SELECTIVITY ASSESSMENT OF CHLORFENVINPHOS REEVALUATED BY INCLUDING PHYSIOLOGICAL AND BEHAVIORAL EFFECTS ON AN IMPORTANT BENEFICIAL INSECT

Anne Alix, Anne Marie Cortesero, Jean Pierre Nénon, and Jean Pierre Anger

Abstract—Selectivity is an important factor in identifying candidate pesticides to be used in crop protection since it characterizes chemicals that, while being effective against target pests, exert an acceptable impact on the other components of the environment. Extrapolated to an integrated pest management (IPM) context, selectivity implies that candidate pesticides may preserve the ability of beneficial insects to significantly control target pest populations. In the present study, we assess the physiological selectivity of the organophosphate chlorfenvinphos, used to protect cruciferous crops against the cabbage root fly, Delia radicum (Diptera: Anthomyiidae), by investigating both the lethal and sublethal effects exerted on its main parasitoid Tryblionographa rapae (Hymenoptera: Figitidae). The comparison of the median lethal doses showed that T. rapae was at least seven times less sensitive than D. radicum to chlorfenvinphos. However, longevity of parasitoids surviving a sublethal dose was reduced by half. The potential fecundity of females was decreased by 9.6 to 22.8%. Chlorfenvinphos also induced important behavioral changes in both sexes and reduced the chances for parasitoids to mate by more than 70%. While most behavioral changes were reversible, effects on mating and on fecundity were not, thereby suggesting long-term effects on the reproduction of the parasitoid. These cumulative effects of chlorfenvinphos would have dramatic consequences on the efficacy of parasitoids contacting such doses of chlorfenvinphos in the field and therefore there is question about the intrinsic selectivity of this insecticide.

Keywords—Physiological selectivity, Sublethal effects, Beneficial insects, Tryblionographa rapae, Delia radicum

INTRODUCTION

Parasitoids and predators of phytophagous insects can play a crucial role in the natural control of pest populations, and their contribution in integrated pest management (IPM) programs has been widely demonstrated [1,2]. However, precisely because of their trophic interactions with pests, these species may be the first nontarget arthropods affected by insecticide exposure.

Considerable efforts have been made to limit the use of pesticides to treatments that demonstrated selective properties. Selectivity commonly characterizes pesticides that will “curb the number of pest species with minimal effects on all other components of the environment” [3]. In an IPM context, the effects of candidate pesticides on entomophagous insects should not compromise their contribution to the control of pest populations. In this context, selective properties of chemicals may be of intrinsic origin, the chemical being less toxic for beneficial insects than for pests. In such a case, selectivity is referred to as physiological selectivity [4]. Selectivity may also rest on ecological parameters that influence the relative exposure of beneficial insects compared with pests. Relative exposure depends on ecological habits of both populations and agricultural practices. In such a case, selectivity is referred to as ecological selectivity [4]. Ecological selectivity is particularly investigated in the case of chemicals that are not physiologically selective [4]. The question of physiological selectivity is therefore of particular importance.

In most cases, the physiological selectivity of pesticides is assessed by comparing lethal dose point estimates for pests and related natural enemies and by determining selectivity ratios and indexes. These ratios are then compiled into databases that provide a useful tool for rapid screening of chemicals according to their acute effects [5]. However, indexes based on lethal effects only are not sufficient to ensure that beneficial insects contacting so-called selective pesticides, and particularly insecticides, will not be affected. Even if they remain far less documented compared with lethal effects, adverse effects induced in parasitoids that are exposed to sublethal doses of insecticides have frequently been reported [6,7]. For sublethal effects, the parasitization capacity of females, which is a key survival factor for parasitoid populations and is critical to the efficacy of biological control, is the most extensively studied parameter [8,9]. Nonetheless, the success of parasitism rests on many physiological and behavioral events that can also be disrupted by insecticides. O’Brien et al. [10], e.g., observed the presence of shriveled and grainy eggs in the ovaries of Bracon mellitor females exposed to azinphos-methyl and chlordimeform so that eggs that were laid were not viable. Elzen et al. [11] showed that the flight activity of Microplitis croceipes females sprayed with a mixture of fenvalerate plus chlordimeform was altered and that the attractiveness of cotton plants sprayed with the same mixture was decreased. In a recent study, Delpuech et al. [12] observed that sublethal doses of the organophosphate chlorpyrifos depressed sex pheromone communication between sexes in Trichogramma brassicae, thereby compromising mating and production of female offspring. These examples demonstrate that, by affecting the...
Chlorfenvinphos selectivity reevaluated

