

Oviposition behaviour and patch-time allocation in two aphid parasitoids exposed to deltamethrin residues

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Abstract

Neurotoxic insecticides are widely used for crop protection. One consequence is that changes in behaviour can be expected in surviving beneficial insects because of an impairment of host perception and motor abilities. Under laboratory conditions, we studied the impact of deltamethrin, a pyrethroid, on the oviposition behaviour of two hymenopterous parasitoids of aphids, *Aphidius matricariae* (Haliday) and *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). They both parasitize *Myzus persicae* (Sulzer) (Homoptera: Aphididae), which is the preferred host of *A. matricariae*, regardless of the host plant, whereas *D. rapae* is a major parasitoid of aphids on Cruciferae crops, including *M. persicae*. After exposure to deltamethrin, the different items of oviposition behaviour and the total time spent on the patch were recorded. The results showed that the patch time allocation by both parasitoid species was not significantly affected by deltamethrin treatment, when compared with the controls. Nor were the frequencies and sequences of behavioural items modified (e.g., frequency of sting). It therefore appeared that the patch use of *A. matricariae* and *D. rapae* on new colonies of *M. persicae* was not disturbed by deltamethrin at the three doses tested. The possibility that parasitoid strains are partially tolerant to deltamethrin is discussed.

Introduction

The importance of parasitoids for the biological control of aphids has been demonstrated on several occasions (Powell, 1983; Chambers et al., 1986). However, crop protection is mostly based on broad-spectrum chemical insecticides that are noxious to beneficial insects. For instance, aphid resurgence or an increase in populations of secondary pests can occur as a result of death or altered parasitoid activities due to insecticides (Hardin et al., 1995; Longley et al., 1997).

Aphid parasitoids can come into contact with insecticides through direct exposure to spray droplets (Jepson, 1989), spray residues on crop foliage (Jepson, 1989; Longley & Jepson, 1996a,b), or dietary exposure when feeding on contaminated water droplets, nectar, or honeydew (Longley & Stark, 1996). Indirect exposure during development within the host can also occur (Süss, 1983; Hsieh & Allen,

1986; Longley, 1999). Exposure to low doses of insecticide residues is highly probable due to their widespread use (Brown, 1989), and may induce sublethal effects in contaminated surviving insects (Elzen, 1989). Sublethal effects are particularly expected, regarding the behaviour of insects exposed to insecticides, because most of these substances are neurotoxic (Haynes, 1988).

Aphid parasitoids spend a significant proportion of their adult life searching for and attacking their hosts. Host searching behaviour involves orientation to habitat and host odours (Vinson, 1998). Once an aphid is detected, the female is able to evaluate host species and quality, allowing her to verify the presence of the physiological conditions necessary for the development and growth of her progeny. Acceptance or rejection of the aphid follows this examination. The behaviour of female parasitoids is based on cues perceived during antennal contact with the host and/or ovipositor insertion (Viggiani, 1984; Hågvar & Hofsvang, 1991). Host recognition relies on multiple sensory cues (Powell & Wright, 1991; Battaglia et al., 1993; Braimah & van Emden, 1994; Powell et al., 1998). From the detection of odours to oviposition, the various behaviours are

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entirely dependent on neural transmissions, which are targeted by neurotoxic insecticides. Therefore, changes in oviposition behaviour by insecticide exposure might be expected.

Few data are available on the sublethal effects of pesticides on oviposition behaviour in aphid parasitoids. Kühner et al. (1985) described the negative effects of a herbicide on the parasitic behaviour of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), which included a reduction in the number of sting attempts and stings after exposure to a pesticide. Desneux et al. (2000) showed that a pyrethroid, lambda-cyhalothrin, could disturb the oviposition behaviour of *Aphidius ervi* parasitizing *Myzus persicae* on oilseed rape. Other studies examining the behavioural effects of insecticide exposure have reported modifications to the foraging pattern of aphid parasitoids (De Jiu & Waage, 1990; Longley & Jepson, 1996a; Umoru et al., 1996). For instance, Irving & Wyatt (1973) described a disturbance of the oviposition behaviour of *Encarsia formosa* on *Trialeurodes vaporariorum* during exposure to pirimicarb. *Trissolcus basalus* females exposed to a low dose of deltamethrin (pyrethroid) reduced their walking speed and the time they spent on host-patches (Salerno et al., 2002). A clear understanding of the possible effects of pesticides on oviposition behaviour in parasitoids is important, particularly in the context of integrated pest management (IPM). Even a small change in the reproductive potential of parasitoids may limit successful biological control.

The aim of the present work was thus to investigate the impact of deltamethrin on the oviposition behaviour and patch time allocation of parasitoids of a major aphid pest, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). The species studied were *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae), and *D. rapae*. *Aphidius matricariae* is an important natural enemy of *M. persicae* on *Brassica* (Bijaya Devi et al., 1999) and on various other crops (Schlinger & Mackauer, 1963). *Diaeretiella rapae* has been reported to play a significant role in preventing aphid outbreaks in Cruciferous crops such as rapeseed and mustard (Bahana & Karhioc, 1986; Ohiman & Kunar, 1986; Souza et al., 1992). We were interested in the effects of deltamethrin because, given its low toxicity to mammals (rapid enzymatic degradation following ingestion, Soderlund & Bloomquist, 1989) it is widely used to protect crops against a variety of insect pests. Moreover, pyrethroids are potent neurotoxins that alter the function of voltage-sensitive sodium channels (Soderlund & Bloomquist, 1989), thus potentially impairing some neural function. Finally, although pyrethroid efficiency is due to a 'knock down' action at the time of spraying, dry residues remain for several days on treated foliage (Mahaut & Deleu, 1997), representing a potential threat to foraging parasitoids.

