figure 40 (plate 5.7) after a petroleum ether wash. The waxes are washed out by petroleum ether. Figures 41-44 (Plate 5.7 and 5.8) shows the adaxial surfaces of tomato leaves after washing with the same solvent. Relatively dense trichomes are shown on the surface. Trichomes are open and therefore can facilitate water movement by capillary action. The arrangement of the basal cells is compact. They are irregular in shape with raised centres and the cell boundaries form deep grooves. In case of sugar cane leaves it is evident that the petroleum ether wash may not remove all the wax (Figure 46, plate 5.8). Compared with Figure 13 (Plate 5.2), the stomata in Figure 46 (Plate 5.8) show that some waxes remain in the close proximity of the opening, whereas the waxes from the surface of the maize leaf seem to be completely removed by the petroleum ether (Figure 48, plate 5.8). Figure 47 (Plate 5.8) shows the adaxial surface of an orange leaf after petroleum ether washing. Here, scattered wax particles are found on the guard cell indicating that waxes of different leaf surfaces may not dissolve completely in petroleum ether, possibly due to the differences in the chemical constituents

of the waxes concerned.

5.4 Discussion

A wide range of variation in structure and wax content was observed among different leaf types. These differences can influence the overall behaviour of pesticides deposits on leaf surfaces. In addition to the amount of wax present, their distribution pattern, chemical orientation and physical structures are also important for interpreting their effects on the pesticide deposits. Variation in wax chemistry can influence the ability of lipophilic chemicals to dissolve in the wax (Holloway, 1970). The physical properties of the leaf surface, in combination with chemical constituents, influence the deposition, distribution and retention of spray chemicals. The considerable differences in the wettability of different leaf surfaces are primarily governed by the nature of chemical group exposed (Adam, 1925). This is further affected by the surface roughness (Wenzell, 1936).

The most important roughness feature is formed by the modification of the cuticle surface by the underlying venation. The prominence of the veins differs widely and in general the more xeromorphic a leaf the less conspicuous is the venation. For example on monocotyledonous leaves, such as barley, maize and wheat, the venation appears as a series of prominent ridges that run parallel to each other along the length of the leaf. On dicotyledonous leaves, the venation is reticulate and varied widely in prominence as is the case for on leaves of cabbage, tomato, rape and orange. This influences the wetting and spreading of liquid deposits. The wide variation in spread factors was noted by Baker *et al.* (1983) between formulations and between leaves of different species and might also be influenced by the roughness arising from the venation system. They also observed that when oil-based formulations were applied to maize leaves, the spray deposits moved readily across the surface and the redistribution proceeded primarily in a lateral direction. On strawberry leaves, these formulations moved mainly along the venation.

Surface topography is also influenced by the shape and size of the underlying epidermal cells, for example on the leaves of maize and tomato (Figures. 37, pl.5.7 and 43, pl.5.8). These surfaces may be flat, convex or papillose. The minute surface ornamentation of the cuticle gives a granular, grooved or ridged appearance (Stace, 1965). Spray droplets landing on a ridged surface will have a tendency to move towards the grooves, especially on surfaces with high wax blooms. Subsequently, an ultimate accumulation of drops will build up along the parallel grooves, leaving the ridged area relatively free of deposits (e.g. on maize leaves Figure 37, pl.5.7). On dwarf bean (Figure 23, pl.5.4) or on tomato leaves (Figure 43, pl.5.8) such movement of deposits along the grooving will be juxtaposed.

Such microscopical roughness of venation and epidermal cells can produce water repellent leaf surfaces. The contact angles on such surfaces are not altered very much by removal of wax with petroleum ether washing (e.g. reduction of contact angle on maize leaves after petroleum ether washing, Chapter 7), although the Figures 37, pl. 5.7, and 48, pl.5.8 confirm the removal of wax.

Intrinsic roughness developed from superficial wax deposits is another important aspect of leaf surface characteristics that has been studied at microscopical and ultramicroscopical magnifications (Hall, 1963, Schieferstein & Loomis, 1959, Scott, *et al.* 1958, Hall, 1966, Bain & Mcbean, 1967). Structural differences between plant species (for example, on barley and sugar cane leaves in Figures 1, pl.5.1 and 13, pl.5.3), and varieties of same species (for example, on rape leaves in Figures 31-36, pl.5.6) were also observed by Hall (1965). Immature leaves carry more waxes and have a greater density of crystalline platelets or rodlets than mature leaves (Baker, *et al.* 1983) such as barley seedlings. Crystalline waxes, as platelets or rodlets, gave high contact angles (Fernandes, 1965; Hall, 1965) and therefore, were less wettable. Superficial waxes that produce bloom (as on cabbage leaves Figure 7, pl. 5.2) also resulted in high contact

angles (Hall, 1966). In both static and dynamic conditions in the laboratory and the field, surface roughness played the more important role in spray retention by influencing the droplet "bounce" (Hartley & Brunskill, 1958) and the magnitude of the contact angle hysteresis (Furmidge, 1962).

Figure 46 (Plate 5.8) shows the surface of a sugar cane leaf after washing with petroleum ether and it is seen that the wax particles in the close vicinity of the stomatal opening have not been washed off completely (compare with Figure 13, plate 5.2). The compact parallel grooving arrangement of basal cells can facilitate droplet spreading along the grooves. Figures 41-43 (Plates 5.7 and 5.8) show the trichomes and other surface topography of dewaxed tomato leaves. Figure 42 (Plate 5.7) shows the topography with a deep valley probably resulting from an underlying venation and dense arrangement of trichomes. From inspection of Figures 41 and 42 (Plate 5.7), it can be hypothesized that a large droplet has a greater chance of suffering distortion due to trichome intervention, but a fine droplet can easily impact and be accommodated in between the trichomes. However, some droplets must land on the tips of the trichomes and be shed more easily from the leaf. Large droplets may cover larger parts of the leaf surface including the trichomes. The role of trichomes in leaf wettability is widely accepted but poorly studied (Holloway, 1970). The influence of 'open' and 'closed' pattern trichomes in leaf wettability (Challen, 1962) is described in Chapter 7.

A comparison between Figures 9 and 44 (Plates 5.2 and 5.8) for tomato and Figures 38 and 48 for maize (Plates 5.7 and 5.8), confirms that wax is removed by petroleum ether washing. In some cases, such removal seems incomplete (compare Figures 13 and 46, plate 5.2 and 5.8). This accords with results which show a reduction in contact angles on some dewaxed leaf surfaces (see chapter 7), suggesting that wax is the main source of hydrophobicity on these leaf surfaces.

Although the frequency and sizes of stomata of individual

leaf surfaces were not quantified in the present studies, it is obvious that differences exist in the number and sizes of stomata on different leaf surfaces. Early studies have shown that stomata play an important role in foliar penetration by liquids; penetration is higher in stomatous than astomatous leaves (Sargent, 1965), thus penetration is correlated with stomatal frequency (Jyung, *et al.* 1965). Schónherr and Bukovac (1972) and Greene and Bukovac (1974) studied the penetration of stomata by liquids as a function of surface tension, wettability and stomatal morphology. Liquids with a surface tension up to 30dyn cm^{-1} gave a zero contact angle and infiltrated stomata spontaneously, while liquids having a surface tension above 30dyn/cm did not wet the leaf surface and can not infiltrate stomata readily. Only a few surfactants, such as organosilicone used in agricultural spray chemicals, are able to effect such surface tension reduction and alter stomatal flooding (Field & Bishop, 1988; Stevens *et al.* 1992). However, this infiltration process has a very brief existence just after spray application when spray deposits are still liquid. Later on, the cuticular penetration depends solely on uptake pathways.

Similarly, structures such as trichomes also have an effect on spray deposits. Pubescence can influence the retention and penetration of spray drops (Ormond & Renney, 1968). Hess *et al.* (1974) showed that large droplets from foliar sprays shatter on impact with its trichomes greatly affecting the amount of [(4-chloro-o-tolyl)oxyl]acetic acid (MCPA) that reached the true epidermis.

The role of epicuticular waxes on leaf wettability, pesticide retention, penetration, spreading etc. was discussed in chapter 1. In addition, surfactants used in spray chemicals can also modify the wax structure and composition (Takeno & Foy, 1974), which in turn influences the overall interaction between spray droplets and leaf surfaces.

Further microscopical studies of leaves after specific spray applications, alongside bioassay and residual data, may provide important information concerning the spreading,

distribution, persistence, uptake and hence overall efficacy of a pesticide.

Thus, a systemic analysis of all these factors will help to build a better understanding of pesticide efficacy.

Chapter 6

The role of leaf epicuticular waxes in the toxicity responses of *F. candida* and *A. colemani* to deltamethrin and dimethoate residues

6.1 Introduction

Recent increases in the use of systemic and residual contact pesticides in pest management practices have emphasized the need for additional information on factors involved in the uptake and transfer process. Differences in leaf sorption have been noted between species, with leaf age, and with the spray chemicals and their formulation (Hull, 1970). The main available routes of uptake, especially for systemic pesticides, are penetration into the cuticle by diffusion, absorption through trichomes and stomatal infiltration by liquid or vapour movement (Sands & Bachelard, 1973a). For contact insecticides, surface retention is more important than penetration, although retention on the exposed surface makes the deposits more vulnerable to degradation by natural weathering.

The role of epicuticular waxes as a barrier to the transcuticular movement of many substances has been well documented (Norris & Bukovac, 1972; Schonherr, 1976), but their role in the retention and ultimate transfer to the insect cuticle is less understood. Although there is no direct correlation between the thickness of the wax layer and rate of penetration, differences between penetration of the adaxial and abaxial surfaces have been attributed to differences in the density and the orientation of wax deposits.

The amount and the chemical and physical nature of waxes varies greatly from species to species, surface to surface, part to part and with age (Baker, 1971; Holloway, 1971; Kolattukudy & Walton, 1972). Variations in physical appearance are related to the quantity, chemical constitution and structure of the epicuticular waxes (Netting *et al.* 1972; Netting & von Weltstein-Knowels, 1973). Environmental

conditions, such as temperature and light intensity, can affect the quantity and the structure of the epicuticular wax (Juniper, 1960; Hallam, 1970; Whitecross & Armstrong, 1972). The visual manifestation of epicuticular wax as glaucous or non-glaucous also reflects the physical and chemical nature of wax constituents and is related to water-use efficiency (Richard *et al.* 1986). Wax quantity is generally lower for non-glaucous than for glaucous genotypes (Clarke *et al.* 1993). Surface waxes of leaves affect drought tolerance, efficacy of chemical spraying and provide protection against microbial entry (Englington & Hamilton, 1967; Hadley, 1981). The effects of genotype and environment have been studied in detail by Jordan *et al.* 1983; Baenziger *et al.* 1983.

In addition to the hydrophobic characteristics of wax constituents, waxes also form microstructures on many leaf surfaces that profoundly affect wettability. This in turn affects how foliar applied chemicals distribute themselves on a leaf surface. Hydrophobicity is the most important physico-chemical property of the epicuticular wax, but if this barrier can overcome, the superficial wax may play an important role in facilitating the passage of lipophilic chemicals into the wax embedded in the cutin layer (Holloway, 1970). Sometimes, if pesticide deposits are retained in the cuticle without further penetration, the solution of the pesticide in the waxy layer be as toxic as contact and more resistant to natural weathering than the crystal deposition on the surface (Martin & Batt, 1958).

For any agricultural chemical sprayed on foliage, physico-chemical conditions such as wetting, spreading, coverage, retention and penetration are important in determining the effectiveness against target invertebrates. These physico-chemical factors are governed by the nature of the active ingredient of the spray chemical and its formulation, and by the leaf surface characteristics, e.g. epicuticular waxes and their ultrastructure (Linskens *et al.* 1965; Crafts & Foy, 1962; Baker *et al.* 1983). Waxes play a definitive role in droplet reflection, which occurs from either dry or uniformly

wetted leaf surfaces and is probably due to air films trapped between the droplet and the surface (Hartley, 1967). On dry surfaces, the causes are more preferentially attributed to the presence of micro-roughness, usually wax crystals or a dense covering of trichomes (Holloway, 1994). Similarly, wax and its ultrastructure affects wettability (Wattanabi & Yamaguchy, 1991). In some studies on retention, attention has been focused on the dynamic surface tension of the spray solution. However, to ensure a formulation is effectively retained on the foliage, a better understanding of leaf surface characteristics is needed.

Leaf characteristics can profoundly alter the whole retention phenomenon (Stock & Davies, 1994) and thereby the residual availability of the active ingredient to the target invertebrate. Wetting of plant surfaces by a foliar applied chemical is regarded as one of the important factors for its effectiveness as pesticide. The documentation on the role of surface characteristics on wettability, retention and penetration by applied chemicals (Zisman, 1964; Possingham *et al.* 1967; Holloway 1969a) makes frequent reference to the nature of the surface waxes and their orientation (Holloway, 1969). In some cases, the quantity of the surface waxes is not critical, providing the entire surface is covered by a monomolecular layer of wax which can sufficiently reduce the wetting (Baker & Bukovac, 1971).

A review of epicuticular waxes and their role in retention, penetration, wetting, distribution, spreading and coverage is in chapter 1. This review shows that there has been little experimental work on the role of these factors on direct toxicity responses of invertebrates.

The present chapter was mainly aimed at determining the relationship between the amount of epicuticular wax and various leaf surface characteristics and the residual toxicity responses of exposed invertebrates to two different pesticides.

6.2 Materials and methods

6.2.1 Selection of plant materials: Fresh leaves were collected from the plants grown in the field and in a glasshouse. Barley seedlings, cabbage, tomato, orange, sugarcane, dwarf bean, maize and three varieties of rape were grown in the glasshouse of the Biology Department of Southampton University. Wheat leaves were collected from a farmers field at Manydown in Hampshire, UK. Pear leaves were collected from plants grown on the campus of Bio-medical School, University of Southampton. Barley seedling leaves used in the experiment were at growth stage 13 (three leaves unfolded). Comparatively older leaves of cabbage, tomato, orange, sugar cane, dwarf bean and pear were collected at the vegetative stage of the plant. Young and comparatively old leaves of maize and rape were also collected at their vegetative stage. Wheat leaves were collected from plants at growth stage 65(anthesis begun).

6.2.2 Determination of residual susceptibilities: These are the same as described in chapter 3 and 4.

6.2.3 Extraction of epicuticular wax : There are several procedures currently used for the extraction of wax from leaf surfaces. The most common one is dipping the leaf with gentle agitation in a suitable solvent for 5-10 sec.(Martin & Batt, 1958, Fernandes *et al.* 1964). Dipping is not appropriate for removal of wax from each leaf surface separately. To extract wax either from adaxial or abaxial leaf surfaces, 5 to 10 ml of solvent were allowed to run over the surface to be dewaxed and collected in a glass beaker or a petridish (Farnandes *et al.* 1964; Holloway, 1969; Baker, 1971). Different workers have used different solvents, including petroleum ether (Fernandes *et al.* 1964; Hallam & Juniper, 1971), chloroform (Holloway, 1969; Baker, 1971; Jeffree *et al.* 1975), and diethyl ether (Purdy & Truter, 1963; Denna, 1970).

Healthy and fresh leaves, free from visible injury and infection and uncontaminated by spray chemicals, were taken from the glasshouse, field and garden. All the leaves were

weighed and the total surface area of each was measured by image analysis on a computer. The waxes from the adaxial surfaces of the leaves were isolated separately by allowing successive portions of solvent (petroleum ether, 5ml each) to run over the surfaces from fine-orifice burettes and collected on pre-weighed glass petridishes. The solutions were filtered and the solvent was evaporated at room temperature in a fume cupboard. The residue was then weighed and the amounts of wax per unit area were calculated.

6.2.4 Scanning electron microscopy of *Folsomia candida*: The fresh and unsprayed leaf samples were attached to chamber 2 of bioassay chamber (as described in chapter 2), their adaxial surfaces facing upward. One bioassay chamber was kept clean without any leaf attached. Living *F. candida* insects (2 to 3 in number), freshly collected from the stock culture, were allowed to stay in all chambers for 24 hours continuously. The insects could not survive in a clean bioassay chamber for a longer period. After 24 hours, the insects were picked up carefully by a fine brush and immersed in test-tubes containing absolute alcohol and marked according to leaf species. They were then dried by a critical point dryer 'CPD' with a similar protocol to that described in Chapter 5 for leaf surfaces. Specimen insects were mounted on 0.5" aluminium pin stubs (Agar, Agar Scientific Ltd, Essex, UK) by means of double-sided adhesive tape, and marked. The specimens were coated with silver paint by a sputter coater (Emscope, London, UK) prior to examination under the scanning electron microscope (SEM). Insects were examined by placing the stubs in the air-locked specimen chamber of an SEM (Hitachi-S-450) at an accelerated voltage of 10kv and with a 50 mw emission current. The images were taken by a camera fixed to the SEM and recorded on Ilford FP4-120 film.

6.2.5 Statistical analysis: Data for residual analysis used

here were those obtained and described in chapter three for *F. candida* and chapter four for *A. colemani*. A linear regression model was fitted to data of log-72h LD_{50} for *F. candida* on different leaf species sprayed with deltamethrin, against the amount of wax extracted per unit area from each leaf type. Another regression model was plotted for the data of log-72h LD_{50} values for *F. candida* on dimethoate treated leaf surfaces against the wax content of different leaf species. A similar analysis was carried out for *A. colemani* exposed on three different leaf types sprayed with deltamethrin.

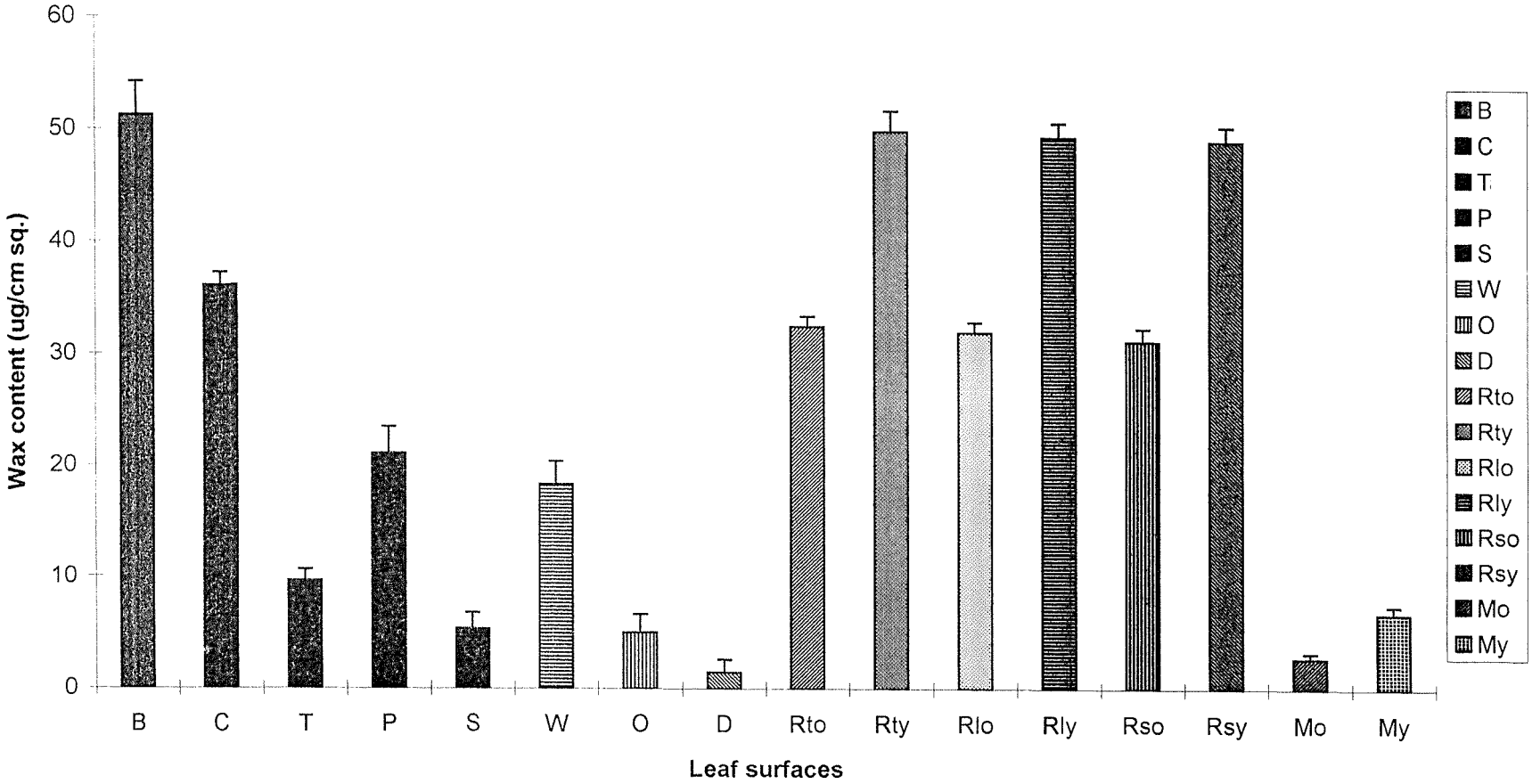
6.3 Results

6.3.1 Wax content of different leaf types and susceptibility ranking of *F. candida*:

Figure 6.1 shows the amount of wax in µg cm^{-2} extracted from the adaxial surfaces of different test leaf types. Barley seedling leaves had the highest amounts of wax present on the adaxial surface. Dwarfbean leaves had the lowest amount wax per unit area. The young and old leaves of three varieties of rape all had very similar amounts of wax. Varietal differences in the amount of wax were less evident than the age differences. Leaves of tomato, sugar cane, orange, maize(young), maize(old) and dwarfbean fell within the range of 1 to 10 µg of wax cm^{-2}. Cabbage (old) leaves had moderately high amounts of waxes, similar in quantity to the old leaves of three varieties of rape. The amount of wax for pear and wheat leaves ranges from 18 to 20 µg cm^{2}.

Table 6.1 shows the ranking relation between 72h LD_{50} values (g AI ha^{-1}) for *F. candida* exposed to deltamethrin residues on different leaf surfaces and the amount of epicuticular wax per unit area. A close relationship was observed between the amount of epicuticular wax and the susceptibilities of *F. candida* to deltamethrin sprayed on different leaf surfaces. However, it was evident from

Figure 6.1 Wax content of adaxial surfaces of different leaf types



Key to test leaf surfaces; B =barley (seedling); C= cabbage (old); T= tomato (old); P=pear (old); S= sugarcane (old); W= wheat (old); O= orange (old); D =dwarfbean (old); Rto = rape v. Tanto(old); Rty = rape v. Tanto(young); Rlo= rape v. Lirawell (old); Rly =rape v. Lirawell (young); Rso =rape v. Starlight (old); Rsy = rape v. Starlight (young); Mo= maize (old); My= maize (young)

Table- 6.1. Ranking relation between 72-h ld50 of *F. candida* for Deltamethrin 2.5 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	LD50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (S)	*H. vulgare*		6.36 (4.58-8.25)	1	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	8.96 (6.18-12.11)	5	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	16.87 (12.48-22.67)	9	09.65 (1.03)	11
Pear (o)	*P. communis*		14.43 (9.82-20.50)	8	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		20.94 (13.98-32.40)	10	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	24.86 (16.38-41.00)	11	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		40.79 (27.15-73.70)	13	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	77.14 (54.45-119.26)	15	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	8.23 (5.82-10.92)	3	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	7.91 (5.92-10.12)	2	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	9.42 (6.77-12.46)	6	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	8.61 (6.58-10.92)	4	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	9.80 (7.25-12.76)	7	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	8.24 (6.07-10.66)	3	49.22 (1.28)	4
Maize (o)	*Z. mays*	Marcia	66.65 (43.44-134.60)	14	2.77 (0.24)	15
Maize (y)	*Z. mays*	Marcia	37.53 (25.94-61.89)	12	6.74 (0.45)	12

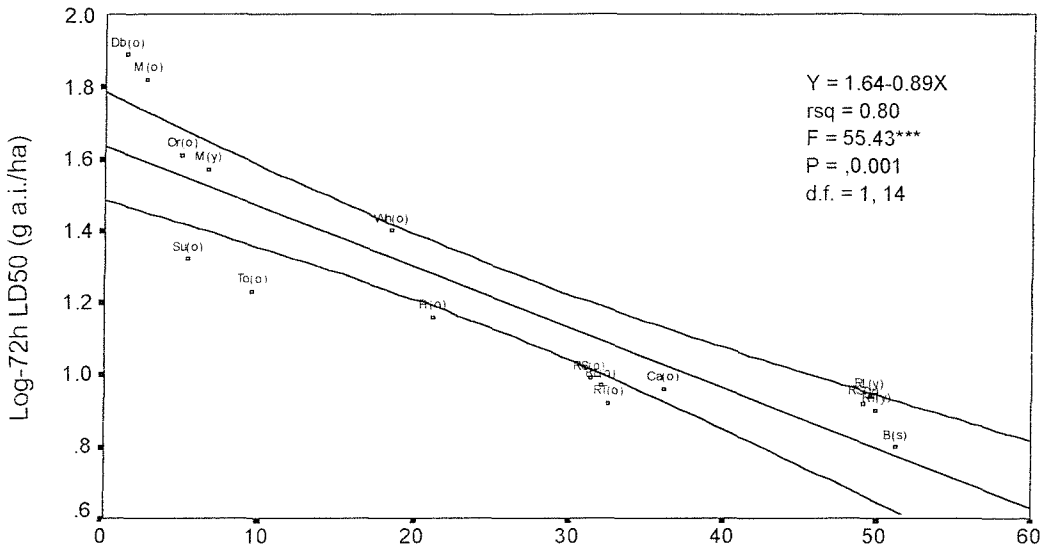
s =seedling, o =old, y =young

Table- 6.2. Ranking relation between 72-h ld50 of *F. candida* for Dimethoate 40 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	LD50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (s)	*H. vulgare*		8.69 (6.99-10.86)	15	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	5.20 (4.16-6.49)	13	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	2.80 (2.13-3.60)	10	09.65 (1.03)	11
Pear (o)	*P. communis*		1.76 (1.26-2.31)	5	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		4.19 (2.92-5.91)	12	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	2.77 (2.17-3.49)	9	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		1.62 (1.08-2.23)	4	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	2.36 (1.83-2.97)	8	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	1.95 (1.58-2.39)	7	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	1.57 (1.20-1.97)	3	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	1.91 (1.55-2.34)	6	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	1.56 (1.18-1.97)	2	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	1.91 (1.55-2.32)	6	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	1.35 (1.02-1.70)	1	49.22 (1.28)	4
Maize (o)	*Zea amys*	Marcia	4.17 (3.25-5.36)	11	2.77 (0.24)	6
Maize (y)	*Zea mays*	Marcia	5.95 (4.67-7.60)	14	6.74 (0.45)	2

s =seedling, o =old, y =young

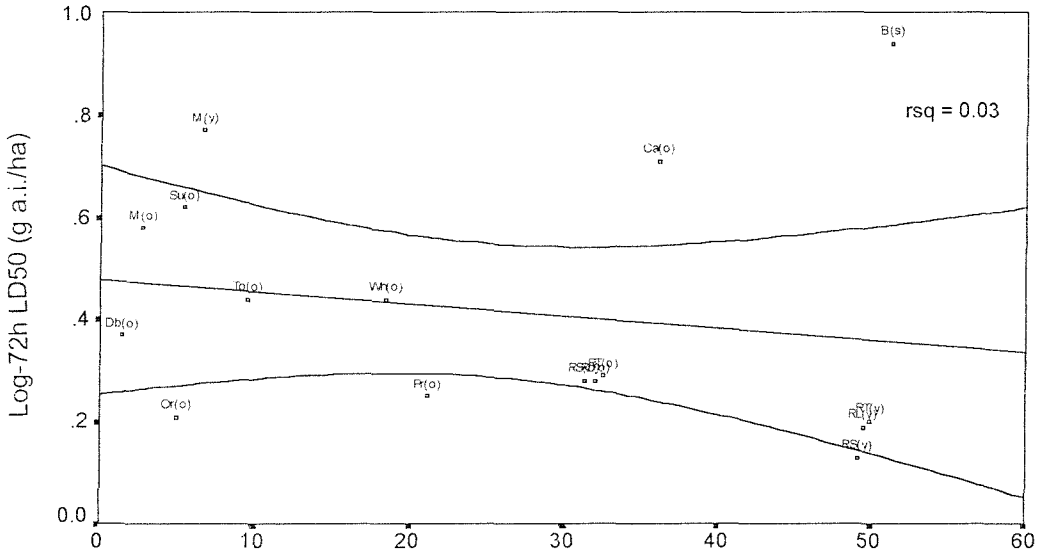
Figure 6.2 Correlation between log-72h LD50 of *F. candida* for deltamethrin and wax content of different leaf surfaces



Wax content (ug/cm. sq.) of different leaf surfaces

Key to test leaf types: B(s), Barley seedling; Ca(o), Cabagge old; RT(o), Rape v. Tanto (old); RT(y), rape v. Tanto (young); RL(o), Rape v. Lirawell (old);RL(y), rape v. Lirawell (young) ,RS(o), Rape v. Starlight (old;)RS(y), Rape v. Starlight (young); Db(o), Dwarfbean (old); Pr(o), Pear(Old); Su(o), Sugarcane (old); Wh(o), Wheat (old); Or(o), Orange (old); M(o) Maize (old); M9y), Maize (young)

Figure 6.3 Correlation between log-72h LD50 of *F. candida* for dimethoate and wax content of different leaf surfaces Key to test types as Fig. 6.2



Wax content (ug/cm. sq.) of different leaf surfaces

Figure 6.4 Correlation between standard deviation of probit slope and log-72h LD50 of *F. candida* for different leaf surfaces sprayed with deltamethrin. Key to test leaf types Fig. 6.2

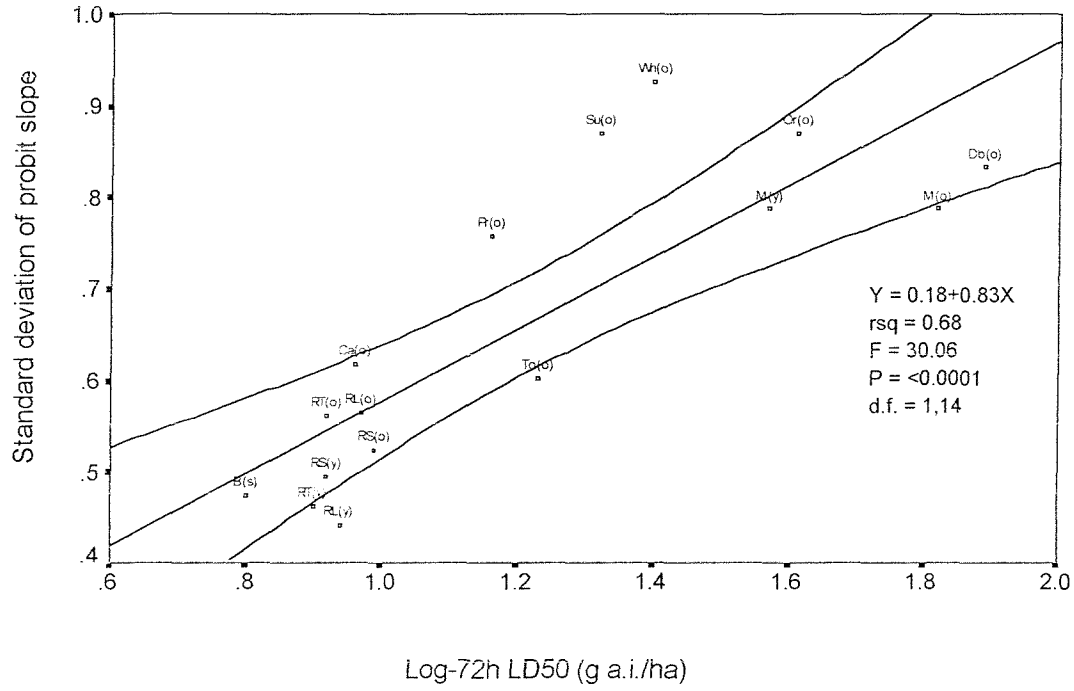


Figure 6.5 Correlation between standard deviation of probit slope and log-72h LD50 of *F. candida* fo different leaf surfaces sprayed with dimethoate. Key to test leaf type as Fig. 6.2

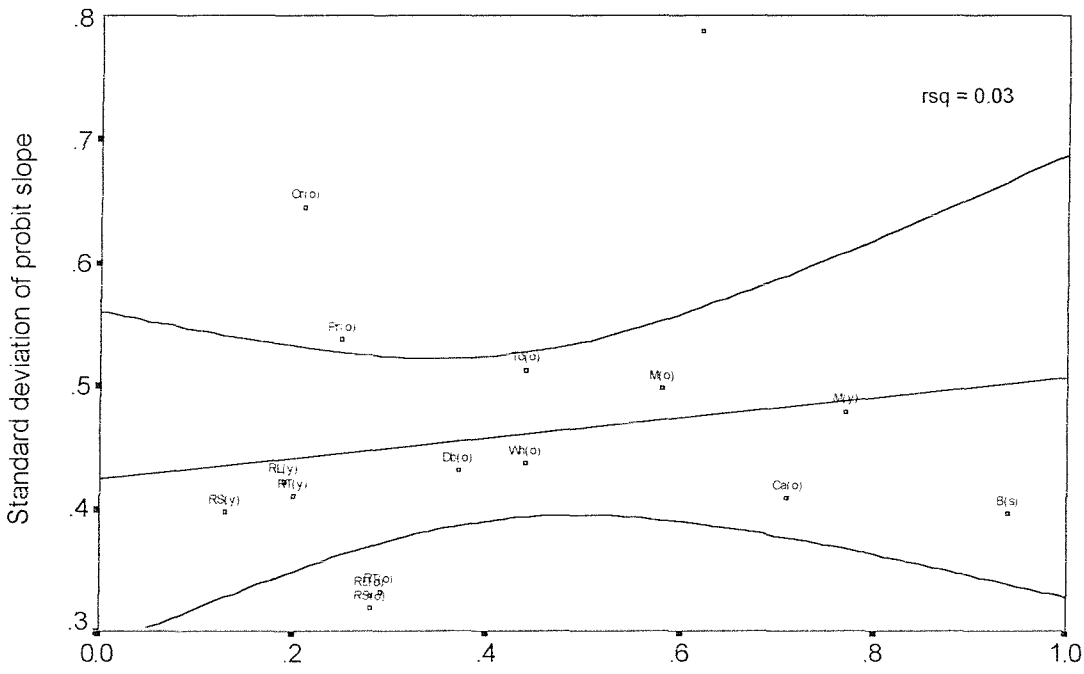


Figure 6.6 Correlation between log-24h LD50 of *A. colemani* for deltamethrin and wax content of different leaf surfaces. Key to test leaf types as Fig. 6.2.

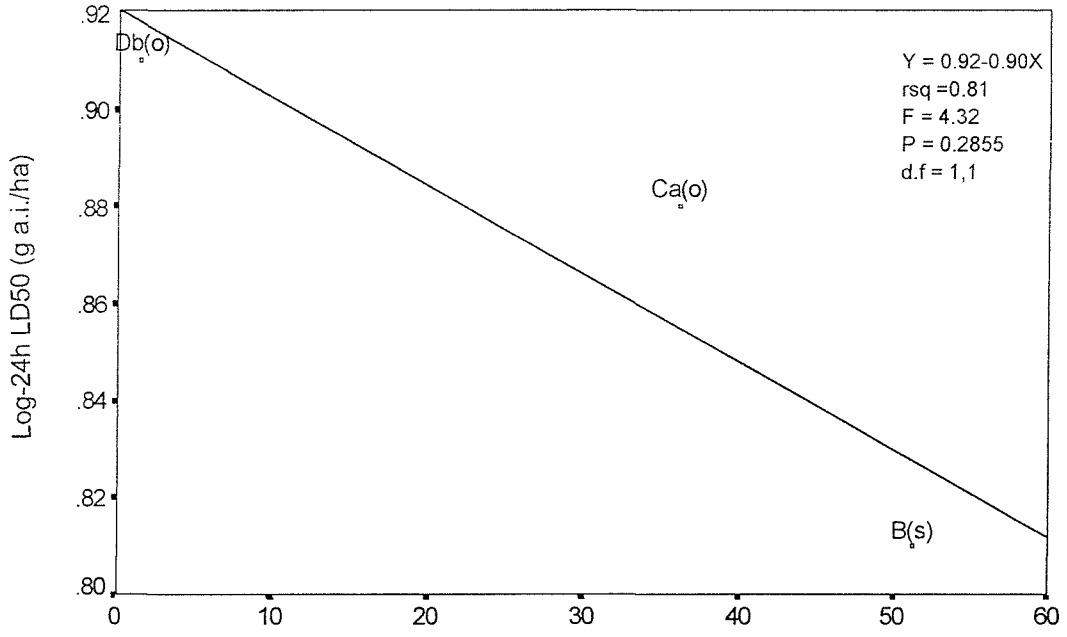


Table-6.2 that no such relationship exists between the amount of wax and the LD_{50} values for *F. candida* on different leaf species treated with dimethoate.

A linear regression model was fitted to data for log-72h LD_{50} (g AI ha^{-1}) of *F. candida* for deltamethrin against the amount of wax (µg cm^{-2}) found on different leaf surfaces (Figure 6.2) giving a significant negative correlation (r^2= 0.80; F= 55.43***; d.f.= 1,14; P<0.001). *F. candida* were less susceptible on leaf surfaces having lower amounts of waxes per unit area.

When a similar regression model was fitted to data for log-72h LD_{50} values of *F. candida* for dimethoate against the amount of wax present on each leaf types (Figure-6.3) no significant correlation was observed (r^2= 0.03). From these results it was evident that the nature of the active ingredient and the formulation are factors which may affect the role of wax in mediation of toxicity from leaf surfaces to invertebrates. Figure 6.4 and 6.5 show the standard deviation of tolerance distribution of *F. candida* on different leaf surfaces for deltamethrin and dimethoate respectively.

