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Analytical Techniques for Quantifying Direct, Residual, and Oral Exposure of an Insect Parasitoid to an Organophosphate Insecticide

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Attempts at ranking the level of risk posed by pesticides to non-target invertebrates in arable crops based on their presence or absence at the time of spray application, their position in the crop canopy, and their diurnal activity patterns have been made (Jepson 1989). However, such rankings arc limited by the lack of quantification of important factors that mediate short-term effects, including the susceptibility and exposure of individual species (Jepson 1988). The three main routes of pesticide exposure for a non-target beneficial species, such as adult aphid parasitoids, are uptake after direct exposure to spray droplets, uptake of residues by contact with contaminated surfaces, e.g. soil or vegetation, and oral uptake from contaminated food sources, e.g. aphid honeydew. These routes of exposure are not necessarily of equal importance, but each pose different threats to parasitoid survival through time. Therefore to extrapolate from laboratory studies to field conditions, quantitative information is needed on the relative importance of these different routes of uptake.

The determination of these exposure routes to non-target invertebrates has been applied successfully to relatively large species such as carabids and coccinellids (Wiles and Jepson 1993) and linyphiids (Jagers op Akkerhuis and Hamers 1992). However, until now it has proven impractical to use aphid parasitoids due to their small size and periodic flight behaviour. This paper reports on laboratory bioassays designed to quantify the direct, residual and oral exposures of the aphid parasitoid *Aphidius ervi* Haliday to an organophosphate insecticide, using two different analytical techniques.

MATERIALS AND METHODS

Parasitoids were cultured on pea aphids, *A cyrthosiphon pisum* (Harris), maintained on broad bean plants, *Vicia fava* (cv. Banner), in an environmental chamber (25±1°C; 78±5% RH; 16:8 L:D photoperiod). Female parasitoids were used in bioassays within

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48 hr of emergence, and unless otherwise stated, fed on a 50% honey solution prior to use.

The organophosphate insecticide, Diazinon Ag500 (399.1mg AI/mL) was obtained through commercial sources, and will hereafter be referred to as the radioinert sample. A ^{14}C-labelled sample of technical Diazinon (0. 150mCi) was obtained from the Ciba Geigy Corporation (Δ-2-^{14}C-Diazinon; 98.8% purity; specific activity=37.2μCi/mg). This was combined with formulated radioinert diazinon in a 1:1 ratio to make a solution of radio-labelled diazinon of field concentration (1.25mg/mL).

A horizontal spraying apparatus was designed according to the dimensions of the conventional vertical Potter spray tower (Potter 1952). This design was necessary to allow the spraying device to be enclosed within a large polythene bag and housed inside a fume cupboard in order to control external contamination by spray drift. A Thomas atomiser, connected to an air compressor, was separated from the object to be sprayed by a rigid transparent acetate column (61.5 cm length, 12 cm diameter). The spray was delivered at 100 kPa for a 3 sec period. The time period was chosen arbitrarily as it provided a fine, even deposit over the sprayed surface. A repeatable mean spray deposit of 7.14ng diazinon/mm^2 was achieved.

The three different bioassays carried out were as follows:
(1) To determine the quantity of insecticide uptake via residual exposure, solutions of radioinert diazinon representing the recommended field concentration (1.25mg in 1mL of water), and half field concentration were made. They were sprayed onto the inner surface of glass Petri-dish bases and lids (9cm-diameter) using the spray apparatus described above. Control dishes were sprayed with distilled water.
For each treatment, female wasps were individually transferred using an aspirator onto a treated Petri-dish once the spray solution had dried (approximately 30 mins), and left exposed to residues for either 0.5, 1, 2.5, 4, 5.5 or 8 mins. Ten wasps were used for each insecticide concentration and exposure time combination. Initial observations indicated these exposure times covered the full range of observable toxic effects in parasitoids exposed to a field concentration of diazinon. At the end of a set exposure period, wasps were promptly removed using clean forceps, and transferred to glass vials for preparation of chemical analysis using gas chromatography, as described below. For a comparison of analytical techniques, an identical set of bioassays was performed using solutions of radio-labelled diazinon.