Materials and methods

Plants

Oilseed rape (*Brassica napus*, winter variety Goéland) was grown under greenhouse conditions in individual plastic pots (6 cm diameter). Plants were infested at the two-leaf stage to provide aphid patches for behavioural experiments.

Insects

All insects were reared in environmental chambers at 23 ± 1 °C, under a L16:D8 photoperiod. *Myzus persicae* was reared on broad bean plants (*Vicia faba* L.); *D. rapae* and *A. matricariae* were reared on *M. persicae* on *B. napus* leaves (field-collected parasitoids were yearly incorporated in the laboratory strain). At the mummy stage, parasitized aphids were removed from the leaves and kept individually in plastic Petri dishes until adult emergence. 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 (80%). The parasitoids used for all experiments were 24–48 h old. The females had never been in contact with plants or aphids prior to the experiments and were used only once.

Insecticide

The active ingredient deltamethrin (certified purity of 98%) was provided by Cluzeau InfoLabo (Sainte-Foy-la-Grande, France). The active ingredient was in the form of crystals. A preliminary experiment was run to determine the range of doses, by exposing insects to deltamethrin at decreasing concentrations from the recommended field application rate (ACTA, 2002) until mortality rates lower than 100% were observed. In order to establish the regression line of mortality, adult parasitoids were exposed to six (*A. matricariae*) or seven (*D. rapae*) doses increasing by a factor of two (30 insects per dose in each species).

Three doses were then estimated from the regression line: a 'Lethal Dose₅₀' (LD₅₀) which is classically used to evaluate the toxicity of pesticides on organisms; a LD₂₀, lower than the 30% threshold recommended for the use of pesticides in IPM (Barrett et al., 1994), and a theoretical lethal dose of 0.1% (LD_{0.1}), i.e., a sublethal dose inducing no significant mortality. In the present study, doses were expressed as ng of active ingredient per cm² because we were studying the effects of residues. Female parasitoids were exposed for 24 h to dry residues of deltamethrin on glass, according to standard methods used to evaluate insecticide toxicity for parasitoids (Barrett et al., 1994; Candolfi et al., 2001).

Parasitoid exposure to pesticide

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 cm²). To obtain a homogeneous deposit, we introduced 200 µl of solution (pure acetone was used as a control) which allowed total coverage of the internal surface of the tube, which was then manually rotated until no more droplets were seen on the glass wall. The tubes were left for 1 h on the bench to allow complete evaporation of the acetone before introducing the parasitoids. As both the internal surface of the tubes and the volume of the solution were fixed, it was possible to express the quantity of insecticide residue per unit of surface area. Ten female parasitoids were placed in each tube. Two drops of honey were deposited on a small plastic strip so that they would not be contaminated by the insecticide. The tubes were closed with a fine nylon gauze to allow air circulation. Pesticide exposure was achieved at 15 ± 1 °C, 65 ± 5% r.h., and under a L12:D12 photoperiod. This temperature prevented the mortality of wasps in the control tubes. Parasitoids also moved actively at this temperature. After a 24-h exposure period, the number of dead parasitoids was counted and the survivors collected and placed individually in Petri dishes (5.3 cm diameter). The behavioural tests were performed within 2 h of the end of exposure.

Aphid patches

When oilseed rape plants were at the two-leaf stage, two *M. persicae* viviparous females were placed on one of the two leaves. Aphids were left for 3 days at 23 ± 1 °C to form colonies of 19–21 individuals. This procedure produced homogenous colonies of aphids on all the plants used in the behavioural experiments.

Behavioural tests

Parasitoid females were placed individually on an aphid patch. Observations were carried out through a binocular microscope. Depending on the behaviour studied, the frequency or duration were recorded using The Observer software (Noldus Information Technology, Wageningen, The Netherlands). The observations were carried out at a temperature of 20 ± 1 °C.

The behaviours recorded were: 'antennal contact' (brief contact between an antenna of the parasitoid and an aphid body), 'antennal examination' (contact between both antennae of the parasitoid and an aphid body), 'sting attempt' (when the ovipositor was extruded next to an aphid, or into an aphid exuvia), and 'sting' (ovipositor insertion into an aphid). Because these behaviours were very brief, only their frequency was recorded. The frequency and duration of four other behaviours were also recorded: 'walking onto aphid patch', 'walking out of aphid patch', 'grooming', and 'time spent immobile'.

The observation period lasted until the parasitoid flew away or left the patch for more than 60 s. The data were thus

uncensored. On each day of observation, 3–4 females per species and per dose of deltamethrin were observed in a randomised order. Each female was tested on an unexploited aphid patch to prevent any effect of previous parasitism.

Data analysis

The linear regression model of mortality curves was determined using WIN DL computer software (CIRAD-CA/MABIS, Montpellier, France), based on a logarithmic transformation of doses and a probit transformation of mortalities (log-probit model, Finney, 1971). Abbott corrected mortalities (Abbott, 1925) allowed us to account for mortalities in the untreated groups. The WIN DL software allowed an estimation of the LD_{0.1}, LD₂₀, and LD₅₀ from the linear model, for each species.

