Effects of deltamethrin on the specific discrimination of sex pheromones in two sympatric *Trichogramma* species

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ABSTRACT

The large amounts of insecticides used for crop protection lead to widespread environmental pollution. Determination of the potential impacts induced by this contamination on key species involved in the equilibrium of ecosystems is therefore a necessity. In this study, we tested the effects of a pyrethroid insecticide, deltamethrin, on the capacity of males from two sympatric *Trichogramma* species to discriminate the sex pheromones emitted by females of their own species (*Trichogramma* are parasitoids of Lepidopterous). The impact of an acute exposure as could occur at field edges was evaluated using a dose inducing 20% mortality (LD 20). The impact of a low exposure corresponding to diffuse environmental pollution was evaluated by applying an LD 0.1 (a dose inducing no apparent mortality).

For *T. semblidis*, deltamethrin decreased the specific recognition of sexual pheromones at the higher dose (LD 20) but had no effect on this recognition at the lower dose (LD 0.1). However, deltamethrin decreased the saturation of pheromone receptors at both doses. For *T. evanescens*, deltamethrin increased the recognition of sexual pheromones at both doses, though not during the same period of observation (at the beginning for the LD 20, at the end for the LD 0.1), but it did not decrease the saturation of the pheromone receptors. These differing results were analyzed considering the behavior of the insects, their level of sensitivity to the insecticide and its mode of action. They provide new insights regarding possible consequences of environmental pollution by insecticides on functional biodiversity.

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1. Introduction

When a crop is sprayed with pesticides, studies have shown that 9.5% of the sprayed pesticides enter the atmosphere through drift or volatilization and can therefore be found in all environmental compartments outside treated areas (De Jong et al., 2008). Although this contamination is more pronounced in areas of intensive use of pesticides, such as agricultural crop areas and their peripheries, it can also affect areas much farther away. These more distant areas, which are believed to be uncontaminated because they are not treated, can harbor natural populations with an essential reservoir function that will therefore be exposed to low doses of pesticides (De Jong et al., 2008).

Conventional toxicological screening and testing methods are useful for detecting overt toxic effects of chemicals, but are generally poor with respect to exploring more subtle effects, such as changes in behavioral responses. However, neurotoxic insecticides, by disrupting

the transmission of nerve impulses in the nervous system, can lead to the death of insects, but they can also cause many other effects without necessarily being lethal (see Desneux et al., 2007 for a review). These effects will affect the behaviors of insects because of their dependence on their nervous system for perception of stimuli, transmission of this perception to the central nervous system, elaboration of a response to the perceived stimulus, and finally, execution of the behavior chosen according to the perceived stimulus (Vosshall and Stocker, 2007). Therefore, beyond the mortality induced by insecticides, these insecticides may impact wildlife health and the equilibrium of ecosystems through an action at sublethal level (Desneux et al., 2007). Pyrethroids are the second most widely used family of insecticides (organophorus being the first, EC, 2007, U.S. EPA, 2011). Deltamethrin (Pyr.), the insecticide tested in this study, is used on many crops (e.g., cereal, corn, crucifer, artichoke, asparagus, beet, salad, tomato, pepper, potato, apple, pear, peach, grape, rice, peas, and onion, cf. Couteux and Lejeune, 2009). It is a neurotoxic insecticide that interferes with the transmission of action potentials along neurons (Soderlund, 2012).

In the wild, insects come into contact with a great variety of olfactory signals. It is of vital importance for them to be able to

distinguish among all of these signals and to differentiate those associated with their life history traits (Ha and Smith, 2009). Specific discrimination of sex pheromones is one of these vital traits because it is an important step in reproduction (Ayasse et al., 2001). *Trichogramma* are oophagous parasitoids. They are key species because they control populations of other insects, including numerous pest species, and they play an important role in the equilibrium of ecosystems. Furthermore, they are beneficial insects and are regularly used for biological control. *Trichogramma* attack over 400 pest species, most of which are Lepidopterous (Li, 1994).

Obtaining a better understanding of the impact of pesticides on beneficial insects, such as parasitoids, is a necessity. This impact must be assessed against a background of acute exposure of beneficial insects that can occur in open fields or field edges, such as during treatment of crops, and that will correspond to exposure to high doses. This impact must also be assessed in the context of the diffuse environmental pollution that can reach ecosystems very distant from treated areas and will, therefore, correspond to exposure to much lower doses that do not necessarily cause any apparent mortality.

