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Insecticide resistance and control failure likelihood among populations of the boll weevil (*Anthonomus grandis*) from Mato Grosso (Brazil)

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ABSTRACT. The cotton producers from southern Mato Grosso are currently experiencing control failure with the use of the use of insecticides against the cotton boll weevil *Anthonomus grandis* Boheman, the main pest species of this commodity. Therefore, the present study was designed to survey insecticide resistance and the associated likelihood of control failure among boll weevil populations in the region. Ten insect populations were sampled during the 2016/2017 season and subjected to time-mortality (contact) bioassays in glass vials impregnated with dried insecticide residues at their respective label rates. The three insecticides most frequently used in the region were surveyed: the organophosphate malathion and the pyrethroids beta-cyfluthrin and zeta-cypermethrin. The survival curves showed estimates of the respective median survival time (LT_{50}) for each combination of insecticide and insect copulation. However, there were no significant differences in susceptibility among populations. The estimates of control failure likelihood for each compound at their respective label rates also indicated negligible risk of control failure with their use. These findings are consistent with time-mortality results indicating the lack of insecticide resistant populations at the surveyed sampling sites, suggesting that the reported field control failures result from other causes such as problems with insecticide application.

Keywords: chemical control; cotton pest; resistance management; survival time; susceptibility and sustainable pest management.

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Introduction

Brazil is among the five largest cotton producers in the world and is one of the largest exporters of cotton (Ridley & Devadoss, 2012; 2014; Food and Agriculture Organization of the United Nation [FAOSTAT], 2013). The central-western region is the main area of cotton production, and the state of Mato Grosso is the largest producer accounting for over 70% of national production (James, 2011). Notwithstanding, one factor limiting cotton expansion in Brazil is reduced yield resulting from pest attacks, particularly by the boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Ribeiro et al., 2010). The boll weevil is devastating because of its high reproductive potential, and it causes damage during its larval stage by forming galleries inside the young fruits and flowerbuds of the cotton plant (Showler, 2008).

The main control method against the boll weevil is the use conventional insecticides, primarily compounds of the organophosphorus group and pyrethroids (Showler, 2007). These groups of insecticides were used to eradicate the boll weevil in the United States (Allen, 2008), and their use also prevails in Brazil is (Azambuja & Degrande, 2014).

Farmers reported control failures using pyrethoid insecticides in response to inquiries made during field collection of swab populations for experimental use. Thus, the presence of the pest in the field following intensive use of phosphorus and pyrethroid insecticides suggests that cotton boll weevil in this region may be resistant to these insecticides, hence, the reported control failures. Therefore, the aim of this work was to survey and assess the occurrence of insecticide resistance, and to evaluate the risk of insecticide failure in populations of boll weevil sampled in the state of Mato Grosso. The organophosphorus malathion and the pyrethroids beta-cyfluthrin and zeta-cypermethrin were tested because they are the most widely used insecticides in the region.

Material and methods

Populations of insects

Inflorescences, young fruits and flowerbuds with oviposition marks were collected in 2016 and 2017 in cotton producing regions of the state of Mato Grosso (Figure 1). The sampling sites were concentrated in the main cotton-growing micro-regions, including the counties of Rondonópolis, Alto Taquari, Itiquira, Serra da Petrovina, Pedra Preta, Juscimeira, and Jaciara (Table 1). The counties of Alto Garças, Campo Verde, Primavera do Leste, Ponte de Pedra, Guiratinga, and Lucas do Rio Verde did not have significant boll weevil outbreaks, which made it impossible to sample populations and perform the bioassays at these areas. After sampled, the structures were placed in 4-liter plastic pots and closed by organza fabric, remaining at ambient temperature until emergence of adult boll weevils. Bioassays were performed with newly emerged adults (<2 days of age).