In order to analyse the oviposition behaviour of the two species on *M. persicae* patches, we first characterized the behavioural sequence for control groups using a quantitative diagram (Figures 1 and 2). We quantified the sum of individual transition frequencies between two successive behaviours in the control group of each species, expressed as a percentage of the total number of successions in the diagram. Then, we defined two sequences to estimate the effect of both insecticide treatment and parasitoid species: (1) the initiation of host handling, and (2) host acceptance determining the issue of the host-handling behaviour. Indeed, even if the number of the different behavioural items might not be affected, an effect could occur in the transition frequencies between behaviours.

The initiation of host handling and host acceptance were defined as follows:

- Initiation of host-handling: 'antennal contact' when followed by either: (a) 'antennal examination', (b) 'sting', or (c) 'sting attempt'. A disturbance of this sequence, for example if 'antennal contact' was followed by 'grooming' or 'walking on the aphid patch', could indicate a disruption in the perception of the host when initiating host-handling at the time of 'antennal contact'.
- Host acceptance: when the 'antennal contact' – 'antennal examination' sequence was followed by either a 'sting' or a 'sting attempt'. A modification by deltamethrin of this sequence could result from an alteration in the ability to accept the host following 'antennal examination'.

The statistical analysis compared frequencies of 'initiation of host handling' and 'host acceptance' sequences. We also analysed the frequency of occurrence of the four items constituting host handling behaviour. Frequencies were expressed per time unit, because individual observations were not all of the same duration.

To analyse the effect of the three doses of deltamethrin on patch residence time, we used a generalized linear model based on a Gamma distribution and log-link function. This

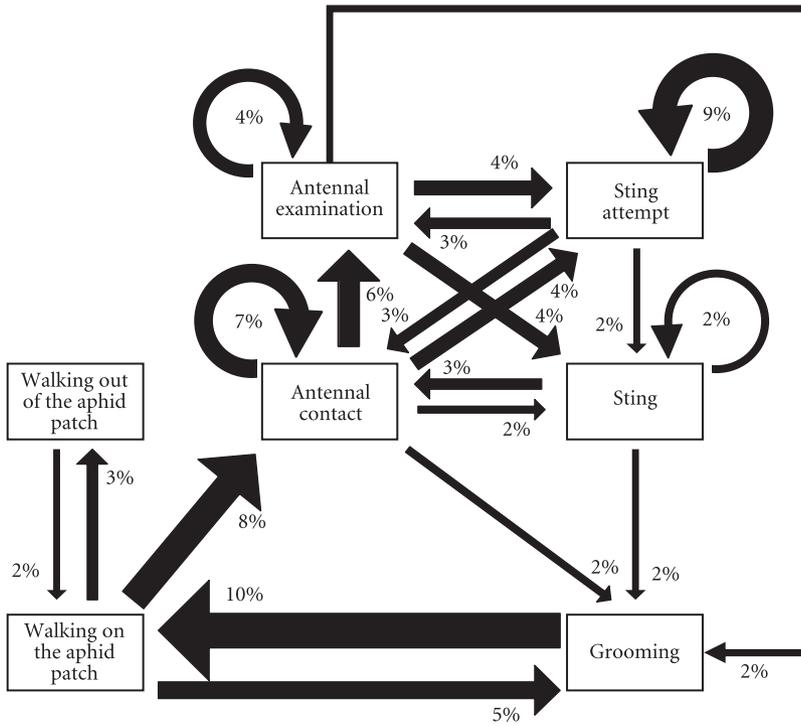


Figure 1 Quantitative diagram of the oviposition behaviour of *Diaeretiella rapae* on *Myzus persicae* patches. The probabilities of transition between behavioural items are indicated and represented by the width of the arrows. Probabilities of transition of less than 1% are not represented.

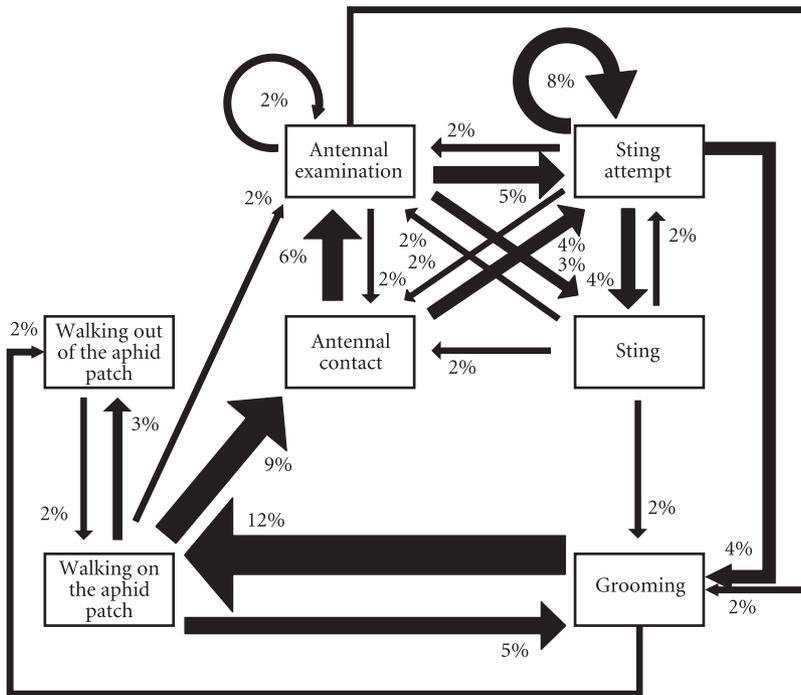


Figure 2 Quantitative diagram of the oviposition behaviour of *Aphidius matricariae* on *Myzus persicae* patches. The probabilities of transition between behavioural items are indicated and represented by the width of the arrows. Probabilities of transition of less than 1% are not represented.

statistical method accounts for the fact that the time variable lacks homoscedasticity and normality. For this, we used Proc Genmod in the SAS statistical package (SAS, 1999).

