ENTOMOPHAGA 35 (1), 1990, 49-56

THE EFFECT OF INSECTICIDES ON THE DISTRIBUTION OF FORACING PARASITOIDS, *DIAERETIELLA RAPAE [HYM. : BRACONIDAE]* ON PLANTS

GU DE JIU ([1]) & J. K. WAAGE ([2])

([1]) South China Agricultural University,
Guangzhou, Peoples Republic of China
([2]) CAB International Institute of Biological Control
Silwood Park, Buckhurst Lane, Ascot, Berks, UK SL5 TA

Foraging aphid parasitoids, *Diaeretiella rapae* M'Intosh, were exposed to sublethal doses of the insecticides pirimicarb, permethrin and malathion on brussells sprouts plants. Observations on wasp distribution over time revealed that wasps spent less time on sprayed plants, relative to controls and, while on these plants, tended to concentrate activity on unsprayed surfaces. For permethrin and malathion, pesticide residues reversed the stereotypic upward foraging pattern of the wasp. Negative consequences of sublethal pesticide doses for parasitoid foraging efficiency are discussed.

KEY-WORDS: Pesticide, parasitoid, foraging behaviour, *Diaretiella rapae,* sublethal effects.

The integration of chemical and biological control is a challenge to pest management, and has generated much interest in the effect of pesticides on natural enemies. Many pesticides are known to be more toxic to natural enemies than to pests (**Croft & Brown,** 1975), and the effect of this differential mortality on the population dynamics of both can be substantial (**Waage** *et al.*, 1986 ; **Waage,** 1989). Most studies on pesticide-natural enemy interactions have concentrated on lethal effects. The fewer studies on sublethal effects concentrate on such life history parameters as longevity, fecundity and searching rate of affected natural enemies (**Croft,** 1977).

This paper examines another possible sublethal effect, that of pesticides on natural enemy foraging behaviour. Recent advances in our understanding of natural enemy foraging, particularly in hymenopterous parasitoids, reveal this process to be complex and to involve responses to several levels of environmental patchiness (**Waage,** 1979 ; **van Alphen & Vet,** 1986). Some parasitoids are known to concentrate foraging time (though not necessarily parasitism) on plants where hosts are abundant (e.g. **Waage,** 1983 ; **Summy** *et al.*, 1985 ; **Smith & Maelzer,** 1986) and to forage on plants in a structured, non-random manner (e.g. **Ayal,** 1987). Because pesticides can affect insect behaviour, because they vary in their distribution on plants, and because they affect the density and distribution of pests within and between plants (**Trumble,** 1985), there is clearly potential for an interaction between pesticide application and parasitoid foraging.

In order to examine the effect of pesticides on natural enemy foraging, a laboratory system was set up to observe the distribution of foraging females of the braconid, *Diaeretiella rapae* M'Intosh on sprayed and unsprayed brussels sprout plants, where they searches for their aphid host, *Brevicoryne brassicae*. This host-parasitoid system was chosen because *D. rapae* is known to exhibit a stereotyped foraging pattern on plants: wasps usually alight on lower leaves, spend a considerable time searching the upper surface of the leaf on which they land, and then move up the stem, investigating successively higher petioles and leaves as they climb. Ayal (1987) has documented this behaviour and argued that it is adaptive to the exploitation of aphid populations in wild brassicas, which usually occur on the flower spikes and produce honey dew which covers the upper surfaces of the lower leaves, where wasps can thereby quickly assess the presence of aphids above.

Three pesticides were used ; malathion, permethrin and pirimicarb, in order to explore a range of both selective and broad-spectrum compounds. Sublethal doses were determined for each, and these were used to study effects on on-plant foraging behaviour.

MATERIALS AND METHODS

D. rapae was reared on the cabbage aphid, *Brevicoryne brassicae*, at 25 ± 1 °C, 68 % RH and an L16 : D8 regime. Aphids were reared on potted seven week old brussels sprouts plants at 20 ± 1 °C. *D. rapae* were standardized for experiments as follows.

Leaves containing mummies of *D. rapae* were collected into butter dished (9.5-10.5 × 4 cm) covered with muslin cloth. A piece of moistened cotton wool with 40 % honey solution was provided as food for emerging wasps. Every day, about 15 newly emerged wasps (approximately equal numbers of each sex) were transferred by aspirator to muslin-covered glass vials (7.5 × 2.5 cm) containing cotton wool soaked with honey solution. These wasps were left for 24 h before experiments to give an adequate period for mating. Wasps used in experiments were therefore 24-48 h old.

