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Side-effects of pesticides on adults of *Aphidius rhopalosiphi* DeStefani-Perez (Hymenoptera: Aphidiidae) in the laboratory: results of the 8th Joint Pesticide Testing Programme

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Abstract: 14 of the 21 products of the 8th JPTP were tested on glass plates on adults of the aphid parasitoid *Aphidius rhopalosiphi* at their maximum recommended field rate. For Insect Growth Regulator products, fertility assessment of surviving females was followed on two successive generations when it was possible, and reduction of beneficial capacity calculated on basis of these results.

Bavistin, Dithane M45, Thianozan and Topsin M were found harmless on glass plates and therefore effects on this beneficial under field conditions are not expected. Captan, Euparen Kumulus, Match, Polo, Scala and Telmion, which were slightly harmful and Admiral, Vertimec and Zolone flow, which were harmful, need further tests, as extended-lab test, semi field or field test for an adequate assessment of their side-effects on *A. rhopalosiphi*.

Effects of IGR products as Match and Polo on reproduction were limited to the first generation of wasps. No effects on mummies production and adults emergence were found on the second generation.

Keywords: *A. rhopalosiphi*, glass plate test, insecticides, fungicides, IGR, joint pesticide testing programme.

Introduction

Since early 70', a great number of pesticides was tested on several beneficial organisms species by the IOBC Working Group „Pesticides and Beneficial Organisms" during seven Joint Pesticide Testing Programmes (Franz *et al.* 1980, Hassan *et al.*, 1983, 1987, 1988, 1991, 1994, Sterk *et al.*, 1999). This very important work has allowed to select pesticides that were harmless for beneficials organisms and can be used in IPM program in different crops. From the 4th to the 7th JPTP, 82 pesticides were tested by Polgar on *Aphidius matricariae* Hal. (Hym.; Aphidiidae), with the help of a specific glass plate method (Polgar, 1982). As the work was stopped with this species and as Aphidiidae were considered as a major aphid natural enemy in several crops, there was an urgent need to test products selected in the 8th JPTP on an aphidiid species.

A. rhopalosiphi is a common and economically important parasitoid of cereal aphids and is distributed in several countries of the Western Europe area (Stary, 1970, Dedryver *et al.*, 1985). According to its high sensitivity to pesticides, *A. rhopalosiphi* is currently used as indicator species for registration studies at European level (Barrett et al., 1994). This species was also routinely tested in Belgium at the Department of Biological Control, in the context of an aphid IPM program in wheat. Over 60 insecticides and fungicides used in wheat were tested on this species by means of a glass plate method derived from Polgar (Jansen, 1996, 1998 & 1999). This adapted method was used to assess the effects of the products selected by the IOBC Working Group „Pesticides and Beneficial Organisms" for the 8th JPTP. Results are presented and discussed, as well as selection of test methods and test species.

Material and methods

Chemicals

Pesticides tested on *A. rhopalosiphi* were all selected for the 8th JPTP. They were studied at a single rate, corresponding to the maximum recommended field rate for fruit/orchard, glasshouse and arable crop. For products to be used in orchards and glasshouse crops 40 % of the maximum field rate were tested as agreed in Barret et al. (1994) (dilution effect in 3-dimensional crops). Dilutions of products were made on the basis of an application of 200 l water/ha. As exposure in the field was not expected, herbicides were not tested. List of products and doses tested (g a.i./ha and % product/water) are given in table 1.

Table 1: List of compound tested on glass plates on adults of *A. rhopalosiphi*. Trade name, active ingredient, formulation, tested rate (g a.i./ha and concentration of formulated product in water (200 l water/ha).

Trade name	active ingredient	formulation	active ingredient/ha	concentration product
1-Insecticides				
Admiral	pyriproxyfen	EC	50 g	0.250 %
Match	lufenuron	EC	50 g	0.500 %
Polo	diafenthiuron	SC	500 g	1.000 %
Telmion	rape seed oil	-	10 l	5.000%
Vertimec	abamectine	EC	13.5 g	0.375%
Zolone flow	phosalone	SC	600 g	1.200 %
2-Fungicides				
Bavistin	carbendazim	DF	400 g	0.400 %
Captan	captan	WP	3400 g	2.050 %
Dithane M45	mancozeb	WP	3200 g	2.000 %
Euparen M	tolyfluanide	WG	2500 g	1.563 %
Kumulus	sulphur	DF	4800 g	3.000 %
Scala	pyrimethamil	SC	1000 g	1.250 %
Thianozan	thiram	WG	2400 g	1.500 %
Topsin M	thiophanate-methyl	WP	2100 g	1.500 %

Application of chemicals

Pesticides were applied on glass plates (10x10cm) with the help of a „Precision Computer Control Sprayer" apparatus (Burkard Manufacturing, Rickmansworth, England). Dilution's of products were made soon before application and calculated on basis on an application volume of 200 l/ha. Apparatus was calibrated to deliver this volume ± 10%. Glass plates were weighed before and immediately after application to make sure that the correct amount of product was applied.

