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Response of different populations of seven lady beetle species to lambda-cyhalothrin with record of resistance

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ABSTRACT

Simultaneous use of biological and chemical controls is a valued and historic goal of integrated pest management, but has rarely been achieved. One explanation for this failure may be the inadequate documentation of field populations of natural enemies for insecticide tolerance or resistance because natural enemies surviving insecticide application do not create problems like resistant pest species. Therefore, this study investigated 31 populations of lady beetles (Coleoptera: Coccinellidae) regarding their susceptibility to lambda-cyhalothrin, a pyrethroid insecticide that is widely used in cotton and other crops to control lepidopteran and coleopteran pests that are not targeted as prey by lady beetles. The study focused on seven coccinellid species common in cotton fields *Coleomegilla maculata* De Geer, *Cycloneda sanguinea* (L.), *Eriopis connexa* Germar, *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, *Olla v-nigrum* (Mulsant), and *Brumoides foudrasi* (Mulsant) and one lady beetle species [*Curinus coeruleus* Mulsant] from a non-cotton ecosystem for comparisons. Dose-mortality curves were estimated after topical treatment of adult lady beetles with lambda-cyhalothrin. Statistically significant variations in lady beetle susceptibility were observed between species and between populations of a given species. Seven and eighteen populations of lady beetles exhibited greater values of LD_{50} and LD_{90}, respectively, than the highest recommended field rate of lambda-cyhalothrin (20 g a.i./hectare≈0.2 g a.i./L) for cotton fields in Brazil. Furthermore, based on LD_{50} values, 29 out of 30 tested populations of lady beetles exhibited ratios of relative tolerance varying from 2- to 215-fold compared to the toxicity of lambda-cyhalothrin to the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). Four populations of *E. connexa* were 10.5–37.7 times more tolerant than the most susceptible population and thus were considered to be resistant to lambda-cyhalothrin, the first record of resistance for this species. These findings demonstrate that field selection for resistance to lambda-cyhalothrin in common lady beetles is occurring, opening up possibilities to effectively integrate biological control where the popular insecticide lambda-cyhalothrin is used.

1. Introduction

Integration of natural enemies and insecticides for pest control in agroecosystems has been a core component of integrated pest management since the concept was first articulated by Stern et al. (1959), but it has rarely been achieved due to obvious incompatibilities (Tabashnik and Johnson, 1999), and those situations in which the insecticides are more toxic to the pests than to their natural enemies are viewed as rare exceptions (Croft, 1990). However, given the widespread reliance on pesticides, it is of great importance that efforts be made to integrate biological and chemical controls in order to reduce sole reliance on chemical control, and to help growers value the contribution of natural enemies to their cropping systems.

Pyrethroid insecticides are widely used against insect defoliators, and the lambda-cyhalothrin is one of the most used pyrethroid against lepidopteran and coleopteran pest species (Ruberson and Tillman, 1999). Otherwise, aphid outbreaks in cotton and other crops following field spraying with pyrethroids such as lambda-cyhalothrin are frequently reported (Hardin et al., 1995; Deguine et al., 2000; Godfrey et al., 2000; Dutcher, 2007; Obrycki et al., 2009), and are usually correlated with a reduction in the populations of aphid predators and parasitoids (Broza, 1986; Kidd and Rummel, 1997; Longley, 1999). Beyond acute toxicity, lambda-cyhalothrin also causes behavioral change and sublethal effects on

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natural enemies (Desneux et al., 2004; Spindola et al., 2013; Ferreira et al., 2013). Thus, conserving and augmenting lady beetle populations can help to suppress aphid outbreaks in crop ecosystems (Obrycki et al., 2009). Lady beetles in the subfamily Coccinellinae are particularly important as predators of aphids (Hodek and Honěk, 1996). Moreover, even naturally occurring levels of lady beetles in a crop field can restrain aphid population growth early in the cropping season and can continue to do so during the critical crop developmental stage (Powell and Pell, 2007). To obtain this pest management benefit, however, practices must be adopted that allow survival of these predators in the field. These require a firm information base upon which to make sound decisions.

Although lady beetle biology, evolution, physiology, behavior, and trophic ecology have been heavily studied (e.g. Hagen, 1962; Hodek and Honěk, 1996; Weber and Lundgren, 2009; Giorgi et al., 2009), lady beetle resistance to insecticides in the field has received little attention. Laboratory-based bioassays for lethal acute toxicity on different developmental stages and adult reproductive and survival output have been largely published (Web of Science®, research topic <Coccinellidae and insecticides> from 1970 to November 2011) for several insecticides based on field-recommended rates or sublethal doses. Such tests are important to define the impact of insecticides on lady beetles for designing pest management programs which can be coupled with resistant strains of lady beetles.

Enhancing preservation of lady beetles in crop ecosystems will also require reducing the negative effects of insecticides (Lu et al., 2012), especially those used (1) to control pests not targeted by lady beetles (such as lepidopteran and coleopteran larvae); and/or (2) against pest species that have reached a harmful economic threshold despite predation by lady beetles (such as some Hemiptera). The most common response in such situations is to apply broad-spectrum insecticides that are at least as toxic to natural enemies as to the pests (Pathan et al., 2008). However, another approach would be to identify, encourage, and even augment those natural biological control agents that are more resistant to important pesticides than the pest species being targeted with a given chemical. Knowledge of the relative toxicity of pesticides for natural enemies and pests sharing a crop system would allow reporting resistant populations. About 600 species of insects and mites have been recorded as resistant to at least one insecticide (Whalon et al., 2012). However, only 44 of these species are classified as natural enemies, and most are mites or Hymenoptera – fifteen and eighteen species, respectively (Croft, 1990; Whalon et al., 2012). Only three lady beetle species have been regarded as resistant or highly tolerant to organophosphate and pyrethroid insecticides: *Coleomegilla maculata* De Geer, *Hippodamia convergens* Guérin-Méneville, *Stethorus gilvifrons* (Muls.), and *Eriopis connexa* Germar (Head et al., 1977; Graves et al., 1978; Ruberson et al., 2007; Kumral et al., 2011; Rodrigues et al., 2013a, 2013c).