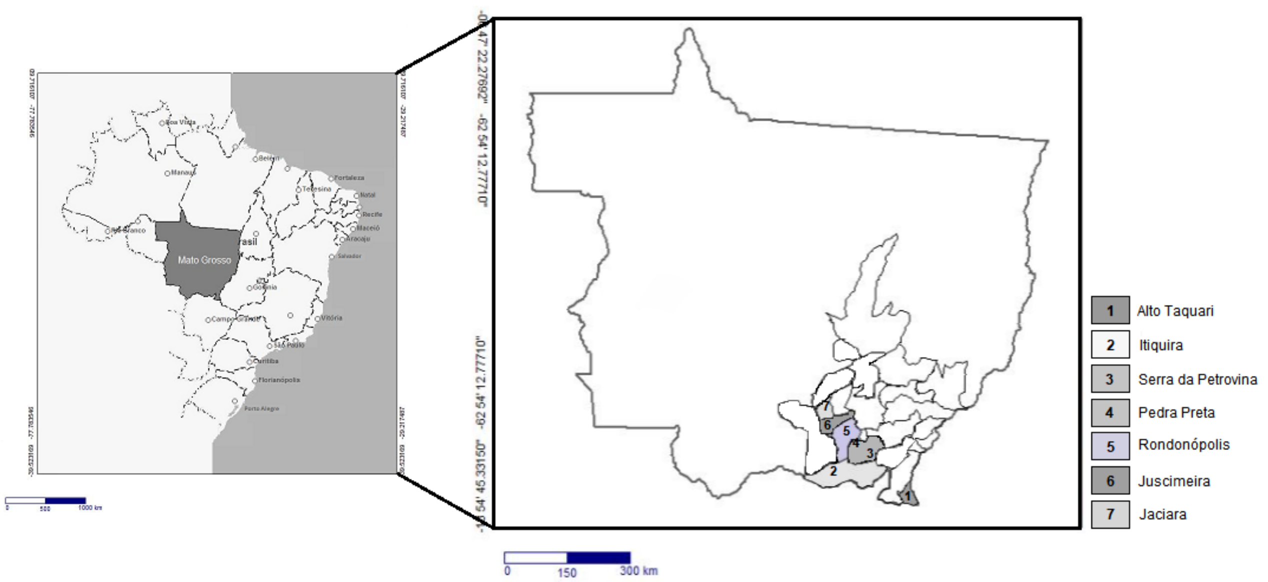

Figure 1. Map illustrating the sampling locations in the state of Mato Grosso, Brazil.

Insecticides

Organophosphate malathion and the pyrethroids beta-cyfluthrin and zeta-cypermethrin were the insecticides used. These insecticides have always been used at their maximum label rates for use in the field considering a spray volume of 100 L ha^{-1}: malathion at 2 L c.p. ha^{-1} (1,000 g a.i. L^{-1}, emulsifiable concentrate, FMC, Uberaba, Minas Gerais State, Brazil), beta-cyfluthrin at 100 mL c.p. ha^{-1} (125 g a.i. L^{-1}, concentrated suspension, Bayer CropScience, Belford Roxo, Rio de Janeiro State, Brazil), and 250 mL c.p. ha^{-1} of zeta-cypermethrin (400 g a.i. L^{-1}, emulsifiable concentrate FMC, Campinas, São Paulo State, Brazil). Distilled water was used as a diluent.

Time-mortality bioassays and control failure estimation

Petri dishes (9 x 1.5 cm) were impregnated with dry insecticide residues (i.e., contact bioassay) following the methodology of Yuan and Chambers (1998). Each petri dish received 1 mL of insecticidal suspension at the desired concentration (Yuan & Chambers, 1998). The insecticidal residue was dried in fresh air for 24 hours, after which 20 adult insects were placed into each Petri dish, with three replicates for each combination of insect population and insecticide. The top of the Petri dish was brushed with odorless talc to prevent insects from escaping. A control treatment (with distilled water only) was used to determine the natural mortality. Insect mortality was assessed every 24 hours until all insects were dead. Insects were recognized as dead when they were unable to walk coordinately, even when touched with a fine hair brush.

Insect mortality at 24 hours was taken as a measure of control efficacy, whereas temporal mortality assessments were used for survival analyses. The results of control efficacy (i.e., mortality at 24 hours) were corrected by the natural mortality observed in the respective controls of each boll weevil population

(Abbott, 1925). These results were subsequently used in the estimation of control failure likelihood (CFL) through the formula (1) proposed by Guedes (2017). The expected mortality is the minimum efficacy threshold expected for the record of insecticides (i.e., 80%; MAPA, 1995). Thus, if the corrected mortality is equal to 80%, the risk of control failure (or control failure likelihood) is zero. If the corrected mortality is greater than 80%, the risk of control failure is less than zero, indicating a negligible risk of failure of control (Guedes, 2017).

$$\text{CFL}(\%) = 100 - \left[\left(\frac{\text{achieved mortality }(\%) \times 100}{\text{expected mortality}}\right)\right] \quad (1)$$

Table 1. Median lethal time estimates (LT_{50}) for each population for insecticide. LT_{50} values followed by the same letter did not differ significantly by the Holm-Sidak test (p < 0.05). The values of χ^2 and *P* refer to the significance of the overall difference among populations populations for the same insecticide.

