

# *Diaeretiella rapae* Limits *Myzus persicae* Populations After Applications of Deltamethrin in Oilseed Rape

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J. Econ. Entomol. 98(1): 9–17 (2005)

**ABSTRACT** In fall, *Myzus persicae* (Sulzer) (Homoptera: Aphididae) may exhibit population resurgence in winter oilseed rape in France. This resurgence may arise from pyrethroid treatments against Coleoptera (*Psylliodes chrysocephala* L.) that either kill parasitoids present during treatment or prevent recolonization by off-crop parasitoids. We studied the impact of *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) on populations of *M. persicae* when parasitoids were introduced on deltamethrin-treated plants at increasing intervals after treatment. Parasitoids were introduced 1, 2, 7, or 14 d posttreatment on individually caged plants infested with established populations of *M. persicae*. Aphids were counted 7, 14 and 21 d after parasitoid introduction. First, we observed that both the pesticide and the parasitoid reduced aphid population growth and that their effects were additive. Second, there was no mortality of parasitoids exposed to treated leaves in a device with a refuge area, and only 20% of mortality without the refuge area. Furthermore, deltamethrin residues had no effect on the reproduction of *D. rapae* females. Compared with the known toxicity of deltamethrin to *D. rapae* on glass, this low mortality may have been due to both the high liposolubility of deltamethrin (leading to a rapid diffusion of residues in the oilseed rape leaf cuticle) and to the existence of a refuge area. This work suggests that *D. rapae* could limit populations of *M. persicae* in the fall, even after pyrethroid treatment, because the presence of deltamethrin residues had little impact on the parasitoid.

**KEY WORDS** aphid parasitoid, pesticide residues, population, toxicity, risk assessment

CROP PROTECTION IS MOSTLY based on broad-spectrum chemical insecticides that are noxious to beneficial insects such as parasitoids (Haskell and McEwen 1998). For example, pest resurgence or an increase in populations of secondary pests can occur as a result of the death or perturbation of beneficial arthropods by pesticides (Hardin et al. 1995, Longley et al. 1997). Parasitoids can be exposed to insecticides through spray droplets (Jepson 1989), through residues on the crop foliage when foraging (Brown 1989; Jepson 1989; Longley and Jepson 1996a,b), or when feeding on contaminated water droplets, nectar, or honeydew (Longley and Stark 1996). Exposure during development in the host also may occur (Süss 1983, Hsieh and Allen 1986, Longley 1999). Exposure to insecticide residues is highly probable due to their widespread use.

Generally, applications of chemical insecticides result in a major initial reduction in the density of phytophagous and parasitoid populations. For example, deltamethrin treatment was shown to result in a 90% reduction in the number of parasitoids and a 78% reduction in the number of aphids (Longley et al. 1997). After insecticide treatment, pest and parasitoid populations may recover differently because their varying susceptibility to insecticides (Croft and Brown 1975, Rajakulendran and Plapp 1982, Weires et al. 1985) and reproductive rates (Longley et al. 1997). This dynamic has been particularly well documented under laboratory conditions (Waage et al. 1985). When recolonizing a crop after insecticide treatment, parasitoids may return to depleted areas (where insecticide treatments have been done) from undepleted surroundings (Longley et al. 1997), a process called "horizontal recruitment." In addition, a process of "vertical recruitment" of parasitoids (i.e., the emergence of adults from mummies) may occur in the treated crop. In both cases, parasitoids are exposed to insecticide residues, the persistency of which may vary from a few days to a few weeks, depending on the substance. In addition to their lethal effect, insecticide residues also can induce sublethal effects on fecundity, longevity (Moriarty 1969), and behavior

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through their neurotoxic activity (Haynes 1988, Elzen 1989).

Cruciferous crops are grown on a large scale in Europe. Oilseed rape fields alone covered >4.6 million ha in 2002 (United Nations Food and Agriculture Organization data). In Europe, the dominant aphid species infesting *Brassica* crops are cabbage aphid, *Brevicoryne brassicae* (L.) (Homoptera: Aphididae), a specialist of cruciferous plants; and green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae), which is polyphagous and can develop on some 300 host plant species (Van Emden et al. 1969). In France, problems related to the green peach aphid on oilseed rape in the fall have been observed since 1997. Populations can pullulate and weaken plants (CETIOM 2002), and *M. persicae* transmits viruses, even at a low population density (Schliephake et al. 2000). The transmission of viruses in oilseed rape has become a problem since 1996–1997 and markedly affects yields (Graichen and Schliephake 1996).

The pyrethroid insecticide Decis micro (i.e., deltamethrin), frequently used to treat oilseed rape for Coleoptera (*Psylliodes chrysocephala* L.) in the fall, has been suspected to affect aphid parasitoid populations. It may be insufficient to kill aphids, first because the rate used (5.0 g [AI]/ha) is lower than that recommended against aphids on oilseed rape (6.25 g [AI]/ha); and second, because aphids are partially resistant to pyrethroids (Foster et al. 2002). However, the insecticide may disturb the action of parasitoids, having a lethal effect on local populations during treatment applications and limiting recolonization by off-crop parasitoids.

The current study aimed to determine the interval that would allow parasitoids to return safely to aphid-infested winter oilseed rape crops after deltamethrin treatment. The work was conducted on the aphid parasitoid *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) because it has been reported to play a significant role in preventing aphid outbreaks in cruciferous crops such as oilseed rape and mustard (Bahana and Karhioc 1986, Ohiman and Kunar 1986, Souza et al. 1992, Bijaya Devi et al. 1999). Under semifield conditions (outdoor potted and caged plants), we assessed the action of *D. rapae* on *M. persicae* populations when the parasitoids were introduced on deltamethrin (Decis micro)-treated plants at increasing intervals after the treatment. We also quantified deltamethrin residues on the foliage and evaluated in the laboratory the acute toxicity of deltamethrin residues to *D. rapae*.

## Materials and Methods

### Semifield Study

**Experimental Plants.** Oilseed rape plants, *Brassica napus* (winter variety Goéland), were grown outside in individual plastic pots (2-liter volume, 17 cm in diameter). At the two-leaf stage, they were covered with individual plastic cages (40 cm in height, 17 cm in diameter). Ventilation was provided through two holes covered with gauze, one on the upper surface

(17.5 cm in diameter), and the other on the side of the cage (8 cm in diameter).

