INFLUENCE OF SOME EXPERIMENTAL FACTORS ON THE EVALUATION OF THE SENSITIVITY OF *APHIDIUS RHOPALOSIPHI* DE STEFANI-PEREZ TO PHOSALONE

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ABSTRACT

Mead-Briggs (1992) proposed a laboratory method to assess the contact toxicity of a pesticide on the cereal aphid parasitoid *Aphidius rhopalosiphi* De Stefani-Perez. The influence of air moisture, photoperiod, sex of insects and storage conditions on the sensitivity of this parasitoid to phosalone was studied. Results showed that insect sensitivity is the most influenced by air moisture. The storage conditions of mummies before use seems also to be an important factor.

INTRODUCTION

The evaluation of side-effects of pesticides on beneficial arthropods is required by the directive 91/414/ECC regulating the authorization of plant protection products in the European Union. One of the recommended test species for this evaluation is the cereal aphid parasitoid *Aphidius rhopalosiphi* De Stefani-Perez (*Hymenoptera, Aphidiidae*).
The directive 96/12/EC defines the kind of assays required in the framework of this new regulation. Regarding the side-effects of pesticides towards beneficial arthropods, it refers to the injunctions of a document entitled "Guidance document on regulatory testing procedures for pesticides with non-target arthropods" (Barrett *et al.*, 1994).
According to this directive, the first stage of a compound toxicity assessment consists on testing it in laboratory conditions on an inert substrate. This method was slightly modified (Mead-Briggs, 1997) and is regularly used in our laboratories (Mahaut & Deleu, 1998; Deleu & Mahaut, 1998).
The purpose of this study is to verify in laboratory conditions if some experimental factors such as air moisture, photoperiod, storage conditions of mummies and sex of the parasitoids, influence or not their sensitivity to the phosalone insecticide.

MATERIALS AND METHODS

Production of the host plant for aphids

Plastic pots of 8 cm of diameter with vermiculite (Sibli rank 3) were humidified to their basis by tap water. Fifteen seeds of wheat (*Triticum aestivum* L. cv. Estica) were sown in each pots. The pots were placed in a controlled room at a temperature of 16,5°C (±1°C) with an air moisture of 80% ±3%. A luminosity of 4500 lux was provided by 11 neon lamps, placed to 80 cm above the pots.

Production of cereal aphid

The wheat plants were removed of the controlled room at the stage first leaf. About 20 cereal aphids (*Sitobion avenae* F. and *Metopolophium dirhodum* Wlk.) at different stages were placed in each pots. These pots were then covered by a Plexiglas® cylinder of 18 cm of high and a diameter of 8 cm. The upper part of cylinders was closed with a gauze and two lateral openings (4×10 cm) were cut out and covered by gauze to ensure aeration. Pots were then stored in a controlled room maintained at a temperature of 18°C (±1°C) and a photoperiod of 16 hours under 5000 lux provided by 14 neon lamps. The air moisture was about 70% (±2%).

Production of *A. rhopalosiphi*

Different cages adapted from Stăry's method (1966) were used for the production of parasitoids. This cages were 61×42×42 cm, with wire-mesh lateral sides. The top was of glass and the front and back closed with nylon gauze. Twelve pots of cereals with aphids were placed in one cage. Twelve pairs of wasps (*A. rhopalosiphi*) were let in this enclosure during 24 hours to allow oviposition. Pots of wheat were then removed and covered with a cylinder. Ten days after the infestation, the "mummies" (or parasitised aphids) were collected and placed individually in small glass tube. These tubes were stored at 20°C (±2°C) or in a controlled room maintained at 2°C (±2°C).

Phosalone spraying

The insecticide formulation used was a suspension concentrate (SC) with 500 g/l phosalone (Zolone Flo®, Rhône-Poulenc Agro). The insecticide was sprayed at the maximum dose rate recommended in cereals (750 g a.i./ha). The preparation was sprayed by a pneumatic sprayer (Caussin, 1993) on glass disks (diameter: 3 cm) at 200 l/ha. Before the disks spraying, the sprayer was calibrate to ensure a good reproducibility of the spraying with a percentage standard deviation of less than 10%. A total of 300 disks were treated with the same spray mixture. Disks were then stored in a dark room at 20°C (±2°C) until their use.

