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MULTISTEP BIOASSAY TO PREDICT RECOLONIZATION POTENTIAL OF EMERGING PARASITOIDS AFTER A PESTICIDE TREATMENT

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Abstract—Neurotoxic pyrethroid insecticides are widely used for crop protection, and lethal and sublethal perturbations can be expected in beneficial insects. Under laboratory conditions, the lethal and sublethal effects of deltamethrin on the aphid parasitoid *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae) were studied at the mummy stage and in emerging adults. Following a multistep bioassay, analyses were aimed at evaluating the effects of deltamethrin at various crucial steps in the recolonization process following a deltamethrin treatment: Parasitoid pupal development (emergence from the mummies), adult survival, and host-searching capacity. A four-armed olfactometer was used to investigate the effect of deltamethrin on host-searching behavior (a range of concentrations causing 0.4–79.4% mortality was tested), and a Potter tower was used to test the deltamethrin effect with a realistic application method (four concentrations were tested: 0.5, 5.0, 6.25, and 50 g active ingredient [a.i.]/ha). Deltamethrin reduced the percentage of emergence from mummies, but only when exposed to the 50 g a.i./ha concentration. However, for all concentrations tested, the insecticide induced a decrease in longevity after emergence from sprayed mummies and significant adult mortality when parasitoids walked on fresh residues on leaves. Indices were defined and predicted a high mortality and, thus, reduction of recolonization capacities. However, deltamethrin had no effect on orientation behavior toward aphid-infested plants for adults that survived a residual exposure to the insecticide. The impact of deltamethrin on recolonization via pupal emergence and interest in the methodology used are discussed.

Keywords—Sublethal effect Potential fecundity Insecticide Toxicity Behavior

INTRODUCTION

Deltamethrin is a synthetic pyrethroid insecticide widely used in the field and valued for its residual activity and high toxicity to terrestrial invertebrates [1]. The registration status of deltamethrin was modified when the European Commission Regulation Amendment 1972/99 requested a re-evaluation of the effects on nontarget animals (including arthropods) of this active ingredient (a.i.) [2] for inclusion in Annex 1 of directive 91/414/EEC [3]. Moreover, identification of effects on nontarget arthropods is of primary importance, because the use of natural enemies in integrated pest-management programs is increasing to limit environmental pollution by pesticides. Many natural enemies of phytophagous insects are hymenopterous parasitoids. They are characterized by a parasitic larval development causing the death of the host. Among pest mortality factors, insect parasitoids cause the strongest mortality [4] and, therefore, are important organisms for biological control.

In crops generally, parasitoids can be exposed to pesticides through direct exposure to spray droplets or to residues on the crop foliage when foraging for hosts [5] or through dietary exposure through feeding on contaminated water droplets, nectar, or honeydews [6]. Indirect exposure during development in the host also can occur [7]. However, several authors have reported that aphid parasitoids could be protected against insecticides during the pupal stage in the mummified host aphid [8,9]. Generally, applications of chemical insecticides result in a major initial reduction in the density of phytophagous and parasitoid populations. Deltamethrin treatment resulted in a 90% reduction in the number of parasitoids [10]. When recolonizing the crop after insecticide treatment, parasitoids return to depleted areas (where insecticide treatments have been made) from undepleted surroundings [10] via the immigration process. In addition, recolonization may occur within the treated crop by parasitoids emerging from exposed mummies (pupal emergence process). In both cases, parasitoids are exposed to insecticide residues (in mummies during treatment or when walking on treated leaves).

Acute toxicity endpoints alone do not provide a sufficient basis for assessment of the effects of insecticides on natural populations of nontarget organisms [11]. Sublethal effects must be assessed, because they can result from residual or direct exposure to insecticides in the environment. These effects are particularly expected in the behavior of insects exposed to insecticides, both because the majority of these insecticides are neurotoxic [12] and because multiple sublethal effects on parasitoids after exposure to synthetic insecticides have been reported [13–15].

Pyrethroids like deltamethrin are neurotoxic insecticides [16] and, therefore, can potentially perturb behaviors such as host searching. Deltamethrin at a sublethal concentration has been shown to increase the arrestment behavior of treated *Trichogramma* males responding to female pheromones [17]. *Trissolcus basalis* females exposed to a low concentration of this insecticide reduced their walking speed and the time spent on host patches [18]. However, to our knowledge, other sublethal effects, such as orientation behavior, which is particu-

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larly important to host and host–plant localization, have not been investigated.

The aim of the present work was to provide a multistep bioassay to assess lethal and sublethal toxicities of deltamethrin on aphid parasitoids. The effects of deltamethrin were assessed experimentally at the following crucial steps in the recolonization via the pupal emergence process after treatment: Parasitoid pupal development and emergence from mummies, adult survival, and host-searching capacity. The toxicity of deltamethrin when applied on mummies was evaluated, as was the subsequent impact on longevity of parasitoids emerging from these mummies. The toxicity of deltamethrin residues applied on oilseed rape leaves was assessed on adult parasitoids. Finally, the impact of deltamethrin was investigated on the host-searching behavior of parasitoids surviving exposure to increasing concentrations based on a previously validated method using a four-arm olfactometer [14,15].

