

## SIDE EFFECTS OF INSECTICIDES ON LARVAE OF THE APHID SPECIFIC PREDATOR *EPISYRPHUS BALTEATUS* (DEGEER) (DIPT. SYRPHIDAE) IN THE LABORATORY

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### ABSTRACT

The effects of 9 insecticides on larvae of the hoverfly *Episyrphus balteatus* were investigated in the laboratory. The compounds examined were all registered to control cereal aphids in summer. They were tested in concentrations equivalent to the maximum field rate recommended for use in Belgium. 2-3 day old hoverfly larvae were exposed until pupation to insecticide residues freshly applied to glass petri dishes. The reproductive performance of the adults obtained from these larvae was later evaluated in a fertility test. Results were related to a water-treated control and the reduction in beneficial capacity was calculated for each compound, in order to classify them according to the scheme approved by the IOBC Working Group "Pesticides and Beneficial Organisms".

Cyfluthrin, cypermethrin, phosalone and pirimicarb caused 100.0 % larval mortality. Only two larvae reached the pupal growth stage with deltamethrin and the adults failed to emerge. A corrected mortality of 78.1 % and a mean production of 52.7 eggs/females were obtained with  $\lambda$ -cyhalothrin but the eggs did not hatch. The reduction in beneficial capacity of these six compounds was equal to 100.0 % and they were rated 'harmful' (category 4) for larvae of *E. balteatus*. Bifenthrin, esfenvalerate and fluvalinate gave much lower mortality rates. Nevertheless the first two pyrethroids reduced the fertility of females and were classified as 'slightly harmful' (category 2). The reproductive performance of adults was not affected by fluvalinate (category 1).

From these results it was concluded that fluvalinate had no effect on the aphid specific predator *E. balteatus* and is suitable for use in Integrated Pest Management of aphids in cereals. Bifenthrin and esfenvalerate also had little effect in our laboratory tests. Such tests can be considered as a worst case study, and it seems likely that the impact of these compounds on hoverflies larvae in the field will be limited. Cyfluthrin, cypermethrin, deltamethrin,  $\lambda$ -cyhalothrin, phosalone and pirimicarb were toxic in the laboratory and must be tested in semi-field or field situations for an adequate assessment of their side effects on hoverflies.

### INTRODUCTION

Hoverfly larvae of the subfamily Syrphinae are effective aphid specific predators. Several authors have noted their importance in the limitation of cereal aphid populations in Western Europe and they are considered, with entomopathogenic fungi and parasitic hymenoptera as a major natural enemy of aphids in wheat in summer (Rautapää, 1976, Dedryver and al., 1985, Chambers and Adams, 1986, Chambers, 1987, Ferran and al., 1987, Latteur and Oger, 1987, Poehling and Borgemeister, 1989, Latteur and Oger, 1991, Hasken and Poehling, 1994, Tenhumberg and Poehling, 1994, Tenhumberg, 1995, Tenhumberg and Poehling, 1995). Unfortunately, like many beneficial arthropods, syrphid larvae may be affected by insecticide treatments, especially those applied to control aphid populations. As a consequence of the elimination of aphid natural enemies or the limitation of their action by non-selective insecticides, aphids are able to rapidly recolonize treated fields. Thus, there is a need to select and to use insecticides that are compatible with their natural enemies.

Data on the toxicity of aphicides registered for use in Belgium in wheat on syrphid larvae are limited. Results of the Joint Pesticides Testing Programmes of the IOBC working group "Pesticides and Beneficial Organisms" showed that phosalone and pirimicarb were moderately harmful and deltamethrin harmful for larvae of *Syrphus vitripennis* Meigen in laboratory trials (Hassan and al., 1987). Gräpel (1982) found pirimicarb toxic for larvae of the hoverfly *Metasyrphus corollae* (F.). Pirimicarb has also been tested in the field where it was toxic for syrphids at doses of 100g and 50g a.s./ha. A certain degree of tolerance appeared only at the lower tested dose of 25g a.s./ha. (Niehoff and Poehling, 1995).

The toxicity of other compounds used in wheat in Belgium to control cereal aphids (bifenthrin, cyfluthrin, l-cyhalothrin, cypermethrin, esfenvalerate, and fluvalinate) was not known. The object of this work was to determine and to compare the toxicity of these insecticides on larvae of *E. balteatus* in the laboratory. This species was selected because it is considered as the commonest one in cereal fields (Dean, 1974, Latteur, 1976, Dedryver and *al.*, Poehling, 1988, Poehling and Borgemeister, 1989, Tenhumberg, 1992, Tenhumberg and Poehling, 1995). The methods that have been used were based on methodology developed by Rieckmann (1988) for *M. corollae* and adapted for *E. balteatus*.