In this study, we tested the sublethal effects of deltamethrin on the capacity of males from two sympatric species, *Trichogramma semblidis* and *T. evanescens*, to discriminate between sex pheromones emitted by females from these species. Males were tested following exposure to two different doses of insecticide, one of which was relatively high and induced 20% mortality (LD 20) and rather corresponded to possible exposure in the vicinity of treated areas, while the other dose induced no apparent mortality (LD 0.1) and corresponded to diffuse environmental pollution reaching ecosystems distant from treated areas.

2. Materials and methods

2.1. Chemicals, equipments and biological material

The insecticide deltamethrin (99% certified purity) was purchased from Cluzeau Info Labo (Sainte-Foy-La-Grande, France). The acetone (99% purity) was purchased from Merck Eurolab (Briare Le Canal, France). The camera (Canon N 50 camera with a 25 mm lens), the video card and the software Cible_p5 were purchased from Secad (Saint Martin du Fresne, France). The detergent Micro-90 was purchased from Bioblock Scientific (Illkirch, France). The *Ephestia kuehniella* eggs killed by UV radiation were purchased from Biotop (Livron-sur-Drôme, France)

2.2. Insects

Strains of two sympatric *Trichogramma* species, *T. evanescens* and *T. semblidis* (Hymenoptera, Trichogrammatidae), kindly provided by B. Pintureau (INRA, Lab. Biologie Fonctionnelle, Insectes et Interactions, Lyon, France), were used for the experiments. These strains were established from insects captured in the vicinity of Lyon (France) at Monsols and Vénissieux, respectively. The insects were reared at 20 °C under a 12L:12D photoperiod on the eggs of a factitious host, *Ephestia kuehniella* (Lepidoptera, Pyralidae), killed by UV radiation.

2.3. Determination of lethal doses

E. kuehniella eggs infested with *Trichogramma* were individually (one egg per vial) isolated in glass vials (3 cm in length, 6 mm in diameter) containing a minute drop of honey to feed the insects when emerging. Approximately 24 h after their emergence, males and females were sexed and males were exposed to the insecticide. For this purpose, 3 μl of deltamethrin diluted in acetone were deposited on pieces of paper (2.2 cm × 4 mm). The 3 μl were deposited on the center of each piece of paper and spread across their surface by capillarity. The pieces of paper were left for 1 h on the lab bench to allow total evaporation of the acetone and then introduced into each vials containing the tested insects that were exposed individually to the insecticide (one insect and one piece of paper per vial). Papers on which pure acetone was deposited were used as controls. Mortality was determined after 24 h of exposure to the treated pieces of paper at 22 °C (contamination occurred via tarsal contact). To calculate regression lines for mortality, five solutions containing increasing concentrations of the insecticide were used, and 20 individuals were tested for each concentration. This test was performed on the males of each species. Mortality data were analyzed via probit analysis (Finney, 1971), and the theoretical 0.1 and 20% lethal doses (LD 0.1, LD 20) to be used for behavioral tests were then estimated by linear regression using the log-probit program of Raymond (1985). The insecticide dilutions were stored at 4 °C between experiments.

2.4. Video tracking of males

In *Trichogramma* species, virgin females deposit pheromones on their substrate that induce arrestment in males (Pompanon et al., 1997; Delpuech et al., 1998a, 1998b; Al Dabel, 2004). To study the specificity of these pheromones, cylindrical arenas 3 cm in diameter and 1 mm in height were used. The arenas were constructed from punched Plexiglas® sheets covered on both sides with a glass sheet.

The arenas were divided in half by a silicon bar (chemically inert). Inside each half arena, one virgin female (approximately two days old) of each species, was introduced and allowed to move around to mark the substrate with its pheromones. Thus, the pheromones of the *T. evanescens* female were deposited on one half of the arena and the pheromones of the *T. semblidis* female on the other half. After five minutes (a sufficient amount of time to enable marking of the entire half of the arena), both the females and the silicon bar were removed. A male was then placed in each arena, and its path was recorded for eight minutes using a computerized (PC compatible) video tracking device. In this test, the arena was placed under a circular fluorescent tube (daylight, 300 lx). Each image (25/s) obtained using a camera was digitized with a video card, and the coordinates of the insect were recorded using Cible_p5 software. The percentage of time spent by each male inside each part of the arena was then calculated. The male response was determined as the percentage of time spent by the male in the half of the arena marked by the sexual pheromones of its conspecific female. When this percentage is higher than 50%, it indicates a male preference for conspecific pheromones. When it is approximately 50%, it indicates the absence of a preference for either of the species pheromones.