In this study, we propose a strategy based on laboratory observations of both lethal and sublethal effects for testing the selectivity of the organophosphate chlorfenvinphos, which is widely used as a means to control Delia radicum L. (Diptera: Anthomyiidae) in cruciferous crops. Trybliographa rapae W. (Hymenoptera: Figitidae) is a major natural enemy of D. radicum [13]. Parasitization rates of up to 60% have been reported in untreated crops, suggesting an important role for this species as a biological control agent [14]. Little information concerning the toxicity of chlorfenvinphos to this parasitoid is available. In a previous work, Hassan [15] found that chlorfenvinphos induced very low mortality rates in adult T. rapae exposed to treated soils in laboratory tests, compared with other compounds such as other organophosphates and organochlorines. However, the author reported a reduced parasitization rate in treated crops. It is therefore important to determine the precise effects that chlorfenvinphos may exert on this beneficial insect and to understand its selective properties.

In this study, we first determined the median lethal doses (LD50) of chlorfenvinphos for adults of D. radicum and T. rapae in order to calculate a selectivity ratio. Sublethal effects were then investigated on T. rapae egg viability, longevity of both sexes, and behavior. Both lethal and sublethal effects were studied in insects contacting known amounts of insecticide through topical applications. Topical applications were chosen because observed effects can subsequently be related to a precise dose, thus facilitating further discussions on the comparison of the sensitivity of the two species and the comparison of behavioral and physiological responses of the parasitoid [9,16,17].

MATERIAL AND METHODS

Insects

Both strains of D. radicum and T. rapae were obtained from the Institut National de la Recherche Agronomique station at Le Rheu (Ille et Vilaine, France). The T. rapae was reared on D. radicum larvae infesting rutabaga (Brassica napus) roots, as described by Neveu et al. [18]. Rearing and experiments were conducted in a climatic room (20 ± 1°C, 16:8 light:dark, and 60 ± 10% relative humidity). Natural mortality can occur a few days after emergence. Therefore, in order to avoid selection of the most vigorous insects, we used 1-d-old adults for all tests. For all tests, T. rapae females were deprived of hosts. For mating tests, virgin males and females were obtained by isolating parasitized pupae in gelatin capsules until emergence.

Insecticide application method

Solutions of chlorfenvinphos (2-chloro-1-(2,4 dichlorophenyl) vinyl diethyl phosphate) were prepared by diluting the commercial preparation (Birlane CE 40, BASF Agro, Levallois Perret, France) in bidistilled water.

Insects were isolated in plastic boxes (7.2 cm high, 11 cm in diameter) provided with four aeration holes (2.5 cm in diameter, two holes on the top of the box and two holes on the side) covered with fine mesh. Before the insecticide treatment, insects were immobilized by a 5-s exposure to carbon dioxide. Preliminary tests showed that this anesthetic compound induced neither mortality nor behavioral changes. Insects were then submitted to a topical application of 0.5 μL of the required insecticide solution on the abdomen. Applications were performed using a 1-μL syringe provided with a bevel point (Hamilton, provided by Sigma Aldrich, St. Quentin Fallavier, France). After being treated, insects were kept in the plastic boxes and were provided with water and honey.

Determination of LD50

The LD50 was determined for both D. radicum and T. rapae. In order to estimate the lethal dose range for the two species, a first series of tests was conducted over a wide range of 1:10 dilutions, including a solution prepared at the recommended field concentration (0.6 g/L). The lethal dose ranges were then determined using seven concentrations, inducing mortality ranging from 0 to 100%. Control insects were submitted to a topical application of bidistilled water. Forty insects of both sexes were tested for each dose and control. Mortality was determined 24 h after treatment.

