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SIDE EFFECTS OF INSECTICIDES ON *APHIDIUS RHOPALOSIPHI* (*HYM.: APHIDIIDAE*) IN LABORATORY

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The toxicity of bifenthrin, cyfluthrin, λ-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fluvalinate, phosalon and pirimicarb have been assessed in laboratory on the parasitic hymenoptera *Aphidius rhopalosiphi*. A strong toxic effect was found with every tested product when adult parasitoids were exposed to freshly applied residues on both glass plates and maize leaves for 24 hours. Only two products, fluvalinate and esfenvalerate, did not kill all the insects. No differences were observed between mortalities on glass plates and leaves. Applied on aphids mummies, cyfluthrin and deltamethrin slightly reduced the emergence of young parasitoids, but not their reproductive performance. The other tested products had no effects on adult emergence. On basis of these results, the insecticides are of comparable toxicity to *A. rhopalosiphi* in laboratory.

KEY-WORDS: pesticides, natural enemies, cereal aphids, *Aphidiidae*.

In open fields, the control of pests with insecticides can lead to pest resurgence (Bartlett, 1958; Horn, 1983). This generally occurs when broad spectrum insecticides that affect natural enemies of pests are applied. In order to avoid this kind of problems, extensive work has been carried out to select insecticides compatible with beneficial organisms. For about twenty years, the working group "Pesticides and Beneficial Organisms" of the International Organization for Biological Control (IOBC) has achieved a considerable work in this field. It has particularly contributed to the development of standard methods to test the side-effects of pesticides on the natural enemies of various pests.

Sitobion avenae (F.) and *Metopolophium dirhodum* (Walker) (Homoptera: *Aphididae*) are two of the major economic injurious cereal pests in Belgium (Latteur & Oger, 1987). These aphids can be controlled by several species of aphidiids. During some years, these hymenoptera are able to maintain aphid populations below their economic injury threshold (Latteur & Oger, 1991). The most commonly found species in cereals is *Aphidius rhopalosiphi* De Stefani-Perez (Starý, 1970). This insect is exposed to pesticides applied to control cereal aphid populations. In Belgium, eight active ingredients are used: six synthetic pyrethroids (bifenthrin, cyfluthrin, λ-cyhalothrin, deltamethrin, esfenvalerate and fluvalinate), one organophosphorous compound, phosalone, and one carbamate, pirimicarb. The effects of a limited number of these insecticides have ever been assessed on *A. rhopalosiphi* in laboratory studies (deltamethrin and λ-cyhalothrin, Krespi *et al.*, 1991; pirimicarb, Borgemeister *et al.*, 1993). Some compounds have been tested on other species of aphidiids occasionally found in cereals (phosalon and pirimicarb on *Diaeretiella rapae* (McIntosh),

Delorme, 1976; deltamethrin and pirimicarb on *Aphidius matricariae* Haliday, Stevenson *et al.*, 1984). However, it is difficult to apply those results for *A. rhopalosiphi* because the possibilities of extrapolations of data's from one species to another are not well known.

In this frame, it is not possible to determine and compare the toxicity of the eight insecticides used in Belgium to control cereal aphids on *A. rhopalosiphi*. The aim of this work is to study under laboratory conditions the side-effects of these insecticides towards this species. In a first time, the toxicity of fresh pesticide residues applied on glass plates and on maize leaves will be assessed to adult parasitoids. In a second series, the products that are toxic to the adults will be tested on aphid mummies. These two studies fulfill the requirements of the standard characteristics of test methods developed by the IOBC working group "Pesticides and Beneficial organisms". They correspond to the "initial laboratory toxicity, susceptible life stage" (glass plates test) and "laboratory toxicity, less susceptible life stage" (mummies test) of the IOBC testing scheme respectively. The maize leaf test has been carried out to compare the toxicity of pesticide residues applied on leaves and on glasses.

