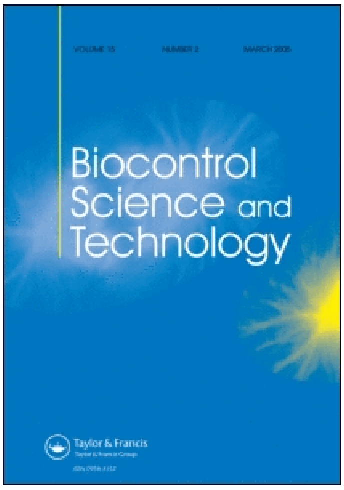

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Effects of deltamethrin on the reproduction of *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae)

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Abstract

The influence of deltamethrin on the reproduction of *Trichogramma cordubensis,* a thelytokous egg parasitoid, was investigated by studying egg maturation and daily fecundity of insecticide treated wasps and offspring emergence rates. The insecticide was applied to the parasitoids at the prepupal stage within its host eggs (*Ephestia kuehniella*). The total number of parasitized eggs per female during the first 7 days was not significantly influenced by the tested concentrations of deltamethrin. Prevalence of parasitism during this period had a similar pattern between deltamethrin treatments and the control. In addition, the mean number of mature eggs observed per female per day was significantly correlated to mean daily fecundity, regardless of the treatments. Offspring emergence was significantly influenced by the insecticide treatments experienced on their progenitors, decreasing significantly at 48 and 72 h for the highest tested concentration of deltamethrin (23.6 mg [a.i.]/L). Despite that, deltamethrin had no adverse effects on the reproduction of treated wasps, particularly when was applied at the concentration recommended by the manufacturer (12.5 mg [a.i.]/L).

Keywords: Trichogramma*, fecundity, egg maturation, offspring emergence, deltamethrin, pyrethroid*

Introduction

Trichogramma cordubensis Vargas & Cabello is a native thelytokous species of São Miguel Island (Azores) (Pintureau et al. 1991). Its effectiveness as a biological control agent for Azorean agricultural pests has been investigated in our laboratory by studying parasitoid biology (Garcia & Tavares 1995, 1997, 2001; Garcia et al. 1995a, 2001, 2002), population dynamics (Garcia et al. 1995b, 1998) and pesticide lethal and sub-lethal effects (Vieira et al. 2001; Pereira 2003).

Many studies have compared the relative toxicity of pesticides, including insecticides, fungicides and herbicides, to *Trichogramma* in screening trials. Laboratory and field studies have shown that *Trichogramma* wasps are highly susceptible to most broad-spectrum insecticides, reducing their efficacy as biological control agents (Bull

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& Colleman 1985; Hagley & Laing 1989; Branco & França 1995; Sterk et al. 1999; Brunner et al. 2001; Hewa-Kapuge et al. 2003). According to several authors, some pyrethroids are considered to be very harmful to *Trichogramma* by adversely affecting emergence rates (Hohmann 1991, 1993; Suh et al. 2000; Takada et al. 2001; Vieira et al. 2001), adult mortality (Jacobs et al. 1984; Suh et al. 2000; Brunner et al. 2001; Vieira et al. 2001; Hewa-Kapuge et al. 2003) and parasitism of host eggs (Jacobs et al. 1984; Hagley & Laing 1989; Hohmann 1993; Hewa-Kapuge et al. 2003).

Deltamethrin, the pyrethroid selected for this study, is considered environmentally safe, and highly vulnerable to enzymatic degradation by *Aedes aegypti* and other insects (Kumar et al. 2002). Several studies report the lethal and sub-lethal effects of deltamethrin on treated adults and immature stages of *Trichogramma* (Hohmann 1991, 1993; Jalali & Singh 1993; Branco & França 1995; Vieira et al. 2001), but nothing is known about the effects on the egg maturation of treated wasps and emergence rates of their offspring.