**Insects.** All insects were reared in environmental chambers at a temperature of  $23 \pm 1^\circ\text{C}$  under a photoperiod of 18:6 (L:D) h. *M. persicae* (R1-strain: partially resistant to insecticides through the overproduction of carboxylesterase) was reared on broad bean, *Vicia fabae* L. *D. rapae* was reared on *M. persicae* on *B. napus* leaves. Mummified aphids containing *D. rapae* were removed from the leaves and stored in plastic petri dishes until parasitoid emergence. Adult females were mated at emergence and then held for 24 h in glass tubes (5 cm in length, 1 cm in diameter, five individuals per tube), where they were supplied with a dilute honey solution (80%) but no aphids or plants. The parasitoids used for all experiments were 24–48 h old.

**Pesticide Treatment.** Insecticide-treated plants were sprayed with formulated deltamethrin (Decis micro), which was diluted with water (to obtain 3 liters of solution) and applied at the recommended field rate against Coleoptera *P. chrysocephala* in the fall (5 g [AI]/ha, corresponding to 300 liters/ha of solution). The treatment was applied using a power-pack aerosol hand sprayer. The nozzles of the sprayer (30 psi, XR8001VS Teejet Spray, Teejet South Europe, Orléans, France) were directed toward the plants from a distance of  $\approx 0.5$  m. The date of insecticide treatment was labeled T = 0.

**Experimental Groups and Procedure.** All individually caged plants were infested by 10 *M. persicae* of mixed stages, 8 d before the deltamethrin treatment. This 8-d period ensured the initial growth of aphid populations before any further manipulations. The plants were then divided into experimental groups of 30 plants each. Groups of plants were distributed according to a 2 by 2 by 4 factorial design with repeated measures. The first two-level factor was the presence or absence of deltamethrin. The second two-level factor was the presence or absence of parasitoids (five males and five females released per plant). The four-level factor was the lag time between deltamethrin treatment and parasitoid release: parasitoids were released on the caged plants either 1 (T + 1), 2 (T + 2), 7 (T + 7), or 14 (T + 14) days after deltamethrin treatment.

To quantify subsequent aphid population dynamics, we counted the number of aphids per plant 7, 14, and 21 d after the introduction of parasitoids into the cage. Because all plants had been treated the same day, aphids were consequently counted on different calendar dates for each group, depending on the lag between deltamethrin treatment and parasitoid introduction. To evaluate *D. rapae* population dynamics, we collected mummies on the plants and counted the number of emerging females. This number provided an index,  $R_0$ , of the net reproductive rate in the parasitoid population (i.e., the number of females produced per female and per generation).

**Analyses of Deltamethrin Residues.** Deltamethrin residues were analyzed on aphid-infested plants treated and handled as described previously. Sampling

**Table 1.** Details of the gas chromatography/mass spectrometer system

Apparatus description	Varian 3400 gas chromatograph, Varian Saturn II ion trap mass spectrometer
Description of column	MDN-5S fused silica capillary column, 30 m × 0.32 mm × 0.25 μm (Supelco, Bellefonte, PA)
Temperature of heated zones	Oven, 220 to 300°C at 20°C/min and 15 min at 300°C; splitless injector SPI type, 290°C
Carrier gas	Helium, 11 psi
Injection volume	1 μl

was carried out for each of the four ages of residues, i.e., 1, 2, 7, and 14 d after treatment. Between 27 and 34 leaves were sampled on several plants from each group. Sampled leaves were held gently by the petiole to avoid any contact with the leaf surface to be analyzed. Hexane and dichloromethane were used to extract the residues because deltamethrin is highly soluble in organic solvents and cuticular waxes are abundant on oilseed rape leaves. The extractions were carried out by soaking and agitating several leaves of the same group in three successive 100-ml baths of hexane (in a 250-ml beaker) for 5, 20, and 60 s, respectively. An ultimate extraction was carried out in 100 ml of dichloromethane for 30 s. These four extracts per sample were then vacuum-evaporated at ambient temperature (Rotavapor), diluted in 5 ml of hexane, and stored at a cold temperature until analysis. After the last soaking, the leaves were dried at ambient temperature and placed on a Bristol-board covered with a transparent plastic sheet. The whole setup was photocopied, photocopies were scanned, and the scanned leaf surface was measured using computer graphics software.

Chemical samples were analyzed by gas chromatography-mass spectrometry (technical details listed in Table 1). The chromatographic conditions made it possible to obtain a relatively fine chromatographic peak for deltamethrin with rapid elution ( $\approx 8$  min). The detection of deltamethrin was based on three specific ions present in the mass spectrum obtained in electronic impact mode: ions at  $m/z$  181, 251, and 253. Quantification was performed by adding up the signals obtained for each of these three ions and comparing this relative peak area with that obtained from standard solutions. The calibration curve (external calibration) was established using five points corresponding to 40, 100, 200, 500, and 1000 pg injected. One microliter of each sample was injected, and two replicates per point were carried out. Under these experimental conditions, the limit of detection was  $\approx 10$  pg of deltamethrin injected and the limit of quantification was  $\approx 20$  pg.

### Toxicity of Deltamethrin Sprayed on Leaves under Laboratory Conditions

First, to complement the semifield experiment, we evaluated the mortality rate of parasitoids when exposed to the four ages of deltamethrin residues on oilseed rape leaves (1, 2, 7, and 14 d after treatment,

the same plants being used as for the analysis of residues). Untreated leaves were used as controls. The stems of the leaves were immersed in a glass vial filled with water, and the leaf was placed inside a small aerated plastic cage (11.8 by 8.8 by 4.7 cm; one 5-cm-diameter hole covered with gauze), placed on the vial, and in which 10 parasitoids were introduced. Five replicates were performed for each age of residues and the controls. Twenty-four hours later, the dead parasitoids were counted. Insecticide exposure was performed at  $20 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and a photoperiod of 12:12 (L:D) h. This device contained untreated areas (i.e., plastic cage wall) similar to the semifield cages.