Determination of sublethal effects

Sublethal effects of chlorfenvinphos were studied for parasitoids only. Effects of chlorfenvinphos on longevity, fecundity, and mating were studied in insects submitted to the application of 300 ng of the insecticide, which results in 10% mortality in females and 30% in males. For insects, this dose also corresponds to contact with 0.5 μL of the insecticide diluted at the recommended field concentration.

Effects of chlorfenvinphos on parasitoid behavior were assessed in males and females both treated with the LD10 (203 ng/insect in males and 300 ng/insect in females) so that no bias related to sexual difference in sensitivity would be induced.

Effects on potential fecundity of T. rapae females were investigated. One hundred fifty mated females were placed individually in plastic boxes with food and water and submitted to chlorfenvinphos at the LD10. Lifetime fecundity was then determined by dissecting 20 females 1, 5, 10, 15, and 20 d after treatment and counting viable and nonviable eggs after neutral red (Merck Eurolab, Fontenay sous Bois, France) coloration of the ovaries. Eggs were observed and counted using an ocular micrometer. Control females (150) treated with bidistilled water were handled similarly.

Effects on longevity were studied for both males and females. After treatment, parasitoids were kept separately in plastic boxes and provided with honey and water. Mortality was checked daily from the day of emergence to determine the longevity of wasps. For both treated and control males and females, 40 replications were made.

Effects on the parasitoid behavior were also studied for both sexes. Control and LD10-treated wasps were kept individually in plastic boxes with water and honey but lacking any other stimuli. Preliminary tests showed that the first intoxication symptoms normally appeared 5 min after treatment and could develop rapidly into prostration. Therefore, behavioral observations were performed immediately after insects recovered from anesthesia and were conducted for 40 min. Behavior,
including walking, preening, resting, and antennating, was recorded and timed. Wasps were considered antennating when they were observed exploring aeration holes with the antennas, perhaps in an attempt to leave the observation box. For both treated and control males and females, 21 repetitions were made.

Finally, effects of chlorfenvinphos on mating were investigated. Adults emerging from isolated pupae were placed in plastic boxes in the combinations of one male and one female both treated with bidistilled water (control), one female treated with chlorfenvinphos and one male treated with bidistilled water, one female treated with bidistilled water and one male treated with chlorfenvinphos, and one male and one female both treated with chlorfenvinphos. Each experiment was repeated 21 times. The observations began immediately after treatment. The effect of chlorfenvinphos on the attractiveness of males toward females was evaluated by considering the number of males that contacted the females and initiated courtship. We called this behavior pairing. When females were receptive to courtship, then mating (copulation) occurred. Observations were conducted until mating occurred. When no mating occurred within 90 min, the observation was terminated. In order to test for long-term effects, the same experiment was repeated 1 d after insecticide exposure.

Data analysis

All statistics were performed using Statgraphics Plus from Manugistic Company. The LD50s were calculated using a regression on probit-transformed data. Confidence intervals on LD50s corresponded to values of probit four and probit six determined from the regression line. Differences between two LD50 values were considered significant (p < 0.05) when no overlap of the 95% confidence limits occurred. Lethal dose ratios were determined by dividing the LD50 for T. rapae by the LD50 for D. radicum, as the regression lines were statistically parallel (95% confidence limits of slopes overlapped). Numbers of eggs in ovaries of control and treated females were compared using Student t tests. Percentages of viable eggs were compared using chi-square tests. Parasitoid longevity and data from behavioral observation were analyzed using Wilcoxon signed rank tests. The number of times insects fell from the top or the sides of the box was compared using a Student t test. Pairing and mating frequencies between the different pairs were compared using chi-square tests.

RESULTS

Lethal doses

The mortality induced by chlorfenvinphos was plotted as a function of the dose applied, and regression lines were calculated. The theoretical doses inducing a mortality rate of 50% for males and females of both species are given in Table 1. The LD50 was significantly lower in D. radicum than in T. rapae. In T. rapae, the LD50 was significantly lower in males than in females. The lethal dose ratios between the parasitoid and the pest were 7.4 for males and 12.2 for females.