When considering the releasing approaches of *Trichogramma*, two different methodologies can be used (Smith 1996): the inundative approach, which tends to view the parasitoid as a fast-action replacement for chemical insecticides, and the inoculative approach, which considers the parasitoid as one aspect of Integrated Pest Management (IPM). In inoculative releases, however, it is the progeny of the released parasitoids that have a later effect on the reduction of the targeted host population. Accordingly, when using pesticides in IPM, we should not only evaluate the lethal and sub-lethal effects on the treated parasitoids, but also on their progeny.

Hence, with this research we aim to evaluate the influence of deltamethrin on the reproduction of *T. cordubensis* by studying egg maturation and daily fecundity of treated wasps and offspring emergence rates.

Material and methods

Insects

The colony of *T. cordubensis* used in this experiment was originally established from parasitized eggs of *Autographa gamma* L. (Lepidoptera: Noctuidae) found at Ribeira do Guilherme (São Miguel island, Azores). Parasitoids were laboratory reared at $20 \pm 1^{\circ}$C, 75 ± 5% r.h. and L16:D8 for approximately 12 generations on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, according to the methods of Tavares and Vieira (1992).

Insecticide treatments

All tests were done with fresh solutions diluted with distilled water of a commercial formulation of deltamethrin (Decis 2.8% EC, Aventis). Two concentrations were tested: one recommended by the manufacturer for the control of lepidopteran pests (12.5 mg [a.i.]/L) and another corresponding to the LC_{50} (23.6 mg [a.i.]/L) for *T. cordubensis* when the insecticide is applied at the prepupal stage (estimated by Pereira, 2003). Controls were treated with distilled water.

For each treatment, 15 groups of 10 female wasps with less then 24 h old were isolated in glass tubes (7×1 cm). A drop of honey solution (10%) was poured in each tube to provide the parasitoids a carbohydrate source. Eggs of *E. kuehniella* were presented to wasps on egg cards prepared by spraying a fixed area (0.8×0.8 cm) of an index card with a water solution of non-toxic glue, and then spreading host eggs

(400 ± 10 eggs) on this surface. The host eggs were less than 24 h old and had previously been irradiated with ultra-violet light for 20 min to prevent the embryo development (Voegelé et al. 1974). Parasitism occurred during 24 h at 22 ± 0.5°C, 70 ± 5% r.h. and 16L:8D.

All treatments were applied 96 h after parasitism, when parasitoids were at the prepupal stage. Cards containing the parasitized eggs were sprayed with 6 mL of the aqueous suspension of the insecticide or distilled water, using a Potter's Tower equipment (Burkard, Rickmansworth, UK) at 2 bar. This resulted in a homogeneous spray coverage of 9.52 ± 2.17 μL (mean ± s.d.) of fluid per cm^2, corresponding to a pulverization pressure of 1000 L/ha. After spraying, egg cards were allowed to dry for 2 h inside a fume hood. Afterwards, each egg card was isolated inside a glass tube (7 × 1 cm) and maintained at the previously described abiotic conditions until emergence of adult wasps. The emerged wasps were then used in the following experiments:

Daily fecundity of treated wasps and offspring emergence rates. For each treatment and immediately after the emergence of the parasitoids, 30 females were individually isolated in glass tubes (4 × 1 cm) containing a card with 100 ± 10 eggs of *E. kuehniella* with a drop of honey solution (10%). Egg cards were prepared as described above and were replaced every 24 h with fresh ones during seven consecutive days to determine fecundity. Cards with parasitized eggs were maintained at 22 ± 0.5°C, 70 ± 5% r.h. and 16L:8D for offspring development. Fecundity was determined by counting the number of parasitized host eggs that turned black (i.e. with parasitoid prepupae) using a dissecting microscope at 25 magnification. Emergence rates were estimated by dividing the number parasitized host eggs with emergence holes by the total number of parasitized host eggs. Females that did not parasitize during the first 24 h were eliminated from the statistical analysis.

Egg maturation. For each treatment and immediately after the emergence of the parasitoids, 15 host-deprived females were dissected and mounted on microscope slides to determine the number of mature (full-sized) eggs present in the four ovarioles of each wasp. Eggs in excess of 71 μm length were considered mature, as the average egg size previously determined for *T. cordubensis* is of 84 × 28 μm. All observations were made under a light microscope at 400 magnification.

