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The role of cuticular waxes and surface roughness in determining the insecticidal efficacy of deltamethrin and dimethoate applied as emulsifiable concentrates to leaf surfaces

A. B. M. N. U. CHOWDHURY[1], PAUL C. JEPSON[2], MARTYN G. FORD[3], & GEOFF K. FRAMPTON[4]

[1]*Environmental Health and Consumer Protection Division, Southampton City Council, Southampton, UK,* [2]*Integrated Plant Protection Centre, Oregon State University, Cordley Hall, Corvallis, OR, USA,* [3]*Centre for Molecular Design, Institute of Biomedical and Biomolecular Sciences and School of Biological Sciences, University of Portsmouth, King Henry Building, Portsmouth, UK, and* [4]*Ecology and Evolutionary Biology Group, School of Biological Sciences, University of Southampton, Southampton, UK*

Abstract
Previous work has shown that insecticide residual toxicity to arthropods on foliage varies strongly with leaf type. Although several aspects of leaf morphology could influence insecticide toxicity, the possible role of leaf waxes, which could influence bioavailability, has not previously been investigated. In this study, the influence of leaf wax cover on the residual toxicity of deltamethrin and dimethoate was investigated using the standard test arthropod *Folsomia candida* Willem (Collembola: Isotomidae) as a surrogate for soft-bodied leaf-dwelling insects. Sixteen leaf types were studied, representing a wide range of crop species. Deltamethrin efficacy increased with increasing leaf surface wax cover. No such relationship was observed, however, for the more polar insecticide dimethoate. Leaf surface roughness was examined using atomic force microscopy and was also observed to influence the efficacy of deltamethrin. The increased efficacy of deltamethrin may be attributed in part to the acquisition of insecticide-contaminated wax particles by *F. candida* walking over treated leaf surfaces. We provide a regression equation to describe the relationship between wax content, surface roughness and the response of *F. candida* to deltamethrin-treated leaf surfaces. We discuss the implications of our findings for the risk assessment of pesticides in IPM, in particular concerning the choice of leaf substrates for use in toxicity screening tests with natural enemies.

Keywords: Folsomia candida, *deltamethrin, dimethoate, wax content, surface roughness*

1. Introduction

Pesticide toxicity to foliage-dwelling non-target arthropods varies with leaf type (Chowdhury et al. 2001). Leaves of different plant species may differ markedly in their wax content, which could affect the contact bioavailability of lipophilic pesticide AI to arthropods in several ways, for example by influencing a pesticide's plant surface penetration, photodegradation or adsorption. So far, however, no studies have determined whether leaf waxes could explain the variation in insecticide toxicity observed between plant species.

The role of cuticular waxes as barriers to the transcuticular movement of many substances has been well documented (Beament 1964; Norris and Bukovac 1972; Schönherr 1976) but their role in transfer of AI between, and retention by, the plant and insect surfaces is less well understood. In addition to their hydrophobic wax content, leaf surfaces are covered by microstructures that profoundly affect surface roughness and wettability. These features in turn affect how foliar applied chemicals are distributed over a leaf surface. If the hydrophobic barrier of epicuticular waxes can be overcome, the superficial wax may also facilitate the passage of lipophilic chemicals into the wax embedded in the cutin layer (Holloway 1970). Plant epicuticular waxes can also modify rates of pesticide photodegradation (Angioni et al. 2004). All of these phenomena are likely to affect the bioavailability of the pesticide at the leaf surface.

A considerable amount of work describing the physico-chemical interactions between leaf surfaces and foliar-applied chemicals has been published (Challen 1962; Crafts and Foy 1962; Kerler and Schönherr 1988a,b; Riederer and Schönherr 1988; Schönherr and Riederer 1989; Bukovac et al. 1990; Gaskin and Holloway 1992; Schreiber and Schönherr 1992, 1993; Schönherr and Bauer 1992; Bukovac and Petracek 1993; Baur et al. 1996). However, the effects of these interactions on exposed invertebrates, including non-target organisms, is less well understood. The present study investigates the effects of plant wax cover and surface roughness on bioavailability to

Correspondence: A. B. M. N. U. Chowdhury, Environmental Health and Consumer Protection Division, Southampton City Council, Southampton, UK.
E-mail: nasirchowdhury@aol.com; nasir.chowdhury@southampton.gov.uk; a.chowdhury@uwe.ac.uk

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exposed organisms and hence the efficacy of foliar-sprayed pesticides. We used the standard test species *Folsomia candida* Willem (Collembola: Isotomidae) (Fountain and Hopkin 2005) as a model non-target arthropod that can be readily exposed to insecticide-treated leaf surfaces under controlled laboratory conditions. The results are used to interpret bioassay results reported earlier (Chowdhury 1998; Chowdhury et al. 2001).

2. Material and methods

2.1. Biological materials and test invertebrates

2.1.1. Folsomia candida Willem (Collembola: Isotomidae) was selected as test species as it has a well-described biology and is used as a 'standard' test species in ecotoxicology, including the risk assessment of pesticides (Fountain and Hopkin 2005). Guidelines for testing the effects of pesticides on *F. candida* as a non-target reference species have also been developed by the International Organisation for Biological Control (IOBC) (Kiss and Bakonyi 1992). A soil-dwelling collembolan, *F. candida*, is closely related to other Collembola (Isotomidae) species that occur on plant surfaces, including those of cereal crops (Frampton 1999). Morphologically, the soft cuticle and relatively short appendages of *F. candida* have similarities with those of some other leaf-dwelling non-target arthropods, such as the larvae of certain Coleoptera, Lepidoptera and Symphyta (Hymenoptera), which might be expected to exhibit a similar type of contact exposure to chemical substances at the leaf surface. *F. candida* is thus appropriate as a study organism in this work, because the focus is on contact exposure.

2.1.2. Culturing of test species. The test invertebrates were cultured and maintained in the School of Biological Sciences at the University of Southampton.