Results

Determination of deltamethrin doses

The log-probit regression equations were $Y = -0.38 + 2.84X$ ($\chi^2 = 6.44$, d.f. = 5, $P = 0.265$) for *D. rapae* and $Y = -0.01 + 1.79X$ for *A. matricariae* ($\chi^2 = 5.58$, d.f. = 4, $P = 0.233$). The data always fitted the linear model (not significant statistical deviance of data from regression equation), allowing a valid determination of the LD₅₀, LD₂₀, and LD_{0.1} for each species. For *D. rapae* and *A. matricariae*, respectively, the doses that induced 50% mortality (LD₅₀) were 1.36 ng cm⁻² and 1.01 ng cm⁻², LD₂₀ was 0.68 ng cm⁻² and 0.34 ng cm⁻², and LD_{0.1} was 0.10 ng cm⁻² and 0.02 ng cm⁻². When using these doses to expose females before behavioural testing, corrected mortalities were equal to 6.20 ± 3.16% for LD_{0.1}, 17.54 ± 6.40% for LD₂₀, and 48.35 ± 3.85% for LD₅₀ in the case of *D. rapae*, and 5.49 ± 3.37% for LD_{0.1}, 17.75 ± 5.67% for LD₂₀, and 46.58 ± 6.66% for LD₅₀ in the case of *A. matricariae*.

Foraging and oviposition behaviour of both parasitoid species

Once released on an aphid patch, the first female behaviour was 'walking on the aphid patch'. *Diaeretiella rapae* and

A. matricariae females moved typically, with continuous antennal drumming on the leaf surface. In most cases, females detected a host by 'antennal contact'. Then, females began the host-handling sequence which finally led to a 'sting' or 'sting attempt' (Figures 1 and 2). The most frequent host-handling sequence was as follows: an 'antennal contact', where females discovered a potential host, an 'antennal examination' of the host, and then a 'sting' or 'sting attempt'. In most cases, the host-handling sequence stopped when the females started 'grooming' (which could occur after any of the host-handling behaviours). Some behaviours could be repeated, particularly 'antennal contact', 'sting attempt', and to a lesser extent 'sting' and 'antennal examination'. After 'grooming', females resumed 'walking on the aphid patch' behaviour. Most females performed several host-handling sequences before leaving the patch. Excursions out of the patch were rare.

Impact of deltamethrin on the behaviour of *Diaeretiella rapae* and *Aphidius matricariae*

In both parasitoid species, there was no significant effect of the three deltamethrin doses on the frequencies of 'antennal contact', 'antennal examination', 'sting', 'sting attempt', and of the two behavioural sequences considered (Table 1). There was no significant difference between the species when we considered the number of behavioural items per minute. However, there was a significant difference

Table 1 Mean number (± SE) of behaviours related to host handling per minute and sequences per minute for *Diaeretiella rapae* and *Aphidius matricariae* females previously exposed to three doses of deltamethrin (LD_{0.1}, LD₂₀, or LD₅₀) for 24 h or a control, on *Myzus persicae* patches. The statistical comparison between control and the three doses for each species is provided

	Average frequencies per min of behaviours and sequences					
	Antennal contact	Antennal examination	Sting attempt	Sting	Initiation of host-handling	Host acceptance
<i>Aphidius matricariae</i>						
Control n = 34	0.57 ± 0.08	0.37 ± 0.08	0.56 ± 0.11	0.30 ± 0.06	0.09 ± 0.04	0.18 ± 0.05
LD _{0.1} n = 33	0.45 ± 0.06	0.65 ± 0.15	0.83 ± 0.19	1.11 ± 0.71	0.14 ± 0.05	0.08 ± 0.02
LD ₂₀ n = 34	0.58 ± 0.11	0.42 ± 0.10	0.75 ± 0.29	0.43 ± 0.15	0.14 ± 0.05	0.23 ± 0.08
LD ₅₀ n = 36	0.83 ± 0.22	0.67 ± 0.25	0.60 ± 0.16	0.39 ± 0.12	0.10 ± 0.06	0.18 ± 0.31
<i>Diaeretiella rapae</i>						
Control n = 35	0.78 ± 0.10	0.70 ± 0.15	0.63 ± 0.17	0.42 ± 0.11	0.11 ± 0.03	0.34 ± 0.06
LD _{0.1} n = 34	0.75 ± 0.11	0.75 ± 0.14	0.81 ± 0.16	0.30 ± 0.06	0.14 ± 0.03	0.30 ± 0.06
LD ₂₀ n = 35	0.93 ± 0.16	0.93 ± 0.19	0.81 ± 0.16	0.38 ± 0.11	0.25 ± 0.08	0.28 ± 0.08
LD ₅₀ n = 35	0.67 ± 0.09	0.62 ± 0.13	1.03 ± 0.29	0.40 ± 0.10	0.08 ± 0.03	0.28 ± 0.05
ANOVA						
Deltamethrin	F _{3,268} = 0.60 (P = 0.618)	F _{3,268} = 0.42 (P = 0.740)	F _{3,268} = 0.71 (P = 0.549)	F _{3,268} = 0.57 (P = 0.636)	F _{3,268} = 0.56 (P = 0.644)	F _{3,268} = 2.01 (P = 0.113)
Species	F _{1,268} = 3.72 (P = 0.055)	F _{1,268} = 3.86 (P = 0.051)	F _{1,268} = 0.96 (P = 0.327)	F _{1,268} = 0.96 (P = 0.327)	F _{1,268} = 9.24 (P = 0.003)	F _{1,268} = 0.55 (P = 0.458)
Species × deltamethrin	F _{3,268} = 1.74 (P = 0.160)	F _{3,268} = 1.23 (P = 0.300)	F _{3,268} = 1.30 (P = 0.275)	F _{3,268} = 0.52 (P = 0.670)	F _{3,268} = 0.72 (P = 0.539)	F _{3,268} = 0.67 (P = 0.572)