To estimate the number of mature eggs present in the ovarioles of *T. cordubensis* after 24, 48, 72, 96, 120, 144 and 168 h of oviposition experience, wasps were allowed to parasitize for different periods of time. For that, and immediately after their emergence, females were individually isolated in glass tubes (4 × 1 cm) containing a card with 100 ± 10 eggs of *E. kuehniella* with a drop of honey solution (10%). Egg cards were prepared as described above and replaced every day with fresh ones. Subsequent to every 24 h of parasitism, batches of 15 females from each treatment were dissected for observation of the mature eggs, following the above-mentioned procedure. Females that did not parasitize during the first 24 h were not considered for statistical analysis.

Statistical analysis

Analysis of variance (ANOVA) were conducted on data regarding the number of mature eggs, fecundity and emergence rates. Where statistical differences existed between data sets ($P<0.05$), Fisher's least significant difference tests (LSD) were used to separate the differing means. An ANOVA repeated measures procedure was used to study the interactions between insecticide treatments and the prevalence of parasitism or offspring emergence rates. To correct heterocedasticity, data concerning emergence rates were transformed by arcsine $\sqrt{(x)}$ and, fecundity and number of mature eggs were transformed by $\sqrt{(x+0.5)}$ prior to analysis of variance (Zar 1996).

For each treatment, a Person's correlation was performed between the average number of mature eggs observed per day and the females' daily fecundity (Zar 1996). All the analyses were performed using SPSS 12.0 for Windows (SPSS Inc. 2003).

Results

Daily fecundity of treated wasps and offspring emergence rates

Results show that the total number of parasitized eggs per female during the 7 days was not significantly influenced by the tested concentrations of deltamethrin ($F=0.034$, df $=2$ & 58, $P=0.966$; one-way ANOVA) (Figure 1).

Wasps parasitized a significantly higher number of hosts on the first day of parasitism (over 60% of the total number of parasitized eggs, for all treatments), sharply decreasing thereafter ($F=158.589$, df $=4.804$ & 54, $P<0.0001$; ANOVA repeated measures) regardless of the tested treatments ($F=0.668$, df $=2$ & 58, $P=0.517$; ANOVA Repeated Measures) (Figure 2). The prevalence of parasitism during the 7 days had a similar pattern between both deltamethrin treatments and the control, as demonstrated by the non-significant interaction between these groups ($F=1.420$, df $=9.608$ & 48, $P=0.174$; ANOVA repeated measures).

Offspring emergence rates were significantly influenced by the time of parasitism ($F=3.681$, df $=5.959$ & 33, $P=0.002$, ANOVA Repeated Measures; Figure 3) and by the interaction between this factor and deltamethrin treatments ($F=1.976$, df $=11.918$ & 27, $P=0.028$, ANOVA repeated measures). Results show that for the higher concentration of deltamethrin (23.6 mg [a.i.]/L) the emergence rates were high in the first 2 days (i.e. at 0 and 24 h), decreasing significantly on the 3rd and 4th day (i.e. at 48 and 72 h) ($P<0.05$, LSD tests; Figure 3). However, from the 5th day (i.e. at 96 h) onwards emergence rates increased, reaching to values similar to the control ($P<0.05$, LSD tests; Figure 3).

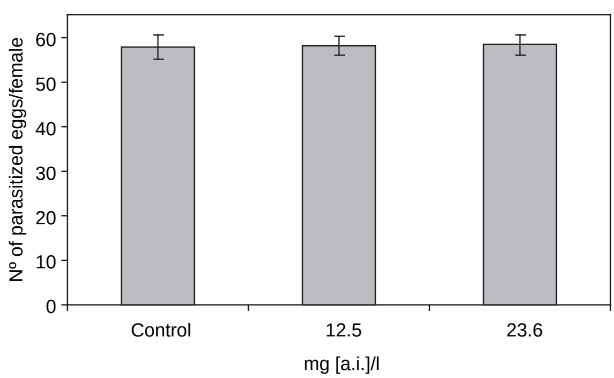

Figure 1. Total number (mean $\pm$s.e.) of parasitized eggs per female of *T. cordubensis* during 7 days, when wasps were exposed to different concentrations of deltamethrin.

