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ORIGINAL PAPER

Spinosyn resistance in the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract Spinosad has been used to control *Tuta absoluta* in Brazil for more than a decade but will eventually be replaced by spinetoram despite the risk of cross-resistance. Therefore, the susceptibility to both molecules and the activity of detoxification enzymes were determined for eight representative populations of *T. absoluta* to assess resistance and the risk of cross-resistance. The LC_{50} values for spinosad varied from 0.007 (Pelotas) to 0.626 mg/L (Sumaré); the LC_{50} values for spinetoram varied from 0.047 (Pelotas) to 0.308 mg/L (Sumaré). The LC_{99} values for spinosad varied from 0.23 (Pelotas) to 11.56 mg/L (Venda Nova do Imigrante); the LC_{99} values for spinetoram varied from 0.55 (Pelotas) to 6.71 mg/L (Iraquara). The resistance levels ranged from 1.0- to 93.8-fold (RR_{50}) and 1.0- to 51.5-fold (RR_{99}) for spinosad and from 1.0- to 6.5-fold (RR_{50}) and 1.0- to 12.1-fold (RR_{99}) for spinetoram. The concentration-mortality responses to spinetoram were more homogeneous than those to spinosad. A strong correlation between the susceptibilities of *T. absoluta* populations to spinosad and spinetoram was observed, showing the similarity of the mode of action of both molecules and producing cross-resistance between them. The β–esterase activity of *T. absoluta* populations was correlated with spinosyn susceptibility, suggesting a potential contribution of the enzyme to evolved spinosyn resistance. The evolution of resistance to spinosyns in *T. absoluta* observed in this study suggests that strategies to mitigate resistance must be carefully implemented over the short term and that rotation with other products is encouraged.

Keywords Enhanced detoxification · Cross-resistance · Spinosad · Spinetoram · Tomato leafminer

Key message

- Insecticide resistance is an increasing phenomenon among pests and impairs the control of invasive pests, particularly to risk-reduced insecticides such as spinosyns.
- High levels of resistance in field populations of *Tuta absoluta* to spinosyns are reported as well as cross-resistance to other spinosyns and detoxificative metabolism.
- The reporting of *Tuta absoluta* resistance to spinosad will lead to further studies for generating tools of resistance monitoring to spinosyns, thus fine-tuning the insecticide resistance programs.

Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), known as the tomato pinworm in Brazil, was restricted to South America, where it caused great damage to Brazilian tomato production, until mid-2000 (Guedes and Picanço 2012). In 2006, however, *T. absoluta* was first observed in eastern Spain (Desneux et al. 2010, 2011). Currently, it is found in almost all European countries, North Africa and the Middle East. The larvae of *T. absoluta* affect the vertical growth of the tomato plant due to the consumption of

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leaf tissue, reducing the productivity of the tomato crop (Desneuxet al. 2010). Chemical control, usually with broad-spectrum insecticides, has been the most widely used method for reducing the losses caused by *T. absoluta*. The spraying of insecticides occurs excessively and intensely every growing season in Brazil (Guedes and Siqueira 2012).

The excessive and intense use of insecticides in tomato crops has selected populations of *T. absoluta* in Brazil that were resistant to several insecticides, and cases of resistance have tended to increase after the introduction of this pest to the countries of Europe, Africa, and the Middle East, particularly due to the initial pressure of population on the crops. Resistance of *T. absoluta* to pyrethroids, organophosphates, abamectin, cartap, and chitin synthesis inhibitors has been found in South America (Siqueira et al. 2000a, b; Salazar and Araya 2001; Siqueira et al. 2001; Lietti et al. 2005; Silva et al. 2011). These studies, aimed at the detection of resistance and the preliminary determination of the mechanism of insecticide resistance, were conducted after several years of insecticide use for the control of *T. absoluta*.

The status of the resistance of *T. absoluta* to insecticides has been neglected, and the association of this resistance with failures of pest control in tomato fields has remained obscure until recently (Silva et al. 2011; Gontijo et al. 2013). However, these studies have shown that *T. absoluta* can develop resistance to many classes of insecticides if resistance management strategies are not properly established. Accordingly, high risks are involved in the use of insecticides based on spinosyns, one of the few classes of insecticides still effective against *T. absoluta* in Brazil.