2.1.3. Leaf types. Plants were selected mainly from crops of economic importance and included examples of leaves having glabrous, sub-glabrous and glossy surfaces. Barley, orange, cabbage, sugarcane, maize, tomato, rape (*Brassica napus*) varieties and dwarf beans were grown under glass. Wheat leaves were collected from a field at Manydown, Hampshire, UK, and pear leaves were collected from plants grown outdoors on the campus of the University of Southampton, Hampshire, UK.

2.1.4. Test chemicals. The insecticides used in this study were the synthetic pyrethroid deltamethrin and the organophosphate dimethoate. Both were applied as the formulated product *Decis* (deltamethrin 25 g l^{-1} EC, Hoechst, UK Ltd.) and *Croptex Dimethoate* (dimethoate 400 g l^{-1} EC, Hortichem Ltd, UK). These represent widely-used broad-spectrum insecticides (Garthwaite et al. 2003) with contrasting chemical properties. Deltamethrin is a lipophilic contact insecticide (log $K_{ow} = 6.2$, water solubility = 0.0002 mg l^{-1} at 20°C, vapour pressure = 0.002 mPa at 25°C, molar volume = $316.1X^3$), whereas dimethoate is a lipophobic systemic insecticide (log $K_{ow} = 0.55$, water solubility = 25 g l^{-1} at 21°C, vapour pressure = 0.29 mPa at 20°C, molar volume = $161.8X^3$).

2.2. Bioassay chamber and protocol

Modified Petri dishes were used to confine the invertebrates on the test leaf surfaces (Chowdhury 1998). The adaxial surface of the leaf substrates were sprayed under a Potter laboratory spray tower (Potter 1952) calibrated to deliver a spray volume equivalent to 2001 ha^{-1}. For each leaf type, five to seven concentrations were applied and the treated substrates allowed to dry for approximately 30 min. Control surfaces were treated with distilled water. Freshly collected *F. candida* ($n = 10$), from the laboratory culture were then placed into each of three replicate dishes per concentration. Mortality data were taken at 24-h intervals for up to 4 days after spraying the insecticides.

Data describing the susceptibility of *F. candida* to deltamethrin and dimethoate are given by Chowdhury et al. (2001).

2.3. Extraction of epicuticular wax

Extraction of wax from leaf surfaces was based on the method of Fernandes et al. (1964). Fresh leaves were taken from the glasshouse, field or garden. The leaves were weighed and the total surface area of each measured by computer-based image analysis. The waxes from the adaxial surfaces of a leaf were extracted by allowing successive aliquots (5 ml per replicate wash) of solvent (petroleum ether) to run over the leaf surface from a fine-orifice burette and collected on a pre-weighed glass Petri dish. The solutions were filtered and the solvent allowed to evaporate at room temperature. The residue was then weighed and the amount of wax per unit area calculated.

2.4. Scanning electron microscopy of F. candida

Leaf samples with their adaxial surfaces facing upward were attached to a bioassay chamber (Chowdhury 1998). One chamber without any leaf attached was prepared as a control surface. Adults of *F. candida* ($n = 2-3$, freshly collected from a stock culture) were allowed to remain in all experimental bioassay chambers for up to 24 h. The insects were then removed from the chamber using a fine brush, immersed for 30 min in absolute ethanol and dried by a critical point dryer (CPD) (BALZERS, Balzers Union Aktiengesellschaft, Liechtenstein). The leaf samples were placed into the specimen pressure chamber of a CPD. The chamber was pre-cooled to a

temperature of 10°C at a pressure of 50 bar or 20°C at a pressure of 40 bar. Transition liquid was introduced into the specimen chamber using six to eight drainage cycles to ensure complete exchange. The chamber was then heated to reach the critical temperature and pressure of the transition liquid (for CO_2 this is 31°C and 73.8 bar). The liquid was then volatilised and the dried specimen removed after a pressure reduction.

The specimens were mounted, either before or after drying, on aluminium pin stubs of diameter 25 mm (Agar, Agar Scientific, Ltd. Essex, UK) by means of double-sided adhesive tape and labelled according to leaf type. The specimens were silver coated using a sputter coater (Emscope, London, UK) prior to examination under the scanning electron microscope (SEM). Specimens were examined by placing the stubs in the air-locked specimen chamber of a SEM (Hitachi-s-40) at an acceleration voltage of 10 kV and with a 50-mW emission current. The images were recorded by a camera fixed to the SEM (using Ilford FP4-120 film).

2.5. Scanning electron microscopy (SEM) of different leaf surfaces

Specimens of leaf types were collected from greenhouses, orchards and the field and preserved in small vials containing absolute alcohol. The specimens were prepared and examined under SEM following the procedure described for *F. candida* (see above).

2.6. Measurement of leaf surface roughness by atomic force microscopy (AFM)

It has been widely reported that leaf surface roughness, which results from a combination of leaf morphology and the extent and organisation of the superficial surface waxes, plays an important role in determining retention (Furmidge 1962), spreading and wetting (Holloway 1970; Baker et al. 1983). Until recently, microscopic and ultramicroscopic roughness of leaf surfaces have been investigated using SEM. Atomic force microscopy (AFM), however, offers an alternative method for characterising surface roughness without subjecting the leaves to the extreme conditions required for SEM (Parsons et al. 1974).

Replicas of leaf surfaces were prepared by placing small pieces (1 cm^{-2}) of cellulose acetate (125 μm thick) soaked in acetone, on the adaxial surface of a freshly sampled leaf. After drying (approx. 10 min), the cellulose acetate sample was carefully removed from the leaf surface and attached to an AFM sample mount using carbon-loaded, double-sided adhesive tape with the impression side of the leaf surface facing upwards. The replica specimens were then scanned using the AFM. The surface topography of a leaf was analysed using TopoMetrix® image analysis software (ThermoMicroscopes, Sunnyvale, CA, USA) and arithmetic roughness averages calculated. Arithmetic surface roughness average, R_a, is the arithmetic average of the absolute values of the measured profile height deviations, given by:

$$Ra = \frac{1}{n}\sum_{i=1}^{n} |Z_i - \bar{Z}|$$

where n = number of height positions along line profile, Z_i = height at position i (nm), and Z = average height (nm). Figure 1 shows a representative image of the wax structures, crevices and valleys that cover a barley leaf together with a surface roughness profile (lateral resolution of AFM images was less than 1 nm and height resolution was less than 1 Å).

