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Lambda-cyhalothrin exposure, mating behavior and reproductive output of pyrethroid-susceptible and resistant lady beetles (*Eriopis connexa*)

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ABSTRACT

Insecticide resistance is a ubiquitous consequence in arthropod pest species subjected to insecticide use in agricultural fields. The widespread use of insecticides also allows selection of insecticide resistance among non-target arthropod species, such as natural enemies. Nonetheless, the potential consequences of sublethal insecticide exposure in resistant natural enemies are frequently neglected. The detected pyrethroid resistance in the lady beetle *Eriopis connexa* (Germar) in Brazilian agricultural fields afford the opportunity of assessing the consequences of the sublethal exposure of the broadly used pyrethroid insecticide lambda-cyhalothrin in the mating behavior and reproductive output of susceptible and resistant populations of this species. Survival bioassays with lambda-cyhalothrin allowed estimation of sublethal exposure times at the maximum labeled rate to assess the reproductive consequences of such exposure in pyrethroid-susceptible and resistant strains of *E. connexa*. Such sublethal exposures led to significant difficulties in female mounting by male of both populations. Pyrethroid exposure also extended the duration of female body tremulation and of coupling, while latency to mate, tremulation, coupling and female shaking to dislodge the males after coupling differed between strains with interacting effect of insecticide exposure only for the latter behavior. As a consequence mainly of latency to mate, progeny production was significantly smaller among pyrethroid-resistant females where lambda-cyhalothrin exposure exhibited a negligible effect. Thus, population rather than exposure itself prevailed in determining reproductive output of *E. connexa* and pyrethroid resistance incurred in reproductive costs in this species that may counterweight the benefits of its survival.

1. Introduction

Insecticide resistance is a frequent consequence of pest control in crop production systems by means of insecticide use, and overuse (Guedes et al., 2016, 2017a,b). This phenomenon is essentially a genetic change in response to selection by insecticide use among individuals of a species (Sawicki, 1987; Whalon et al., 2008; IRAC, 2017). The management shortcoming associated with insecticide resistance is that its development can potentially compromise chemical control used against a pest species (Sawicki, 1987; Whalon et al., 2008; IRAC, 2017; Guedes, 2017). Nonetheless, the phenomenon may also take place among species not targeted by the insecticide application, including among them natural enemies of the pest species (Croft and Morse, 1979; Hoy, 1990; Bielza, 2016).

Selection for insecticide resistance is usually associated with the use of lethal insecticide concentrations eliminating susceptible individuals. However, sublethal concentrations are also important in selecting resistant genotypes favoring the survival and reproduction of resistant individuals (Guedes et al., 2017b). Natural enemies are frequently subject to sublethal insecticide concentrations as non-target species. Furthermore, sublethal exposure may also be achieved due to the peculiar behavioral traits of the non-targeted species that may minimize exposure as compared with that of the target pest species (Cordeiro et al., 2010; Lima et al., 2013).

Lady beetles (Coleoptera: Coccinelidae) are common aphid predators in agricultural fields and used as a pest management tactic in aiding the management of several aphid species. An example is the lady beetle species *Eriopis connexa* (Germar), an important aphid predator widely distributed in various crop ecosystems in South America (e.g., maize, sorghum, soybean, wheat) and introduced into North America, which is frequently exposed to pyrethroid insecticides (Rodrigues et al., 2013a; Costa et al., 2017). This scenario has led to a relatively high

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frequency of insecticide resistance among populations of *E. connexa* in field crops (Costa et al., 2017). As a consequence, pyrethroid resistance was observed in *E. connexa* with an autosomal and semi-dominant pattern of inheritance associated with enhanced activity of detoxification enzymes (Rodrigues et al., 2013a, 2013b, 2014). Life-history and behavioral differences were also reported in pyrethroid resistant *E. connexa* and the resistance was not restricted to a single pyrethroid, but extended to different members of this insecticide group (Torres et al., 2015). However, the effect of sublethal exposure on pyrethroid-resistant *E. connexa* remains unknown, particularly regarding the species reproductive behavior, what we targeted in the present study.

Survival bioassays with the pyrethroid lambda-cyhalothrin were performed in a pyrethroid-susceptible and a resistance population of the lady beetle *E. connexa.* Such bioassays allowed the determination of a suitable sublethal length of exposure to the insecticide label rate to assess the potential behavioral effects of such exposure and its reproductive consequences in both populations. We hypothesized that the pyrethroid was likely to impair mating in both populations compromising their progeny production, particularly in the susceptible population, as the resistant population would be better able to cope with the sublethal stress imposed.

2. Material and methods

2.1. Insects

The two populations (lambda-cyhalothrin resistant and susceptible) of the lady beetle *E. connexa* used in the experiments were obtained from populations maintained at the Entomology Unit of the Federal Rural University of Pernambuco in Recife (State of Pernambuco, Brazil). Both populations were originally field-collected by 2009 and have been maintained in laboratory with periodical introduction of newly-collected field insects and tested for insecticide resistance, as described elsewhere (Rodrigues et al., 2013a, 2014; Torres et al., 2015).