Populations	Malathion			
	LT_{50} (hours)	Fidulcial interval	χ^2	*P*
Pedra Preta	0.27 a	0.24 - 0.29	309.08	<0.001
Alto Taquari	0.20 b	0.19 - 0.21		
Juscimeira	0.20 b	0.19 - 0.22		
Serra da Petrovina	0.13 bc	0.09 - 0.18		
Itiquira	0.07 d	0.06 - 0.07		
Rondonópolis 2016	0.07 d	0.05 - 0.08		
Rondonópolis 2017	0.13 c	0.12 - 0.15		
Jaciara 1	0.13 c	0.12 - 0.15		
Jaciara 2	0.13 c	0.12 - 0.14		
Jaciara 3	0.13 c	0.12 - 0.15		
Populations	**Zeta-cypermethrin**			
	LT_{50} (hours)	Fidulcial interval	χ^2	*P*
Pedra Preta	0.33 de	0.297 - 0.369	271.74	<0.001
Alto Taquari	0.4 cd	0.389 - 0.411		
Juscimeira	0.367 bc	0.343 - 0.391		
Serra da Petrovina	-	-		
Itiquira	0.75 b	0.719 - 0.781		
Rondonópolis 2016	0.267 e	0.246 - 0.287		
Rondonópolis 2017	0.4 b	0.384 - 0.416		
Jaciara 1	0.4 bcd	0.386 - 0.414		
Jaciara 2	0.4 b	0.384 - 0.416		
Jaciara 3	0.4 b	0.384 - 0.416		
Populations	**Beta-cyfluthrin**			
	LT_{50} (hours)	Fidulcial interval	χ^2	*P*
Pedra Preta	0.47 a	0.436 - 0.497	257.69	<0.001
Alto Taquari	0.43 c	0.411 - 0.456		
Juscimeira	0.47 b	0.442 - 0.492		
Serra da Petrovina	0.75 a	0.711 - 0.789		
Itiquira	0.75 a	0.665 - 0.835		
Rondonópolis 2016	0.20 d	0.137 - 0.263		
Rondonópolis 2017	0.23 bcd	0.209 - 0.257		
Jaciara 1	0.47 bc	0.435 - 0.498		
Jaciara 2	0.23 cd	0.212 - 0.254		
Jaciara 3	0.23 cd	0.200 - 0.267		

Statistical analyses

The survival results of the time-mortality bioassays were subjected to survival analysis using Kaplan-Meier estimators to obtain the respective median survival times (LT_{50}) for each population and insecticide. The survival curves of each population for each insecticide were compared using the Holm-Sidak test (p < 0.05) (SigmaPlot 12.5, 2013, Systat, San Jose, CA, EUA).

The results of risk of failure of control would be compared with 0% using the test of Z (p < 0.05) with continuity correction, to show the populations where such risk of failure was significant (Dângelo, Michereff-Filho, Campos, Silva, & Guedes, 2018). However, as the risks of insecticide control failure were negligible, no such testing was required.

Results and discussion

The survival curves obtained showed significant differences for the different insecticides and control regardless of the insect population considered (χ^2 = 2959.81, df = 3,p < 0.001) (Figure 2), but responses among populations also differed significantly in terms of survival without exposure to insecticides (χ^2 = 430, df = 9, p < 0.001) (Figure 3). The median lethal times (LT_{50}) for each population and insecticide are shown in Table 1. These estimates allowed the calculation of the respective resistance ratios considering the most susceptible population (i.e., with a lower LT_{50}), as the susceptibility standard (Table 2). The observed resistance ratios ranged from 1 to 4 and were not significantly different. Consequently, the estimated risks of control failure were negligible (Table 2), making the formal statistical tests of comparison unnecessary.

