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Differential impacts of six insecticides on a mealybug and its coccinellid predator

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ABSTRACT

Broad-spectrum insecticides may disrupt biological control and cause pest resurgence due to their negative impacts on natural enemies. The preservation of sustainable pest control in agroecosystems requires parallel assessments of insecticide toxicity to target pests and their key natural enemies. In the present study, the leaf dipping method was used to evaluate the relative toxicity of six insecticides to the striped mealybug, *Ferrisia dasylirii* (Cockerell) (Hemiptera: Pseudococcidae) and its predator, *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae). Three neurotoxic insecticides, lambda-cyhalothrin, methidathion and thiamethoxam, caused complete mortality of both pest and predator when applied at their highest field rates. In contrast, lufenuron, pymetrozine and pyriproxyfen caused moderate mortality of third-instar mealybug nymphs, and exhibited low or no toxicity to either larvae or adults of the lady beetle. At field rates, lufenuron and pymetrozine had negligible effects on prey consumption, development or reproduction of *T. notata*, but adults failed to emerge from pupae when fourth instar larvae were exposed to pyriproxyfen. In addition, pyriproxyfen caused temporary sterility; *T. notata* females laid non-viable eggs for three days after exposure, but recovered egg fertility thereafter. Our results indicate that the three neurotoxic insecticides can potentially control *F. dasylirii*, but are hazardous to its natural predator. In contrast, lufenuron and pymetrozine appear compatible with *T. notata*, although they appear less effective against the mealybug. Although the acute toxicity of pyriproxyfen to *T. notata* was low, some pupal mortality and reduced egg fertility suggest that this material could impede the predator's numerical response to mealybug populations.

1. Introduction

Many herbivorous arthropods in Brazilian cotton, *Gossypium hirsutum* L., commonly reach pest status and cause economic losses, thus requiring appropriate management strategies. Transgenic *Bt*-cotton has significantly reduced insecticide applications against various lepidopteran pests (Lu et al., 2012; Naranjo, 2011), but other pests unaffected by *Bt* require additional management tactics. In Brazil, an everincreasing catalogue of pesticides is registered against key cotton pests: 177 against *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), 129 against *Aphis gossypii* Glover (Hemiptera: Aphididae), 98 against *Chloridea* (= *Heliothis*) *virescens* (F.) (Lepidoptera: Noctuidae), 86 against *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), 37 against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), and 30 against *Bemisia tabaci* biotype B (Gennadius) (Hemiptera: Aleyrodidae) (Agrofit, 2017). At present, no insecticide is registered in Brazil to control mealybugs (Hemiptera: Pseudococcidae) on cotton, and only a few active ingredients are recommended for controlling them on other crops (Agrofit, 2017). Thus, mealybug management to date has relied on either natural biological control, or collateral mortality due to insecticide applications targeting other pests.

Mealybugs are a large and diverse group that exploit some 250 plant families (Ben-Dov, 1994; Morales et al., 2016) around the world (Miller, 2005). They cause direct damage to plants by sucking phloem sap from both vegetative and reproductive structures (Oliveira et al., 2014a; Walton et al., 2009). Mealybugs excrete surplus sugars as honeydew, which promotes the growth of sooty mold that, in turn, can impair photosynthetic productivity (Saeed et al., 2007; Silva et al., 2014). Depending on infestation intensity, mealybugs can reduce cotton yield as much as 40–50% (Nagrare et al., 2009), and even kill plants (Oliveira et al., 2014a).

In northeastern Brazil, the mealybugs *Phenacoccus solenopsis* Tinsley (Silva, 2012) and *Ferrisia dasylirii* (Cockerell) (Silva-Torres et al., 2013) have both emerged recently as new threats to cotton. Oliveira et al. (2014a) reported that *F. dasylirii* could increase its numbers 412-fold in a single generation under greenhouse conditions, and that fecundity

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increased by 37% and 18% as a function of water stress and nitrogen fertilization, respectively (Oliveira et al., 2014b). Each female can produce > 390 crawlers in ca. 16 days at 27–28 °C (Oliveira et al., 2014c). Thus, *F. dasylirii* has explosive population growth potential in the semi-arid cotton-growing region of Brazil, especially under conditions of drought or heavy fertilization.