(2) To determine the quantity of insecticide uptake via oral exposure, twenty individual wasps, previously denied food for 24 hr, were offered a food source combining a 50:50 mixture of honey and a solution of radioinert diazinon at field concentration. The honey solution was presented through the narrow end of a disposable pipette tip (1-200μL volume range). This ensured that only the head and mouthparts of wasps contacted the solution. Time spent feeding (taken as time spent with head inserted in end of pipette tip) was recorded. Once feeding had ceased, wasps were immediately removed using forceps and processed for chemical analysis. For comparison, identical

bioassays were conducted, this time substituting a field concentration solution of the radio-labelled diazinon into the food source.

(3) To determine the degree of insecticide diffusion through a mummy case (direct exposure), 150 aphid mummies containing late larval stages were positioned onto glass cover slips with double-sided adhesive tape. Single 0.5µL droplets of a radio-labelled solution of field concentration diazinon, with a 0.2% solution of wetting agent (Triton X100), were applied to the dorsal surface of the mummies using a Hamilton repeating microapplicator fitted with a 25µL Hamilton syringe. At time intervals of 1, 2, 4, 8, 16 and 24 hr after treatment, 25 mummies were randomly selected and their dorsal surfaces removed using fine insect pins. Parasitoids were carefully extracted using clean forceps. Subsequently, two separate chemical analyses were conducted:
(a) internal extraction - to determine the quantity of chemical that had penetrated through the mummy case and become absorbed in the developing parasitoid, batches of five extracted larvae were placed into single scintillation vials;
(b) external extraction - to determine the quantity of chemical that remained unabsorbed on the mummy case, each batch of five coverslips, empty mummy cases and adhesive tape from (a) were placed in a single scintillation vial. Vials from (a) and (b) were then processed and analysed in a scintillation counter as described below.

In all bioassays, quantities of radioinert and radio-labelled diazinon in parasitoid tissue was analysed using gas chromatography and liquid scintillation counter techniques, respectively. A description of each analytical technique is given below.

Gas chromatography sample preparation.
To kill and preserve the wasps prior to analysis, 1mL of acetone was added to a 2mL glass vial containing 5 wasps. The contents were transferred into a 4mL Wheaton v-vial and 4 x 250µL rinses of acetone were quantitatively transferred from the first vial to the Wheaton vial. The contents of the vial were evaporated using a gentle stream of air within a fume cupboard. After an addition of 1mL of ethyl alcohol, the vials were placed in a Bransonic Ultrasonic cleaner for 30 mins to extract diazinon. Afterwards, the insect mixture was allowed to settle for 10 mins and then transferred into a 10mL syringe filter fitted with an Acrodisc 3CR PTFE (3mm HPLC certified 0.45pm) and filtered into an autosampler vial. A volume of 1µL of the filtrate (a mixture of diazinon and ethyl alcohol) was injected into a gas chromatography column (details in Table 1). A calibration curve was established by injecting a range of known quantities of radioinert diazinon, enabling subsequent readings from bioassays to be converted to µg of active ingredient of diazinon.

Scintillation counter sample preparation.
Batches of five parasitoids were placed into scintillation vials and kept frozen until analysis. Once thawed, wasps were dissolved in 250µL of tissue solubiliser (Tissue Solubiliser TS-2, Research Products International), and left in a waterbath at 50°C for

Table 1. Details of the gas chromatography column.

Apparatus description:	HP5890 Series II Gas Chromatograph equipped with Nitrogen-Phosphorus Detector (NPD), automatic sampler and ChemoStation program.
Column description:	Phase: DB-17; Length: 30 metres; Internal diameter: 250mm; Film thickness: 0.25 microns.
Temperature of heated zones:	Oven: 90°C to 260°C, at 35°C/min; Injector: 260°C; Detector: 275°C (NPD).
Flow rate of gases:	Carrier gas: Helium, 5ml/min; Make-up gas: Helium, 25ml/min; Detector gases (1) Air: 110ml/min; (2) Hydrogen: 3ml/min.
Injection volume:	1µl

12 hr. A volume of 20mL of Aquasol-2 (Packard Instrument Company) was added to each scintillation vial and the solution was radioassayed. Radiocarbon in the parasitoid samples was determined by use of a Searle Delta 300LSC model 6890 (Searle Analytic Inc., Illinois, U.S.A.). The data was converted from counts per minute (cpm) to disintegrations per minute (dpm) by correcting for background radiation, dilution, quench and counting efficiency. A calculation of dpm per quantity of diazinon of the original insecticide solution enabled the subsequent conversion of dpm readings to µg/active ingredient of diazinon in parasitoid samples.