Bioassays description

Eight arenas (5 treated and 3 untreated) were used for each test (Figure 1). Ten insects of the same sex were placed in each arenas for 24h. After this period dead insects were counted to evaluate the toxicity of phosalone. The mortality observed was corrected for control mortality using Abbott's formula (Abbott, 1925).
The arenas used in the experiment (figure 1) consist of a circular ring (2×5 cm diam.) with 6 circular holes. Four holes were covered by gauze to allow ventilation. The other two were closed by a cotton-wool plug. One of them was humidified with water and the second with a solution of honey and water (1/1 v/v). Each cell was connected to a pump to changed the air two times/minute. A glass plate (6×9 cm) was covered by a treated disk with the untreated face of the disk placed on the plate. The insects were anaesthetised and laid on the treated face of the disk. A second disk was then placed on the ring with the treated face orientated inside the arenas. This disk was covered by a second glass plate. The different components were kept together by a rubber band.

The control arenas were built according to the same design with untreated disks.

Each arenas was then connected to the pump by a rubber tube. Temperature and air moisture were recorded by a sonde placed in an arena with a precision of ±3% for air moisture and ±0.3°C for the temperature. The recorder was programmed by Escort Software (version 1.00). The interval of time between two consecutive records was one minute.

Phosalone residues analysis

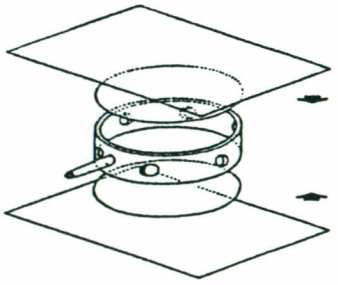

Figure 1: Bioassay arena

As glass disks were stored until their use, residues of phosalone were measured at three different times during the experiments to ensure that the residues level was stable. These measures were performed by High Performance Liquid Chromatography (HPLC). Before each chromatographic determination, the insecticide residues were extracted by 10 ml of the mixture acetonitrile/H_3PO_4 0,1% (70/30 v/v).

Material

HPLC:	BECKMAN 166+118
Automatic injector:	AS 507
Volume injected:	20 µl

Column: MN NUCLEOSIL 100-5C18 (125 × 4 mm)
Eluant: acetonitrile/H_3PO_4 0,1% (70/30 v/v), isochratic
Flow: 1.1 ml/minute
Detector: UV at 201 nm
Data provided by GOLD 8.01

Studied factors

The aim of this study was to evaluate the influence of air moisture, photoperiod during bioassays, storage conditions of the mummies and sex of parasitoids on the insect mortality. There were two levels for each considered factor.

The influence of air moisture was assessed at 60 and 85% (±5%).

The influence of photoperiod was estimated by comparison of the results for a photoperiod of 16 h light (1000 lux) and with the results when bioassays are carried out in the darkness.
The insects used for the experiments came from mummies stored at 20°C (±2°C) or stored between 10 and 20 days at 2°C (±2°C).

The difference of sensitivity between females and males was also evaluated.

The experimental design was a factorial design, each level of a factor being associated with each level of the others factors. One bioassay was carried out for each of the 16 combinations of factors. These bioassays were carried out separately in the time because two preliminary tests demonstrated that the population of the insects remained stable all the time. It was a completely randomized design with five replicates per test. However, for 4 bioassays, there were only 3 replicates due to a lack of insects.

Analysis of results

The average corrected mortality obtained for each combination are given in the table 1.