MATERIALS AND METHODS

REARING OF *A. RHOPALOSIPHI*

The rearing of *A. rhopalosiphi* was established from field-collected mummies in May-June 1993. It was conducted in woodframe cages (40 × 40 × 60 cm) with two perspex and two fine metallic mesh walls. The front and the back walls were made of nylon gauze. The front wall was adapted to allow the introduction and the exit of biological material (plants, aphids, parasitoids...). Young parasitoids were periodically released into the woodframe cage. Young seedlings of wheat infested by cereal aphids (*S. avenae* and *M. dirhodum*) were introduced into the cage for 24 to 48 hours. After this period, they were removed and covered with a cylindrical perspex cage (Ø: 8 cm, h: 18 cm, with the top covered with nylon gauze) for the incubation of the parasitoids. All these operations were done in a climatic chamber (20 ± 1°C, 80 ± 10% RH, light: 10,000 to 15,000 lux, neon tubes, 18 h light/6 h dark photoperiod). Ten to 12 days after egg laying, aphid mummies were taken off the plants with flexible tweezers and individually placed in glass tubes (Ø: 10 mm, L: 40 mm) closed with a piece of cotton wool till adult emergence. In these conditions, *A. rhopalosiphi* life cycle, (from egg to adult) was about 15 ± 1 days long.

TEST PRODUCTS

Nine commercial formulations have been used in this set of experimentation. Eight of them are registered in Belgium to control cereal aphid populations in spring-early summer: Baythroid EC 050 (cyfluthrin), Decis EC 2.5 (deltamethrin), Karate 25 (λ-cyhalothrin), Mavrik 2F (fluvalinate), Pirimor G (pirimicarb), Sumi Alpha (esfenvalerate), Talstar Flo (bifenthrin) and Zolone Flo (phosalon). Cymbush DG (cypermethrin) is only used to control BYDV vector aphids in winter. All the insecticides have been tested at a single dose, corresponding to their maximum recommended field rate, which is generally used by the farmers.

INITIAL TOXICITY ON GLASS PLATES-ADULTS

Application of pesticides

The insecticidal formulations were applied on rectangular glass plates (9 × 6 cm) with a Burgerjon type Potter-tower apparatus (Burgerjon, 1956). The apparatus was calibrated to provide an homogenous deposit of 2 ± 0.2 mg/cm^2, corresponding to a field application of

200 ± 20 l/ha. The glass plates were weighed before and immediately after the application of pesticides to make sure that a correct deposit was obtained. All insecticidal dilutions were made in tap water soon before application.

Exposure unit

The exposure units were made of an aluminum ring frame and two rectangular glass plates that have previously been treated with insecticides. The glass plates were fitted with a rubber band onto the ring frame (Ø: 50 mm, h: 18 mm) as floor and ceiling, the treated glass face was turned inside. The ring was pierced with seven holes. Two of them were used to feed the insects with a honey/water solution (30% w/v) offered on two pieces of cotton wool. Four ventilation holes were covered with a fine nylon gauze and the last hole was used to connect a peristaltic pump. The pump was regulated to renew the air in the cage once every minute and prevent possible accumulation of insecticides fumes.

Conduct of trials

Adult parasitoids used for the test were 24-hours old. Some 24-48 hours specimens were used to complete the populations only if they have been previously kept in a cool place, between 5 and 7°C. Preliminary experiments have shown that longer stocking periods lowered the fertility of females. For each exposure unit, 12 parasitoids (6 males, 6 females) were collected from their emergence tube with the help of a small aspirator, slightly anaesthetized with CO_2 and transferred into the cage. The cage was then closed and immediately connected to the pump. For each product, 5 replicates of one cage were made. Five cages assembled with water treated glass plates were used as control for each set of experiments. All assays were conducted in a climatic chamber (20 ± 1°C, 80 ± 10% RH) under continuous diffuse light.

After 24 hours of exposure, the number of dead parasitoids was counted and the survivors collected with a small aspirator in order to determine the side-effect of the insecticide residue on the fertility performance of the insects. The moribunds were counted as dead if they exhibited signs of severe intoxication two hours after opening of the cage. The mortality observed in each cage was corrected with the corresponding control mortality using Abbott's formula (1925). Statistical analysis of the results was made with the help of a Student's t-test at $p = 0.05$ level (Dagnelie, 1970).

The assessment of the fertility performance of the parasitoids was done by releasing couples of surviving *Aphidius* (1 male, 1 female) for a 24 hour period in perspex cylindric cages covering wheat seedlings infested by 60-100 cereal aphids (a mix of *S. avenae* and *M. dirhodum* of all growth stages). These cages were similar to those described for aphid production. After 12 days, the number of aphids mummies was counted and compared to control with the help of a Student's t-test at $p = 0.05$ level (Dagnelie, 1970).