In this study we investigated dose-mortality responses of 31 populations of eight lady beetle species from different localities in Brazil to the widely used insecticide lambda-cyhalothrin. We examined the tolerance ratio among populations of different lady beetles species and potential resistance ratio response based on the results for populations of the same species in relation to lambda-cyhalothrin. Further, the tolerance ratio was calculated for lady beetles based on the recommended field rates for spraying cotton fields and the lethal doses calculated for controlling the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). This procedure aimed to show the impact of spraying lambda-cyhalothrin to control, the most important cotton pest in Brazil and in other regions where it occurs relative to response determined for the lady beetles studied.

2. Material and methods

2.1. Lady beetle collections

Between March 2009 and September 2012, we collected all developmental stages (but mainly adults) of lady beetle species belonging to the Coccinellinae and Chilocorinae subfamilies in various Brazilian localities (Table 1) and transported them to the Biological Control Laboratory of the Universidade Federal Rural de Pernambuco (UFRPE), Recife, PE.

Two species of lady beetles that are not aphid predators were collected and used in the bioassays as a source of comparisons (see Table 1). These were *Brumoides foudrasi* (Mulsant) collected while preying on cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in a commercial cotton field located in Surubim County, PE, and *Curinus coeruleus* (Mulsant) collected while preying on psyllid, *Euphalerus clitoriae* Burckhardt and Guajará (Hemiptera: Psyllidade) on pea trees, *Clitoria fairchildiana* Howard (Leguminosae: Papilionoideae), located at the UFRPE campus, Recife, PE. The other lady beetle species are predominantly predators of aphids, but also consume other cotton pests, such as lepidopteran eggs, scales, and mites. As common predators in crop fields, these species were easily identified at the species level (Dr José Adriano Giorgi, Universidade Federal do Pará, PA, Brazil).

2.2. Maintenance of lady beetles

All collected species were reared separately under laboratory conditions of 25 ± 1 °C and 12:12 h (L:D) photoperiod. Nymphs and adults of the striped mealy bug, *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae), were used as prey for *B. foudrasi*. Adults of *C. coeruleus* were maintained with psyllids collected from pea trees. The remaining lady beetle species were reared with aphids when available (cotton aphid, green peach aphid, or brown citrus aphid), especially during the early adult stage to stimulate egg maturation and oviposition; in the absence of aphids, we provided eggs of the Mediterranean flour moth, *Anagasta* (=*Ephestia*) *kuehniella* (Zeller) (Lepidoptera: Pyralidae). These lady beetles adults also were provided with a supplementary diet composed of a 1:1 mixture of honey and yeast. Both the aphids and the supplementary diet were offered ad libitum.

Due to difficulties in distinguishing gender in lady beetles, 4–6 adults were kept together to guarantee mating. They were held in transparent 1-L plastic containers with an organdy-covered opening on the lid to allow ventilation, and a piece of paper towel as an oviposition substrate. Paper towel pieces with egg batches were transferred in groups to transparent 500-mL plastic containers and eggs of *A. kuehniella* were provided on the third day to prevent cannibalism after eclosion. Later, 2-d old larvae were transferred to 80-mL transparent plastic cups (3 larvae per cup). According to the instar, larvae were provided ad libitum with eggs of *A. kuehniella* from a colony maintained in the same laboratory using the rearing methodology of Torres et al. (1995). The lady beetle larvae were reared until pupation and adult emergence under these conditions, after which the newly emerged adults were maintained as described above or were assayed between 5- and 8-day old.

2.3. Dose mortality response

The lambda-cyhalothrin used was the commercial product Karate Zeon [lambda-cyhalothrin 5 percent w/v −50 g/L SC (soluble concentrated); Syngenta S.A., Brazil] purchased at the local specialized market. For each population of adult lady beetles, we conducted preliminary bioassays with various doses of the commercial product diluted in distilled water, starting with a concentration of 20 g of a.i./ha (0.1–0.2 g of a.i./L), based on the highest recommended field rate (http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons) to control lepidopteran larvae, such as the cotton bollworm, *Heliothis virescens* (Fabr.) (Lepidoptera: Noctuidae). A total of 5–6 doses of lambda-cyhalothrin were tested, according the preliminary bioassays results, which produced a range of responses between 0 percent and 100 percent mortality.

The adults (control or treated) were subjected to topical treatment using a minimum of twenty insects per dose and a range of 127–337 insects per species/population to define each dose mortality response. The bioassays were repeated at least two times on different days. A total of 6622 lady beetles from 31 populations collected across different locales and crop systems were assayed. Topical treatment consisted of applying 0.5 μl of the diluted product on the ventral part of the insect abdomen with a Hamilton™ syringe of 25 μL volume. For the control group only water was applied. After treatment, the insects were placed in filter-paper lined Petri dishes (12 cm diameter × 1.5 cm height), given a paste of 1:1 honey:yeast as food, and kept at 25 ± 1 °C, 12:12 h (L:D) photoperiod and 55–68 percent RH. Mortality was assessed 24 h after insecticide application, and adults were considered to be dead if it was unable to turn upright and begin to walk after being placed on its dorsum at the respective intervals of evaluation.

In September 2011, a dose-mortality curve was determined for a field population of the boll weevil, *A. grandis*. At the end of the season cotton buds and bolls exhibiting signs of holding an immature weevil inside were collected from a