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The sublethal effects of endosulfan on the circadian rhythms and locomotor activity of two sympatric parasitoid species

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HIGHLIGHTS

- Endosulfan (organochlorine insecticide) largely contributes to environmental pollution.
- Parasitoids are important species in ecosystems, as they control insect populations.
- Endosulfan at LC 0.1 modified the rhythm of locomotor activity of two parasitoid species.
- Endosulfan synchronized the diurnal activity of the two parasitoid species.
- By increasing competition between these species, this could impact the whole ecosystem.

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ABSTRACT

The organochlorine insecticide endosulfan is dispersed worldwide and significantly contributes to environmental pollution. It is an antagonist of the neurotransmitter gamma-aminobutyric acid (GABA), which is also indirectly involved in photoperiodic time measurement. In this study, we show that endosulfan at a dose as low as LC 0.1 modified the rhythm of locomotor activity of two sympatric parasitoid species, *Leptopilina boulardi* and *Leptopilina heterotoma*. The insecticide strongly increased the nocturnal activity of both species and synchronized their diurnal activity; these activities were not synchronized under control conditions. Parasitoids are important species in ecosystems because they control the populations of other insects. In this paper, we discuss the possible consequences of these sublethal effects and highlight the importance of such effects in evaluating the consequences of environmental pollution due to insecticides.

1. Introduction

Endosulfan, a cyclodiene organochlorine insecticide, is a semi-permanent organochlorine pollutant. Its half-life in atmospheric ozone has been estimated to be approximately 320 d (US-ATSDR, 2013). Endosulfan has been used in large quantities in nearly every country worldwide. For example, the average use of endosulfan worldwide was 12,450 t year^{-1} from 2000 through 2004 (Weber et al., 2010). Although its use has recently been addressed by new regulations in several countries and will be phased out by 2016 (USEPA, 2010), it remains in use and will continue to be used after this date in countries (e.g., India, China) that do not regulate it. Because endosulfan is a remanent insecticide and because it can travel very long distances via transport through the atmosphere, it will continue to participate in environmental pollution for some time to come. For example, a recent study by Lavin et al. (2012) found that endosulfan originating from Australia was transported to a national park in the Southern Alps in New Zealand. The authors determined the origin of the insecticide using trace element profiles in atmospheric particulate matter and demonstrated that endosulfan had travelled approximately 3000 km via the atmosphere and wind currents. It is therefore important to determine the effects of endosulfan on the life history traits of free-living organisms that are not the intended targets of the insecticide.

The insecticide endosulfan is a highly toxic convulsant that causes the hyperexcitation of the nervous system by antagonizing

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the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Bloomquist, 1996; Chen et al., 2006). Endosulfan binds to GABA postsynaptic receptors and blocks chloride ion channels, thereby impeding the chloride flux that is normally activated by GABA to produce an inhibitory effect on nervous transmission in postsynaptic neurons (Bloomquist, 1996; Chen et al., 2006). However, Vieira et al. (2005) showed that GABA may also play a regulatory role in melatonin synthesis in insects, as has been demonstrated in mammals. Melatonin is a highly conserved molecule that is involved in photoperiodic time measurement in both invertebrates and vertebrates. The production of melatonin is maximal during the scotophase in both invertebrates (Vieira et al., 2005) and vertebrates (Pevet, 2000), and it is through the periodic secretion of melatonin that the central nervous system integrates photoperiodic information (Pevet, 2000). Therefore, endosulfan, by competing with GABA, which is involved in the synthesis of melatonin, may not only impair nervous transmission but also modify the circadian rhythm of insects.

In this paper, we assess the effects of sublethal doses of endosulfan on the circadian rhythms of two parasitoid species that attack Drosophila larvae, *Leptopilina boulardi* and *Leptopilina heterotoma*. The differences in the rhythms of activity of these two species are known to be important for the equilibrium of the populations of the two species in sympatry (Fleury et al., 2000). Furthermore, parasitoid species play an important role in the equilibrium of ecosystems because they control the populations of the insects they infest.

2. Materials and methods

2.1. Biological material

A strain of *L. boulardi* (Hymenoptera, Eucoilidae) and a strain of *L. heterotoma* (Hymenoptera, Eucoilidae) were used for the experiments. They were trapped in an orchard located in southeastern France (Domain of Gotheron, INRA, Saint-Marcel-lès-Valence, Drôme, France, lat. 44°58′21.74″N, long. 4°55′38.41″E). The strains were reared on *Drosophila melanogaster* larvae at 25 °C.