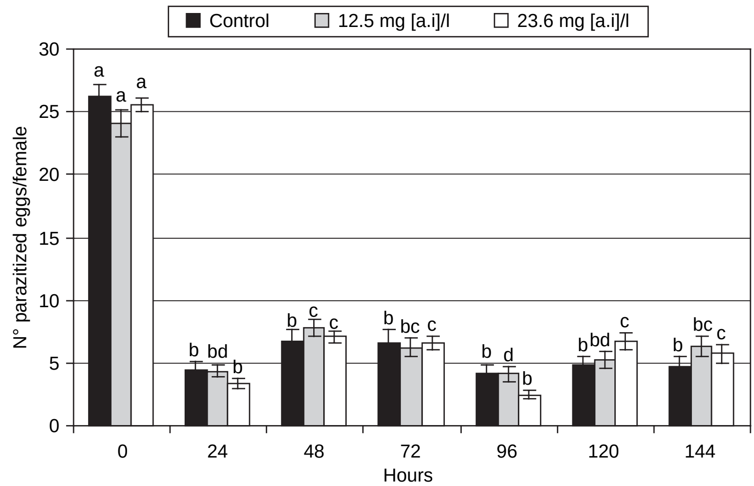

Figure 2. Daily fecundity (mean ± s.e.) of *T. cordubensis*, when wasps were exposed to different concentrations of deltamethrin; Bars regarding the same treatment with different letters are significantly different at $P < 0.05$ (LSD procedure within ANOVA).

Egg maturation

After 24 h of oviposition, the mean number of mature eggs per female decreased significantly ($F = 53.448$, df = 7 & 108, $P < 0.0001$, control; $F = 70.565$, df = 7 & 103, $P < 0.0001$, 12.5 mg [a.i.]/L; $F = 26.607$, df = 7 & 101, $P < 0.0001$, 23.6 mg [a.i.]/l); one-way ANOVA), becoming relatively stable at low values throughout the following days, regardless of the treatments (Figure 4).

In all treatments, the mean number of mature eggs per female per day was significantly correlated to mean daily fecundity ($R^2 = 0.997$, $P < 0.0001$, control; $R^2 =$

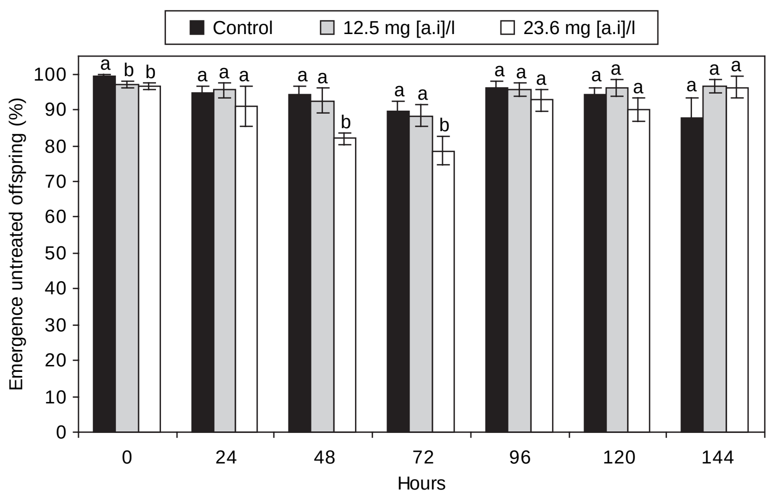

Figure 3. Offspring emergence rates (mean ± s.e.) of *T. cordubensis,* when progenitors were exposed to different concentrations of deltamethrin. Bars regarding treatments within each hour with different letters are significantly different at $P < 0.05$ (LSD procedure within ANOVA).