TOXICITY ON MAIZE LEAVES-ADULTS

The assessment of the toxicity on maize leaves was made in the same way as the glass plates test. The chemicals were applied on pieces of maize leaves (about 6 × 7 cm) by means of a Burgerjon Potter-tower. The leaves were cut from plants cultured in glasshouse without pesticide applications. Before insecticide application, the two extremities of the central leaf nervure were cut to adjust the leaf piece size to the exposure cage. The exposure units were assembled using these treated leaves, covered with untreated glass plates to make handling of the cages easier. A slightly humidified piece of filter paper was

introduced between the maize and the glass plate to avoid desiccation and deformation of the leaves. All other manipulations were similar to those described for the glass plates test.

TEST ON MUMMIES

Sections of wheat leaves bearing mummified aphids originating from the *A. rhopalosiphi* rearing were carefully attached on rectangular glass plates with the help of double-side sticky tapes. The mummies were of uniform age, 2-3 days old. The glass plates with sections of leaves were thus treated with a Burgerjon Potter-tower. Two hours after the insecticide application, treated mummies were removed from the leaves, placed individually in emergence tubes and kept at $20 \pm 1°C$, $80 \pm 10\%$ RH until adult emergence. For each test product, five replicates of one plate bearing 20 to 22 mummies were made. Five plates bearing 20 to 22 mummies were treated with water as control for each set of experiments.

The mummies were observed twice a day and the percentage emergence was calculated for each replicate. Most adults emerged 2 to 4 days after spraying, but some of them were found dead in their tube. They were taken in account in the expression of the results: the "total percentage emergence" is calculated with alive and dead parasitoids, the "alive percentage emergence" only with alive insects.

The fertility of the emerging parasitoid was assessed for three couples in each replicate when it was possible to associate males and females of the same age. The fertility test was conducted in a manner similar to the methods previously described. The percentage of emergence of treated mummies and the number of mummies produced per female were compared to controls by means of a Student t-test at $p = 0.05$ level (Dagnelie, 1970).

RESULTS

TOXICITY ON GLASS PLATES AND ON MAIZE LEAVES-ADULTS

All the insecticides tested on glass plates and on maize leaves were toxic for parasitoid adults (table 1). For bifenthrin, cyfluthrin, λ-cyhalothrin, cypermethrin and deltamethrin, a severe and irreversible knock-down effect was observed within a few minutes after the start of the experiments.

On glass plates, esfenvalerate and fluvalinate were slightly less toxic (88.3 and 93.4% respectively) as compared to 100% mortality of the other products. On maize leaves, mortality percentages of all different compounds were not statistically different. However the variability of the results was higher. Hence, no difference in toxicity was observed between glass plates and maize leaves for each insecticide.

For insecticide treatment, fertility performances could not be evaluated because all the females were found dead. The control gave a mean of 16.4 mummies produced per female, with results ranging for 0 to 35 mummies/female.

TEST ON MUMMIES

The results of the test on mummies are listed in table 2 (total percentage emergence, alive percentage emergence and fertility assessment). With the exception of deltamethrin and cyfluthrin, no other insecticides affected emergence of treated mummies related to the control. For these two products, the reduction of emergence was probably due to the toxicity of residues of pesticides present on the mummies. If dead adults parasitoids are counted in the calculation of emergence (total emergence), any differences appears between the different objects.

TABLE 1

Toxicity of insecticidal residues applied on glass plates and on maize leaves on adults of A. rhopalosiphi. *Percentage of corrected mortality (mean of 5 replicates) ± standard deviation*

active ingredient	dose (a.i./ha)	corrected mortality ± sd*	
		glass plates	maize
bifenthrin	7.5 g	100.0 ± 0.0 a	100.0 ± 0.0 a
cyfluthrin	15 g	100.0 ± 0.0 a	100.0 ± 0.0 a
cypermethrin	20 g	100.0 ± 0.0 a	100.0 ± 0.0 a
1-cyhalothrin	5 g	100.0 ± 0.0 a	100.0 ± 0.0 a
deltamethrin	5 g	100.0 ± 0.0 a	100.0 ± 0.0 a
esfenvalerate	5 g	88.3 ± 8.5 b	89.7 ± 8.1 ab
fluvalinate	36 g	93.4 ± 3.3 b	80.0 ± 19.5 ab
phosalon	750 g	100.0 ± 0.0 a	100.0 ± 0.0 a
pirimicarb	125 g	100.0 ± 0.0 a	100.0 ± 0.0 a

* Numbers followed by the same letter are not statistically different (t-test, p = 0.05 level).

