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The sublethal effects of deltamethrin on *Trichogramma* behaviors during the exploitation of host patches

Jean-Marie Delpuech *, Maxime Delahaye

Université de Lyon, CNRS, Université Claude Bernard Lyon 1, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France

HIGHLIGHTS

► *Trichogramma* species are parasitoids of numerous species of Lepidoptera.
► Deltamethrin decreased the level of infestation of healthy host eggs.
► Deltamethrin increased the level of infestation of unsuitable, previously infested hosts.
► Deltamethrin increased antennal and ovipositor rejection of previously infested hosts.
► These effects may impact the equilibrium of natural ecosystems.

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ABSTRACT

Trichogramma and parasitoids as a whole are key species because they regulate natural populations of other insects. As any non-target species, this parasitoid can be exposed to insecticides by environmental pollution. This study identified the effects of an LD 20 of deltamethrin (a pyrethroid) on the behavior of *Trichogramma brassicae* females infesting a patch of host eggs. The study found that females that survived exposure to the insecticide infested fewer host eggs; spent more time on unsuitable, previously infested host eggs; and infested more previously infested host eggs than controls. The insecticide also induced an increase in antennal and ovipositor rejection of previously infested host eggs. These results are discussed in the light of the mode of action of pyrethroid insecticides. The findings of the study highlight sublethal effects that reduce the fitness of parasitoids and that could consequently modify the equilibrium of natural ecosystems.

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1. Introduction

When a *Trichogramma* female arrives at a host patch, she receives information about the quality of this patch by perceiving different stimuli that can emanate from chemical mediators, such as the kairomones of her host, which furnish information on the suitability of the host, and pheromones left by her congeners, which furnish information on the quality of the patch by informing her of the possibility that some host eggs are already infested. She also receives information from physical mediators that inform her of the size of the host eggs and, therefore, of host characteristics, including the number of her eggs that can develop in each host. From all this information, the female can evaluate the quality of the patch, decide to stay or leave, and select a strategy of infestation: how many eggs to lay, in how many hosts, and to infest every host eggs or not. The acquisition of information from the perceived stimuli, the transmission of this information to the central nervous system, the elaboration of an adapted response to the perceived information and, finally, the execution of the behavior chosen according to the perceived information all depend on the transmission of nerve impulses in the nervous system (Vosshall and Stocker, 2007). For this reason and because the majority of insecticides are neurotoxic (Casida, 2009) and interfere with the transmission of nerve impulses in insects, every behavior described above may be disrupted by insecticides. Insecticides can produce numerous sublethal effects on the life history traits of insects (see Desneux et al., 2007 for a review), particularly on those traits involved in reproduction. Deltamethrin, the insecticide used in this study, is a neurotoxic insecticide that interferes with the transmission of action potentials along neurons (Soderlund, 2012). It belongs to the pyrethroids, the second most widely used family of insecticides (organophosphates are the most-used family: EC, 2007; US, EPA, 2011). Deltamethrin is used on many crops (e.g., cereal, maize, crucifers, artichoke, asparagus, beet, salad vegetables, tomato, pepper, potato, apple, pear, peach, grape, rice, peas, and onion, cf. Couteux and Lejeune, 2009).

Trichogramma parasitoids are key species because they regulate the natural populations of other insects, including pests. Furthermore, they are the most widely used insect natural enemy in the world

* Corresponding author at: Lab Biometrie Biologie Evolutive UMR CNRS 5558, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. Tel.: +33 4 72 43 29 12; fax: +33 4 72 43 13 88.
E-mail address: jean-marie.delpuech@univ-lyon1.fr (J.-M. Delpuech).

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(Knutson, 1998). Nine species of *Trichogramma* are reared around the world and released annually on an estimated 80 million acres of agricultural crops and forests in 30 countries (Knutson, 1998). *Trichogramma* are released to control 28 different caterpillar pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets, fruit trees and pine and spruce trees (Smith, 1996; Yu and Byers, 1994).

Due to their intensive use in modern agriculture and urban protection, insecticides produce environmental pollution, to which non-target insects, such as *Trichogramma* parasitoids, may be exposed. The impact of environmental pollution resulting from insecticides on this type of insect will therefore have ecological consequences because it can modify the equilibrium of natural ecosystems, but it will also have economic consequences because of the pest populations that the insects naturally control. In this study, we tested the effects of a 20% lethal dose (LD 20) of deltamethrin on the foraging behavior of *Trichogramma brassicae* infesting a patch of host eggs.

2. Materials and methods

2.1. Insects

A strain of *T. brassicae* Bezdenko (Hymenoptera, Trichogrammatidae) was used for the experiments. This strain was reared on *Ephestia kuehniella* eggs (Lepidoptera, Pyralidae) killed by UV radiation to prevent non-parasitized eggs from emerging. Host eggs were supplied in excess. Therefore, only one *T. brassicae* egg was laid per *E. kuehniella* egg. The rearing and experiments were conducted at 21 °C under a 12 L:12D photoperiod (light phase from 7:30 am to 7:30 pm).