2.7. Statistical analysis

The mortality data of *F. candida* used for analysis of the efficacy of residues on plant surfaces were those (Table I) already reported by Chowdhury et al. (2001), and for which LD_{50} values were already estimated using probit analysis (Finney 1971). Multiple linear regression (Minitab v.12.01;

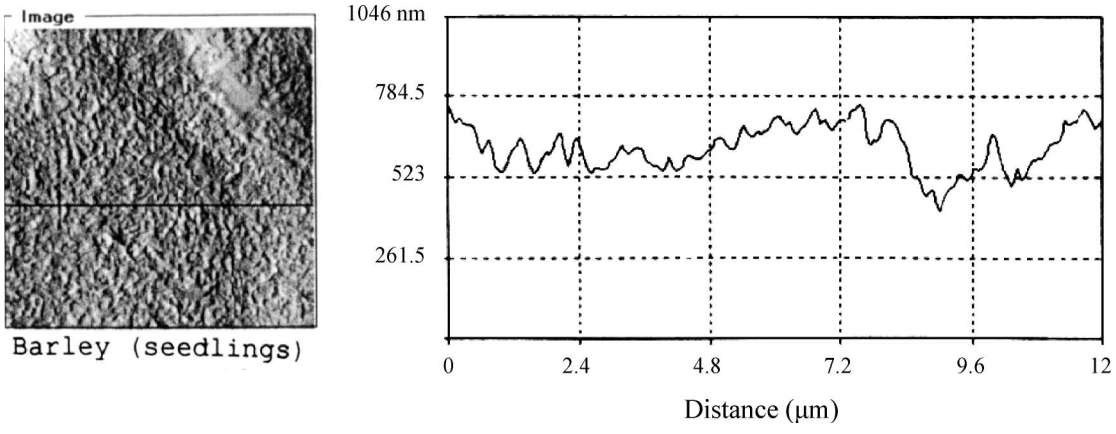

Figure 1. A typical atomic force microscopy image of a barley seedling leaf and a transect showing its surface roughness (Central resolution is less than 1 nm and height resolution is less than 1 Å).

Minitab, USA) was used to investigate the relationship between: (i) the $-\log LD_{50}$ values for *F. candida* estimated for different leaf species sprayed with either deltamethrin or dimethoate; (ii) the wax content extracted per unit area (WC); and (iii) the surface roughness (R_a) of the leaf measured by AFM. These properties are presented in Tables I and II.

3. Results

3.1. The wax cover of different leaf types

The amount of wax ($\mu g\ cm^{-2}$) extracted from the adaxial surfaces of different leaf types are ranked in Figure 2. Barley seedling leaves had the highest amounts of wax, whereas dwarf bean leaves had the least. Young leaves tended to be covered by larger amounts of wax than older leaves. Leaves of tomato, sugar cane, orange, maize (young), maize (old) and dwarf bean fell within the range of $1-10\ \mu g$ of wax cm^{-2}. In contrast, cabbage, barley and rape leaves were covered in large amounts of waxes ($30-55\ \mu g\ cm^{-2}$) that formed blooms on the leaf surface. Intermediate amounts of wax were observed for pear and wheat leaves.

3.2. The relationship between leaf properties and insecticidal efficacy

The efficacy of deltamethrin increased exponentially with the quantity of surface wax encountered on a leaf surface by an insect (Table I, Figure 3). No such relationship, however, was observed for *F. candida* exposed to leaf surfaces treated with the

Table I. Ranking of 72 h LD_{50} of *F. candida* for deltamethrin 2.5EC and wax content of different leaf types.

Leaf types	Scientific name	Variety	LD_{50} (g AI ha^{-1}) (95% CI)	Rank	Wax ($\mu g\ cm^{-2}$) (SE)	Rank
Barley (s)	*Hordeum vulgare*		6.36 (4.58–8.25)	1	51.33 (2.89)	1
Cabbage (o)	*Brassica oleracea*	Prixie	8.96 (6.18–12.11)	5	36.12 (1.13)	5
Tomato (o)	*Lycopersicon esculentum*	Money maker	16.87 (12.48–22.67)	9	09.65 (1.03)	11
Pear (o)	*Pyrus communis*		14.43 (9.82–20.50)	8	21.17 (2.38)	9
Sugarcane (o)	*Saccarum officinerum*		20.94 (13.98–32.40)	10	5.40 (1.38)	13
Wheat	*Triticum aestivum*	Hereward	24.86 (16.38–41.00)	11	8.42 (2.04)	10
Orange	*Citrus sp.*		40.79 (27.15–73.70)	13	5.06 (1.62)	14
Dwarf bean	*Phaseolus vulgaris*	Sutton	77.14 (54.45–119.26)	15	1.46 (1.13)	16
Rape (o)	*Brassica 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)	*Zea mays*	Marcia	66.65 (43.44–134.60)	14	2.77 (0.24)	15
Maize (y)	*Zea mays*	Marcia	37.53 (25.94–61.89)	12	6.74 (0.45)	12

(s) = seedlings; (o) = old; (y) = young.

Table II. Ranking of 72 h LD_{50} of *F. candida* for dimethoate 40EC and wax content of different leaf types.

