Environmental Pollution 230 (2017) 709–717

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Sublethal insecticide exposure affects reproduction, chemical phenotype as well as offspring development and antennae symmetry of a leaf beetle☆

Thorben Müller*, Alexander Prosche, Caroline Müller

Department of Chemical Ecology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

ARTICLE INFO

Article history:
Received 21 April 2017
Received in revised form
6 July 2017
Accepted 6 July 2017

Keywords:
Cuticular hydrocarbon profile
Transgenerational effects
Pyrethroid
Lambda-cyhalothrin
Phaedon cochleariae

ABSTRACT

The area of agriculturally used land and following to that the use of pesticides are steadily increasing. Insecticides do not only reduce pest organisms on crops but can also affect non-target organisms when present in sublethal concentrations in the environment. We investigated the effects of an exposure to sublethal pyrethroid (lambda-cyhalothrin) concentrations, at doses 20 and 60 times lower than the LC_{50}, respectively, on reproductive traits and adult cuticular hydrocarbon (CHC) profiles of a leaf beetle (*Phaedon cochleariae* Fabricius). Furthermore, we tested for effects on growth and antennae symmetry of the offspring generation that was not exposed to the insecticide. Sublethal insecticide concentrations decreased the egg number produced by the adults and the hatching rate. Moreover, the chemical phenotype (CHC profile) of adults was altered in dependence of the insecticide treatment, with sex-specific effects. In the unexposed offspring of insecticide-exposed parents, a prolonged development time and a fluctuating asymmetry of the females' antennae were detected, revealing transgenerational effects. The insecticide effects on the CHC profiles of the parental generation might have been caused by changes in CHC precursors, which were potentially induced by the insecticide treatment of the insect diet. Such altered CHC pattern may have implications for intraspecific communication, e.g., in mate choice, as well as in an interspecific way, e.g., in interactions with other arthropod species. The observed detrimental transgenerational effects might be explainable by a reduced investment in the offspring, maternal transfer or epigenetic processes. An asymmetry of the antennae may lead to defects in the reception of chemical signals. In conclusion, the results disclose that, besides detrimental (transgenerational) effects on reproduction and development, an exposure to sublethal insecticide concentrations can impair the chemical communication between individuals, with impacts on the sender (i.e., the CHC profile) and the receiver (i.e., caused by asymmetry of the antennae).

1. Introduction

Areas of agriculturally used land are steadily increasing and recent estimations suggest that globally additional areas of 200–300 ha will be cultivated in the next 40 years (Chaplin-Kramer et al., 2015; Kareiva and Marvier, 2012; Schmitz et al., 2014). In parallel, the use of insecticides rises to counteract the risk of crop loss due to pest species (Guedes et al., 2016). The accumulation of insecticides in the soil and their entrance into aquatic ecosystems entail a high threat of environmental pollution (Arias-Estévez et al., 2008; Ippolito et al., 2015). Consequently, insecticides do not exclusively impact pest species on crops, but non-target organisms can also be impaired by sublethal concentrations in the proximity (Desneux et al., 2007). Thereby, entire animal communities of a habitat (Frampton & van den Brink, 2007; Lee et al., 2001) and, in the long term, also population-dynamics of insects can be influenced by insecticides (Guedes et al., 2016; Stark and Banks, 2003). Thus, more knowledge is needed to understand the consequences of (sublethal) insecticide exposure on multiple life-history traits of non-target organisms.

An exposure to sublethal insecticide concentrations can induce various (costly) detoxification mechanisms, like oxidation and hydrolysis processes (James and Xu, 2012; Li et al., 2007) and can cause oxidative damage (Rodrigues et al., 2015). Consequently, the

☆ This paper has been recommended for acceptance by Charles Wong.

* Corresponding author.
E-mail address: thorben.mueller@uni-bielefeld.de (T. Müller).

http://dx.doi.org/10.1016/j.envpol.2017.07.018
0269-7491/

growth and reproduction as well as morphological and physiological parameters are impaired (Ceuppens et al., 2015; Farnesi et al., 2012; Guo et al., 2013; Zhu et al., 2012). Likewise, locomotion activities (Tooming et al., 2014), host- and mate-finding abilities (Desneux et al., 2004; Tappert et al., 2017) and life history strategies (Debecker et al., 2016) can be affected by insecticide exposure. However, little is known on insecticide effects on the chemical phenotype of exposed insects, i.e., their cuticular hydrocarbon (CHC) or sex pheromone composition. CHCs are decisive for intra- and interspecific communication in insects (Howard and Blomquist, 2005). Thus, alterations of the CHC profile due to insecticide exposure might have far-reaching implications for mate choice and for interactions between arthropod species.

Moreover, sublethal insecticide concentrations may not only target the exposed generation but can act in a transgenerational way, influencing the unexposed offspring (Costa et al., 2014; Guo et al., 2013). Many studies on transgenerational impacts of insecticides mainly investigated egg development and hatching success as response variables (Guo et al., 2013; Pan et al., 2014; Tan et al., 2012). Besides these short-term parameters, also long-term transgenerational insecticide effects on distinct life history traits over the entire ontogeny should further be researched, as partly conducted by Costa et al. (2014). Transgenerational effects of parental insecticide exposure may be caused by a reduced ability of the parents to invest in their offspring or a maternally driven transfer, as known for tolerance transmission (Rahman et al., 2010) and immune priming processes (Moreau et al., 2012; Sadd and Schmid-Hempel, 2009).