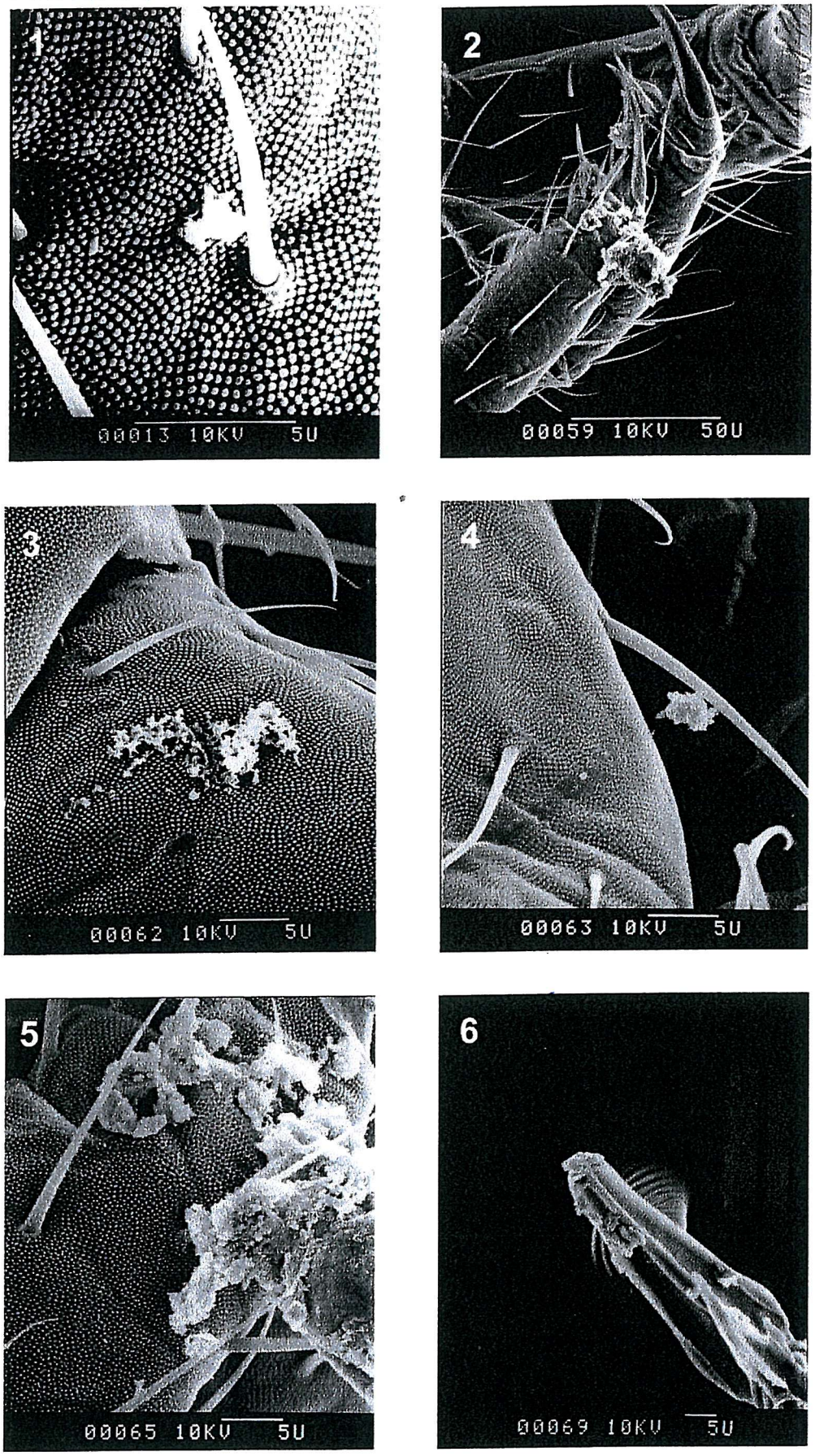
There was a strong correlation between the susceptibilities of *F. candida* and the amount of wax on different leaf surfaces treated with deltamethrin. The parasitoid *A. colemani* was tested on a smaller number of leaf species to see if there was a similar correlation. The three leaf species had low, medium and high wax contents with amorphous, semiglaucous and glaucous surfaces. They were dwarf bean (amorphous and low in wax), cabbage (glaucous and medium in wax) and barley seedlings (semiglaucous and high in wax). A linear regression model fitted to data for log-72h LD_{50} values (g AI ha^{-1}) of *A. colemani* on different leaf surfaces against the amount of wax present again gave a strong negative correlation with an r^2 value of 0.81. (Fig.6.6)

6.3.2 Scanning electron microscopy of *F. candida*: Figures 1 to 30 (plates 6.1-6.5) show the different body parts of *F. candida* exposed on untreated adaxial surfaces of various leaf species and clean plastic (petridish) surface for 24 hours. The SEM was used to investigate whether exposed *F. candida* pick up epicuticular waxes from leaf surfaces. There was no conclusive evidence that the structures, presumably not an integral part of insect cuticle, which were found on the body of some exposed *F. candida* were wax particles. However, three criteria can be applied: a) the magnification at which these particles were observed; b) the structure of those particles and c) the location of such particles on the body of *F. candida* exposed on wax-free clean surfaces.

Figures 1-3 (pl. 6.1) shows some wax-like structures on the leg of *F. candida* exposed on a barley seedling leaf at different magnifications. The structures in Figure 3 (pl. 6.1) were observed at a magnification of 3.3k. They have similarities with the wax structures found on the adaxial surface of barley seedlings leaves (see Figs. 1 and 2, pl. 5.1 in chapter 5). Figure 6 (pl. 6.1) shows the dorsal side of the tarsi with an accumulation of such structures at the tips. Figures 7-12 (pl. 6.2) show various parts of *F. candida* exposed on a cabbage leaf at various magnifications. The solitary structure at the tip of a body hair shown in Figure 9 (pl.6.2) and the structures shown on tarsi in Figure 10 (pl. 6.2) and on leg muscle in Figure 12 (pl. 6.2) were viewed at magnifications of 1.8k, 3.3k and 3.4k respectively. These structures also showed some similarity with the wax bloom observed on cabbage leaves (see Fig. 7, pl. 5.2 in chapter 5).

The structures found on the body of *F. candida* exposed on a tomato leaf (Figures 13 and 14, pl. 6.3) showed no close similarities with the wax particles observed on tomato leaves (see Figures 8-11, pl. 5.2 in chapter 5). In those

PLATE. 6·1



Figures 1 -6 Scanning electron microscopy of *Folsomia candida* after 24 hrs of exposure on different leaves

Explanation of Plate 6.1.

Fig. 1. Leg of *F. candida* exposed on barley leaf, 7.9k.

Fig. 2. Leg of *F. candida* exposed on barley leaf, 720x.

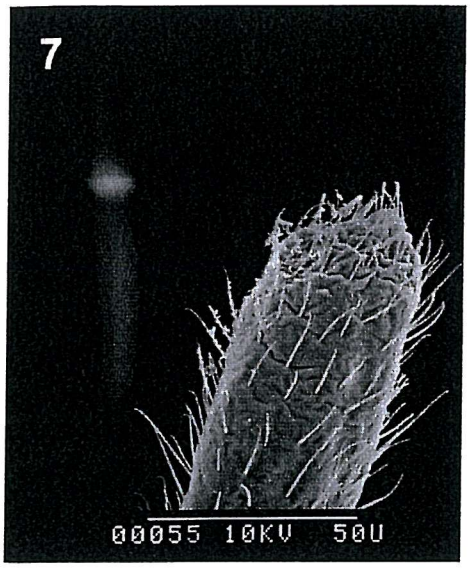
Fig. 3. Leg of *F. candida* exposed on barley leaf, 3.3k.

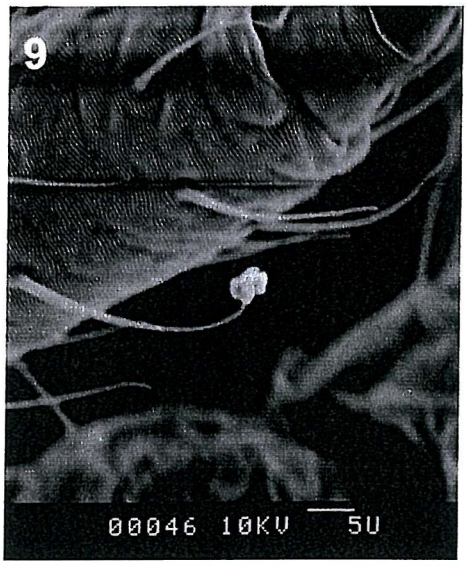
Fig. 4. Body hair of *F. candida* exposed on barley leaf, 3.4k.

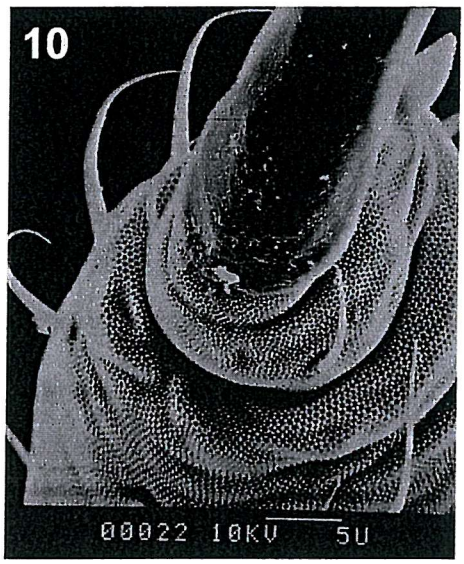
Fig. 5. Leg of *F. candida* exposed on barley leaf, 2.9k.

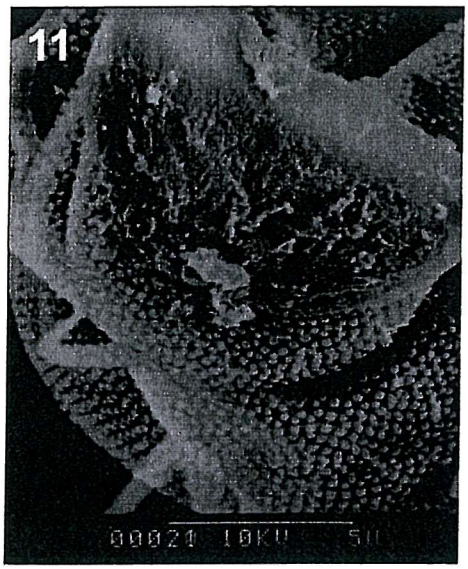
Fig. 6. Leg tip of *F. candida* exposed on barley leaf, 2.7k.

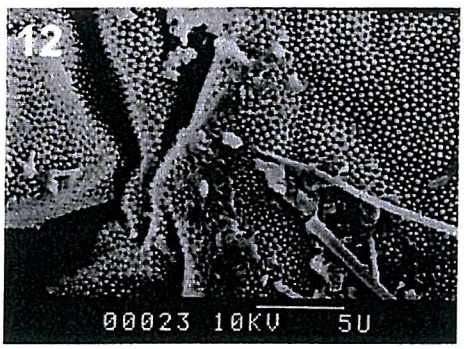
PLATE 6·2











Figures 7-12 Scanning electron microscopy of *Folsomia candida* after 24 hrs of exposure on different leaves

Explanation of Plate 6.2.

Fig. 7. Antennae of *F. candida* exposed on cabbage leaf, 820x.

Fig. 8. Leg of *F. candida* exposed on cabbage leaf, 1.0k.

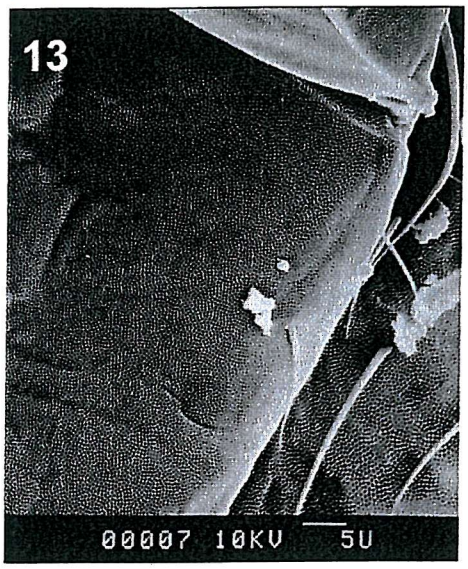
Fig. 9. Hair tip on leg of *F. candida* exposed on cabbage leaf, 1.8k.

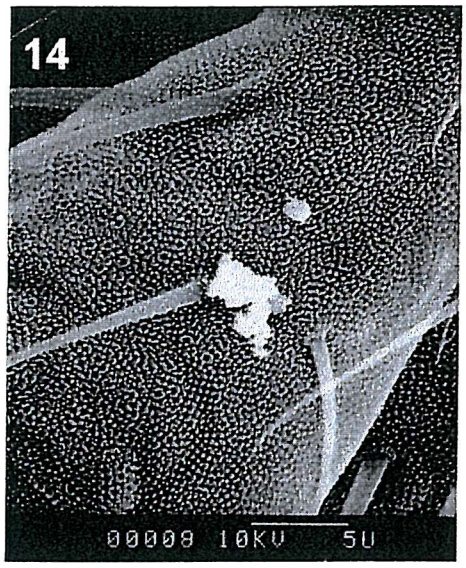
Fig. 10. Leg of *F. candida* exposed on cabbage leaf, 3.3k.

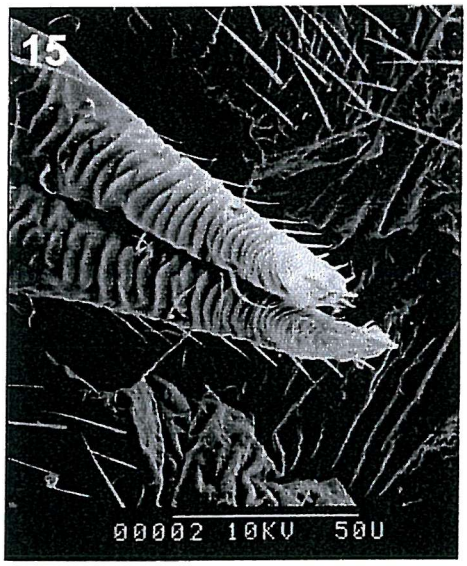
Fig. 11. Leg of *F. candida* exposed on cabbage leaf, 7.3k.

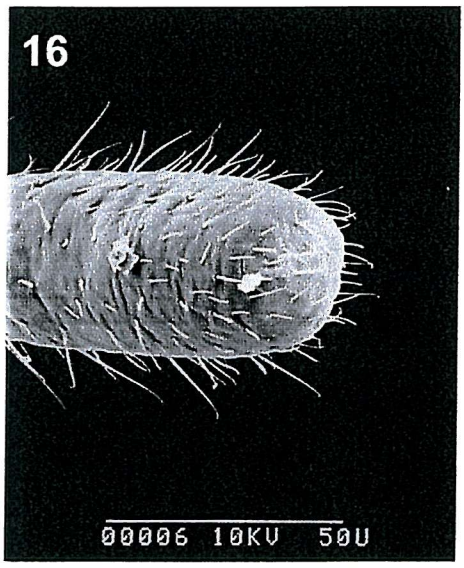
Fig. 12. Leg of *F. candida* exposed on cabbage leaf, 3.4k.

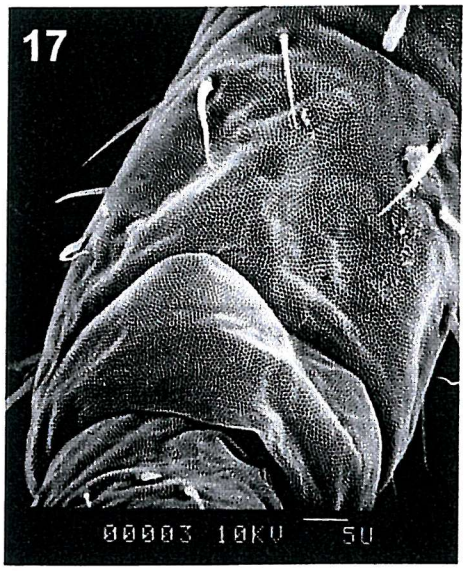
PLATE 6-3

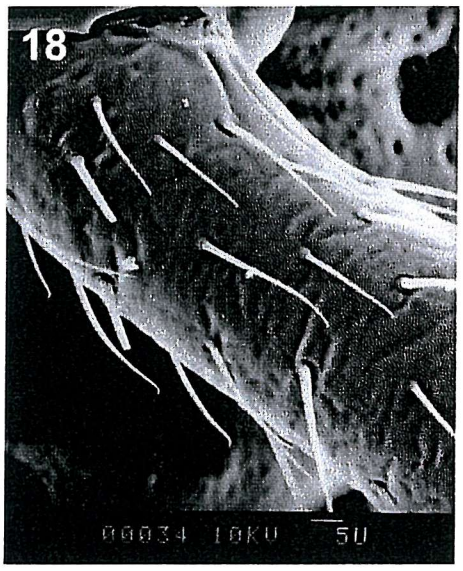












Figures 13-18 Scanning electron microscopy of *Folsomia candid a* after 24 hrs of exposure on different leaves

Explanation of Plate 6.3.

Fig. 13. Tail of *F. candida* exposed on tomato leaf, 1.7k.

Fig. 14. Tail of *F. candida* exposed on tomato leaf, 3.9k.

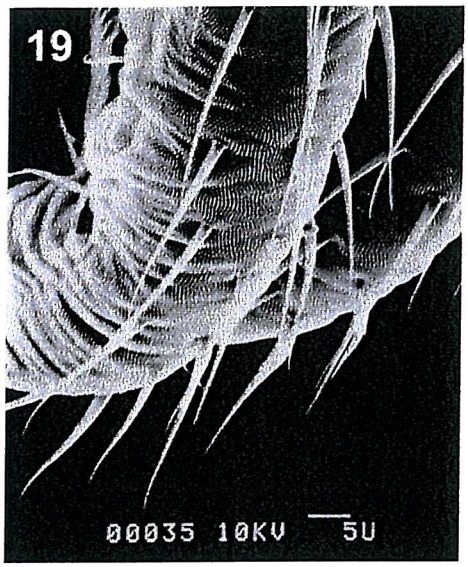
Fig. 15. Tail tip of *F. candida* exposed on sugarcane leaf, 740x.

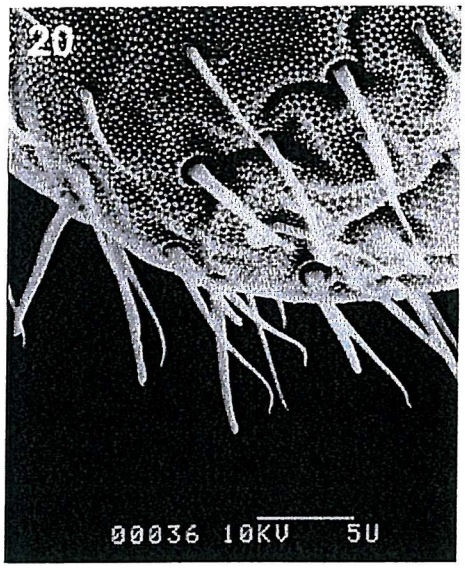
Fig. 16. Antennae of *F. candida* exposed on sugarcane leaf, 950x.

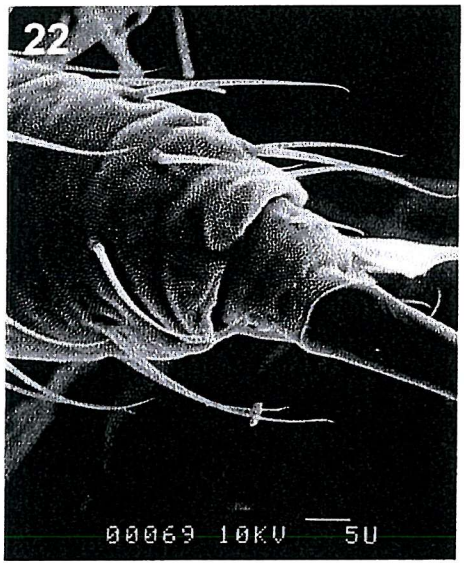
Fig. 17. Leg of *F. candida* exposed on sugarcane leaf, 1.8k.

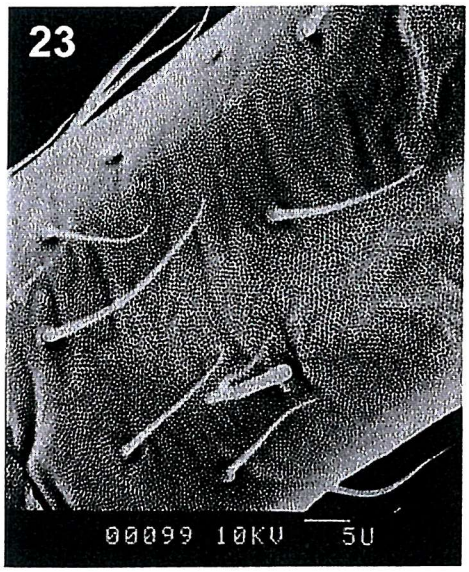
Fig. 18. Leg of *F. candida* exposed on orange leaf, 1.2k.

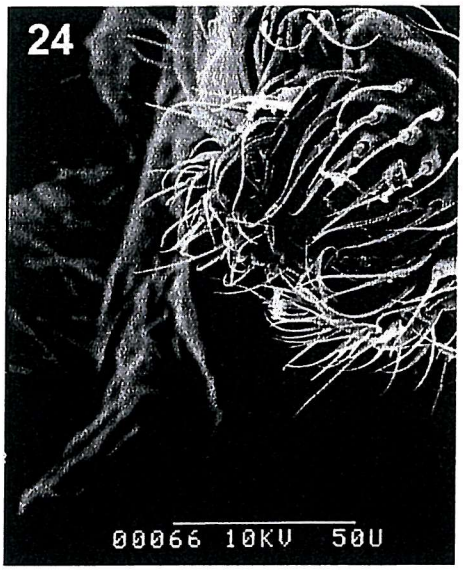
PLATE 6·4











Figures 19-24 Scanning electron microscopy of *Folsomia candida* after 24 hrs of exposure on different leaves

Explanation of Plate 6.4.

Fig. 19. Tail of *F. candida* exposed on orange leaf, 1.6k.

Fig. 20. Tip of antennae of *F. candida* exposed on orange leaf, 3.8k.

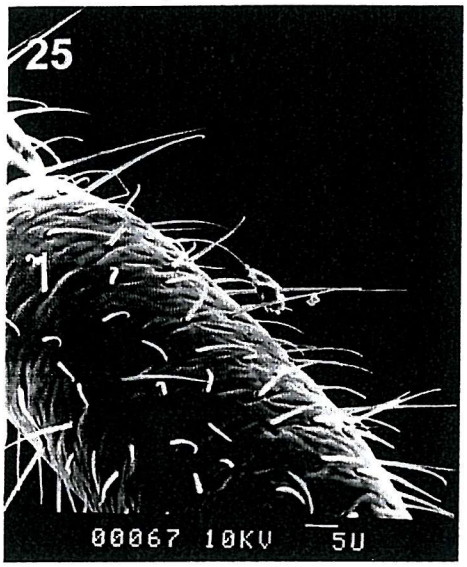
Fig. 21. Antennae of *F. candida* exposed on dwarfbean leaf, 710x.

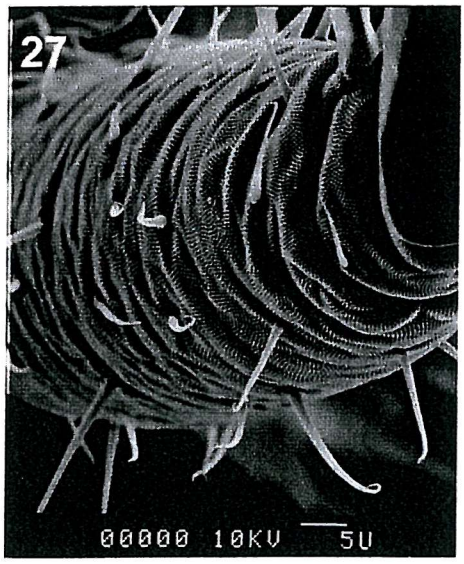
Fig. 22. Leg of *F. candida* exposed on dwarfbean leaf, 1.7k.

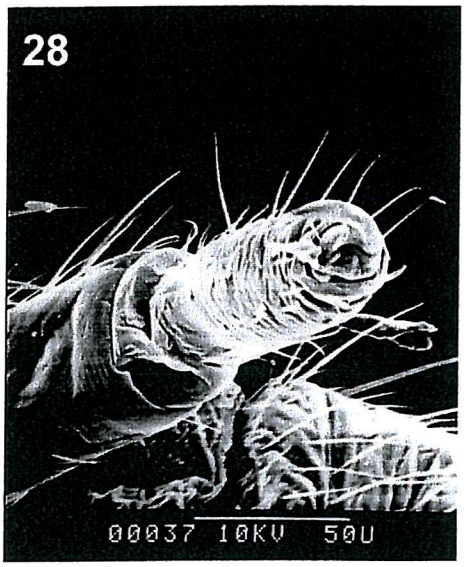
Fig. 23. leg of *F. candida* exposed on dwarfbean leaf, 1.8k.

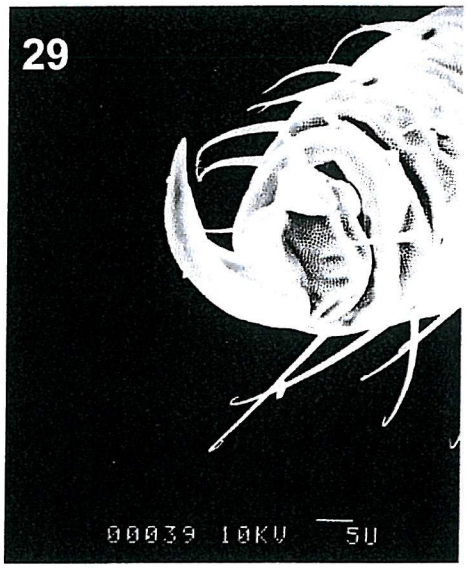
Fig. 24. Mouth of *F. candida* exposed on rape var. tanto (young) leaf, 720x.

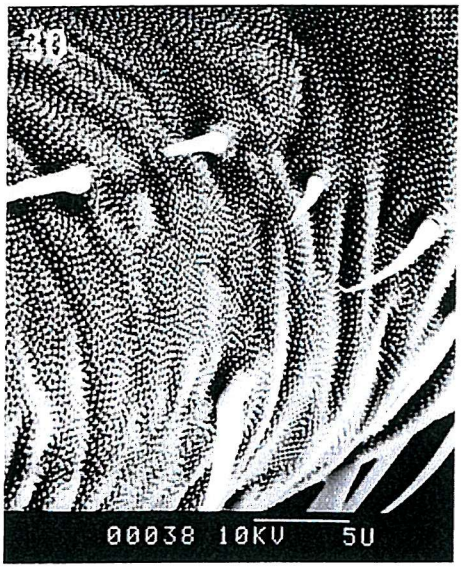
PLATE 6•5











Figures 25-30 Scanning electron microscopy of *Folsomia candida* after 24 hrs of exposure on different leaves

Explanation of Plate 6.5.

Fig. 25. Antennae of *F. candida* exposed on rape var. tanto (old) leaf, 1.2k.

Fig. 26. Tail of *F. candida* exposed on maize (old) leaf, 740x.

Fig. 27. Leg of *F. candida* exposed on maize (young) leaf, 1.8k.

Fig. 28. Leg of *F. candida* exposed on clean petridish, 620x.

Fig. 39. Leg of *F. candida* exposed on clean petridish, 1.4k.

Fig. 30. Leg of *F. candida* exposed on clean petridish, 3.8k.

figures, waxes were observed as scattered tiny crystals. However it is possible that these particles were picked up on the body of the insect as clusters.

Figure 16 (pl. 6.3) shows the antennae of a *F. candida* exposed on a sugarcane leaf, at a magnification of 950X. Here, the structures on setae and cuticle showed a close similarity with those observed on the adaxial surface of sugarcane leaves (see Figure 13, pl.5.3 in chapter 5). The structures found on the body of *F. candida* exposed on orange leaf (Figure 18, pl.6.3) also had similarities with those observed on orange leaves (see Figure 20, pl. 5.4). In both cases the magnification was 1.2k. The low frequency of such structures on different body parts of *F. candida* exposed on sugar cane, orange, dwarf bean (Figures 15-23, plates 6.3 and 6.4) and on maize (Figures 26 and 27, pl. 6.5), suggests that the insects encounter less wax and it is known that lower amounts of wax are present on the adaxial surfaces of such leaves.

The most convincing evidence for pick-up of wax by the insects was that no such structures were observed on the body of *F. candida* exposed on a clean surface for 24 hours (Figures 28-30, pl. 6.5).

6.4 Discussion

Insect may pick up superficial waxes from treated leaf surfaces already contaminated with insecticide deposits. Earlier studies suggested that insecticide deposits are transferred through a process of diffusion to the insect body or to the plant surface (Lewis and Hughes, 1957). For lipophilic substances, there is competition between the leaf surface and the insect body and the one which possesses more waxes has a greater chance of accumulating insecticide deposits. Thus if the leaf surface has more waxes than the insect cuticle it is more likely that the insecticide will be less available to the insect (Lewis and

Hughes, 1957). But the whole transfer may be as described. The theory of transfer through diffusion, until a steady state is reached is more appropriate when both the surfaces are more or less static. Here, although the leaf surface was more or less static the exposed *F. candida* was moving. In addition to surface chemical properties, the physical properties of the cuticle and the behavioural activity of the insect will influence pick-up and therefore affect the transfer process. During behavioural activities such as walking, grooming, rubbing etc. the insects exert extra forces on the leaf surface which disrupt the normal diffusion process. The exposed and active insect can easily pick up or rub off superficial substances from the leaf surface. Epicuticular waxes are the most likely to be affected. In the present experiment leaves of barley seedlings and the species of the family Brassicae had comparatively high epicuticular wax contents. Deltamethrin deposits, due to high lipophilicity, can accumulate in leaf surfaces with high wax levels and can be retained intact without further penetration or loss by volatilization. The exposed *F. candida* then are more likely to pick up the deposits not only by simple diffusion but also by scraping up the epicuticular waxes where the active ingredients are stored. Once picked up the contaminated wax, the active ingredient of the insecticides can slowly be released and transfer to the site of action by diffusion.

The primary and predominant sites of particle attachment are the ventral body parts and legs, which come in direct contact with the treated surface. However, it is quite possible that during cleaning movements, the legs, antennae and setae with their numerous spines act as instruments of distribution of particles from the contaminated area to apparently uncontaminated parts of the body. As this cleaning movement take place frequently, a proportion of particles is constantly being transferred to different

parts of the body (Lewis and Hughes, 1957).

Deltamethrin residues on leaves with very low amounts of waxes, such as dwarf bean, orange, maize and sugar cane may not be retained successfully on the surface. Because of their lipophilicity they could leach further via stomatal infiltration or absorption through trichomes and other surface structures, into the cuticular waxes. Even the deposits that are retained on the surface are more vulnerable to degradation and volatilization by natural "weathering".

In the present experiments it was also observed that, after spraying in a Potter Tower, leaf surfaces with large amounts of epicuticular wax (such as those of barley seedlings and species of the Brassicae family) spray deposits remained as individual tiny droplets with uniform distribution for some period. In contrast, on leaf surfaces with little wax, (such as orange, dwarf bean, sugar cane and maize leaves) the deposit drops join together a short time after landing, to make several large isolated drops of irregular shape, leaving some areas free of liquid deposition.

To utilise an insecticide to its full potential, the mechanism which results in increased availability of insecticide to the target insect, and the way this mechanism changes on substrate, must be explored.

Usually, surface hydrophobicity determines the extent of adhesion on that particular surface. For example, when oil-based formulations of pirimicarb are sprayed on a polar surface of broad bean leaves (*Vicia fabae*), it is observed that the droplet remains available on the leaf surface without spreading. Black bean aphids (*Aphis fabae*), which come into contact with such a droplet, become enveloped in a thin film which spreads, pulling the legs and antenna

into close proximity with the body (Hart, 1979). Although the spread of a droplet on the plant surface increases the chance of a moving insect encountering the deposit, the chance of it being transferred to the exposed insect is also reduced as the material becomes more intimate with the relatively more polar underlying surface (Ford & Salt, 1987). The presence of epicuticular wax makes the surface hydrophobic. Lipophilic insecticides spread into a thin film on waxy surfaces of both waxy leaves and the insect cuticle, but remain as discrete droplets proud of the surface when placed on a hydrophilic surface, such as leaves of broad bean (Hart, 1979).

The efficacy of a contact insecticide depends upon its availability on the plant surface. The accumulation of active ingredient by the exposed insect will be reduced by transfer of that a.i. into the underlying tissues of the leaves, which makes systemic insecticides more effective. It can be predicted that a nonpolar insecticide has a better chance of being retained on a hydrophobic surface. Scanning electron microscopy provides some support for this. It shows evidence for pick-up of contaminated wax particles from the leaf surface and thus an increased chance of active ingredient transfer.

Low susceptibilities of *F. candida* for dimethoate treated leaf surfaces of barley, cabbage, sugar cane, maize, wheat and tomato seem to be more attributable to surface properties such as roughness and glaucousness rather than the amount of their epicuticular waxes. The above surfaces are mostly non-glaucous, having macro-roughness (for example leaves of tomato with dense trichomes) and micro- and ultra-microscopic roughness (such as cell shape and arrangements of sugar cane and maize and waxes of barley and cabbage). Leaf surfaces on which *F. candida* was found to be most susceptible are comparatively smooth and glossy (such as orange) and amorphous (for example dwarf bean

leaf). Cabbage and rape leaves both had glaucous surfaces, but the waxes of cabbage leaves are more rough and process occasional blooms (see Figures 4 and 7, plates 5.1 and 5.2), differing from the thin crystalline waxes of rape (see Figures 31-36, pl. 5.6) which has few wax granules mainly concentrated on and around the stomata.

Although the stomatal and trichome frequency were not quantified in the present studies, stomatal infiltration and diffusion through trichomes can also play a role in pesticide availability along with the systematic properties of dimethoate. Further studies with other systemic and non-systemic OPs and stomata and trichome frequencies along with surface roughness could provide important information regarding the toxicity responses of *F. candida* on different leaf surfaces.

Chapter 7

Leaf surface wettability and its role in the mediation of toxicity to *Folsomia candida* Willem (Collembola:Isotomidae) exposed on different leaf surfaces

7.1 Introduction

In the previous chapters (Chapters, 1 and 6) it is revealed that almost every plant surface posesses hydrophobic properties to variable degrees. To deliver active ingredient of any foliar applied pesticide to the plant surfaces, a liquid carrier, mainly water, is added to increase the volume of spray chemical to cover large areas. Foliar applied pesticides therefore need to overcome the barrier of surface hydrophobicity, to achieve successfull coverage, retention and penetration. Due to the differential nature of the hydrophobicity on different plant surfaces careful consideration must always given to the wetting properties of different pesticides and formulations. Adding surface active agents increases the wetting properties of a particular pesticide. Once impacted, the behaviour of foliar sprayed deposits and subsequent transfer to the target invertebrates is influenced considerably by the affinity of the deposit for the plant surface (Ford & Salt, 1987). Surface tension and the force of adhesion are the two main factors which determine such affinity of a deposit and thereby the wettability. The role of surface active agents is therefore to reduce the surface tension of the liquid drops applied on the plant surface and to increase their retention by reducing the chance of droplet reflections. This again depends on droplet size. There is a critical droplet diameter and critical surface tension above or below which retention is either very low or very high (Brunskill, 1956; Hartley & Brumskill, 1958). The sigmoidal relationship between surface tension and retention was also confirmed by Anderson and Hall, 1989. Once spray droplets have been retained by foliage and reached equilibrium, then the degree of spreading and coverage will mainly be governed by the surface tension forces of the liquids and the surface characteristics. Interactions between spray droplets and plant surfaces

involve a series of events that affect application efficiency and performance of agrochemicals. These includes impaction, reflection, retention, spreading, drying and deposit formation (Hartley & Graham-Bryce, 1980). Spray interaction with plant surfaces as affected by spray volume and surfactants is reviewed with special reference to droplet:plant surface relationships during impaction, drying and deposit formation by Bukovac, *et al.* (1995). There is a substantial body of work on leaf surface characteristics and deposit behaviour, such as wetting (Holloway, 1969; 1970), impaction and redistribution (Baker *et al.* 1983) retention (Furmidge, 1962; Holloway, 1994), spreading (Boize *et al.* 1976; Baker *et al.* 1983), penetration (Baker *et al.* 1992). Some of the work is reviewed in detail and discussed in Chapters 1, 3 and 4 and 6.

Most of these publications focused on efficiency in spray application and physico-chemical behaviour of leaf surfaces and sprayed chemicals. No or little attention was given to the ultimate toxicity responses of exposed invertebrates in relation to those interactions.

To a large extent, wettability of leaf surfaces plays an important role in the pre-penetration processes, such as, deposition, distribution and retention of spray chemicals (Holloway, 1970). The variation in the contact angles represents the rate of adhesion on both polar and hydrophobic surfaces. In Chapter 2 and 3 relationships between the hydrophobic chemicals (such as wax) and the toxicity responses of exposed invertebrates to lipophilic pesticide, encouraged the present study, as hydrophobicity of plant surfaces governed the wettability, an important consideration in pesticide formulation.

In this study wettability of leaf surfaces by water (i.e. intrinsic wettability) and by two different spray solutions is examined and the physical behaviour of droplets is studied in relation to leaf surface characteristics to explore their role in ultimate mortality responses of exposed invertebrates

and to address the following questions:

1) Is there a direct relationship between the inherent wettability of leaf surfaces and the toxicity responses of exposed organisms ?

2) Can the inherent wettability of leaf surfaces by water be used in the interpretation of toxicity responses ?

3) How far can the formulation and spray solution change the wettability of the surface and how do deposits behave on different leaf surfaces after impaction?

4) How far do these changes affect the toxicity responses of exposed organisms to pesticide residues on different leaf surfaces.

7.2 Materials and methods

7.2.1 Plant materials

Plants were selected mainly from crops of major economic importance and included examples of leaves having glaucous, sub-glaucuous and glossy surfaces. They comprised of orange, cabbage, barley, wheat, sugarcane, maize, tomato, pear, rape var. Tanto, rape var. Lirawell, rape var. Starlight and dwarf bean. Barley leaves were collected from seedlings. Leaves from plants that had reached the vegetative stage were collected from glasshouse grown orange, cabbage, tomato, sugarcane and dwarfbean plants. Both old and young leaves were used from maize and three varieties of rape. These were collected, at the vegetative stage, from plants grown in the glasshouse. Pear leaves were collected from the campus surrounding the Biology Department at the University of Southampton, Hampshire UK. Wheat leaves were collected from a farmer's field in Manydown, Hampshire, UK. The research was undertaken in the Department of Physical Chemistry at Portsmouth University and the Department of Biology at Southampton University. All the glasshouse grown plants were taken to the laboratory in their pots before each experiment. Fresh leaves were taken from the plants just before mounting and observation. Leaves of orange, sugarcane, pear and wheat were not carried down to the laboratory due to their size and field growth, and were collected from the their place of origin and taken to the laboratory. Care being taken to avoid

any physical damage.

7.2.2 Preparation of sample leaves

Leaves were cut into appropriate sizes for the specimen chamber of the contact angle goniometer and the sample leaf cuttings were attached to a specially-designed leaf attachment base. This was make from two rectangular plastic plates, each 3.5 X 5 cm. One of the plates was cut in the middle to make a central rectangular hole of 1.4 X 3.6 cm. The cut portion of the plate was then slightly filed along the edges, so that it could fit within the hole of the original plate smoothly and with enough space to accommodate the thickness of the leaf. The cut portion was then glued onto the middle of the 2nd plate. Before observation, leaf samples were placed on the 2nd plate. The 1st plate (with the hole) was then placed over the leaf sample and was slid down gently and carefully so that the middle portion of the sample leaf became exposed through the cut hole. The purpose of this technique was to make the sample leaves as flat as possible without touching the portion of the sample where the contact angle of liquid drop was to be measured. This plastic platform was then placed in the specimen chamber of a contact angle goniometer (Kernco Instruments Co Ltd. Texas, USA). A series of 1 to 2 µl droplets of double-distilled water, deltamethrin 2.5EC (0.5mg/ml) and dimethoate 40EC (0.212mg/ml) were placed separately on the different leaf types and species from a gas-tight microsyringe (Series II Syringe, SGE, Australia). Before each working session, the specimen chamber of the goniometer was saturated with water vapour by pouring distilled water into the bottom of the chamber and replacing the lid for 30 to 60 minutes.