2.2. Determination of lethal doses

E. kuehniella eggs parasitized by *Trichogramma* were individually (one egg per vial) isolated in glass vials (3 cm in length, 5 mm in diameter) containing a minute drop of honey to feed the insects upon emergence. Approximately 24 h after their emergence, the males and females were sexed. The females were individually exposed to the insecticide. For this purpose, 3 µl of deltamethrin (99% certified purity; Cluzeau Info Labo, Sainte-Foy-La-Grande, France) diluted in acetone was deposited on pieces of paper (2.2 cm × 4 mm), which were left for 1 h on the lab bench to allow the total evaporation of acetone to occur. The pieces of paper were then introduced into each vial containing a tested female. Papers on which pure acetone was deposited were used as controls. Mortality was determined after 24 h of exposure to the treated piece of paper at 21 °C, photoperiod 12:12 (contamination occurred via tarsal contact). To calculate regression lines for mortality, 5 groups of 50 individuals were exposed to a control solution and 4 solutions of increasing concentrations of insecticide. The mortality data were analyzed with a probit analysis (Finney, 1971), and the LD 20 to be used for behavioral tests was then estimated with a linear regression using the log-probit program of Raymond (1985). The dilution corresponding to the LD 20 was stored at 4 °C between behavioral experiments.

2.3. Protocol for the observation of the behavior of insects on host patches

Two days prior to emergence, *E. kuehniella* eggs infested by *Trichogramma* were individually isolated in glass vials (3 cm in length, 5 mm in diameter) with a minute drop of honey. The males and females were sexed 48 h after the first emergences, and each female was then placed with one male for fertilization. After at least 1.5 h, the males were removed, and the females were exposed to an LD 20 of deltamethrin as described in "Determination of lethal doses" section. The females were left in their exposure vial until their behavior on a patch of host eggs was observed. Accordingly, the exposure time to the insecticide ranged from 22 h to 27 h. For all observations, a control female was always observed after a treated female. The females were fertilized but naive (i.e., they had never been in contact with host eggs and therefore had no ovipositional experience). The females were one to three days old at the moment of the observation of their behavior. A new host patch was used for each female. Each host patch was made by gluing two groups of 9 *E. kuehniella* eggs with water onto the center of a 10 cm × 10 cm square sheet of paper. These host eggs had previously been killed by UV radiation. The first group of eggs consisted of 9 eggs infested by *T. brassicae*. The second group consisted of 9 healthy (not infested) eggs. The two groups were approximately 5 mm apart. Within each group, the eggs were approximately 1 mm apart. The eggs of the group of infested eggs were infested 24 h before the experiment by leaving about 100 *E. kuehniella* eggs, killed by UV radiation, with about 10 *T. brassicae* females in a closed Petri dish until the use of the eggs for the experiment. Only insects that were able to move normally after their exposure to the insecticide were used for the experiment (the discarded insects represented less than 10% of the total). For the experiment, one parasitoid female was placed on the center of the group of infested eggs and was free to move inside the patch, to leave it or to leave the sheet of paper. The female's behavior was observed with a stereomicroscope. The observations were performed at 21 °C in a quiet room.

2.4. Behavior of the parasitoid in control conditions

After a female is released inside the group of infested eggs, she generally leaves it and enters inside the group of healthy eggs. She then walks over an egg and moves her antennae rapidly up and down. This stage is defined as the drumming stage. The female then pierces a hole into the egg by rotating her ovipositor alternately left and right, the stage defined as the drilling stage. Once the chorion of the egg is pierced, the female can decide either to pull her ovipositor out of the egg and feed from the hemolymph that exudes or to insert her ovipositor completely into the egg and lay an egg of her own. After infesting the first host egg several times, the female leaves it and continues to drum on the substrate with her antennae. Once she arrives at another egg, the female screens it by drumming on it with her antennae. She can then decide either to drill it with her ovipositor or to leave it. If she leaves without drilling, this event is termed an antennal refusal. If the female has drilled the egg, she can then either infest it by completely inserting her ovipositor or pull her ovipositor out and not infest it (ovipositor refusal). Generally, an egg refusal (antennal or ovipositor refusal) occurs if the egg is already infested. The female continues to perform these behaviors on several eggs, sometimes momentarily leaving the patch and then returning to it. Finally, after several ovipositions or rejections, she concludes by leaving the patch.

2.5. Observed behaviors

The following behaviors were counted and their duration recorded with JWatcher software (Blumstein et al., 2006):

- Entry into the group of healthy (not infested) eggs
- Entry into the group of infested eggs
- Climbing onto an egg
- Drumming on an egg
- Drilling an egg
- Antennal rejection (the egg is left after antennal drumming)
- Ovipositor rejection (the egg is left after drilling without infesting it)
- Egg rejection (sum of the last two behaviors)
- Oviposition
- Moving intra-patch (the parasitoid walks between eggs)
- Exit from the group of healthy eggs
- Exit from the group of infested eggs

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