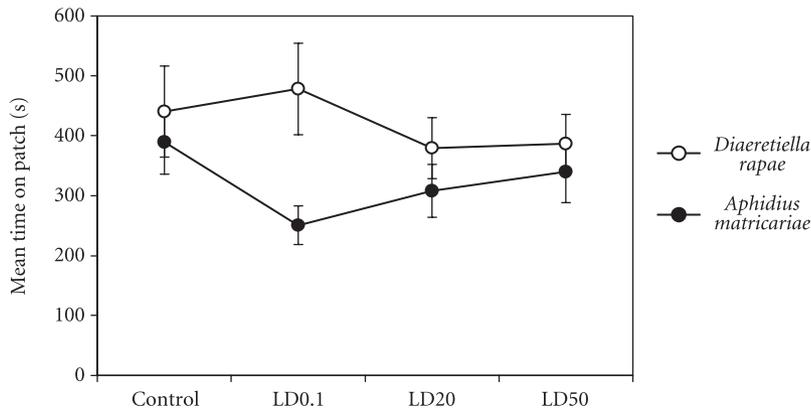


Figure 3 Mean total time in seconds (\pm SE) on patches of *Myzus persicae* for *Diaeretiella rapae* and *Aphidius matricariae* females previously exposed to three doses of deltamethrin (LD_{0.1}, LD₂₀ or LD₅₀) for 24 h, or for control females. There was no significant difference between the three doses of deltamethrin and the control ($\chi^2 = 2.27$, d.f. = 3, $P = 0.518$), but the difference between the two species was highly significant ($\chi^2 = 7.28$, d.f. = 1, $P = 0.007$). The interaction between deltamethrin doses and the species effects was not significant ($\chi^2 = 4.45$, d.f. = 3, $P = 0.127$) (likelihood ratio test of a general linear model).

between the two species regarding the 'initiation of host handling' sequence, but not the 'host acceptance' sequence.

The three deltamethrin doses did not significantly modify patch residence time (Figure 3). There was nonetheless a significant difference in the patch time allocation between *A. matricariae* and *D. rapae*. The latter species stayed about 1.5-fold longer on patches of *M. persicae* on oilseed rape leaf.

Discussion

This study demonstrated no effect of the three doses of deltamethrin tested on oviposition behaviour and patch time allocation in two aphid parasitoid species, *A. matricariae* and *D. rapae*. After exposure to deltamethrin residues on glass for 24 h, the survivors did not exhibit altered frequencies and sequences of different behaviours, and the females could still parasitize aphids. Neither was patch time allocation altered by exposure to deltamethrin.

An effect of deltamethrin exposure on parasitism was expected in the two species tested because this insecticide had previously been reported to disturb both sensory perception and motor functions (in addition to toxic and lethal effects). For example, the male locomotor response to female sex pheromones was altered in the wasp *Trichogramma brassicae*, even when treated at a low LD_{0.1} dose of deltamethrin (Delpuech et al., 1999). An impairment of learned olfactory responses was reported in deltamethrin-treated honeybees (Abramson et al., 1999). Walking speed was reduced in *Trissolcus basalis* exposed to a LD₂₀ (Salerno et al., 2002), and also in honeybees exposed to LD₂₀ and LD₅₀ (Rafalimanana, 2003). Moreover, it has been shown that other pyrethroids modify oviposition behaviour in the parasitoids *Aphidius colemani* (Ahmad & Hogson, 1998) and *Aphidius ervi* (Desneux et al., 2000; Desneux et al., 2004). In the latter case, the insecticide was

lambda-cyhalothrin, which is a type-II pyrethroid-like deltamethrin, with the same molecular and cellular targets, i.e., voltage-sensitive sodium channels and the GABA receptor-chloride ionophore complex (Soderlund & Bloomquist, 1989).

The lack of effect of the three deltamethrin doses used in our study could have two explanations: either the deltamethrin molecules did not alter the functions necessary for host-handling behaviour, or the surviving insects were less susceptible. The second explanation would suggest that the levels of toxic molecules in surviving insects were too low to induce nervous disorders. This hypothesis implies some heterogeneity of susceptibility to deltamethrin within the *A. matricariae* and *D. rapae* populations. According to Croft (1990), tolerance to pesticides may be a direct result of an organism's short- and long-term exposure to toxins in the natural environment. Variable levels of tolerance may reflect differences in vigour or in genetically determined susceptibility to pesticides (such as detoxification mechanisms, Bergé et al., 1996). The latter has been reported in some other hymenopteran parasitoid species (Hoy, 1990). Both sources of variability were thus likely to occur in our two parasitoid populations. Vigour may vary as a function of characteristics that were not controlled precisely in our experiment, such as size, stress, and general physiological state. Genetic variability of sensitivity to insecticides may have resulted from the yearly incorporation of field-collected females to the mass-reared strain. Indeed, exposure to different pesticides residues occurs over time under field conditions, and it is possible that less susceptible parasitoids had been selected.