Second, we determined the toxicity of fresh deltamethrin residues without refuge areas. We used the formulated insecticide Decis micro. It was applied to cut leaves (7 cm in diameter) by using a Burgerjon-type Potter-tower (Burgerjon 1956). Two different doses were tested: 0.5 g (AI)/ha (corresponding to the residue rate 1 d posttreatment; Fig. 3) and 5 g AI/ha (field application rate); water-sprayed leaves were used as controls. The exposure units, slightly modified from those developed by Jansen (1996), were assembled as follows: two treated leaves covered with glass plates were fitted with a rubber band onto a plastic ring (5 cm in diameter, 2 cm in height) as the floor and ceiling, with the treated side turned inside. The ring was pierced with four holes. Two were used to feed the insects, one with water, one with a honey/water solution (80%) offered on two pieces of cotton wool. Two ventilation holes were provided, covered with fine nylon gauze. Ten parasitoids were introduced per unit. There were five replicates per dose and a control. After 24 h, the dead parasitoids were counted. Pesticide exposure was performed at  $20 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and a photoperiod of 12:12 (L:D) h.

### Statistical Analyses

For the semifield experiment, we tested the effect of deltamethrin, parasitoids, and day lag between insecticide treatment and parasitoid release on 1) the number of aphids averaged over 3 wk (average density) and 2) the evolution of aphid density over the 3-wk period (factor time). For this, we used a generalized linear model for repeated measure designs based on a Poisson distribution, a log-link function, and an exchangeable correlation matrix. Such generalized linear models allow for deficiencies in 1) normality, 2) homoscedasticity, and when analyzing the number of aphids, 3) statistical independence among counts made on the same plant but at different sampling dates. In practice, we used Proc Genmod in the SAS statistical package, with the GEE option to analyze repeated measures (SAS Institute 1999). To evaluate the impact of deltamethrin on *D. rapae* offspring production as a function of the number of aphids, we used a type 2 functional response (Holling 1959) applied to the number of emerging females ( $nf$ ):

$$nf = \frac{Q \cdot nh \cdot T}{1 + Q \cdot th \cdot nh}$$

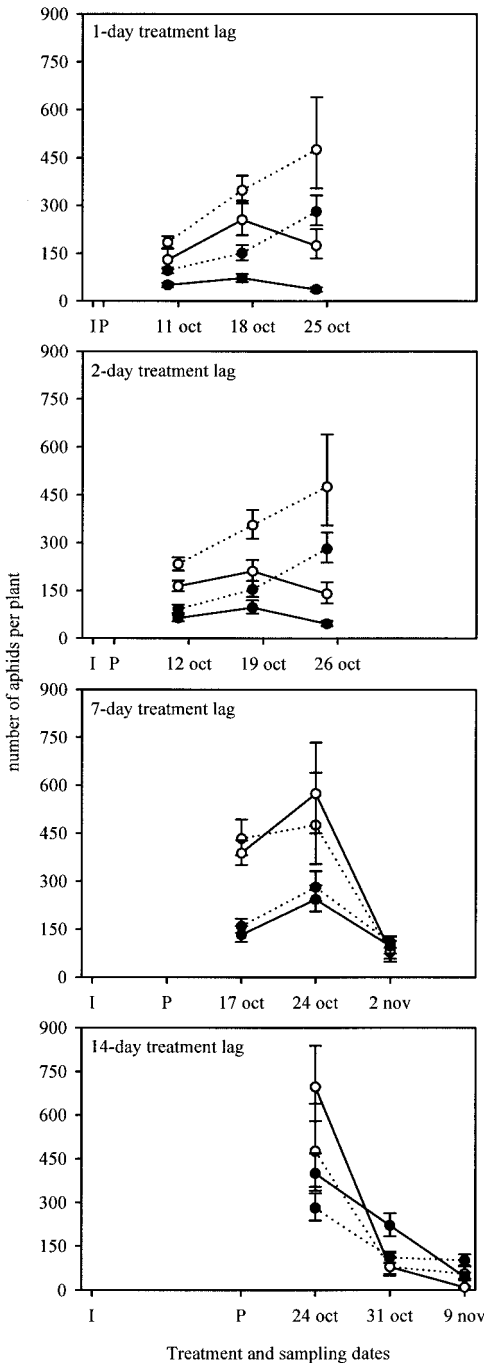


Fig. 1. Mean number of aphids per plant plotted as a function of time elapsing between insecticide (deltamethrin) treatment (I) and dates of parasitoid release (P). Each plot displays the data for a given day lag between parasitoid release and deltamethrin treatment. Continuous lines, plants with parasitoids; dashed lines, without parasitoids; filled circles, plants with insecticide; open circles, without insecticide. All x-axes are aligned on the number of days since the initial deltamethrin treatment, common to all experimental units. Plotted means and standard errors were back-transformed from statistics computed on log-transformed data.

Table 2. Type-3 statistics from the repeated measure generalized linear model used to analyze the number of aphids in the 1- and 2-d lag between deltamethrin treatment and parasitoid introduction

Source of variation	df	Chi-square	P value
Effects of day lag between groups			
Lag × deltamethrin × parasitoids × time	2	0.92	0.5318
Lag × deltamethrin × parasitoids	1	1.80	0.1802
Lag × deltamethrin × time	2	2.97	0.2258
Lag × parasitoids × time	2	0.21	0.9005
Lag × deltamethrin	1	1.07	0.3005
Lag × parasitoids	1	0.19	0.5541
Lag × time	2	2.94	0.2303
Lag	1	0.05	0.8247
Effects of deltamethrin and parasitoids on average aphid density			
Deltamethrin × parasitoids	1	5.47	0.0193
Deltamethrin	1	77.46	<0.0001
Parasitoids	1	55.72	<0.0001
Effects of deltamethrin and parasitoids on the evolution of aphid density			
Deltamethrin × parasitoids × time	2	4.40	0.1108
Deltamethrin × time	2	8.99	0.0111
Parasitoids × time	2	59.29	<0.0001
Time	2	82.60	<0.0001

The time factor was used to measure the impact of deltamethrin and parasitoids on the evolution of aphid density throughout the three week period.

where  $Q$  is the quest constant (i.e., parasitoid efficiency),  $th$  is the handling time (i.e., the time needed for each parasitoid to pursue and parasitize each aphid),  $nh$  is the number of hosts, and  $T$  is the available time (we used  $T = 1$ , for one generation of released parasitoids).