RESULTS AND DISCUSSION

The residual uptake of diazinon by *A. ervi* was dependent on the concentration of the residues and exposure time (Figures 1a and 1b). Uptake increased progressively with exposure time, with periods of most rapid uptake occurring during the first 4 mins and 5.5 mins of exposure to field concentration and half field concentration residues, respectively. Beyond these time periods, total uptake either slowed down or ceased. This may have resulted from the sites of uptake of residual deposits, mainly the tarsal receptors or setae (Hartley and Graham-Bryce 1980), reaching maximum loading and/or subsequent parasitoid grooming behaviour removing insecticide particles from the exterior of the body.

The two different analytical techniques were found to retrieve similar quantities of diazinon from parasitoids tested on both radioinert and radio-labelled residues. Additional methods, not used in this study but outlined in Bull et al. (1987), can be used to quantify the amount of insecticide actually penetrated into a parasitoid over time. This involves an external rinse of the wasp to obtain an outer fraction, subsequently followed by an inner fraction of pesticide extracted by grinding up the insect body as described above.

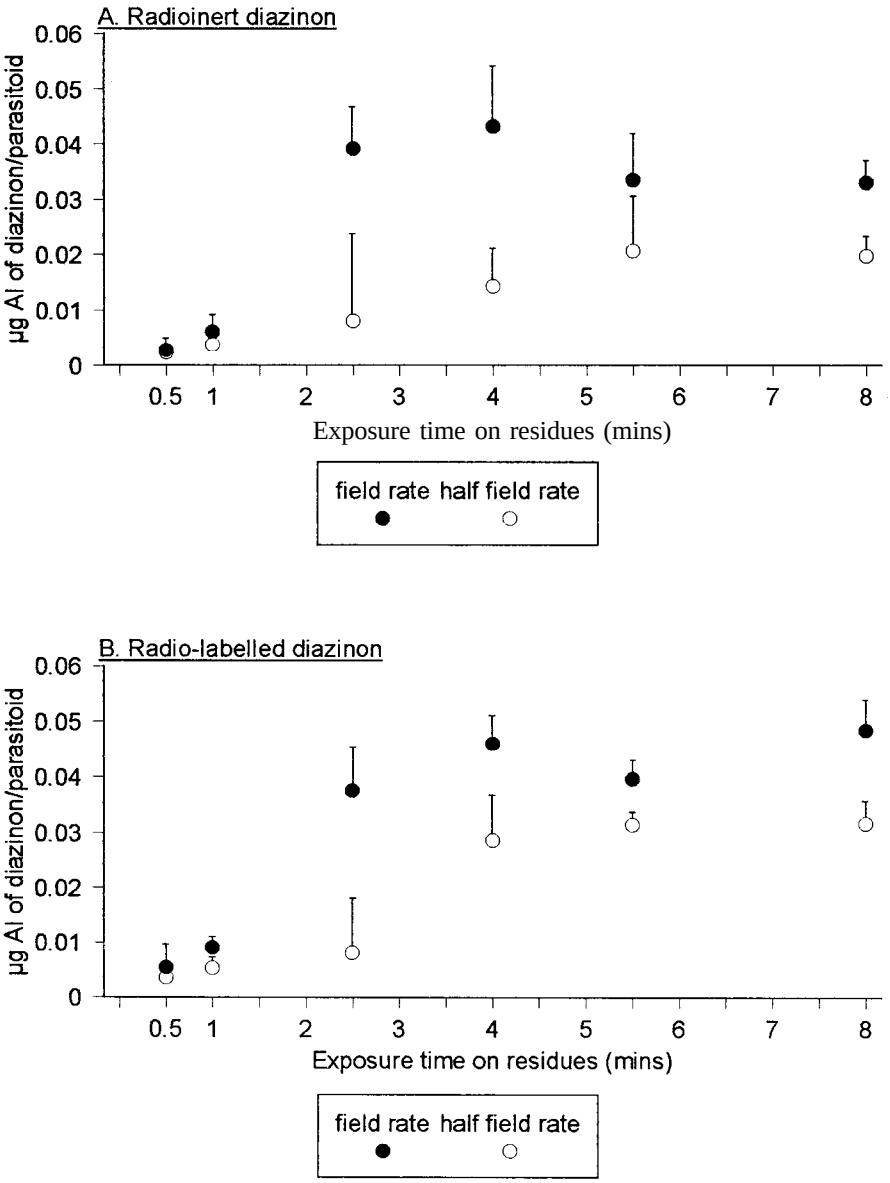

Figure 1. Quantity of insecticide active ingredient (± 95% C.L.) taken up by wasps via exposure for different time periods to residues of field rate and half field rate of (A) radioinert and (B) radio-labelled diazinon.

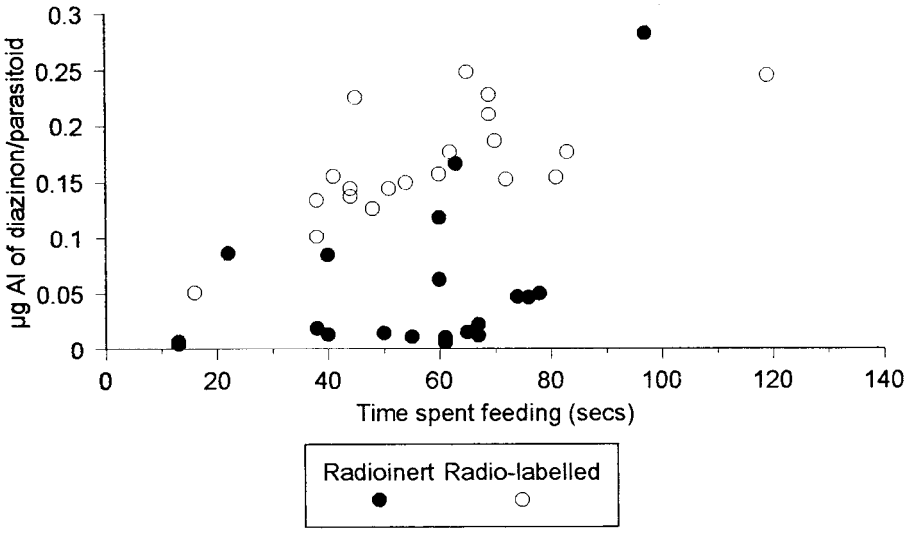

Figure 2. Quantity of insecticide active ingredient ingested by wasps during periods of feeding on honey solutions containing field concentrations of radioinert and radio-labelled diazinon.

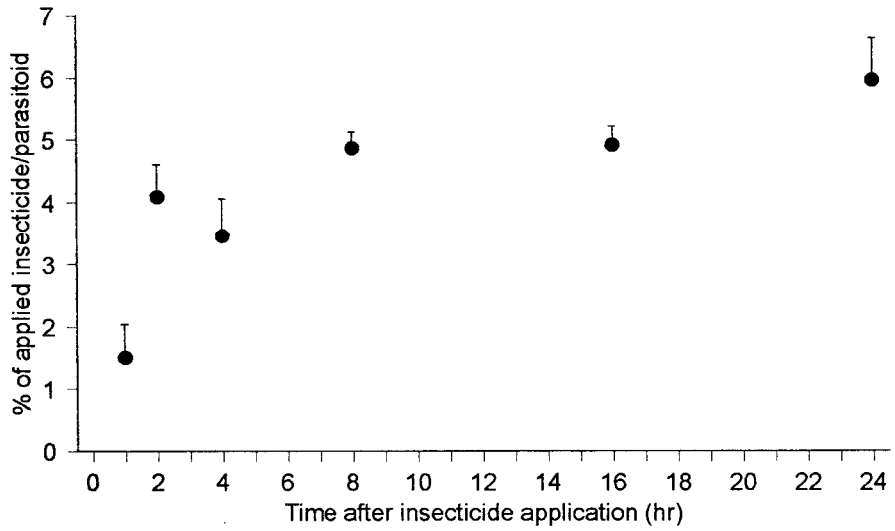

Figure 3. Percentage of total insecticide (± 95 C.L.) applied to mummies (field concentration of radio-labelled diazinon) retrieved from internal parasitoids at intervals after application.