The suitability of *F. dasylirii* as prey for indigenous and exotic natural enemies has been explored in the hopes of improving understanding of its biological control. A number of insect predators develop and reproduce successfully on this species, including the lady beetles *Tenuisvalvae notata* (Mulsant) (Barbosa et al., 2014a) and *Cryptolaemus montrouzieri* Mulsant (Marques et al., 2015) (Coleoptera: Coccinellidae), and the lacewings *Chrysoperla externa* (Hagen) and *Ceraeochrysa everes* (Banks) (Neuroptera: Chrysopidae) (Tapajós et al., 2016). In northeastern Brazil, *T. notata* is often found feeding within mealybug colonies on cotton plants and bioassays indicate that all larval instars prey successfully on both immature and adult mealybugs. An exclusive diet of *F. dasylirii* yielded 83% larval survival of *T. notata* and a reproductive rate of 7.6 eggs / female / day with ca. 62% egg viability (Barbosa et al., 2014a). Females of *T. notata* exhibited a type III functional response when preying upon *F. dasylirii* crawlers, and a type II response preying upon third instar nymphs and adult females (Barbosa et al., 2014b). Thus, the beetle would seem to be a good candidate biological control agent.

Unfortunately, most of the insecticides used in Brazilian cotton have broad-spectrum activity (Agrofit, 2017), and thus the potential to disrupt biological control. We hypothesized that neurotoxic insecticides commonly used in Brazilian cotton, such as organophosphates, pyrethroids and neonicotinoids, would be harmful to *T. notata*, whereas insect growth regulators (IGRs) and antifeedants would be more compatible with mealybug biological control. Therefore, the present study evaluated the relative toxicity of six insecticides to both *F. dasylirii* and its predator, *T. notata*, and monitored any surviving lady beetles for sub-lethal effects. These insecticides represented six modes of action: an acetylcholinesterase inhibitor, a sodium channel modulator, a nicotinic acetylcholine receptor competitive modulator, a juvenile hormone mimic, a chordotonal organ modulator, and a chitin biosynthesis inhibitor (Insecticide Resistance Action Committee, 2017).

2. Materials and methods

2.1. Insect colonies

Stock colonies of the mealybug *F. dasylirii* and its predator *T. notata* were established in a climate-controlled room set to 25 ± 2 °C, 60–70% RH, and a 13:11 (L:D) photoperiod, at the Laboratory of Ecology and Biological Control of the 'Universidade Federal Rural de Pernambuco' (UFRPE), Recife, Pernambuco State, Brazil. All experiments were carried out in climate-controlled growth chambers set to these same physical conditions.

2.1.1. Ferrisia dasylirii

Cotton leaves and stems infested with nymphs and adult mealybugs were collected in the Experimental Area of the Crop Protection Unit of the 'UFRPE', and reared in the laboratory following method of Sanches and Carvalho (2010). Weekly, ca. 50 reproductive mealybug females are removed from an old infested pumpkin fruit of *Cucurbita moschata* Duchesne var. 'Jacarezinho' (~ 1 kg each) and used to infest a fresh one on a plastic tray lined with paper towel, which serves to absorb the excreted honeydew. Under the physical conditions described above, the pumpkins are completely infested within 30 days, whereupon they are placed into transparent Plexiglass cages (30 × 40 × 20 cm) and provide a continuous food supply for lady beetles for another 30 days.