For the different experimental situations,  $Q$  and  $th$  were estimated via a nonlinear regression based on the least squares method and an iterative procedure (PROC NLIN in the SAS statistical package, SAS Institute 1999).

For toxicity experiments under laboratory conditions, we tested the effect of chemical treatments on the mortality rate by using the Mann-Whitney test.

### Results

**Impact of Deltamethrin on Aphid Populations and Parasitoid Efficiency in Semifield Conditions.** We first analyzed the impact of deltamethrin and parasitoids

Table 3. Type-3 statistics from the repeated measure generalized linear model used to analyze the number of aphids in the 7-d lag between deltamethrin treatment and parasitoid introduction

Source of variation	df	Chi-square	P value
Effects of deltamethrin and parasitoids on average aphid density			
Deltamethrin × parasitoids	1	0.19	0.6612
Deltamethrin	1	16.29	<0.0001
Parasitoids	1	0.27	0.6046
Effects of deltamethrin and parasitoids on the evolution of aphid density			
Deltamethrin × parasitoids × time	2	3.52	0.1718
Deltamethrin × time	2	4.71	0.0948
Parasitoids × time	2	0.63	0.7315
Time	2	45.38	<0.0001



on the density of aphids averaged over 3 wk for each group. We then assessed the impact of deltamethrin and parasitoids on the dynamic of aphid populations. The results are plotted in Fig. 1. Statistical results are summarized in Tables 2 and 3. The statistical results did not indicate a lag effect between the 1- and 2-d lag groups (Table 2, effects of time lag between both groups). This lack of lag effect can be visualized from the general similarity between the two top plots in Fig. 1. Thus, both groups were gathered to analyze the effects of deltamethrin and parasitoids on aphid populations.

In the 1- and 2-d lag groups, the average densities of aphid populations were significantly reduced by deltamethrin and parasitoids (Table 2, effects of deltamethrin and parasitoids on average aphid density). The proper effect of the deltamethrin treatment, of the parasitoid, and of the interaction of these two covariates was significant. Fewer aphids were present after deltamethrin treatment or after the introduction of parasitoids compared with the control group (without deltamethrin and without parasitoids). In 7-d lag groups, there was a significant effect of deltamethrin on the average density of aphid populations, but no effect of parasitoids (Table 3, effects of deltamethrin and parasitoids on average aphid density). In all 14-d lag groups, there was a marked reduction in aphid populations during the 3-wk period (Fig. 1). This was probably due to weather conditions (the average temperatures were around 5–6°C during this period, versus 11–13°C previously). Thus, under these conditions, it was not possible to evaluate the effect of parasitoids and deltamethrin on aphid populations.

As for the impact of deltamethrin and parasitoids on aphid population dynamics, first, in 1- and 2-d lag groups, time affected aphid densities, with an overall density (all combinations) that rose during the 3 wk of the experimental period (Table 2, effects of deltamethrin and parasitoids on the evolution of aphid density). Interactions between time and deltamethrin effects, and between time and parasitoid effects were both significant. In the control group (without deltamethrin and without parasitoids), aphid populations grew continuously through the first, second, and third weeks (Fig. 1). When treated with deltamethrin, aphid numbers increased in the same way but with smaller populations. In the groups where parasitoids were introduced, aphid populations increased from the first to the second week but not from the second to the third week. Thus, deltamethrin and parasitoids affected the population dynamics of *M. persicae*, but differently. These two factors acted independently (Table 2, deltamethrin  $\times$  parasitoids  $\times$  time; N.S.). In 7-d lag groups, aphid population dynamics was different (Table 3). Aphid densities varied over time with a similar pattern for all aphid populations (no deltamethrin  $\times$  parasitoids  $\times$  time interaction). After 1 and 2 wk, there were about one-half fewer aphids on plants sprayed with insecticide than on control plants, whether or not parasitoids had been released on the plants (Fig. 1). However, between the second and

third weeks, aphid populations fell markedly, resulting in a similar average aphid density on all plants.

**Impact of Deltamethrin on Net Reproductive Rate of Parasitoids.** The relationship between female offspring production and the number of aphids is shown in Fig. 2 for 1-, 2-, and 7-d lag groups. In all cases, the data were satisfactorily explained by a type 2 functional response ( $P < 0.001$ ; Table 4). The reduction in aphid populations observed in the 14-d lag groups did not allow assessment of the effect of deltamethrin on *D. rapae* offspring production.

When parasitoids were introduced 1 d after deltamethrin treatment (1-d lag), we observed a greater production of females per aphid when the plants were treated with deltamethrin. However this effect was not significant because the confidence limits of the estimated parameters of the model ( $Q$ , quest constant; and  $th$ , handling time) overlapped. In the 2- and 7-d lag

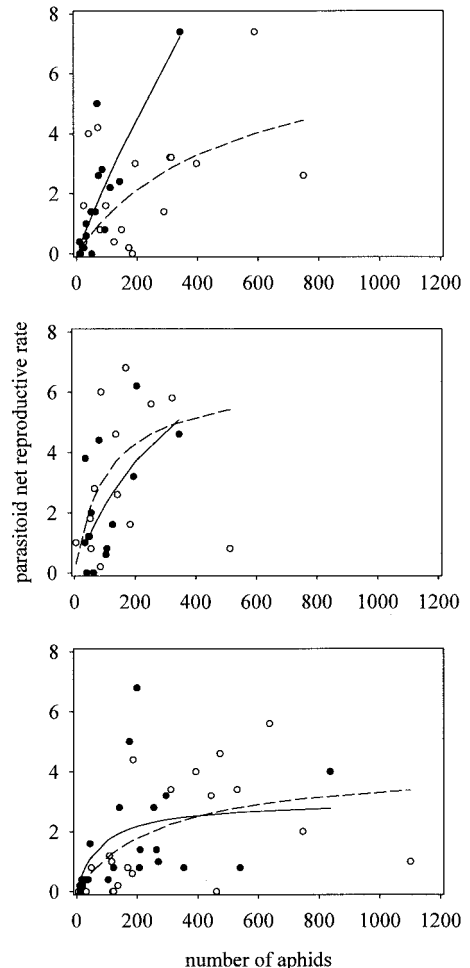


Fig. 2. Regression model of the mean number of offspring parasitoid females per plant as a function of aphid numbers and deltamethrin treatment. Open circles and dotted lines, without deltamethrin; filled circles and continuous lines, with deltamethrin (for lag 1, 2, and 7).