Leaf types	Scientific name	Variety	LD_{50} (g AI ha^{-1}) (95% CI)	Rank	Wax ($\mu g\ cm^{-2}$) (SE)	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)	*L. 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	5.40 (1.38)	13
Wheat	*T. aestivum*	Hereward	2.77 (2.17–3.49)	9	8.42 (2.04)	10
Orange	*Citrus sp.*		1.62 (1.08–2.23)	4	5.06 (1.62)	14
Dwarf bean	*P. vulgaris*	Sutton	2.36 (1.83–2.97)	8	1.46 (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)	*Z. mays*	Marcia	4.17 (3.25–5.36)	11	2.77 (0.24)	15
Maize (y)	*Z. mays*	Marcia	5.95 (4.57–7.60)	14	6.74 (0.45)	12

(s) = seedlings; (o) = old; (y) = young.

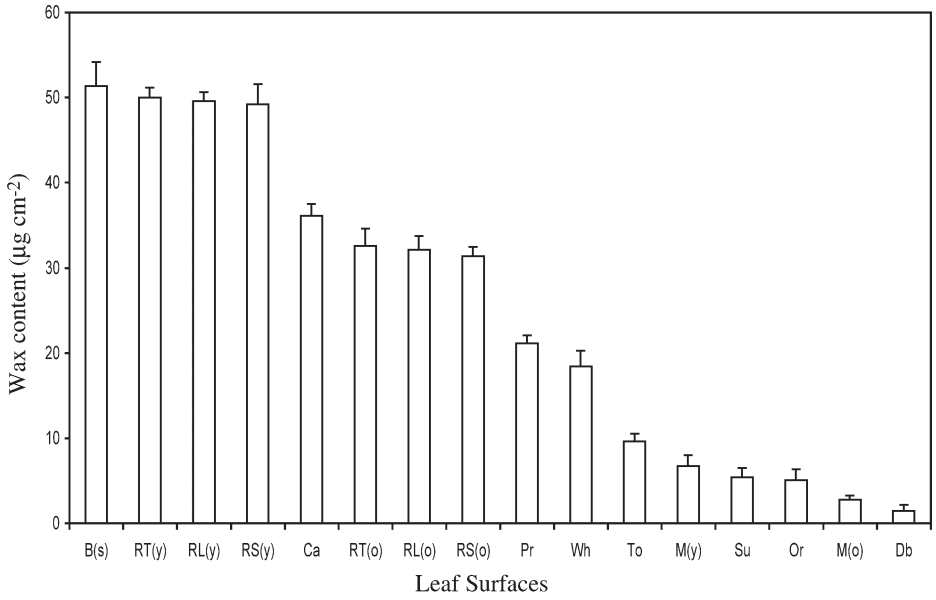

Figure 2. Mean wax content (+SE) of adaxial surfaces of 16 different leaf types. Key to test leaf types: B, barley; Ca, Cabbage; To, Tomato; RT, rape v. tanto; RS, Rape v. starlight; RL, Rape v lirawell; Db, Dwarf bean; Pr, Pear; Su, Sugarcane; Wh, Wheat; Or, Orange; M, Maize; (s), seedlings; (o), old; (y), young.

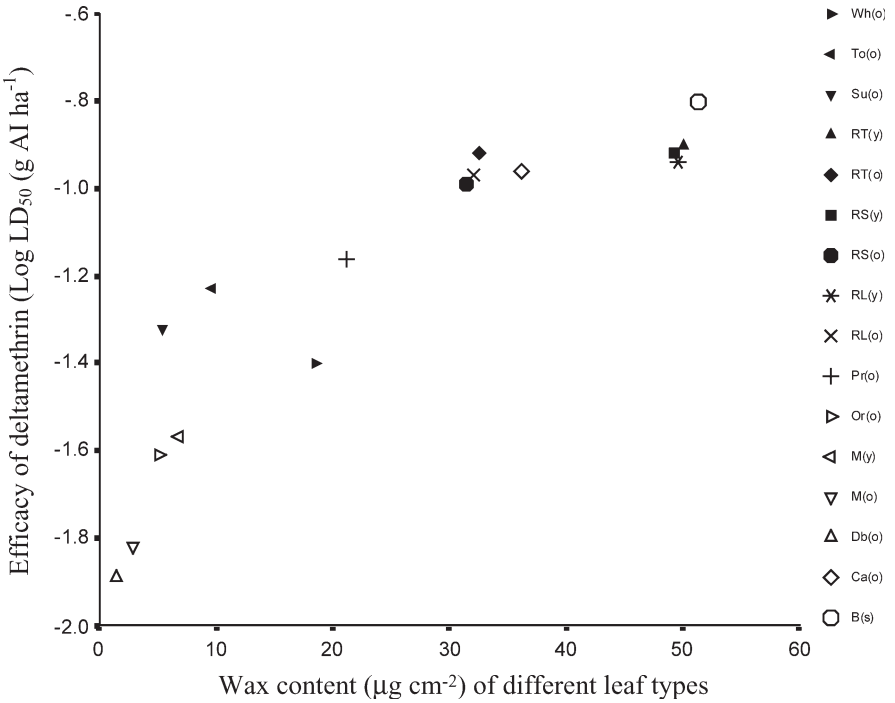

Figure 3. Relationship between the efficacy of deltamethrin for *F. candida* and wax content of different leaf types. Key to test leaf types are as in Figure 2.

substantially more polar insecticide dimethoate (Table II, Figure 4). The nature of the active ingredient therefore affects the role that the surface wax plays in mediating the toxicity of insecticide residues to invertebrates exposed to treated leaf surfaces. Although the comparison between the intrinsic toxicity of dimethoate and deltamethrin for *F. candida* has not been evaluated in the present study, previous work showed that both products cause similar intrinsic toxic effects to predatory Coleoptera (Jepson et al. 1995). Therefore, a comparison between the residual toxicity of two insecticides with contrasting properties should provide information on the role of substrate properties in mediating the toxicity to exposed invertebrates.