DETERMINATION OF AND APPROPRIATE INSECTICIDE DOSE

Preparations of each insecticide were sprayed with a Hudson X-Pert hydraulic sprayer at a pressure of 2.5 kg/cm^2 and flow rate of 1.2 l/min onto grease-proof paper. The nozzle was moved over the paper at a height of 1 m above the target and a standard walking speed of approximately 1 m/sec. Grease-proof paper was used to simulate the waxy leaves of the brussels sprout.

When the spray had dried, the paper was cut into 8.8 × 7.3 cm pieces and rolled into 7.5 × 2.5 glass vials, such that sprayed paper covered the inner surfaces of all but the ends of the vial.

The insecticides used were Pirimicarb ("APHOX" 50 % w/w, from ICI), Permethrin ("AMBUSH", 25 % EC, ICI) and Malathion (60 % w/v, Murphy Chemical Ltd). Agral 90 (ICI) at 0.3 ml/l water was used as a sticker. Tap water was used as a control treatment.

Between 14 and 17 wasps were introduced into each tube, which was covered with muslin to allow ventilation and reduce any fumigant effect. The wasps were left in the tube for 1 h at 25 ± 1 °C and 68 % RH. They were then transferred by aspirator to clean vials with cotton wool soaked in honey solution and left 24 h, after which mortality was recorded.

Five replicates were made for each insecticide at each of a range of concentrations, starting with the recommended field dose and repeated, where necessary, by dilutions of 50 % until a sublethal dose was found.

DISTRIBUTION OF SPRAYED DROPLETS ON BRUSSELS SPROUT PLANTS

To determine the distribution of insecticide on potted plants, a total of 28 white paper cards, 1 × 2 cm, were attached to different parts of a seven-leaved, seven week old brussels sprout plant. One card was placed on the upper and lower surface of each leaf, and on the petiole and stem adjacent.

Plants were sprayed as above with a 1 : 10 solution of Saturn Yellow dye (H. Haeffner & Co.) in water. The sprayed was moved at 1 m/s. at 1 m above the plant. After spraying, the number of droplets per card were counted under an UV lamp. The experiment was replicated with 5 plants.

WASP OBSERVATIONS ON SPRAYED PLANTS

Seven-leaved, seven week old potted brussels sprouts plants were sprayed as above with sublethal concentrations of each insecticide. For the controls, plants were sprayed with tap water. After drying, plants were put in a 45 × 45 × 50 cm cage with muslin sides and top and clear perspex front, at 20 ± 1 °C and 68 % RH.

Prior to each experiment, a standardized wasp was placed for 0.5 h in a 7.5 × 2.5 cm vial containing a leaf was then brought in contact with the upper surface of the 2nd leaf of the caged plant, and the wasp was allowed to walk across. At 5 min intervals over the following hour, the position and activity of the wasp on the plant was recorded. Each record gave the level of the leaf on the plant (1 = bottom leaf, 7 = top leaf) and whether the wasp was on the upper or lower surface, petiole or stem immediately below the leaf. Wasps which had left the plant and landed on the side of the cage were recorded as being "off the plant". This experiment was replicated 10 times (plants and wasps) for each insecticide.

Contingency table tests were used for statistical analysis of all experiments.

RESULTS

Table 1 gives the effect of various concentrations of insecticides on wasps, as determined by one hour exposure to sprayed grease-proof paper in vials. Malathion was found to be far more toxic than pirimicarb or permethrin, the LD_{50}s being, respectively, .004 ml/m^2, .150 g/m^2 and .065 ml/m^2. To use a dose likely to be sublethal in parasitoid foraging experiments on plants, pirimicarb and permethrin were used at the recommended field concentrations of 280 g/ha and 200 ml/ha, respectively, while malathion was used at one fiftieth the recommended concentration of 2.1 l/ha.

In order to determine the distribution of pesticide droplets on a plant, cards from each plant level and position were classified in category 1 (0-20 droplets per card), 2 (21-60 droplets) and 3 (more than 60 droplets). The distribution of cards in different categories did not differ significantly between the 5 replicates ($\chi_8^2 = 3.6$ $p > 0.8$). Considering the plant as possessing 7 levels (i.e. leaves) with each level possessing 4 cards (upper leaf, lower leaf, petiole and adjacent stem), there was no significant difference in the degree of coverage at different levels ($\chi_{12}^2 = 5.4$, $p > 0.9$). This is to be expected as the leaves on such young open plants do not shade each other. The distribution of cards with different numbers of droplets did differ however between plant surfaces ($\chi_6^2 = 150.3$, $p < 01$). Coverage on petioles and stems was relatively low, while coverage of upper leave surfaces was uniformly high, and of lower leaf surfaces uniformly low.

