DEVELOPMENT OF A SEMI-FIELD METHOD FOR TESTING THE SIDE-EFFECTS OF PESTICIDES ON THE HOVERFLY *EPISYRPHUS BALTEATUS* (DE GEER)

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SUMMARY

A semi-field method was developed to evaluate the effects of pesticides on the hoverfly *Episyrphus balteatus* (Diptera, Syrphidae). The effects of deltamethrin (DECIS EC 2,5) and phosalon (ZOLONE FLO) were tested on 2-3 day-old larvae, on young potato and broad bean plants. Trials were carried out indoors at 20°C and outdoors under cover.
The analytical quantification of pesticide residues using chromatographic analysis was an original component of this work which required the collaboration of biologists and chemists. The results suggest that in these experimental conditions the larvae are exposed to less residue than in laboratory trials with larvae confined in Petri dishes or other small arenas.

INTRODUCTION

The hoverfly *Episyrphus balteatus* (Diptera, Syrphidae) is one of the most important natural enemies of aphids in many crops in Europe. In terms of numerical abundance, this species is the commonest syrphid found in cereals in Central Europe (KRÖBER & CARL, 1991).
As a consequence of this major role in the agrocenose, *E. balteatus* has been selected as a recommended species for testing the effects of pesticides on beneficial arthropods for regulatory purposes (BARRETT *et al.*, 1994). The working group "Pesticides and Beneficial Organisms" of the International Organization for Biological Control (IOBC), West Palearctic Regional Section (WPRS) developed standard laboratory methods to test the side-effects of pesticides on important natural enemies, including syrphid species (RIECKMANN, 1988) and other foliage dwelling predators like lacewings (BIGLER, 1988) and ladybirds (PINSDORF, 1989). However few methods are available to evaluate the side-effects of pesticides on non-target leaf dwelling insects in semi-field and field conditions. BIGLER & WALDBURGER (1988) proposed a semi-field method for larvae of the green lacewing *Chrysoperla carnea* and more recently, a procedure was developed for the ladybird *Coccinella septempunctata* (SCHMUCK *et al.*, 1997). For syrphids, TORNIER & DRESCHER (1992) proposed a semi-field testing method for the evaluation of the effects of pesticides on the adults of the hoverfly *E. balteatus* but no specific method under semi-field conditions has been developed for larval stages of this species. Within this context, we studied the effects of deltamethrin (DECIS EC 2,5) and phosalon (ZOLONE FLO) on 2-3 day-old *E. balteatus* larvae. Both pesticides

were applied on young potato and broad bean plants. Trials were carried out either indoors in controlled conditions at 20°C or outdoors under cover.

MATERIAL AND METHODS

Plant production

To obtain plants of regular sizes, the following procedure was carried out :
Potato (var. Kennebec) and broad bean (var. Tic) seeds were sown individually in 9 cm diameter mould pots and placed in a growth room at 20°C or outdoors under cover.
Approximately one week after emergence, the broad bean seedlings were pinched out so as to retain only the two first leaflets on the stem. For potatoes, one stem per pot was kept and cut above the third compound leave. All the lateral leaflets were removed to retain only the terminal ones.

Insect production

2-3 day-old larvae of *E. balteatus* were produced from eggs collected on broad bean plants infested with *Aphis fabae* and placed in a mating cage for 24 hours. Young larvae were fed on *Acyrthosiphon pisum* reared on broad bean. All the production was performed at 20°C.

Pesticide spraying

Potato or broad bean plants were sprayed with an electronic sprayer at rates of 5 g deltamethrin/ha and 750 g phosalon/ha in water at 200 l/ha.
Before each use, the sprayer was calibrated to ensure a good reproducibility of the spraying with a percentage standard deviation of less than 10%.
Preliminary trials with phosalon were carried out to study the distribution of the pesticide residues on the upper and lower leaf surfaces and on the stem of sprayed plants.

Bioassay description

After the treated plants had dried, two young larvae were placed on the stem of each plant. Ten treated plants and ten control plants were prepared for each treatment. Pea aphids (*Acyrthosiphon pisum*) were added every day on seedlings to provide a food source for the larvae. Pots were placed in a plastic tray inside a test cage. The cages were 61×42 and 42 cm high, with wire-mesh lateral sides. The top was of glass and the front and back closed with nylon gauze. For the potato trials, each cage contained five plants. For broad beans, all the treated seedlings were placed in one cage and the untreated ones in another. A thin water film was maintained in each tray to prevent the escape of larvae. Test cages were placed either in a controlled environment at 20°C or outdoors under cover.

Every twenty-four hours, dead and living larvae were counted and their position was noted. The mortality observed was corrected for control mortality using the equation of ABBOTT (1925).
The trials were stopped as soon as all larvae on treated plants had died or after a maximum of ten days. When pupation occurred, pupae were kept until the adults emerged and their sex was determined.
At the end of the trials, plants were collected and pesticide residues quantified by chromatographic analysis ((GC)2-ECD for deltamethrin and HPLC-UV for phosalon).

RESULTS

Preliminary trials

Preliminary tests with phosalon showed that more than 90 % of insecticide residues were distributed on the upper surfaces of potato leaflets (Table 1). Only 2,2% and 4,5% of the residues were found on the undersides of the leaflets and on the stem respectively. In the case of broad bean, about 80% of the residues were present on the upper surfaces, the rest of the deposit was found under the leaflets and on the stem in equal parts.