Table 1: Average corrected mortality in the treated arenas (%)

Air moisture	60 %				85 %			
Sex	females	males	females	males	females	males	females	males
Photo-period	light	light	dark-ness	dark-ness	light	light	dark-ness	dark-ness
2°C	93	67	93	86	100	100	100	100
20°C	40	57	10	30	100	96	94	98

An important difference of mortality was observed between 85% and 60% of air moisture. When the bioassays were carried out with 85% of air moisture, the corrected mortality was about 100% with a low variability between replicates.

On the other hand, the corrected mortality was more variable when the tests were performed with 60% of air moisture.
Because of the differences observed between both humidity conditions and the high variability in the results, the analysis of the variance was only performed with the results for the bioassays carried out at 60% of air moisture (Table 2). An angular transformation was firstly done on mortality percentages to fulfill the variance equality requirements (Dagnelie, 1998).

Table 2: Three-way analysis of variance

Sources of variation	Degrees of freedom	F observed	Probabilities
Sex	1	0.00	0.965
Storage conditions	1	60.10	0.000 *
Photoperiod	1	2.73	0.111
Sex × Storage conditions	1	9.08	0.006 *
Sex × Photo-period	1	1.40	0.249
Storage conditions × Photoperiod	1	10.15	0.004 *
Sex × Storage conditions × Photoperiod	1	0.12	0.729
Error	24		
Total	31		

When the tests were performed at 60% of air moisture, a very high significant difference was observed for the storage conditions of mummies. No significant differences were obtained for the "sex" and "photoperiod" factors. There was a significant interaction between the factors "storage conditions × sex" and the factors "storage conditions × photoperiod". Therefore, two separated analysis were achieved for both combination of factors (Table 3).

Table 3: Two-way analysis of variance

Sources of variation	Degrees of freedom	Storage conditions 20°C		Storage conditions 2°C	
		F observed	Probabilities	F observed	Probabilities
Sex	1	3.48	0.087	6.72	0.024 *
Photoperiod	1	8.71	0.012 *	1.79	0.206
Sex × Photoperiod	1	0.26	0.621	1.79	0.206
Error	12				
Total	15				

For tests carried out at 60% of air moisture and for mummies stored at 20°C, there was a significant difference for photoperiod, a highest mortality being observed when the bioassays were performed with a photoperiod of 16 hours (average of corrected mortality = 49%) rather than obscurity (average of corrected mortality = 12%).
When the tests were performed at 60% of air moisture with mummies stored at 2°C, there was a significant difference for sex factor. The average corrected mortality of females (95%) was higher than the average corrected mortality of males (79%).

DISCUSSION

The analysis of phosalone residues by HPLC demonstrated that the level residues remained stable throughout the experiment. The average concentration of phosalone residues was 6.09 µg/cm² (s.d. = 3.8%) the first day of experiment; 6.37 µg/cm² (s.d. = 2.9%) the 21th day of experience and 6.29 µg/cm² (s.d. = 4.4%) the last day. The average concentration of residues found on the disks used during the experiment was 6.25 µg/cm² (s.d. = 4.3%).

The most important factor tested was the air moisture during the experiments. Indeed, mortality obtained whilst tests were carried out at 85% was close to 100% and could hide the influence of the other studied factors. Therefore the knowledge of the humidity conditions during laboratory bioassays with *A. rhopalosiphi* seems to be needed before discussion of the results.

While bioassays were performed at 60% of air moisture, storage conditions of mummies were important because mortality obtained was greater for insects coming from mummies stored at 2°C (±2°C). This higher mortality of insects could be explained by the "cold shock"theory. According to Dendelinger *et al.* (1991), a quick exposition at low positive temperatures alters cellular membranes. When bioassays were performed with mummies stored cold, the mummies went immediately from 20 to 2°C without progressive temperature decrease.

Photoperiod had an influence when the bioassays were carried out at 60% of air moisture with insects stored at 20°C. Sex of insects influence also their sensitivity to phosalone whilst mummies are stored at 2°C and the bioassays performed at 60% of air moisture. The influence of photoperiod and sex in such conditions would be confirmed.

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