7.2.3 Working Principle of Goniometer

The method used for measuring the contact angle was as described by Gückel and Synnatscke (1975). In brief, the method was as follows- a drop of liquid (1), from a micrometer syringe (2), was placed on a small piece of leaf (3) lying horizontally, and the contact angle which was set

up in the course of time was measured with the angle goniometer telescope (4). A lamp (5) illuminated the specimen chamber (6). It was important that the cell space was saturated with water vapour. In order to measure the internal temperature of the measuring space a temperature sensor can be inserted through the aperture (7).(see Fig. 7.1)

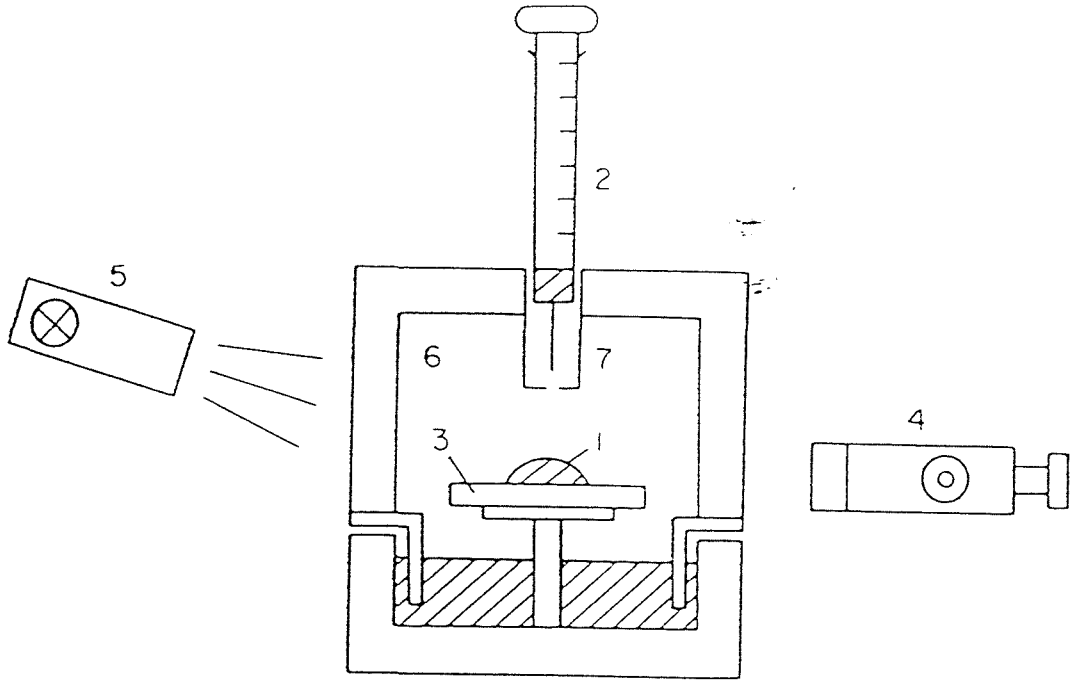


Figure 7.1 Measuring procedure for the contact angle goniometer (adapted from Gückel and Synnatschke, 1975)

7.2.4 Photography of droplets using the goniometer

The original eye-piece was removed from the attached compas.. apparatus of the goniometer. A Kyawa SD-Z eyepiece-to- camera adaptor was then fitted in its place using a specially made PVC sleeve.

A Nikon camera was fitted to the other end of the Kyawa adaptor and focused through the camera's eyepiece using the telescope focus control. A test film was shot to calculate

the correct exposures. The film used was Kodak Gold 400 ISO to keep exposure time as short as possible. A cable release was used to minimize the vibration. Pictures were taken with or without placing different colour filters on the illumination source of the goniometer.

7.2.5 Statistical analysis

Residual susceptibility data for *F. candida* described in Chapter 3 were used. Regression analysis was undertaken to establish correlations between the contact angle and the LD_{50} values of *F. candida* for deltamethrin and dimethoate. Individual regression models were fitted for the correlation between wax content (wax content data described in Chapter 6) and contact angle of water, deltamethrin and dimethoate solution on different leaf surfaces.

7.2.6 Selection of deltamethrin and dimethoate concentration

In the present bioassay a series of doses were used to determine the residual susceptibilities of *F. candida* to deltamethrin and dimethoate (see chapter 3). Due to the extensive nature of the experiments, only the highest concentrations of pesticide used in the bioassays were selected for contact angle studies.

7.3 Results

7.3.1 Comparative studies on the initial contact angle of water, deltamethrin and dimethoate solution on different leaf surfaces

Comparative determinations of contact angles of water, deltamethrin and dimethoate solution at the concentration of 0.5mg ml^{-1} and 0.212mg ml^{-1} respectively are shown in Table 7.1. Instead of presenting the data for one angle of the drop or an average of the left and right angles, both the angle readings are shown to facilitate the further interpretation of deposit behaviour on different leaf surfaces from the time of impaction, and during subsequent changes until the apparent drying of the deposit.

The highest contact angles of water were observed on barley

Table- 7.1. Contact angle of water, deltamethrin and dimethoate solution at impaction on different leaf surfaces.

Leaf species	Water drop (1ul) (SD±)			Deltamethrin 2.5EC solution (1ul) drop (@ 0.5mg/ml (SD ±)			Dimethoate 40 EC solution (1ul) drop (@ 0.212 mg/ml) (SD ±)		
	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average
Barley (S)	147.27 (2.01)	147.53 (2.06)	147.40 (1.99)	89.87 (0.72)	89.93 (0.85)	89.90 (0.73)	106.93 (3.45)	106.47 (3.91)	106.70 (3.61)
Cabbage (o)	132.33 (7.62)	132.07 (7.28)	132.20 (7.39)	48.2 (3.71)	48.33 (3.38)	48.27 (2.71)	114.20 (10.71)	115.53 (11.19)	114.87 (10.78)
Tomato(o)	111.00 (7.09)	111.60 (9.71)	111.30 (7.63)	56.00 (5.05)	55.47 (5.64)	55.73 (3.71)	91.27 (6.23)	91.20 (5.90)	91.23 (5.56)
Pear(o)	92.33 (2.60)	91.93 (2.29)	92.13 (2.26)	43.00 (3.14)	42.93 (3.21)	42.97 (3.11)	71.27 (5.23)	71.20 (5.09)	71.23 (5.12)
Sugarcane (o)	78.73 (10.12)	78.40 (11.32)	78.57 (10.50)	44.20 (2.53)	44.07 (2.46)	44.13 (2.35)	67.40 (5.76)	69.00 (6.57)	68.20 (6.03)
Wheat (o)	103.67 (9.07)	103.20 (7.18)	103.43 (7.89)	62.27 (4.85)	60.60 (5.70)	61.10 (4.85)	88.80 (8.02)	88.40 (7.91)	88.60 (7.90)
Orange (o)	82.27 (4.63)	82.47 (4.57)	82.37 (4.50)	37.87 (2.47)	37.47 (3.28)	37.67 (2.88)	74.20 (5.61)	75.70 (5.97)	74.97 (5.70)
Dwarfbean (o)	40.00 (3.363)	40.60 (2.92)	40.27 (3.21)	36.87 (3.76)	36.27 (4.09)	36.57 (3.68)	38.33 (1.66)	38.33 (1.74)	38.33 (1.70)
Rape v. Tanto (0)	123.00 (1.67)	123.00 (1.67)	123.00 (1.67)	50.00 (1.67)	50.00 (1.41)	50.00 (1.41)	104.40 (2.94)	104.20 (2.63)	104.30 (2.79)
Rape v. Tanto (y)	140.20 (3.54)	140.20 (3.54)	140.20 (3.54)	72.80 (1.72)	72.80 (1.72)	72.80 (1.72)	114.80 (2.56)	114.80 (2.56)	114.80 (2.56)
Rape v. Lirawell (0)	120.80 (2.04)	120.80 (2.04)	120.80 (2.04)	48.80 (2.32)	48.80 (2.32)	48.80 (2.32)	105.00 (2.28)	104.00 (2.58)	104.70 (2.40)
Rape v. Lirawell (Y)	139.00 (3.41)	139.00 (3.41)	139.00 (3.41)	69.80 (2.79)	70.60 (2.33)	70.20 (2.48)	114.00 (2.76)	113.60 (3.20)	113.80 (2.98)
Rape v. Starlight (o)	124.60 (1.62)	124.60 (1.62)	124.60 (1.62)	50.20 (1.60)	51.00 (2.19)	51.00 (2.19)	108.00 (2.00)	108.00 (2.00)	108.00 (2.00)
Rape V. Starlight (Y)	142.60 (3.01)	142.60 (3.01)	142.60 (3.01)	75.20 (2.79)	75.40 (2.87)	75.30 (2.82)	117.80 (1.72)	117.60 (1.85)	117.70 (1.78)
Maize (0)	68.30 (6.94)	67.80 (7.41)	68.05 (7.10)	43.73 (2.91)	43.67 (3.05)	43.70 (2.88)	62.47 (5.85)	63.70 (6.88)	63.10 (6.34)
Maize (Y)	68.30 (5.93)	67.80 (5.93)	68.30 (5.93)	60.80 (4.28)	61.80 (4.25)	61.17 (4.43)	62.27 (4.34)	62.67 (4.04)	62.47 (4.11)

s, Seedling; o, Old; y, Young

seedlings, followed by rape v. Starlight (young), rape v. Tanto (young), rape v. Lirawell (young), cabbage (old), rape v. Starlight (old), rape v. Tanto (old), rape v. Lirawell (old), tomato (old), wheat (old), orange (old), sugar cane (old), maize (young), maize (old) and dwarf bean (old). Glaucous and semi-glaucous surfaces showed higher contact angles than smooth and non-glaucous or amorphous surfaces. Large differences were observed between the old and young leaves. Significant differences in contact angle resulting from varietal differences were not observed.

In comparison with the contact angle of water on different leaf surfaces, contact angles of both insecticides were lower probably because of the surfactant/adjuvants that were incorporated in the formulation for better wetting. Leaf surfaces forming high contact angles with water and having high amounts of wax (see chapter 6) showed much greater reduction in contact angle with deltamethrin solution. In many cases, such as cabbage , rape v. Tanto (old), rape v. Lirawell (old), rape v. Starlight (old), tomato and pear, this reduction was more than 50%. This range of reduction of contact angles produced by deltamethrin, which is lipophilic, provides evidence of the role of fatty substances in increasing the wetting by other lipophilic chemicals. This hypothesis is supported further by the contact angle data for dimethoate on leaf surfaces with high amounts of wax (Table-7.1). With dimethoate, contact angles were much greater on leaf surfaces with high amounts of wax than was the case for deltamethrin. In some cases, the reduction in contact angles was only around 13-22% (approx.) (Figure 7.3), indicating that surfaces with high hydrophobicity were much less wettable and spreadable by dimethoate than deltamethrin.

Another interesting feature of the study was that leaf surfaces with lower contact angles of water did not show high levels of reduction with either of the insecticides tested, although there are still differences between them. For example, the contact angle of water on dwarf bean was 40.27 and those of deltamethrin and dimethoate were 36.57 and 38.33

respectively. Similarly, the contact angles of water on maize (old) and maize (young) were 68.05 and 68.30 respectively and those of deltamethrin and dimethoate were 43.70 and 61.17, and 63.10 and 62.47 respectively.

It can be concluded from these results that the wettability of leaf surfaces with high hydrophobicity was increased by the formulation for both insecticides, although deltamethrin produces greater wettability responses than dimethoate. In contrast, the wettability of leaf surfaces with low water contact angles (i.e. more wettable) remained more or less unchanged with the pesticides.

7.3.2 Time series studies on the reduction of advancing contact angle of water

In Table-7.2 comparative figures are given for reduction of advancing contact angle of water over time after initial deposition on the different leaf surfaces. On orange and dwarf bean leaves the contact angles reduced to less than 15° after 60 minutes of impaction in the static conditions of the specimen chamber of the goniometer. Water drops were found to be least spreadable on barley seedlings and still showed high contact angles of 140° after 60 minutes of impaction, indicating only a small reduction of 4.4% in the initial contact angle. The 2nd place in percentage reduction of advancing contact angle was occupied by the young leaves of rape v. Starlight (Figure 7.2) followed by the leaves of rape v. Tanto (young), cabbage, rape v. Lirawell (young), rape v. Tanto (old), rape v. Starlight (old), rape v. Lirawell (old), tomato, maize (young), maize (old), wheat, sugarcane and pear. Wheat, sugarcane and pear showed more than 50% reduction in their advancing contact angles after 60 minutes of impaction and those for orange and dwarf bean were greater than 80%.

The test *F. candida* were introduced to the sprayed leaf surface after 30 minutes (Chapter 3), and it may therefore be more appropriate to look at the percentage reduction in advancing contact angles of water on each leaf type after 30

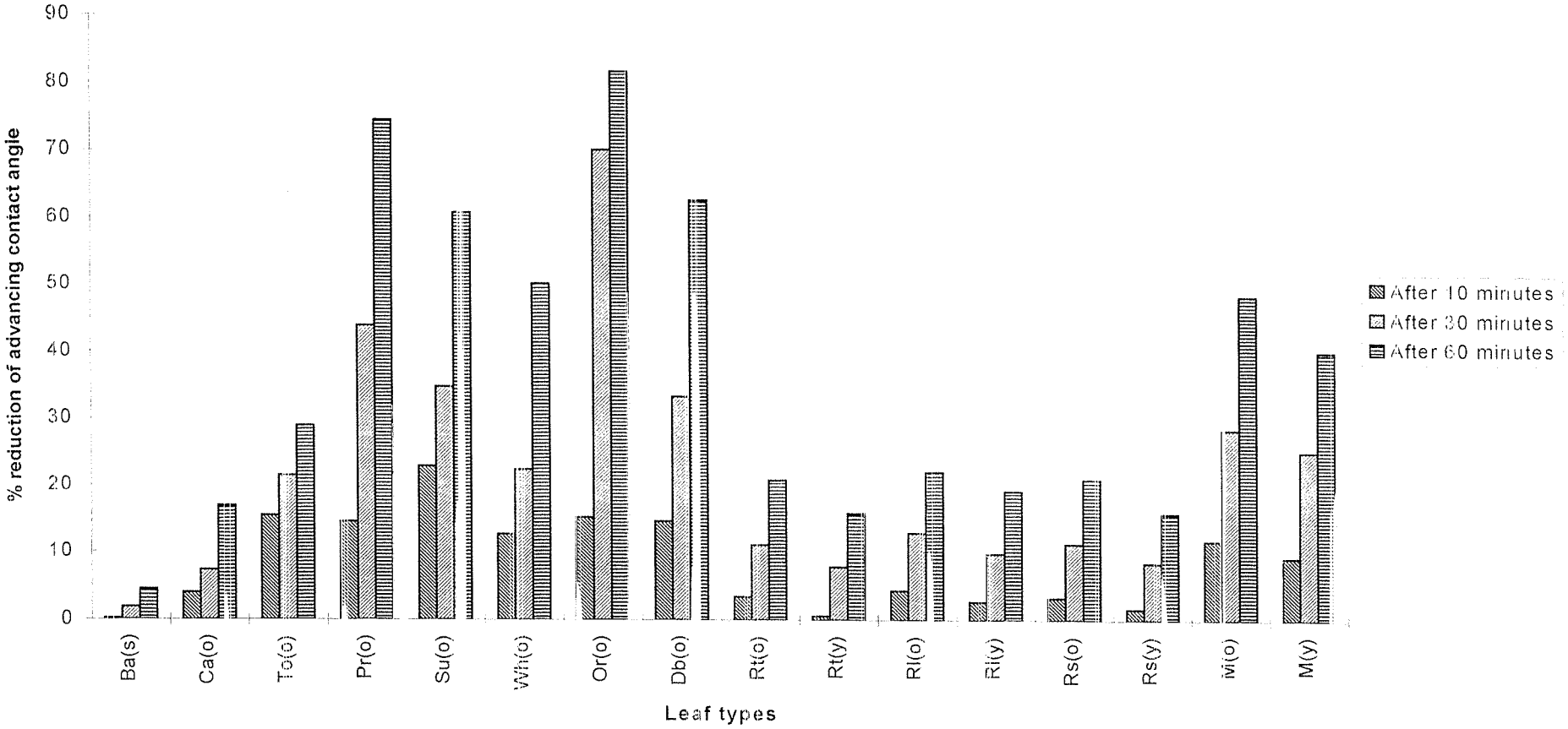
Table-7.2. Time series studies on the advancing contact angle of water on various leaf types

Leaf species	After 0-30 secs of impaction (SD ±)			After 10 minutes (SD ±)			After 30 minutes (SD ±)			After 60 minutes (SD ±)		
	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average
Barley(s)	147.27 (2.01)	147.53 (2.06)	147.40 (1.99)	147.13 (1.67)	147.13 (1.67)	147.13 (1.67)	144.60 (1.62)	144.60 (1.62)	144.60 (1.62)	140.93 (2.57)	140.87 (2.60)	140.87 (2.49)
Cabbage(o)	132.33 (7.62)	132.07 (7.28)	132.20 (7.39)	128.28 (5.97)	125.71 (6.29)	127.00 (5.95)	124.82 (13.68)	120.18 (15.32)	122.50 (14.21)	110.36 (16.17)	109.36 (14.18)	109.86 (14.70)
Tomato (o)	111.00 (7.09)	111.60 (9.71)	111.30 (7.63)	95.50 (8.17)	92.75 (8.52)	94.13 (7.93)	88.62 (4.55)	86.25 (7.82)	87.44 (5.69)	81.36 (19.97)	76.64 (16.70)	79.00 (17.96)
Pear (o)	92.33 (2.60)	91.93 (2.29)	92.13 (2.26)	76.33 (6.51)	75.53 (6.30)	75.93 (6.29)	51.73 (4.27)	51.73 (4.01)	51.73 (4.05)	23.40 (1.78)	23.60 (1.58)	23.50 (1.62)
Sugarcane (o)	78.73 (10.12)	78.40 (11.32)	78.57 (10.50)	60.07 (10.79)	61.00 (11.82)	60.53 (11.23)	51.20 (10.96)	52.60 (12.39)	51.23 (11.14)	30.33 (11.43)	31.27 (11.86)	30.80 (11.57)
Wheat (o)	103.67 (9.07)	103.20 (7.18)	103.43 (7.89)	90.20 (7.08)	90.47 (6.57)	90.33 (6.66)	79.87 (6.84)	80.47 (7.14)	80.17 (6.90)	52.60 (8.64)	50.93 (8.42)	51.50 (8.30)
Orange (o)	82.27 (4.63)	82.47 (4.57)	82.37 (4.50)	69.87 (5.18)	69.80 (4.71)	69.83 (4.88)	24.53 (2.60)	24.60 (2.87)	24.57 (2.66)	<15	<15	<15
Dwarfbean (o)	40.00 (3.63)	40.60 (2.92)	40.27 (3.21)	34.20 (3.69)	34.53 (3.38)	34.37 (3.02)	27.00 (3.88)	26.67 (2.75)	26.83 (3.00)	<15	<15	<15
Rape v. Tanto (o)	123.00 (1.67)	123.00 (1.67)	123.00 (1.67)	118.80 (1.17)	118.80 (1.17)	118.80 ((1.17)	109.40 (0.80)	109.40 (.080)	109.40 (0.80)	97.40 (1.50)	97.40 (1.50)	97.40 (1.50)
Rape v. Tanto (y)	140.20 (3.54)	140.20 (3.54)	140.20 (3.54)	139.40 (3.14)	139.40 (3.14)	139.40 (3.14)	129.20 (2.79)	129.20 (3.12)	129.20 (2.94)	118.00 (2.45)	118.00 (2.45)	118.00 (2.45)
Rape v. Lirawell (o)	120.80 (2.04)	120.80 (2.04)	120.80 (2.04)	115.60 (1.74)	115.60 (1.74)	115.60 (1.74)	105.20 (1.72)	105.20 (1.72)	105.20 (1.72)	94.00 (2.00)	94.20 (2.14)	94.10 (2.03)
Rape v. Lirawell (y)	139.00 (3.41)	139.00 (3.41)	139.00 (3.41)	135.20 (3.87)	135.20 (3.87)	135.20 (3.87)	125.20 (3.43)	125.40 (3.72)	125.30 (3.57)	112.20 (3.72)	112.20 (4.17)	112.30 (3.92)
Rape v. Starlight(o)	124.60 (1.62)	124.60 (1.62)	124.60 (1.62)	120.60 (1.74)	120.40 (1.62)	120.50 (1.67)	110.40 (0.80)	110.80 (1.17)	110.60 (0.97)	98.60 (1.36)	98.40 (1.36)	98.50 (1.22)
Rape v. Starlight (y)	142.60 (3.01)	142.60 (3.01)	142.60 (3.01)	140.40 (3.26)	140.20 (3.37)	140.30 (3.31)	130.60 (2.73)	130.60 (2.73)	130.60 (2.73)	120.60 (4.59)	120.00 (3.69)	120.30 (4.12)
Maize (o)	68.30 (6.84)	67.80 (7.41)	68.05 (7.10)	60.00 (6.74)	60.10 (6.67)	60.05 (6.70)	48.70 (7.50)	48.60 (7.34)	48.65 (7.42)	35.00 (5.29)	35.20 (4.75)	35.10 (5.00)
Maize (Y)	68.30 (5.93)	68.30 (5.93)	68.30 (5.93)	62.00 (5.04)	62.00 (5.27)	62.00 (5.15)	51.00 (4.52)	51.10 (4.32)	51.05 (4.42)	40.90 (3.75)	40.80 (3.57)	40.85 (3.65)

S = seedling; o = old; y = young

minutes of impaction. It must be noted, however, that the conditions in the goniometer specimen chamber and the insectary were not the same. In the specimen chamber, the air was saturated with moisture and there was relatively low air movement which allowed the drop to remain without drying for an extended period. In the insectary, due to different moisture levels and air movement, most of the drop deposits on leaf surfaces apparently dried within 30 to 60 minutes after impaction. Drop size also needs to be taken into consideration. For contact angle measurements drops were mainly in the 1 to 2µl size range, whereas spray drops from the Potter Laboratory Spray Tower were much smaller in size and more subject to rapid drying. In Figure 7.2, a comparative picture of the percentage reduction of contact angles of water drops on different leaf surfaces after 10, 30 and 60 minutes of impaction is given. On the basis of the percentage reduction of contact angle (representing the relative spreading of the impacted drops on the leaf surfaces), sixteen leaf surfaces tested can be categorised as showed in Table 7.3. Water drops were found to be highly unspreadable on the leaves of barley seedlings at the end of 60 minute observation periods. There were large shifts of category among the leaf surfaces observed from 30 minutes to 60 minutes. After 30 minutes of impaction, water drops were found to be highly non-spreadable on the leaves of barley, cabbage, and three varieties of rape (both old and young) and showed a reduction of advancing contact angles ranging from 1-15%. Leaves of tomato, sugarcane, wheat, dwarfbean and maize (both old and young) fell into the second category and water drops were found to be moderately non-spreadable on those leaf surfaces, while on pear and orange leaves water drops were found moderately spreadable and spreadable respectively after 30 minutes of impaction. On none of the leaf surfaces tested, water drops were found highly spreadable after 30 minutes of impaction. After 60 minutes, considerable shifs in these positions was observed. With the exception of barley leaves, water drops on all the other leaves in the highly spreadable catagory shifted to the moderately non-spreadable category. On tomato leaves, water

Figure 7.2 Percenage reduction of advancing contact angle of water after 10, 30 and 60 minutes of impaction on different leaf types



Key to test leaf types: Ba(s)=Barley; Ca=Cabbage; To=Tomato; Pr=Pear; Su=Sugarcane; Wh=Wheat; Or=Orange; Db=Dwarf bean; Rt=Rape v. Tanto; Rl=Rape v. Linawell; Rs=Rape v. Starlight; M=Maize; (s)=Seedlings; (o)=Old; (y)=Young

Table 7.3 Classification of different leaf surfaces on the basis of percent reduction of advancing contact angles of water drop after 60 and 120 minutes of impaction.

Catagory	% reduction of advancing contact angles	Leaf types after 30 minutes	Leaf types after 60 minutes
Highly non-wettable	1-15%	Barley (s), Cabbage, Rape v. Tanto (old), Rape v Tanto (young), Rape v. Starlight(old), Rape v. Starlight (young), Rape v. Lirawell (old), Rape v. Lirawell (young)	Barley (s)
Moderately non-wettable	15-35%	Tomato, Sugarcane, Wheat, Dwarfbean, Maize (old), Maize (young)	Cabbage, Tomato, Rape v. Tanto (old), Rape v. Tanto (young), Rape v. Starlight (old), Rape v. Starlight (young), Rape v. Lirawell (old), Rape v. Lirawell (young),
Moderately wettable	35-50%	Pear	Maize (old), Maize (young)
Wettable	50-80%	Orange	Wheat, Dwarfbean, Pear, Sugarcane
Highly wettable	>80%		Orange

drops still remained as moderately non-spreadable. Maize (old and young) shifted to moderately spreadable, whereas wheat and dwarf bean shifted one step further to the spreadable category. Pear leaves shifted from moderately spreadable to spreadble and orange leaves were found to be highly spreadable.

This can be interpreted in relation to the micro- and macro-structures of individual leaf types. In the case of tomato leaves, trichomes were seem to be the major conributor. For sugarcane, wheat and maize the structures of the basal cells may have played a major role. The cell structures of these three genera were more elongated with deep grooving along the joining edges of the adjacent cells (see the SEM pictures in chapter 5) which facilitates drop spreading along cell boundaries. The role of trichomes, especially on tomato leaves, will become clear from analysis of contact angle photographs. The maximum reduction of the water drops on pear, orange and dwarf bean was due to the their surface nature (smooth and amorphous) and the low amounts of wax present. The overall response of contact angle at the time of impaction and subsequent reduction in advancing contact angles are seem to be mainly influenced by the amount of wax present, especially on the leaf surfaces above 90^0. These results reveal that the behaviour of impacted deposits changes according to the nature of leaf surface characteristics, which have an impact on deposit spreading and coverage.

7.3.3 Time series studies on the advancing contact angles of deltamethrin solution

Table 7.4 shows the results of time series studies on the reduction of advancing contact angles of deltamethrin solution (0.5mg ml^{-1}) on different leaf types. Broadly, the sixteen leaf types tested can be grouped into two categories; 1) leaf surfaces on which the contact angles of impacted drops fell below $10-15^0$ within 60 minutes of impaction and 2) leaf surfaces where the angles fell to $15-35^0$ after 60 minutes. Pear, sugarcane, wheat, orange, dwarf bean, maize

Table-7.4. Time series studies on the advancing contact angle of deltamethrin 2.5EC solution (0.5mg/ml of water) on various leaf types

Leaf species	After 0-30 secs of impaction (SD ±)			After 10 minutes (SD ±)			After 30 minutes (SD ±)			After 60 minutes (SD ±)		
	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average
Barley (s)	89.87 (0.72)	89.93 (0.85)	89.90 ((0.73)	85.13 (1.26)	84.67 (1.19)	84.90 (1.19)	55.13 (2.50)	54.80 (2.51)	54.93 (2.47)	17.43 (2.13)	17.21 (2.14)	17.32 (2.10)
Cabbage(o)	48.20 (3.71)	48.33 (3.38)	48.27 (2.71)	39.93 (2.43)	40.93 (2.46)	40.43 (2.27)	31.53 (2.33)	32.67 (2.18)	37.57 (3.24)	22.93 (2.23)	23.87 (2.30)	23.20 (2.99)
Tomato (o)	56.00 (5.05)	55.47 (5.64)	55.73 (3.71)	45.07 (6.56)	45.07 (7.52)	45.23 (5.83)	36.93 (4.78)	36.27 (5.82)	36.60 (4.51)	24.87 (5.55)	23.93 (4.12)	24.40 (3.71)
Pear (o)	43.00 (3.14)	42.93 (3.11)	42.97 (3.11)	31.80 (3.19)	32.13 (3.01)	31.97 (3.06)	20.53 (2.75)	21.00 (2.94)	20.77 (2.82)	<15	<15	<15
Sugarcane (o)	44.20 (2.53)	44.07 (2.46)	44.13 (2.35)	33.13 (2.44)	33.00 (2.39)	33.07 (2.39)	22.53 (1.93)	21.93 (1.91)	22.23 (1.87)	<15	<15	<15
Wheat (o)	62.27 (4.85)	60.60 (5.70)	61.10 (4.85)	35.07 (5.81)	34.27 (2.69)	35.33 (6.13)	19.47 (1.41)	19.53 (1.78)	19.50 (1.52)	<10	<10	<10
Orange (o)	37.87 (2.47)	37.47 (3.28)	37.67 (2.36)	31.60 (2.78)	31.40 (2.85)	31.50 (2.68)	22.60 (2.15)	22.60 (2.21)	22.60 (2.15)	<15	<15	<15
Dwarfbean (o)	36.87 (3.76)	36.27 (4.09)	36.57 (3.68)	18.47 (4.45)	17.93 (4.14)	18.20 (4.23)	<10	<10	<10	*	*	*
Rape v. Tanto (o)	50.00 (1.67)	50.00 (1.41)	50.00 (1.41)	38.40 (1.85)	38.20 (1.83)	38.30 (1.83)	28.00 (2.76)	28.00 (2.76)	28.00 (2.76)	16.80 (1.17)	16.80 (1.17)	16.80 (1.17)
Rape v. Tanto (y)	72.80 (1.72)	72.80 (1.72)	72.80 (1.72)	67.60 (2.24)	67.60 (2.24)	67.60 (2.24)	51.60 (2.06)	51.80 (2.40)	51.70 (2.23)	33.40 (1.85)	33.40 (1.85)	33.40 (1.85)
Rape v. Lirawell (o)	48.80 (2.32)	48.80 (2.32)	48.80 (2.32)	38.20 (2.14)	37.80 (2.48)	38.00 (2.30)	27.40 (2.65)	27.20 (2.79)	27.30 (2.71)	16.00 (2.10)	16.00 (2.61)	16.00 (2.35)
Rape v. Lirawell (y)	69.80 (2.79)	70.60 (2.33)	70.20 (2.48)	65.00 (2.97)	65.00 (2.97)	65.00 (2.97)	53.20 (3.12)	53.00 (3.41)	53.10 (3.26)	34.40 (2.50)	34.60 (2.73)	34.50 (2.61)
Rape v. Starlight(o)	50.20 (1.60)	51.00 (2.19)	51.00 (2.19)	40.00 (1.10)	40.00 (1.10)	40.00 (1.10)	27.60 (2.32)	27.20 (2.32)	27.40 (2.15)	17.00 (3.41)	17.00 (3.41)	17.00 (3.41)
Rape v. Starlight (y)	75.20 (2.71)	75.40 (2.87)	75.30 (2.82)	68.60 (2.33)	68.60 (2.33)	68.60 (2.33)	50.40) (2.87)	50.40 (2.87)	50.40 (2.80)	35.20 (0.98)	35.80 (2.04)	35.50 (1.34)
Maize (o)	43.73 (2.91)	43.67 (3.05)	43.70 (2.88)	33.33 (2.98)	32.80 (3.27)	33.07 (3.02)	16.40 (1.40)	16.93 (1.44)	16.67 (1.37)	<10	<10	<10
Maize (Y)	60.80 (4.28)	61.80 (4.25)	61.17 (4.43)	50.07 (3.73)	50.27 (3.70)	50.17 (3.70)	23.53 (2.39)	24.13 (2.22)	23.83 (2.25)	<10	<10	<10

* = The droplets loose their normal shape into the complex topographical structure of the leaf (especially the trichomes) and become impossible to get the correct angle of the droplets s = seedling; o = old; y = young

(old) and maize (young) fell within group one. All those leaf types had low amounts of waxes ranging from 2-20µg cm^{-2} (see chapter 6). The other leaves fell within group two, and had high amounts of wax ranges from 30-50 µg cm^{-2}.

Deltamethrin solution showed a high reduction of contact angle (40%-60% approx.) in comparison with that of water drops on almost all the leaf surfaces, except dwarf bean, maize (old) and maize (young), where the percentage reductions were 9.19, 35.79 and 10.44 respectively. This was due to the surfactants used in the formulation and may also have been affected by the lipophilic nature of the deltamethrin. An important step was to look at the percentage reduction of the advancing contact angle in comparison with that of a water drop. On the surfaces where water drops were highly non-spreadble and moderately non-spreadable, reductions of adavncing contact angles, after 60 minutes of impaction, were extraordinary. For example, on the surface of barley seedlings the reduction of the advancing contact angle of the deltamethrin solution was around 80% after 60 minutes of impaction.

7.3.4 Time series studies on the advancing contact angles of dimethoate solution

Further interesting results were observed for the drops of dimethoate solution(0.212mg ml^{-1}; Table 7.5). Here, on leaf surfaces with high wax content and where water drops were highly non-spreadable and moderately spreadable (such as barley, cabbage and all varieties of rape), the reduction of advancing contact angles showed a more or less similar trend and none of them fell below 15^0, even after 60 minutes of impaction. The reductions in initial contact angles on these surfaces were much lower in comparison with deltamethrin. The initial contact angles of dimethoate drops were higher than 90^0 on all the surfaces where the initial contact angle of water was above 90^0. That is, the dimethoate solution did not exhibit a reduction in initial contact angle on hydrophobic surfaces below 90^0. However, interestingly, these reductions were still much greater on those surfaces in comparison with

Table-7.5. Time series studies on the advancing contact angle of dimethoate 40EC solution (0.212 mg/ml of water) on various leaf types

Leaf species	After 0-30 scecs of impaction (SD ±)			After 10 minutes (SD ±)			After 30 minutes (SD ±)			After 60 minutes (SD ±)		
	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average	Left angle	Right angle	Average
Barley (s)	122.33 (2.55)	122.53 (1.71)	122.43 (2.06)	121.33 (2.24)	121.60 (1.58)	121.47 (1.87)	106.93 (3.45)	106.47 (3.91)	106.70 (3.61)	90.33 (7.46)	90.07 (7.50)	90.02 (7.44)
Cabbage(o)	114.20 (10.71)	115.53 (11.19)	114.87 (10.78)	104.60 (11.92)	105.60 (11.95)	104.83 (11.74)	92.00 (11.67)	91.53 (11.22)	91.80 (11.53)	56.73 (10.31)	57.13 (9.62)	56.93 (9.95)
Tomato (o)	91.27 (6.23)	91.20 (5.90)	91.23 (5.56)	78.73 (5.69)	79.40 (5.98)	79.07 (5.60)	65.20 (5.00)	66.47 (5.00)	65.83 (4.84)	51.47 (4.01)	51.13 (4.16)	51.23 (3.89)
Pear (o)	71.27 (5.23)	71.20 (5.09)	71.23 (5.12)	47.40 (5.17)	47.80 (6.96)	46.27 (6.88)	31.87 (4.22)	31.20 (4.37)	31.53 (4.11)	<15	<15	<15
Sugarcane (o)	67.4 (5.76)	69.00 (6.57)	68.20 (6.03)	53.93 (5.92)	54.06 (4.92)	54.00 (4.90)	28.60 (4.18)	28.47 (4.00)	28.54 (3.94)	<15	<15	<15
Wheat (o)	88.80 (8.02)	88.40 (7.91)	88.60 (7.90)	77.00 (7.74)	75.87 (8.36)	76.43 (7.97)	64.33 (8.38)	64.27 (8.57)	64.23 (8.43)	35.27 (4.58)	35.20 (4.87)	35.23 (4.69)
Orange (o)	74.20 (5.61)	75.70 (5.97)	74.97 (5.70)	57.00 (6.30)	57.80 (6.02)	57.39 (6.07)	25.80 (3.80)	26.47 (3.67)	26.13 (3.70)	<15	<15	<15
Dwarfbean (o)	38.33 (1.66)	38.33 (1.74)	38.33 (1.74)	12.00 (2.03)	12.60 (2.12)	12.30 (1.96)	<10	<10	<10	*	*	*
Rape v. Tanto (o)	104.40 (2.94)	104.20 (2.94)	104.30 (2.79)	95.60 (3.38)	96.00 (2.12)	95.80 (3.06)	85.40 (1.85)	85.00 (1.67)	85.20 (1.75)	67.80 (1.72)	67.80 (1.72)	67.80 (1.72)
Rape v. Tanto (y)	114.80 (2.56)	114.80 (2.56)	114.80 (2.56)	109.40 (2.94)	109.40 (2.94)	109.40 (2.94)	99.20 (2.48)	99.20 (2.85)	99.20 (2.66)	83.20 (2.23)	83.20 (2.23)	83.20 (2.23)
Rape v. Lirawell (o)	105.00 (2.28)	104.00 (2.58)	104.70 (2.40)	95.80 (0.98)	95.80 (0.75)	95.80 (0.81)	84.40 (1.20)	84.60 (1.36)	84.50 (1.26)	72.80 (1.72)	73.00 (2.00)	72.90 (1.85)
Rape v. Lirawell (y)	114.00 (2.76)	113.60 (3.20)	113.80 (2.98)	108.80 (2.48)	109.00 (2.76)	108.90 (2.62)	97.80 (3.19)	98.20 (3.06)	98.00 (3.11)	85.80 (4.02)	86.60 (4.32)	86.20 (4.17)
Rape v. Starlight(o)	108.00 (2.00)	108.00 (2.00)	108.00 (2.00)	98.00 (1.90)	98.00 (1.90)	98.00 (1.90)	85.20 (2.64)	85.20 (2.64)	85.20 (2.58)	70.20 (2.79)	70.60 (2.80)	70.40 (2.78)
Rape v. Starlight (y)	117.80 (1.72)	117.60 (1.85)	117.70 91.78)	111.60 (1.85)	112.00 (2.10)	111.80 (1.94)	101.40 (1.74)	101.40 (1.74)	101.40 (1.74)	86.20 (2.48)	86.20 (2.48)	85.90 (2.33)
Maize (o)	62.47 95.85)	63.73 (6.88)	63.10 (6.34)	50.93 (5.51)	51.00 (4.72)	50.83 (5.14)	23.40 (2.87)	24.00 (2.58)	23.70 (2.59)	<15	<15	<15
Maize (Y)	62.27 (4.34)	62.67 (4.04)	62.47 (4.11)	51.20 (3.99)	51.27 (4.11)	51.23 (4.00)	24.07 (2.46)	24.33 (2.75)	24.30 (2.41)	<15	<15	<15

* = The droplets loose their normal shape into the complex topographical structure of the leaf (especially the trichomes) and become impossible to get the correct angle of the droplets s = seedling; o = old; y = young