In the analysis of the effects of insecticides on the distribution of foraging wasps two distinct effects were considered : the effect of insecticides on (1) time spent on the plant and

TABLE 1

Toxicity of 3 insecticides to D. rapae

Insecticide	Dose-mortality function*	r	LC50 (as multiple of R)*	Rate applied in experiment
Pirimicarb	y = 2.85 + 9.9R	0.98	5.3R	R
Permethrin	t = 4.1 + 14.2R	0.99	3.2R	R
Malathion	y = 34.7 + 853.9R	0.83	.02R	.02R

* In this function, y represents the percent mortality of *D. rapae*, and R the recommended field rate of pirimicarb (280 g/ha, 50 % w/w formulation), permethrin (200 ml/ha of 25 % EC) and malathion (2.1 l/ha, 60 % w/v formulation). The function is calculated for 4 (pirimicarb) or 5 (permethrin, malathion) doses with 5 replicates per dose (see text).

TABLE 2

Effects of sublethal dose of three insecticides on the distribution of observations of wasps between plant and cage wall

Treatment	Wall	Plant	χ^2 (against control)
Pirimicarb	61	59	66.17*
Permethrin	39	81	32.75*
Malathion	45	75	41.03*
Control	4	116	—

* $p < .01$ with Yates correction.
Overall $\chi^2 = 67.5$ ($p << .01$).

(2) patterns of distribution while on the plant. Table 2 shows the number of observations of wasps, pooled over all replicates and hours, on and off the plants for the different treatments. A significant difference was found between each treatment and the control in the number of observations on and off plants. The percentage of total observations on the plant were 50.8, 32.5 and 37.5 for pirimicarb, permethrin and malathion, respectively, as compared to 96.7 for the control. A significant difference was found between the insecticide treatments in the distribution of time on and off plants ($\chi_2{}^2 = 11.32$, $p < .01$). For wasps on plants, the presence of insecticides had a clear effect on the wasp distribution. The distribution of observations at different levels (= leaves), pooled across replicates is shown in table 3. Wasps in the control treatment spent an average of 40.5 % of on-plant observations above leaf 2 (on which they were released), while this percentage was only 22 %, 17.2 % and 18.6 % for the pirimicarb, permethrin and malathion treatments, respectively. This difference with respect to controls was significant for permethrin and malathion, but not pirimicarb (observations on levels 5, 6, 7 pooled for analysis). The overall pattern suggests that the insecticides permethrin and malathion caused wasps not to move up the plant during foraging, and even to move down the plant, relative to controls.

Table 4 shows the effects of insecticides on the time spent on different plant surfaces. A significant difference was found between insecticide treatments and control for permethrin and malathion, but not for pirimicarb. With respect to upper and lower leaf surfaces, wasps spent a greater proportion of observations on lower leaf surfaces for pirimicarb (62.5 %) and malathion (80 %), and about the same proportion for permethrin (48.8 %), in comparison to the control (41.6 %).

TABLE 3

Effects of sublethal doses of three insecticides on the distribution of wasp observations at different levels on the plant (wasps placed on the upper surface of the second leaf). Observations on levels 5, 6 and 7 were pooled for analysis.

Treatment	Level (leaf number)							χ^2 against control
	1	2	3	4	5	6		
Pirimicarb	8	38	3	4	2	1	3	0.12 NS
Permethrin	36	31	6	3	3	2	0	40.82*
Malathion	31	30	6	4	2	2	0	35.16*
Control	8	61	16	13	11	2	5	

* $p << .01$.
Overall $\chi^2 = 59.5$ ($p << .01$).

TABLE 4

Effects of sublethal doses of three insecticides on the distribution of observations of wasps between various plant surfaces

Treatment	Section of plant				
	Upper surface	Lower surface	Petiole	Stem	χ^2 (against control)
Pirimicarb	15	25	12	7	6.53*
Permethrin	22	21	17	21	13.36**
Malathion	5	20	16	30	43.25**
Control	52	37	15	12	—

* $p < .08$.
** $p < .01$.
Overall $\chi^2 = 52.9$ ($p << .01$).