**Table 4.** Impact of deltamethrin on *D. rapae* female offspring production as a function of aphid numbers (model developed by Holling 1959)

Model adjustment			
Control		Deltamethrin	
Effects of deltamethrin and aphids on female offspring production in 1-d lag group			
Parameters	Confidence limits	Parameters	Confidence limits
$Q =$	$-0.0170 < 0.0737 < 0.1644$	$Q =$	$0.0586 < 0.1192 < 0.1798$
$th =$	$-0.0159 < 0.0269 < 0.0697$	$th =$	$-0.0109 < 0.0048 < 0.0206$
$F = 15.7$	$P < 0.001$	$F = 45.36$	$P < 0.001$
Effects of deltamethrin and aphids on female offspring production in 2-d lag group			
Parameters	Confidence limits	Parameters	Confidence limits
$Q =$	$-0.1386 < 0.2505 < 0.6396$	$Q =$	$0.0024 < 0.1292 < 0.2560$
$th =$	$0.0009 < 0.0282 < 0.0555$	$th =$	$-0.0189 < 0.0170 < 0.0530$
$F = 18.37$	$P < 0.001$	$F = 19.48$	$P < 0.001$
Effects of deltamethrin and aphids on female offspring production in 7-d lag group			
Parameters	Confidence limits	Parameters	Confidence limits
$Q =$	$-0.1012 < 0.0733 < 0.1586$	$Q =$	$0.1757 < 0.1584 < 0.4925$
$th =$	$0.0083 < 0.0461 < 0.0839$	$th =$	$0.0038 < 0.0616 < 0.1194$
$F = 25.29$	$P < 0.001$	$F = 45.36$	$P < 0.001$

groups, the confidence limits of both parameters also overlapped, and there was no visible difference in the production of females between untreated and treated groups, the regression lines being almost superimposed.

**Quantity of Deltamethrin Residues Present on Leaves.** Increasing periods of soaking in hexane and dichloromethane were used to extract the deltamethrin residues from successive layers of the leaf cuticle. The first 5-s hexane soaking was supposed to extract only the deltamethrin present on the upper surface of the leaves and thus likely to contaminate parasitoids walking on them (Riederer and Schneider 1989, Stammitti et al. 1996). Other soaking times (20 s in hexane, 60 s in hexane, and 30 s in dichloromethane) made it possible to determine whether deltamethrin had penetrated the layers of the leaf cuticle. The results are shown in Fig. 3.

One day after insecticide treatment (1-d lag), most of the total extracted residues were contained in the first 5 s soaking. Only a small quantity of deltamethrin remained in the subsequent soakings. The total amount of extracted residues was close to one-tenth ( $\approx 5$  ng [AI]/cm<sup>2</sup> corresponding to 0.5 g [AI]/ha) of the applied rate. Two days after insecticide treatment (2-d lag), the same pattern of extraction was observed, but a smaller amount of residue was obtained from the first soaking (2.7 ng [AI]/cm<sup>2</sup> deltamethrin). At this date, the quantity present in the second soaking in hexane (20 s) was higher than 1 d after treatment, with  $\approx 1.2$  ng (AI)/cm<sup>2</sup>. Seven and 14 d after insecticide treatment (7- and 14-d lag), little deltamethrin was found on the leaves ( $< 0.5$  ng (AI)/cm<sup>2</sup> for all soaking times and on both dates).

**Toxicity of Deltamethrin-Sprayed Leaves (Device with Refuge Areas).** Mortality was similar for parasitoids exposed to deltamethrin-treated plants and parasitoids exposed to control plants (Table 5).

**Toxicity of Deltamethrin-Sprayed Leaves (Device without Refuge Areas).** When parasitoids were confined on dried deltamethrin residues, the doses used induced a mean percentage of mortality of  $20.0 \pm 4.5\%$  (dose of 0.5 g [AI]/ha),  $44.3 \pm 13.1\%$  (dose of 5.0 g [AI]/ha), and  $2.9 \pm 2.9\%$  (untreated leaves). Both application doses induced significant parasitoid mortality compared with untreated leaves (0.5 g [AI]/ha: Mann-Whitney, MW = 2.5,  $P = 0.004$ ; 5.0 g [AI]/ha: MW = 2.5,  $P = 0.002$ ).

## Discussion

This study shows an effect of deltamethrin and parasitoids on aphid populations, during the fall and under semifield conditions. In groups of chemically treated plants, we observed a significant reduction in aphid populations compared with the control groups (without insecticide and without parasitoids). Although the recommended field rate against oilseed rape coleopterans is lower than the recommended rate against *M. persicae*, it nonetheless reduces aphid populations. In the presence or absence of deltamethrin residues, *D. rapae* also limits aphid population growth, suggesting that pesticide residues did not prevent the action of parasitoids under experimental conditions. Moreover, parasitoids and insecticide treatment had additive impacts on aphid populations. In fact, the deltamethrin spraying reduced aphid populations whose growth was subsequently limited by parasitoids introduced 1 or 2 d after treatment. At the

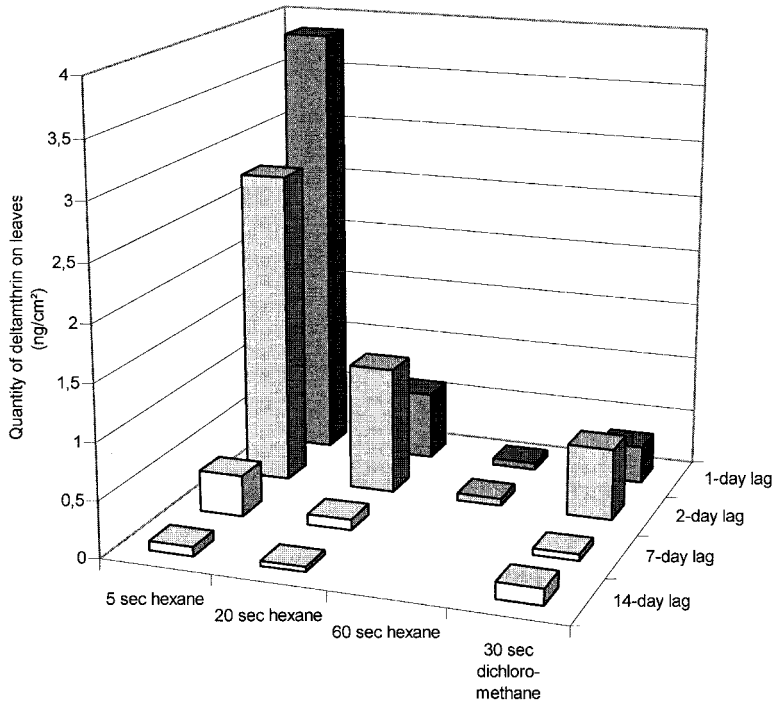


Fig. 3. Deltamethrin residues on oilseed rape as a function of days after plant treatment (1-, 2-, 7-, and 14-d lag) and time of soaking in organic solvents (hexane and dichloromethane).