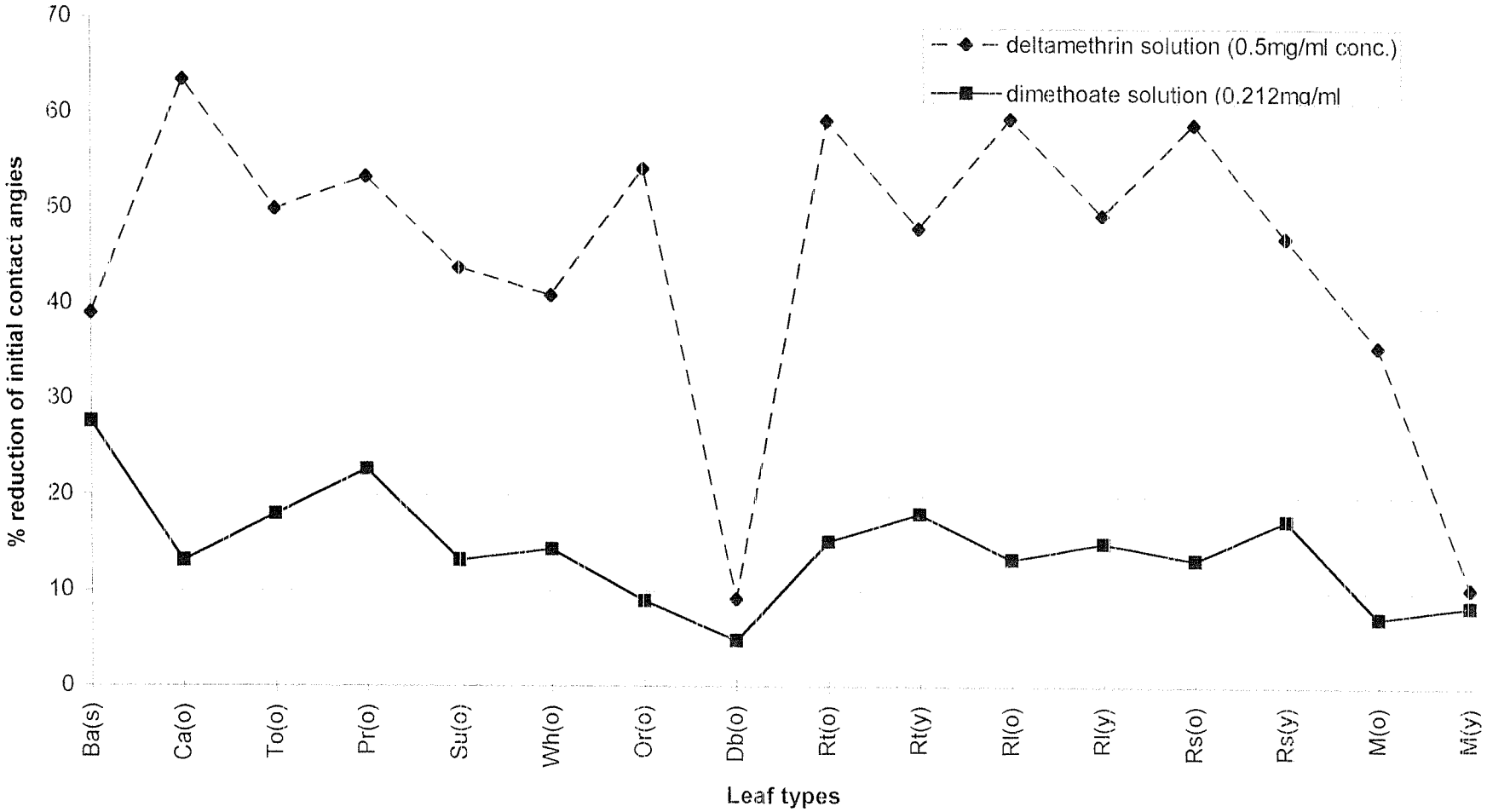


Figure 7.3 Percentage reduction of initial contact angles of deltamethrin and dimethoate solution in comparison to initial conatct angles of water on different leaf types

Key to test leaf types as Fig.7.2

Table 7.6 Percent reduction of advancing contact angles of deltamethrin and dimethoate solution after 10, 20 and 30 minutes of impaction

Leaf types	% reduction of advancing contact angles of deltamethrin (@ 100 g a.i./ha)			% reduction of advancing contact angles of dimethoate (@ 42.50 g a.i./ha)		
	after 10 minutes	after 30 minutes	after 60 minutes	after 10 minutes	after 30 minutes	after 60 minutes
Barley (s)	5.56	38.90	80.73	0.78	13.04	26.47
Cabbage (o)	16.24	22.17	51.93	8.74	20.08	50.44
Tomato (o)	18.84	34.33	56.22	13.33	27.84	43.85
Pear (o)	25.60	51.66	*	35.04	55.73	*
Sugarcane (o)	25.06	49.63	*	20.82	58.15	*
Wheat (o)	42.18	68.09	**	13.74	27.51	60.24
Orange (o)	16.38	40.01	*	23.45	65.15	*
Dwarfbean (o)	50.23	**	***	67.91	**	***
Rape v. Tanto (o)	23.40	44.00	66.40	8.15	18.31	35.00
Rape v. Tanto (y)	7.14	28.98	54.12	4.70	13.59	27.53
Rape v. Lirawell (o)	22.13	44.06	67.21	8.50	19.29	30.37
Rape v. Lirawell (y)	7.41	24.36	50.71	4.31	13.88	24.25
Rape v. Starlight (o)	21.57	46.27	66.67	9.26	21.11	34.81
Rape v. Starlight (y)	8.90	33.06	52.86	5.01	13.84	27.02
Maize (o)	24.32	61.85	**	19.45	62.44	*
Maize (y)	17.98	61.04	**	17.99	61.10	*

s = seedling; o = old; y = young; * = contact angle reached below 15^0; ** = contact angle reached below 10^0; *** = drop loose their normal shape into the complex topographical structure of the leaf or almost flattened and become impossible to get the correct reading

Table 7.7 Differences of contact angles of water between waxed and dewaxed leaf surfaces

Leaf types	on waxed leaf surface			on dewaxed leaf surface		
	Left angle (SD ±)	**Right angle (SD ±)**	**Average (SD ±)**	**Left angle (SD ±)**	**Right angle (SD ±)**	**Average (SD ±)**
Barley (seedling)	147.27 (2.01)	147.53 (2.06)	147.40 (1.99)	100.73 (0.88)	100.67 (0.70)	100.70 (0.79)
Cabbage (old)	132.33 (7.62)	132.07 (7.18)	132.20 (7.39)	101.90 (3.11)	102.05 (3.05)	101.98 (3.08)
Cabbage (young)	141.40 (2.65)	141.37 (2.64)	141.39 (2.65)	103.23 (4.06)	103.13 (4.30)	103.18 (4.18)
Dwarfbean (old)	40.00 (3.63)	40.60 (2.92)	40.27 (3.21)	92.40 (3.00)	93.20 (3.96)	92.80 (3.48)
Maize (old0	68.30 (6.94)	67.80 (7.41)	68.05 (7.10)	64.00 (5.40)	60.80 (7.44)	62.40 (6.42)

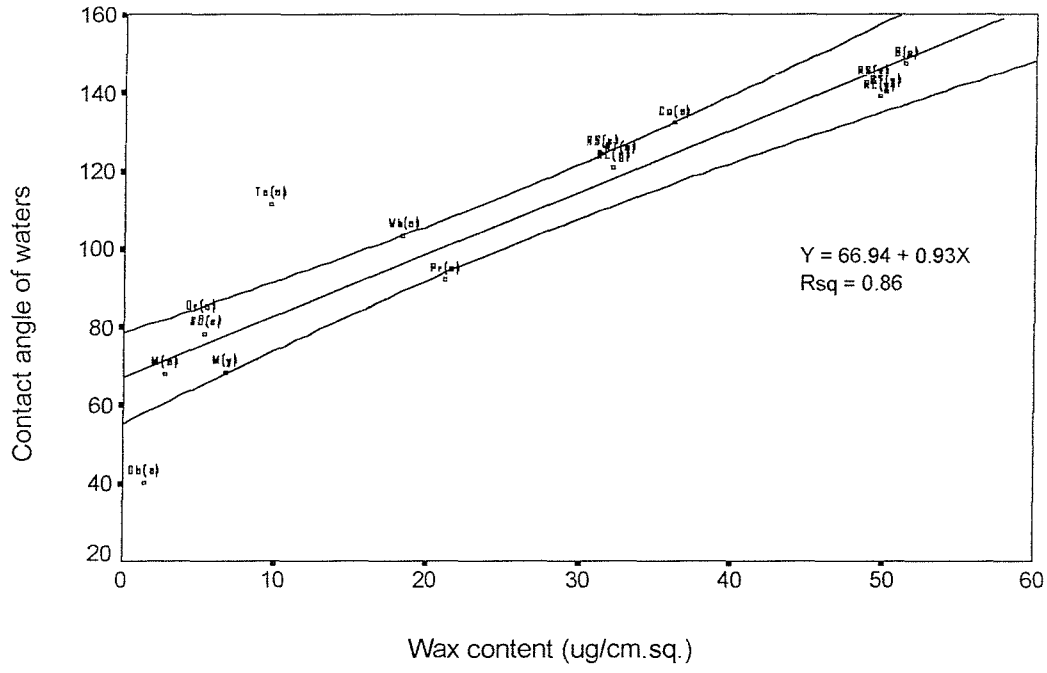


Figure 7.4 Correlation between contact angle of water and wax content of different leaf surfaces Key to leaf types: B, Barley; Ca, Cabbage; RT, Rape v. Tanto; Rs, Rape v. Starlight; Rl, Rape v. Lirawell, Db, Dwarfbean, To, Tomato, Pr, Pear; Su, Sugarcane; Wh, Wheat; Or, Orange; M, Maize; (s), Seedlings; (o), old; (y), Young

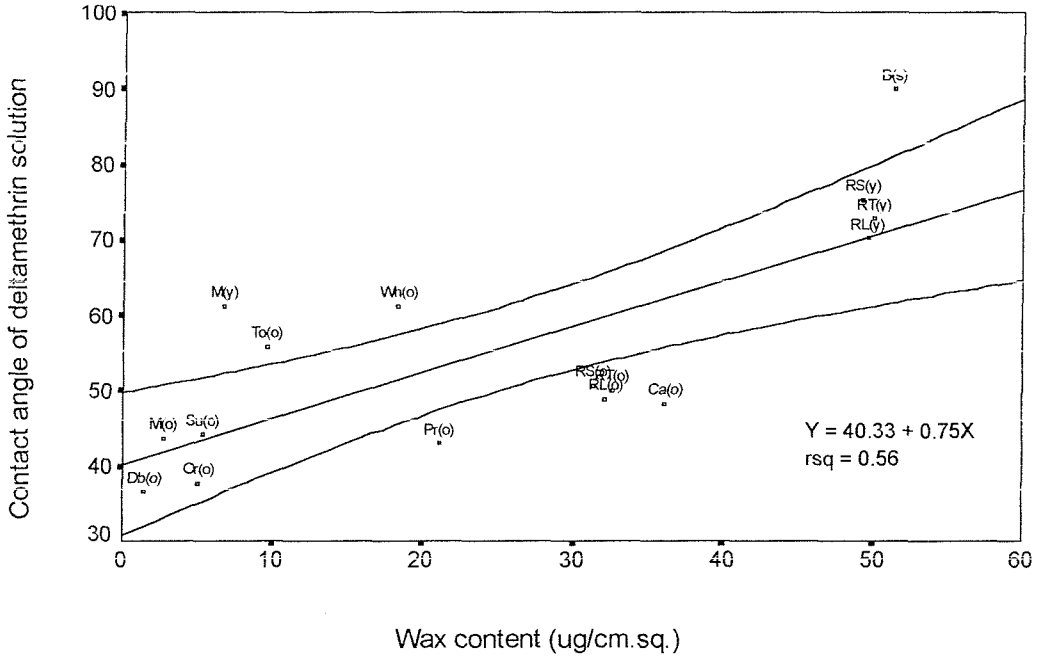


Figure 7.5 Correlation between contact angle of deltamethrin solution (mg/ml of water) and wax content of different leaf surfaces Key to test leaf types are as Figure 7.5

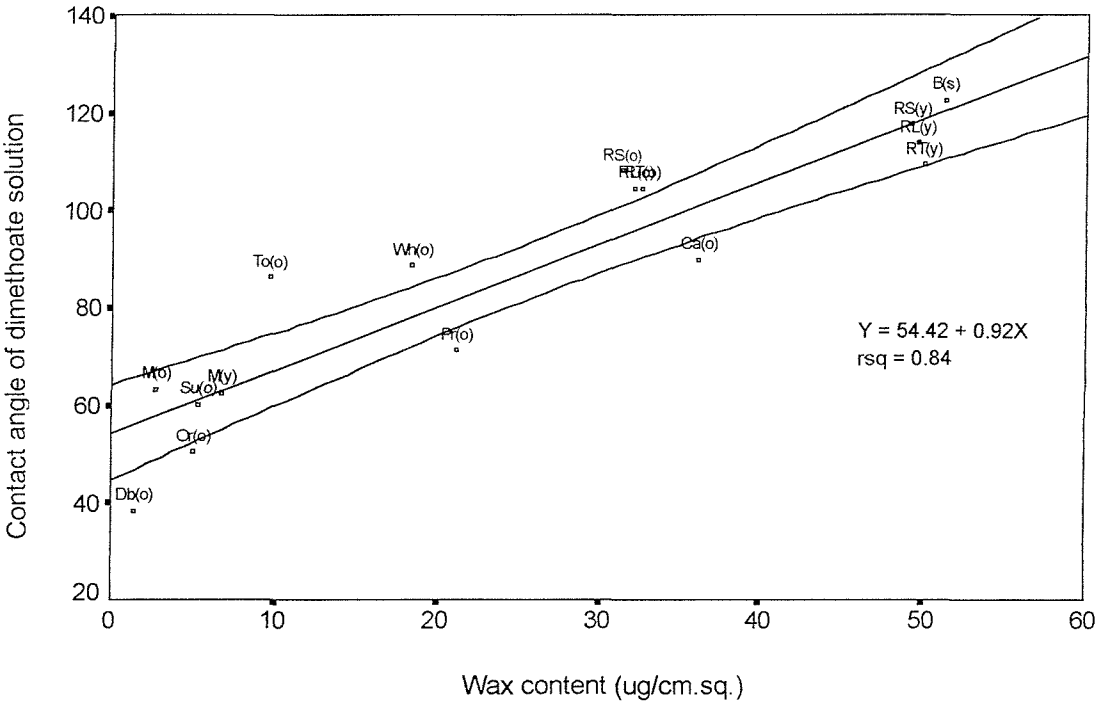


Figure 7.6 Correlation between contact angle of dimethoate solution (0.212 mg/ml of water) and wax content of different leaf surfaces Key to test leaf types are as figure 7.4

the reduction on surfaces having an initial contact angle of water below 90^0. For example on sugarcane, orange, dwarfbean, maize (old), and maize (young) the reduction of initial contact angle of dimethoate from those for water on these surfaces were 13.20, 8.98, 4.82, 7.27 and 8.53% respectively (Fig.7.3). That is, the dimethoate formulation, in addition to its hydrophilic nature contributed little to the hydrophilic nature of the surfaces.

Subsequent reductions of advancing contact angle for dimethoate on hydrophilic surfaces were seen to be much greater than those on hydrophobic surfaces. For example, on the leaf surfaces of dwarf bean, maize (old and young), orange, pear and sugar cane, the advancing contact angles of dimethoate solution reached below 15^0 after 30 minutes of impaction (Table 7.5) and made those surfaces highly spreadable.

7.3.5 Contact angle and wax content

A linear regression model was fitted to data of initial contact angles of water, deltamethrin and dimethoate against the wax content of different leaf types. Figure 7.4 shows the correlation between contact angles of water and the wax content of different leaf types, giving a significant positive correlation (r^2 = 0.86; F = 84.52; d.f. 1,14; P = <0.001). A similar regression line between the contact angles of deltamethrin and wax content (Figure 7.5) gives again a positive correlation (r^2 = 0.56; F = 18.00; d.f. 1,14; P = 0.0008). Figure 7.6 shows the same for dimethoate, giving a positive correlation between contact angles and the wax content (r^2 = 0.84; F = 75.83; P = <0.001). It was evident from the regression analysis that wax played an important role in wettability, although the degree of relationship varied according to the nature of liquid drops.

7.3.6 Impacting behaviour of drops on different leaf surfaces: a photographic study

It is well-established from earlier reviews and the results of the present studies that contact angles of a droplet can

vary according to leaf species. In addition to this, in the current study it was also observed that the contact angle can vary according to position, drop size and under the influence of other prominent leaf structures such as trichomes. The two sides of the droplet (left and right) can also differ according to landing position. This will become clear if we go through the contact angle data and the pictures of droplets taken with the goniometer telescope using a specially modified SLR camera attachment. For example droplet(s) of water, deltamethrin and dimethoate solution on barley leaves shown in Figures 7.7, 7.19 and 7.29 respectively, had stable positions with both angles lying on a more or less horizontal line. This may have reduced the tendency of the droplet to spread because of the apparent balance in the forces of gravitation on both sides of the drop. In contrast, the droplet angles on cabbage leaves (Figs. 7.8, 7.9, and 7.10), were not horizontally equal, making one angle more spreadable by the force of gravitation. As a result of trichome structures, droplets on tomato leaves (Figs. 7.11, 7.12, 7.21 and 7.31) were interrupted, and differed in angles from drop to drop. In Figures 7.12 and 7.31, the left angle was disrupted by interference with surface structures. Some trichomes were smaller than the drop and became trapped inside it. The trapped trichomes, in the course of drop spreading, punctured the intact drop. At certain stages of advancing contact angle, when the forces of adhesion and surface tension were more active, trapped trichomes disrupt the drop's coherency forces and allow it to spread rapidly with an increase in advancing contact angle. Drops on orange (Figures 7.15, 7.23 and 7.33) and dwarf bean (Figures 7.16, 7.24 and 7.34) showed marked differences in their contact positions due to leaf topography and the positions of the droplets. Water drops on hydrophobic surfaces such as barley, cabbage and rape were intact and proud of the surface. However, the deltamethrin solution was able to reduce this proudness considerably, whereas dimethoate did so, but to a much lower extent. These pictures show a very close agreement with the contact angle data of all the three liquids on different leaf surfaces.

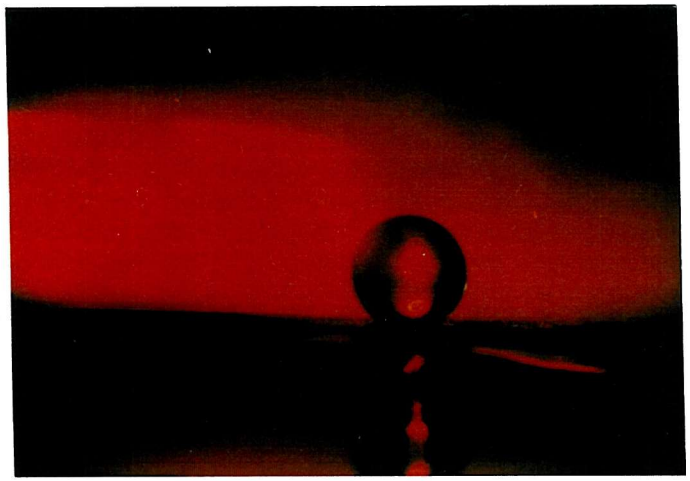


Figure 7.7 Water drop on barley I(seedling) leaf

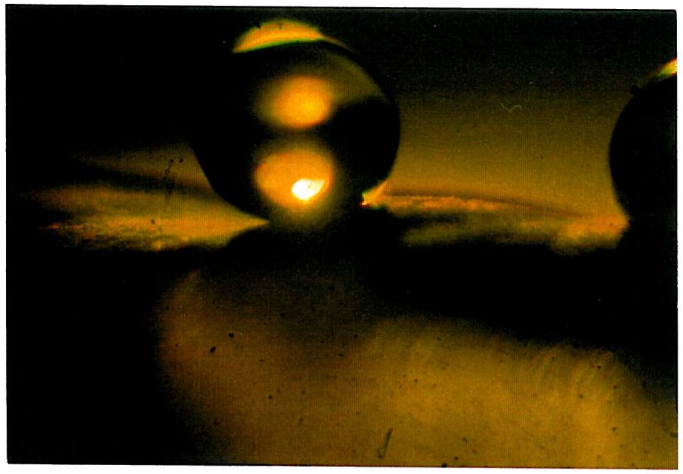


Figure 7.9 Water drop on cabbage leaf (old)

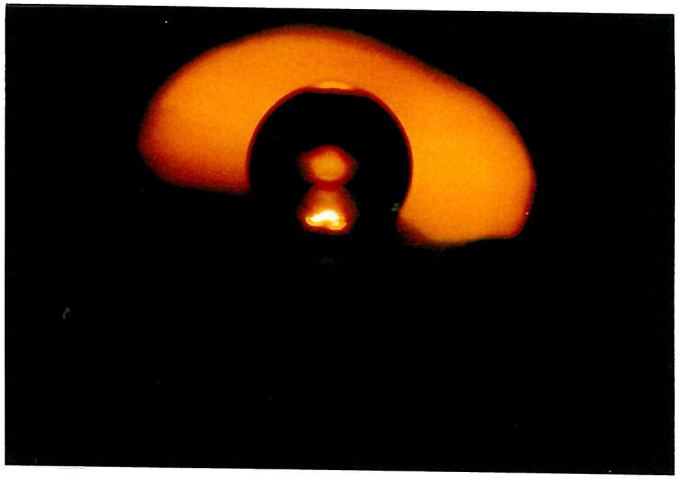


Figure 7.8 Water drop on cabbage leaf (old)

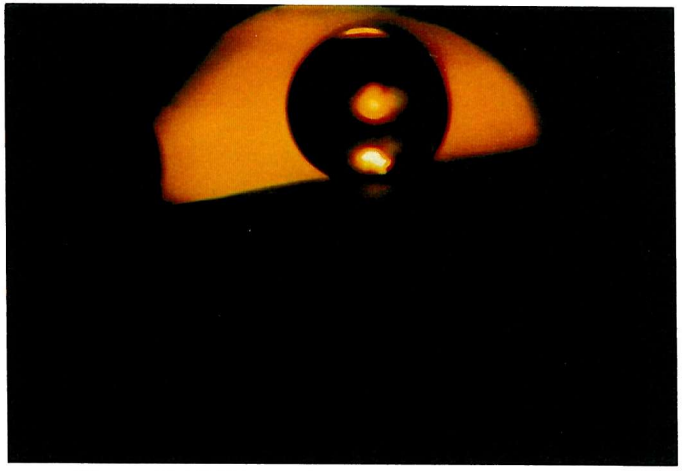


Figure 7.10 Water drop on cabbage leaf (old)

Figure 7.11 Water drop on tomato leaf (old)

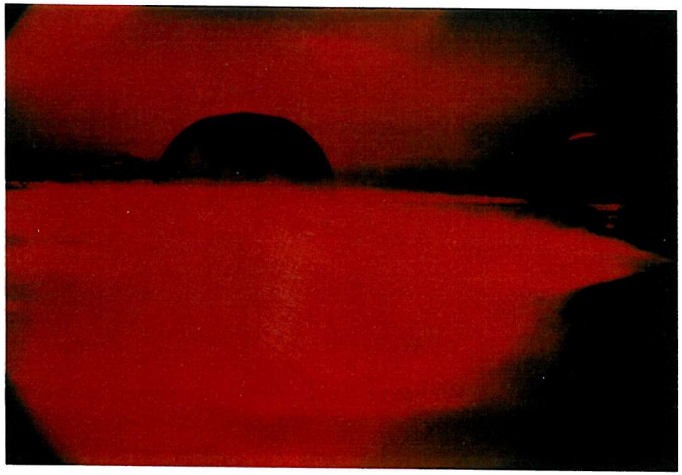


Figure 7.13 Water drop on sugarcane leaf (old)

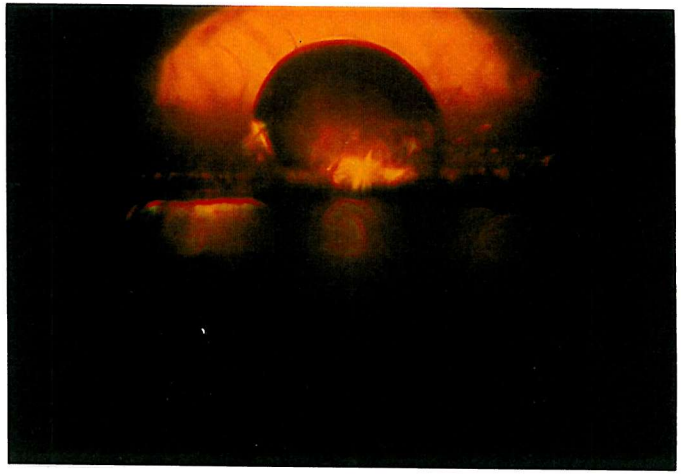


Figure 7.12 Water drop on tomato leaf (old)

Figure 7.14 Water drop on wheat leaf (old)

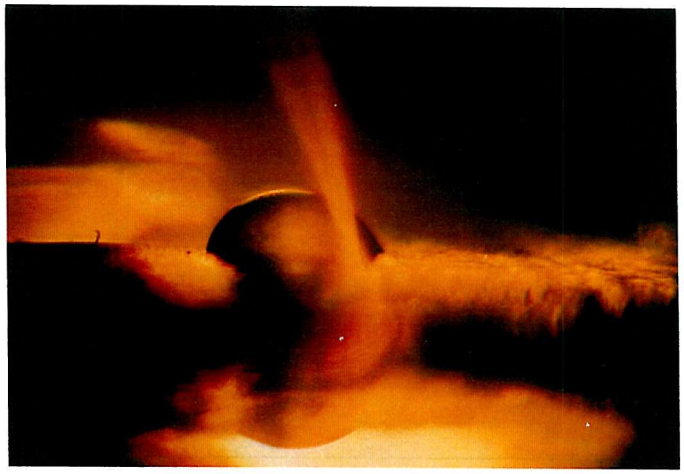


Figure 7.15 Water drop on orange leaf (old)

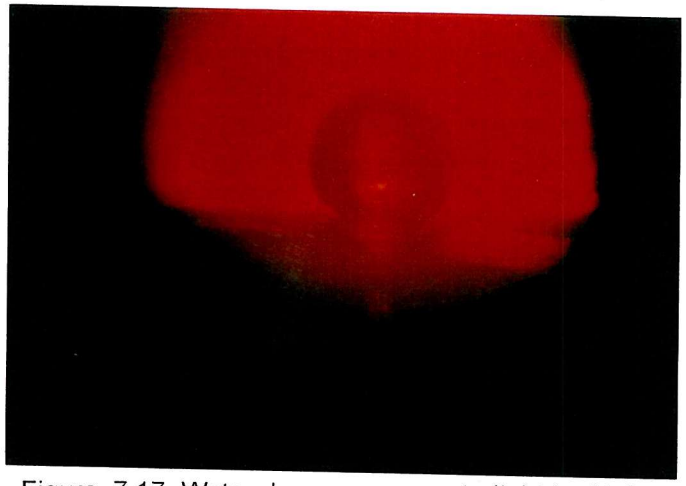


Figure 7.17 Water drop on rape v. starlight leaf (old)

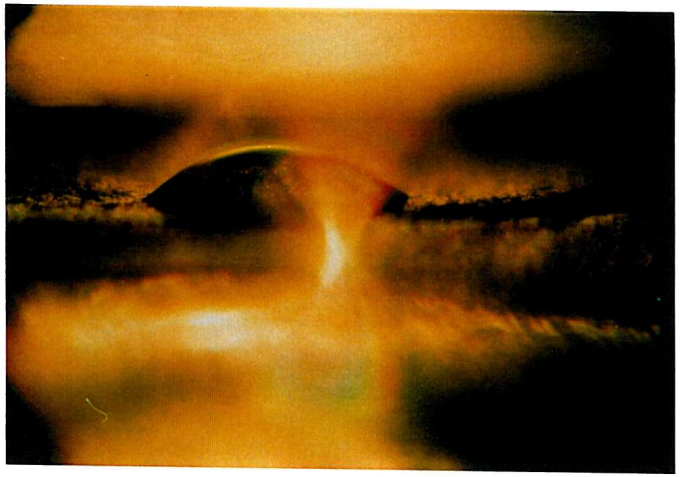


Figure 7.16 Water drop on dwarfbean leaf (old)

Figure 7.18 Water drop on rape v. starlight leaf (young)

Figure 7.19 Deltamethrin drop on barley leaf (seedling)

Figure 7.21 Deltamethrin drop on tomato leaf (old)

Figure 7.20 Deltamethrin drop on cabbage leaf (old)

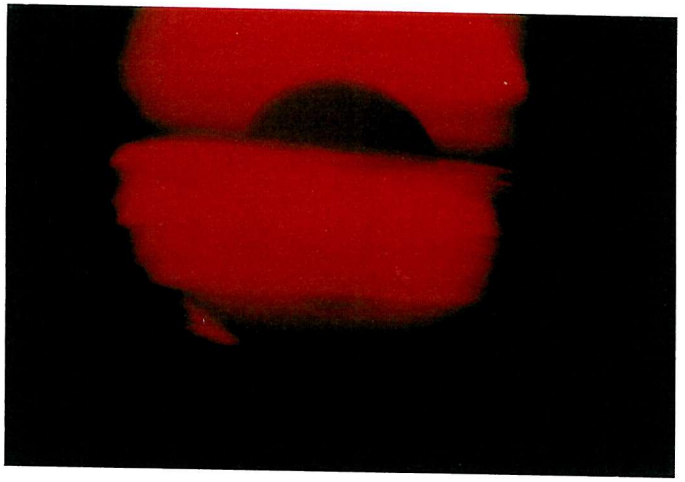


Figure 7.22 Deltamethrin drop on sugarcanel eaf (old)

Figure 7.23 Deltamethrin drop on orangeleaf (old)

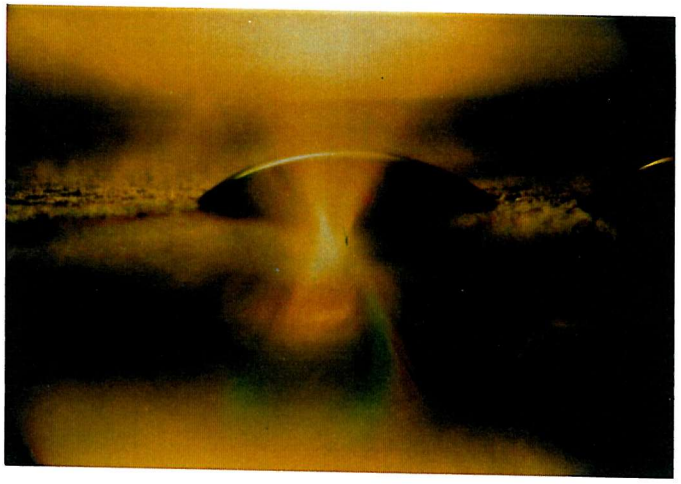


Figure 7.24 Deltamethrin drop on dwarfbean leaf (old)

Figure 7.25 Deltamethrin drop on rape v. starlight leaf (old)

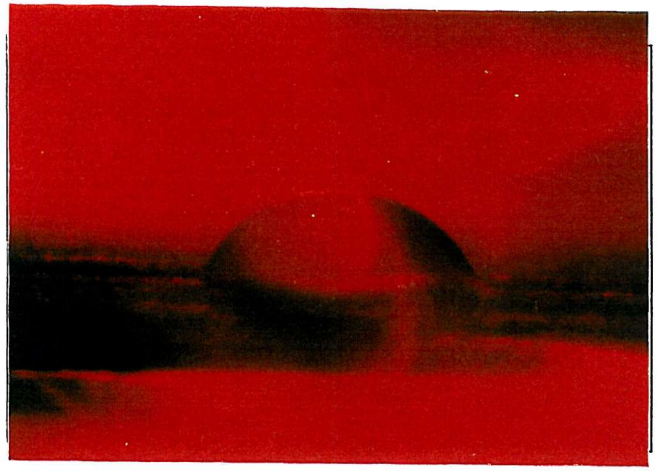


Figure 7.26 Deltamethrin drop on rape v. starlight l eaf (young)

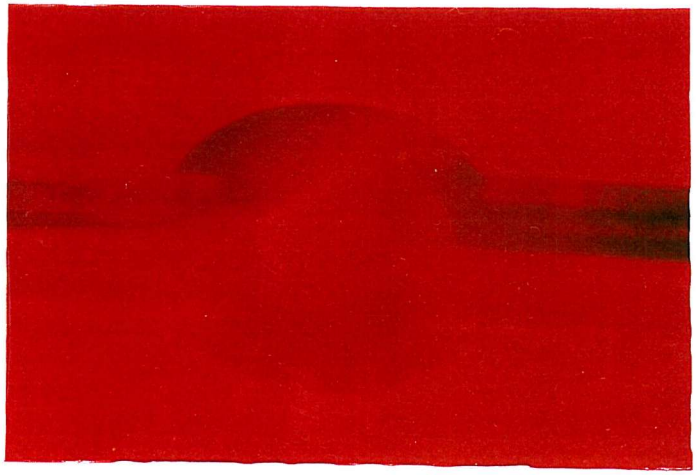


Figure 7.27 Deltamethrin drop on maize leaf (old)

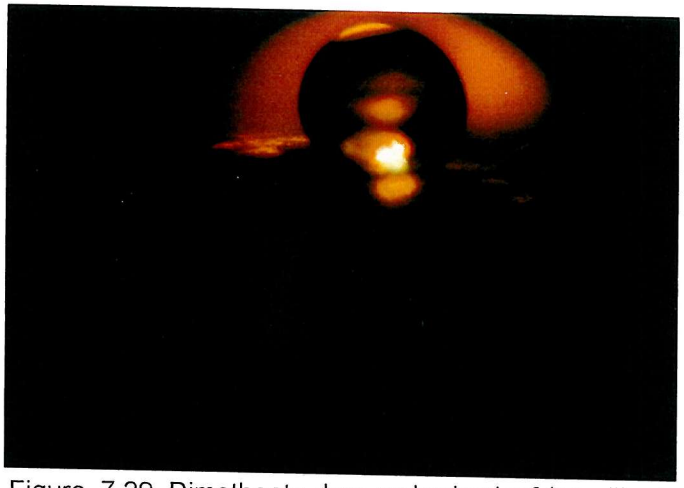


Figure 7.29 Dimethoate drop on barley leaf (seedling)

Figure 7.28 Deltamethrin drop on maize leaf (young)

Figure 7.30 Dimethoate drop on cabbage leaf (old)

Figure 7.31 Dimethoate drop on tomato leaf (old)

Figure 7.33 Dimethoate drop on orange leaf (old)

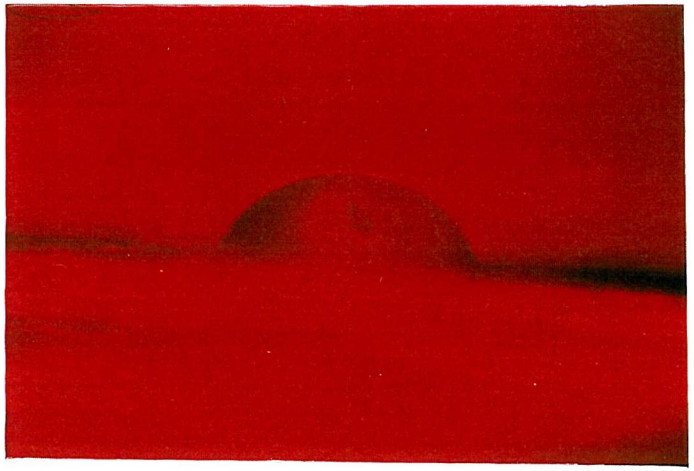


Figure 7.32 Dimethoate drop on sugarcane leaf (old)

Figure 7.34 Dimethoate drop on dwarfbean leaf (old)

Figure 7.35 Dimethoate drop on rape v. starlight leaf (old)

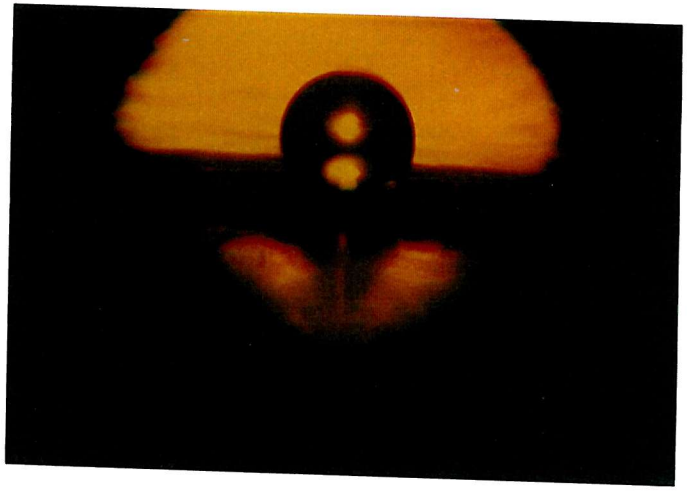


Figure 7.36 Dimethoate drop on rape v. starlight leaf (young)

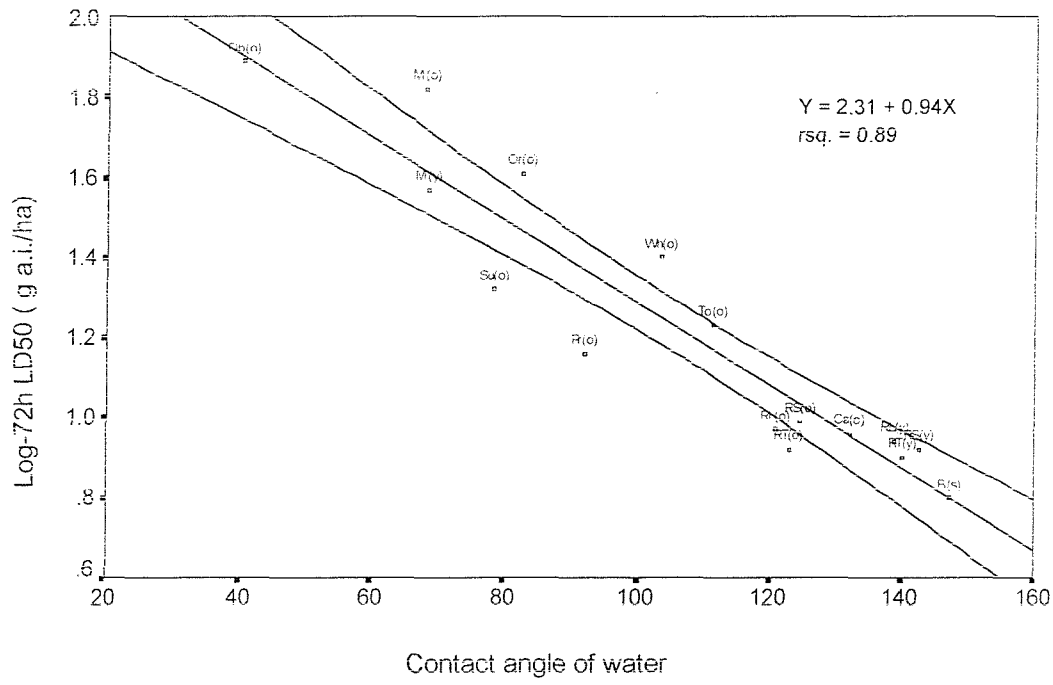


Figure 7.37 Correlation between contact angle of water (1 ul drop) and log-72h LD50 of *F. candida* for deltamethrin on different leaf surfaces Key to test leaf types are as Figure 7.4