Conduct of trials

Glass plate tests on *A. rhopalosiphi* were conducted in two steps, following IOBC standard procedure for testing side-effects of pesticides on beneficial arthropods (Hassan, 1992). Adult wasps were exposed for a 24h-period to dried pesticide residues applied to glass plates and subsequently, the reproductive performance of surviving females was assessed. All these operations, as well of the method of rearing of *A. rhopalosiphi* were detailed in previous publications (Jansen, 1996, 1998), except pesticide application procedure which was changed. Products were tested by set of 4 or 5 products with a water-treated control. Four exposure units of 10 wasps were tested for each product and for control. The reproductive performance of 10 females or of all surviving females when less than 10 survived was assessed. The reduction of the beneficial capacity (E) was calculated by means of Overmeer-Van Zon formula (1982):

$$E (\%) = 100 \% - ((100 \% - Mc) \times Rt/Rc)$$

Mc = mean corrected mortalities of wasps (initial exposure), Rt = mean reproductive performance of treated wasps, Rc = mean reproductive performance of control.

According to the degree to which they reduced the beneficial capacity, pesticides were classified in one of the four IOBC categories relative to their initial toxicity on inert surfaces (Hassan, 1992).

Specific considerations with IGR products

For products that acted as Insect Growth regulator, fertility assessment of surviving female was followed on a second generation, assuming that it was possible. Mummies harvested at the end of the first fertility assessment were kept and percentage of adults that emerged were calculated. 10 females and males of this generation and of the same product (not necessary from the same replicate) were associated in couples and released in fertility assessment units as used before to assess the effects of products on a second generation. After 24h, adults wasps were removed and mummies left to developed 10 to 12 days in the same environmental conditions that as previously described. Thus, mummies that were formed were harvested, counted and adults of the second generation left to emerged. Any abnormalities or malformation of adults of the 1st and 2nd generation were noted. Reproductive ratio were calculated and reduction of beneficial capacity calculated on basis on an adapted version of Overmeer-Van Zon formula :

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

$$R = (Rt1/Rc1)*(Rt2/Rc2)$$

Mc = mean corrected mortalities of wasps (initial exposure),
Rt1 and Rt2 = mean number of living adults/female exposed to test product at the 1st and 2nd generation
Rc1 and Rc2 = mean number of living adults/female in the control at the 1st and 2nd generation.

Results

Results of the 3 set of experiment are listed in table 2. Control mortalities ranged between 0.0 and 5.0 %. With Bavistin, Dithane M, Topsin (set 1) and Thianozan (set 3), no or little direct effects on adult wasps and on reproductive performance of surviving females were observed and these products were classified as harmless at maximum recommended field rate. With Kumulus (set 1), Captan, Euparen (set 2) and Match (set 3), mortalities were low and close to control value, but reproductive performance were reduced and products classified as slightly harmful. Scala (set 2), Polo and Telmion (set 3) were also considered as slightly harmful, with effects on both survival and fertility. With Zolone flow (set 1), Admiral and Vertimec (set 3), no females survived to exposure and fertility assessment was not possible. These products were classified as harmful.

Table 2: Glass plate tests with adults of *A. rhopalosiphi*. Observed, corrected mortalities (4x10 wasps), mummies produced/female (10 female/product), reduction of beneficial capacity (E) and IOBC rating for initial toxicity on inert surfaces.

test product	observed mortality	corrected mortality	mummies/ female	E	IOBC class
Set 1					
Control	0.0%	-	22.9	-	
Bavistin	2.5%	2.5%	23.7	-3.5%	1
Dithane M45	12.5%	12.5%	23.6	9.8%	1
Kumulus	5.0%	5.0%	13.4	44.4%	2
Topsin M	10.0%	10.0%	19.0	25.3%	1
Zolone flow	100.0%	100.0%	-	100.0%	4
Set 2					
Control	2.5%	-	40.6	-	-
Captan	5.0%	2.5%	27.5	32.1%	2
Euparen M	14.3%	12.3%	29.9	35.4%	2
Scala	27.5%	26.1%	23.9	56.5%	2
Thianozan	10.0%	8.1%	32.0	27.5%	1
Set 3					
Control	5.0%	-	22.1	-	-
Admiral	92.5%	92.5%	-	100.0%	4
Match	7.5%	1.9%	4.7	79.6%	2
Polo	27.1%	21.9%	10.1	66.7%	2
Telmion	47.5%	46.3%	9.5	77.4%	2
Vertimec	95.0%	95.0%	-	100.0%	4

Results of fertility assessment on first and second generation of adults that survived to the exposure to Match and Polo are given in table 3. At first generation, number of mummies produced by surviving females were reduced compared to control. However, no difference in adult emergence was observed between control and both products. No adult abnormalities or malformation was observed, except two wingless adults, one in the control and one with Match. At the second fertility assessment, production and emergence of mummies with Match and Polo were comparable to control values, even a little bit higher. Comparison of reduction capacity of wasps calculated on basis of Overmeer-Van Zon formula (E1, 1st generation results only) and of adapted formula (E2, 1st + 2nd generation) did not lead to a different IOBC classification.