Spinosad and spinetoram belong to the spinosyn group of insecticides (Group 5, IRAC mode of action classification), a family naturally derived from macrocyclic lactones (Salgado and Sparks 2005). Spinetoram, a mixture of two synthetically modified spinosyns (Spinosyn J and L), was recently introduced as a new control agent for insects, with a greater speed and action potential than spinosad (Spinosyn A and D) (Sparks et al. 2008). Spinosad primarily activates the nicotinic receptors of acetylcholine (Salgado and Saar 2004) and has been shown to be metabolized by cytochrome P450-dependent monooxygenases (Reyes et al. 2012; Geng et al. 2013). Therefore, these factors may lead to cross-resistance among spinosyns that will limit the lifetimes of new spinosyns. The knowledge of the patterns of cross-resistance and resistance mechanisms allows the development of an accurate resistance management program that may prevent or minimize the development of resistance in insect populations (Scott 1989).

The establishment of the susceptibility baseline of *T. absoluta* for spinosyns is essential to delay the evolution of pest resistance to insecticides (Siegfried et al. 2005). This information serves as a reference for susceptibility monitoring. Therefore, management programs addressing resistance to more efficient insecticides can be developed and implemented. Spinosad was registered in Brazil in the early 2000s to control a wide variety of pests, including *T. absoluta* (MAPA 2013). No instances of resistance to spinosad have subsequently been reported for *T. absoluta*, although a recent study conducted in Chile reported the survival of individuals exposed to a diagnostic concentration of 1 mg a.i./L, suggesting the development of resistance to spinosad (Reyes et al. 2012). However, this concentration may not be sufficient to discriminate individuals resistant to spinosad, particularly because there is no characterization of spinosad resistance in *T. absoluta*. In the current study, a survey of resistance in *T. absoluta* to spinosad and cross-resistance to spinetoram was conducted; moreover, the possible role of enhanced detoxification as a mechanism contributing to the survival of *T. absoluta* populations exposed to spinosyns was assessed.

Materials and methods

Insects

Leaves, stems, and fruit infested with larvae of *T. absoluta* were collected in commercial and experimental fields during 2010 and 2011 in four regions of Brazil (Table 1) and sent to the Laboratory of Insect-Toxic Interactions (LITI) at the Federal Rural University of Pernambuco (UFRPE, Recife—PE). After delivery, the material was immediately transferred to wooden cages lined with anti-

Table 1 Collection sites of *Tuta absoluta* populations in Brazilian tomato crops

Population	Initials	Geographic coordinates	Collection date
Guaraciaba do Norte—CE	GBN	4°10′01″S, 40°44′51″W	Fev/2010
Venda Nova do Imigrante—ES	VDN	20°20′23″S, 41°08′05″W	Ago/2011
Tianguá—CE	TNG	3°43′56″S, 40°59′30″W	Fev/2010
Paulínia—SP	PLN	22°45′40″S, 47°09′15″W	Ago/2010
Pelotas—RS	PLT	31°46′19″S, 52°20′33″W	Nov/2011
Sumaré—SP	SMR	22°49′19″S, 47°16′01″W	Set/2011
Iraquara—BA	IRQ	12°14′55″S, 41°37′10″W	Nov/2011
Anápolis—GO	ANP	16°29′46″S, 49°25′35″W	Dez/2011

aphid screening. Each population was maintained in four cages, three for the breeding of larvae (45 × 45 × 45 cm) and one for adults (30 × 30 × 30 cm). The populations of *T. absoluta* were reared in the laboratory at 25 ± 1 °C at a relative humidity of 65 ± 5 % and under a 12 h photophase.

Insecticides

The insecticides used in the experiments were spinosad (Tracer® 480 g a.i./L concentrated suspension, Dow AgroSciences Industrial Ltda, Franco da Rocha, SP, Brazil) and spinetoram (250 g a.i./L dispersible granules, Dow AgroSciences, Franco da Rocha, SP, Brazil). The latter insecticide is still in the process of registration for use in the control of *T. absoluta*.