## MATERIAL AND METHODS

### Mass rearing of *E. balteatus*

Adults of *E. balteatus* were kept in woodframe cages (85x60x60cm) with three fine metallic mesh sides. The top and the front were made of perspex. An opening (40x60cm) covered with nylon gauze was made in the front door to allow the introduction and the removal of materials and manipulations in the cages. The cages were placed in a controlled environment chamber ( $20\pm 2^{\circ}\text{C}$ ,  $70\pm 20\%$  HR) under neon light providing about 10.000 lux with a 16 h light / 8 h dark photoperiod.

Adults of hoverflies were continuously fed with cubes of crystallized sugar and freshly ground pollen collected by honeybees. The pollen was kept at  $4^{\circ}\text{C}$  for periods when honeybees were not active. Water was offered *ad libitum* on pieces of cotton wool. Before oviposition (preoviposition period), flowers (*Fagopyrum esculentum*, *Phacelium tanacetifolium*, *Cosmos* sp.) were offered to young hoverflies to complement the sugar and pollen and to increase the flight activity. This was particularly important since mating of *E. balteatus* occurs only during flight (Tornier and Drescher, 1992). The flowers were cultured in glasshouses, untreated with pesticides.

After 8-10 days, flowers were removed and replaced by 10-15 cm high plants of broad bean (*Vicia fabae* L.) infested by black bean aphids (*Aphis fabae* Scopoli) for egg-laying (oviposition period). These plants were periodically replaced by new ones according to the needs in syrphid larvae. For the test, broad bean plants were put in the oviposition cage for 24 hours to obtain larvae of the same age. The plants bearing syrphid eggs and aphids were cut and put in plastic trays (18x12x12cm) closed with nylon gauze. The newly hatched larvae were first fed with *A. fabae* and 2-3 days later transferred in clean plastic trays (30 to 40 young larvae per cage) and fed with green pea aphids (*Acyrtosiphon pisum* (Harris)) offered on cut broad bean plants. Cages and food were changed three times a week. When they appeared, pupae were removed from the trays and put in petri dishes lined with moistened filter paper to facilitate hatching. Eggs, larvae and pupae were kept in a controlled environment chamber ( $20\pm 2^{\circ}\text{C}$ ,  $70\pm 20\%$  HR).

### Performance of the test

According to the IOBC standard for the evaluation of the side effects of pesticides on beneficial arthropods in the laboratory, tests with syrphids were divided in two parts. First, young larvae were exposed to pesticides applied in glass petri dishes and kept there until they pupated. Afterwards, the reproductive performance of the young adults emerging from these pupae was assessed.

The insecticides that have been tested in this study are registered in Belgium to control cereal aphid populations in summer. Commercial formulations have been used. All the insecticides have been tested at a single dose, corresponding to their maximum recommended field rate. The formulations, active ingredients and doses tested are listed in table 1.

**Table 1** : List of the chemicals tested on larvae of *E. balteatus*: trade name of products, active substance and recommended field dose (a.i./ha).

trade name	active substance	recommended field dose
BAYTHROID EC050	cyfluthrin	15 g
CYMBUSH DG	cypermethrin	20 g
DECIS EC25	deltamethrin	5 g
KARATE 25	$\lambda$ -cyhalothrin	5 g
MAVRIK 2F	fluvalinate	36 g
PIRIMOR G	pirimicarb	125 g
SUMI ALPHA	esfenvalerate	5 g
TALSTAR FLO	bifenthrin	7.5 g
ZOLONE FLO	phosalone	750 g

### Exposure of larvae to pesticides

Glass petri dishes ( $\varnothing$  100 mm, h 15 mm) served as the exposure unit for syrphid larvae. Pesticides were applied to both dishes and lids with a Burgerjon spray tower (Burgerjon, 1956). Control dishes were sprayed with water. The apparatus was calibrated to deliver a homogeneous deposit of  $2 \pm 0.2 \mu\text{l}/\text{cm}^2$ , corresponding to a field application of  $200 \pm 20 \text{ l/ha}$ . Each lid was pierced with a hole ( $\varnothing$ : 5 mm) used to connect the petri dishes to a peristaltic pump. Air was sucked from the dishes to avoid the accumulation of pesticide fumes. The pump was regulated to renew the air of the dishes 1 to 2x per minute.