Table 3: Results of fertility assessment on 1st and 2nd generation with IGR products: mummies and living adults produced/female at 1st and 2nd assessment, reduction of beneficial capacity calculated on basis of 1st and 2nd generation results.

	F1 generation		F2 generation			
Test product	Mummies/ female	adults/ female	mummies/ female	adults/ female	E2	IOBC class
Control	22.1	18.9	24.2	22.1		
Match	5.0	4.7	32.0	28.0	69.1 %	2
Polo	10.1	9.1	32.3	29.0	50.7 %	2

Discussion

According to IOBC testing scheme, glass plate tests are only used to prove harmlessness of compounds. If effects greater than 30% are observed, further testing, like extended-lab test on natural substrate, tests with less susceptible life stage, semi-field or field test have to be conducted for an appropriate assessment of side-effects of products under practical conditions. According to results that were obtained with products of the 8th JPTP, it can be concluded that Bavistin, Dithane M45, Thianozan and Topsin M are harmless to adults of *A. rhopalosiphi* in the laboratory at maximum recommended field rate. Therefore, toxic effect in practical conditions are not expected. For other products, further tests are required. However, it could be useful to differentiate products as Kumulus, Captan or Euparen which were only slightly harmful and products as Admiral, Vertimec and Zolone flow which totally reduced beneficial capacity of the wasps.

Fertility assessment of females wasps surviving the residual glass plate tests with IGR compounds as Polo or Match, showed that reduction of reproductive performances was limited to the first generation. No abnormalities or malformation was observed in the progeny during two successive generations. Thus, effects observed at the first fertility assessment could probably be attributed to a reduction of fitness of wasps after exposure to tested products rather than a true „IGR" activity. Possible effects of these compounds on other aphidiid life stages, like larvae inside aphids or nymphs inside mummies, were nevertheless not determined. Concerning selection of test method and test species, there is currently several questions. Different glass plate methods with an aphidiid species have been published (*Diaeretiella rapae* (M'Intosh), Kühner *et al.*, 1985, *A. matricariae*, Polgar, 1988, *A. rhopalosiphi*, Mead-Briggs 1992, Jansen, 1996). These methods fulfil IOBC requirements for testing side-effects of pesticides on beneficial organisms on inert surfaces and only differ by details, like fertility assessment adapted to the biology of test species. Exposure of wasps to treated glass plates lasted 24h, except with Mead-Briggs method with 48h of exposure. This last method is specifically used for registration studies. In the context of selection of products for IPM purpose, glass plate test are only used as a worst case study and to prove harmlessness of compound. In this case, 24h of exposure can be considered as sufficient. Comparison of results of glass plate test, extended lab test with plants treated till run-off or treated in the field with the same products at the same doses showed that there was an important overestimation of toxicity with glass plates test (Jansen, 1998 and 2000). Therefore, 24h of exposure can be considerate as sufficient in an IPM context. However, it could be interesting to compare the same products tested with 24 and 48h of exposure and according results eventually adapt the method for forthcoming JPTP.

The selection of 24h of exposure instead of 48 has also be made to link the results obtained with *A. rhopalosiphi* with those published in the previous JPTP with *A. matricariae*, tested with 24h of exposure. However, there is little information available on the comparison of sensibility of different aphidiid species. Bayoun *et al.* (1995) have compared sensitivity of *D. rapae* and *Lysiphlebus testaceipes* (Cresson), two aphidiid parasiting the Russian Wheat aphid *Diuraphis noxia* (Kurdjumov), to 14 insecticides. They have found that *D. rapae* was in a general way more tolerant to insecticides than *L. testaceipes*, but differences appeared according to chemical classes of tested products. Maise *et al.* (1997) found that *A. rhopalosiphi* was more sensitive than *A. matricariae* and *A. colemani*, but only one product, dimethoate, was tested. Influence of origin and handling of test organisms was not taken into consideration. Thus, if *A. rhopalosiphi* seems to be a suitable indicative species for aphidiids at this moment, several problems as choice of test species and time of exposure must be clarified.

Conclusions

According to results obtained in this study, Bavistin, Dithane M45, Thianozan and Topsin M were found harmless to adults of *A. rhopalosiphi* on glass plates. Captan, Euparen Kumulus, Match, Polo, Scala and Telmion, which were slightly harmful and Admiral, Vertimec and Zolone flow which were harmful need further tests, as extended-lab test, semi field or field test for an adequate assessment of their possible side-effects on *A. rhopalosiphi.*

Two Insect Growth Regulator, Match and Polo, reduced fertility of females wasps exposed 24h to fresh residue of products. However, reduction was limited to the first generation and no true "IGR" activity was noted.

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