end of the experiment,  $\approx 900$  aphids were found per untreated plants without parasitoids, 300–400 on plants that were either treated or with parasitoids, and 100 on treated plants with parasitoids. Thus, the combination of deltamethrin and released parasitoids was 3–4 times more efficient on aphid populations than when used separately. In plants where parasitoids were introduced 7 or 14 d after treatment, the action of parasitoids could not be observed because the aphid populations declined continuously due to falling temperatures toward the end of fall.

Data on *D. rapae* reproduction did not indicate any significant effects of deltamethrin on the relationship between female offspring production and aphid numbers, although the rate of female emergence relative to aphid numbers tended to be higher in the deltamethrin-treated groups (1-d lag group). This was probably due to the presence of fewer aphids on treated plants, because it has been shown that aphid parasitoids display better control of small aphid populations (Rabasse and Van Steenis 1999). The lack of

a significant effect of deltamethrin was in accordance with the findings of Krespi et al. (1991), who studied another aphid parasitoid, *Aphidius rhopalosiphi* (De Stefani-Perez), and reported an absence of deltamethrin effects on the parasitism rate when adults were exposed to the insecticide for 10 min or 1 h. Under our semifiel experimental conditions, exposure also may have been limited to the time spent parasitizing, because the parasitoids could escape the treated surface by flying to uncontaminated leaves or cage areas. The absence of a deltamethrin effect on the production of female offspring also suggested that parasitoid behavior was not disturbed, or in any case insufficiently to reduce parasitic efficiency. However, other studies have reported modifications to foraging behavior and foraging patterns in aphid parasitoids when plants were treated with deltamethrin (Longley and Jepson 1996a,b), or permethrin, another pyrethroid (Jiu and Waage 1990), and also pirimicarb (carbamate) (Umoru et al. 1996). In addition, Salerno et al. (2002) demonstrated that *Trissolcus basal*

Table 5. Toxicity of different ages of deltamethrin residues in a device with refuge areas

		Age of deltamethrin residues (days after treatment)			
		T + 1	T + 2	T + 7	T + 14
Mean % of mortality ( $\pm$ SE) <sup>a</sup>	Control leaves	0.0 $\pm$ 0.0a	2.0 $\pm$ 2.0a	0.0 $\pm$ 0.0a	2.0 $\pm$ 2.0a
	Treated leaves	0.0 $\pm$ 0.0a	8.0 $\pm$ 2.0a	2.0 $\pm$ 2.0a	2.0 $\pm$ 2.0a

Treated leaves were taken from plants treated in the semifiel experiment.

<sup>a</sup>Mortality after 24 h of exposure. Mean of five replicates ( $n = 10$  parasitoids per replicate).

(Wollaston) parasitoid females exposed to a low dose of deltamethrin reduced their walking speed and the time spent on host patches. However, female offspring production was not measured in all these studies.

Measurements of deltamethrin residues showed that only one-tenth of the applied dose was present on the leaves, up to 2 d after treatment, and that 7 and 14 d after the insecticide treatment, little deltamethrin remained on the leaves. This agreed with the findings of El-Ansary and El-Zogby (1992) who described persistence 3–4 d after treatment, and Ruzo and Casida (1979), who indicated a half-life of 7–8 d on cotton leaves in glasshouses and a more rapid degradation rate under field conditions.

The measured residual rate of 5 ng (AI)/cm<sup>2</sup> had no effect on the action of introduced parasitoids under semifield conditions, although during a previous study we had found an LD<sub>50</sub> value of 1.4 ng (AI)/cm<sup>2</sup> on *D. rapae* exposed on glass (unpublished data). Two explanations are possible for our measurements of mortality due to residues on leaves with and without refuge areas. First, in agreement with Mahaut and Deleu (1997), mortality was lower when deltamethrin residues are on leaves. Indeed, when parasitoids were confined to dried deltamethrin residues (only 1 h after treatment), at a rate of 0.5 g (AI)/ha (i.e., 5 ng [AI]/cm<sup>2</sup>), a mortality rate of only 20% was found, whereas at a rate of 5.0 g (AI)/ha (50 ng [AI]/cm<sup>2</sup>), i.e., >35 times the LD<sub>50</sub> on glass, the rate reached 44.3%. One possible explanation was that deltamethrin was adsorbed into the waxy layer of the oilseed rape leaf cuticle because commercial formulations of deltamethrin exhibit marked lipophilicity (Ro  and Pastre 1990), and was thus less available to parasitoids. Second, refuge areas were available in the cages used under semifield conditions, and we found no parasitoid mortality when they were exposed, in a device with refuge areas, to leaves from treated plants, even on the first day after deltamethrin treatment (corresponding to an exposure to 5 ng [AI]/cm<sup>2</sup> deltamethrin residues; Table 5). In outdoor caged plants, parasitoids did not remain in the uncontaminated refuge areas (cage wall, abaxial leaf surfaces, or vertically aligned parts of leaves; Cilgi and Jepson 1992, Koch and Weisser 2001) because they clearly parasitized the aphids. But because of the known repulsive action of pyrethroids on parasitoids (Longley and Jepson 1996a, Perera 1982, Hoy and Dahlsten 1984), they may have flown to the cage walls and thus recovered from insecticide exposure.