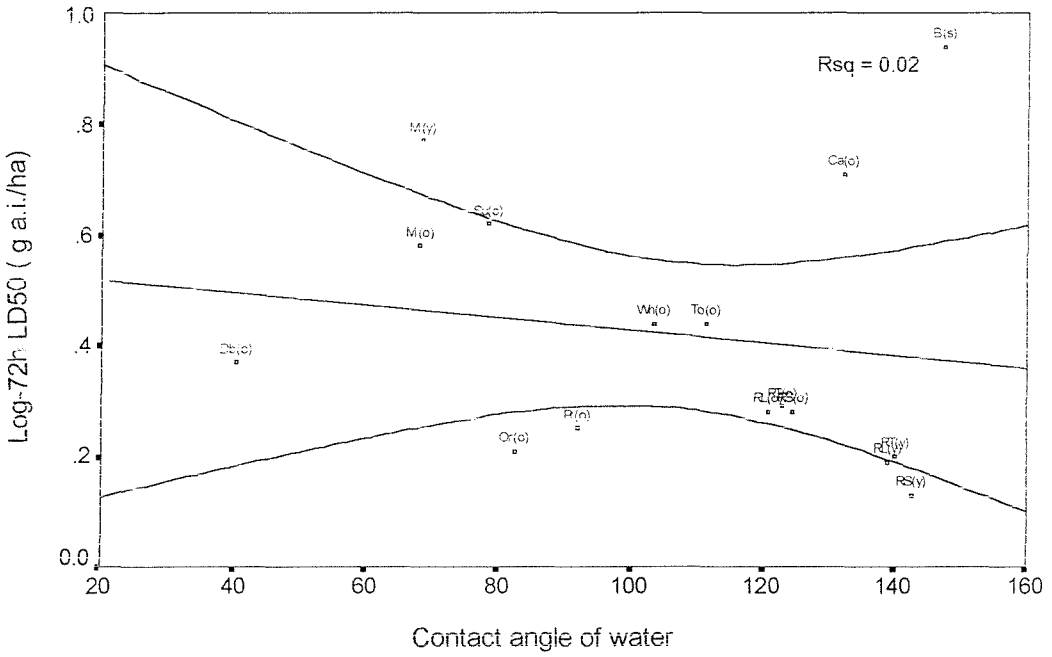


Figure 7.38 Correlation between contact angle of water (1 ul drop) and log-72h LD50 of *F. candida* fordimethoate on different leaf surfaces Key to test leaf types are as Figure 7.4

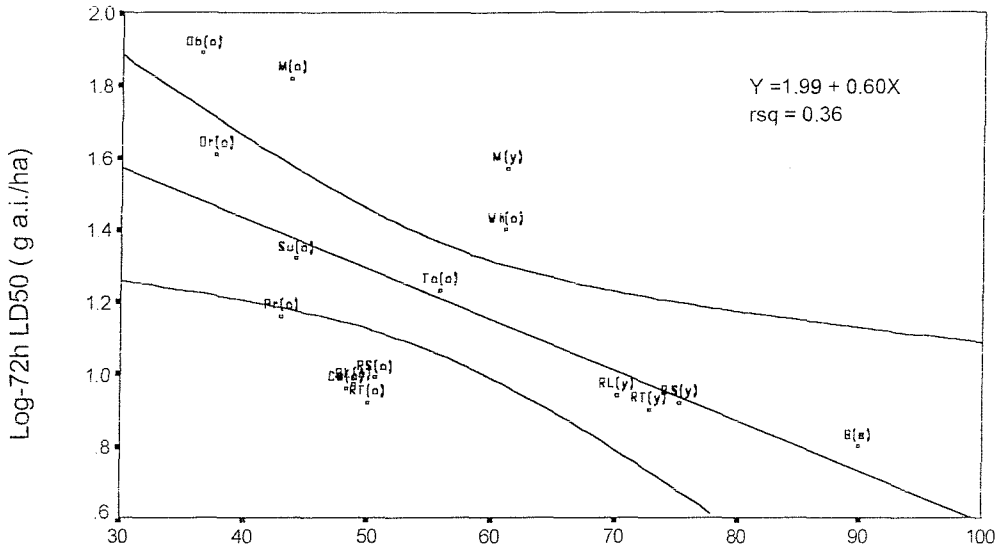


Figure 7.39 Correlation between contact angle of deltamethrin solution (0.5mg/ml of water) (1 ul drop) and log-72h LD50 of *F. candida* for deltamethrin on different leaf surfaces Key to test leaf types are as Figure 7.4

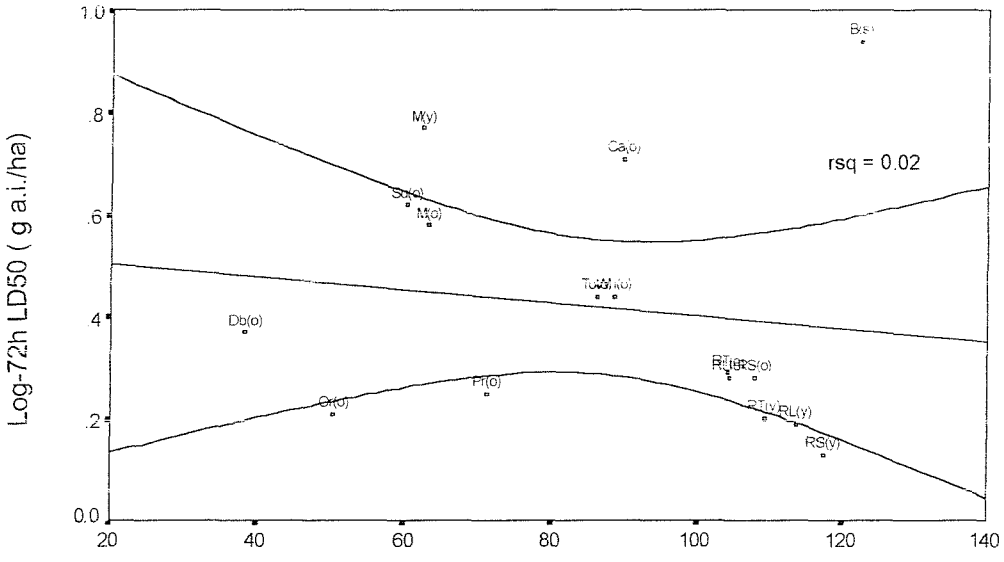


Figure 7.40 Correlation between contact angle of dimethoate solution (0.212mg/ml of water) (1 ul drop) and log-72h LD50 of *F. candida* fordeltamethrin on different leaf surfaces Key to test leaf types are as Figure 7.4

7.3.7 Toxicity responses of *F. candida* and the contact angle of water

The contact angles of pure distilled water were taken as an indicator of inherent wettability. Although these inherent wettabilities for each leaf type can be changed quite considerably by the formulation of the chemical concerned, the trend of change still depends upon inherent wettability (i.e. the wettability determined by distilled water). Therefore, for analysing the relationship between wettability and the susceptibility of *F. candida* on different leaf surfaces, the contact angle of water on those surfaces was used.

Linear regression models were fitted to data for Log-72h LD_{50} values of *F. candida* both for deltamethrin and dimethoate, against contact angle (Figures 7.37 and 7.38). Log 72h LD_{50} values of *F. candida* for deltamethrin sprayed on different leaf types showed a significant negative correlation against the contact angle for different leaf surfaces ($r^2 = 0.89$; $F = 111.20^{***}$; d.f. 1,14; $P = <0.001$). No correlation was observed between log 72h LD_{50} of *F. candida* for dimethoate sprayed on different leaf types and the contact angle of water ($r^2 = 0.02$).

7.4 Discussion

Contact angles above 110^0 on different leaf surfaces showed a close and positive correlation with the amount of wax, corresponding with the earlier findings of Holloway (1969). However, the latter author emphasised the fact that the role of waxes are prominent where contact angles were above 90^0 and attributed importance to the additional factors which also become prominent and act in combination with the quantity of wax to increase the overall hydrophobicity and greater contact angles. Contact angles for smooth films of isolated waxes from leaf surfaces account for only 50-60% of the contact angle measured on leaf surfaces. It follows that, if roughness is a result of wax ultrastructure, then also leaves with high wax coatings may have greater surface roughness. Directly or indirectly, the amount of wax must play an

important role in the formation of the contact angle. In the present experiment, contact angles for water, on waxed and dewaxed leaf surfaces (Table 7.7), indicate that contact angles above 100° reduce substantially after petroleum ether washing (e.g. cabbage (old) and barley seedlings). These results are supported by the findings of Challen (1960) for the leaves of *Agropyron repens* and suggest that the dominant factor affecting wetting of the surface are the surface chemicals and their ultra-microscopic roughness. Contact angles for water on dewaxed maize leaves remained largely unchanged. Similar results were also observed by Challen (1960) on the less waxy lower surfaces of *Papaver orientale* where low contact angles on the surface were not influenced by ether washing. On dwarf bean leaves the contact angle was increased rather than decreased after solvent washing. Holloway (1969) found similar results for a number of leaf species (e.g *Acer pseudoplatanus*) after chloroform washing. No rational explanation has yet emerged for this phenomenon. In this study it was observed that dwarf bean leaves, after petroleum ether washing lost their smooth and soft appearance. Although, dwarf bean leaves contain only a small amount of wax (1-2µg cm^{-2}). This may cover the whole surface as a very thin layer. In the absence of this layer, probably the leaf microstructures such as cell shape and arrangement, which were ridged, irregular and convex (see Figs. 5.24 and 5.25 in chapter 5), become more prominent features in governing contact angle formation.

The contact angles observed on tomato leaves strongly support the role of other characteristics features of the leaf surface (such as trichomes) rather than the amount of wax. Even contact angles above 90^0 did not correlate with the amount of wax present. Tomato leaves contained only 9.65 µg of wax per square centimetre, far less than barley, cabbage and rape and very close to that of sugarcane, maize and orange, whereas the contact angle on tomato is much higher than that of sugarcane, maize and orange and much closer to that of mature leaves of rape varieties. Leaf surfaces devoid of large amounts of epicuticular wax maintain their

hydrophobicity by other complex structures resulting from the underlying venation, projecting wax plates and the dense arrangement of trichomes which decreases the area of contact between the impacting droplet and the leaf surface (Adam, 1963). According to Challen (1962) trichomes that are arranged in a 'close pattern' produce highly water repellent structures which trap air-films, for example, the lower surfaces of apple and raspberry, whereas 'open pattern' trichomes facilitate wetting probably by their capillary action.

Tomato leaves have irregular and small cells (see Figs. 5.41-5.44 in chapter 5) giving a grooved network that can give rise to microscopic roughness (Boize, *et al.* 1976). In addition, in the present experiment, it was observed that trichome structures altered the contact position of the droplet (Figs. 7.11. 7.12, 7.21 and 7.31). In many cases, the contact point of the impacting droplet tended to restrict the formation of the true contact angle and also the spreading by the projecting trichome. In such a situation, as the droplet arch rests on projecting trichomes of different shapes, it was sometimes impossible to measure the true contact angle. If the droplet is larger than the tiny trichome(s), then trichomes that are trapped within the droplet project from it in due course, causing complete distortion of droplet shape and increasing the chance of sudden and rapid spreading.

Convex cell surfaces of rape and tomato cause higher contact angles. Holloway (1971) found the adaxial surface of *Trifolium repens* is highly water repellent with a convex cell surface with crystalline wax deposits. Basal cell arrangement can also affect spreading. Small drops, and a proportion of larger drops, tend to spread readily on dicotyledon leaves following the reticulate pattern of epidermal grooves (e.g. in dwarf bean and tomato) (see Figs. 5.23-5.25 and 5.41-5.44 in chapter 5), whereas, on monocotyledon leaf surfaces, spreading tends to follow the uninterrupted grooves between the longitudinally aligned rectangular epidermal cells (e.g. sugarcane and maize). It would be better if, in future

studies, the measurement of contact angles of drops were taken both longitudinally and transversely on such leaves. However, contact angle measurements alone may not be sufficient to provide good predictions for spreading although the time series studies of advancing contact angles may be able to minimise this shortfall to a certain extent. Spreading is a much more complex phenomenon than wetting. Surface tension, critical micelle concentration and contact angles are all involved in the spreading of spray droplets. Simple measurement of these properties can not adequately predict the spreading of commercially formulated complex adjuvant/surfactants on leaf surfaces (Abbott *et al.* 1990).

The classification of leaf surfaces on the basis of percentage reduction in advancing contact angle over time and also the initial contact angle of water on different leaf types showed a close agreement with wax content. However, it is not sufficient to correlate these directly. For example, barley and young varieties of rape were close to each other in amounts of wax, but the percentage reduction of advancing contact angles were not close. Rape leaves were less glaucous than the young leaves of barley seedlings. Barley leaves have projecting crystalline wax platelets in a dense pattern (see Figs. 5.1-5.3 in chapter 5) covering almost the whole cell surface, whereas those on rape leaves are scattered and dense, just on and around stomata (see Figs. 5.31-5.36 in chapter 5). Increasing glaucousness is correlated with increasing contact angle (Netting & von Wetlstein-Knowels, 1973), and also with an increasing proportion of β-diketones and hydroxy-β-diketones in waxes and an increasing proportion of wax tubes.

In the present experiment, the wheat leaves used were non-glaucous and had waxes around the stomata (see Figs. 5.17 and 5.18 in chapter 5). The amount of wax was not as high as that found in other studies (Netting & von-Wettestein-Knoweles, 1973). Earlier studies showed high contact angles on wheat ($>130^0$) and were ascribed to the wax structures present (Throughton & Hall, 1967). However, variation in the

magnitude of contact angles may result from changes in density, distribution and morphology of the wax structures, such as crystalline tubes or plates. Johnson, *et al.* (1983) showed large differences in surface structures and amounts between glaucous and non-glaucous selections of wheat.

The contact angles of both deltamethrin and dimethoate solution were lower than that of water and must mainly be due to the presence of surfactants/adjuvants in the formulation which increase the efficiency of delivery to the target plant, uptake, redistribution and persistence (Gaskin & Holloway, 1992).

It can be concluded from these results that the wettability of leaf surfaces with high hydrophobicity was increased by the formulations of both insecticides and also that deltamethrin had greater wettability responses than dimethoate. The wettability of leaf surfaces with low contact angles (i.e. more wettable) remains more or less unchanged by the pesticides.

The results for residual susceptibility of *F. candida* on different leaf surfaces also indicated that the higher toxicity of dimethoate on different leaf surfaces compared with deltamethrin was not a function of wettability, but of the independent effects of the active ingredient, its formulation and the dose rate used in the experiment (Figures 7.37 and 7.38).

The toxicological responses of *F. candida* to deltamethrin on different leaf surfaces were not merely a function of leaf wettability. It was observed, in this experiment, that barley leaves were less wettable by deltamethrin than other leaf surfaces but more toxic to *F. candida*. Other factors including sorption and retention may also important. It is possible that deltamethrin was bound to the superficial wax layer and that it was than picked up by the insect. Thus more waxy surfaces contribute more pesticide-contaminated wax particles to the exposed organisms, increasing the

susceptibility of organism concerned.

The evaporative loss of deltamethrin is very low on waxy surfaces compared with non-waxy surface. Boehncke *et al.* (1990) observed only 12% evaporative loss of deltamethrin on the waxy surface of kohlrabi, whereas the loss from less waxy summer wheat and lettuce was 69% and 44% respectively.

The uptake of some chemicals is also higher on waxy surfaces. This was supported by the work of Baker, *et al.*(1992). In their experiment, it was found that the uptake of test chemicals into waxy rape and strawberry leaves was higher than into the less waxy sugar beet leaves. Leaves with low contact angles had greater coverage by spray droplets than leaves with high contact angle. However, retention and coverage can be conflicting (Furmidge, 1962) in their effects. Holloway (1993) showed that the organosilicone 'Silwet L77', which reduced dynamic surface tension, caused a significant reduction in spray retention on the leaves of sugar beet. Therefore, if the coverage of a leaf surface is effective (characterised by a low advancing contact angle) mean retention per unit area can be considerably reduced. Leaves with high contact angles (such as barley, cabbage and rape can therefore retain pesticide droplets more effectively then the leaves with low contact angles, such as dwarfbean, maize and orange.

It is possible to conclude from this experiment that, although the wetting of leaf surfaces by water droplet has some relation with the amount of wax, other leaf characteristics may also play a role.

From this study four points arise

i) the reduction of contact angles of deltamethrin on different leaf surfaces was faster than that of water,

ii) the trend of reduction of contact angles of pesticides according to leaf types was different from that of water,

iii) there is some relationship between reduction of contact angles and the amounts of wax and leaf structures (trichomes etc.) at least during initial reduction after 10 and 30

minutes (e.g in tomato)
and iv) high toxicity responses of *F. candida* for deltamethrin on different leaf types showed no direct relationship with the high reduction rate of deltamethrin droplets. For example, the contact angle reduction on dwarf bean was far greater than that of barley, whereas barley was more toxic to *F. candida* with deltamethrin than was dwarf bean. This finding again supported the previous hypothesis that toxicity responses in this study were not accelerated by wettability of the deltamethrin solution, rather than opposite. Figure 7.37 showed that higher toxicity was observed on leaf surfaces with lower wettability. That is in transfer of toxic ingredient of deltamethrin to exposed *Folsomia candida*, there is a negative correlation with wettability.

For dimethoate all the leaf types with high and moderate amounts of wax (such as leaves of cabbage and both old and young leaves of rape) showed a more or less similar trend in contact angle reduction and none of them reached angles of less than 10° after 60 minutes of impaction. However, the contact angles of dimethoate on dwarf bean reached less than 10° just after 10 minutes of impaction, making the drop highly spreadable, followed by the reduction of advancing contact angle below 15^0 after 30 minutes of impaction on orange, pear, maize and sugarcane (Table-7.5).

Therefore, from this experiment it can be confirmed that dimethoate has less wetting properties than deltamethrin and the initial angle at impaction was much closer to that of water (Table 7.1). The high toxicity of dimethoate in this study was not a direct function of wettability but of the dose concentration and properties of original toxicant (AI).

CHAPTER 8

General discussion

8.1 General overview of the experimental goals and findings

The major aim of the thesis was to provide information on the role of leaf surface characteristics in mediating pesticide toxicity to exposed invertebrates. To reach the goal, a series of experiments was systematically conducted which involved selection of a suitable laboratory test species, development of general methodology, and validation of the preliminary findings with a beneficial invertebrate. To achieve the goal of the project the followings steps were taken:

a) development of a general methodology for using *F. candida* as a test invertebrate in laboratory bioassays with pesticide deposits on different leaf substrates;

b) determination of the residual susceptibility of *F. candida* to deposits of deltamethrin and dimethoate;

c) modification of a standardized laboratory test procedure for the aphid parasitoid *A. colemani* to provide more wide-ranging bioassay data;

d) determination of residual susceptibility of *A. colemani* to deltamethrin on a range of leaf surfaces;

e) identification of leaf surface characteristics that play an important role in mediating pesticide residual toxicity to test invertebrates.

The implication of these findings have been discussed in detail in the respective chapters. The background of the present studies was included with an extensive review of the literature (Chapter 1). The following section will therefore discuss only the overall implications of the results and how they may be used in aiding the understanding of the pesticide-plant surface-invertebrates interactions, which in turn can be used to improve pesticide formulation and application and thereby ensure maximum efficacy with minimum hazard risks. The results of the present experiments will also help in the development of suitable laboratory procedures for toxicological studies on different substrates

using a standard laboratory test species.

8.2 Trends in pesticide usage and innovative approaches: a brief note on the background of the present studies

The striking progress in pesticide development ensures the increasing agricultural commodities and resources. After a period of explosive growth till the 1980's (Graham-Bryce, 1987), innovative approaches have been taken to reduce environmental hazards and increase the efficacy of pest control. One direct result of such approaches is the development of pesticides with low application rates such as deltamethrin, and ultra low volume and other formulations. This has led to a substantial decrease in average field application rates since the 1950's (Graham-Bryce, 1987). Such reduction however, does not reflect the reduction in pesticide usage. Since the use of first generation pesticides (mainly plant derivatives and inorganic compounds) the subsequent development of second (organic compounds) and third (anti-juvenile hormone) generation insecticides led to fourth generation pesticides, the "growth regulators" which resulted from fundamental studies of insect-plant chemical interactions (Bowers, 1987).

Experience with broad-spectrum pesticides and studies of their environmental fate not only led to the search for new generation pesticides but also encouraged development of new techniques and methods of pesticide formulation and application along with environmental impact studies. Such studies have included the effects of lower dose rates (Cross & Berrie, 1990; Currier & Wilkowski, 1988); protection of natural enemies, use and combination of biological and chemical methods of pest control (Bartlett, 1964; Bosch & Stern, 1962; Brown *et al.* 1983; Carter & Sotherton, 1983; Basedow *et al.* 1985; Brust *et al.* 1986; Brown, 1989;); short and long term risk assessment (Jepson, 1988, 1989, 1993a; Burn, 1989, 1992); environmental fate of pesticides (Arnold & Briggs, 1990); formulation (Crisp, 1971; Graham-Bryce, 1983); application methods (Free, *et al.* 1967; Matthews, 1979); ecological selectivity (Ripper *et al.* 1951); and

physiological selectivity (Croft & Brown, 1975; Stevenson & Walters, 1983). Physiological selectivity has recently been considered with great interest. Large differences in the relative toxicity of compounds to different species has been observed (Graham-Bryce, 1975). The underlying basis for these differences in susceptibility can be manipulated to minimise beneficial environmental impact.

Some pesticides (especially the broad-spectrum types) targeted on a pest can also affect the pest of low population (secondary pest), which normally controlled by their natural enemies in absence of pesticides. Injudicious application of DDT on wheat killed the predators of spider mites, a secondary pest, and in the absence of their natural enemies an outbreak of secondary pest populations took place (Graham-Bryce, 1987). Again a pesticide becomes less effective against a pest but still higly lethal to its natural enemies. In the absence of natural control agents the pest population can increase to a higher level than before, causing pest resurgence. The possibility of frequent pest outbreaks has been demonstrated experimentally by Burn, (1987). The use of broad-spectrum pesticides (especially synthetic pyrethroids) to control European corn borer on maize resulted in syrphid and coccinellid mortality (Mestes & Cabanetts, 1985) and consequently may have increased the outbreak frequency of *R. padi* (Naibo & Seyer, 1985).

In addition, there are also problems of pest resistance to pesticide, which can make the pesticide less effective. By the early 1980's, resistant strains in over 400 species had been recorded from areas where chemical control was practised intensively (Georghiou, 1981). There are also examples of cross-resistance. The gene *kdr* in houseflies confers knockdown, giving moderate resistance to DDT (Farnham, 1977). In Danish population of houseflies *kdr* was selected by the use of DDT in 1940's. After the withdrawl of DDT it remained dormant until the 1970's. With the use of synthetic pyrethroids it rapidly then re-emarged, resulting in strong pyrethroid resistance (Sawicki & Denholm, 1984).

Overuse of pesticides also causes environmental pollution. One example of such pollution resulted from the attempt to control wheat bulb fly (*Delia coarc tata*). Seed treatments with organophosphate against this pest have recently caused deaths in wild birds especially geese (Sheaill, 1985).

Application methods and efficient targetting of the pesticides could play an important role in limiting the damaging effects on polyphagous predators and parasitoids. Conventional hydraulic spraying is a relatively inefficient method of applying aphicide during summer, as it allows much of the pesticide to penetrate the canopy and reach the soil. Electrostatic charging systems deliver a greater proportion of the spray to the upper parts of crops and may be more effective in controlling pests mainly active in the leaf canopy, such as cereal aphids.

Determination of appropriate droplet size is also important. Small drops (100 µm diameter) may not contain enough dose to kill the target insect, whereas large drop sizes of 300 µm diameter contain enough dose to be lethal, but over 90% of the content would be wasted even it contacts the insect. Therefore optimum size should be smaller (Graham-Bryce, 1977a). However, any drop below 50 µm in diameter will again increase the chance of drift hazards and will depend on the movement of surrounding air (Graham-Bryce, 1987).

Most of the work cited above is concerned with pesticides and invertebrates. In crop ecosystems, adjusting application and formulation methods to take advantage of the characteristics of the plant surface can lead to an increase in pesticide efficacy and also helps to reduce the side-effects of pesticides on beneficial invertebrates.

8.3 Determining the roles of leaf surface characteristics in mediating pesticide toxicity to exposed invertebrates

Leaf surface characteristics play an important role in the physico-chemical activity of foliar-applied chemicals (Chapter 1). The results in chapters 3, 4 and 6 show how they

affect mortality responses of exposed invertebrates. The majority of published work has focused upon the physics and chemistry of leaf surfaces and the pesticide, and commented on factors of ecological importance (e.g. Holloway, 1970; Baker *et al.* 1983; Holloway, 1994;). The main focus has been given to wetting, retention, coverage, spreading, penetration and uptake of spray liquids in relation to the leaf surface characteristics and the chemical properties of the sprayed liquid. Only a few studies have been conducted on direct toxicity to exposed invertebrates in relation to substrate characteristics (e.g. Lewis & Hughes, 1957; Hart, 1979;).

To proceed further with the development of hypotheses concerning the role of leaf surface characteristics in residual toxicity, it was essential to develop sensitive methods that could be used to establish whether or not any significant differences in pesticide availability exists between the leaf types. This approach could then be used to determine on which leaf surface types invertebrates are likely to be most or least susceptible to particular pesticides. For example, in Table 8.1, the LD_{50} values for *F. candida* indicated difference in pesticide availability on different leaf substrates sprayed with deltamethrin. These bioassay results provided a basis for further experimentation on the role of leaf surface characteristics. Differences in pesticide availability must result from interaction between leaf surface characters and the applied chemical, which determine the transfer of active ingredients to the organism and ultimately to the site of action.

8.4 Role of epicuticular wax content in mediating pesticide availability to *F. candida* and *A. colemani*

It is evident from the results that the epicuticular wax content is correlated with deltamethrin availability to *F. candida* (Chapter 3). The quantities of wax extracted from several leaf types are very similar to those recorded by Baker *et al.* 1983; Denna, 1970 and Martin & Batt, 1958. For a given dose level, mortality increased with increase in the quantity of epicuticular wax. For example, *F. candida* was

Table 8.1. Log LD_{50} values of *P. candida* at different intervals of exposure on different leaf surfaces treated with deltamethrin and dimethoate

Leaf types	24hrs after treatment log LD_{50} @ g a.i./ha (95% CL)		48hrs after treatment log LD_{50} @ g a.i./ha (95% CL)		72hrs after treatment log LD_{50} @ g a.i./ha (95% CL)		96hrs after treatment log LD_{50} @ g a.i./ha (95% CL)	
	delta.	dimeth.	delta.	dimeth.	delta.	dimeth.	delta.	dimeth.
Barley (s)	1.14 (1.02-1.19)	1.23 (1.11-1.37)	0.94 (0.81-1.05)	1.06 (0.96-1.16)	0.80 (0.66-0.92)	0.94 (0.84-1.04)	0.63 (0.46-0.75)	0.85 (0.75-0.95)
Cabbage (o)	1.33 (1.19-1.47	1.03 (0.93-1.15)	1.08 (0.93-1.21)	0.87 (0.76-0.99)	0.96 (0.79-1.08)	0.71 (0.62-0.81)	0.73 (0.51-0.88)	0.53 (0.43-0.62)
Tomato (o)	1.70 (1.56-1.89)	0.55 (0.44-0.66)	1.33 (1.21-1.45)	0.49 (0.38-0.60)	1.23 (1.10-1.36)	0.44 (0.33-0.56)	1.01 (0.88-1.12)	0.40 (0.29-0.50)
Pear (o)	1.59 (1.41-1.85)	0.44 (0.22-0.63)	1.26 (1.11-1.41)	0.30 (0.13-0.44)	1.16 (0.99-1.31)	0.25 (1.10-0.36)	0.99 (1.39-1.13)	0.18 (0.04-0.29)
Sugarcane (o)	1.73 (1.56-2.01)	1.15 (1.02-1.32)	1.51 (1.35-1.73)	1.01 (0.87-1.18)	1.32 (1.15-1.51)	0.62 (0.46-0.77)	1.22 (1.06-1.39)	0.54 (0.39-0.68)
Wheat (o)	1.73 (1.56-2.00)	0.73 (0.60-0.85)	1.61 (1.42-1.87)	0.58 (0.48-0.68)	1.40 (1.21-1.61)	0.44 (0.34-0.54)	1.23 (1.03-1.42)	0.38 (0.26-0.48)
Orange (o)	1.88 (1.70-2.18)	0.59 (0.48-0.71)	1.85 (1.66-2.21)	0.39 (0.24-0.52)	1.61 (1.43-1.87)	0.21 (0.03-0.35)	1.38 (1.22-1.57)	0.13 (-0.03-0.26)
Dwarfbean (o)	2.27 (2.14-2.48)	0.86 (0.76-0.97)	2.19 (2.01-2.46)	0.73 (0.63-0.83)	1.89 (1.74-2.08)	0.37 (0.26-0.47)	1.73 (1.58-1.91)	0.31 (0.22-0.40)
Rape v. Tanto (o)	1.49 (1.33-1.69)	0.52 (0.42-0.62)	1.13 (0.99-1.26)	0.40 (0.30-0.51)	0.92 (0.77-1.04)	0.29 (0.20-0.38)	0.81 (0.68-0.92)	0.21 (0.12-0.29)
Rape v. Tanto (y)	1.26 (1.14-1.37)	0.57 (0.47-0.66)	1.02 (0.89-1.14)	0.41 (0.31-0.51)	0.90 (0.77-1.01)	0.20 (0.08-0.30)	0.67 (0.51-0.77)	0.13 (0.04-0.22)
Rape v. Lirawell (o)	1.50 (1.35-1.68)	0.57 (0.46-0.67)	1.18 (1.05-1.30)	0.38 (0.29-0.47)	0.97 (0.83-1.10)	0.28 (0.19-0.37)	0.75 (0.59-0.87)	0.26 (0.16-0.35)
Rape v. Lirawell (y)	1.30 (1.17-1.44)	0.54 (0.44-0.63)	1.04 (0.91-1.16)	0.32 (0.20-0.42)	0.94 (0.82-1.04)	0.19 (0.07-0.29)	0.69 (0.55-0.80)	0.09 (-0.03-0.19)
Rape v. Starlight (o)	1.44 (1.31-1.60)	0.53 (0.42-0.63)	1.19 (1.06-1.31)	0.35 (0.24-0.45)	0.99 (0.86-1.11)	0.28 (0.19-0.37)	0.84 (0.71-0.95)	0.25 (0.17-0.33)
Rape v. Starlight (y)	1.24 (1.11-1.36)	0.52 (0.42-0.61)	0.99 (0.84-1.11)	0.37 (0.27-0.47)	0.92 (0.78-1.03)	0.13 (0.009-0.23)	0.67 (0.52-0.78)	0.10 (-0.007-0.19)
Maize (o)	1.95 (1.75-2.31)	1.06 (0.96-1.16)	1.98 (1.77-2.36)	0.58 (0.47-0.69)	1.82 (1.64-2.13)	0.62 (0.51-0.73)	0.81 (0.68-0.92)	0.77 (0.66-0.89)
Maize (y)	1.88 (1.69-2.21)	1.29 (1.19-1.42)	1.77 (1.58-2.04)	0.96 (0.84-1.09)	1.57 (1.41-1.79)	0.77 (0.67-0.88)	0.67 (0.51-0.77)	0.62 (0.51-0.72)

s = seedlings; o = old; y = young

found highly susceptible on surfaces of barley leaves followed by rape var. tanto (young), rape v. Starlight (young), rape v. Tanto (old), rape v. Lirawell (young), cabbage (old), rape v. Lirawell (old), rape v. Starlight (old), pear (old), tomato (old), sugar cane (old), wheat (old), maize (young), orange (old), maize (old) and dwarf bean (old) (chapter 3). The LD_{50} values for *F. candida* on these leaf surfaces were significantly correlated with the quantity of epicuticular waxes extracted from their adaxial surfaces (chapter 6).

There are two possible explanations for the results above. First, deltamethrin is comparatively lipophilic, which permits the pesticide to bind successfully with other lipophilic substances, such as the epicuticular wax. The active ingredient then is more likely to be retained on the leaf surface and is protected from rapid evaporative loss. Decreases in evaporative losses of deltamethrin from waxy leaf surfaces were shown by Boehncke *et al.* (1990). If further penetration of active ingredient into the leaf is restricted by successful adhesion to epicuticular and cuticular waxes, a solution of pesticide in the waxy layer may retain contact toxicity and be more resistant to natural weathering than crystalline deposits on the surface (Martin & Batt, 1958).

Secondly, the alternative possibility is that waxes may provide a different route of uptake for the active ingredient. For invertebrates with relatively low behavioural activity, such as some lepidopteran larvae, the most important route of pesticide transfer is adhesion and diffusion. In such cases, there is competition for lipophilic compounds between the wax of leaf surfaces and that of the insect cuticle that results in a steady accumulation of active ingredient by the insect, and finally establishes a steady state, when the rate of pick-up by the insects is equivalent to the rate of detachment (Ford & Salt, 1984). Additionally, insects may be exposed to pesticides by accumulating whole wax particles contaminated with pesticide

residues from leaf surfaces. With insects like *F. candida*, not only do certain body parts make direct contact (for example the legs and the end of the abdomen) but also the body parts which do not touch the surface can be contaminated by the antennae, mouthparts and legs during activities such as walking, grooming, cleaning, rubbing. This pick-up process may be more or less continuous, and during cleaning movements, the legs, antennae with their spines and setae may act as instruments of distribution and transfer of particles from primary sites of particle attachment to apparently uncontaminated parts of the body (Lewis & Hughes, 1957). In this way, the rate of encounter with the residual toxicant can increase several-fold. Scanning electron microscopy of *F. candida* exposed to different leaf surfaces for 24h provided some evidence for this pick-up and transfer.

As predicted earlier, the leaf surface characteristics can be greatly affected by the nature of the pesticide concerned. This was evident in the results obtained in bioassays with dimethoate, an organophosphate with systemic properties (Chapter 3). No direct correlation was observed between the susceptibility of *F. candida* and the amount of epicuticular wax on various leaf types. However, the most important observation was that significant differences exist between the LD_{50} values for *F. candida* exposed to different leaf surfaces treated with dimethoate. It is obvious that the interaction between the leaf surface characteristics and the applied pesticide has been changed.

Although it is not possible to answer all questions with few experiments, nevertheless, the present studies provide some important information and a basis for further study. The low toxicity of dimethoate deposits on the leaf surfaces of barley, cabbage, sugarcane, maize, wheat and tomato may be attributed to other surface properties such as roughness and glaucousness, rather than with amounts of epicuticular wax. These surfaces are mostly non-glaucous, with macroscopic (e.g. dense trichomes on tomato leaves), microscopic (e.g. basal cell structures and arrangements on sugarcane, wheat

and maize leaves) and ultramicroscopic (e.g. dense crystalline wax plates on barley and wax blooms on cabbage leaves) roughness. Leaf surfaces on which *F. candida* were found to be highly susceptible to dimethoate were comparatively smooth and glossy (orange leaves) or amorphous (dwarf bean). Stomatal infiltration and diffusion through trichomes may have played an important role in pesticide uptake, along with the systemic properties of dimethoate.

The magnitude of differences in susceptibility of *F. candida* on different leaf surfaces treated with the same pesticide and application rate makes it important to consider the dosages applied to the invertebrates in relation to the type of crop. During formulation of pesticides, emphasis should be placed on the impact of the plant surface on the efficacy of the pesticides.

Further experimentation with a range of invertebrate species and plant surfaces can give a better understanding of the processes involved in pesticide-surface-invertebrate interactions. Such tests would be valuable with pests of major economic importance, such as cereal aphids, and their natural enemies, such as coccinelid beetles. The current recommended single rates of application for such common pests should be re-evaluated in relation to the crop involved.

To verify the hypothesis of pick-up of waxes from the plant surface, chemical analysis of *F. candida* exposed to leaf surfaces could be conducted.

8.5 Extrapolation of *F. candida* as a standard laboratory test species

F. candida is suitable for bioassay studies in the laboratory. The ranking in table 8.2 for *F. candida* and *A. colemani* shows a similar trend in susceptibility to deltamethrin residues on three different leaf surfaces in relation to the quantity of wax present on the surfaces, despite large differences in the relative toxicity. To explore the possibilities of extrapolating the results to

Table 8.2. Ranking relation between 24-h LD_{50} values of *F. candida* and *A. colemani* for deltamethrin and wax content of three different leaf species

Leaf species	24-h log LD_{50} values of F. candida for deltamtehrin @ g a.i./ha (95% CL)	Rank	24-h log LD_{50} values of A. colemani for deltamethrin @ g a.i./ha (95% CL)	Rank	Wax content (ug per cm. sq.) (SE±)	Rank
Barley (s)	1.14 (1.02-1.19)	1	0.80 (0.76-0.84)	1	51.33 (2.89)	1
Cabbage (o)	1.33 (1.19-1.47)	2	0.87 (0.84-0.91)	2	36.12 (1.13)	2
Dwarfbean (o)	2.27 (2.14-2.48)	3	0.91 (0.87-0.94)	3	01.46 (1.13)	3

s = seedling; o = old; y = young

other economically important species, further experiments should be conducted with a number of predators and parasitoids, such as the members of Coccinelidae, Carabidae, Staphylinidae and Aphidiinae.

8.6 Further validation of findings: The primary and most important goal of the thesis was to explore the role of leaf characteristics in the mediation of pesticide toxicity to an exposed invertebrate. For this purpose, an appropriate laboratory test species, *F. candida* was used. To verify the results further, similar bioassays were conducted with the parasitoid, *A. colemani*. Three leaf species were selected as representatives of species with low, medium and high wax content on their epicuticle and were treated with deltamethrin. In most cases the LD_{50} values were much lower than those obtained for *F. candida* exposed to deltamethrin (Table 8.2). However, the susceptibility trend supports the previous findings, that the amount of epicuticular wax was negatively correlated with the LD_{50} values of *A. colemani* exposed to deltamethrin. The higher susceptibility of *A. colemani* in comparison to *F. candida* on these three leaf species may result from differences in intrinsic detoxifying ability and differences in behaviour that may affect exposure.