In these experiments, insects were tested only once, and only insects that were able to walk were used (i.e., we did not use moribund insects) to limit interference between the effects of the insecticide and mobility capacity. Prior to being used, the glass sheets were washed with detergent (Micro-90, Bioblock Scientific, Illkirch, France) in an ultrasonic bath for one hour, then rinsed in distilled water and dried at 120 °C.

2.5. Effects of deltamethrin on the specific recognition of sex pheromones by males

For each species, the responses of males surviving the exposure to the insecticide (i.e., males exposed either to an LD 0.1 or LD 20 of deltamethrin using the method detailed in *2.2*

Determination of lethal doses) to conspecific pheromones (in a choice situation) were compared to the responses of control males. Insects were left into their exposure vials until they were tested for their response to pheromones, therefore the exposure time to the insecticide lasted from 22 h to 27 h. For all the tested insects, the testing of a control insect always followed the testing of a treated insect. The kinetics of the responses were plotted by calculating for each 30-s period the mean percentage of time spent by males in the area marked by conspecific pheromones during the 8 min of observation.

2.6. Statistical analysis

Student's *t*-tests were performed after arcsine (square-root) transformation of the percentages.

The responses of males to conspecific pheromones as a function of the time were analyzed by linear regression whose validity was tested by ANOVA after arcsine (square-root) transformation of the percentages (linearity test and comparison of the slope to 0). Regression lines of treated and controls were compared by testing the equality of their slopes. When the regression lines were found parallel (slopes not significantly different), their intercepts were compared by ANCOVA.

3. Results

3.1. Lethal doses

The theoretical doses that induce 20 and 0.1% mortality (LD 20 and LD 0.1, respectively) and their 95% confidence intervals are given in Table 1. These doses were used for testing the sublethal effects of deltamethrin on the responses of males to the female sexual pheromones of the two sympatric species.

3.2. Specificity of the recognition of sexual pheromones

For both *T. semblidis* and *T. evanescens*, the percentage of time spent by males in the area marked by conspecific pheromones was significantly greater than 50% under control conditions (means of 84% (SE: 1.69) and 62% (SE: 1.37) of time spent in the conspecifically marked area, respectively; $t=17.0$, $n=72$, $p < 0.001$ for *T. semblidis*, and $t=7.89$, $n=69$, $p < 0.001$ for *T. evanescens*). Therefore, the males of both species showed a clear preference for and recognition of conspecific female pheromones. However, the response of *T. semblidis* males was greater than that of *T. evanescens* males and therefore was more specific ($t=10.2$, $df=139$, $P < 0.001$).

3.3. Effects of deltamethrin on the recognition of conspecific sexual pheromones by treated males

3.3.1. Kinetics of T. semblidis
The responses of *T. semblidis* males to conspecific pheromones as a function of the time when the males were exposed to an LD 20 of

Table 1
Lethal doses of deltamethrin applied to the males of the two *Trichogramma* species.

	T. semblidis	*T. evanescens*
LD 20 (95% CI)	8.77 (0.18–17.3)	688 (305–1060)
LD 0.1 (95% CI)	0.496 (0.000–3.046)	15.8 (0.51–65.5)
Slope of the regression line (SE)	1.80 (0.686)	1.37 (0.296)