Species of the Aphidiinae subfamily are indicator organisms that have been selected for the evaluation of chemical product toxicity on nontarget arthropods [19,20] as part of the registration requirement [3]. Thus, we worked on the Aphidiinae species *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae). The oilseed rape was considered given its importance in Europe and frequent treatment by deltamethrin [21]. The aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae) was selected because it has been a serious pest of oilseed rape since 1996 [22] and is parasitized by *D. rapae* in that crop [23].

MATERIALS AND METHODS

Insects

All insects were reared in environmental chambers at 23 ± 1°C (mean ± standard deviation throughout) under a 18:6-h light:dark photoperiod. *Myzus persicae* was reared on broad bean plants (*Vicia fabae* L.); *D. rapae* was reared on *M. persicae* transferred on *Brassicae napus* leaves (field-collected parasitoids were incorporated yearly in the laboratory strain). At the mummy stage, parasitized aphids were removed from the leaves and kept individually in plastic Petri dishes until emergence of adults. Adult females were mated at emergence and then stored in groups of five in glass tubes (5 × 1 cm) for 24 h. During this time, they were supplied with a dilute honey solution in water (80%). The female parasitoids used for all experiments were 24 to 48 h old. The females had never been in contact with plants or aphids before the experiments and were used only once.

Experiment 1—Effects of deltamethrin on parasitoid emergence and longevity

Oilseed rape leaves bearing mummified aphids parasitized by *D. rapae* (mummies of uniform age, 2–3 d) were carefully attached on rectangular glass plates with double-sided sticky tape. The glass plates with leaves were thus treated with a formulated deltamethrin used for application in crops (emulsifiable formulation, 25,000 mg a.i./L; Decis micro®; Bayer Crop Science France, Lyon, France). Insecticide was applied using a Burgerjon-type Potter tower (National Institute for Agricultural Research, Versailles, France), producing a deposit of 1.69 ± 0.1 μl/cm^2. The sprayed volume was kept constant (4 ml). Four concentrations of the insecticide were tested: 0.5 g a.i./ha (corresponding to a residual deposit), 5 g a.i./ha (frequent field application rate in oilseed rape), 6.25 g a.i./ha (field application rate against *M. persicae* in oilseed rape), and 50 g a.i./ha (10-fold the field rate). Water-sprayed leaves were used as controls. After each spray, the apparatus was carefully washed with 70% ethanol and then with water. Two hours after the insecticide application, treated mummies were removed from the leaves and placed individually in gelatin capsules to check rapidly for emergence (because of both small size and transparency). Mummies were kept at 20 ± 1°C and 65% ± 5% relative humidity until emergence. For each tested concentration (and water control), seven replicates of one leaf bearing 10 ± 1 mummies were made. The mummies were observed twice a day, and the percentage of emergence was calculated for each group. Then, emerging adults were placed individually in Petri dishes (diameter, 5.3 cm) with access to food (dilute honey solution, 80%). The parasitoids were observed twice a day, and the longevity in days was calculated for each group.

Experiment 2—Toxicity of deltamethrin on leaves

Diaeretiella rapae females were exposed to deltamethrin residues on leaves. The insecticide was applied to leaves using the Burgerjon-type Potter tower. The concentrations were those used for the mummy treatment. Water-sprayed leaves served as controls. The exposure units, slightly modified from those developed by Jansen [9] and recommended by Mead-Briggs et al. [24] for ecotoxicological tests on Aphidiinae, were assembled as follows: Each unit consisted of two deltamethrin-treated leaves placed on glass plates, facing each other, and separated by a transparent plastic ring (diameter, 5 cm; height, 2 cm) to form an enclosed arena. The parasitoids were contained in this arena and exposed to the dried residues on the treated leaves. The base and ceiling of each arena consisted of the glass plate plus treated leaves, with the leaves facing inward, into the enclosed arena. Rubber bands were placed around the upper and lower rims of the ring to improve the seal with the glass. The ring was pierced with four holes. Two were used to feed the insects, one with water and one with a dilute honey solution (80%) offered continuously on two pieces of cotton wool. The two remaining holes were covered with fine nylon gauze for ventilation. The leaves were sprayed and allowed to dry for 1 h before being used. Ten parasitoids were introduced per unit, with five to seven replicates per concentration and control. After 24 h, the dead parasitoids were counted. Pesticide exposure was performed at 20 ± 1°C and 65% ± 5% relative humidity under a 12:12-h light:dark photoperiod.