2.1.2. Tenuisvalvae notata

A colony of *T. notata* was established from approximately 50 adult beetles collected on cotton plants infested with *F. dasylirii* and the cotton mealybug, *P. solenopsis*, in Surubim County (07° 49′ 59″ S, 35° 45′17″ W), Pernambuco State, Brazil. The colony of *T. notata* was maintained by caging approximately 100 adult lady beetles with a mealybug-infested pumpkin in a transparent Plexiglass cage (as above). These cages had screened windows (ca. 2 mm nylon mesh) for ventilation, and were lined with paper towel to absorb honeydew. A full description of the rearing of *T. notata* and its life cycle can be found in Barbosa et al. (2014a).

2.2. Insecticide solutions

Assuming application in 150 L of water per hectare, we prepared solutions of the following insecticides at their highest field rates (FRs) recommended against cotton pests in Brazil (brand name, FR, active ingredient concentration ≈ g a.i.·L^{-1}): lambda-cyhalothrin (Karate Zeon 50 CS®, 400 mL ha^{-1} ≈ 0.13 g a.i.·L^{-1}), lufenuron (Match 50 EC®, 800 mL ha^{-1} ≈ 0.27 g a.i.·L^{-1}), methidathion (Suprathion 400 EC®, 1.000 mL ha^{-1} ≈ 2.67 g a.i.·L^{-1}), pymetrozine (Chess 500 WG®, 400 g ha^{-1} ≈ 1.33 g a.i.·L^{-1}), pyriproxyfen (Tiger 100 EC®, 400 mL ha^{-1} ≈ 0.27 g a.i.·L^{-1}), and thiamethoxam (Actara 250 WG®, 200 g ha^{-1} ≈ 0.33 g a.i.·L^{-1}) (Agrofit, 2017). These concentrations were prepared by diluting each insecticide in tap water plus 0.05% of the surfactant Wil Fix® (Charmon Destyl Chemical Industry Ltda, Campinas, SP, Brazil); this solution alone served as the control treatment.

2.3. Residual toxicity to Ferrisia dasylirii

Leaf discs (8 cm diam) were cut from cotton leaves using a metal cylinder, then rinsed with a 1% hypochlorite solution, and again in tap water, before dipping them for 30 s into a treatment solution. Leaf discs were left to air-dry for one hour at room temperature before being placed into glass Petri dishes (10 cm diam) lined with moistened filter paper. Mealybug nymphs (≤ 24 h after the second molt) were then transferred from pumpkins to each leaf disc (n = 10 per disc) and the dishes were covered with plastic film (Royal Pack®) to prevent escapes. Mortality was assessed for five consecutive days; nymphs were considered dead if they failed to move when prodded with a soft brush. The experiment employed a randomized design with seven treatments of 10 replicates each.

2.4. Residual toxicity to Tenuisvalvae notata adults and sublethal effects

Cotton leaf discs were treated and placed into glass Petri dishes (as described above in 2.3). Five adult lady beetles (≤ 24 h old post-emergence) and 10 third-instar nymphs of *F. dasylirii* were introduced into each Petri dish (replicate), which was then covered with plastic film (Royal Pack®). Beetle mortality and mealybug consumption were both assessed daily for five days, with mealybugs restored daily. Lady beetles were recorded as dead if they failed to right themselves within five minutes after being placed upside down on their elytra. The experiment was a randomized design with seven treatments of 10 replicates each.

2.5. Residual toxicity to Tenuisvalvae notata larvae and sublethal effects

Preliminary tests revealed that only pymetrozine, lufenuron and pyriproxyfen would produce enough surviving larvae to assess their biological performance as adults, so only these materials were examined for sub-lethal effects. Cotton leaf discs were again treated and placed into glass Petri dishes (as described above in 2.3). A single fourth-instar *T. notata* larvae (ca. 24 h after the third molt) was transferred to each Petri dish (n = 35 per treatment) along with three adult mealybugs to serve as food. Previous work has shown this to be an abundant food supply for this life stage (Barbosa et al., 2014a). After a