Table 1 : Distribution of phosalon residues on potato and broad bean plants. Percentages of total deposit found on the different parts of 15 plants. (Percentage standard deviation in parentheses).

Plant	Upper leaf surface	Lower leaf surface	Stem
Potato	93,9% (1,5%)	2,2% (50,6%)	4,5% (22,8%)
Broad bean	83,5% (6,5%)	8,2% (42,1%)	8,3% (42,9%)

Bioassays with *E. balteatus*

The results of the toxicity bioassays are summarised in table 2.

Table 2 : Cumulative corrected mortalities obtained with *Episyrphus balteatus* on potato and broad bean plants using indoor or outdoor trials. Pesticide residues found on upper leaf surfaces (ULF), lower leaf surfaces (LLF) and stems (S) of test plants at the end of trials.

Test plants	active sub-stances	type of trials	average corrected mortalities on days (1-10) (%)										pesticide residues (µg/cm²)		
			D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	ULF	LLF	S
Potato	Deltamethrin	Indoor	35	50	69	94	100						0.129	0.003	0.007
		Outdoor	3	25	32	36	25	46	70	80	79	85	0.068	0.002	0.006
	Phosalon	Indoor	63	68	75	88	94	94	93	92			11.46	0.27	0.78
		Outdoor	34	54	89	100							10.63	0.25	0.82
Broad bean	Deltamethrin	Indoor	X	39	56	63	69	73	73	85	85	85	0.027	0.003	0.006
		Outdoor	25	41	47	45	45	48	55	55	65	80	0.032	0.003	0.004
	Phosalon	Indoor	20	42	59	60	60	54	46				4.29	0.42	0.65
		Outdoor	16	32	47	50	94	X	94	94	94		6.18	0.61	1.27

X: no observation

Effects of deltamethrin applied on potato

In controlled conditions, all the larvae were dead five days after spraying and the relationship between mortality and time was a straight line. In the outdoor trial, 30% of larvae were still alive after one week and the mortality slope was shallower. The surviving larvae pupated and produced normal adults.

Effects of deltamethrin applied on broad bean

Larvae on treated broad bean plants also died slightly sooner in controlled conditions than on outdoor plants. Corrected mortalities on day 5 were about 70 and 50% respectively. The indoor trials show clearly that mortality occurred faster on potato than on broad bean.

Effects of phosalon applied on potato

The toxic effects of phosalon were a little faster in controlled conditions than outside but 5 days after treatment 5% of larvae from the inside trial and none from the outside trial survived. It seems that mortality occurs mainly during the first few days after exposure and most larvae which were still alive after four days pupated normally.

Effects of phosalon applied on broad bean

In controlled conditions, phosalon was moderately toxic for *E. balteatus* with only 60% corrected mortality after 5 days. Variable results were obtained outside under cover with 95% of dead larvae after 3 days in one trial and less than 50% by day 5 in a second trial. Perhaps variations in weather conditions, especially temperature inside the cages, explain such results.

Cumulative mortality in controls

No significant differences occurred between the cumulative mortality on untreated broad bean seedlings and that on potatoes. Further, there were no significant differences between the results in indoor or outdoor conditions. About 96%, 92% and 85% of larvae were alive respectively after 24, 48 and 72 hours. After five days, the average mortality in controls was slightly greater than 20%.

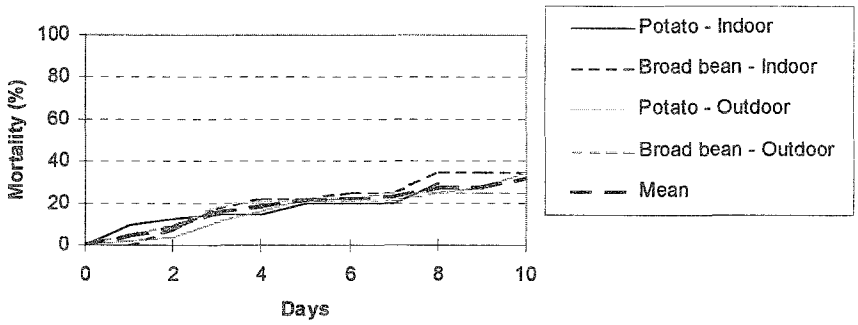

Figure 1 : Cumulative mortality in controls (*Episyrphus balteatus*)

DISCUSSION

It appears from these initial results that this semi-field method using young potato or broad bean plants, exposes syrphid larvae to less residue than laboratory trials with larvae confined in Petri dishes or other small arenas. For a similar residue level per unit area, the mortality after 24 or 48 hours was less than that we obtained on glass or detached leaves in previous work (MAHAUT & DELEU, 1997).

Tests with potatoes gave slightly higher mortalities than those with broad bean seedlings. One explanation could be found in the higher insecticide residue level on the upper leaf surfaces of potato plants than of broad beans. It seems also that syrphid larvae need to move a little more on potato plants to find pea aphids, which tend to disperse on the non-compatible host. On broad bean seedlings, the aphids occupy the underside of the leaflets and young syrphid larvae probably find their food more easily.

Results obtained with untreated plants showed that the mortality level in the controls remained acceptable during a minimum of five days.

Further work is needed to improve or validate this method but this preliminary work confirms that results obtained in a controlled environment can be rather different from those of outdoor trials where weather conditions, especially temperature, are variable.

ACKNOWLEDGEMENTS

This work was sponsored by the Research and Development Department (DG6) of the Ministry of Middle Classes and Agriculture, Brussels, Belgium.

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