Two hours after pesticide application, the petri dishes were assembled and connected to the pump. Five 2-3 day old larvae were put in each dish and fed with *A. fabae* and *A. pisum* offered ad libitum. For each tested product and for the water control, five dishes were assembled. Exuviae and dead aphids were cleaned from the dishes with brushes each day. Further aphids were added twice a day. Larval mortality was checked daily until pupation and dead larvae were removed. The pupae were kept in the petri dishes until adult emergence. Pupae that did not emerge within 15 days were considered as dead. Observed preimaginal mortalities were obtained by the addition of pupal and larval mortalities. Preimaginal mortalities were corrected using the value of the corresponding control mortalities, according to the Abbott formula (1925).

### Fertility assessment of adults

Young adults that emerged from pupae were released in woodframe cages similar to those previously described for mass rearing. The sex of any hoverflies and the date of emergence were noted. All the adults from the five petri dishes treated with the same product were released in the same cage. Their subsequent treatment was divided into the preoviposition period and oviposition period. During the preoviposition period, they were fed in a similar way as described for mass rearing. An equal quantity of flowers of the same species was put into each cage. Ten days after the mean emergence date of the adults, the flowers were removed and the oviposition period was initiated by the introduction of three pots containing four to five 10-15cm high broad beans plants infested by *A. fabae*. These plants were replaced by new ones three times a week until no eggs were found. The number of eggs laid on each plant was counted. If the number of eggs for the three pots was higher than 1000, the number of broad bean plants was increased.

Three samples of 25-30 eggs per oviposition cage were put into plastic petri dishes with aphids for the assessment of the hatching rate. The number of young larvae in each petri dish was counted daily and new aphids were added to avoid egg cannibalism. The hatching rate was used to calculate the number of viable eggs produced per female at each count. The mean total number of viable eggs produced per female was thus calculated in each oviposition cage and the R ratio determined (viable eggs/female in the treated group divided by viable eggs/female in the control group).

The fertility assessment was made in a controlled environment chamber, under the same conditions of temperature, humidity and lighting as describe for the mass rearing. Under these conditions, the oviposition period generally lasted 30 to 40 days.

#### Calculation of reduction of beneficial capacity

The reduction in beneficial capacity (E) of larvae of hoverflies exposed to an insecticide was assessed by the formula of Overmeer and Van Zon (1982):

$$E = 100\% - (100\% - M) \times R$$

Where M = corrected larval + pupae mortality and R = reproductive ratio.

According to the E value, insecticides were classified in the four IOBC categories for laboratory trials (Hassan, 1992): class 1, "harmless",  $E < 30\%$ ; class 2, "slightly harmful",  $30\% \leq E < 80\%$ ; class 3, "moderately harmful",  $80\% \leq E \leq 99\%$ ; class 4, "harmful",  $E > 99\%$ .

#### RESULTS

The results of the experiments (preimaginal mortality, fertility assessment and reduction in beneficial capacity) are listed in table 2. The evolution of larval mortality during the exposure phase is given in figure 1.

All syrphid larvae exposed to glass petri dishes treated with cyfluthrin, pirimicarb, phosalone and cypermethrin were killed. For the first three compounds, 100.0% toxicity was observed after only 2 to 3 days of exposure, indicating that these insecticides were highly toxic for *E. balteatus* on glass plates. Effects of cypermethrin were slower and about 40% of larvae survived after 7 days of exposure. However, they were unable to feed and finally died after 9 days. In the same set of treatments, control larvae reached the pupal stage 5 to 7 days after treatment. In glass petri dishes treated with deltamethrin, two larvae out of 25 pupated but no adults emerged. Corrected mortality of hoverflies exposed to  $\lambda$ -cyhalothrin reached 78.1% and adults emerged normally from the pupae that developed. However, few eggs were laid and they did not hatch. Thus, the reduction in the beneficial capacity of *E. balteatus* exposed to  $\lambda$ -cyhalothrin reached 100.0%. According to the testing scheme developed by the working group "Pesticides and Beneficial Organisms" of the IOBC, cyfluthrin,  $\lambda$ -cyhalothrin, cypermethrin, deltamethrin, phosalone and pirimicarb were classified as "harmful" (class 4) for larvae of *E. balteatus* on glass plates.