Mean LD 20 and LD 0.1 values in ng expressed as the quantity of active ingredient deposited per piece of paper. Slopes of the regression lines for mortality.
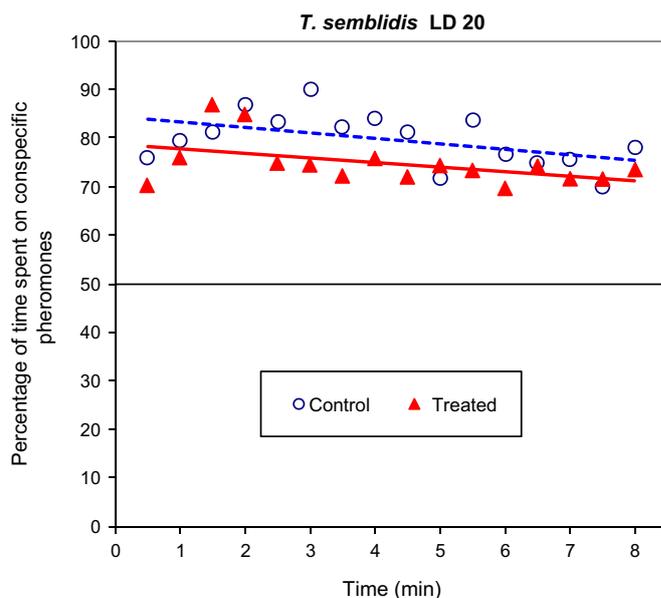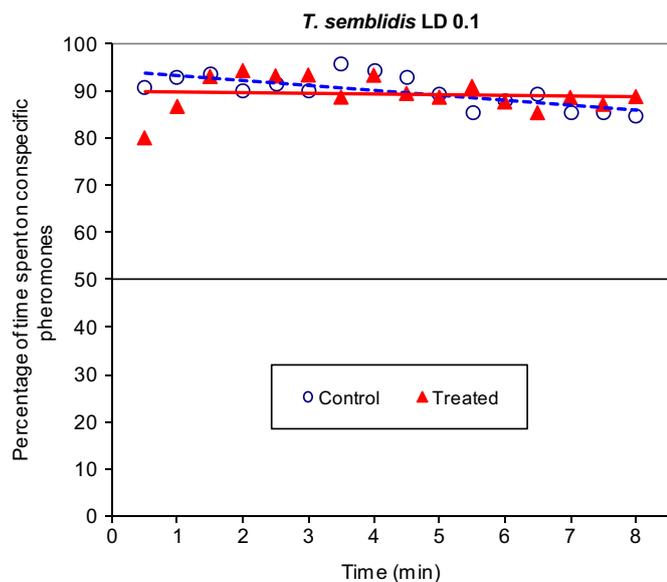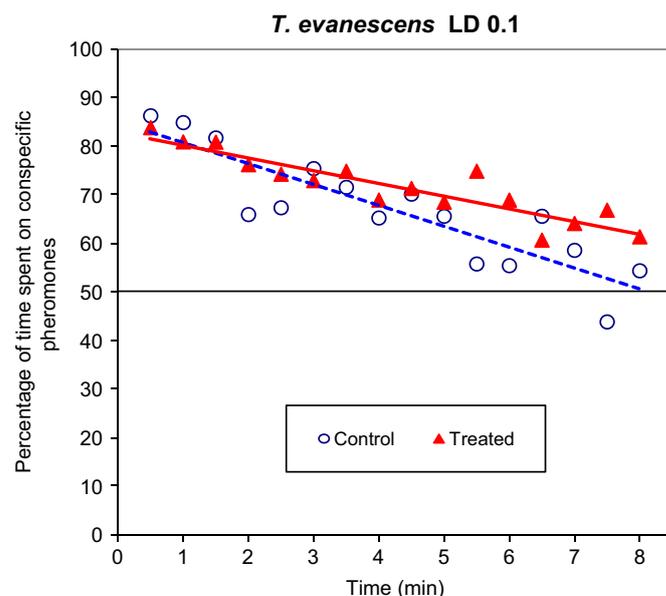

Fig. 1. Recognition of conspecific pheromones by *T. semblidis* males. Percentage of time spent by males in the area marked with female pheromones from the same species. Means calculated each 30 s for the males exposed to an LD 20 of deltamethrin (triangular dots, solid line) and the control males (circle dots, dashed line). Regression lines were calculated on means; 50% corresponds to an absence of recognition of conspecific pheromones.

deltamethrin and for controls are presented in Fig. 1. The linearity test shows that the distribution of observed values is not significantly different from a line, regression lines are therefore a good representation of the kinetics of these responses (linearity test: treated ($n=40$) $F_{14,624}=0.82$, not significant; control ($n=41$) $F_{14,640}=1.51$, not significant). Whereas the decrease over time of the responses of control was statistically significant, the decrease for treated males was not (slope of the regression line: -1.11 for controls, significantly different from 0, $F_{1,640}=6.42$, $P < 0.02$; and -0.91 for treated males, not significantly different from 0, $F_{1,624}=3.09$, not significant). However, the slopes of the regression lines do not differ significantly between controls and treated males ($F_{1,1292}=0.092$, not significant), though their intercepts do differ significantly ($F_{1,1293}=10.04$, $P < 0.01$), showing that the insecticide significantly decreased the response of males to conspecific pheromones. Nevertheless, this response was still significantly greater than 50% (mean of 75% (SE:2.7) of time spent in the conspecifically marked area; $t=8.38$, $n=40$, $P < 0.001$). The recognition of sexual pheromones was decreased in males exposed to deltamethrin, but they were still able to recognize conspecific pheromones.