For the contact insecticide deltamethrin, there is a significant and positive association between the mean and standard deviation of the tolerance distribution of *F. candida* exposed to treated leaf surfaces (Figure 5), whereas no association was observed for the systemic

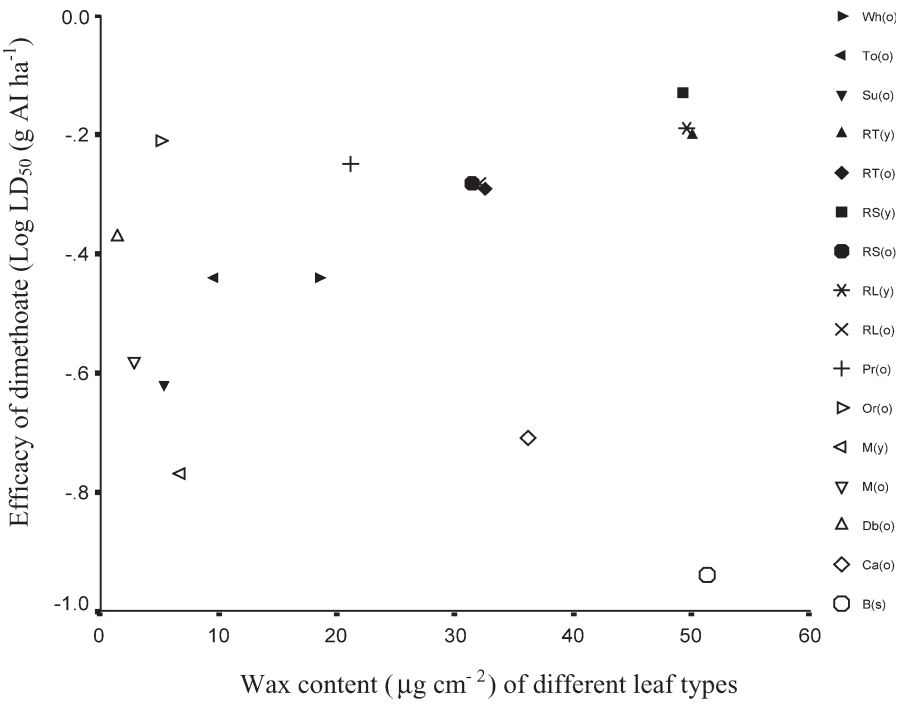

Figure 4. Relationship between the efficacy of dimethoate for *F. candida* and wax content of different leaf types. Key to test leaf types are as in Figure 2.

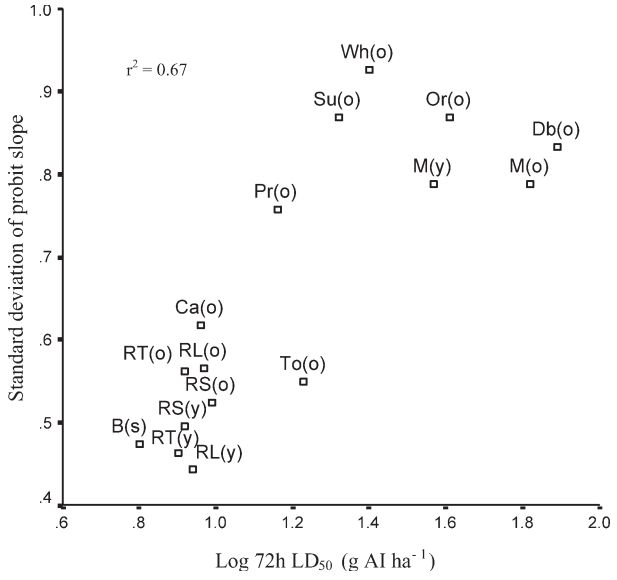

Figure 5. Relationship between the standard deviation of the probit slope and 72 h Log LD_{50} of *F. candida* exposed on different leaf surfaces treated with deltamethrin. Key to test leaf types are as in Figure 2.

and relatively volatile insecticide dimethoate (Jepson et al. 1995) (Figure 6).

3.3. Scanning electron microscopy (SEM) studies

SEM was used to demonstrate whether exposed organisms had picked up superficial waxes from leaf surfaces. Three criteria have been used in attempting to determine the identity of the wax-like structures observed in the scanning electron micrograph. These are: (a) the magnification at which these particles were observed; (b) the structural form of the particles; and (c) the presence or absence of these structures on the body of *F. candida* exposed to the wax-free surface of clean Petri dishes.

Based on these criteria, superficial wax-like structures were observed on the leg of *F. candida* exposed to barley leaves (Figure 7a) at a magnification of $\times 3300$. The structures have similarities with the wax structures seen on the adaxial surfaces of barley seedling leaves (Figure 7b) also observed at $\times 3300$. Superficial structures found on the antennae of a *F. candida* (Figure 8a) exposed to sugarcane leaf observed at a magnification of $\times 950$ are similar to the wax observed at $\times 930$ on the adaxial surface of sugarcane leaf (Figure 8b). Similar results were also observed with other leaf types. The most notable observation was that no such superficial structures were found on the body parts of *F. candida* exposed to clean Petri dishes (Figure 9a – c) observed at magnifications of $\times 620$, $\times 1400$ and $\times 3800$, respectively.

3.4. Atomic force microscopy (AFM) studies

AFM was used to measure the microscopic and ultramicroscopic roughness of a subset of eight leaf surfaces, i.e. roughness arising from the presence of wax crystals and wax blooms, the topography of the underlying cell wall and any microcrystalline inclusions of oxalic acid or silica, etc. Microscopic roughness excludes trichomes and cell wall boundaries. Dwarf bean and barley show the greatest microscopic roughness whereas pear and tomato are microscopically smooth (Figure 10).