Experiment 3—Effects of deltamethrin on orientation behavior

Parasitoid exposure to pesticide. In this experiment, the potential sublethal effects of pure deltamethrin (not the formulated product) were addressed, because it has known, specific targets in the nervous system and facilitates reproducible exposure on glass. Because adjuvants are thought to kill insects through suffocation at the time they are sprayed [25,26], the use of pure deltamethrin versus formulated product should not bias results of residual exposure effects on specific behavior. The a.i., deltamethrin (certified purity, 98%), was provided by Cluzeau InfoLabo (Sainte-Foy-la-Grande, France) in crystalline form. A preliminary experiment was run to determine the range of concentrations, by exposing insects to deltamethrin at decreasing concentrations from the recommended field application rate, until mortality rates lower than 100% were ob-

served. Then, adult parasitoids were exposed to four concentrations, increasing by a factor of two, which provided levels of mortality ranging from 0 to 80% (concentration range, 0.29–2.34 ng/cm^2).

Acetone solutions of deltamethrin were applied to the inner surface of glass tubes (length, 9.3 cm; diameter, 2.3 cm; internal surface, 67.4 cm^2). Pure acetone was used as control. To obtain a homogeneous deposit, 200 μl of solution were introduced using a Microman® pipette (Gilson, Middleton, WI, USA), and the tube was then manually rotated until no more droplets were seen on the glass wall, which allowed a total coverage of the internal surface of the tube. Tubes were left for 1 h at room temperature to ensure complete evaporation of the acetone before introducing parasitoids. Both the internal surface of the tubes and the volume of solution were fixed; therefore, it was possible to express the quantity of insecticide residue per unit of surface (ng a.i./cm^2). Ten parasitoid females were introduced per tube, with two drops of honey on a small plastic strip. Tubes were closed with a fine nylon gauze to allow air circulation. Exposure was performed at 15 ± 1°C and 65% ± 5% relative humidity under a 12:12-h light:dark photoperiod. Control mortality being higher in a glass tube than in the presence of foliage, the temperature was set at 15°C to reduce mortality in the control tubes. Thus, control mortality remained less than 10%, which is recommended as a validity criterion when evaluating insecticide toxicity [27]. We checked every hour for the first 10 h that parasitoids were active in the tubes. After a 24-h exposure period, the number of dead parasitoids was counted, and the survivors were collected and placed individually in Petri dishes (diameter, 5.3 cm). The behavioral tests were performed within 2 h following the end of exposure.

Behavioral tests. Oriented responses toward aphid-infested plant odor were investigated in a four-armed olfactometer. Pressurized and humidified air flowed into the central chamber through four arms (200 ml/min/arm) and was extracted from the center of the chamber so that four air fields of equal area were established. The odor source constituted of oilseed rape stems (*Brassica napus* var Goeland, kept in water), with a total of seven to eight leaves infested by *M. persicae* (400–500 aphids after 7 d of infestation). The device was placed on a light table providing a homogeneous fluorescent light (800 lux) in a room at 70% relative humidity and 25°C (a temperature commonly used to study insect olfactory responses in laboratory devices). Previous experiments [14,15] indicated that this temperature ensures both active locomotion of aphid parasitoids and a high odor-release rate from aphid-infested plants. In the experiments, only one field of the olfactometer was odorized at a time. To deliver the odor into one of the four fields of the olfactometer, the corresponding arm was connected to an air-tight glass jar (height, 25 cm; diameter, 11 cm) containing the odor source.

A female parasitoid was introduced into a vial connected to the center of the four-armed olfactometer, from which it could walk out freely. Observations started when the female freely entered the chamber, which occurred 12.4 ± 3.6 s after introduction into the olfactometer, and lasted for 1 min. That time was sufficient to observe a statistically significant attraction to the aphid-infested plant odor [14,15]. The position of the female (fields numbered clockwise from one to four) was recorded continuously on a computer using Observer event-recorder software (Noldus Information Technology, Wageningen, The Netherlands) to compute the overall time spent in each field. After approximately 20 individual observations, the olfactometer was carefully washed with ethanol, and the position of the odorized field was changed. On each day of an experiment, females exposed to the different deltamethrin concentrations were tested in a randomized order. Sample sizes are reported on the figures.

Additionally, to confirm the results obtained with *D. rapae*, the behavior of another Aphidiinae aphid parasitoid species, *Aphidius matricariae* Haliday (Hymenoptera: Braconidae), was studied using the protocol described above. This species also occurs on *M. persicae* on *Brassica* leaves [23].

Data analysis

A Mann–Whitney test with Bonferroni adjustment was used to compare mortality and longevity between groups to evaluate the following: Toxicity of deltamethrin to adults on sprayed leaves, impact of deltamethrin on emergence when mummies were sprayed, and effect of deltamethrin on longevity of emerged individuals from sprayed mummies. A chi-square test was used to compare the percentage of individuals that died during the 48 h following emergence from deltamethrin-treated mummies and the control. Analysis was performed on this postemergent period, because the highest oviposition rate of Aphidiinae aphid parasitoids occurs at the beginning of adult life [28,29] and, thus, mortality at this age might impair parasitoid fitness more than at a later age.