TABLE 2

Toxicity of insecticides on A. rhopalosiphi *mummies. Percentage of total and alive emergence ± standard deviation and reproductive performance of females emerging from treated mummies (n = number of females)*

active ingredient	dose (a.i./ha)	% total emergence ± sd*	% alive emergence ± sd*	reproductive performance	
				n	mummies/ femmale ± sd*
control		89.4 ± 9.8 a	89.4 ± 9.8 ab	48	14.2 ± 13.8 ac
bifenthrin	7.5 g	88.6 ± 9.1 a	85.5 ± 10.8 abcd	15	14.7 ± 10.6 ab
cyfluthrin	15 g	81.6 ± 14.3 a	64.0 ± 15.9 c	8	20.5 ± 21.5 ab
1-cyhalothrin	5 g	92.9 ± 2.4 a	92.9 ± 2.4 a	15	12.1 ± 13.2 ab
cypermethrin	20 g	86.5 ± 5.4 a	79.8 ± 10.4 bc	8	16.4 ± 19.2 ab
deltamethrin	5 g	83.4 ± 8.6 a	71.6 ± 13.2 cd	13	10.5 ± 16.9 ab
esfenvalerate	5 g	95.0 ± 3.2 a	91.4 ± 7.9 ab	15	24.0 ± 19.5 b
fluvalinate	36 g	92.0 ± 8.7 a	92.0 ± 8.7 ab	13	22.4 ± 17.1 bc
phosalon	750 g	92.0 ± 8.7 a	92.0 ± 8.7 abd	15	9.5 ± 11.3 a
pirimicarb	125 g	88.4 ± 7.8 a	88.4 ± 7.8 abd	15	21.3 ± 18.1 bc

* Numbers followed by the same letter are not statistically different (t-test, p = 0.05 level).

Related to the results of the control, no products have affected the fertility performance of parasitoids emerging from treated mummies. In the case of esfenvalerate, the production of mummies was even significantly higher than control. However, these results must be carefully interpreted because of the great data variability.

DISCUSSION

This set of experiments aims at determining and to comparing the toxicity of 9 insecticides used in Belgium to control cereal aphids on adults and pupae of *A. rhopalosiphi*. Few differences have appeared between the chemicals. Fluvalinate and esfenvalerate were the

less toxic insecticides for adult parasitoids while deltamethrin and cyfluthrin were the only products that affected the emergence of mummies. The comparison of these results with those previously published (Krespi *et al.*, 1991; Borgemeister *et al.*, 1993) is difficult. Indeed, if some of the tested products are similar, the methods that have been used and the doses that have been tested are not.

When pesticides are toxic on glass plates, the IOBC testing scheme recommends to test the pesticides on a natural substrate. In this study, the insecticides have been tested on both glass plates and maize leaves placed in similar experimental conditions. No differences of toxicity were observed for all the products that have been studied. These results suggest that the use of a natural substrate can afford little information more than artificial substrate in the case of contact insecticides (all the products that have been tested are contact insecticides except pirimicarb which is known to have a slight translaminar activity).

If the sensibility to insecticides of adults and pupae parasitoids is compared, the protection of the *Aphidius* against pesticide effects by mummified aphids is clearly demonstrated. This protection was previously described by several authors (Stevenson *et al.*, 1984; Borgemeister *et al.*, 1993) and can play a considerable benefit rule for aphidiids populations in field. Indeed, when products that are toxic for aphidiid adults are applied, their populations can be restored by newly emerging parasitoids protected by aphids mummies against pesticides effects. However, these young parasitoids are also exposed to the pesticide residues and can be killed if the product does not rapidly lose its toxic activities. In this way, the determination of the persistence of the chemicals that have been tested in this study is essential. Different methods already exist for some parasitoids species (Hassan, 1988; Oomen, 1988; Polgar, 1988; Bellows *et al.*, 1993) but techniques for *A. rhopalosiphi* need to be developed.

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RÉSUMÉ

Effets secondaires au laboratoire de quelques insecticides sur l'hyménoptère parasitoïde *Aphidius rhopalosiphi* (*Hymenoptera : Aphidiidae*)