3.5. Multiple regression analysis

The relationship between wax content, surface roughness and the efficacy ($-\log LD_{50}$) of *F. candida* on deltamethrin-treated leaf surfaces is:

$$-\text{Log } LD_{50} = -1.49(\pm 0.083) + 0.023WC(\pm 0.002) - 0.0015R_a(\pm 0.0004) \quad (1)$$

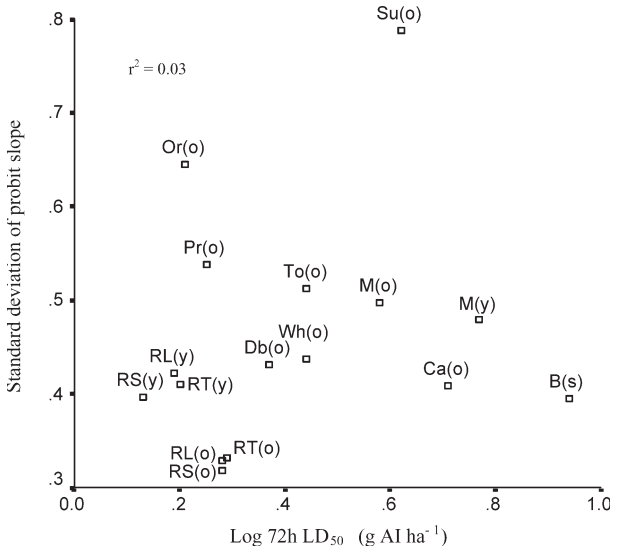

Figure 6. Relationship between the standard deviation of the probit slope and 72 h Log LD_{50} of *F. candida* exposed on different leaf surfaces treated with dimethoate. Key to test leaf types are as in Figure 2.

Where WC is the wax content (μg cm^{-2}) and R_a is the arithmetic surface roughness average (nm), $n = 8$, $R^2 = 0.97$, $F_{2,5} = 102.22$, $P < 0.001$.

4. Discussion

The factors that affect the bioavailability of insecticides applied to plant surfaces, their uptake by passing insects and hence their efficacy are poorly understood. Investigation of these processes may lead to a better understanding of the basis of AI transfer and perhaps to improved application and formulation. This study confirms, using a model arthropod species, that leaf waxes are an important component of leaf morphology that affects bioavailability of lipophilic insecticides.

4.1. The role of diffusion and adhesion in the distribution of active ingredient

Early studies suggested that insecticide deposits penetrate into the insect body or the plant surface through a process of diffusion (Boize et al. 1976; Schőnherr and Baur 1994). Transfer from the plant to the insect surface, however, depends on differences in surface adhesion and formulation viscosity (Ford and Salt 1987; Crease et al. 1987). Lipophilic insecticides such as deltamethrin (Log $P = 6.2$) will adhere preferentially to the most hydrophobic surface, usually the insect epicuticle. Surface forces are therefore likely to dominate the AI transfer process and diffusion will play a less significant role.

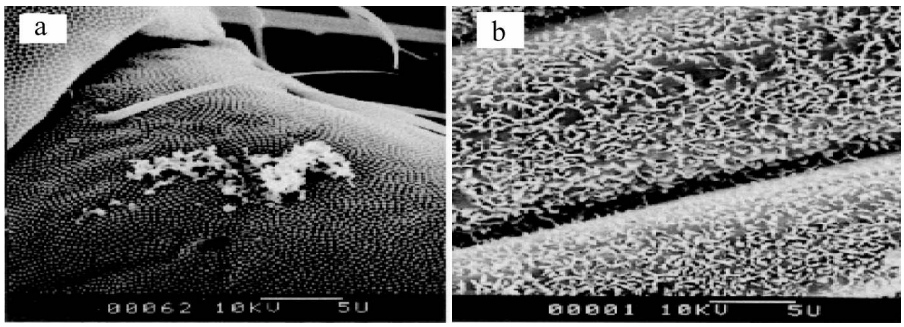

Figure 7. Scanning electron micrograph of (a) leg of *F. candida* and (b) adaxial surface of barley leaf showing prominent wax structures.

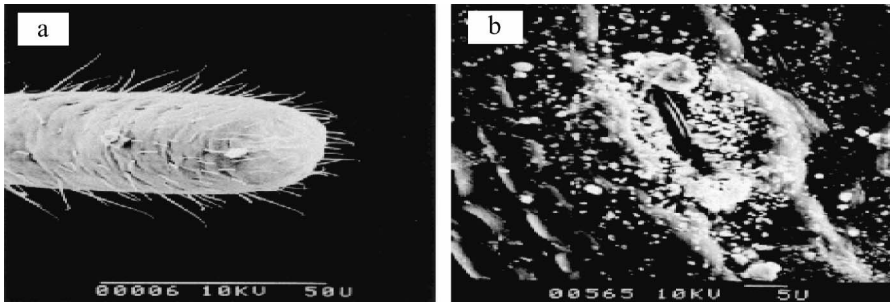

Figure 8. Scanning electron micrograph of (a) antenna of *F. candida* and (b) adaxial surface of sugarcane leaf showing prominent wax structures.

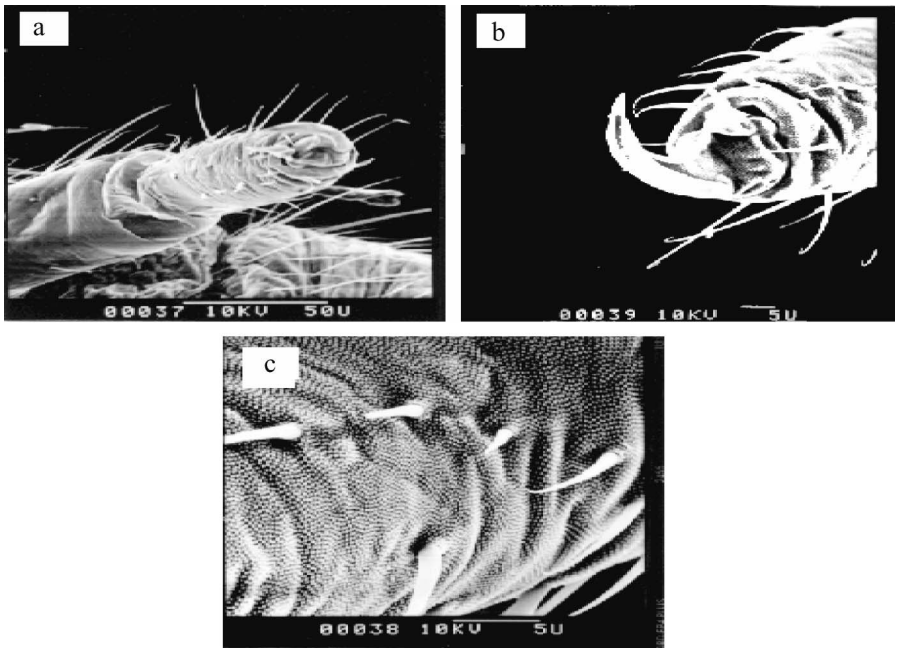

Figure 9. Scanning electron micrograph of (a) leg of *F. candida* exposed on clean petri dishes at magnification: (a) ×620; (b) ×1400; and (c) ×3800.