To evaluate the impact of insecticide exposure on orientation behavior, three statistical analyses were carried out. First, Friedman analysis of variance on ranks was performed for each experimental situation. The Friedman test statistic, if significant, allows rejection of the null hypothesis (equal time spent in each field of the olfactometer). Second, to investigate the existence of a concentration–response relationship, a logistic regression of the percentage of time spent in the odor as a function of deltamethrin concentration (at which parasitoids were previously exposed) was carried out [15]. In this regression, the deviation of the observed data is calculated relative to a linear model under the assumption that no linear concentration–effect relationship exists. Third, to detect any effect of a given concentration, the Kolmogorov–Smirnov statistic was used to compare the time spent in the odorized field between the control group and each treated group. All statistical analyses were performed using S-Plus software (Insightful, Seattle, WA, USA).

Two indices based on results from experiments 1 and 2 also were estimated to obtain a quantified toxicity assessment of deltamethrin on aphid parasitoids, taking into account the different possibilities of mortality and effects. The first index, the reproductive potential, was based on the study by Hag Ahmed [29], who reported the age-specific fecundity of *Aphidius* spp. We estimated the number of eggs laid per day in the control and used this to evaluate the reduction of reproductive potential as a function of the longevity of parasitoids from treated mummies. This was undertaken to evaluate the number of eggs potentially laid per day as a function of the percentage of live individuals. The results were analyzed by comparing the average number of total eggs laid per female in groups exposed to deltamethrin and in the control group using a Wilcoxon sign-rank test.

The second index, the population survival index, expressed the percentage of individuals that would be able to recolonize the treated crop via the pupal emergence process. It resulted from the multiplication of four values. The first value consid-

ered was the percentage of mummified parasitoids, this stage being the only one surviving direct field spraying [10]. This value was fixed at 0.2 (for any given time, 20% of the Aphidiinae population is at the mummy instar [30]). The second was the percentage of emergence from the mummies. The third was the percentage of adults that survived more than 48 h (adults that survived contact with their contaminated mummy). The last value considered was the percentage of adults that survived residual exposure on leaves, such exposure being highly probable when treatment occurs at the mummy stage.

RESULTS

Experiment 1—Effects of deltamethrin on emergence and longevity

Percentage of *D. rapae* adult emergence significantly decreased only after exposure to the strongest concentration of deltamethrin (i.e., 50 g a.i./ha) when compared to the control group. At the other concentrations, including those used in the field with oilseed rape, the emergence percentage was not affected by exposure at the mummy stage (Fig. 1A). The longevity was significantly and equally reduced by all concentrations (Fig. 1B).

After emergence, 2.9% of individuals died during the first 48 h in the control group (Fig. 2). Slightly more individuals died during this period when emerging from mummies treated with 0.5 and 5.0 g a.i./ha of deltamethrin, with mortality of 8.6 and 8.0%, respectively; this difference was not significant. When emerging from mummies treated with the 6.25 g a.i./ha concentration or with the strongest concentration of deltamethrin (50.0 g a.i./ha), significantly more individuals died than in the control group (24.0 and 17.3%, respectively).

Experiment 2—Toxicity of deltamethrin on leaves

All concentrations of deltamethrin tested on leaves were toxic to *D. rapae* adults (Fig. 3). Indeed, all concentrations induced significant mortality compared to the control group. Moreover, mortality increased significantly across the range of concentrations tested.

Experiment 3—Effects of deltamethrin on orientation behavior

For the olfactometer tests, the concentrations used induced 0.4% ± 3.0% to 79.4% ± 6.2% of corrected mortality in *D. rapae* and 13.1% ± 2.5% to 72.7% ± 4.1% of corrected mortality in *A. matricariae* (Fig. 4). All groups exhibited a significant attraction toward the aphid-infested plant odor (Friedman analysis of time allocation to the four fields). The relative time spent in the odorized field by *D. rapae* and *A. matricariae* females exposed to increasing concentrations of deltamethrin (Fig. 4) was not significantly different from that of control females. The logistic regression of the percentage of time spent in the odor as a function of the pesticide concentration was not significant. Statistical results of the three analyses for the two species are reported in Table 1.

Reproductive potential and population survival index

The reproductive potential of *D. rapae* (Fig. 5) was significantly reduced for parasitoid populations exposed to all the different concentrations of deltamethrin at the mummy instar ($p < 0.05$). The number of eggs potentially laid by a female during her entire life was approximately 209, 170, 178, 153, and 122 for the control group and those exposed to 0.5, 5.0, 6.25, and 50 g a.i./ha, respectively. Thus, percentages of reproductive potential were 81, 85, 73, and 58% for the 0.5, 5.0, 6.25, and 50 g a.i./ha groups (relatively to the control), respectively. Thus, a strong effect of longevity reduction by deltamethrin on reproductive capacities of aphid parasitoid females is predicted.