It was shown that the action of parasitoids on aphid populations was possible when they were introduced after deltamethrin treatment under semifield conditions. Because adult parasitoids are killed by the spray, mummies may constitute a reservoir for aphid parasitoids. Stary (1970) reported that the pupal stage within a mummified aphid was the least susceptible to insecticide. Krespi et al. (1991) and Jansen (1996) reported an absence of deltamethrin effects on the emergence rate of *A. rhopalosiphii*. However, Hsieh and Allen (1986) reported an effect of permethrin, a pyrethroid, on the emergence rate of *D. rapae*. So, the sensitivity of the mummy stage may depend on the

combination of aphid parasitoid species and pesticide considered.

In conclusion, and in a perspective of integrated pest management, our results show that deltamethrin residues on oilseed rape foliage had no effect on the limitation of aphid populations by parasitoids recolonizing treated plants, even only 1 d after treatment. Indeed, both pesticide and parasitoids reduced aphid population growth, and their effects were additive. Because the spray is known to kill a large proportion of aphid parasitoids, the limitation of aphid growth after treatment will then be mostly dependent upon the number of mummified or off-crop parasitoids able to recolonize the crop.

### Acknowledgments

We thank Minh-H  Pham-Del gue for scientific advice; Claude Mayer, Axel Decourty, and Hichem Azzouz for experimental help; Jean-Michel Rabasse for useful discussion of the experiments; Jacques Einhorn and Christian Malosse for help and discussion of the residue analyses; and Laurent Gillot for providing biological materials. This work benefited from a grant from CETIOM/Bayer Crop Science France.

### References Cited

- Bahana, J., and G. K. Karhioc. 1986. The role of *Diaeretiella rapae* in population control of cabbage aphid. *J. Insect Sci.* 7: 605–607.
- Bijaya Devi, P., T. K. Singh, and H. Jiten Singh. 1999. Studies on the natural enemy complex of the green peach aphid, *Myzus persicae* (Sulzer), on knol-khol, *Brassica oleracea gongylodes*. *Ann. Plant Protection Sci.* 7: 37–40.
- Brown, R. A. 1989. Pesticides and non-target terrestrial invertebrates: an industrial approach, pp. 19–42. *In* P. C. Jepson [ed.], *Pesticides and non-target invertebrates*. Intercept, Wimborne, United Kingdom.
- Burgerjon, A. 1956. Pulv risation et poudrage au laboratoire par des pr parations pathog nes insecticides. *Ann. Epiphyt.* 4: 675–684.
- [CETIOM] Centre Technique Interprofessionnel des Ol agineux M tropolitains. 2002. Le colza d'hiver, les techniques culturales, le contexte  conomique. Edition CETIOM, Thiverval-Grignon, France.
- Cilgi, T., and P. C. Jepson. 1992. The use of tracers to estimate the exposure of beneficial insects to direct pesticide spraying in cereals. *Ann. Appl. Biol.* 121: 239–247.
- Croft, B. A., and A. W. A. Brown. 1975. Responses of arthropod natural enemies to insecticides. *Annu. Rev. Entomol.* 20: 285–336.
- El-Ansary, O., and F. El-Zogby. 1992. Toxicity and residual hazards of some insecticides to honey bees. *Alexandria J. Agric. Res.* 37: 259–275.
- Elzen, G. W. 1989. Sublethal effects of pesticides on beneficial parasitoids, pp. 129–150. *In* P. C. Jepson [ed.], *Pesticides and non-target invertebrates*. Intercept, Wimborne, United Kingdom.
- Foster, S. P., I. Denholm, and A. L. Devonshire. 2002. Field-simulator studies of insecticide resistance to dimethylcarbamates and pyrethroids conferred by metabolic- and target site-based mechanisms in peach-potato aphids, *Myzus persicae* (Hemiptera: Aphididae). *Pest Manage. Sci.* 58: 811–816.
- Graichen, K., and E. Schliephake. 1996. Auftreten, Symptome und Vektoren des Wasserr benvergilbungsvirus