The responses of males to conspecific pheromones as a function of the time when they were exposed to an LD 0.1 of deltamethrin and for controls are presented in Fig. 2. As previously, the distribution of observed values is not significantly different from a line, regression lines are therefore a good representation for the kinetics of these responses (linearity test: treated ($n=33$) $F_{14,512}=1.52$, not significant; control ($n=31$) $F_{14,480}=0.54$, not significant). Whereas the responses of the controls significantly decreased over time, the decrease was not significant for treated males (slope of the regression line: -0.98 for controls, significantly different from 0, $F_{1,480}=7.69$, $P < 0.01$; and -0.2 for treated males, not significantly different from 0, $F_{1,512}=0.34$, not significant). However, the slopes of the regression lines for controls and treated males do not differ significantly ($F_{1,1020}=3.14$, not significant) nor do their intercepts ($F_{1,1021}=0.198$, not significant). Therefore, deltamethrin at LD

Fig. 2. Recognition of conspecific pheromones by *T. semblidis* males. Percentage of time spent by males in the area marked with female pheromones from the same species. Means calculated each 30 s for the males exposed to an LD 0.1 of deltamethrin (triangular dots, solid line) and the control males (circle dots, dashed line). Regression lines were calculated on means; 50% corresponds to an absence of recognition of conspecific pheromones.

Fig. 4. Recognition of conspecific pheromones by *T. evanescens* males. Percentage of time spent by males in the area marked with female pheromones from the same species. Means calculated each 30 s for the males exposed to an LD 0.1 of deltamethrin (triangular dots, solid line) and the control males (circle dots, dashed line). Regression lines were calculated on means; 50% corresponds to an absence of recognition of conspecific pheromones.
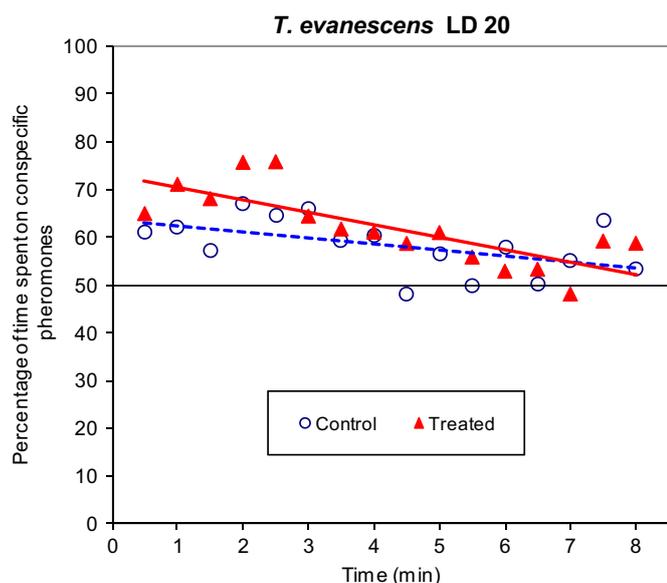
Fig. 3. Recognition of conspecific pheromones by *T. evanescens* males. Percentage of time spent by males in the area marked with female pheromones from the same species. Means calculated each 30 s for the males exposed to an LD 20 of deltamethrin (triangular dots, solid line) and the control males (circle dots, dashed line). Regression lines were calculated on means; 50% corresponds to an absence of recognition of conspecific pheromones.

0.1 had no significant effect on the recognition of conspecific pheromones by *T. semblidis* males.

3.3.2. Kinetics of T. evanescens

The responses of *T. evanescens* males to conspecific pheromones as a function of the time when they were exposed to an LD 20 of deltamethrin and for controls are presented in Fig. 3. The linearity test shows that the distribution of observed values is not significantly different from a line, regression lines are therefore a

good representation for the kinetics of these responses (linearity test: treated ($n=31$) $F_{14,480}=0.68$, not significant; control ($n=35$) $F_{14,544}=1.65$, not significant). The responses of both controls and treated males significantly decreased during the observation time (slope of the regression line: -1.23 for controls, significantly different from 0, $F_{1,544}=7.73$, $P<0.01$; and -2.58 for treated males, significantly different from 0, $F_{1,480}=14.56$, $P<0.001$). The slopes of the regression lines do not significantly differ between controls and treated males ($F_{1,1052}=3.01$, not significant), but their intercepts do differ significantly ($F_{1,1053}=4.29$, $P<0.04$). This difference between the intercept values is due to the higher values for the attraction of males by conspecific pheromones during the first half of the observations when they were exposed to the insecticide (Fig. 3).