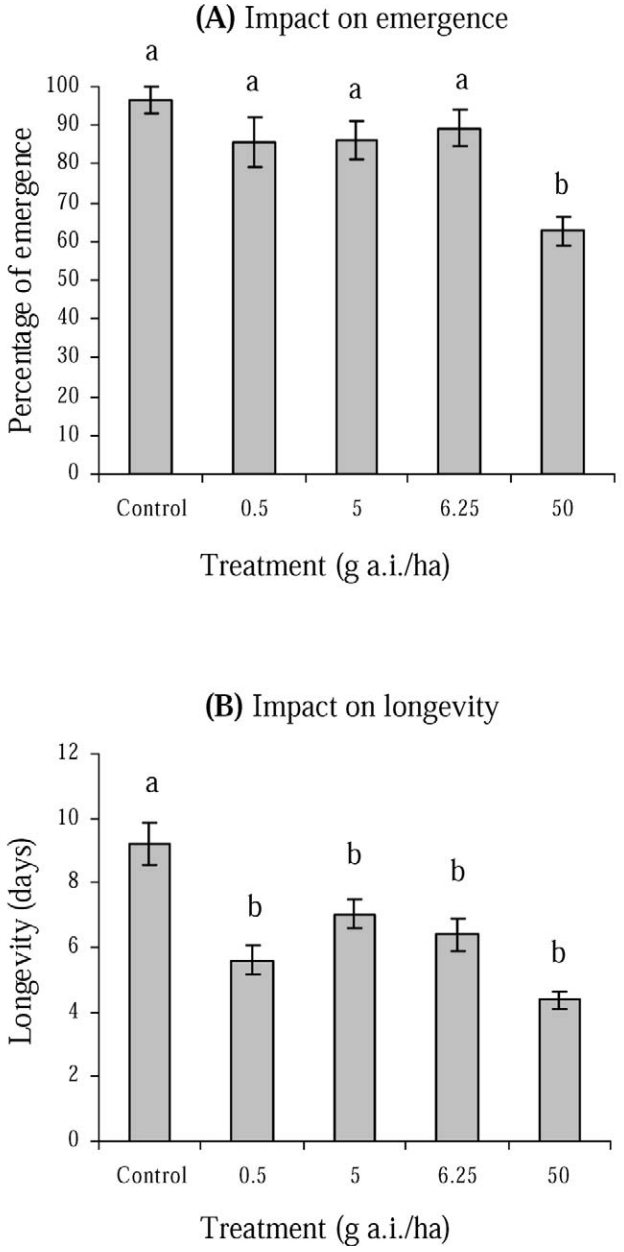

Fig. 1. Effect of four concentrations (g active ingredient [a.i.]/ha; water used for control) of deltamethrin (commercial formulation; Decis micro®; Bayer Crop Science France, Lyon, France) on (**A**) mean percentage of emergence (± standard error [SE]) and (**B**) mean longevity in days (± SE) of emerged parasitoids. Values with the same letter are not significantly different at the $p < 0.05$ level (Mann–Whitney test with Bonferroni adjustment method).

The population survival index indicated a high toxicity of the different concentrations of deltamethrin for the aphid parasitoids. Indeed, in the pupal emergence process, during which mummies acted like a reservoir for the parasitoid population, approximately 13, 9, 3, and 0.2% of the entire population would survive after deltamethrin application at concentrations of 0.5, 5.0, 6.25, and 50 g a.i./ha, respectively, based on the present experimental conditions.

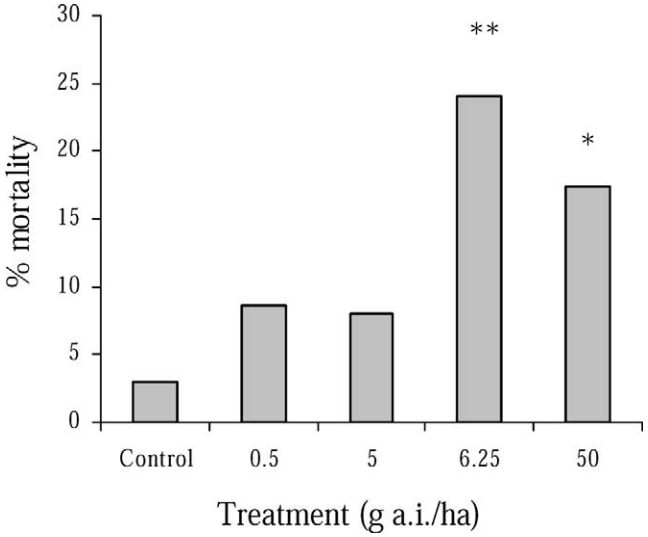

Fig. 2. Percentage of individuals dying during the first 48 h after emergence from deltamethrin-treated mummies (four concentrations in g active ingredient [a.i.]/ha; water used for control). A chi-square test with Yates correction was used to compare concentration results to the control (n = 47–73; *$p < 0.05$, **$p < 0.01$).