The principal studies on the detoxification mechanisms of deltamethrin were carried out in rodents (Ruzo et al., 1978, 1979). The main metabolic reactions were the hydroxylation and ester cleavage of deltamethrin. Both reactions were revealed in insects through studies in cell

cultures, and inactivated the neurotoxic action of deltamethrin molecules (Casida & Ruzo, 1980; Ruzo et al., 1988). This was confirmed by Pilling et al. (1995) in the honeybee. These authors showed that the principal detoxification of lambda-cyhalothrin (pyrethroid) occurred through hydroxylation and ester cleavage. Thus, during our experiments, parasitoids surviving deltamethrin exposure may have recovered their behavioural abilities through a detoxification of the insecticide molecule. This conclusion is reinforced by other experiments on three Aphidiinae species, *D. rapae*, *A. matricariae*, and *A. ervi*, which showed that orientation behaviour towards aphid-infested plants was not disturbed following exposure to increasing doses of deltamethrin (inducing from 0 up to 80% of mortality; N. Desneux, unpubl.). Thus, partial tolerance to deltamethrin may possibly be linked to previous exposure to pyrethroids in the environment where the females were collected to initiate the laboratory colonies. Indeed, for our experiments, they were collected in two areas where large areas of crops such as oilseed rape are regularly treated with deltamethrin.

Our experiments did not demonstrate any significant difference in the frequencies of behavioural items between *A. matricariae* and *D. rapae* and only one significant difference in the frequency of the 'Initiation of host handling' sequence. However, *D. rapae* females remained about 1.5-fold longer on *M. persicae* patch on oilseed rape leaf than *A. matricariae* females, and were therefore likely to be attacking more aphids. Although *D. rapae* have been reported on a variety of crops (Pike et al., 2000), it is clear from the literature that it is mainly found on cruciferous plants (Bahana & Karhioc, 1986; Ohiman & Kunar, 1986; Souza et al., 1992; Vaughn & Antolin, 1998; Bijaya Devi et al., 1999), and particularly on *Brevicoryne brassicae* (Mackauer & Kambhampati, 1984; Gabrys et al., 1998). When *M. persicae* is found on cruciferous crops, it is also reported as a potential host for *D. rapae* (Lyon, 1968). Studies have shown that *D. rapae* is attracted by characteristic odours from Cruciferae (allylthiocyanate) (Read et al., 1970; Sheehan & Shelton, 1989; Vaughn et al., 1996). On the other hand, *A. matricariae* has no particular plant preference. Thus, the specificity of *D. rapae* towards cruciferous plants may be responsible for longer patch time allocation.

Finally, in the context of integrated pest management, this study shows that aphid parasitoids contaminated by deltamethrin residues, if they survive, do not seem to exhibit altered behaviour, or can recover their ability to parasitize hosts when foraging in untreated areas (such as refuge areas). In this case, *D. rapae* and *A. matricariae* could constitute important species for the control of insecticide-resistant aphid populations and/or aphids protected from insecticide treatment in refuge areas.

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References

- Abbott WS (1925) A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265–267.
- Abramson CI, Aquino IS, Ramalho FS & Price JM (1999) The effect of insecticides on learning in the Africanized Honey Bee (*Apis mellifera* L.). *Archives of Environmental Contamination and Toxicology* 37: 529–535.
- ACTA (2002) Index Phytosanitaire, 37eme edn. ACTA, Paris.
- Ahmad M & Hodgson CJ (1998) The searching efficiency of *Aphidius colemani* Viereck after visiting insecticide treated plants at different time intervals. *Entomon* 23: 185–189.
- Bahana J & Karhioc GK (1986) The role of *Diaeretiella rapae* in population control of cabbage aphid. *Journal of Insect Science* 7: 605–607.
- Barrett K, Brandy N, Harrisson EG, Hassan S & Oomen P (1994) Guidance document on regulatory testing procedures for pesticides with non-target arthropods. SETAC, Brighton, UK.
- Battaglia D, Pennacchio F, Marincola G & Tranfaglia A (1993) Cornicle secretion of *Acyrtosiphon pisum* (Homoptera, Aphididae) as a contact kairomone for the parasitoid *Aphidius ervi* (Hymenoptera, Braconidae). *European Journal of Entomology* 90: 423–428.
- Bergé JB, Chevillon C, Raymond M & Pasteur N (1996) Resistance of insects to insecticides. Molecular mechanisms and epidemiology. *Comptes Rendus de la Société de Biologie* 190: 445–454.
- Bijaya Devi P, Singh TK & Jiten Singh H (1999) Studies on the natural enemy complex of the green peach aphid, *Myzus persicae* (Sulzer) on Knol-Khol, *Brassica oleracea gongyloides*. *Annals of Plant Protection Science* 7: 37–40.
- Braimah H & van Emden HF (1994) The role of the plant in host acceptance by the parasitoid *Aphidius rhopalosiphii* (Hymenoptera: Braconidae). *Bulletin of Entomological Research* 84: 303–306.
- Brown RA (1989) Pesticides and non-target terrestrial invertebrates: an industrial approach. *Pesticides and Non-Target Invertebrates* (ed. by P C Jepson), pp. 19–42. Intercept, Wimborne, UK.
- Candolfi MP, Barrett KL, Campbell P, Forster R, Grandy N, Huet MC, Lewis G, Oomen PA, Schmuck R & Vogt H (2001) Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. SETAC/ESCORT 2 Workshop Report, 21–23 March 2000, Wageningen, The Netherlands.
- Casida JE & Ruzo LO (1980) Metabolic chemistry of pyrethroids-insecticides. *Pesticide Science* 11: 257–269.
- Chambers RJ, Sunderland KD, Stacey DL & Wyatt IJ (1986)