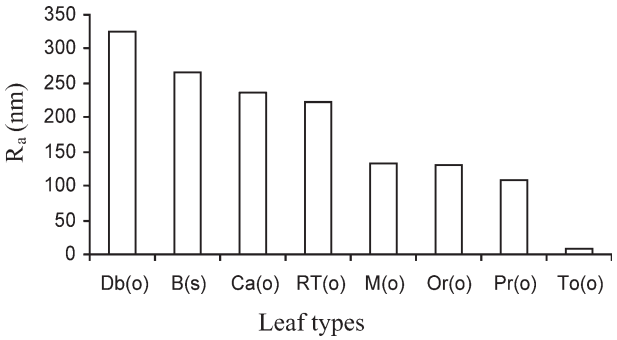

Figure 10. Leaf surface microscopic roughness (R_a) of eight different leaf types. Key to test leaf types are as in Figure 2.

Distribution of insecticide by diffusion is more likely to be important when both the plant and insect surfaces are static and in continuous contact. In the case of moving insects that walk on leaf surfaces, this occurs only when the organism is at rest. For sedentary targets including eggs, sucking and piercing insects such as whitefly larvae, and fungal spores, continuous and prolonged contact is normal and under these conditions, the rate of AI transfer may be diffusion controlled.

4.2. Behavioural aspects of AI transfer

The behavioural activity of the insect will also influence AI transfer. During walking, grooming and rubbing, the insects exert additional forces that result in transfer of superficial substances from the leaf surface. Plant waxes, for example, can be transferred in this way (Figures 7 and 8). These materials are similar in chemical composition to, and therefore have affinity for, the epicuticular waxes that cover the insect cuticle. However, they are less tightly bound to the plant surface than the insect waxes are to the insect integument, and appear to be readily transferred on contact with a passing insect (Figures 7a and 8a).

The transfer of insecticides from the surface of treated leaves to the insect surface will be affected by a number of factors. These include anatomy and mode of locomotion which affect the area of leaf contacted in a given distance covered, the rate of locomotion and the surface features of those parts of the insect which contact the leaf (Salt and Ford 1984). In this study the insect used was *F. candida*, but its behaviour may not be typical of that of leaf feeding insects since it is a soil scavenger. However, there is a wide range of morphology and locomotory behaviour among plant-feeding insects, and this species was regarded as a suitable model to determine the main factors involved in AI transfer (see above).

4.3. Encounter, availability and AI transfer

The efficacy of a contact insecticide sprayed onto a leaf surface will depend on both the probability of encounter and the availability of the active ingredient to a contacting insect (Ford and Salt 1987). Both processes combine to determine the dose of insecticide that is transferred from the treated leaf surface to the surface of the insect integument.

Efficacy can be expressed in terms of the reciprocal of the LD_{50} or $-\log LD_{50}$ ($\log 1/LD_{50}$) where $1/LD_{50}$ has units of ha g AI^{-1}. If the efficacy of deltamethrin is plotted for 16 different leaf types against the quantity of wax observed at the leaf surface, a curvilinear relationship is observed, with high wax

content associated with high efficacy (Figure 3). Because it is lipophilic and exerts a low vapour pressure, deltamethrin will accumulate at leaf surfaces such as barley and rape which have a high wax cover and be retained intact without further penetration into the leaf or significant loss by volatilisation. Exposed *F. candida* are then likely to encounter and pick up plant waxes contaminated with insecticide (Figure 7b). Once transferred, the insecticides can be released slowly from the wax, penetrate the insect integument and move to the site of action as a result of diffusion, and internal circulation of haemolymph. The data described in Table I also suggest a dependence of efficacy on surface roughness, an observation confirmed by the regression model (equation (1)) estimated from eight leaf surfaces. This result emphasises the importance of leaf surface characteristics such as wax cover and surface roughness for the effectiveness of dried EC formulations of non-polar insecticides such as deltamethrin.

No such relationship could be observed for dimethoate (Figure 4). In fact, the efficacies of dried EC deposits of this organophosphate applied to different leaf surfaces were not significantly different (Table II). Thus, the efficacy of dimethoate, a polar insecticide, appears to be less sensitive to changes in leaf surface properties than more hydrophobic materials such as deltamethrin. This may be because of the low concentration of this compound in the plant waxes, and/or the greater affinity of the compound for plant cuticle.

The increased efficacy observed for deltamethrin may also arise as a result of other factors. Immediately after spraying deltamethrin EC, leaf surfaces with large amounts of epicuticular wax (e.g., barley seedlings and rape) were covered with discrete droplets of uniform distribution. In contrast, on leaf surfaces with low wax content (e.g., orange, dwarf bean, sugarcane and maize leaves), the droplets coalesced soon after landing, to form several large isolated drops of irregular shape, leaving areas completely free of insecticide deposits. These features may result in differences in area covered by the active ingredient and could therefore modify the probability of encounter in terms of both its magnitude and variability. This could explain why a relationship between the mean and slope of the tolerance distribution was observed for deltamethrin EC treatment but not for dimethoate EC (Chowdhury et al. 2001). The more uniform placement of the pyrethroid on waxy leaf surfaces could explain its higher efficacy compared to that of the organophosporous insecticide. For dimethoate, the heterogeneity of placement and dosing probably masks any underlying association that might exist between the tolerance distribution parameters.