8.7 Leaf wettability and the toxic response: Increases in leaf wettability, (measured on the basis of the contact angles of distilled water on individual leaf types), did not result in increased toxicity to *F. candida* on different leaf surfaces treated with deltamethrin. Contact angles observed on several leaf types agree with those recorded by Holloway, 1969, 1970; Richard *et al.* 1986. *F. candida* was more susceptible on surfaces with low wettability (high contact angles) (Chapter 7). Theoretically, wettability has always been considered to be one of the most important factors for determining pesticide efficacy. During the pre-penetration process, it influences the deposition, distribution and retention of spray chemicals (Holloway, 1970). This does not always mean that higher wettability produces higher toxicity,

although in pesticide formulation, wetting of the leaves is taken to be a priority for increasing efficacy and surfactants are used to increase the wetting properties.

Efficacy is a term which covers, especially in field, a number of factors such as spray application, total coverage, volume and mortality and is affected by precise control over spray application and coverage, and not simply by the quantity of pesticide on the leaf surface, whereas toxicity is a more unified concept, mainly measured by mortality, especially in the laboratory. However, such concepts are not as simple as they may appear. In addition to wettability, the behaviour of spray droplets on the leaf surface is also important. Formulation and droplet sizes can contribute to the overall wetting and spreading of the chemical, especially in field conditions. The kinetic energy of the air-borne drop is dissipated as damped oscillations of the droplet which spread and recoil after impaction on plant surfaces. The extent of the oscillation is determined by the kinetic energy of the drop and its surface tension. After the oscillation stops, liquid deposits tend to spread across the surface at an initial rate determined by the drop viscosity (Crease *et al.* 1985). Large drops (>200µm in diameter) are normally more influenced by gravity than smaller drops (<80µm in diameter) (Ford & Salt, 1987). As the deposits dry with the loss of volatile carrier, dynamic changes in surface tension make the process of substrate-deposit interactions more complex. This in turn affects the distribution of the active ingredient. Slow-drying deposits may be less uniform than quick drying deposits (Hartley & Graham-Bryce, 1980). In the present experiments it was observed that, although the leaf surfaces of orange and dwarf bean were found to be highly wettable, the tiny droplets of spray liquid joined together after a few minutes of impaction and formed large, isolated drops of irregular shape. This behaviour of spray drops may result in the freeing up of certain areas from spray deposits. On leaf surfaces with low wettability (such as barley, cabbage and rape) spray droplets remain as fine, tiny drops, with continuous and uniform patterns over the surface remaining

for considerable periods. This may ensure maximum coverage and retention.

However, spreading is a much more complicated phenomenon than wetting. Surface tension, critical micelle concentration and contact angle are all involved in the spreading of spray droplets. Single measurements of contact angle can not adequately predict the spreading of commercially formulated complex adjuvant/surfactants on leaf surfaces (Abbott *et al.* 1990). Again, coverage and retention can be conflicting (Furmidge, 1962). Organosilicone ' Silwet L77', which reduces dynamic surface tension, caused a significant reduction in spray retention on leaves of sugarbeet (Holloway, 1993). Therefore if the coverage is effective, mean retention per unit area can be considerably reduced. Leaves with high contact angles (such as barley, cabbage and rape) are able to retain the pesticide droplets more effectively than leaves with low contact angles (such as dwarfbean, maize and orange). These factors can modify the physical nature of the deposit and therefore the availability on the surface. Providing the proportion of the active ingredient and the plant surface are fixed, modification of formulation can enhance the pesticide availability on the target foliage. The role of formulation and substrate is strikingly evident from the work of Chadwick (1985). When permethrin was sprayed as wettable powder (WP), 100% mortality was observed after 5 minutes of application both on Plywood and emulsion paint. When the same pesticide was applied as an emulsifiable concentrate (EC) at the same rate of deposition, 16% and 100% mortality of *Blattella germanica* was observed after 5 minutes and 6h respectively on plywood; whereas on emulsion paints 14% and 4% mortality respectively of the same species was observed after a similar period of time.

Addition of surface-active agents to pesticide formulations considerably changes the contact angle (Ebeling, 1939; Ford *et al.* 1965). This increases the spreading and coverage. By understanding the action of leaf surface characteristics in relation to the particular pesticide, better spray

formulation and application methods can be achieved. Equally good pest control can often be achieved with spray volumes of less then 20 l ha^{-1} compared with conventional higher volumes of 200 l ha^{-1} (Matthews, 1973; Taylor & Merritt, 1974)

It was also observed that dimethoate has less wetting properties than deltamethrin (chapter 7). However, the high toxicity responses of *F. candida* to dimethoate residues showed no relationship with wettability of the leaf surfaces. Therefore, the toxicity responses of *F. candida* on leaf surfaces treated with dimethoate were not a direct function of wettability but of the dose concentration and properties of original toxicant.

8.8 Future work on leaf surface roughness, wettability and the pesticide availability

In the present studies, microscopic and ultra-microscopic roughness of leaf surfaces was documented only by imaging using the Scanning Electron Microscope. Earlier reviews and findings revealed the importance of leaf surface roughness in the deposit-surface interactions, such as wetting, spreading and coverage and retention. Variation in retention was found to be closely related to the irregularities of the leaf surface (Furmidge, 1962). Higher retention is found on the lower surface of black currant leaves whose surface contours show a series of sharp peaks corresponding to every prominent vein structures where considerable quantities of liquid can be trapped between the veins. The upper surface of the leaves of the same species is irregular, but the veins form depressions that will not retain as much liquid as the lower surface, so the measured retention was found to be lower (Furmidge, 1962). In the present studies (Chapter 7) and according to various previous authors (Holloway, 1970; Baker *et al.* 1983), it was found that most spreading of liquids occurs on smoother surfaces and is represented by the low advancing contact angle. Such increase in spreading results in low retention (Boize *et al.* 1976). Therefore, the relative importance of spreading and retention on a particular crop-pest situation and information on the leaf surface roughness

will help with formulation and application strategies.

The roughness described above is the 'macroscopic- roughness' based on sharp peaks or depressions that are associated with the pattern of leaf venation and the presence of pubescence. The 'microscopic' and 'ultramicroscopic' roughness arises from irregularities in the cuticle and may be added to by the wax structures present. Scanning electron microscopy of different leaf surfaces in Chapter 5 shows this roughness and several micrgraphs are similar to those of other authors (Harr, *et al.* 1991, Jeffree, *et al.* 1976).

Further quantification of microscopic and ultra-microscopic roughness of leaf surfaces may be possible by using the Atomic Force Microscope (AFM). During the present studies this was tested, probably for the first time, to measure the leaf surface roughness (Figure 8.1 and Table 8.3). There seems to be no established protocol of leaf sample preparation for AFM as there is for SEM. Before recent progress in preparation of biological samples for SEM, replica techniques were used. As with SEM, it is not practicable to observe fresh and hydrated botanical specimens under the AFM. Such attempts failed due to distortion and instability of the scanned image, probably due to the moisture content of the leaf surfaces. Therefore, replicas of leaf surfaces were prepared by placing small pieces of cellulose acetate (125µm thick) soaked in acetone, on the leaf specimen with the surface to be observed facing downwards. After drying off the acetone (approx. 10 minutes), the cellulose acetate sample was carefully removed from the leaf surface and attached to an aluminium stub (Agar Scientific Ltd. UK.) with the side with the impression of the leaf surface facing upwards.

Atomic Force Microscopy of leaf surfaces shows the micro- and ultra-microscopic roughness both graphically and arithmetically. This approach has interesting potential for the study of leaf surface roughness. The advantage of AFM over SEM is that, in addition to the surface image, relative

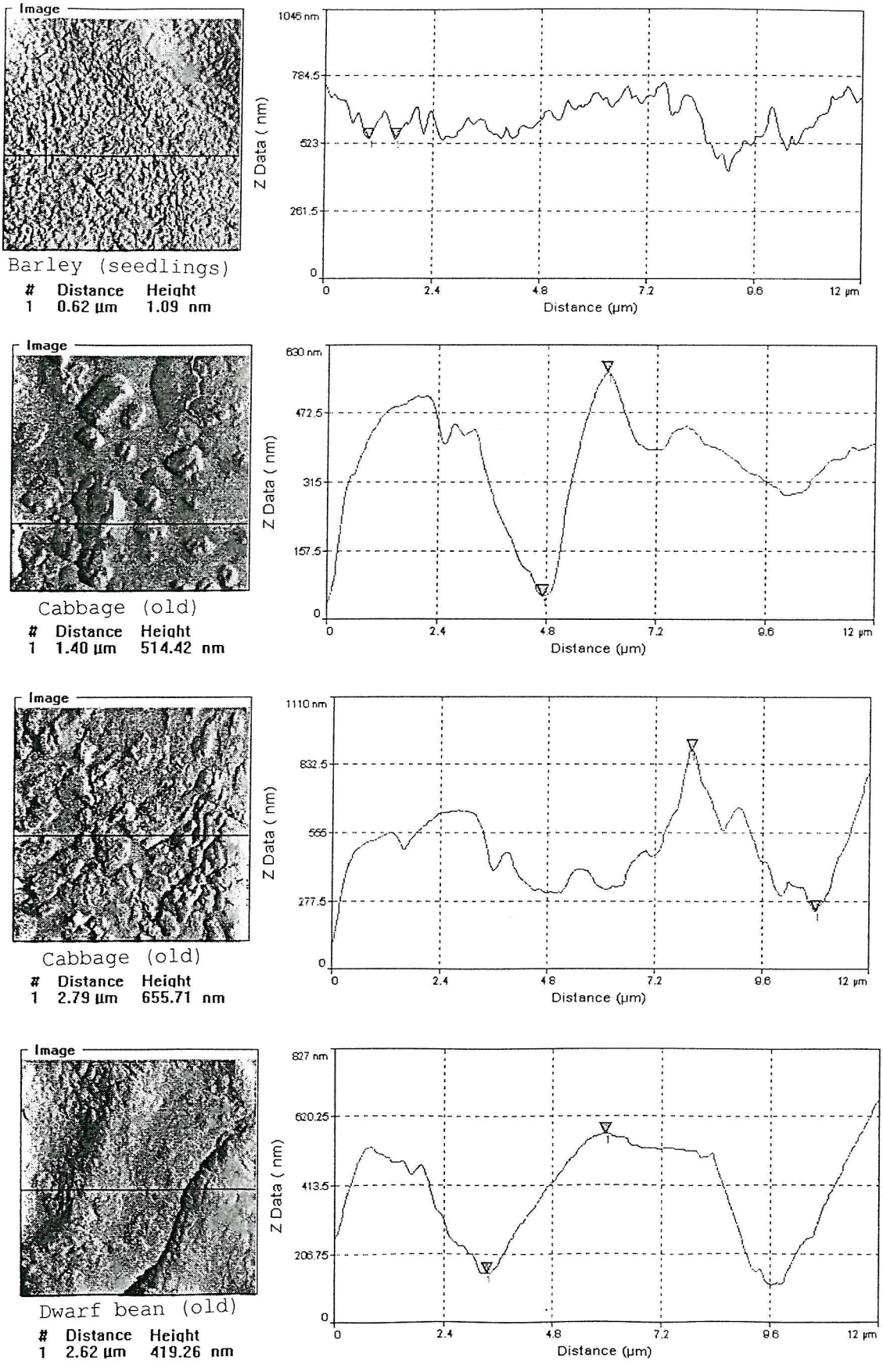


Figure 8.1 Measurement of leaf surface roughness of various leaf types by using Atomic Force Microscopy

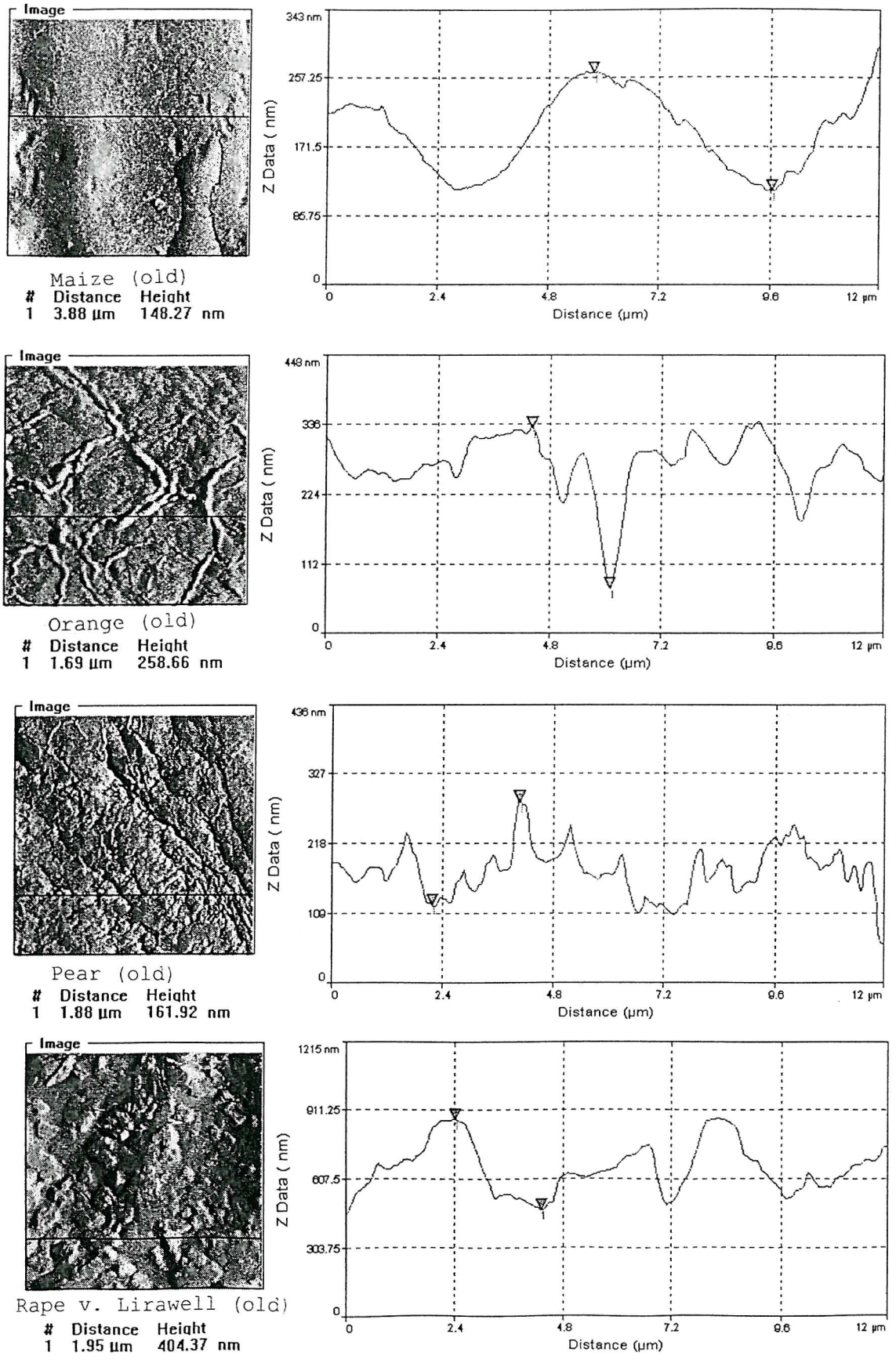


Figure 8.1 (cont.)Measurement of leaf surface roughness of various leaf types by using Atomic Force Microscopy.

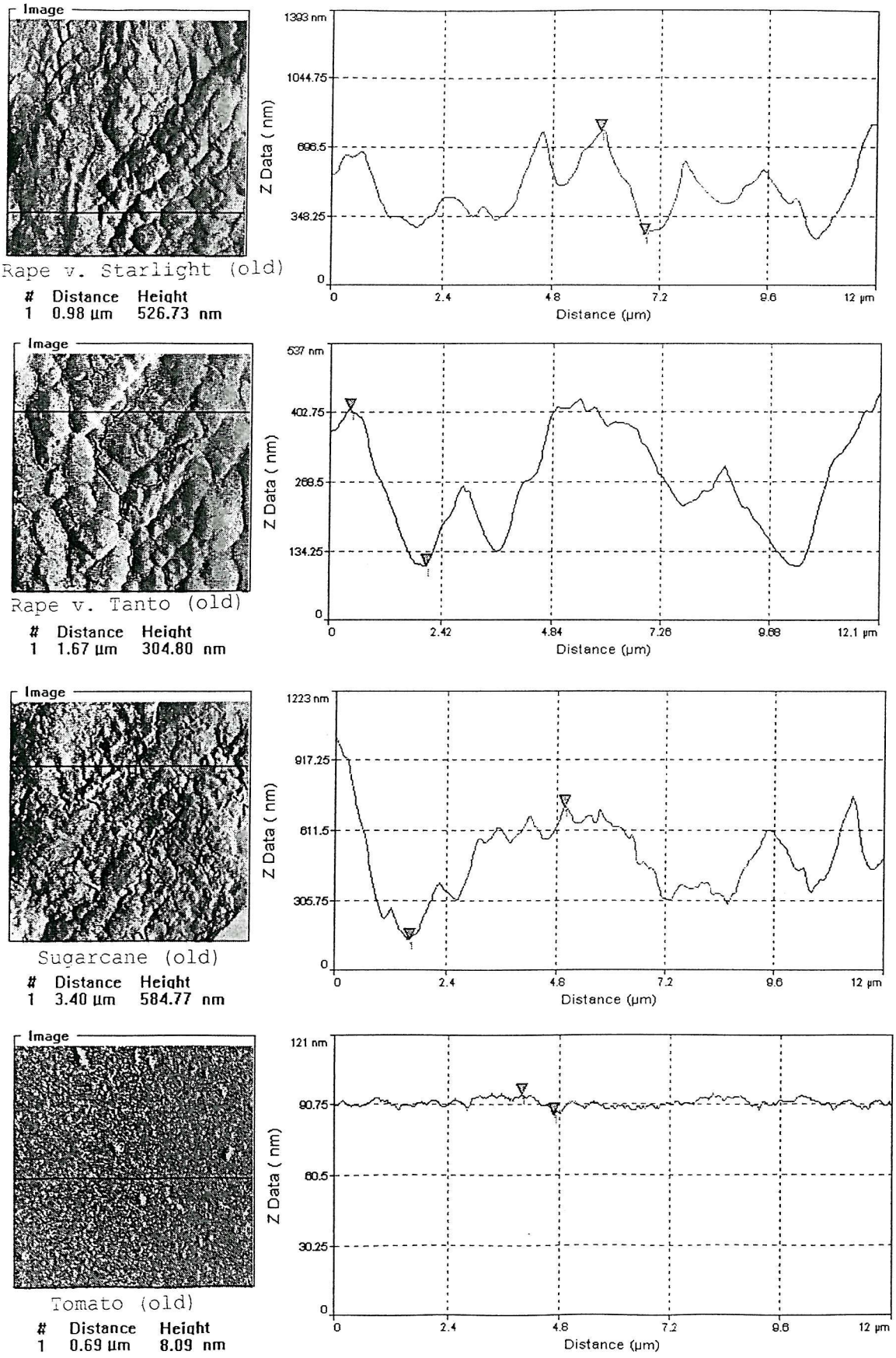


Figure 8.1 (cont.) Measurement of leaf surface roughness of various leaf types by using Atomic Force Microscopy.

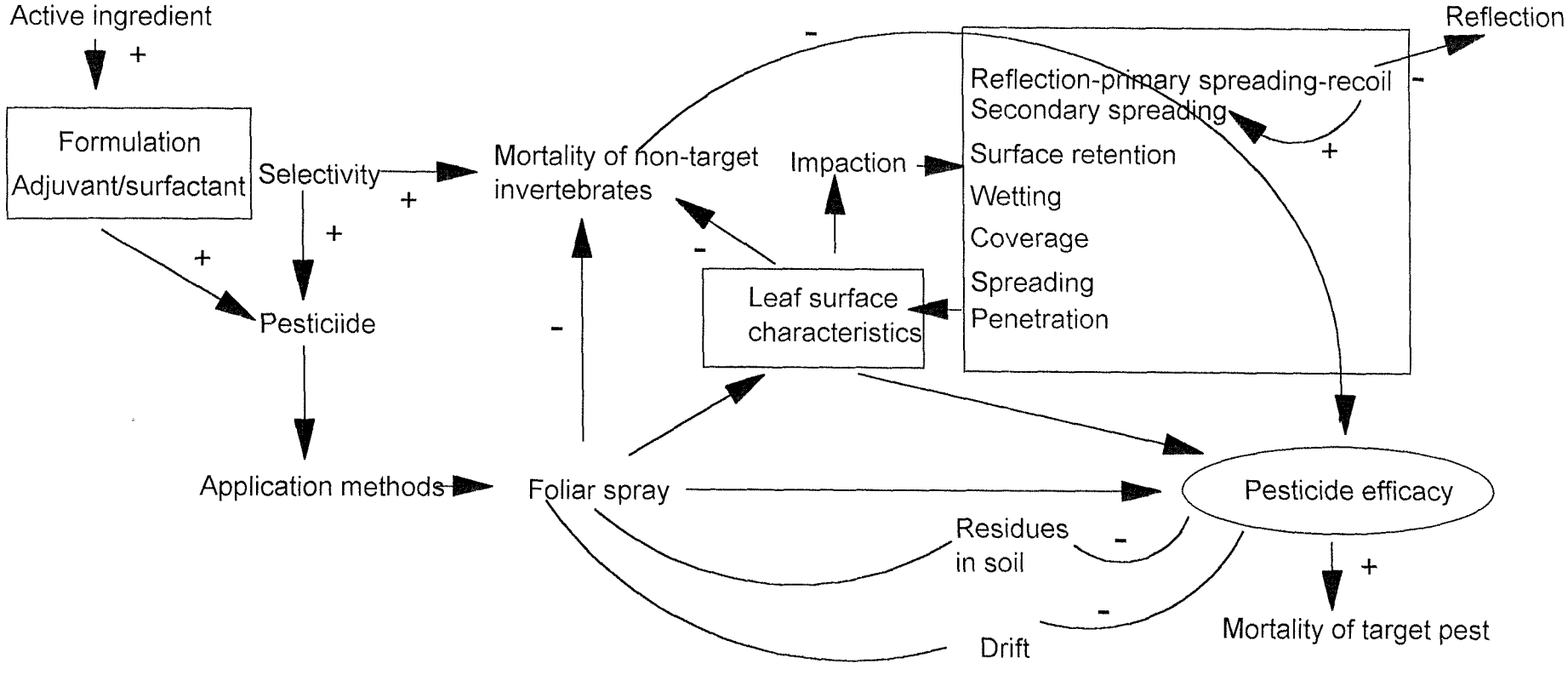


Figure 8.2 Fate of pesticide and its efficacy to target invertebrates

peaks and depressions and arithmetic roughness averages (Ra) (Table 8.3) can be determined by using a suitable image analysis programme such as Topo Metrix® image analysis software (TopoMetrix Corporation, Saffron Waldon, UK.).

Microscopic roughness arises from the cell shape, the arrangement of which determines the geometry of grooves, which is also important in retention and spreading of liquid drops. In general, the spread on leaves of drops >20µm diameter will be governed by macroscopic and microscopic roughness since they contain sufficient volume to be conducted along the relatively wide grooves in the epidermis (Boize *et al.* 1976). Greater understanding of the roles of leaf surface roughness will help to explain the way in which pesticides of different formulations form deposits.

8.9 Conclusions

To achieve thegoal of an effective and robust approach to ensure maximum efficacy of a pesticide with minimum side-effects on non-target species, the ecotoxicolgical risk assessment experiments must also include the effects of plant surface characteristics in mediating toxicity. A flow chart shown in Figure 8.2 highlighted the fate of foliar applied pesticides and some of the topics covered in the boxed area has been investigated in the present study.

Residual susceptibility data for different invertebrate species to different dose rates should be generated with special reference to plant surfaces. Such data will provide a means of selecting appropriate chemicals and dosages for specific crop-invertebrate systems. Currently, most of the research on the interactions between plant surfaces and pesticide are laboratory-based and focused on the physical and chemical behaviour of both components. Direct toxicological studies on invertebrates in relation to the physics and chemistry of pesticides and plant surfaces should be incorporated in current testing programmes. Such studies of plant surfaces and pesticide toxicity associated with pests and their natural enemies may provide greater insights

Table 8.3 Arithmetic roughness average (Ra) of various leaf types

Leaf types	obs. 1	obs. 2	obs. 3	obs. 4	obs. 5
Rape v. Tanto	202	202	234	240	240
Cabbage	342	270	188	234	148
Maize	124	141	..	..	..
Orange	90	151	171	110	126
Pear	97	49	103	125	162
Tomato	10	8	6	4	9
Dwarf bean	436	214	..	..	..
Rape v. Starlight	509	280	191	..	..
Rape v. Lirawell	288	..	..	..	..
Barley (ssedlings	283	252	264	..	..

.. obervation did not taken

into the overall management of pesticides in specific agroecosystems.

More research is urgently needed on the role of plant surfaces in making pesticide available to pests and their natural enemies to reduce application rates of pesticides and thereby protect the beneficial invertebrates with minimum crop loss.

Overall laboratory test method have been developed in this study to aid such researches, with a standard test species and a parasitoid may be adapted to many other chemicals, parasitoid species and plant surfaces. A greater understanding of basic surface-pesticide-invertebrate interactions is still needed to identify which parameters are most important. Further information with semi-field and field experiments can lead to a significant development in pest management research with better ecological insight.

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Appendix-1. 24-h Probit statistics of responses to Deltamethrin 2.5 EC for *F. candida* and different leaf types

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	1.96 (.253)	1.14 (1.02-1.19)	3.841 (4) ns
Cabbage (*Brassica oleracea*) v. Prixie	1.52 (.220)	1.33 (1.19-1.47)	0.803 (4) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	1.62 (.251)	1.70 (1.56-1.89)	0.549 (4) ns
Pear (*Pyrus communis*)	1.12 (.208)	1.59 (1.41-1.85)	0.757 (4) ns
Sugarcane (*Saccarum officinerum*)	1.22 (.221)	1.74 (1.55-2.01)	0.461 (4) ns
Wheat (*Triticum aestivum*) v. Hereward	1.25 (.222)	1.73 (1.55-1.98)	0.861 (4) ns
Orange (*Citrus spp.*)	1.40 (.251)	1.88 (1.70-2.18)	1.096 (4) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	1.86 (.315)	2.27 (2.14-2.48)	4.289 (4) ns
Rape (*Brassica napus*) v. Tanto (Mature)	1.28 (.212)	1.49 (1.33-1.69)	0.551 (4) ns
Rape (*B. napus*) V. Tanto (Young)	1.87 (.242)	1.26 (1.14-1.37)	0.633 (4) ns
Rape (*B. napus*) v. Lirawell (Mature)	1.38 (.218)	1.50(1.35-1.68)	1.706 (4) ns
Rape (*B. napus*) V. Lirawell (Young)	1.59 (.223)	1.30 (1.17-1.44)	1.219 (4) ns
Rape (*B. napus*) v. Starlight (Mature)	1.55 (.226)	1.44 (1.31-1.60)	0.913 (4) ns
Rape (*B. napus*) v. Starlight (Young)	1.74 (.223)	1.24 (1.11-1.36)	0.307 (4) ns
Maize (*Zea mays*) v. Marcia (Mature)	1.04 (.301)	1.95 (1.75-2.31)	0.983 (4) ns
Maize (*Z. mays*) V. Marcia (Young)	1.30 (.293)	1.88 (1.69-2.21)	0.293 (4) ns

a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

Appendix-2. 48-h Probit statistics of responses to Deltamethrin 2.5 EC for *F. candida* and different leaf types

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	2.01 (.275)	0.94 (0.87-1.05)	1.460 (4) ns
Cabbage (*Brassica oleracea*) v. Prixie	1.52 (.223)	1.08 (0.92-1.21)	0.665 (4) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	1.82 (.239)	1.33 (1.21-1.45)	0.680 (4) ns
Pear (*Pyrus communis*)	1.41 (.214)	1.26 (1.11-1.41)	0.720 (4) ns
Sugarcane (*Saccarum officinerum*)	1.20 (.209)	1.51 (1.35-1.73)	0.181 (4) ns
Wheat (*Triticum aestivum*) v. Hereward	1.11 (.208)	1.60 (1.42-1.87)	0.459 (4) ns
Orange (*Citrus spp.*)	1.15 (.223)	1.85 (1.65-2.21)	0.634 (4) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	1.19 (.185)	2.19 (2.01-2.46)	4.966 (4) ns
Rape (*Brassica napus*) v. Tanto (Mature)	1.67 (.231)	1.13 (0.99-1.26)	0.911 (4) ns
Rape (*B. napus*) v. Tanto (Young)	1.81 (.247)	1.02 (0.89-1.14)	0.639 (4) ns
Rape (*B. napus*) v. Lirawell (Mature)	1.71 (.233)	1.18 (1.05-1.30)	0.507 (4) ns
Rape (*B. napus*) V. Lirawell (Young)	1.59 (.223)	1.04 (0.91-1.16)	1.219 (4) ns
Rape (*B. napus*) v. Starlight (Mature)	1.72 (.233)	1.19 (1.06-1.31)	0.709 (4) ns
Rape (*B. napus*) v. Starlight (Young)	1.77 (.247)	0.99 (0.84-1.10)	0.038 (4) ns
Maize (*Zea mays*) v. Marcia (Mature)	1.31 (.252)	1.98 (1.77-2.36)	0.595 (4) ns
Maize (*Z. mays*) V. Marcia (Young)	1.24 (.224)	1.76 (1.58-2.04)	0.269 (4) ns

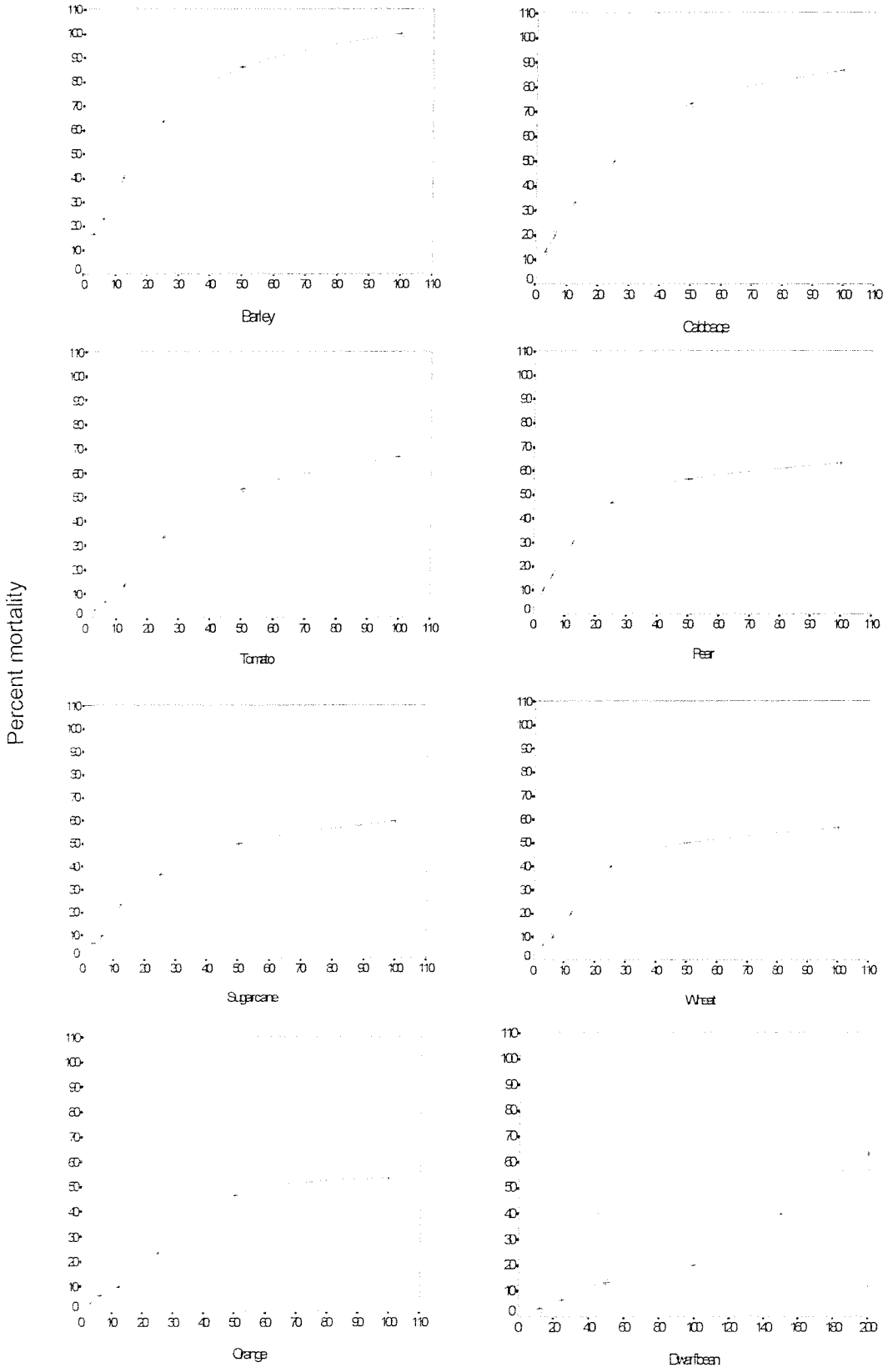
a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

Appendix 3.. 96-h Probit statistics of responses to Deltamethrin 2.5 EC for *F. candida* and different leaf types

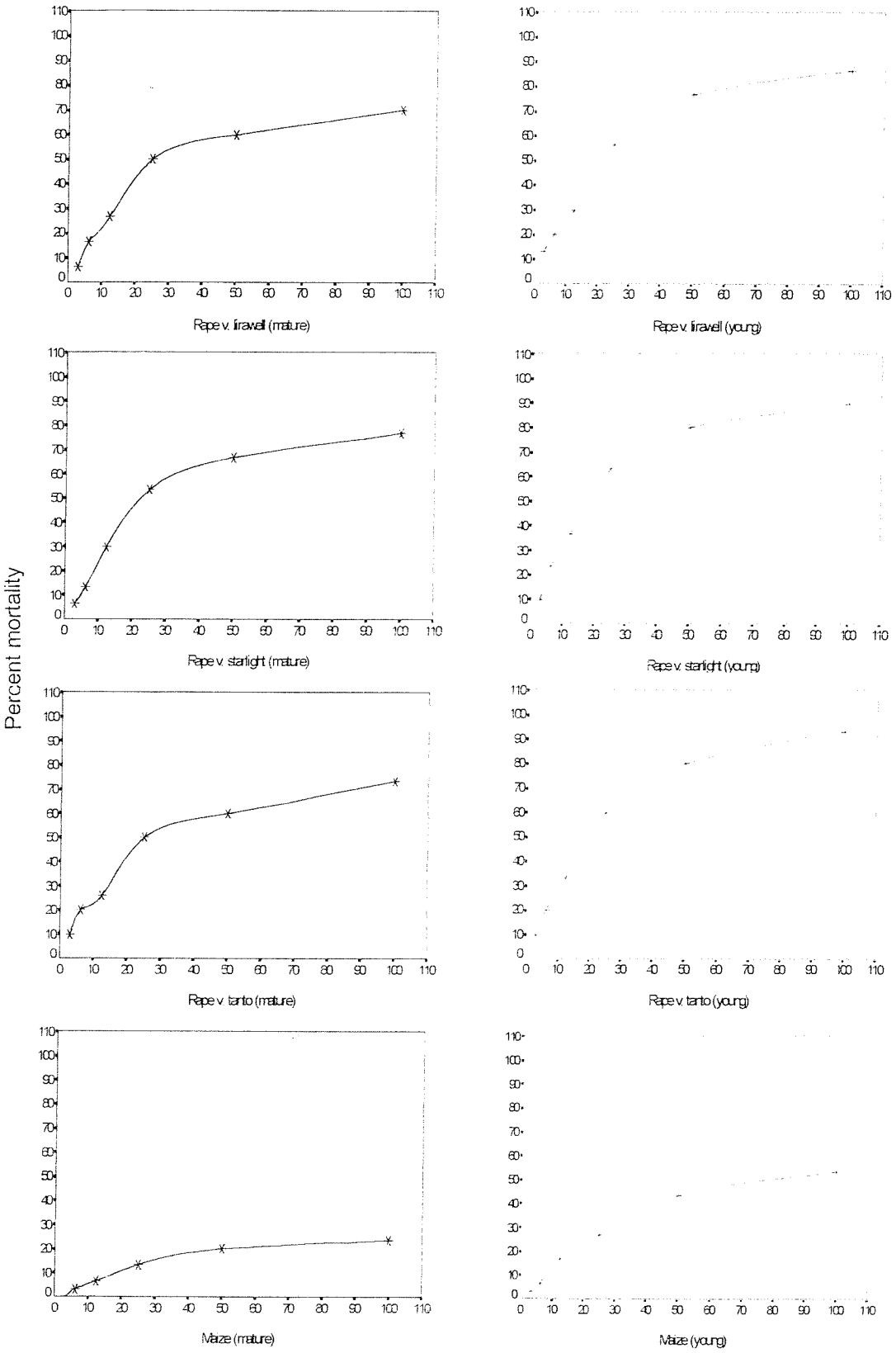
Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	2.33 (.405)	0.63 (0.46-0.75	1.564 (4) ns
Cabbage (*Brassica oleracea*) v. Prixie	1.55 (.252)	0.73 (0.51-0.88)	0.421 (4) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	1.97 (.260)	1.01 (0.88-1.12)	0.885 (4) ns
Pear (*Pyrus communis*)	1.15 (.222)	0.99 (0.81-1.13)	1.616 (4) ns
Sugarcane (*Saccarum officinerum*)	1.26 (.207)	1.22 (1.06-1.39)	0.851 (4) ns
Wheat (*Triticum aestivum*) v. Hereward	1.06 (.200)	1.23 (1.03-1.42)	0.320 (4) ns
Orange (*Citrus spp.*)	1.25 (.207)	1.38 (1.22-1.57)	1.663 (4) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	1.15 (.156)	1.73 (1.58-1.91)	2.345 (4) ns
Rape (*Brassica napus*) v. Tanto (Mature)	2.21 (.321)	0.81 (0.68-0.92)	0.250 (4) ns
Rape (*B. napus*) V. Tanto (Young)	2.44 (.411)	0.67 (0.51-0.77)	1.819 (4) ns
Rape (*B. napus*) v. Lirawell (Mature)	2.05 (.313)	0.75 (0.59-0.87)	0.240 (4) ns
Rape (*B. napus*) V. Lirawell (Young)	2.46 (.404)	0.69 (0.55-0.80)	1.238 (4) ns
Rape (*B. napus*) v. Starlight (Mature)	2.18 (.310)	0.84 (0.71-0.95)	3.225 (4) ns
Rape (*B. napus*) v. Starlight (Young)	2.52 (.423)	0.67 (0.52-0.78)	0.785 (4) ns
Maize (*Zea mays*) v. Marcia (Mature)	1.25 (.219)	1.71 (1.53-1.97)	1.725 (4) ns
Maize (*Z. mays*) V. Marcia (Young)	1.20 (.207)	1.44 (1.28-1.64)	0.185 (4) ns

a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

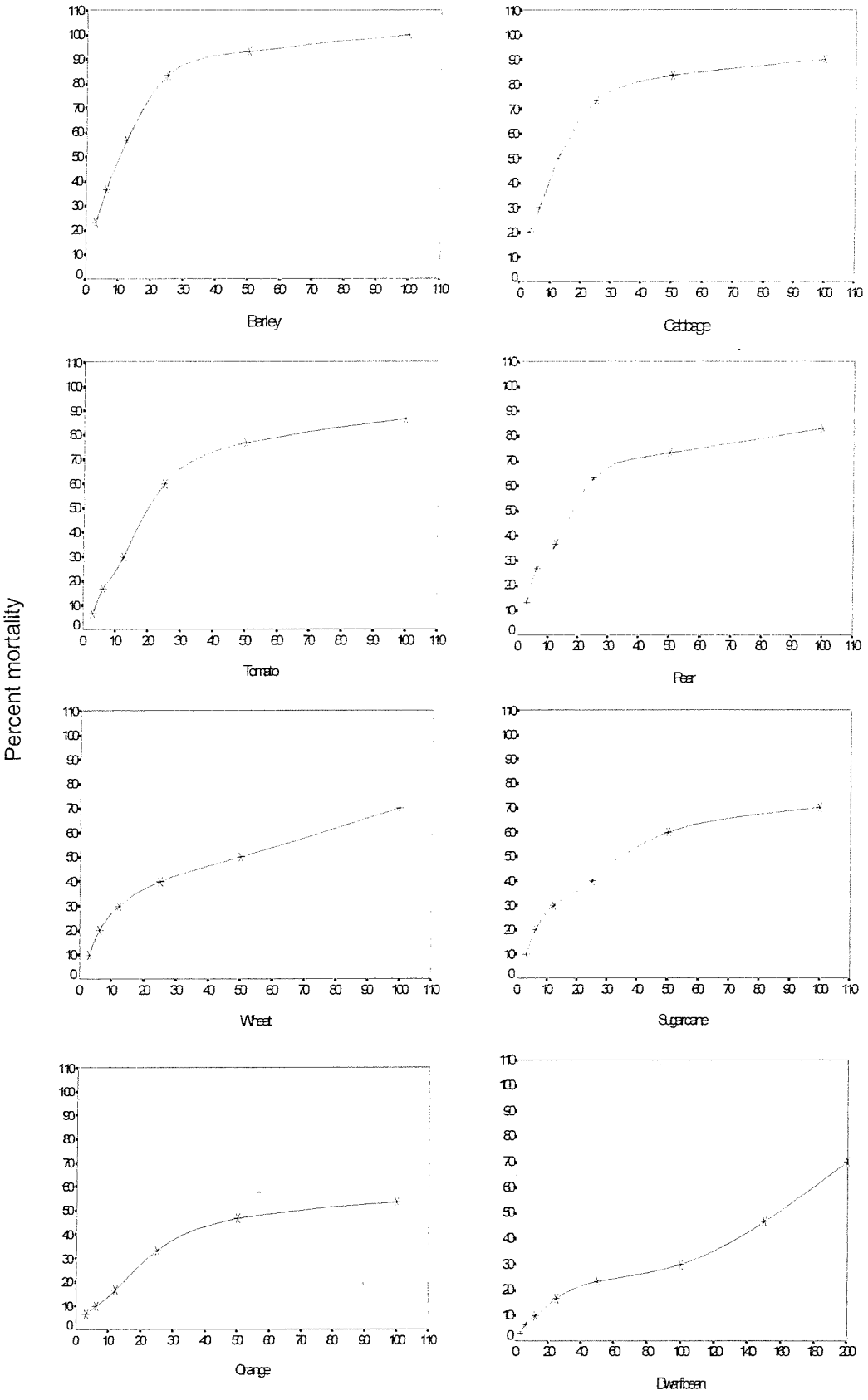
Appendix 4 . Dose response curve of *F. candida* on different leaf types after 24 hrs of treatment with deltamethrin 2.5 EC