**Table 2:** Toxicity of insecticides on the syrphid *E. balteatus*. Mortality of larvae placed in glass petri dishes treated with insecticides, assessment of the fertility of adults obtained from the surviving larvae, reduction of beneficial capacity and classification according the IOBC standard for testing side-effects of pesticides in laboratory. (NA = Not assessed)

active ingredient	exposure phase			fertility assessment			final evaluation	
	larval mortality (%)	pupae mortality (%)	corrected mortality (M) (%)	eggs/female	hatching rate (%)	viable eggs/female	red. beneficial capacity (E) (%)	IOBC classification
<b>set 1</b>								
control	4.3	4.3	-	253.9	45.8	116.9	-	-
bifenthrin	20	4	16.8	160.5	28.3	45.4	67.7	2
cypermethrin	100	-	100	NA	NA	NA	100	4
$\lambda$ -cyhalothrin	66.6	12	78.1	52.7	0	0.0	100	4
<b>set 2</b>								
control	4	4	-	NA	NA	NA	-	-
cyfluthrin	100	-	100	NA	NA	NA	100	4
deltamethrin	92	8	100	NA	NA	NA	100	4
phosalone	100	-	100	NA	NA	NA	100	4
<b>set 3</b>								
control	4	16	-	444.8	35.8	159.1	-	-
esfenvalerate	12	0	-10	294.0	16.8	49.4	65.8	2
fluvalinate	4	12	-5	1270.0	19.7	250.0	0	1
pirimicarb	100	-	100	NA	NA	NA	100	4

Preimaginal mortalities of insects exposed to residues of bifenthrin, esfenvalerate and fluvalinate were very low, and in the case of fluvalinate and esfenvalerate even lower than the control values. Females obtained from larvae exposed to esfenvalerate and bifenthrin laid fewer eggs than control females and the mean hatching rate was also reduced. Reduction in the beneficial capacity relative to the control was equal to 67.7 % and 65.8 % for bifenthrin and esfenvalerate, respectively. These two compounds were rated as "slightly harmful" (class 2) for larvae of *E. balteatus* on glass plates.

Females obtained from larvae exposed to fluvalinate laid more eggs than the control females, but with a lower mean hatching rate. Despite this, the number of viable eggs produced by females was higher than that of control females. Thus fluvalinate had no effect on the reproductive performance of hoverflies. There was no reduction in the beneficial capacity and this compound was classified as "harmless" (class 1) for larvae of *E. balteatus* on glass plates.

## DISCUSSION

Results obtained with insecticides used to control cereal aphids on larvae of *E. balteatus* in the laboratory showed that these compounds can be classified in different categories. Cyfluthrin,  $\lambda$ -cyhalothrin, cypermethrin, deltamethrin, phosalon and pirimicarb were "harmful" in the laboratory for *E. balteatus* and need further testing for an adequate assessment of their toxic effects in field conditions. Indeed, as test conditions are more severe in the laboratory than in field, laboratory tests should be considered a worst case study and the results obtained can only be used to prove the harmlessness of a compound. Pesticides found to be harmless to a beneficial organism in the laboratory are most likely to be of low risk to populations of the same organism in the field and no further testing is recommended. In the case of pesticides with harmful effects in the laboratory, only field testing will reveal if or to what extent the harmfulness remains under practical conditions (Vogt and Hassan, 1994).

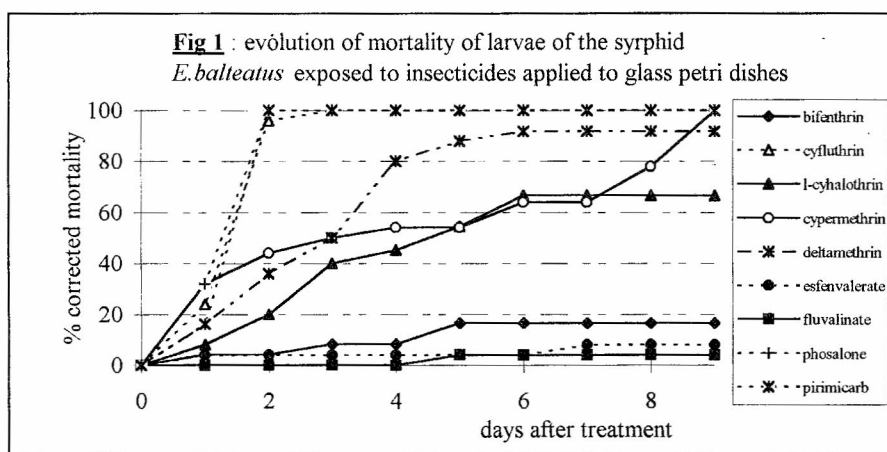


Fig. 1: Evolution of mortality of larvae of the syrphid *E. balteatus* exposed to insecticides applied to glass petri dishes