In T. rapae, a topical application of 300 ng (0.5 μl of the recommended field concentration) of chlorfenvinphos resulted in a mortality of 10% in females and 30% in males. As sublethal effects must be investigated using doses that inflict a maximum of 30% mortality [7], this dose was used in experiments to detect the effects of the insecticide on the fecundity, the longevity, and the behavior of T. rapae.

<table>
<thead>
<tr>
<th>Species</th>
<th>LD50 (95% confidence limit)</th>
<th>Slope (95% confidence limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. radicum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>52.12 (11.19) A</td>
<td>3.41 (0.49)</td>
</tr>
<tr>
<td>Females</td>
<td>56.75 (12.07) A</td>
<td>3.59 (0.29)</td>
</tr>
<tr>
<td>T. rapae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>387.63 (51.67) C</td>
<td>4.56 (0.92)</td>
</tr>
<tr>
<td>Females</td>
<td>690.06 (106.64) D</td>
<td>3.98 (0.22)</td>
</tr>
</tbody>
</table>

Effects on potential fecundity

The number of eggs in the ovaries was significantly lower in chlorfenvinphos-treated females than in control females from the 5th day until the 20th day after treatment (p < 0.05, t test) (Table 2). This difference did not increase over time (the slopes of the regression lines relating the number of eggs as a function of age were 0.068 ± 0.149 and 0.514 ± 0.260 for control and treated females, respectively; df = 198, t = 1.488). This suggests that the loss of eggs occurred in the first 5 d after exposure. No differences were found in numbers of nonviable eggs between treated and control females.

Effects on longevity

Chlorfenvinphos reduced longevity by half in treated T. rapae males and females (p < 0.05, Wilcoxon signed rank test) compared with controls (Table 3). In both control and treated wasps, a significant difference in longevity between sexes was observed. Females lived about twice as long as males (p < 0.05).

Effects on parasitoid behavior

The total times engaged in walking, preening, searching, and resting by control and treated wasps were determined. Control wasps of both sexes exhibited similar behavior in the test arena (Table 4). Males and females spent half of their time resting; the remaining time was mostly spent walking and preening. Relatively little time was spent antennating.

In treated males, the time engaged in walking was increased about threefold compared with controls (p < 0.05), whereas the time engaged in resting was significantly decreased (p < 0.05).

Chlorfenvinphos-treated females spent significantly more time preening and less time resting and antennating than control females (p < 0.05) (Table 4), but no statistical difference in walking time was observed.
Treated male and female wasps also showed uncoordinated leg movements that began $18.44 \pm 2.4$ (mean $\pm$ standard error [SE]) min and $32.48 \pm 2.19$ min after treatment, respectively (Wilcoxon signed rank test, $p < 0.05$). The most important uncoordinated movements often lead to the collapse of the treated insect. The number of times treated males and females fell was significantly increased compared with control ($p < 0.05$), and males fell more often than females ($p < 0.05$).

Treated males were seen making attempts to preen while walking, a behavior that was never observed in control males. Some treated females were observed with their ovipositor extruded, often while walking. This behavior was never observed in control females. These two behaviors were not systematically observed and therefore were not analyzed statistically.

Effects on mating behavior

Chlorfenvphos significantly decreased mating success immediately after treatment but also 1 d after treatment (Table 5). This decrease was observed no matter which sex was treated. The frequency of pairing was also significantly decreased by the treatment ($p < 0.05$).

**DISCUSSION**

Our results suggest that *T. rapae* adults may survive after contacting a dose of chlorfenvphos that kills *D. radicum* adults. Nonetheless, the results from the sublethal experiments indicate that chlorfenvphos should not be considered as a physiologically selective insecticide.