In the present study, we aimed to investigate the consequences of two sublethal insecticide concentrations on various parameters of an exposed parental and an unexposed offspring generation of *Phaedon cochleariae* F. (Chrysomelidae: Coleoptera), using a pyrethroid. Pyrethroids, with lambda-cyhalothrin (LCT) as representative example (Ceuppens et al., 2015), are non-systemic insecticides, which are extensively used in agriculture to control insect pests (Kutluyer et al., 2015; Tooming et al., 2014). LCT induces a knock-down effect by acting on sodium, calcium and chloride channels in the nervous system of insects after consumption and/or absorption through the tarsi (Burr and Ray, 2004; Ceuppens et al., 2015; Roberts and Hutson, 1999). Furthermore, this synthetic chemical can accumulate in biological membranes inducing further oxidative damage (Abbassy et al., 2014). The leaf beetle *P. cochleariae* can be considered as non-target organism when feeding on non-crop plants in habitats next to agriculturally used areas (Müller and Müller, 2016) and as target pest organism when feeding on crops belonging to the Brassicaceae (Finch and Kienegger, 1997; Uddin et al., 2009). All stages of this beetle are herbivorous and can thus be exposed to (sub)lethal insecticide concentrations when feeding on treated plants. In addition to studying effects of environmental pollution on more established study species, like beneficial insects (Desneux et al., 2004; Moscardini et al., 2015; Potts et al., 2010) or disease vectors (Alves et al., 2010; Farnesi et al., 2012), it is important to broaden our knowledge on non-target herbivores.

In the insecticide-exposed parental generation, we studied growth and reproduction traits as well as so far unexplored insecticide impacts on the CHC profile. Thereby, we expected a decreased body mass and reproductive output of the insecticide-exposed parental generation, explainable by the toxicity of pyrethroids, even in sublethal concentrations (Desneux et al., 2007). CHC profiles of adult *P. cochleariae* are shaped in dependence of their host plant species (Geiselhardt et al., 2012) or the fatty acid composition of their diet (Otte et al., 2015). Consequently, we hypothesised distinct CHC profiles of beetles fed on leaves treated with different insecticide concentrations. As transgenerational parameters, we analysed the growth until adulthood and antennae symmetry. Here, we expected negative effects of the parental insecticide treatment on growth and antennae symmetry of the unexposed offspring, potentially explainable by a decreased investment in their offspring or a maternal insecticide transfer. A fluctuating asymmetry might be caused by stressful environmental conditions of the parents, which can induce a reduced developmental homeostasis in their offspring (Beasley et al., 2013; Ribeiro et al., 2007).

2. Material and methods

2.1. Study organism

As study organism we used the mustard leaf beetle *Phaedon cochleariae*. In our laboratory at Bielefeld University, a breeding stock of this species is well established and reared under constant conditions within a climate cabinet (20 °C; 65% r.h., L16:D8) for more than 50 generations. The stock was regularly crossed with beetles collected in the field. Beetles were kept in several ventilated plastic boxes (20 × 20 × 6.5 cm) in groups of about 100–200 individuals and fed *ad libitum* with leaves of 8–10 weeks old non-flowering *Brassica rapa* L. ssp. *pekinensis* var. Michihili (seeds from Kiepenkerl, Bruni Nebelung GmbH, Konken Germany), which were cultivated in 12 cm diameter pots in composted soil in a greenhouse (60% r.h., L16:D8).

2.2. Experimental set-up

Freshly hatched larvae were randomly chosen from the breeding stock and used as directly insecticide-exposed parental study generation. Larvae were reared in Petri dishes (9.5 cm or 14.5 cm diameter) on discs (2.5 cm diameter) of middle-aged leaves of 8–10 weeks old cabbage plants until pupation. Pupae were separated and after adult eclosion, the sex of the beetles was determined. Adults were either kept alone or in pairs in Petri dishes (5.5 cm diameter) lined with moistened filter paper and were offered cabbage leaf discs. At the second day after adult emergence, the single beetles and the pairs were randomly assigned to one of three treatment groups and either offered cabbage leaf discs (2.5 cm diameter) treated with 80 µl of a “low” or “high” sublethal LCT concentration (0.2 mg/L methanol considered as “low” and 0.6 mg/L methanol as “high”) or with 80 µl methanol only (as control treatment), covering the upper surface of the leaf discs completely. The solvent was evaporated before leaf discs were offered to the beetles. Based on the procedure described above, the amount of insecticide each leaf disc received was highly standardised. When sprayed in agriculture, insecticides mainly cover the upper leaf surface of treated crops. The LCT concentrations were chosen based on an acute test, which was conducted following the OECD guideline 423 (OECD, 2001). The high concentration, representing the lowest-observed effect concentration (LOEC), was nearly 20 times lower, whereas the low concentration, representing the no-observed effect concentration (NOEC), was 60 times lower than the average LC_{50}, the concentration that induces 50% lethality (11.83 mg/L, unpublished pre-test). LCT was extracted from the insecticide TRAFO WG (Syngenta Agro GmbH, Maintal, Germany) that contains 5% LCT as active component. To prepare a stock solution of 300 mg/L LCT in methanol, the insecticide was diluted in methanol (HPLC-grade, VWR International GmbH, Darmstadt, Germany) for one min and centrifuged for one min at 13,200 rpm. The supernatant was used to prepare the respective treatment concentrations.

Adults were reared under these distinct conditions for almost three weeks. At day 15 and 17 of adult lifetime, females of all three groups, which had been kept in pairs, were offered untreated cabbage leaves for oviposition. Larvae that hatched from these eggs