The responses of males to conspecific pheromones as a function of the time when they were exposed to an LD 0.1 of deltamethrin and for controls are presented in Fig. 4. As previously, the distribution of observed values is not significantly different from a line, regression lines are therefore a good representation of the kinetics of these responses (linearity test: treated ($n=36$) $F_{14,560}=0.42$, not significant; control ($n=34$) $F_{14,528}=1.43$, not significant). The responses of both controls and treated males significantly decreased over time (slope of the regression line: -4.35 for controls, significantly different from 0, $F_{1,528}=72.76$, $P<0.001$; and -2.50 for treated males, significantly different from 0, $F_{1,560}=26.87$, $P<0.001$). The slopes of the regression lines differ significantly between controls and treated males ($F_{1,1116}=5.99$, $P<0.02$), and this difference is due to the higher attraction of males to conspecific pheromones during the second half of the observations when they were exposed to the insecticide.

4. Discussion

The insecticide deltamethrin induced an alteration of the recognition by *Trichogramma* males of the sexual pheromones of their conspecific females. The effects were different according to the species and dose tested. For *T. semblidis,* an LD 20 of

deltamethrin induced a decrease in the preference of the parasitoid for the sexual pheromones of a conspecific female during a choice situation, whereas an LD 0.1 had no significant effect on this preference. However, both doses induced a decrease in the saturation of the receptors with the pheromones. For *T. evanescens*, an LD 20 of deltamethrin induced an increase in the preference of the parasitoid for the sexual pheromones of a conspecific female in the choice situation during the first half of the 8 min of recording, whereas an LD 0.1 induced an increase in this preference during the second half of the 8 min recording period. However, neither of the doses had an effect on the rate of saturation of the receptors with the pheromones.

Our results confirm the specificity of the recognition of substrate-borne pheromones by *T. semblidis* and *T. evanescens*: males of each species spent more time in the area marked by the female pheromones from their own species. However, this specific recognition was higher for *T. semblidis* males, which restricted more of their movements to the area marked by conspecific pheromones compared to *T. evanescens* males. This difference in specific recognition was also observed by Dupont et al. (2010). It can be explained by the fact that *T. semblidis* males are weaker and have a shorter lifespan than *T. evanescens* males; hence, a stronger ability to discriminate sexual pheromones is important to enable them to locate females for mating prior to their death.

Within each species, controls had a different response to pheromones (i.e., they spent a different amount of time in the area marked by female pheromones) whether they were controls of the LD 20 or LD 0.1. We have no formal explanation for this, except that these controls were tested at different days and that behaviors are subject to important variations within days due to possible environmental and genetic differences. However, the comparison between treated and controls of a same experiment (e.g., LD 20 with *T. semblidis*) is accurate because treated insects were tested at the same time than their controls and were therefore subject to same environmental and genetical (same generation) variations. The only difference between treated and controls of a given dose was the exposure to the insecticide.

Trichogramma are used in biological control for the protection against several pests. One of their main use is the protection against the European corn borer, *Ostrinia nubilalis*, in maize fields (Knutson, 1998). In conventional agriculture, deltamethrin can be used in maize fields against the European corn borer and is then applied at 12.5 g/ha (Couteux and Lejeune, 2009). The two doses tested in this work, LD 20 and LD 0.1, correspond respectively to 0.08 and 0.005 times the field application rate of deltamethrin for *T. semblidis* but it corresponds to 6.26 and 0.14 times the field application rate for *T. evanescens,* respectively.

At an LD 20, deltamethrin significantly decreased the discrimination of sexual pheromones by *T. semblidis* males. Treated males spent less time in the area marked by conspecific pheromones than controls from the beginning to the end of the observations. Furthermore, the kinetics of their response were also modified. Indeed, the time spent in the area marked by conspecific pheromones decreased over time for both controls and treated males, but this decrease (slope of the line) was not significant for the treated males, whereas it was significant for the controls. This regular decrease of the regression line was most probably due to the progressive saturation of receptors with pheromones (Delpuech et al., 1998a). In control conditions, as their receptors become progressively saturated, males become less efficient in distinguishing pheromones from females of their own species and consequently spend less time in the area marked by them, this phenomenon being materialized by a negative slope of the regression line. When males were exposed to the insecticide, they spent less time in the area marked by conspecific pheromones right at the beginning of the observations. Therefore,