Lethal doses

Comparison of lethal dose ratios showed that *T. rapae* was at least seven times less sensitive than *D. radicum*. This difference in sensitivity between species would be about five times greater when considering the mean weight of insects. Indeed, weight of males and females of *T. rapae* are $1.08 \pm 0.02$ (n = 299) and $1.23 \pm 0.02$ mg (± SE) (n = 300), respectively, whereas weight of males and females of *D. radicum* are $4.20 \pm 0.11$ (n = 251) and $6.80 \pm 0.14$ (n = 213) mg (± SE), respectively.

The observed lethal dose ratios were also higher than the mean lethal dose ratio for natural enemies and associated pests of 2.51 that was determined by Theiling from the SELECTV database [5]. This suggested a rather low sensitivity of the parasitoid compared with its host. Such results were not expected when considering data from the literature. Exposure to sprayed residues of chlorfenvphos (0.132% v/v) induced a high mortality (100%) in most of the standard species tested by the International Organization for Biological Control/West Palaearctic Regional Section (http://iobc.ethz.ch/) working group [19]. Contact with chlorfenvphos was also harmful for other natural enemies of *D. radicum* such as carabids and staphylinids [19,20]. In bees, the LD$50$ of chlorfenvphos was 4,100 ng/insect when administered topically but only 1.64 ng/insect when administered orally, which is very close to the quantitative structure–activity relationship–predicted value of 1.26 ng/insect [21]. The higher toxicity of pesticides administered orally compared with those administered topically has also been demonstrated in parasitoids [22] and may be explained by a more rapid increase of amounts of the chemical in the hemolymph and in organs. However, an oral exposure was not applicable for *T. rapae* since, to our knowledge, neither this parasitoid nor its host feed on cruciferous crops.

Effects on potential fecundity

Chlorfenvphos significantly reduced the potential fecundity of treated females. Similar effects have been described in *Braccon hebetor* (Bracodidae) females exposed to carbarsyl [23]. In the case of these females, the egg loss was due to an insecticide-induced egg resorption. Egg resorption is common in hymenopterans and occurs mainly in the case of host deprivation or when females are starved [24]. Therefore, we tested this hypothesis in *T. rapae* females that were starved for 5 d and deprived of hosts. The mean number of eggs (± SE) in fed females was 98.4 ± 1.77, being not different from the one recorded in starved females of 96.4 ± 1.44 (df = 38, $t = 0.87$). Longer starvation periods only inflicted high mortality in females. These results suggest that *T. rapae* may not exhibit osorption.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Control</th>
<th>Chlorfenvphos</th>
<th>Control</th>
<th>Chlorfenvphos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td>21.57A</td>
<td>58.00B</td>
<td>19.16A</td>
<td>27.06A</td>
</tr>
<tr>
<td>Preening</td>
<td>21.70A</td>
<td>27.21A</td>
<td>20.67A</td>
<td>36.95B</td>
</tr>
<tr>
<td>Resting</td>
<td>46.84A</td>
<td>14.30B</td>
<td>53.39A</td>
<td>33.50C</td>
</tr>
<tr>
<td>Antennating</td>
<td>9.69A</td>
<td>0.49B</td>
<td>6.78A</td>
<td>2.49A</td>
</tr>
<tr>
<td>Number of falls</td>
<td>0.68 (0.29)A</td>
<td>4.55 (1.09)B</td>
<td>0.14 (0.10)A</td>
<td>1.27 (0.32)C</td>
</tr>
</tbody>
</table>

Table 4. Mean percentage of time engaged in various behaviors and number of falls observed in males and females of *Trybliographa rapae* receiving a topical application of water (control) or chlorfenvphos; values compared within each behavior; values followed by the same letter are not significantly different at the 0.05 level (Wilcoxon signed rank test for time; $t$ test for number of falls, 40 df); SE = standard error.
Another hypothesis might explain the egg loss observed in *T. rapae* females. Chlorfenavinphos could induce uncontrolled egg laying. In previous work, Ramade [25] observed uncontrolled ovipositions in house fly females exposed to parathion and, as for *T. rapae*, flies were not submitted to any stimulus. The author suggested a relationship between the neurotoxic activity of parathion and this phenomenon. In flies, the egg loss occurred within the first hours after treatment, when the internal dose of insecticide in insects was probably the highest [16]. In *T. rapae*, the egg loss appeared to occur within the first 5 d following treatment. Thus, even when not affecting the viability of eggs, chlorfenavinphos affects the potential fecundity of females by inducing a reduction in egg number that can reach 22.8% (based on the difference in the average egg content of the ovaries between treated and control females 15 d after treatment). Since, in *T. rapae*, females are proovigenic and thereby emerge with a definitive stock of eggs [26], such an effect is not reversible.