Prior to the experiments, the emerged parasitoids were stored 2–5 d in vials (2.5 cm × 10 cm) containing sweet agar–agar and honey as a diet at 18 °C under a 12L:12 D photoperiod.

2.2. Determination of lethal doses

The experimental multi-arenas were each composed of a black polycarbonate sheet (22 cm × 13 cm, thickness 2 mm) containing 15 holes (each 3 cm in diameter) and covered with a glass sheet on both sides. The multi-arenas were held together with sticky tape. Accordingly, each experimental multi-arena contained 15 arenas, each 3 cm in diameter and 2 mm in height. For each experiment, 12 of these multi-arenas were used to test a total of 180 female parasitoids in each arena (one female per arena). For insecticide exposure, five increasing concentrations of the cyclodiene insecticide endosulfan (70% alpha-endosulfan, 30% beta-endosulfan, 99.5% certified purity; Cluzeau Info Labo, Sainte-Foy-La-Grande, France) diluted in acetone and a control solution (pure acetone) were used. A total of 30 µl of the tested solution was deposited in each arena (15 µl on the upper and lower glass sheets covering each arena). The glass sheets were left for one hour on the lab bench to let the acetone completely evaporate. A small drop of honey was then deposited on the wall of each arena to feed the insects, and one parasitoid female was placed in each arena. The arenas were then closed, and the experimental multi-arenas were placed in pairs in a closed transparent plastic box containing 10 petri dishes, 4 cm in diameter, filled with distilled water and placed in sets of 5 on each side of the plastic box for humidification. Mortality was determined after 4 d at 20 °C under a 12L:12 D photoperiod (contamination occurred via tarsal contact). To calculate regression lines for mortality, 5 groups of 30 individuals were exposed to 5 solutions of increasing concentrations of insecticide, and one group of 30 individuals was exposed to the control solution (a total of 180 individuals were used in each test). The mortality data were analysed using a probit analysis (Finney, 1971), and the LC 0.1 to be used for the behavioural tests was then estimated via linear regression using the log-probit program of Raymond (1985). The dilution corresponding to LC 0.1 was stored at 4 °C between behavioural experiments.

2.3. Recording of locomotor activity

Female parasitoids were placed in the same experimental multi-arenas used for the determination of lethal doses. A small drop of honey and one female were placed in each arena. The multi-arenas were placed in pairs in transparent plastic boxes containing 10 petri dishes filled with distilled water for humidification. These plastic boxes, with the multi-arenas, were placed in an air-conditioned room at 20 °C under a 12L:12 D photoperiod. They were placed above an infrared light source (an incandescent lamp filtered with an infrared filter (Kodak filter 88 A, Kodak, Lyon, France), leaving only light with a wavelength higher than 720 nm (infrared light) to be transmitted). A camera sensitive to infrared (Canon CI-20PR, Secad, Saint Martin du Fresne, France) was placed above the multi-arenas to record the movements of each female at night and during the day (see Allemand et al., 1994 for details). The continuous infrared illumination did not affect the dark phase because insects are not sensitive to infrared light (Saunders, 1982).

The activity of two groups of 60 females was recorded. One group was exposed to an LC 0.1 of endosulfan placed in each arena, as described in the section specifying the determination of the lethal doses. The other group was exposed to the control solution. The movements of each female were successively recorded 12 times per hour over 4 d. For each recording, each arena was successively scanned using the camera, always in the same order. The information recorded was the presence or absence of movement by the female relative to the preceding scan. The mean hourly rate of activity, corresponding to the number of times that the female moved between two scans divided by the total number of scans in an hour (12), was calculated. The values obtained on the first day (24 h) of recording were discarded because one day was necessary for the insects to become accustomed to the experimental arenas and to recover from human handling. The values from the insects that died prior to the end of the experiment were discarded because rhythms of activity are modified preceding death. The values from insects that showed no activity during the entire experiment were also discarded because they were most likely due to a failure of the camera to detect the insect.

The values obtained for both groups of insects (exposed (treated) or not exposed (controls) to the insecticide) were compared using a two-way repeated-measures ANOVA with species and endosulfan treatment as sources of variation and time as repeated-measures. The rates of activity were compared between the treated and control animals within each species for their two peaks of activity (at the beginning and at the end of the photophase) and for one hour at the middle of the scotophase (hour 1–2, Student's *t*-test). The mean maximum locomotor activity durations were also compared using Student's *t*-tests. The statistical tests for the rates of locomotor activity were performed after an arcsine square root transformation to normalize the distribution of the values; however, the means presented in the figures and the text are not transformed values.