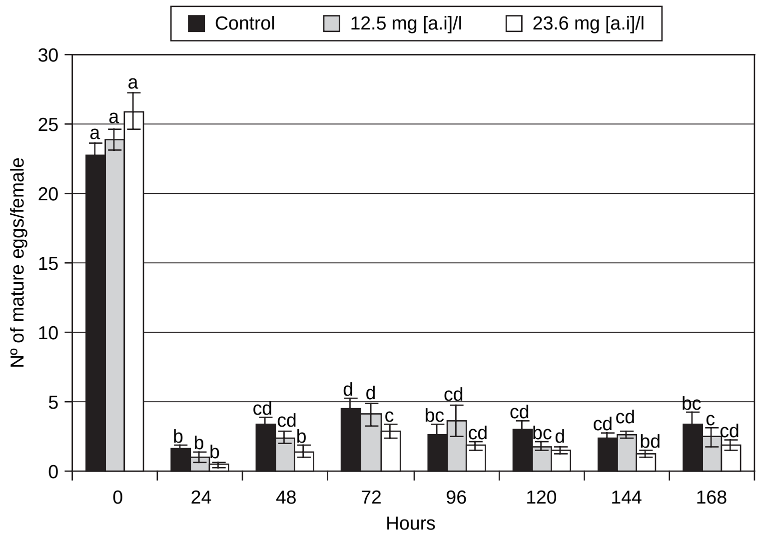

Figure 4. Number (mean ± s.e.) of mature eggs present in the ovaries *T. cordubensis* following 0, 24, 48, 72, 96, 120, 144 and 168 h of oviposition, when wasps were exposed to different concentrations of deltamethrin. Bars regarding the same treatment with different letters are significantly different at $P < 0.05$ (LSD procedure within ANOVA).

0.980, $P < 0.0001$, 12.5 mg [a.i.]/l; $R^2 = 0.977$, $P < 0.0001$, 23.6 mg [a.i.]/l) (Figures 2 and 4).

Discussion

The effects of pesticides caused by direct contact with the toxin are manifested as short-term mortality or relatively long-term sub-lethal consequences, generally having greatest impact on natural enemies (Williams & Price 2004). Several studies report the high susceptibility of *Trichogramma* wasps to deltamethrin, assuming it adversely affects emergence rates (Hohmann 1991, 1993; Vieira et al. 2001), adult survival (Vieira et al. 2001) and parasitism of host eggs (Hohmann 1993; Branco & França 1995).

According to the IOBC standard methods, deltamethrin is classified as harmful for adults and as slightly harmful for pupae of *Trichogramma* (Hassan 1994). Our results showed that deltamethrin, even at a high concentration, did not have a harmful influence on the fecundity *T. cordubensis* when wasps were treated during the prepupal stage. Furthermore, the prevalence of parasitism during the 7 days had a similar pattern between both deltamethrin treatments and the control, indicating that egg production and maturation in *T. cordubensis* was not altered by both deltamethrin treatments. Although *Trichogramma* females emerge with a full complement of eggs, with the onset of oogenesis and vitellogenesis occurring during the prepupal and pupal development (Volkoff & Daumal 1994), some thelytokous species continuously produce eggs after adult emergence, such as *T. cordubensis* (as our results evidence), *T. minutum* (Wang & Smith 1996) and *T. cacoeciae* (Volkoff & Daumal 1994).

According to Vieira et al. (2001), *T. cordubensis* adults exposed to field recommended rates of deltamethrin (12.5 mg [a.i.]/l) have 25% mortality within 48 h; if exposed during the prepupal stage, emergence rates are reduced by 30%. Saber et al. (2005) concluded that adults of *Trissolcus grandis* (Hymenoptera: Scelionidae) exposed to an equivalent concentration of deltamethrin (12.5 μg [a.i.]/mL) have 100% mortality; however, if exposure to the insecticide occurs during the preimaginal stages, emergence rates are reduced by 34% and, longevity and fecundity of emerged wasps (treated at the pupal stage) are not significantly affected. Jalali and Singh (1993) observed that the fecundity of *Trichogrammatoidea armigera* (Hymenoptera: Trichogrammatidae) varied with the preimaginal stage of the parasitoid treated with deltamethrin (Decis 10 EC at 0.002%): when treatments were applied during the pupal stage, fecundity decreased significantly; if deltamethrin was applied during the parasitoid larval stages fecundity was only slightly reduced. Hewa-Kapuge et al. (2003) also observed a decrease in the fecundity of *Trichogramma* nr. *brassicae* (Hymenoptera: Tricogrammatidae) when adults were exposed to residues of another pyrethroid, tau-fluvalinate (0.096 g [a.i.]/l). Still, in this last case, wasps were not protected by the chorion of the host's egg, therefore being more susceptible to the pyrethroids, as observed by these authors and by Vieira et al. (2001).