The insecticide-susceptible population was originally collected from cotton fields in Frei Miguelinho county (07°55′90.1″S and 35°51′45.6″W; State of Pernambuco, Brazil). The pyrethroid resistant population was originally collected from cabbage fields subject to intensive pyrethroid use in Viçosa county (20°75′73″S and 42°86′96″W; State of Minas Gerais, Brazil). The former population has always been maintained free from insecticide exposure and its susceptibility status is periodically checked. The pyrethroid resistant population also periodically received field insects, and the resistance status is also periodically checked with eventual selection for resistance to the pyrethroid lambda-cyhalothrin to maintain the original levels of insecticide resistance; the level of pyrethroid resistance has remained around 40-fold compared with the susceptible population (Rodrigues et al., 2014; Spíndola et al., 2013; Torres et al., 2015).

Both lady beetle populations were maintained apart from each other in Viçosa under controlled environmental conditions of 25 ± 1 °C temperature, 70 ± 10% relative humidity, and 12:12 h (L:D) photoperiod. The insects were provided with eggs of the Mediterranean flour moth [*Ephestia* (= *Anagasta*) *kuehniella* (Zeller) (Lepidoptera: Pyralidae)] *ad libitum*, and collard green leaves infested with cabbage aphids (*Brevicoryne brassicae* (L.) (Hemipera: Sternorrhyncha: Aphididae)) were provided every other day. In addition, 10% honey solution was also provided during the adult stage of the lady beetles to enhance reproduction.

2.2. Survival bioassays

Adult lady beetles (5–7 days-old) were subjected to time- and concentration-mortality bioassays with a commercial formulation of the pyrethroid insecticide lambda-cyhalothrin (Karate® 50 EC; 50 g a.i./L, encapsulate suspension; Syngenta Prot. Cult., São Paulo, SP, Brazil). The aqueous insecticide solution (2 mL) was applied to glass vials (250 mL volume; 178.15 cm^2 of inner surface), which were maintained in a heavy-duty rotator (Roto-Torque model 7637, ColeParmer, Vernon Hills, IL, USA) for rotation until drying to coat the inner walls of each jar with insecticide residue. The upper portion of each glass vial was coated with Teflon PTFE (DuPont, Wilmington, DE, USA) to prevent the insects from escaping. Ten adult insects were placed in each vial at the concentrations of 0, 10, 75, 150, 300 and 600 mL commercial formulation/ha, corresponding to 0, 5.0, 37.5, 75.0, 150.0 and 300.0 ng a.i./cm^2 of lambda-cyhalothrin; the highest concentration corresponds to the maximum label rate for the agriculture field use of this insecticide in Brazil (MAPA, 2017). Mortality was recorded every 15 min for the 1st hour, then at 2, 4, 8, 24 and 48 h. The insects surviving longer than 48 h were transferred to 500 mL plastic containers (used for the regular rearing of the insects) and their mortality was recorded daily until no more live insects remained. The 48 h upper threshold of exposure was used because lambda-cyhalothrin is a fast-acting (pyrethroid) insecticide that requires short exposure for insecticidal activity (Sunderland, 2010; Casida and Durkin, 2013). Insect mortality was recorded until no more live insects remained and the insects were considered as dead when unable to respond to prodding with a fine hairbrush (Santos et al., 2016). The bioassay was replicated three times for each population (and insecticide concentration), with a vial of 10 insects considered as a replicate.

2.3. Reproductive bioassays

Each adult insect was maintained separate from other insects from emergence until the females started exhibiting abdomen enlargement enabling their sex-recognition (7-days after emergence), as no sexual dimorphism is evident in this species. Subsequently, virgin females and males of the lady beetle (7 days-old) were exposed to the maximum label rate of lambda-cyhalothrin (i.e., 30 g a.i./ha corresponding to 300 ng a.i./cm^2) for either 45 min or 48 h depending on the population (susceptible or resistant, respectively), which were the longer lengths of exposure not compromising the survival of each population, as observed in the survival bioassays previously described. The use of different lengths of exposure with the same concentration allowed for a similar level of sublethal effect for both populations. Suitable water-exposed insects were used as controls in each assessment of each exposure time.

The insecticide exposure followed the methods described for the survival bioassays using a single insecticide concentration and exposure periods specific for each population. At least 20 virgin couples were obtained for assessing their mating behavior and the female reproductive output. However, after insecticide exposure the insects were removed and couples were maintained individually in Petri dish arenas (9 cm diameter) with their bottoms covered with filter paper and inner walls coated with Teflon. The mating behavior of each couple was digitally recorded from their initial release in the arena until the eventual separation after copulation using a digital video camcorder (HDR-XR520V, Sony, Tokyo, Japan). The behaviors were recorded based on preliminary observations and included: walking (i.e., latency to interact), contact between female and male, mounting of female by male, female body tremulation, copulation, female body shaking with mounted male, and separation of the couple. If the male initially paired with the female did not start interaction within 15 min, it was replaced, as were the females that failed to mate with three consecutive males offered.

The males of each coupling pair were discarded after the mating and the females were daily observed until they did not lay eggs for a succession of 10 consecutive days. The eggs laid by each female were removed from the Petri dish and observed for up to 10 days after hatching started in each egg cluster.