- Control of cereal aphids in winter wheat by natural enemies: aphid-specific predators, parasitoids and pathogenic fungi. *Annals of Applied Biology* 108: 219–231.
- Croft BA (1990) Pesticide resistance: documentation. *Arthropod Biological Control Agents and Pesticides* (ed. by BA Croft), pp. 357–382. Wiley, New York.
- De Jiu G & Waage JK (1990) The effect of insecticides on the distribution of foraging parasitoids, *Diaeretiella rapae* (Hym: Braconidae) on plants. *Entomophaga* 35: 49–56.
- Delpuech JM, Legallet B, Terrier O & Fouillet P (1999) Modification of the sex pheromonal communication of *Trichogramma brassicae* by a sub-lethal dose of deltamethrin. *Chemosphere* 38: 729–739.
- Desneux N, Noel B & Kaiser L (2000) Sublethal effect of a pyrethroid on orientation behaviour of the parasitic wasp *Aphidius ervi* (Hymenoptera: Aphidiidae) in response to odour from oilseed rape infested by the aphid *Myzus persicae*. *Bulletin IOBC/WPRS* 23: 55–64.
- Desneux N, Pham-Delègue MH & Kaiser L (2004) Effect of a sublethal and a lethal dose of lambda-cyhalothrin on oviposition experience and host searching behaviour of a parasitic wasp, *Aphidius ervi*. *Pest Management Science* 60: 381–389.
- Elzen GW (1989) Sublethal effects of pesticides on beneficial parasitoids. *Pesticides and Non-target Invertebrates* (ed. by PC Jepson), pp. 129–150. Intercept, Wimborne, UK.
- Finney DJ (1971) *Probit Analysis*. Cambridge University Press, Cambridge, UK.
- Gabrys B, Gadomski H, Sobota G & Halarewicz-Pacan A (1998) Reduction of the cabbage aphid, *Brevicoryne brassicae* (L.), population by *Diaeretiella rapae* (McIntosh) on oilseed rape, white mustard, and *Brassica* vegetables. *Bulletin IOBC/WPRS* 21: 197–203.
- Hågvar EB & Hofsvang T (1991) Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. *Biocontrol News and Information* 12: 13–41.
- Hardin MR, Benrey B, Coll M, Lamp WO, Roderick GK & Barbosa P (1995) Arthropod pest resurgence: and overview of potential mechanisms. *Crop Protection* 14: 3–18.
- Haynes HF (1988) Sublethal effects of neurotoxic insecticides on insect behavior. *Annual Review of Entomology* 33: 149–168.
- Hoy MA (1990) Pesticide resistance in arthropod natural enemies: variability and selection responses. *Pesticide Resistance in Arthropods* (ed. by RT Roush & BE Tabashnik), pp. 203–236. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Hsieh CY & Allen WW (1986) Effects of insecticides on emergence, survival, longevity, and fecundity of the parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) from mummified *Myzus persicae* (Homoptera: Aphididae). *Journal of Economic Entomology* 79: 1599–1602.
- Irving SN & Wyatt IJ (1973) Effects of sublethal doses of pesticides on the oviposition behaviour of *Encarsia formosa*. *Annals of Applied Entomology* 75: 57–62.
- Jepson PC (1989) The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. *Pesticides and Non-Target Invertebrates* (ed. by PC Jepson), pp. 95–127. Intercept, Wimborne, UK.
- Kühner C, Klingauf F & Hassan SA (1985) Development of laboratory and semi-field methods to test the side effect of pesticides on *Diaeretiella rapae* (Hym. Aphidiidae). *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 50: 531–538.
- Longley MA (1999) A review of pesticide effects upon immature aphid parasitoids within mummified hosts. *International Journal of Pest Management* 45: 139–145.
- Longley M & Jepson PC (1996a) Effects of honeydew and insecticide residues on the distribution of foraging aphid parasitoids under glasshouse and field conditions. *Entomologia Experimentalis et Applicata* 81: 189–198.
- Longley M & Jepson PC (1996b) The influence of insecticide residues on primary parasitoid and hyperparasitoid foraging behaviour in the laboratory. *Entomologia Experimentalis et Applicata* 81: 259–269.
- Longley M, Jepson PC, Izquierdo J & Sotherton N (1997) Temporal and spatial changes in aphid and parasitoid populations following applications of deltamethrin in winter wheat. *Entomologia Experimentalis et Applicata* 83: 41–52.
- Longley M & Stark JD (1996) Analytical techniques for quantifying direct, residual, and oral exposure of an insect parasitoid to an organophosphate insecticide. *Bulletin of Environmental Contamination and Toxicology* 57: 683–690.
- Lyon JP (1968) Remarques préliminaires sur les possibilités d'utilisation pratique d'hyménoptères parasites pour la lutte contre les pucerons en serre. *Annales des Epiphyties* 19: 113–118.
- Mackauer M & Kambhampati S (1984) Reproduction and longevity of cabbage aphid *Brevicoryne brassicae* (Homoptera: Aphididae) parasitised by *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *The Canadian Entomologist* 116: 1605–1610.
- Mahaut T & Deleu R (1997) Relation entre le comportement chimique et la toxicité de pesticides à l'égard d'*Aphidius rhopalosiphii* De Stephani-Perez, *Adalia bipunctata* (L.) et *Episyrphus balteatus* (De Geer): premiers résultats. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 62: 573–580.
- Ohiman SC & Kunar V (1986) Biological control of mustard aphid, *Lipaphis erysimi* by using the parasitoid *Diaeretiella rapae*. Influence of parasitoids on aphids. *Environmental Entomology* 16: 219–222.
- Pike KS, Sary P, Miller T, Graf G, Allison D, Boydston L & Miller R (2000) Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of northwest USA. *Proceedings of the Entomological Society of Washington* 102: 688–740.
- Pilling ED, Bromleychallenor KAC, Walker CH & Jepson PC (1995) Mechanism of synergism between the pyrethroid insecticide lambda-cyhalothrin and the imidazole fungicide procloraz, in honeybee (*Apis mellifera* L.). *Pesticide Biochemistry and Physiology* 51: 1–11.
- Powell W (1983) The role of parasitoids in limiting cereal aphid populations. *Aphid Antagonists* (ed. by R Cavalloro), pp. 50–56. Balkema, Rotterdam, The Netherlands.
- Powell W, Pennacchio F, Poppy GM & Tremblay E (1998) Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control* 11: 104–112.