Bioassays

The toxicological bioassays were based on the method developed by Silva et al. (2011) with a few modifications. The bioassays were conducted according to a completely randomized design with two replicates per concentration, and the entire bioassay was repeated once. First, preliminary testing was conducted to determine the "all or nothing" response to establish a concentration gradient for estimating the concentration–response curves. The concentrations for spinosad and spinetoram ranged from 7.81 to 180.00 µg a.i./L and 0.02 to 1.50 mg a.i./L, respectively. The insecticide solutions were diluted with water + 0.01 % Triton X-100, and the control treatment used only distilled water + 0.01 % Triton X-100. The tomato leaflets were immersed for 30 s in each insecticide solution, dried for 2 h, and then placed in Petri dishes (8 cm diameter × 1.5 cm height) containing filter paper moistened with 500 µL of distilled water. Ten second-instar larvae were transferred to each Petri dish and then placed in a growth chamber at 25 ± 0.5 °C temperature, 65 ± 5 % relative humidity and 12:12 (L:D) photoperiod. Larval mortality was assessed after 48 h exposure. Mortality evaluations were performed with the aid of a light source and magnifying glass (Olympus SZ61, Olympus®, Center Valley, PA, USA). The mortality criterion was based on the movement of larvae following prodding with a soft brush (Tabashnik et al. 1990). Larvae were carefully removed from the galleries of tomato leaflets, and those larvae that could not move at least one body length were considered dead.

Enzyme activity

Third-instar larvae of *T. absoluta* were collected for the analysis of detoxifying enzymes. Three samples were obtained for each population, and each sample contained ten third-instar larvae of *T. absoluta*. The samples for the esterase and glutathione *S*-transferase assays were homogenized in 200 µL of sodium phosphate buffer (0.02 M, pH 7.2) and sodium phosphate buffer (0.1 M, pH 7.5), respectively, using a Potter-Elvehjem homogenizer (Sigma-Aldrich, St Louis, MO,USA). Homogenates were centrifuged (Eppendorf 5810R) at 15,000×g_{max} and 4 °C for 15 min. The supernatants were collected and stored at −80 °C. For cytochrome P450-dependent monooxygenase assays, the samples were processed and homogenized in phosphate buffer (0.1 M, pH 7.8), added to 1 mM EDTA, 1 mM DTT, 1 mM PTU, 1 mM PMSF, and 20 % glycerol (Wright et al. 2000). The homogenate was first centrifuged at 10,000×g_{max} for 20 min at 4 °C. The supernatant was further ultracentrifuged at 100,000×g_{max} for 1 h at 4 °C. The microsomal fraction was processed in 500 µL sodium phosphate resuspension buffer (0.1 M, pH 7.8) + 20 % glycerol, aliquoted, and preserved at −80 °C until use. Total protein was quantified by the bicinchoninic acid method using bovine serum albumin (BSA) as the standard (Smith et al. 1985).

The methodology described by van Asperen (1962) and adapted to microplate technique was used to assess esterase activity. α-naphthyl and β-naphthyl acetates were used as substrates. Standard curves were prepared with α-naphthol and β-naphthol for activity determination. The specific activity of esterase was calculated in nmols naphthol × min^{-1} × mg of $protein^{-1}$.

The conjugation activity of reduced glutathione to the substrate CDNB (1-chloro-2,4-dinitrobenzene) was determined according to Habig et al. (1974). An extinction coefficient of 9.6 mM^{-1} × cm^{-1} was used to determine the amount of GS-DNB conjugate using the slope of the curve (absorbance/min) to obtain the unit of glutathione *S*-transferase activity.

The activity of cytochrome P450-dependent monooxygenase (*O*-dealkylation) was measured through oxidation of *p*–nitroanisole (O_2N–C_6H_4–O–CH_3) to *p*-nitrophenol by the Netter and Seidel (1964) method. The activity (nmols *p*-nitrophenol × min^{-1} × mg of $protein^{-1}$) of cytochrome P450-dependent monooxygenase per sample was determined from the regression line of the *p*-nitrophenol standard curve.