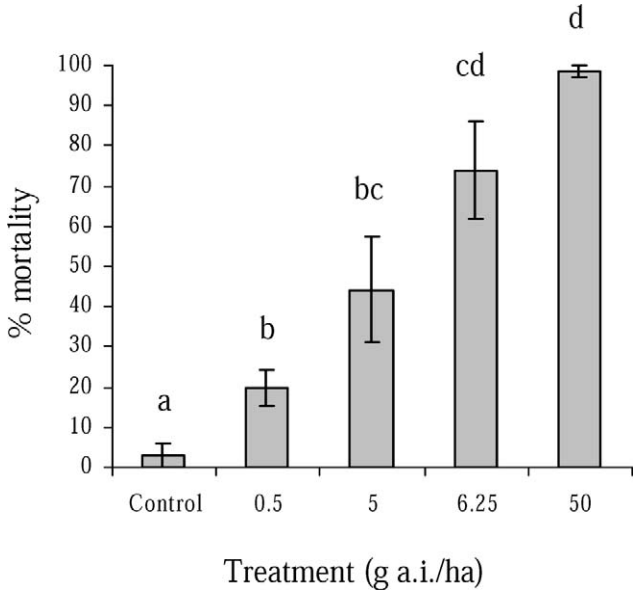

Fig. 3. Toxicity of four concentrations (four concentrations in g active ingredient [a.i.]/ha; water for control) of deltamethrin (commercial formulation; Decis micro®; Bayer Crop Science France, Lyon, France) on leaves for *Diaeretiella rapae* adults. Mean percentage (± standard error) of mortality (mean of 5–7 replicates, n = 10 per replicate). Values followed by different letters are significantly different at the $p < 0.05$ level (Mann–Whitney test with Bonferroni adjustment).

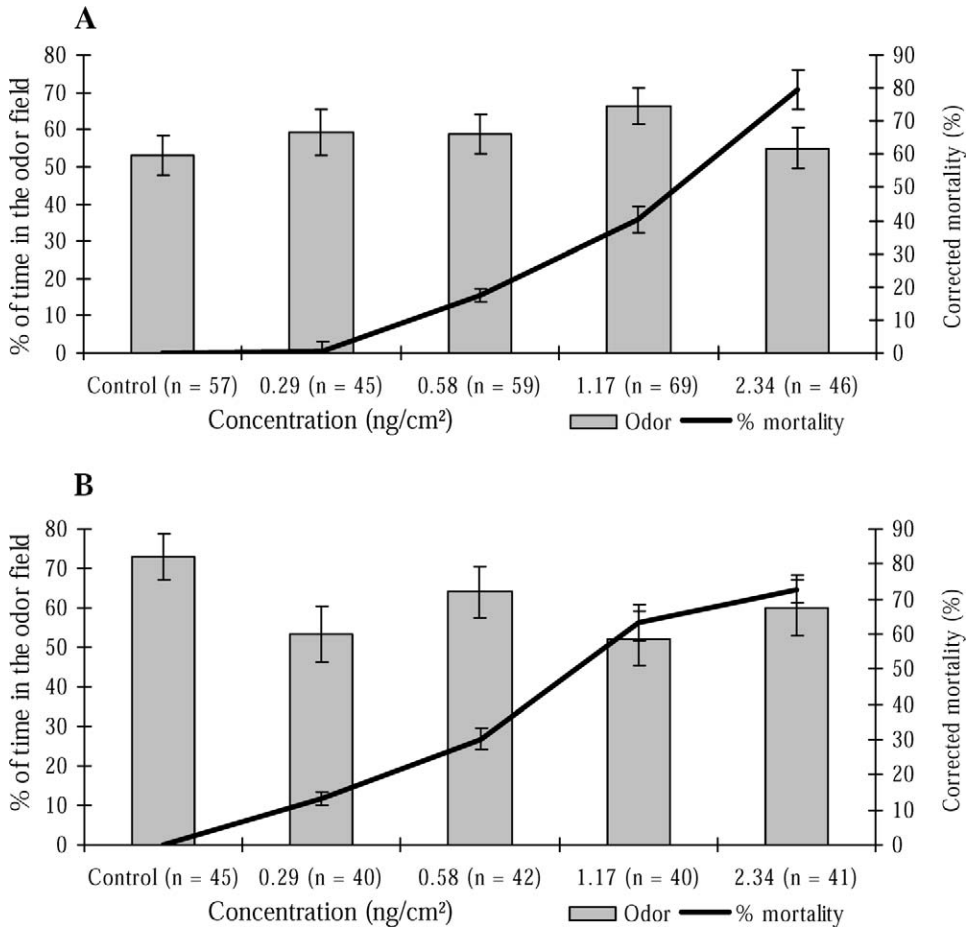

Fig. 4. Effect of four concentrations (ng/cm²) of deltamethrin on the orientation behavior of (**A**) *Diaeretiella rapae* and (**B**) *Aphidius matricariae* females observed at the end of the exposure. Percentage of time (± standard error [SE]) spent in the odorant flow field after 1 min of observation (gray bars) is shown. The corrected mortality (± SE) associated with each concentration is represented by the black curve.