**Effects on longevity**

Chlorfenavinphos significantly reduced the longevity of both male and female *T. rapae*. Such a reduction of longevity has already been observed in parasitoids developing inside hosts that were exposed to insecticides [27,28] and also in parasitoids feeding from insecticide-contaminated nectar [29]. The mechanisms by which insecticides reduce the lifetime of exposed insects remain unknown. It is likely that these chemicals induce irreversible physiological changes that lead to premature death in exposed individuals. The modifications that such changes may have on the susceptibility of insects to pathological agents encountered remains to be investigated.

The reduced longevity induced by chlorfenavinphos in *T. rapae* females and males may greatly affect the beneficial capacity of the species. In females, this premature death may worsen the effects already observed on the potential fecundity since they usually lay eggs during their whole lifetime [26]. In males, this effect may decrease their fitness, limiting the number of females with which they can mate during their lifetime [30].

**Effects on parasitoid behavior**

Chlorfenavinphos induced important changes in the parasitoid general behavior, mainly an increased mobility in males and an extended time engaged preening in females. Increased mobility has been observed in insects after exposure to organophosphates [25,31], pyrethrins [32,33], and amid [34]. In these studies, treated insects showed hyperactivity, which lead to significantly increased mobility occurring in the absence of any stimuli. Such an effect is very likely related to the neurotoxical action exerted by organophosphate insecticides on the insect central nervous system [35].

An increase in the time engaged in preening has also been described in the housefly exposed to parathion [25] and in worker bees exposed to pyrethrins [33]. Longley [36] reported similar behavior in the parasitoid *Aphidius rhopalosiphi* treated with deltamethrin and suggested a reflex response induced by the irritation of chemoreceptors or mechanoreceptors located on the insect body surface.

In our study, males and females showed different behavioral reactions to insecticide exposure, and this, to our knowledge, has never been reported before. Since both sexes were submitted to a LD10 application, these differences are probably not related to a sexual difference in sensitivity. In a recent study, Jagers op Akkerhuis et al. [37] suggested that behavioral symptoms occurring within the first hours after a topical treatment may be separated into two phases, depending on diffusion of the toxicant in insects. In the first phase, which starts within a few minutes after the treatment, insects respond to a peripheral action of the insecticide, which concerns the nerves located just below the body area where the insecticide was applied. During this phase, insects may show increased preening behavior. The second phase starts when the toxicant has diffused into the insect body toward the central nervous system, leading to the uncoordinated behavior observed in treated insects. When considering these suggestions, it can be postulated that the time needed for chlorfenavinphos to reach the central nervous system of *T. rapae* may be shorter in males than in females. This hypothesis may explain the shorter time span that was observed until the uncoordinated movements appeared in males compared with females. This may also explain why males fell more than females since this phenomenon may well be related to the uncoordinated movements.

The most unusual behavior observed in treated females was the extrusion of the ovipositor. As for flies exposed to parathion [25], this symptom was observed after the appearance of uncoordinated movements and could therefore relate to effects of the insecticide on the central nervous system. This effect may explain the loss of eggs we previously described.

Seven observations carried on over a few days after the treatment revealed that all these behavioral changes were reversible within about 24 h. Some of these temporary changes, however, may lead to permanent effects on parasitoid survival and/or efficacy, as in the case of potential fecundity, assuming that behavioral disruption was in part involved in the egg loss observed. In addition to effects on potential fecundity, behavioral changes may also severely alter the females’ effective fecundity by disrupting their ability to search for, reach, and parasitize their host.