their receptors were less exposed to pheromones and probably did not saturate (the slope of the line for treated males is not significantly different from 0). By decreasing the discrimination of sexual pheromones, it appears that deltamethrin prevented saturation of the males' pheromone receptors by decreasing their contact with the odorant molecule. It could be argued that when males were not in the area marked by conspecific females, they were in the area marked by females from the other species (*T. evanescens*) and therefore also in contact with odorant molecules. Nevertheless, sexual pheromone receptors are known to be very specific and are tuned to bind only to their target molecule (Ha and Smith, 2009; Carey and Carlson, 2011). Thus, when males were in the area marked by the other species, their pheromone receptors would not have bound the odorant molecule, and their pheromone perception would not have become saturated or would have saturated later. Alternative explications of the regular decrease of the regression line would be a progressive desensitization of pheromone receptors or an habituation phenomenon. They cannot be totally ruled out.

The decrease in their discrimination of pheromones when males were exposed to an LD 20 of deltamethrin can be due to (i) a decrease in the recognition of conspecific pheromones, which implies a problem in olfaction. (ii) A correct olfaction but a wrong treatment of the information linked to this stimulus. (iii) A correct olfaction and treatment of information but an impairment of the neural processes involved in the behavioral response. Although their discrimination of pheromones was decreased, males exposed to an LD 20 of deltamethrin were still able to discriminate between the odorants produced by the different species and continued to spend more time in the area marked by the pheromones of their own females than in the other area.

An LD 0.1 of deltamethrin had no significant effect on the discrimination of sexual pheromones by *T. semblidis* males, though it did decrease the saturation of their pheromone receptors (the slope of the line was not significantly different from 0 for treated males). Although the difference in the time spent in the conspecific pheromone area between controls and treated males was not significant, treated insects tended to spend less time in their conspecific pheromone area, at least at the beginning of observations. This could explain the decrease in the saturation of the pheromone receptors.

For *T. evanescens*, deltamethrin had significant, but different effects at the two tested doses. At LD 20, it increased the preference of males for the area marked by their conspecific females during the first half of the observations, whereas at LD 0.1, it increased this preference only during the second half of the observations. However, at both doses, the saturation of pheromone receptors was maintained (the slopes of both regression lines where negative and significantly different from 0).

Then, deltamethrin had an opposite effect on *T. semblidis* and *T. evanescens*. For *T. semblidis*, deltamethrin decreased the specific recognition of sexual pheromones at the higher dose (LD 20) but had no effect on this recognition at the lower dose (LD 0.1). However, deltamethrin decreased the saturation of the pheromone receptors at both doses. For *T. evanescens*, deltamethrin increased the recognition of sexual pheromones at both doses, though not during the same period in the observations, but it did not decrease the saturation of the pheromone receptors.

These opposite effects of deltamethrin on the two studied species could be due to differences in their resistance to the insecticide. Indeed, whereas *T. semblidis* showed a sensitivity to deltamethrin equivalent to that observed for *T. brassicae* in previous studies (Delpuech et al., 1999), *T. evanescens* was 78 times more resistant than *T. semblidis* based on a comparison of their LD 20 values. Therefore, the tested strain of *T. evanescens* is much more resistant to deltamethrin than *T. semblidis*, which appears to be a regular

sensitive strain. Because of this resistance, *T. evanescens* was exposed to much higher doses than *T. semblidis* to obtain both of the target levels of mortality (20 and 0.1%). This could explain why the insecticide had an effect at LD 0.1 on *T. evanescens* and not on *T. semblidis*, as the amount of insecticide corresponding to an LD 0.1 was much higher for the former species than for the latter. However, this does not explain why opposite effects were observed in the two species (i.e., a decrease of recognition for *T. semblidis* and increase of recognition for *T. evanescens*).

When the same strains of these two species were exposed to the organophosphorus insecticide chlorpyrifos, it was observed to have the same type of effect on both species, i.e., causing a decrease in pheromone discrimination (Dupont et al., 2010). However, in this case, the two species showed the same rate of sensitivity to the insecticide; neither had developed resistance to chlorpyrifos. Thus, this supports the idea that the difference in the effect of deltamethrin observed between the two species may be due to resistance of *T. evanescens* to this insecticide.

As noted above, another difference between the two species is that under control conditions, *T. semblidis* exhibits a much higher preference for conspecific pheromones than *T. evanescens* (*T. semblidis* males spent more time in the area marked by *T. semblidis* females than *T. evanescens* males spent in the area marked by *T. evanescens* females). Could this difference be involved in the difference in the sublethal effects of deltamethrin on the behavior of these species? Probably not because, when testing chlorpyrifos on these two same species, same strains, the same type of sublethal effect was obtained on both species, i.e., a decrease in the discrimination of pheromones (Dupont et al., 2010), whereas they were also observed to have the same difference in their preference for conspecific pheromones, *T. semblidis* males being more arrested by conspecific pheromones than *T. evanescens* males.