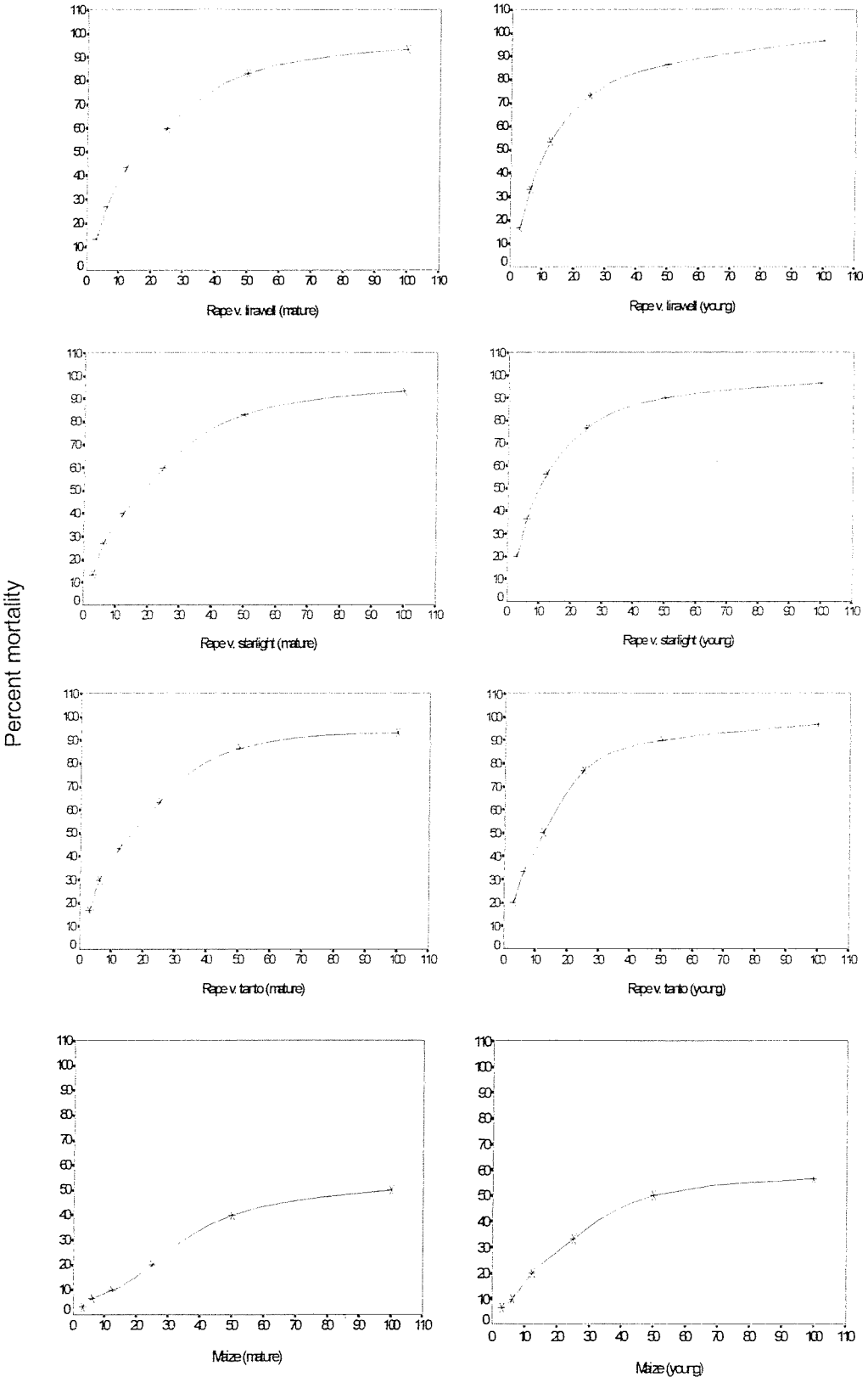
Appendix 4 .(cont.). Dose response curve of *F. candida* ' . on different leaf types after 24 hrs of treatment with deltamethrin 2.5 EC



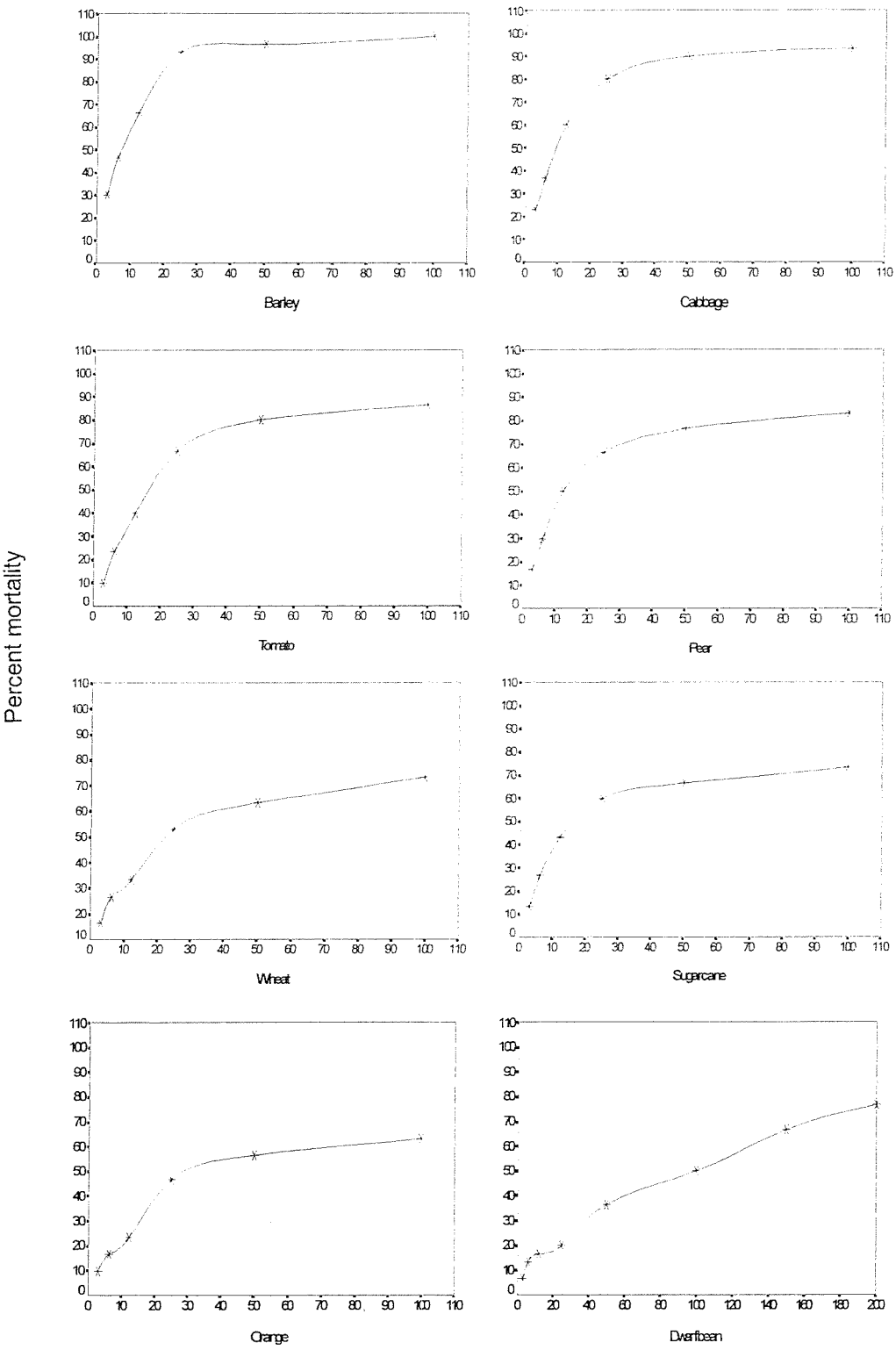
Appendix 5. Dose response curve of *F. candida* ... on different leaf types after 48 hrs of treatment with deltamethrin 2.5 EC



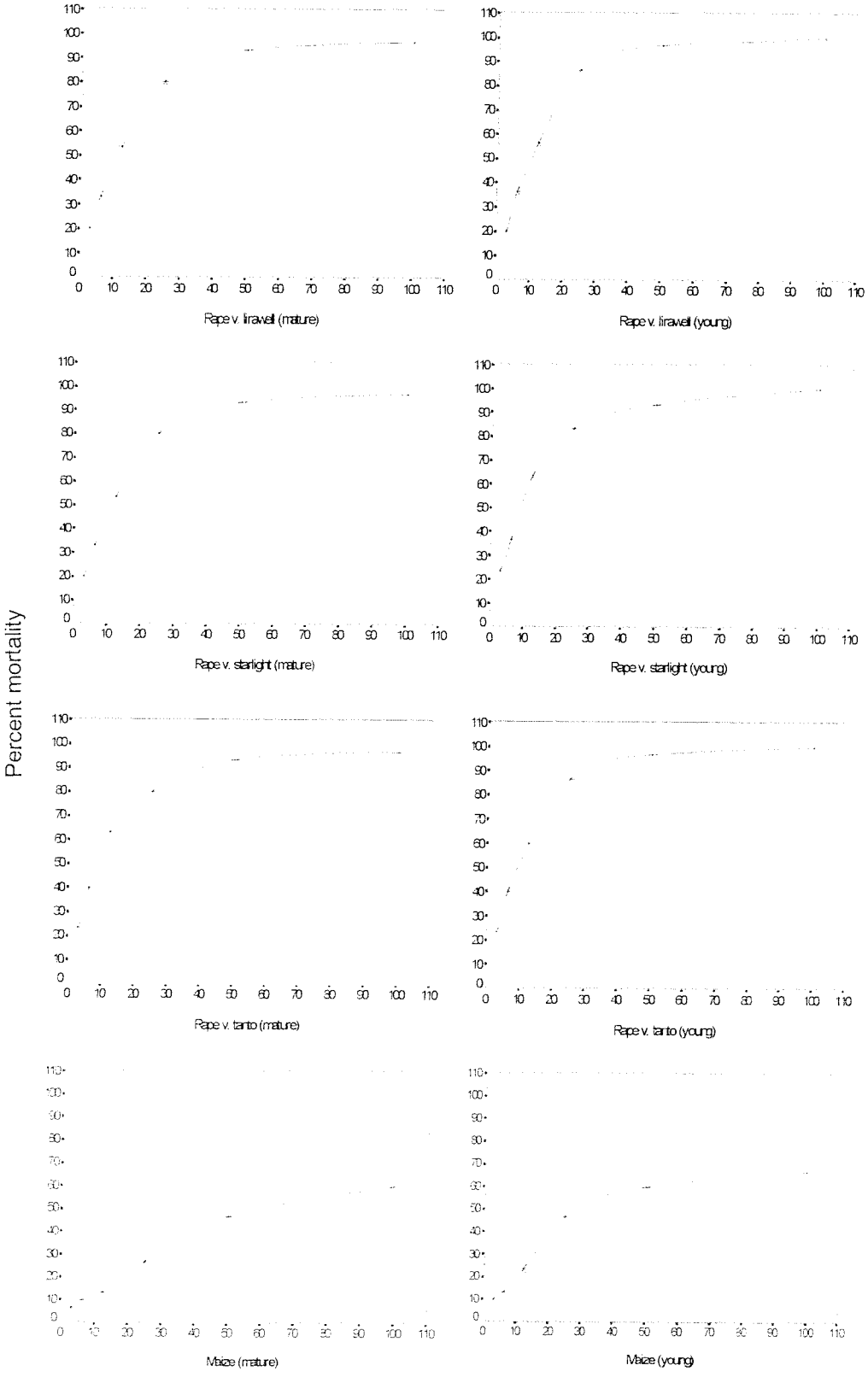
Appendix 5(cont.). Dose response curve of *F. candida* on different leaf types after 48 hrs of treatment with deltamethrin 2.5 EC



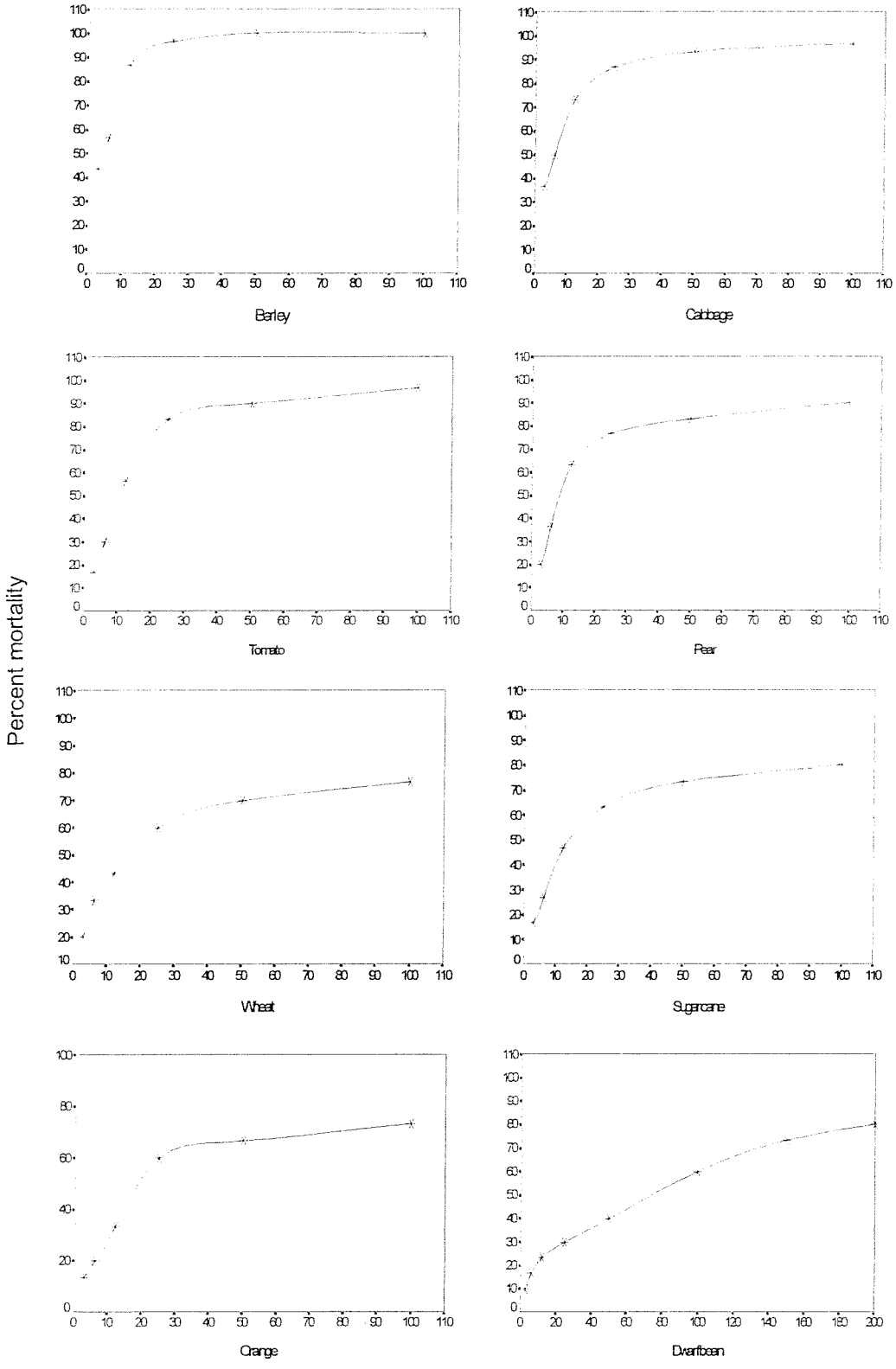
Appendix 6. Dose response curve of *F. candida* . on different leaf types after 72 hrs of treatment with deltamethrin 2.5 EC



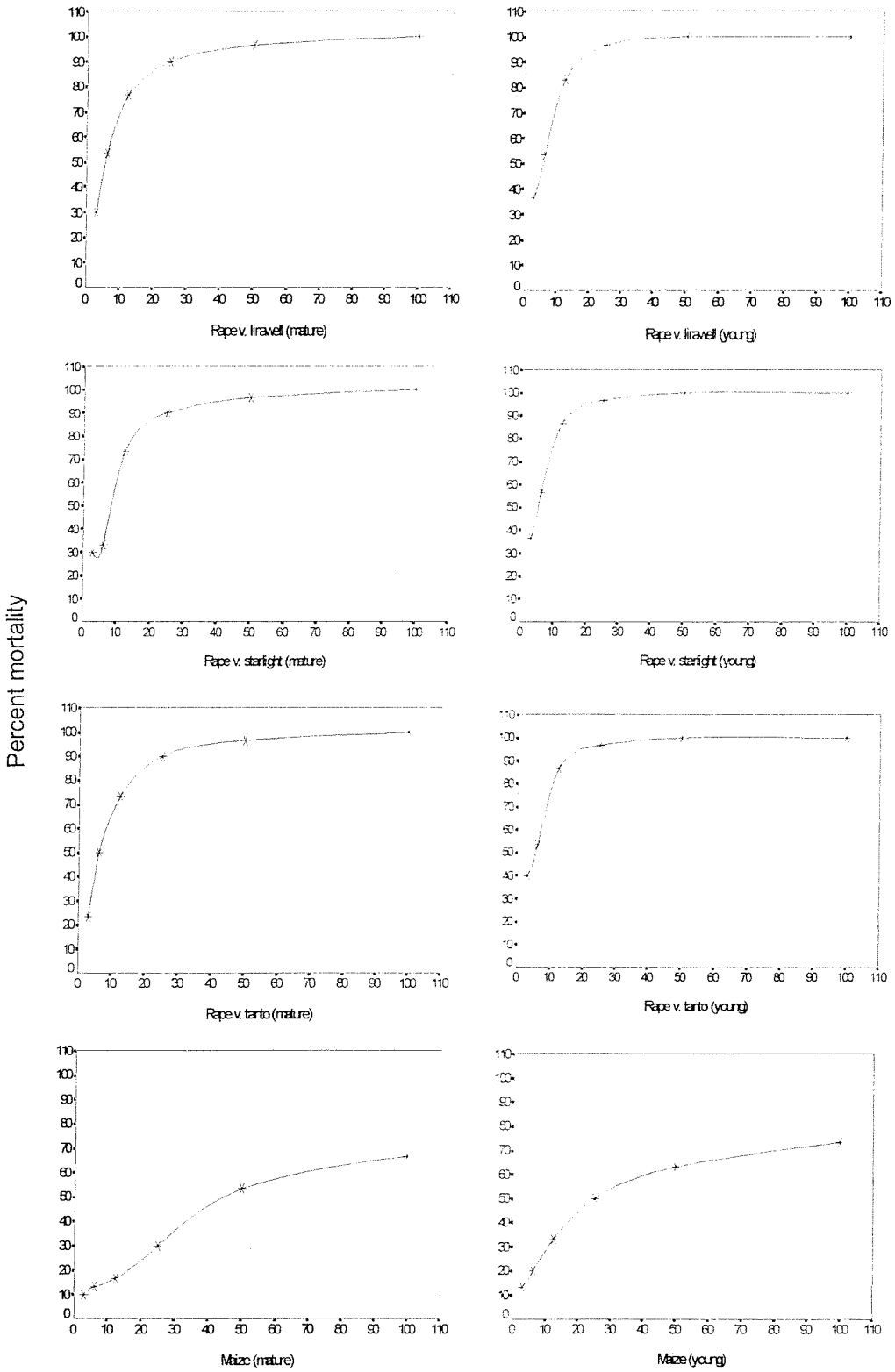
Appendix 6(cont.). Dose response curve of *F. candida* on different leaf types after 72 hrs of treatment with deltamethrin 2.5 EC



Appendix 7. Dose response curve of *F. candida* on different leaf types after 96 hrs of treatment with deltamethrin 2.5 EC



Appendix 7(cont.). Dose response curve of *F. candida* on different leaf types after 96 hrs of treatment with deltamethrin 2.5 EC



Appendix 8. 24-h Probit statistics of responses to Dimethoate 40 EC for *F. candida* and different leaf types

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	1.90 (0.25)	1.23 (1.11-1.37)	2.459 (5) ns
Cabbage (*Brassica oleracea*) v. Prixie	2.11 (0.25)	1.03 (0.93-1.15)	5.490 (5) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	1.95 (0.23)	0.55 (0.44-0.66)	1.701 (5) ns
Pear (*Pyrus communis*)	1.03 (0.16)	0.44 (0.22-0.63)	1.947 (5) ns
Sugarcane (*Saccarum officinerum*)	1.59 (0.21)	1.15 (1.02-1.32)	2.378 95) ns
Wheat (*Triticum aestivum*) v. Hereward	1.71 (0.20)	0.73 (0.60-0.85)	7.969 (5) ns
Orange (*Citrus spp.*)	1.85 (0.22)	0.59 90.48-0.71)	1.497 (5) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	2.19 (0.24)	0.86 (0.76-0.97)	4.622 (5) ns
Rape (*Brassica napus*) v. Tanto (Mature)	2.30 (0.26)	0.52 (0.42-0.62)	6.288 (5) ns
Rape (*B. napus*) V. Tanto (Young)	2.44 (0.28)	0.57 (0.47-0.66)	1.901 (5) ns
Rape (*B. napus*) v. Lirawell (Mature)	2.10 (0.24)	0.57 (0.46-0.67)	3.020 (5) ns
Rape (*B. napus*) V. Lirawell (Young)	2.51 (0.29)	0.54 (0.44-0.63)	2.050 (5) ns
Rape (*B. napus*) v. Starlight (Mature)	2.32 (0.26)	0.53 (0.42-0.63)	5.692 (5) ns
Rape (*B. napus*) v. Starlight (Young)	2.52 (0.29)	0.52 (0.42-0.61)	1.611 (5) ns
Maize (*Zea mays*) v. Marcia (Mature)	2.48 (0.30)	1.06 (0.96-1.16)	4.273 (5) ns
Maize (*Z. mays*) V. Marcia (Young)	2.37 (0.33)	1.29 (1.19-1.42)	0.914 (5) ns

a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

Appendix 9. 48-h Probit statistics of responses to Dimethoate 40 EC for *F. candida* . and different leaf types

Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	2.43 (0.29)	1.06 (0.96-1.16)	2.584 (5) ns
Cabbage (*Brassica oleracea*) v. Prixie	1.94 (0.22)	0.87 (0.76-0.99)	3.105 (5) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	2.06 (0.24)	0.49 (0.38-0.60)	1.561 (5) ns
Pear (*Pyrus communis*)	1.47 (0.20)	0.30 (0.13-0.44)	1.053 (5) ns
Sugarcane (*Saccarum officinerum*)	1.36 (0.18)	1.01 (0.87-1.18)	5.525 (5) ns
Wheat (*Triticum aestivum*) v. Hereward	2.29 (0.26)	0.58 (0.48-0.68)	5.038 (5) ns
Orange (*Citrus spp.*)	1.54 (0.30)	0.39 (0.24-0.52)	2.165 (5) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	2.37 (0.26)	0.73 (0.63-0.83)	4.269 (5) ns
Rape (*Brassica napus*) v. Tanto (Mature)	2.44 (0.29)	0.40 (0.30-0.50)	2.385 (5) ns
Rape (*B. napus*) V. Tanto (Young)	2.40 (0.29)	0.41 (0.31-0.51)	1.115 (5) ns
Rape (*B. napus*) v. Lirawell (Mature)	2.85 (0.35)	0.38 (0.29-0.47)	2.433 (5) ns
Rape (*B. napus*) V. Lirawell (Young)	2.14 (0.27)	0.32 (0.20-0.42)	1.801 (5) ns
Rape (*B. napus*) v. Starlight (Mature)	2.43 (0.30)	0.35 (0.24-0.45)	3.299 (5) ns
Rape (*B. napus*) v. Starlight (Young)	2.50 (0.30)	0.37 (0.27-0.47)	2.642 95) ns
Maize (*Zea mays*) v. Marcia (Mature)	1.80 (0.21)	0.62 (0.51-0.73)	2.088 (5) ns
Maize (*Z. mays*) V. Marcia (Young)	1.79 (0.21)	0.96 (0.84-1.09)	1.392 95) ns

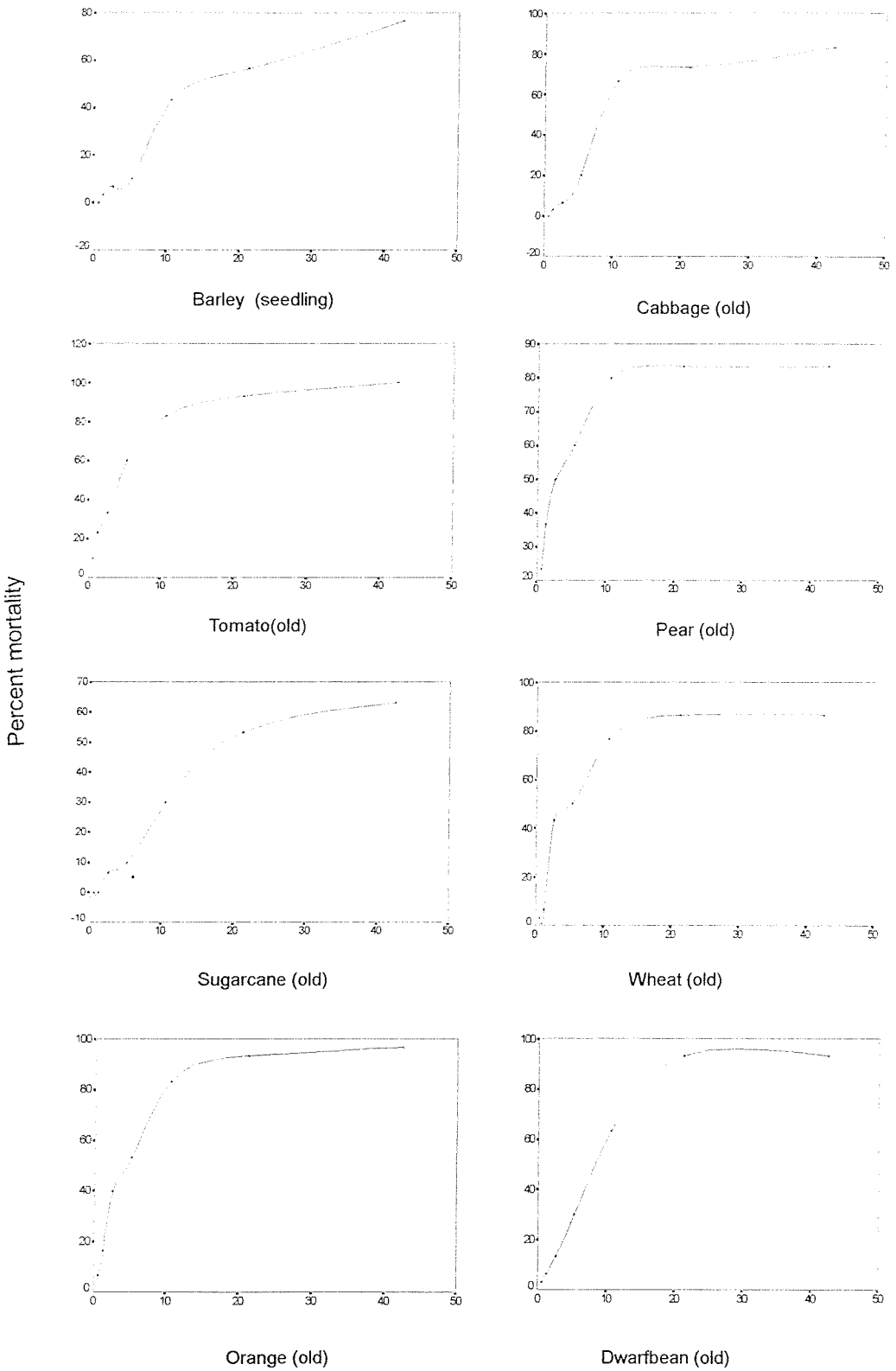
a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

Appendix 10. 96-h Probit statistics of responses to Dimethoate 40 EC for *F. candida* and different leaf types

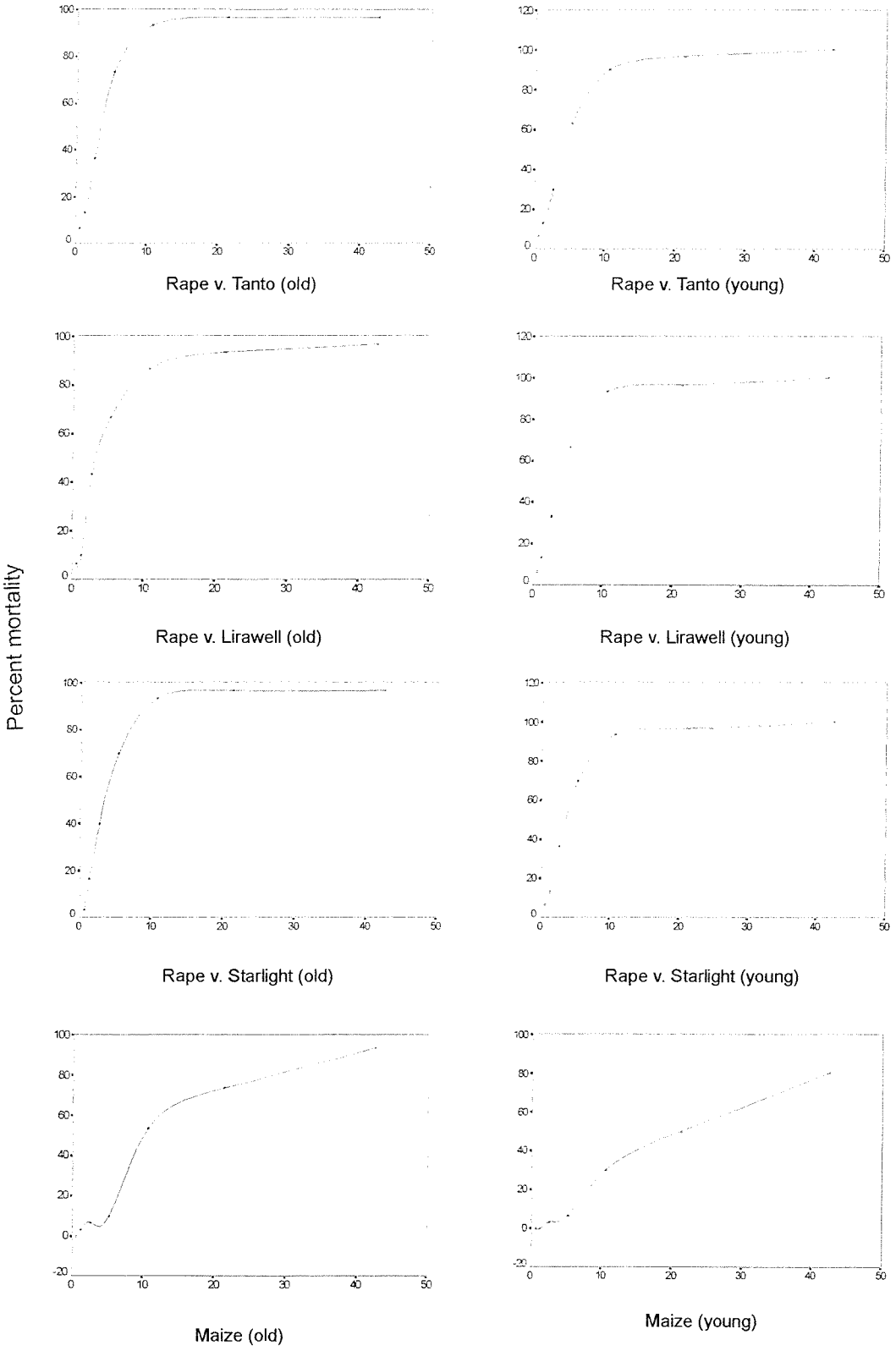
Leaf species	Probit slope (SE ±)	Log LD50 (95%cl) (ga.i./ha)	Heterogeneity x^2 (d.f.) significance a
Barley (*Hordeum vulgare*)	2.37 (0.26)	0.85 (0.75-0.95)	7.399 (5) ns
Cabbage (*Brassica oleracea*) v. Prixie	2.59 (0.30)	0.53 (0.43-0.62)	3.489 (5) ns
Tomato (*Lycopersicon esculentum) v. Moneymaker*	2.15 (0.26)	0.40 (0.29-0.50)	2.793 (5) ns
Pear (*Pyrus communis*)	2.05 (0.28)	0.18 (0.04-0.29)	3.278 (5) ns
Sugarcane (*Saccarum officinerum*)	1.38 (0.18)	0.54 (0.39-0.68)	2.259 (5) ns
Wheat (*Triticum aestivum*) v. Hereward	2.16 (0.26)	0.38 (0.26-0.48)	3.028 (5) ns
Orange (*Citrus spp.*)	1.83 (0.26)	0.13 (-0.03-0.26)	2.073 (5) ns
Dwarfbean (*Phaseolus vulgaris*) v. Sutton	3.07 (0.40)	0.31 (0.22-0.40)	1.265 (5) ns
Rape (*Brassica napus*) v. Tanto (Mature)	3.34 (0.47)	0.21 (0.12-0.29)	0.937 (5) ns
Rape (*B. napus*) V. Tanto (Young)	3.21 (0.47)	0.13 (0.04-0.22)	1.737 (5) ns
Rape (*B. napus*) v. Lirawell (Mature)	2.92 (0.38)	0.26 (0.16-0.35)	4.174 (5) ns
Rape (*B. napus*) V. Lirawell (Young)	2.68 (0.41)	0.09 (-0.03-0.19)	0.615 (5) ns
Rape (*B. napus*) v. Starlight (Mature)	3.68 (0.51)	0.25 (0.17-0.33)	2.228 (5) ns
Rape (*B. napus*) v. Starlight (Young)	3.03 (0.46)	0.10 (-0.007-0.19)	0.951 (5) ns
Maize (*Zea mays*) v. Marcia (Mature)	1.98 (0.23)	0.77 (0.66-0.89)	4.443 (5) ns
Maize (*Z. mays*) V. Marcia (Young)	2.16 (0.25)	0.62 (0.51-0.72)	4.401 (5) ns

a+ Significance level, ns= not significant, $P<0.05$, d.f.= degrees of freedom

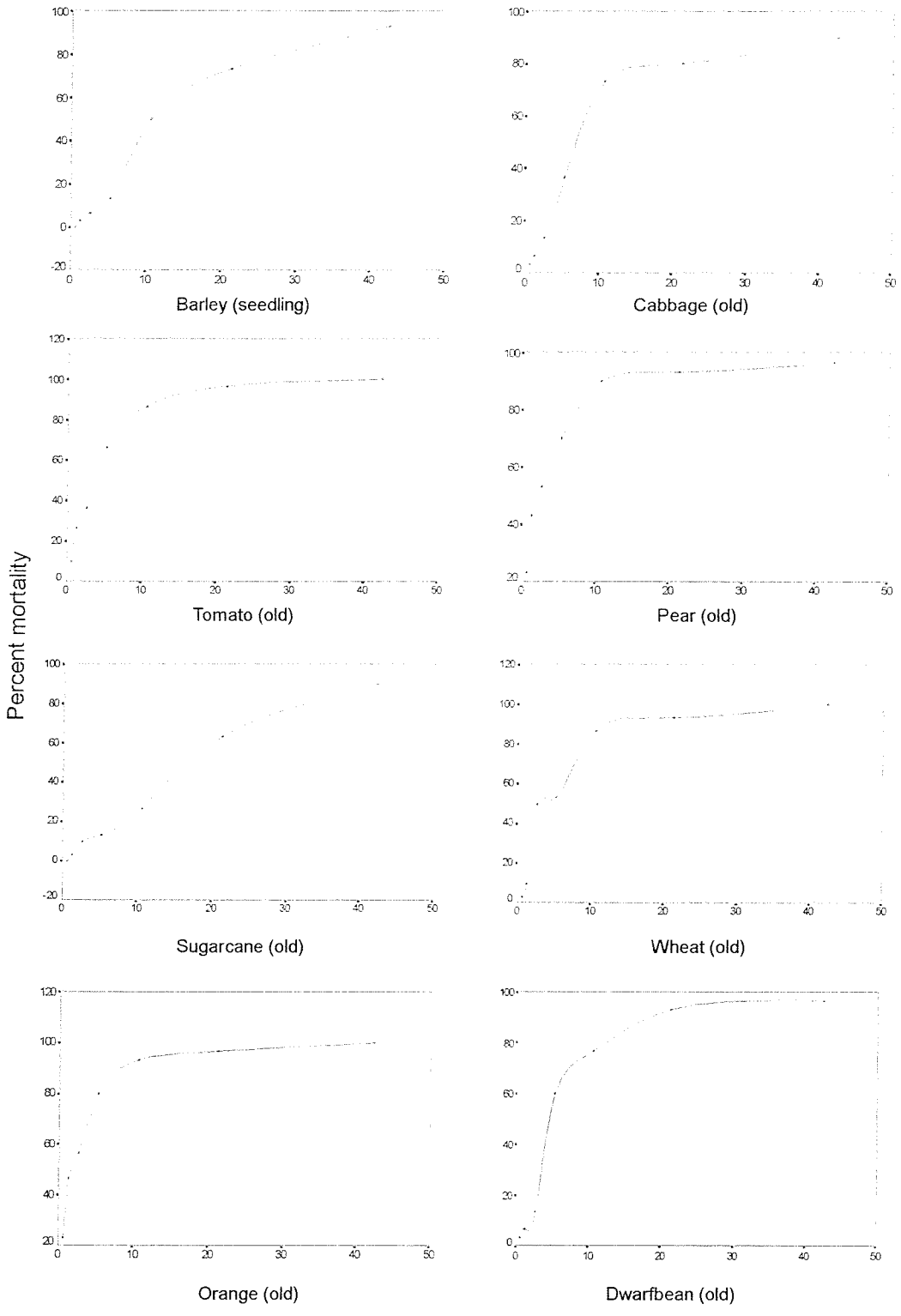
Appendix 11. Dose response curve of *F. candida* on different leaf species after 24hrs of treatment with Dimethoate 40EC.