Moreover, the possible consequences of behavioral disruptions on the interactions with other species, such as predators, should not be overlooked. It is likely that the induction of

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### Table 5. Mating and pairing frequencies in *Trybliographa rapae* where males or females received a topical application of water (−−) or chlorfenavinphos (++); an asterisk indicates values significantly different from control (−−) at the 0.05 level (χ² test, 1 df)

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>−−</th>
<th>+−</th>
<th>−+</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pairing</td>
<td>100</td>
<td>66.66*</td>
<td>80.95*</td>
<td>71.43*</td>
</tr>
<tr>
<td>Mating</td>
<td>100</td>
<td>28.57*</td>
<td>14.28*</td>
<td>4.76*</td>
</tr>
<tr>
<td>1 d posttreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pairing</td>
<td>100</td>
<td>76.19*</td>
<td>57.14*</td>
<td>71.43*</td>
</tr>
<tr>
<td>Mating</td>
<td>100</td>
<td>9.52*</td>
<td>4.76*</td>
<td>9.52*</td>
</tr>
</tbody>
</table>
uncoordinated movements and frequent falls would make the exposed wasps easy prey for predators.

Effects on mating behavior

Chlorfenvinphos severely inhibited mating in T. rapae, no matter which partner was exposed. This inhibition was demonstrated both immediately and 1 day after treatment. Both pairing and mating were inhibited, suggesting that several events of the mating behavior may have been affected. In parasitoids, pairing mainly depends on the emission of calling pheromones by females and on the response of males to these pheromones [38]. Delpuech et al. [12] showed that both phenomena were disrupted in Trichogramma brassicae exposed to chlorpyriphos at the LD20. Similar effects of chlorfenvinphos on the pheromone communication in T. rapae may explain the reduced pairing frequencies we recorded when either the male or the female was treated.

In our study, we observed that mating often failed after courtship when at least one partner was treated. This suggests that both courtship and females’ receptivity to courtship were affected. However, we occasionally observed that the receptivity of T. rapae females did not entirely depend on a perfectly executed courtship. Several females accepted a mate following a disturbed courtship of recently treated males, while other females refused to mate following a courtship that appeared apparently less disturbed, mostly in the case of males tested 24 h after treatment. Therefore, it is likely that courtship in T. rapae also involves chemical stimuli, as is the case for a wide range of hymenopteran parasitoids [30], and that exposure to the insecticide may lead to a disruption of their production.

Regardless of the origin of this inhibition, it is important to note that the effects of chlorfenvinphos on mating appear irreversible since we observed similar results in wasps tested 24 h after treatment. This lasting effect of chlorfenvinphos on mating could have permanent consequences on populations of wasps contacting such a dose in the field. Indeed, in T. rapae, females reproduce by arphenotokous parthenogenesis [26] and virgin females bear only male progeny. Furthermore, in T. rapae, mating occurs soon after emergence [26] and, like in a number of parasitoid species, receptivity of females decreases quickly, no matter if they are mated or not [39]. Therefore, a lasting effect, such as demonstrated in our study, may lead to immediate consequences on the sex ratio of the progeny of treated wasps in the field.

Our study demonstrates that assessing a lethal dose ratio for pest and related natural enemy is greatly insufficient to determine the physiological selectivity of an insecticide. The effects of chlorfenvinphos on the physiology and behavior of T. rapae and their possible consequences on intraspecific interactions do not fit an assumption of physiological selectivity since, in the hypothesis of parasitoids contacting such a dose in the field, these effects would be sufficient to compromise their ability to reproduce.

The possible effects of chlorfenvinphos on interspecific interactions should now be investigated. It is likely that the observed disruption of both the physiology and behavior of the parasitoid may also disrupt other events of a parasitoid beneficial capacity, such as host location, host finding, and parasitization efficiency.

The study of any of these aspects of insecticide toxicological impact, including the possibility of cumulative effects, could give a better prediction of their actual selective properties. Such studies may also be helpful tools to interpret the reduced beneficial capacity observed in parasitoids foraging in treated crops.

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