In the present study, among compounds that were classified as harmful, it is possible to distinguish between products that were highly toxic and killed all the larvae after 2-3 days of exposure (cyfluthrin, phosalone, and pirimicarb) and those that acted more slowly ( $\lambda$ -cyhalothrin, cypermethrin, and deltamethrin). Results with deltamethrin, phosalone and pirimicarb are similar to those obtained in the "Joint Pesticide Testing Programme" of the IOBC (Hassan and *al.*, 1987) for another syrphid species (*S. vitripennis*) and to results cited by Gräpel (1982) for pirimicarb on *M. corollae*. Pirimicarb is the only product that has been tested in field conditions. It was found toxic for syrphid larvae in wheat at the recommended field rate (Niehoff and Poehling, 1995). Phosalone and cyfluthrin, which in our laboratory study gave similar results to those for pirimicarb, could, therefore, be equally toxic in the field.

Two compounds, esfenvalerate and bifenthrin were slightly harmful for *E. balteatus*. They now need to be tested in semi-field or field condition, but as their toxicity on larvae during the exposure phase was low (below 20 %), high toxicity in the field would be surprising. Finally, fluvalinate, was harmless in this worst-case study and effects in the field on syrphids, other than by the reduction of food availability, are not expected. This compound, and to a lesser extent, bifenthrin and esfenvalerate, are therefore possible candidates for Integrated pest management of aphid in wheat. However, natural control of cereal aphid populations is not only the result of the action of syrphid larvae and the effect of these products on the other important aphid natural enemies must also be determined.

#### ACKNOWLEDGEMENTS

This research was financially supported by the "Fonds budgétaire des Matières premières" (Ministère des Classes moyennes et de l'Agriculture, DG4 - Qualité des matières premières et analyses).

#### RESUME

Les effets des produits autorisés en Belgique pour le contrôle des pucerons des céréales en été ont été évalués au laboratoire vis-à-vis des larves du syrphid *Episyrphus balteatus*. Les produits ont été testés à la dose maximale recommandée au champ. Des larves de syrphes âgées de 2-3 jours ont été exposées jusqu'à leur pupaison à des résidus d'insecticides fraîchement appliqués sur des boîtes de pétri en verre. La capacité de reproduction des adultes issus de ces larves a ensuite été évaluée. Les résultats ont été comparés à un témoin (traitements réalisés à l'eau) et la réduction de capacité bénéfique a été calculée de manière à classer les produits selon les critères spécifiés par le groupe de travail de l'OILB "Pesticides et Organismes Utiles".

La cyfluthrine, la cyperméthrine, la phosalone et le pirimicarbe ont entraînés une mortalité de toutes les larves. Deux larves exposées à des résidus de deltaméthrine se sont transformées en pupe, mais aucun adulte n'en est sorti. Une mortalité corrigée de 78.1 % et une production moyenne de 52.7 œufs/femelles ont été obtenues avec la  $\lambda$ -cyhalothrine, mais aucun œuf n'a éclos. La réduction de la capacité bénéfique de ces six produits est égale à 100 % et ils sont classés comme "toxique" (classe 4). La bifenthrine, l'esfenvalérate et le fluvalinate ont donné lieu à des taux de mortalité beaucoup moins importants. Les deux premiers pyrethrinoides ont cependant réduit la fertilité des femelles et ils ont été classés comme "légèrement toxique" (classe 2). La capacité de reproduction des adultes n'a pas été diminuée par le fluvalinate et ce produit a été classé comme "non-toxique" (classe 1).

En conclusion, ces résultats indiquent que le fluvalinate n'a que peu d'effets sur le prédateur aphidiphage *E. balteatus* et que son utilisation dans le cadre de la lutte intégrée en céréale contre les pucerons est envisageable. La bifenthrine et l'esfenvalérate n'ont pas eu d'effets marqués en laboratoire. Comme ces tests sont considérés comme un "worst-case study", il est probable que leur effets sur les syrphes en champ sera très limité. La cyfluthrine, la cyperméthrine, la deltaméthrine, la  $\lambda$ -cyhalothrine, la phosalone et le pirimicarbe se sont avérés toxiques en laboratoire et devront être évalués en semi-champ ou en plein champ, afin de permettre une évaluation de leurs effets sur les syrphes dans des conditions plus proches de la réalité.

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