Table 1. Statistics from the Friedman (null hypothesis equal time spent in each field of the olfactometer), Kolmogorov–Smirnov, and logistic regression tests used to compare time allocation in the four fields of the olfactometer, to compare the time spent in the odorized field between the control group and each treated group (null hypothesis no different from the control), and to test for a concentration–response relationship (null hypothesis no relationship), respectively

Friedman	Control	0.29 (ng/cm²)	0.58 (ng/cm²)	1.17 (ng/cm²)	2.34 (ng/cm²)
Diaeriella rapae					
F	29.26	36.26	48.48	76.09	29.13
p	<0.001	<0.001	<0.001	<0.001	<0.001
Aphidius matricariae					
F	58.47	18.74	35.63	27.95	22.18
p	<0.001	<0.001	<0.001	<0.001	<0.001

Kolmogorov–Smirnov	0.29 (ng/cm²)	0.58 (ng/cm²)	1.17 (ng/cm²)	2.34 (ng/cm²)	Logistic regression	
D. rapae						
Z	0.13	0.10	0.20	0.12	Chi-square	0.07
p	0.62	0.76	0.11	0.74	*p*	0.79
A. matricariae						
Z	0.27	0.24	0.25	0.19	Chi-square	0.51
p	0.07	0.12	0.10	0.21	*p*	0.48

DISCUSSION

The present study demonstrated a high risk of deltamethrin treatments for aphid parasitoids that may recolonize the treated crop when emerging from the mummies (pupal emergence process). When mummies were sprayed, deltamethrin induced a decrease in the longevity of emerged individuals but a reduction of emergence from the mummies only when exposed to the highest (and nonrealistic) concentration (50 g a.i./ha). Furthermore, approximately 20% of adults died during the first 48 h when mummies were sprayed at 6.25 g a.i./ha (recommended field rate) or more. High percentages of mortality also were shown for parasitoid adults that walked on treated leaves. The estimation of the reproductive potential and population survival index permitted the prediction of a low recolonization by parasitoids from the pupal emergence process because of high mortalities at the different levels and reduction of longevity. Nevertheless, no effect of deltamethrin on orientation behavior toward aphid-infested plants was observed when adults survived a residual exposure. Indeed, after exposure to deltamethrin residues for 24 h, the survivors did not exhibit modification of time spent in the odor field (also indicating that their capacities for locomotion were not affected), and females could still detect aphid-infested plants.

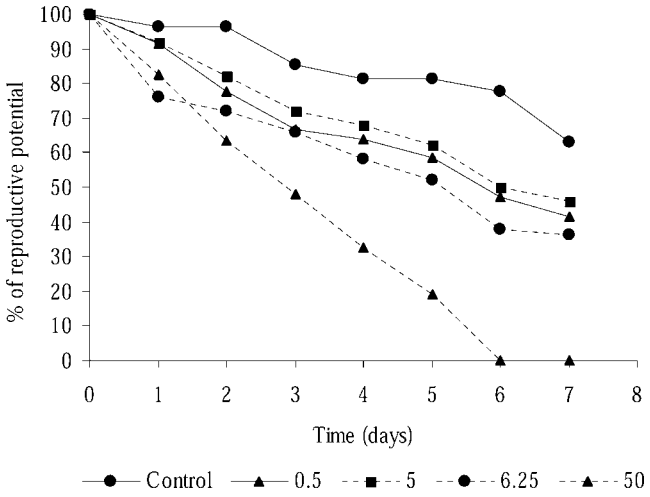

Fig. 5. Impact of four concentrations of deltamethrin (g active ingredient [a.i.]/ha; water for control) on the reproductive potential of *Diaeretiella rapae* females after emergence from sprayed mummies. The reproductive potential is expressed as a percentage of the maximum reproductive potential (no mortality) and is estimated from the number of eggs laid over time [38] and daily mortality.

Effects on pupal development and emergence

In line with other studies, we found no evidence of reduced emergence after spraying realistic concentrations of deltamethrin [8,9]. Indeed, Starý [31] reported that the pupal stage within a mummified aphid was the least susceptible to insecticides. Nevertheless, in agreement with these authors, our results showed a reduction in longevity after exposure to deltamethrin at the mummy stage. The longevity of emerging individuals was reduced in all groups exposed to deltamethrin by approximately 30 to 50%. Moreover, approximately 20% premature mortality was observed at a concentration of 6.25 g a.i./ha. Premature mortality would result from exposure by contact with the outside, treated surface of the mummy at the time of emergence. Such a critical period of intoxication in the case of mummy treatment was reported previously for organophosphates [32] and for deltamethrin [8]. Indeed, emerging adults cut the mummy with their mandibles and examine it by walking and antennal tapping [33]. This premature mortality can have an important impact on population fitness and parasitic efficiency, both because parasitoids of the Aphidiinae family are pro-ovigenic (full complement of mature eggs at emergence) and because the number of eggs laid per day is maximum during the first 2 d after emergence [28,29] and decreases according to a Poisson curve.