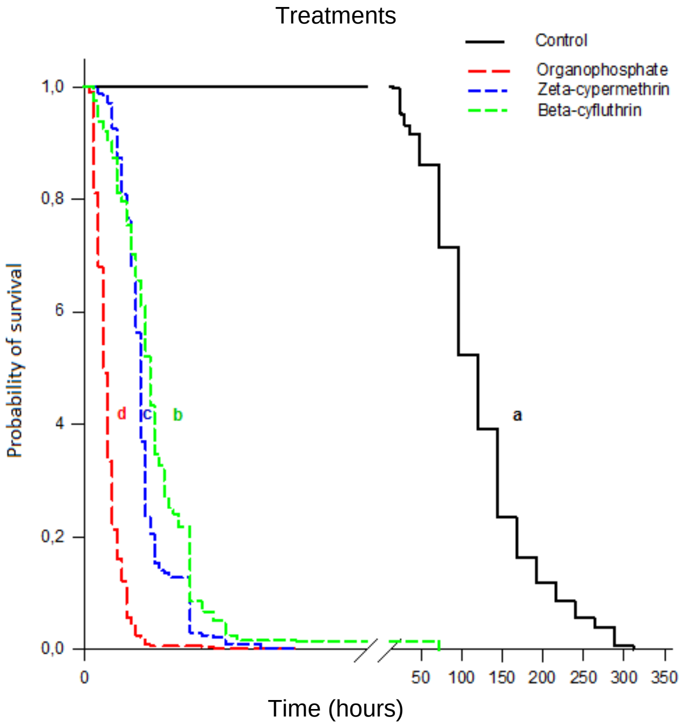

Figure 2. Survival curves of the evaluated insecticides, regardless of the population. The different letters indicate a significant difference among population according to the Holm-Sidak test (p < 0.05).

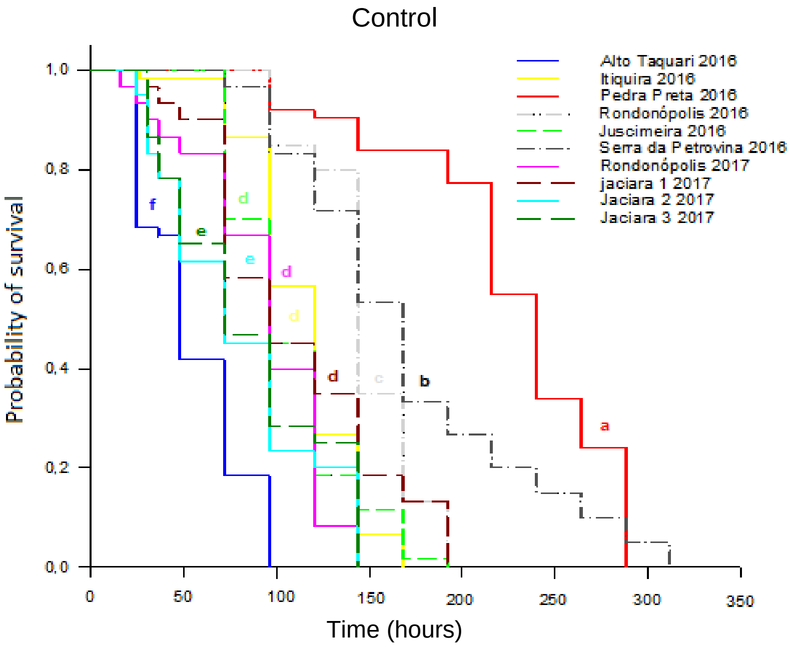

Figure 3. Survival curves of the control treatments for the different populations. The different letters indicate significant difference among populations according to the Holm-Sidak test (p < 0.05).

Table 2. Estimation of resistance ratio and risk of control failure for cotton boll weevil populations and the three insecticides evaluated.

	Organophosphate		Zeta-cypermethrin		Beta-cyfluthrin	
Population	Ratio of resistance ($LT_{50\,R}/LT_{50\,S}$)	Risk of control failure	Ratio of resistance ($LT_{50\,R}/LT_{50\,S}$)	Risk of control failure	Ratio of resistance ($LT_{50\,R}/LT_{50\,S}$)	Risk of control failure
Pedra Preta	4.00	0%	1.25	0%	2.335	0%
Alto Taquari	3.00	0%	1.50	0%	2.165	0%
Juscimeira	3.00	0%	1.37	0%	2.335	0%
Serra da Petrovina	1.99	0%	-	-	3.75	0%
Itiquira	1.00	0%	2.81	0%	3.75	0%
Rondonópolis	1.00	0%	1.00	0%	1	0%
Rondonópolis 2017	1.99	0%	1.50	0%	1.165	0%
Jaciara1	1.99	0%	1.50	0%	2.335	0%
Jaciara2	1.99	0%	1.50	0%	1.165	0%
Jaciara3	1.99	0%	1.50	0%	1.165	0%

The management of *A. grandis* has mostly been carried out via the use of conventional insecticides (Allen, 2008). Among the most commonly used insecticides are pyrethroids and organophosphates (Showler, 2007). Even with intensive application (Silva & Ramalho, 2013), the presence of the cotton boll weevil in cultivated cotton areas remains persistent.