The quantity of insecticide taken up via ingestion was positively correlated with time spent feeding (Figure 2). The results suggest that diazinon showed little or no repellent properties to feeding *A. ervi* and could therefore represent an important route of exposure to aphid parasitoids in the field. A comparison of the two chemical analyses suggests that the use of radio-labelled diazinon samples enabled a greater, or more precise, detection of quantities of ingested active ingredient per unit time of feeding. However, the lower level of diazinon found in the radioinert samples could also be indicative of diazinon metabolism. The isotope study measures radioactive label, irrespective of the structure of the compound, while gas chromatography analysis only measures parent compound.

Diazinon penetration through the mummy case and into the immature parasitoid was dependent on time after insecticide application (Figure 3). The proportion of the total insecticide applied to mummies, which was extracted from parasitoids by chemical analysis, showed a progressive increase over time. Even though percentages remained below 6% throughout the 24 hr post-treatment period, this concentration has been demonstrated to be sufficient to kill 100% of aphid parasitoids before emergence (Hsieh and Allen 1986). The amounts of diazinon detected on mummy cases showed a progressive decrease over time, correlated with the amounts lost through absorption.

The analytical techniques devised in this study have demonstrated that quantitative assessments of the different routes of pesticide exposure to relatively small non-target invertebrates can be made, albeit in a simplified system. Future applications of these techniques could be used, for example, to quantify the effects of pesticide residues on a whole parasitoid-host complex. A quantification of residual exposures to insecticides by primary parasitoids, hyperparasitoids and aphids moving around treated plants could be undertaken in association with behavioural studies to aid in the interpretation of toxicological data. Further work could investigate the reported wide variation in the activity of insecticides from several classes against aphid parasitoids within mummies (e.g. Hsich and Allen 1986; Abo El-Char and El-Sayed 1992). The degree of active ingredient penetration through the mummy case, determined by variables such as octanol/water partition coefficients and vapour pressures (Graham-Bryce 1987) could be quantified, aiding risk predictions in the field and the utilisation of selective insecticide compounds in the future.

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REFERENCES

Abo El-Ghar GES, El-Sayed AM (1992) Long-term effects of insecticides on *Diaeretiella rapae* (M'Intosh) a parasite of the cabbage aphid. Pest Sci 36:109-114

Bull DL, Pryor NW, King EG (1987) Pharmacodynamics of different insecticides in *Microplitis croceipes* (Hymenoptera: Braconidae), a parasite of Lepidopteran larvae. J Econ Entomol 80:739-749

Graham-Bryce IJ (1987) Chemical Methods. In: Burn AJ, Coaker TH, Jepson PC (eds) Integrated Pest Management. Academic Press, pp.113-159

Hartley GS, Graham-Bryce IJ (1980) Physical Principles of Pesticide Behaviour. Academic Press, New York

Hsieh CY, Allen WW (1986) Effects of insecticides on emergence, survival, longevity, and fecundity of the parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) from mummified *Myzus persicae* (Homoptera: Aphididae). J Econ Entomol 79:1599-1602

Jagers op Akkerhuis GAJM, Hamers THM (1992) Substrate-dependent bioavailability of deltamethrin for the epigeal spider *Oedothorax apicatus* (Blackwall) (Aranaea, Erigonidae). Pest Sci 36:59-68

Jepson PC (1988) Ecological characteristics and the susceptibility of non-target invertebrates to long-term pesticide side-effects. In: Field Methods for the Study of the Environmental Effects of Pesticides. BCPC Publications, Thorton Heath, Surrey, UK, pp. 191-200

Jepson PC (1989) The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. In: Jepson PC (ed) Pesticides and Non-target Invertebrates. Intercept Ltd, Andover, Hampshire, UK, pp. 95-128

Potter C (1952) An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomised spray fluids. Ann Appl Biol 39:1-29

Wiles JA, Jepson PC (1993) An index of the intrinsic susceptibility of non-target invertebrates to residual deposits of pesticides. In: Donker MH, Eijsackers H, Heimbach F (eds) Ecotoxicology of Soil Organisms. Lewis Publishers, Chelsea, Michigan, pp. 287-301