Based on these results, it appears that the sublethal effects of deltamethrin vary according to several factors. At a high dose (LD 20), the sublethal effect of deltamethrin was a function of the species tested (or the resistance status of the species). Indeed, for *T. semblidis* (the species susceptible to deltamethrin), the insecticide had a disadvantageous effect due to decreasing specific pheromone recognition, whereas for *T. evanescens* (resistant to deltamethrin), the insecticide had an advantageous effect via increasing its recognition of sexual pheromones, which increases the probability of finding the female of its species. Insects will be more likely exposed to this type of high dose near treated areas, such as at field edges, which are also sites where resistant populations are more likely (De Jong et al., 2008).

At a low dose (LD 0.1), the effect of the insecticide also varied according to species (or its resistance status), but it was always advantageous. For *T. semblidis*, deltamethrin treatment resulted in an absence of pheromone saturation, which will enable recognition to occur for a longer period. In *T. evanescens*, it increased recognition of the pheromone, thus increasing the probability of finding an appropriate female. It is at sites located far from treated areas that low doses of insecticides will be found (De Jong et al., 2008). If these effects do not modify the equilibrium of insect populations, they will contribute to better maintenance of reservoir areas of these beneficial species.

The decrease in the specific discrimination of sex pheromones we demonstrated in this work at the high deltamethrin dose (LD 20) for *T. semblidis* may lead to confusion of males that could result in attempts to copulate with *T. evanescens* females. This would have strong consequences for both species. Indeed, this would represent an obvious loss of time and opportunities for males because interspecific matings are sterile (Delpuech et al., 2010). Furthermore, *Trichogramma* males possess a finite number of spermatozoids (they are prospermatogenic) and can mate

only approximately 20 times (Boivin et al., 2005). Therefore, interspecific matings would represent an important fitness cost for *T. semblidis* males. Moreover, it has also been shown that interspecific matings between *T. semblidis* and *T. evanescens* cause irreversible infertility in females, such that offspring production is reduced and consists of only males (Delpuech et al., 2010). This is because parasitoid wasps are haplodiploid, with males coming from unfertilized, haploid eggs, while females are produced from fertilized, diploid eggs (Cook, 1993). In the case of interspecific matings, only unfertilized eggs develop, while fertilized eggs, which would have given rise to females, degenerate. Furthermore, it has also been shown that the fertility of females mated interspecifically cannot be restored by a new intraspecific mating; i.e., they irreversibly lose fecundity (Delpuech et al., 2010). Thus, interspecific matings would represent an even more important fitness cost for *T. evanescens* females than for *T. semblidis* males and would be very costly for both species.

The sublethal effects of deltamethrin that we recorded in this work certainly may extend to the other pyrethroid insecticides because they share the same mode of action. These effects can be added to the many other known effects of insecticides on the behaviors of parasitoids involved in their reproduction. For example, insecticides have been shown to modify behaviors leading to mating (Delpuech et al., 1998a, b, 1999, 2001; 2010), behaviors involved in the search for hosts (Stapel et al., 2000; Komeza et al., 2001; Desneux et al., 2004; Delpuech et al., 2005), and behaviors involved in the infestation of hosts (Brunner et al., 2001; Rafalimanana et al., 2002; Tran et al., 2004; Delpuech and Leger, 2011). Finally, insecticides have also be shown to modify the sex ratio of the offspring of parasitoids (Rosenheim and Hoy, 1988; Krespi et al., 1991; Delpuech and Meyet, 2003).

Due to economic and agricultural constraints, the use of pesticides, particularly insecticides, in crop protection is currently a necessity and will continue to be for a long time to come. However, the effects demonstrated in this study together with the many others demonstrated in other studies shows that the potential ecological impacts of the use of these substances cannot be ignored. However, these impacts may be dependent on many factors (e.g., dose, species, resistance status) and, thus, difficult to predict or extrapolate. Parasitoids of insects are keystone species because they control populations of other insects. They are essential to maintain a balanced and sustainable ecosystem (Van Veen et al., 2006). Furthermore, numerous pest species are controlled by parasitoids, which also gives economic importance to these species. Any impact on this type of species would therefore have strong effects on the equilibrium of the entire ecosystem and could, thus, have important ecological or economic consequences.

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