Offspring emergence rates of treated *T. cordubensis* were significantly influenced by the interaction between the time of parasitism and the deltamethrin treatments: for the higher concentration of deltamethrin (23.6 mg a.i./l) the emergence rates decreased significantly at 48 and 72 h compared to control. These results indicate that parasitoids starting vitellogenesis upon emergence from their host (i.e. ready to be laid at 48 and 72 h after emergence) were most probably affected (contaminated) by deltamethrin residues resting on the chorion of the host egg, doubtless more concentrated for the upper concentration of pesticide. According to Volkoff and Daumal (1994) the onset of vitellogenesis (i.e. the transfer of cytoplasmatic macromolecules to the oocytes – egg maturation) by *T. cacoeciae* occurs on the 7th day of preimaginal development and takes place on the following 48 h, schedules that corroborate our hypothesis.

According to Williams et al. (2003) some pyrethroids have a high residual toxicity and Xiong et al. (1986) suggested that the inhibition of adult emergence of *Trichogramma japonicum* (Hymenoptera: Trichogrammatidae) seems to be caused by deltamethrin residues on the host egg chorion. Saber et al. (2005) also suggested that *T. grandis* failed to emerge when in contact with the deltamethrin residues while chewing the host egg chorion to make the exit holes.

Narayana and Babu (1992) when treating *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) with different growth regulators during the preimaginal development of the wasps verified that parasitoids exposed to growth regulators on the 7th day of preimaginal development parasitized significantly less hosts than non-treated wasps. In contrast, those exposed to treatments on the 1st and 4th day of preimaginal development had parasitism rates similar to the control group. These authors hypothesize that the decrease in parasitism could be attributed to an adverse effect of insect growth regulators during egg differentiation at that prepupal stage, despite the fact that they did not made any study regarding the wasp's egg maturation process.

On the other hand, emergence rates of *T. cordubensis* wasps from eggs laid at 0 and 24 h, as well from those oviposited from 96 h onward were not affected by either delthametrin treatment. In the first case, when egg maturation occurred (i.e. during the preimaginal development) wasps were still inside the parasitized host, therefore being protected from the pyrethroid by the chorion of the host egg; in the second case, when wasps emerged from the host, the eggs to be laid from 96 h onward were most probably still in an early stage of egg maturation (previous to vitellogenesis, according to Volkoff and Daumal (1994)) and, consequently not susceptible to the pyrethroid residues found on the chorion of the host egg.

Vieira et al. (2001) showed that applying deltamethrin at the concentration recommended by the manufacturer (12.5 mg [a.i.]/l) reduces the emergence rates of *T. cordubensis* wasps exposed to this insecticide in the prepupal stage, but it does not alter their developmental rates and longevities. Additionally, our results have shown that deltamethrin does not affect both fecundity and egg maturation applied to *T. cordubensis* prepupae, but emergence of their offspring can be reduced by high concentrations of insecticide applied on the progenitors. Nevertheless, the magnitude of such reduction can be considered of negligible importance (offspring emergence rates decreased from about 95 to 80%) for the reproductive success of the wasps, particularly when used in inoculative approaches. Additionally, under field conditions many of the parasitized host eggs are found on the underside of the leaves therefore would receive a lower dose of insecticide, attenuating the adverse effects of the chemical treatments.

Even so, further studies are required to determine whether deltamethrin can be used safely under field conditions without disrupting the activity of *Trichogramma* populations, as Scholz et al. (1998) observed in corn fields treated with an equivalent concentration (i.e., 12.5 g [a.i.]/ha) of this insecticide to control *Helicoverpa armigera* (Lepidoptera: Noctuidae).

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