- Powell W & Wright AF (1991) The influence of host food plants on host recognition by four Aphidiine parasitoids (Hymenoptera: Braconidae). *Bulletin of Entomological Research* 81: 449–453.
- Rafalimanana HJ (2003) Evaluation des effets d'insecticides sur deux types d'hyménoptères auxiliaires des cultures, l'abeille domestique (*Apis mellifera* L.) et des parasitoïdes de pucerons: études de terrain à Madagascar et de laboratoire en France. Thesis, INA Paris-Grignon, pp. 206.
- Read DP, Feeny PP & Root RB (1970) Habitat selection by the aphid parasite *Diaeretiella rapae* (Hymenoptera: Braconidae) and hyperparasite *Charips brassicae* (Hymenoptera: Cynipidae). *The Canadian Entomologist* 102: 1567–1578.
- Ruzo LO, Cohen E & Capua S (1988) Comparative metabolism of the pyrethroids bifenthrin and deltamethrin in the bulb mite *Rhizoglyphis robini*. *Journal of Agricultural and Food Chemistry* 36: 1040–1043.
- Ruzo LO, Engel JL & Casida JE (1979) Decamethrin metabolites from oxidative, hydrolytic, and conjugative reactions in mice. *Journal of Agricultural and Food Chemistry* 27: 725–731.
- Ruzo LO, Unai T & Casida JE (1978) Decamethrin metabolism in rats. *Journal of Agricultural and Food Chemistry* 26: 918–925.
- Salerno G, Colazza S & Conti E (2002) Sub-lethal effects of deltamethrin on walking behaviour and response to host kairomone of egg parasitoid *Trissolcus basalıs*. *Pest Management Science* 58: 663–668.
- SAS Institute Inc (1999) SAS/Stat User's Guide, Version 8. SAS Institute, Cary, NC.
- Schlinger EJ & Mackauer MJP (1963) Identity, distribution, and hosts of *Aphidius matricariae* Hal., an important parasite of green peach aphid, *Myzus persicae* (Hymenoptera: Aphidiidae – Homoptera: Aphidoidea). *Annals of the Entomological Society of America* 56: 648–653.
- Sheehan W & Shelton AM (1989) The role of experience in plant foraging by the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *Journal of Insect Behavior* 2: 743–759.
- Soderlund DM & Bloomquist JR (1989) Neurotoxic actions of pyrethroid insecticides. *Annual Review of Entomology* 34: 77–96.
- Souza BM, Bueno VHP & Paes Bueno VH (1992) Parasitoids and hyperparasitoids of mummies of *Brevicoryne brassicae* (Linnaeus.). *Revista de Agricultura Piracicaba* 67: 55–62.
- Süss L (1983) Survival of pupal stage of *Aphidius ervi* Hal. in mummified *Sitobion avenae* F. to pesticide treatment. *Aphid Antagonists* (ed. by R Cavalloro), pp. 129–134. Balkema, Rotterdam, The Netherlands.
- Umoru PA, Powell W & Clark SJ (1996) Effect of pirimicarb on the foraging behaviour of *Diaeretiella rapae* (Hymenoptera: Braconidae) on host-free and infested oilseed rape plants. *Bulletin of Entomological Research* 86: 193–201.
- Vaughn TT & Antolin MF (1998) Population genetics of an opportunistic parasitoid in an agricultural landscape. *Heredity* 80: 152–162.
- Vaughn TT, Antolin MF & Blostad LB (1996) Behavioral and physiological responses of *Diaeretiella rapae* to semiochemicals. *Entomologia Experimentalis et Applicata* 78: 187–196.
- Viggiani G (1984) Bionomics of the Aphelinidae. *Annual Review of Entomology* 29: 257–276.
- Vinson SB (1998) The general host selection behavior of parasitoid hymenoptera and comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control* 11: 79–96.