Finally, table 5 shows the number of on-plant observations spent walking, cleaning and resting for the different treatments. The percentage of observations during which wasps were walking was 70 %, 73.5 % and 65.8 % for pirimicarb, permethrin and malathion, respectively, but only 58.3 % for the control. Wasps spent less time resting in insecticide treatments, but this was significant with respect to controls only for permethrin. With at least permethrin, therefore, sublethal doses of insecticides appear to have an excitatory effect on wasps causing them to spend less time resting and cleaning.

DISCUSSION

The aim of this study was to understand wasp behaviour on sprayed plants. The experiments measured positions of wasps on sprayed plants at successive intervals, not foraging behaviour *per se*. However, such measurement does provide an approximation of foraging behaviour and has become a useful method for estimating parasitoid movements on complex surfaces (**Waage**, 1983 ; **Summy** *et al.*, 1985 ; **Smith & Maelzer**, 1986). *D. rapae* moves fairly slowly on plants (**Ayal**, 1987), and observations at 5 min intervals are likely to capture major changes of position.

TABLE 5

Effects of sublethal dose of three insecticides on the number of observations of wasps on plants engaged in different activities

Treatment	Activity of wasps		
	Walking	Non-walking (cleaning + resting)	χ^2 (against control)
Pirimicarb	42	18 (17 + 1)	$\chi^2 = 1.84$ NS
Permethrin	61	22 (21 + 1)	$\chi^2 = 4.25$*
Malathion	48	25 (23 + 2)	$\chi^2 = 0.76$ NS
Control	67	48 (37 + 11)	—

* $p < .05$ with Yates correction.
Overall $\chi^2 = 5.56$ (pc.13).

In this study, it has been shown that all 3 insecticides tested increase the probability of wasps leaving and/or not returning to a plant. Besides this repellent effect, permethrin caused wasps remaining on plants to spend a greater proportion of their time walking, hence, it appeared to act as a locomotory stimulant.

A repellent effect has been shown for the parasitoid *Encarsia formosa* responding to sublethal doses of various insecticides on real and artificial leaves (**Irving & Wyatt,** 1973; **Perera,** 1982). However, wasps in these experiments were not foraging naturally on plants, and it is not clear that abandonment of search would mean abandonment of the plant under natural conditions or simply movement to other plant parts. **G. Elzen** (pers. comm.) has shown that cotton plants sprayed with certain insecticides are less attractive to foraging *Microplitis croceipes* than unsprayed plants and the same may be true for *D. rapae* (**Wheehan,** pers comm.). Hence it may be that wasps in the present experiment, having left sprayed plants, were less inclined to return due to a reduced attractiveness. The tendency of *D. rapae* which remain on plants to search more on the underside of leaves (which received less pesticide) in permethrin and malathion treatments also indicates a repellent or locomotory stimulant effect which causes wasps to concentrate activity on unsprayed surfaces.

The most striking effect of pesticides in the present study is their interference with the normal upwards foraging pattern of *D. rapae* on plants, demonstrated in table 3 for permethrin and malathion. Studies of droplet distribution showed it to be uniform at all levels, eliminating the possibility that this change in behaviour merely reflects a repellent response to higher pesticide concentrations at higher levels in the plant. Rather, it appears that encounter with pesticides alone is causing the parasitoid to change its sterotyped strategy for searching plants. No doubt this effect would be enhanced on mature plants in the field by the tendency of deposits to be concentrated at the top of plants.

Much variability was found between the effects of different insecticides and no clear evidence emerges from this study that pesticides less toxic to *D. rapae* also exhibit less severe sublethal effects on foraging. Thus, for instance, while pirimicarb does not appear to affect the upward movement pattern of foraging on plants (table 3), it has a strong effect on the tendency to remain off the plant (table 2) and on unsprayed surfaces while on the plant (table 4).

In order to identify unambiguously the effects of pesticides on foraging, this study has not used plants infested with aphid hosts. On sprayed, infested plants, it is likely that arrestant stimuli associated with honeydew (**Ayal**, 1987) and perhaps oviposition would compete with stimulant effects of pesticides shown here. **Perera** (1982) has shown for *Encarsia formosa* that deterrent effects of a pyrethroid were overcome when host density was sufficiently high. However, in sprayed crops where post-spray pest populations may be low relative to the amount of arrestant stimulus present from pre-spray populations, the overall effect is likely to be one of even greater decrease in parasitoid searching efficiency, as parasitoids continue to be arrested by kairomones on plants where few or no live hosts live.