The efficacy of a contact insecticide such as deltamethrin depends upon its availability at the plant surface. Movement of the AI deep into the underlying tissues of the leaves will reduce accumulation of active ingredient by the exposed insect through body contact. However, such penetration is important for systemic pesticides such as dimethoate. A non-polar material such as a plant wax or a pyrethroid will have a better chance than a more polar material such as an organophosphate of being transferred to, or retained on, a hydrophobic surface such as the insect integument. Scanning electron microscopy showing evidence of pick up of potentially contaminated wax particles from the leaf surface (Figures 7 and 8) provides experimental support for this argument.

The surface roughness (microscopic and ultramicroscopic) can also play a role in determining the nature and extent of pesticide/leaf surface interactions. The degree of retention, for example, is related to the irregularities of the leaf surface, with retention increasing with increasing roughness (Furmidge 1962). Higher retention may be favoured by a roughened surface topography characterised by sharp peaks and valleys and on which liquid can be trapped in niches where it is unavailable for transfer to passing insects. Spreading of liquids is facilitated by smooth surfaces on which a low advancing contact angle can be observed. Such spreading is reported to result in poor retention (Boize et al. 1976). Macroscopic roughness arising from the leaf cell boundaries, the arrangement of which determines the orientation and geometry of surface grooves, is also important in retention and spreading of liquid drops. In general, the spread on leaves of drops $>20\,\mu$m diameter will be governed by both macroscopic and microscopic roughness. Such drops are likely to contain sufficient volume to be conducted along the relatively wide grooves in the epidermis (Boize et al. 1976). Therefore, better understanding of the relative importance of spreading, retention and AI transfer and the influence on these properties of wax cover and surface roughness should assist in the development of improved formulation and application strategies.

4.4. Relevance to integrated pest management (IPM)

An aim of the International Organisation for Biological Control (IOBC) Working Group on Pesticides and Beneficial Organisms is to develop a sequential pesticide risk assessment scheme for assessing the safety of pesticides to natural enemies of crop pests in IPM (Hassan 1998a). At the first tier of the scheme, glass plates or quartz sand are used as test substrates to ensure maximum exposure of the test organism to the pesticide, under standardised 'worst-case' test conditions (Dohmen 1998; Hassan 1998b). This system aims to screen out harmless pesticides at an early stage of testing, so that only harmful substances require further evaluation (Bakker 1998). Pesticides identified as harmful at the lower tier are subjected to further testing using progressively more realistic substrates (Dohmen 1998; Hassan 1998b).

Leaves from a wide variety of plant types could be used as relevant substrates in toxicity tests (Hassan 1998a) but in practice relatively few 'standard' species are currently preferred. Leaves of the Dwarf French bean (*Phaseolus vulgaris* L.) are usually used in tests with arable arthropods, for example with ladybird beetles (Coccinellidae) (Schmuck et al. 1998), lacewings (Chrysopidae) (Bigler and Waldburger 1998) and predatory mites (Acari: Phytoseiidae) (Oomen et al. 1991). In specific situations, barley plants (*Hordeum vulgare* L.) may be used instead, for example in tests with aphid parasitoids (*Aphidius* spp.) (Hymenoptera: Aphidiinae). The higher-tier laboratory tests should simulate 'realistic worst case' conditions of exposure to enable the risk of pesticides to be ascertained (Dohmen 1998).

Although our findings concern a collembolan test species, they highlight a possible limitation with the choice of dwarf French beans as preferred leaf substrate in IPM toxicity tests for arable arthropods. Dwarf beans have a relatively low epicuticular wax content, meaning that the bioavailability and efficacy of lipophilic insecticides might be underestimated if the results of tests on dwarf beans are extrapolated to a wider range of crops. In particular, the dwarf bean substrate may not fulfil the criterion of representing a 'realistic worst case' scenario of exposure for lipophilic pesticides. The relatively high epicuticular wax content of barley leaves on the other hand suggests that this might be a more appropriate choice of substrate if the focus is on toxic effects of lipophilic pesticides such as synthetic pyrethroids. This class of insecticides is of particular relevance in IPM toxicity screening studies due to the high frequency and scale of usage (e.g., Garthwaite et al. 2003) and relatively high toxicity to a wide range of beneficial arthropods, including natural enemies (e.g., Frampton 1999).

The Collembola model demonstrates that epicuticular waxes may be dislodged from leaf surfaces by leaf-dwelling insects. This raises questions about the subsequent fate of any pesticide residues that are in the waxes and possible implications for exposure both of leaf-dwelling arthropods and their predators. For instance, it is unclear whether waxes dislodged from the leaf surface might be redistributed to other areas of the arthropod body (for example by grooming of appendages), or to other parts of the ecosystem (for example when leaf-dwelling arthropods fall to the ground or into spider webs). Collembola are relevant in this context because they are abundant in arable crops (Frampton 1999) and widely preyed upon by other arthropods, including natural enemies such as spiders that are highly sensitive to synthetic pyrethroids (e.g., Wiles and Jepson 1992).

Many factors besides those we investigated can affect the exposure of test organisms. However, our findings suggest that where effects of lipophilic insecticides are of interest, an informed choice of leaf type for use as a substrate would be appropriate in IPM toxicity tests. If we assume that the responses of Collembola are broadly representative of the responses of other test organisms, a logical choice of substrate would be to use both barley leaves and dwarf bean leaves in toxicity tests, to capture the range of wax content, and hence insecticide efficacy, found in the main arable crops. Verification of the Collembola findings using other test organisms would, of course, be preferable. Our findings also suggest that differences in the bioavailability of polar and lipophilic compounds should also be considered when evaluating sequential testing schemes for risk assessment in IPM. This could be achieved simply by ensuring that, where feasible, tests are performed with representatives of both polar and lipophilic pesticides.

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