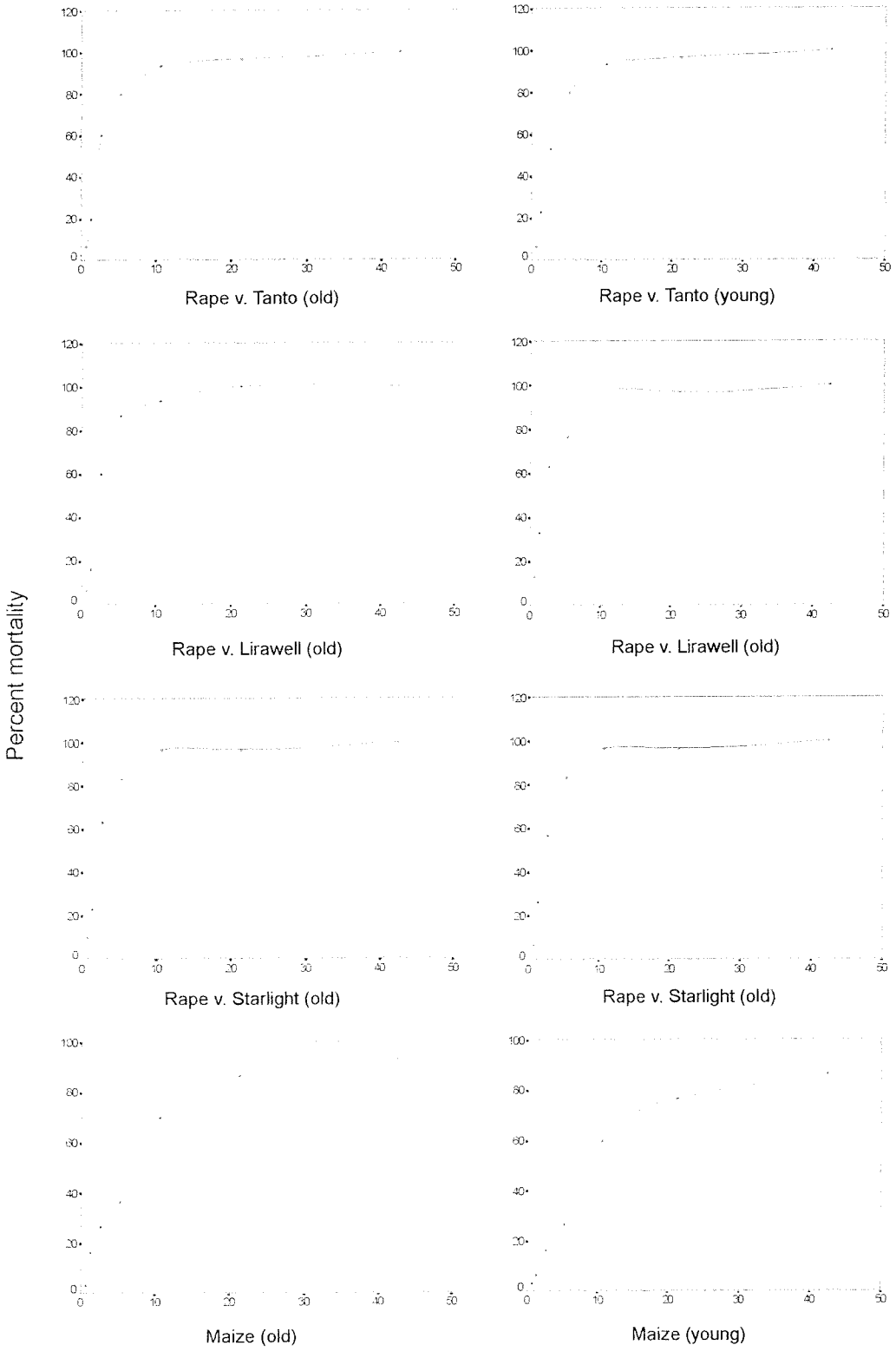
Appendix 11 (cont.). Dose response curve of *F. candida* on different leaf species after 24hrs of treatment with Dimethoate 40EC.



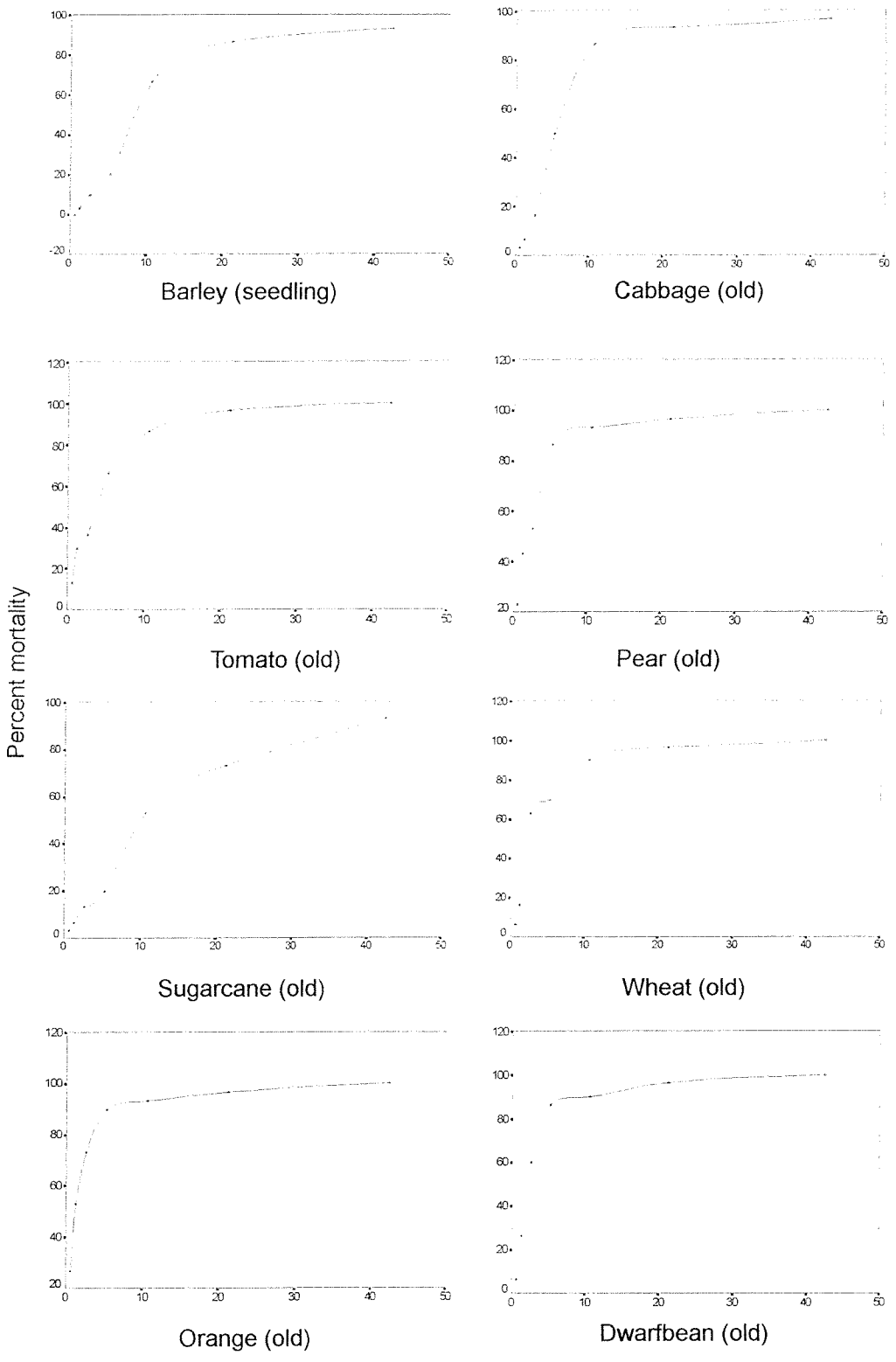
Appendix 12. Dose response curve of *F. candida* on different leaf species after 48hrs of treatment with Dimethoate 40EC.



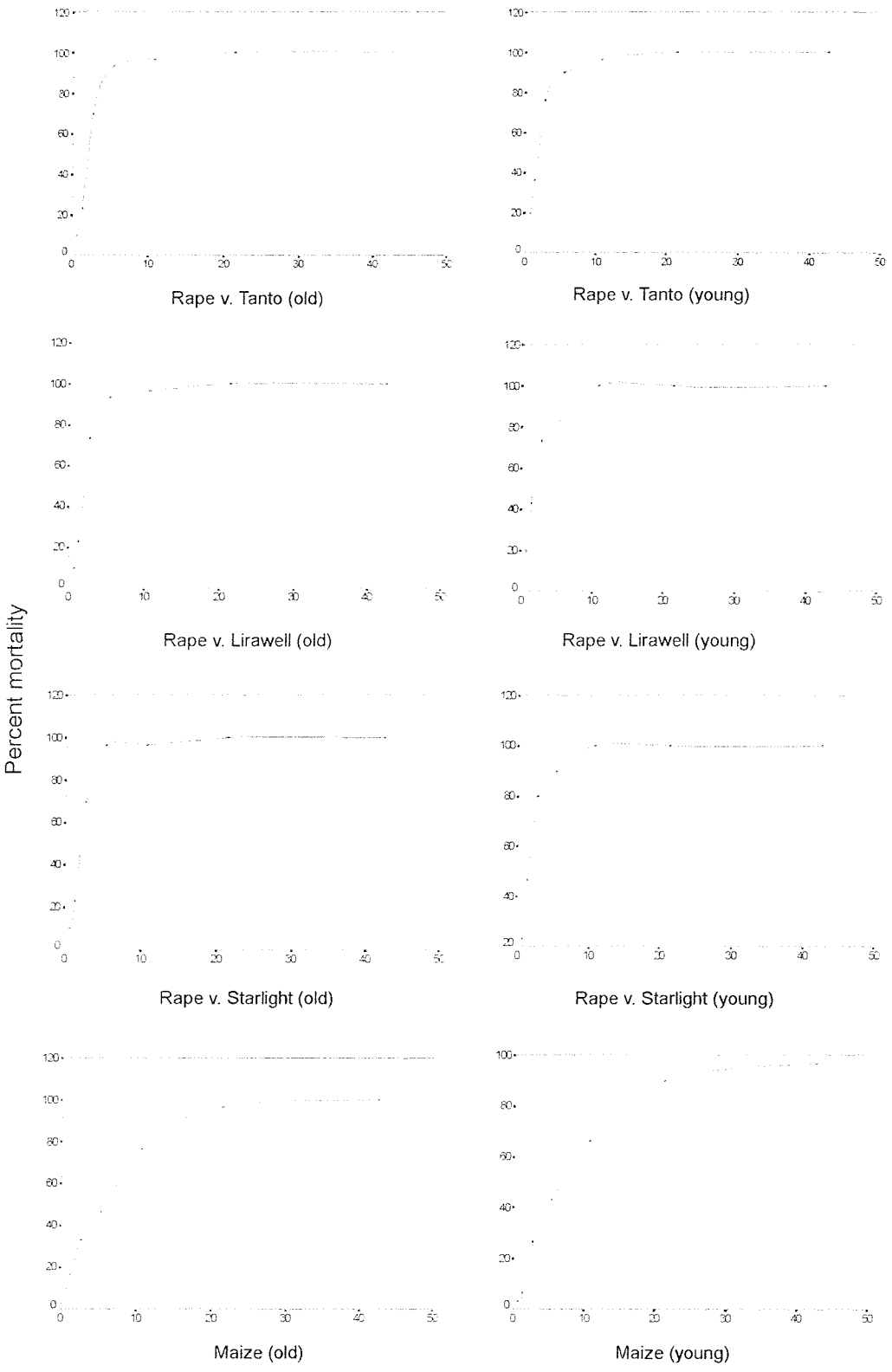
Appendix 12 (cont.). Dose response curve of *F. candida* on different leaf species after 48hrsof treatment with Dimethoate 40EC.



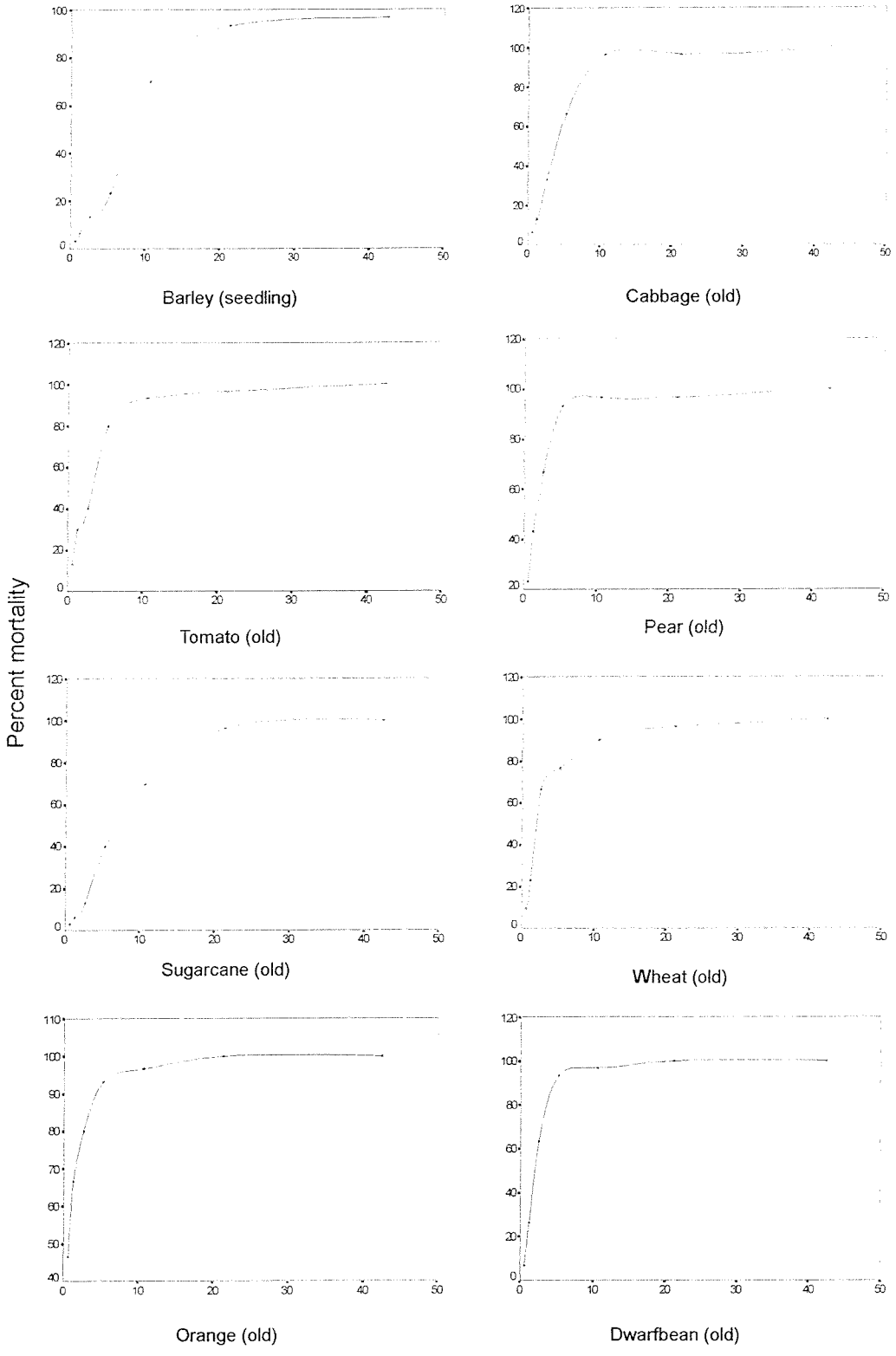
Appendix 13. Dose response curve of *F. candida* on different leaf species after 72hrs of treatment with Dimethoate 40EC.



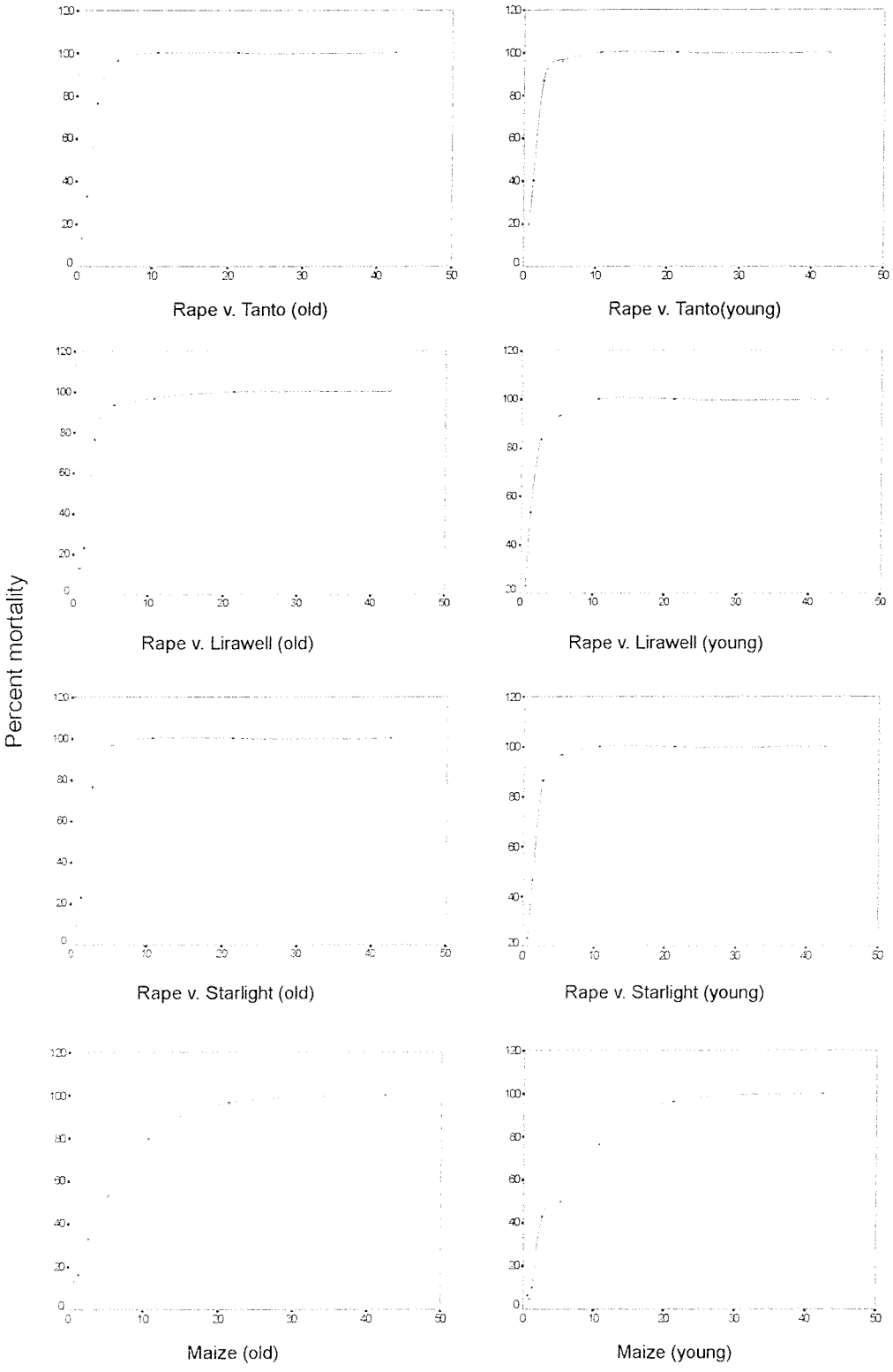
Appendix13 (cont.). Dose response curve of *F. candida* on different leaf species after 72hrs of treatment with Dimethoate 40EC.



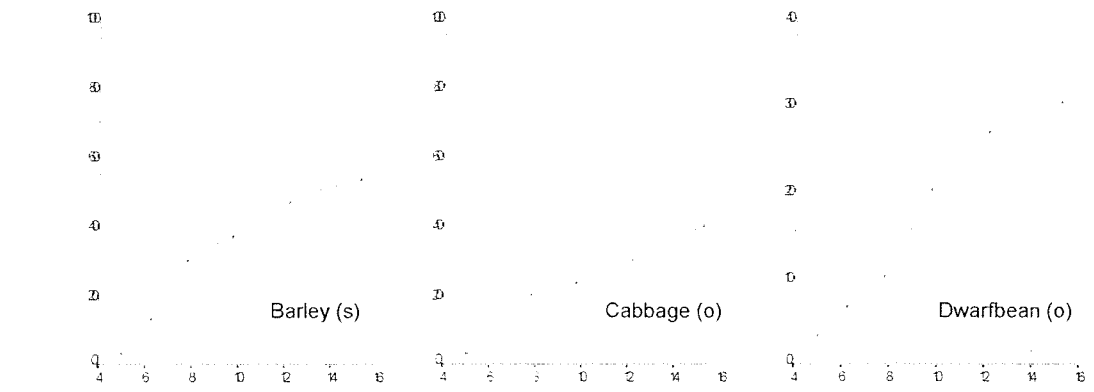
Appendix 14. Dose response curve of *F. candida* on different leaf species after 96hrs of treatment with Dimethoate 40EC.



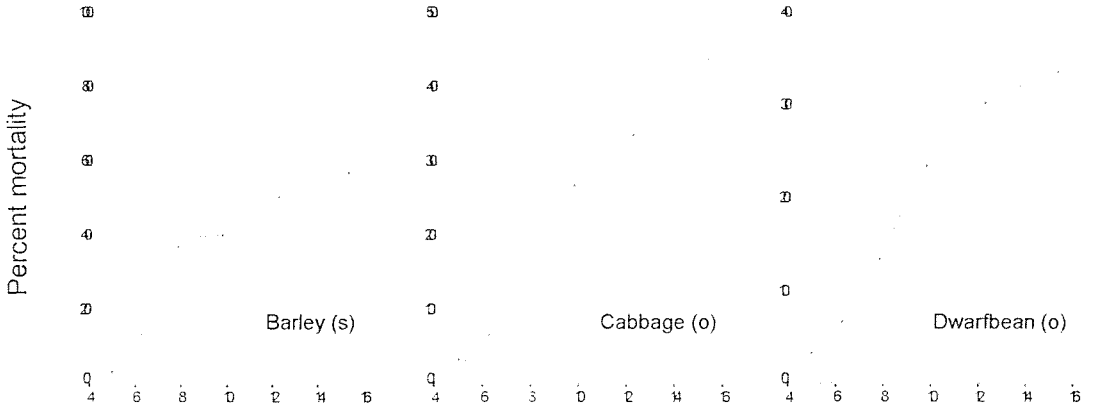
Appendix 14 (cont.). Dose response curve of *F. candida* on different leaf species after 96hrs of treatment with Dimethoate 40EC.



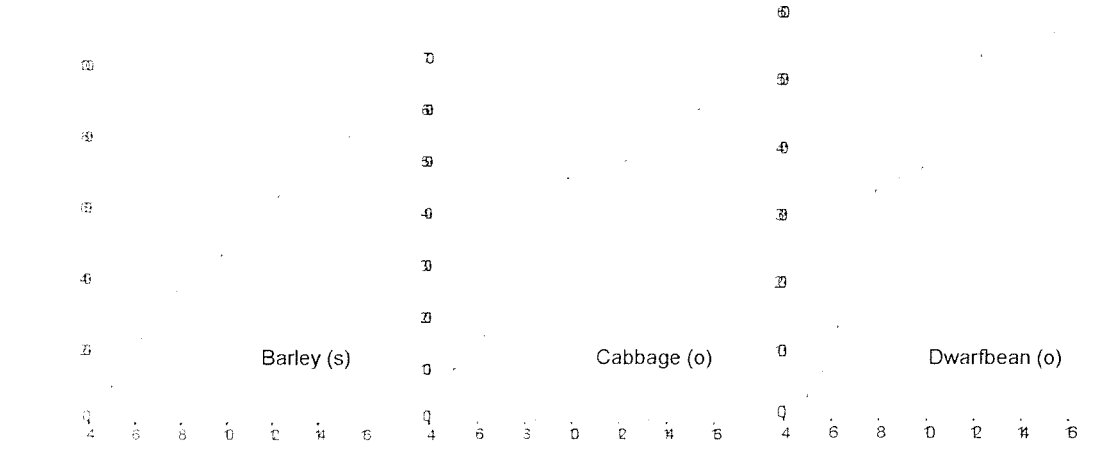
Appendix 15. Dose response curve of *A. colemani* on different leaf surfaces after 2, 8 and 16 hrs of treatment with deltamethrin (test method 2)



After 2hrs of treatment



After 8 hrs of treatment

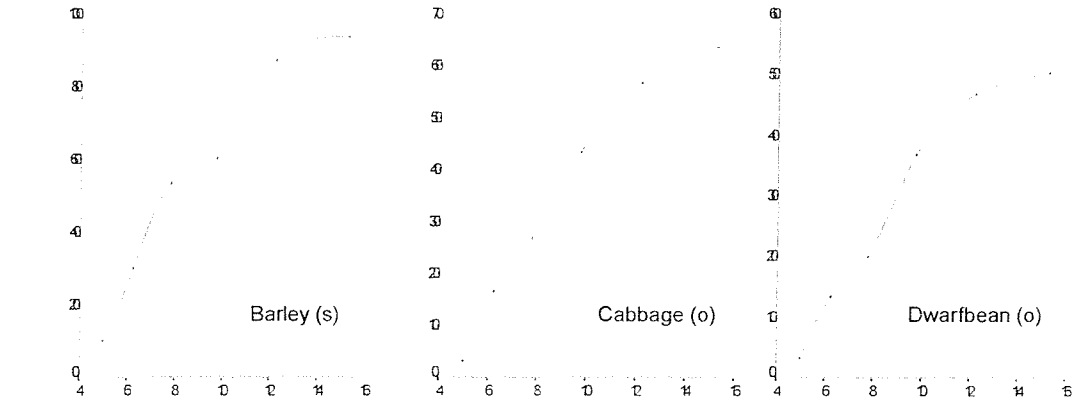


After 16 hrs of treatment

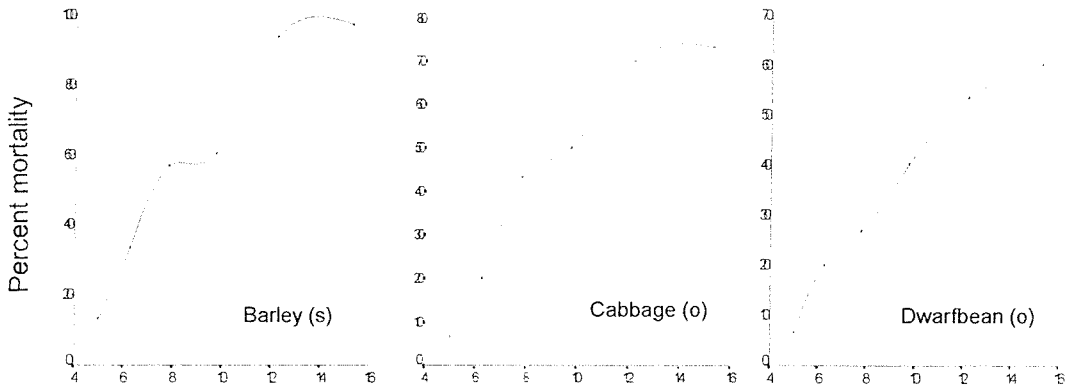
Percent mortality

Doses (g a.i./ha)

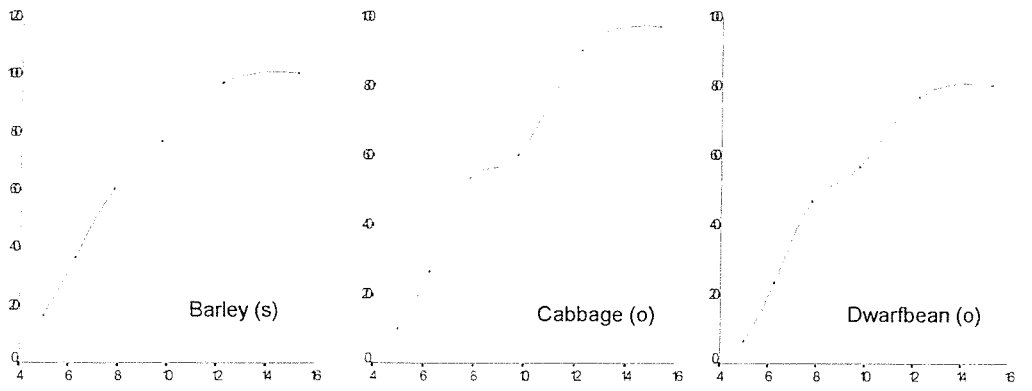
Appendix 16. Dose response curve of *A. colemani* on different leaf surfaces after 2, 8 and 16 hrs of treatment with deltamethrin (test method 3)



After 2hrs of treatment



After 8 hrs of treatment



After 16 hrs of treatment

Doses (g a.i./ha)

Appendix 17.. Ranking relation between 24-h ld50 of *F. candida* for Deltamethrin 2.5 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	Log ld50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (s)	*H. vulgare*		1.14 (1.02-1.19)	1	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	1.33 (1.19-1.47)	5	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	1.70 (1.56-1.89)	10	09.65 (1.03)	11
Pear (o)	*P. communis*		1.59 (1.41-1.85)	9	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		1.73 (1.56-2.01)	11	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	1.73 (1.56-2.00)	11	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		1.88 (1.70-2.18)	12	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	2.27 (2.14-2.48)	14	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	1.49 (1.33-1.69)	7	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	1.26 (1.14-1.37)	3	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	1.50 (1.35-1.68)	8	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	1.30 (1.17-1.44)	4	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	1.44 (1.31-1.60)	6	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	1.24 (1.11-1.36)	2	49.22 (1.28)	4
Maize (o)	*Z. mays*	Marcia	1.95 (1.75-2.31)	13	2.77 (0.24)	15
Maize (y)	*Z. mays*	Marcia	1.88 (1.69-2.21)	12	6.74 (0.45)	12

s =seedling, o =old, y =young

Appendix 18. Ranking relation between 48-h ld50 of *F. candida* .. for Deltamethrin 2.5 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	Log ld50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (o)	*H. vulgare*		0.94 (0.81-1.05)	1	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	1.08 (0.93-1.21)	5	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	1.33 (1.21-1.45)	10	09.65 (1.03)	11
Pear (o)	*P. communis*		1.26 (1.11-1.41)	9	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		1.51 (1.35-1.73)	11	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	1.61 (1.42-1.87)	12	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		1.85 (1.66-2.21)	14	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	2.19 (2.01-2.46)	16	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	1.13 (0.99-1.26)	6	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	1.02 (0.89-1.14)	3	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	1.18 (1.05-1.30)	7	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	1.04 (0.91-1.16)	4	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	1.19 91.06-1.31)	8	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	0.99 (0.84-1.11)	2	49.22 (1.28)	4
Maize (o)	*Z. mays*	Marcia	1.98 (1.77-2.36)	15	2.77 (0.24)	15
Maize (y)	*Z. mays*	Marcia	1.77 (1.58-2.04)	13	6.74 (0.45)	12

s =seedling, o =old, y =young

Appendix 19.. Ranking relation between 96-h ld50 of *F. candida* for Deltamethrin 2.5 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	Log ld50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (s)	*H. vulgare*		0.63 (0.46-0.75	1	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	0.73 (0.51-0.88)	5	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	1.01 (0.88-1.12)	10	09.65 (1.03)	11
Pear (o)	*P. communis*		0.99 (1.39-1.13)	9	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		1.22 (1.06-1.39)	11	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	1.23 (1.03-1.42)	12	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		1.38 (1.22-1.57)	13	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	1.73 (1.58-1.91)	16	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	0.81 (0.68-0.92)	7	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	0.67 (0.51-0.77)	2	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	0.75 (0.59-0.87)	6	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	0.69 (0.55-0.80)	4	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	0.84 (0.71-0.95)	8	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	0.67 (0.52-0.78)	3	49.22 (1.28)	4
Rape (o)	*B. napus*	Tanto	0.81 (0.68-0.92)	7	2.77 (0.24)	6
Rape (y)	*B. napus*	Tanto	0.67 (0.51-0.77)	2	6.74 (0.45)	2

s =seedling, o =old, y =young

Appendix 20. Ranking relation between 24-h ld50 of *F. candida* . . . for Dimethoate 40 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	Log ld50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (s)	*H. vulgare*		1.23 (1.11-1.37)	15	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	1.03 (0.93-1.15)	12	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	0.55 (0.44-0.66)	6	09.65 (1.03)	11
Pear (o)	*P. communis*		0.44 (0.22-0.63)	1	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		1.15 (1.02-1.32)	14	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	0.73 (0.60-0.85)	10	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		0.59 (0.48-0.71)	9	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	0.86 (0.76-0.97)	11	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	0.52 (0.42-0.62)	3	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	0.57 (0.47-0.66)	7	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	0.57 (0.46-0.67)	8	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	0.54 (0.44-0.63)	5	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	0.53 (0.42-0.63)	4	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	0.52 (0.42-0.61)	2	49.22 (1.28)	4
Rape (o)	*B. napus*	Tanto	1.06 (0.96-1.16)	13	2.77 (0.24)	6
Rape (y)	*B. napus*	Tanto	1.29 (1.19-1.42)	16	6.74 (0.45)	2

s =seedling, o =old, y =young

Appendix 21. Ranking relation between 48-h ld50 of *F. candida* for Dimethoate 40 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	Log ld50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (s)	*H. vulgare*		1.06 (0.96-1.16)	16	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	0.87 (0.76-0.99)	13	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	0.49 (0.38-0.60)	9	09.65 (1.03)	11
Pear (o)	*P. communis*		0.30 (0.13-0.44)	1	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		1.01 (0.87-1.18)	15	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	0.58 (0.48-0.68)	10	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		0.39 (0.24-0.52)	6	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	0.73 (0.63-0.83)	12	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	0.40 (0.30-0.50)	7	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	0.41 (0.31-0.51)	8	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	0.38 (0.29-0.47)	5	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	0.32 (0.20-0.42)	2	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	0.35 (0.24-0.45)	3	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	0.37 (0.27-0.47)	4	49.22 (1.28)	4
Rape (o)	*B. napus*	Tanto	0.62 (0.51-0.73)	11	2.77 (0.24)	6
Rape (y)	*B. napus*	Tanto	0.96 (0.84-1.09)	14	6.74 (0.45)	2

s =seedling, o =old, y =young

Appendix 22. Ranking relation between96-h ld50 of *F. candida* for Dimethoate 40 EC and wax content of different leaf species

Leaf species	Scientific name	Variety	Log ld50 g.a.i./ha (95%cl)	Rank	Wax (SE±) (ug/cm²)	Rank
Barley (s)	*H. vulgare*		0.85 (0.75-0.95)	16	51.33 (2.89)	1
Cabbage (o)	*B. oleracea*	Prixie	0.53 (0.43-0.62)	12	36.12 (1.13)	5
Tomato (o)	*S. esculentum*	Money maker	0.40 (0.29-0.50)	11	09.65 (1.03)	11
Pear (o)	*P. communis*		0.18 (0.04-0.29)	5	21.17 (2.38)	9
Sugarcane (o)	*S. officinerum*		0.54 (0.39-0.68)	13	05.40 (1.38)	13
Wheat (o)	*T. aestivum*	Hereward	0.38 (0.26-0.48)	10	08.42 (2.04)	10
Orange (o)	*Citrus spp.*		0.13 (-0.03-0.26)	4	05.06 (1.62)	14
Dwarfbean (o)	*P. vulgaris*	The sutton	0.31 (0.22-0.40)	9	01.46 (1.13)	16
Rape (o)	*B. napus*	Tanto	0.21 (0.12-0. 12-0.29)	6	32.57 (0.89)	6
Rape (y)	*B. napus*	Tanto	0.13 (0.04-0.22)	3	50.03 (1.87)	2
Rape (o)	*B. napus*	Lirawell	0.26 (0.16-0.35)	8	32.12 (0.87)	7
Rape (y)	*B. napus*	Lirawell	0.09 (-0.03-0.19)	1	49.60 (1.28)	3
Rape (o)	*B. napus*	Starlight	0.25 (0.17-0.33)	7	31.35 (1.11)	8
Rape (y)	*B. napus*	Starlight	0.10 (-0.007-0.19)	2	49.22 (1.28)	4
Rape (o)	*B. napus*	Tanto	0.77 (0.66-0.89)	15	2.77 (0.24)	6
Rape (y)	*B. napus*	Tanto	0.62 (0.51-0.72)	14	6.74 (0.45)	2

s =seedling, o =old, y =young