Effects on adult survival

When the toxicity of deltamethrin for *D. rapae* adults on oilseed rape leaves was estimated, a 20% mortality was found after exposure to the concentration corresponding to a residual deposit that can be observed within a few days after oilseed rape treatment (0.5 g a.i./ha) [34] and 44 to 74% mortality for field concentrations (5.0 and 6.25 g a.i./ha). In agreement with

Mahaut and Deleu [35], mortality was lower when deltamethrin residue was on leaves compared to mortality observed on glass. One probable explanation is that deltamethrin was absorbed into the waxy layer of the oilseed rape leaf cuticle as a result of its very high lipophilicity (log K_{ow} = 6.5 [36]).

Effects on parasitoid behavior

Concerning the orientation behavior of adults, an effect of deltamethrin exposure was anticipated, because this insecticide was reported previously to disturb both sensory perception and motor functions in insect parasitoids [17,18]. That was not the case in the present study or in a previous study [37], in which oviposition behavior of *D. rapae* and *A. matricariae* on aphid-infested plants and patch-time allocation were not disturbed following exposure to a similar range of deltamethrin concentrations. Greater vigor in the surviving insects [38] may explain the lack of effect on orientation behavior of the deltamethrin concentrations used in the present study. Vigor may vary as a function of characteristics that were not explicitly controlled in our experiment, such as size, stress, and general physiological state. Alternatively, the olfactometer tests were run at 25°C, which might have reduced deltamethrin toxicity, because it also was reported for another insecticide from the pyrethroid family [39]. However, another study demonstrated that for deltamethrin, the toxicity on insects did not decrease when temperature increased from 17 to 27°C [40]. Our results show that at 25°C, at which host-searching activity is high, intoxication after exposure to deltamethrin residues will not affect olfactory orientation to host-infested plants.

Overall impact on recolonization through mummies

The present study demonstrates that even if the mummy stage is more protected from deltamethrin compared with the adult stage, recolonization from the pupal emergence process may be limited by side effects of deltamethrin on longevity, adult survival, and thus, potential fecundity. From the estimation of the population survival index that included mortality in the treated mummy, mortality during mummy examination by emerged parasitoids, and the residual deltamethrin effect on adults, a strong effect of deltamethrin treatment on emergent aphid parasitoids should be expected. From our data with two recommended field application rates (5.0 and 6.25 g a.i./ha), only 9 and 3%, respectively, of the parasitoid populations would be able to recolonize the treated field through the pupal emergence process. Moreover, we estimated the reproductive potential of the remaining parasitoid females at 73 to 81% relative to the value of control females, leading to a decrease of the recolonization potential of these individuals.

Exposure conditions to deltamethrin used in our laboratory multistep bioassay may overestimate the toxicity. Mummies could be more or less protected by the plant canopy in realistic field conditions [41], and adults were exposed to fresh residues. However, the age of residues and, hence, their toxicity will depend on the time elapsed between the treatment and the emergence of adults. Therefore, measurement of these indices under seminatural or actual field conditions would enable us to establish if toxicity was overestimated under laboratory conditions or to confirm the effects of deltamethrin on pupal emergence process.

The hypothesis about the effects of deltamethrin on recolonization via immigration process might be stated from present and previous studies of the effects of deltamethrin on aphid parasitoids [34,37]. During that process, parasitism efficiency might not be reduced because of unaffected key behaviors, such as olfactory orientation toward hosts (present study) and oviposition [37]. Moreover, an establishment of immigrant parasitoids into a deltamethrin-treated field 1 d after the treatment has been demonstrated [34]. Thus, it could be hypothesized that recolonization of a field via the pupal emergence process may be minimal in the global recolonization process.

Relevance of the multistep bioassay to assess lethal and sublethal effects of pesticides

Current standard assessment methods are based on acute median lethal concentrations [20,42], which may not reflect the global toxicity of pesticides [11] because of lacking detection of multiple sublethal effects. A solution would be to use a demographic approach to estimate toxicity [11]. However, that method presents some limitations, because a lack in detecting perturbations of crucial behaviors, such as long-range detection of host-infested plants by parasitoids, likely results from the small caging conditions used. The use of a multistep bioassay to evaluate the potential effects of an insecticide on parasitoids can help to assess toxicity in a more complete way by including evaluation of pesticide effects on various crucial behaviors of the parasitism process rather than only considering a mortality endpoint. It can be useful in both regulatory assessment of pesticides and pesticide screening in integrated pest-management programs because of the feasibility under laboratory conditions. The results presented here allow calculation of indices predicting recolonization potential from emerging parasitoids after a pesticide treatment, and they contrast with the general assumption that mummies constitute a good reservoir for parasitoid populations following a treatment [8,9]. The proposed procedure is applicable to various aphid parasitoid species, which is important because the Aphidiinae subfamily is one of the two entomophagous families selected as indicators for evaluating the toxicity of new pesticide products on nontarget arthropods before registration [19]. Finally, the method is rather simple to carry out, is easily standardized, and uses low-cost devices.

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