Finally, add to these effects the possibly negative effects on parasitoid foraging strategies of the lower density and changed spatial distribution of the post-spray pest population and the potential impact of sublethal pesticide doses on parasitoid foraging efficiency begins to emerge. The present study has only scratched the surface of this complex and challenging problem.

RÉSUMÉ

Effet des insecticides sur la répartition des parasitoïdes, recherchant leur nourriture *Diaeretiella rapae* [*Hym.* : *Braconidae*] sur les plantes

Les parasitoïdes aphidiphages, *Diaeretiella rapae* M'Intosh, ont été exposés à des doses subléthales des insecticides : pirimicarb, permethrin et malathion sur des plants de Choux de Bruxelles. Les observations sur la répartition des hyménoptères en fonction du temps révèlent qu'ils passent moins de temps sur les plantes traitées que sur les témoins et que sur les premières, ils tendent à concentrer leur activité sur les surfaces non traitées. Pour la permetrine et le malathion, les résidus des produits inversent le modèle stéréotypé de recherche de la nourriture chez l'hyménoptère. Les conséquences négatives des doses subléthales de pesticides pour l'efficacité de recherche du parasitoïde sont discutées.

MOTS CLÉS : Pesticide, parasitoïde, comportement de recherche de la nourriture, *Diaeretiella rapae*, effets subléthaux.

Received : 13 July 1988 ; Accepted : 14 November 1988.

REFERENCES

Van Alpen, J. J. M. & Vet, L. E. M. — 1986. An Evolutionary Approach to Host Finding and Selection. In : Insect Parasitoids (**J. Waage & D. Greathead**, eds), 23-61.

Ayal, Y. — 1987. The Foraging Strategy of *Diaeretiella rapae* I. The concept of the elementary unit of foraging. — *J. of Anim. Ecol.*, 56, 1057-1068.

Croft, B. A. — 1977. Susceptibility surveillance to pesticides among arthropod natural enemies : models of uptake and basic responses. — *Z. Pflanzenkr. Pflanzenschutz*, 80, 140-157.

Croft, B. A. & Brown A. W. A. — 1975. Responses of arthropod natural enemies to insecticides. — *Annu. Rev. Entomol.*, 20, 285-336.

Delorme, R. — 1976. Evaluation en laboratoire de la toxicite pour *Diaeretiella rapae [Hym. : Aphidiidae]* des pesticides utilisés en traitement des parties aériennes des plantes. — *Entomophaga*, 21, 19-29.

Irving, S. N. & Wyatt, I. J. — 1973. Effects of sublethal doses on the oviposition behaviour of *Encarsia formosa*. — *Ann. Appl. Biol.*, 75, 57-62.

Perera, P. A. C. R. — 1982. Some effects of insecticide deposit patterns on the parasitism of *Trialeurodes vaporariorum* by *Encarsia formosa*. — *Ann. Appl. Biol.*, 101, 239-244.

Smith S. D. & Maelzer, D. A. — 1986. Aggregation of parasitoids and density-independence of parasitism in field populations of the wasp *Aphytis melinus* and its host, the red scale *Aonidiella aurantii*. — *Ecol. Entomol.*, 11, 425-434.

Summy, K. R., Gilstrap, F. E. & Hart, W. G. — 1985. *Aleurocanthus woglumi [Hom. : Aleyrodidae]* and *Encarsia poulenta [Hym. : Encyrtidae]* : density-dependent relationship between adult parasite aggregation and mortality of the host. — *Entomophaga*, 30, 107-112.

Trumble, J. T. — 1985. Implications of changes in arthropod distribution following chemical application. — *Res. Popul. Ecol.*, 27, 277-285.

Waage, J. K. — 1979. Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescens*. — *J. Anim. Ecol.*, 48, 353-371.

Waage, J. K. — 1983. Aggregation in field parasitoid populations : foraging time allocation by a population of *Diadegma [Hymenoptera, Icheumonidae]*. — *Ecol. Entomol.*, 8, 447-453.

Waage, J. K., Hassell, M. P. & Godfray, H. C. J. — 1986. The Dynamics of Pest-Parasitoid-Insecticide Interactions. — *J. Appl. Ecol.*, 22, 825-838.

Waage, J. K. — In press. The population ecology of pest, pesticide, natural enemy interactions. In : Pesticides and Non-Target Invertebrates (**P. Jepson**, ed.). — *Intercept*, Winborne, Dorset, UK.