La toxicité de 9 insecticides homologués en Belgique pour le contrôle des pucerons des céréales (bifenthrine, cyfluthrine, λ-cyhalothrine, cypermethrine, deltamethrine, esfenvalerate, fluvalinate, phosalone et pirimicarbe) a été évaluée au laboratoire vis-à-vis de l'hyménoptère parasitoïde *A. rhopalosiphi.* Tous les insecticides testés se sont montrés très toxiques vis-à-vis des parasitoïdes adultes lorsque ceux-ci étaient exposés pendant 24 heures à des résidus fraîchement appliqués sur des plaques de verre et des feuilles de maïs. Seuls le fluvalinate et l'esfenvalérate n'ont pas tué tous les insectes mis en expérimentation. Appliqués sur les stades prépupaux et pupaux de l'hyménoptère (pucerons momifiés), la cyfluthrine et la deltaméthrine ont réduit légèrement l'émergence des jeunes adultes, mais leur fertilité n'a pas été affectée. Les autres produits n'ont eu aucun effet négatif sur l'émergence des parasitoïdes et leur fertilité. Sur la base de ces résultats, la toxicité des insecticides testés vis-à-vis d'*A. rhopalosiphi* apparaît comme très semblable.

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REFERENCES

Abbott, S. W. — 1925. A method of computing the effectiveness of insecticides. *J. Econ. Entomol.*, 18, 265-267.

Bartlett, R. B. — 1958. Laboratory studies on selective aphicides favoring natural enemies of the spotted alfalfa aphid. *J. Econ. Entomol.*, 51, 374-378.

Bellows, T. S., Morse, J. G. & Gaston, L. K. — 1993. Residual toxicity of pesticides used for lepidopteran insect control on citrus to Aphytis melinus Debach (Hymenoptera: *Aphelinidae*). *Can. Ent.*, 125, 995-1001.

Borgemeister, C., Poehling, H.-M., Dinter, A. & Höller, C. — 1993. Effects of insecticides on life history parameters of the aphid parasitoid *Aphidius rhopalosiphi* (*Hym.: Aphidiidae*). *Entomophaga,* 38, 245-255.

Burgerjon, A. — 1956. Pulvérisation et poudrage au laboratoire par des préparations pathogènes insecticides. *Ann. Epiph.*, IV, 675-684.

Dagnelie, P. — 1970. Théorie et méthodes statistiques, vol. 2. *Editions J. Duculot,* S.A., Gembloux.

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

Hassan, S.A. — 1988. Guideline for testing the side effect of pesticides on the egg parasitoid *Trichogramma cacoeciae*. Sequential testing scheme: laboratory contact test 1 — on adults, 2 — on pupae within host, semi-field persistance test. *IOBC/WPRS Bull.*, 1988/XI/4, 3-18.

Horn, D. J. — 1983. Selective mortality of parasitoids and predators of *Myzus persicae* on collards treated with malathion, carbaryl, or *Bacillus thuringiensis. Entomol. exp. appl.*, 34, 208-211.

Krespi, L., Rabasse J. M., Dedryver, C. A. & Nenon J. P. — 1991. Effect of three insecticides on the life cycle of *Aphidius uzbekistanicus* LUZ. (Hym., *Aphidiidae*). *J. Appl. Ent.*, 111, 113-119.

Latteur, G. & Oger, R. — 1987. Principes de base du système d'avertissement relatif à la lutte contre les pucerons des froments d'hiver en Belgique. *Annales ANPP*, 1987, n° 6, Vol.II/III: Conférence internationale sur les ravageurs en Agriculture, 1-2-3 décembre 1987, 129-136.

Latteur, G. & Oger, R. — 1991. Winter wheat aphids in Belgium: prognosis and dynamics of their populations. *IOBC/WPRS Bull.*, 1991/XIV/4, 13-34.

Oomen, P.A. — 1988. Guideline for the evaluation of side-effects of pesticides on *Encarsia formosa*. Sequential testing scheme: laboratory tests: 1 - residual contact test on adults, 2 - direct contact test on pupae, persistence test on adults, semi-field test on adults. *IOBC/WPRS Bull.*, 1988/XI/4, 19-28.

Polgar, L. — 1988. Guideline for testing the effect of pesticides on *Aphidius matricariae* Hal. (Hym., *Aphidiidae*): laboratory contact tests: 1 — on adults, 2 — on aphid mummies, semi-field test on adults. *IOBC/WPRS Bull.*, 1988/XI/4, 29-34.

Stary, P. — 1970. Biology of Aphid parasitoids (Hymenoptera: *Aphidiidae*) with respect to integrated control. Series Entomologica, *Dr W. Junk.*, Den Haag.

Stevenson, J. H., Smart, L. E. & Walters, J. H. H. — 1984. Laboratory assessment of insecticide selectivity-practical relevance. — *Proceedings British Crop Protection Conference - Pests and Diseases*, 1, 355-358.