- (Syn. Westliches Rübenvergilbungsvirus) am Winter-  
raps. Nachrichtenbl Deut Pflanzenschutzd. 48: 186–191.
- Hardin, M. R., B. Benrey, M. Coll, W. O. Lamp, G. K. Rod-  
erick, and P. Barbosa. 1995. Arthropod pest resurgence:  
and overview of potential mechanisms. Crop Protection  
14: 3–18.
- Haskell, P. T., and P. McEwen. 1998. Ecotoxicology—pes-  
ticides and beneficial organisms. Kluwer Academic Pub-  
lishers, London, United Kingdom.
- Haynes, H. F. 1988. Sublethal effects of neurotoxic insecticides  
on insect behavior. Annu. Rev. Entomol. 33: 149–168.
- Holling, C. S. 1959. Some characteristics of simple types of  
predation and parasitization. Can. Entomol. 91: 386–398.
- Hoy, J. B., and D. L. Dahlsten. 1984. Effects of malathion  
and Staley's bait on the behaviour and survival of parasitic  
Hymenoptera. Environ. Entomol. 13: 1483–1486.
- Hsieh, C. Y., and W. W. Allen. 1986. Effects of insecticides  
on emergence, survival, longevity, and fecundity of the  
parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae)  
from mummified *Myzus persicae* (Homoptera: Aphidi-  
dae). J. Econ. Entomol. 79: 1599–1602.
- Jansen, J. P. 1996. Side effects of insecticides on *Aphidius*  
*rhopalosiphii* (Hym. Aphididae) in laboratory. Entomophaga  
1: 37–43.
- Jepson, P. C. 1989. The temporal and spatial dynamics of  
pesticide side-effects on non-target invertebrates, pp. 95–  
127. In P. C. Jepson [ed.], Pesticides and non-target in-  
vertebrates. Intercept, Wimborne, United Kingdom.
- Jiu, G. D., and J. K. Waage. 1990. The effect of insecticides  
on the distribution of foraging parasitoids, *Diaeretiella rapae*  
(Hym: Braconidae) on plants. Entomophaga 35: 49–56.
- Koch, H., and P. Weisser. 2001. Spray deposits of crop pro-  
tection products on plants—the potential exposure of non-  
target arthropods. Chemosphere 44: 307–312.
- Krespi, L., J. M. Rabasse, C. A. Dedryver, and J. P. Nenon.  
1991. Effect of three insecticides on the life cycle of  
*Aphidius uzbekistanicus* Luz. (Hym., Aphidiidae). J. Appl.  
Entomol. 111: 113–119.
- Longley, M. A. 1999. A review of pesticide effects upon  
immature aphid parasitoids within mummified hosts. Int.  
J. Pest Manage. 45: 139–145.
- Longley, M., and P. C. Jepson. 1996a. Effects of honeydew  
and insecticide residues on the distribution of foraging  
aphid parasitoids under glasshouse and field conditions.  
Entomol. Exp. Appl. 81: 189–198.
- Longley, M., and P. C. Jepson. 1996b. The influence of in-  
secticide residues on primary parasitoid and hyperpara-  
sitoid foraging behaviour in the laboratory. Entomol. Exp.  
Appl. 81: 259–269.
- Longley, M., and J. D. Stark. 1996. Analytical techniques  
for quantifying direct, residual, and oral exposure of an  
insect parasitoid to an organophosphate insecticide. Bull.  
Environ. Contam. Toxicol. 57: 683–690.
- Longley, M., P. C. Jepson, J. Izquierdo, and N. Sotherton.  
1997. Temporal and spatial changes in aphid and para-  
sitoid populations following applications of deltamethrin  
in winter wheat. Entomol. Exp. Appl. 83: 41–52.
- Mahaut, T., and R. Deleu. 1997. Relation entre le compor-  
tement chimique et la toxicité de pesticides à l'égard  
d'*Aphidius rhopalosiphii* De Stephani-Perez, *Adalia bipunc-  
tata* (L.) et *Episyrphus balteatus* (DeGeer): premiers res-  
ultats. Med. Fac. Landbouww. Univ. Gent. 62: 573–580.
- Moriarty, F. 1969. The sublethal effects of synthetic insec-  
ticides on insects. Biol. Rev. 44: 321–357.
- Ohiman, S. C., and V. Kunar. 1986. Biological control of  
mustard aphid, *Lipaphis erysimi* by using the parasitoid  
*Diaeretiella rapae*. Influence of parasitoids on aphids.  
J. Environ. Entomol. 16: 219–222.
- Perera, P.A.C.R. 1982. Some effects of insecticide deposit  
patterns on the parasitism of *Trialeuroides vaporariorum*  
by *Encarsia formosa*. Ann. Appl. Biol. 101: 239–244.
- Rabasse, J. M., and M. Van Steenis. 1999. Biological control  
of aphids, pp. 235–243. In R. Albajes, M. L. Gullino, J. C.  
Van Lenteren, and Y. Elad [eds.], Integrated pest and  
diseases management in greenhouse crops. Kluwer,  
Dordrecht, Germany.
- Rajakulendran, V. S., and F.W.J. Plapp. 1982. Comparative  
toxicities of five synthetic pyrethroids to the Tobacco  
Budworm (Lepidoptera: Noctuidae), an ichneumonid  
parasite, *Campolestis sonorensis*, and a predator, *Chrysopa*  
*carnea*. J. Econ. Entomol. 75: 769–772.
- Riederer, M., and G. Schneider. 1989. Comparative study of  
the composition of waxes extracted from isolated leaf  
cuticles and from whole leaves of citrus: evidence for  
selective extraction. Physiol. Plant 77: 373–384.
- Roã, L., and P. Pastre. 1990. The control of insect pests in  
oilseed rape: deltamethrin file. Roussel Uclaf Division  
Agrovet, Paris, France.
- Ruzo, L. O., and J. E. Casida. 1979. Degradation of delta-  
methrin on cotton plants. J. Agric. Food Chem. 27: 572–575.
- Salerno, G., S. Colazza, and E. Conti. 2002. Sub-lethal ef-  
fects of deltamethrin on walking behaviour and response  
to host kairomone of egg parasitoid *Trissolcus basalus*. Pest  
Manage. Sci 58: 663–668.
- SAS Institute. 1999. SAS/Stat user's guide, release 8. ed. SAS  
Institute, Cary, NC.
- Schliephake, E., K. Graichen, and F. Rabenstein. 2000. In-  
vestigations on the vector transmission of the Beet mild  
yellowing virus (BMVY) and the Turnip yellows virus  
(TuYV). J. Plant Dis. Prot. 107: 81–87.
- Souza, B. M., V.H.P. Bueno, and V. H. Paes Bueno. 1992.  
Parasitoids and hyperparasitoids of mummies of *Brevicoryne*  
*brassicae* (Linnaeus). Rev. Agric. Piracicaba. 67: 55–62.
- Stammitti, L., S. Derridj, and J. P. Garrec. 1996. Leaf  
epicuticular lipids of *Prunus laurocerasus*: importance of  
extraction methods. Phytochemistry 43: 45–48.
- Stary, P. 1970. Biology of aphid parasites (Hymenoptera:  
Aphidiidae) with respect to integrated control. Junk, The  
Hague, The Netherlands.
- Süss, L. 1983. Survival of pupal stage of *Aphidius ervi* Hal. in  
mummified *Sitobion avenae* F. to pesticide treatment, pp.  
129–134. In R. Cavalloro [ed.], Aphid antagonists.  
Balkema, Rotterdam, The Netherlands.
- Umoru, P. A., W. Powell, and S. J. Clark. 1996. Effect of  
pirimicarb on the foraging behaviour of *Diaeretiella rapae*  
(Hymenoptera: Braconidae) on host-free and infested  
oilseed rape plants. Bull. Entomol. Res. 86: 193–201.
- Van Emden, H. F., V. F. Eastop, R. D. Hugues, and M. J. Way.  
1969. The ecology of *Myzus persicae*. Annu. Rev. Entomol.  
14: 197–270.
- Waage, J. K., M. P. Hassell, and H.C.J. Godfray. 1985. The  
dynamics of pest-parasitoid-insecticide interactions.  
J. Appl. Ecol. 22: 825–838.
- Weires, R. W., J. R. Leeper, W. H. Reissig, and S. E. Lienk.  
1985. Toxicity of several insecticides to the spotted ten-  
tiform leafminer (Lepidoptera: Grancillaridae) and its  
parasite *Apanteles ornigis*. J. Econ. Entomol. 75: 680–684.

Received 1 August 2003; accepted 28 March 2004.