Despite concerns raised by the producers and the intense applications of insecticide against cotton boll weevil, all assessed weevil populations have shown to be susceptible to these insecticides. The results obtained in this work confirm previous results reported by Bleicher, Jesus, and Almeida (1990) and Soares, Busoli, Yamamoto, and Braga Sobrinho (1994). These authors worked on insecticide efficacy tests with organophosphates and pyrethroids and obtained efficiency superior to 80% against the cotton boll weevil.

The management practices of the farms visited may be contributing to the delay of the evolution of insecticide resistance. According to the field research carried out, the chemical control begins when the cotton is in phase B1, often even before the boll weevil is found during monitoring. Insecticide applications are performed sequentially with the same insecticide, every 3 or 5 days. On average, 15.25 malathion applications were made up until 100 cycle days. Subsequently, pyrethroid insecticides were applied 8.1 times. Pyrethroid insecticides and carbamate, or pyrethroid and pirimiphos-methyl in admixture are also applied in sequential form, albeit in smaller amounts. This type of management is characterized as saturation management, which entails the constant and intense application of the same insecticides in short intervals of time. Thus, maintaining high dosage (Ambethgar, 2009).

Malathion exhibited lower LT_{50}, indicating its higher toxicity when compared to the pyrethroids evaluated. When comparing only the pyrethroids, the insecticide zeta-cypermethrin pexhibited slightly lower mortality than the insecticide beta-cyfluthrin. This result can be explained by the type of formulation used in this insecticide. Zeta-cypermethrin is marketed as an oil-in-water emulsion, unlike beta-cyfluthrin, which is marketed as a concentrated suspension that promotes faster penetration of the former relative to the latter. This seems to be the case because formulations that use oil in its composition (e.g. emulsifiable concentrate), whether vegetable or mineral, usually favor the permeability of the insecticide conveyed through the epicuticle of the insect favoring the toxicity of the insecticide. This seems be the case of zeta-cypermethrin with *A. grandis* (Stadler & Buteler, 2009; Nicetic, Cho, & Rae, 2011).

The cotton boll weevil has been successfully controlled by organophosphorus and pyrethroid insecticides for decades without resistant populations having been observed (Snodgrass & Scott 2003). Among the processes that may lead to resistance to insecticides, metabolic resistance is the most studied (Salvador et al., 2014). The main enzymes capable of sequestering or breaking down the pesticide molecules belong to three families: cytochrome P450 (CYP) dependent monooxidases, glutathione-S-transferases (GST), and carboxylesterases (COEs) (Ramoutar, Cowles, & Alm, 2009; Yu, Lu, Li, Xiang, & Zhang, 2009; Siegwart et al., 2011). However, although these enzymes are present in the cotton boll weevil, their activity is relatively low, indicating the weak metabolic defense in the boll weevil, particularly in the adult phase (Salvador et al., 2014). It seems that even based on the rare incidence of insecticide resistance in populations of the cotton boll weevil, the genetic variability for resistance to insecticides is low in this species.

Thus, despite the high dispersion capacity and intra- and interspecific genetic diversity (Martins, Ayres, & Lucena, 2007; Choi et al., 2011), the low genetic variability for selection for insecticide resistance and the lack of insecticide detoxification mechanisms seem to prevail among *A. grandis* populations. The applications of organophosphorus (i.e. malathion) and pyrethroid insecticides for decades without serious problem of resistance (Snodgrass & Scott, 2003) provides support for this observation.

Conclusion

No insecticide resistance was observed among populations of the cotton boll weevil for the insecticides tested. This led to a negligible risk of insecticide control failure for the compounds assessed. Thus, insecticide control failures reported in the field by producers and consultants are likely due to other factors, such as application or inappropriate use of insecticidal compounds.

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