Data analysis

Mortality data obtained from concentration–response bioassays were corrected with the mortality observed in the control treatment (Abbott 1925) and analyzed by probit analysis at $P > 0.05$ (Finney 1971) using the program Polo-Plus® (LeOra-Software 2005). The resistance ratios were calculated with the lethal ratio test and were considered significant if 95 % confidence interval (CI) did not include the value 1.0. The population from Pelotas-RS

(PLT), with the lowest LC_{50} for spinosyns, was used as a reference to perform comparisons with other populations. Data on the activity of esterases, glutathione *S*-transferases, and cytochrome P450-dependent monooxygenases were analyzed with SAS (SAS Institute 2001). The assumptions of normality and homoscedasticity were tested using PROC UNIVARIATE and PROC GLM (SAS Institute 2001). The activity data were subjected to an analysis of variance (ANOVA) using PROC ANOVA and the Tukey's test (HSD) at $P < 0.05$ for grouping the means (SAS Institute 2001). A Pearson correlation analysis at $P < 0.05$ was performed to investigate the relationship between the enzymatic activities and the average susceptibilities (LC_{50}) of populations to each insecticide using PROC CORR, and regression analysis of the spinosad and spinetoram LC_{50}s was performed with PROC REG (SAS Institute 2001).

Results

Susceptibility

The *T. absoluta* population of Pelotas-RS (PLT) had the lowest LC_{50} for spinosad (0.007 mg a.i./L) and spinetoram (0.047 mg a.i./L) (Tables 2, 3). The remaining populations of *T. absoluta* showed LC_{50} values ranging from 0.060 mg a.i./L (Paulínia-SP) (PLN) to 0.63 mg a.i./L (Sumaré-SP) (SMR) for spinosad. Consequently, the resistance ratios (RR_{50}) for spinosad ranged gradually among populations from 8.9 to 93.8 times. The LC_{99} values for spinosad ranged from 0.23 mg a.i./L (Pelotas-RS) (PLT) to 11.56 mg a.i./L (Venda Nova do Imigrante—ES) (VDN). Consequently, the resistance ratios (RR_{99}) among populations for spinosad ranged gradually from 2.6 (Paulínia-SP) to 51.5 times (Venda Nova do Imigrante—ES). The *T. absoluta* populations of Sumaré-SP (SMR) and Venda Nova do Imigrante—ES (VDN) showed the highest and the lowest regression slope for spinosad, respectively, indicating the highest and the lowest homogeneity of the populations, respectively, for spinosad (Table 2).

The LC_{50} estimates for spinetoram ranged from 0.047 mg a.i./L (Pelotas—RS) to 0.31 mg a.i./L (Sumaré—SP), whereas the LC_{99} values ranged from 0.56 mg a.i./L (Pelotas—RS) to 6.71 mg a.i./L (Iraquara—BA). The corresponding resistance ratios (RR_{50}) ranged from 1.02 (Paulínia—SP) to 6.51 times (Sumaré—SP), whereas the RR_{99} values ranged from 1.2 (Tianguá—CE) to 12.1 times (Iraquara—BA) (Table 3). The population of Paulínia-SP (PLN) showed the curve with the greatest slope (2.98) and, thus, the most homogeneous response among the populations. In contrast, the population of Tianguá—CE showed the curve with the lowest slope (1.28), suggesting a more heterogeneous response of the individuals to spinetoram (Table 3). The pooled data provide the mean response of the eight populations of *T. absoluta*. The curve slope (1.52) and resistance ratio (2.01) were both low in the pooled data (Table 3), suggesting the relative low variation of the populations to spinetoram.

Enzyme activity

Esterase activity differed significantly among populations of *T. absoluta* according to the assays using α- and β-naphthyl acetate substrates (Fig. 1a). For α-naphthyl

Table 2 Relative toxicity of spinosad to 2nd-instar larvae of *Tuta absoluta*

Population	n[a]	DF[b]	Slope ± SE[c]	LC_{50} (CI95 %)[d]	LC_{99} (CI95 %)[d]	χ^{2}[e]	RR_{50} (CI95 %)[f]	RR_{99} (CI95 %)[f]
PLT	323	6	1.52 ± 0.19	0.007 (0.005–0.009)	0.23 (0.12–0.62)	1.34	1.0 (0.8–1.2)	1.0 (0.3–3.1)
PLN	405	5	2.33 ± 0.26	0.060 (0.048–0.073)	0.60 (0.39–1.12)	3.72	8.9 (8.7–9.2)*	2.6 (1.3–4.0)*
TNG	257	5	1.61 ± 0.23	0.129 (0.087–0.176)	3.63 (1.85–11.37)	3.89	19.3 (18.9–19.8)*	16.1 (14.0–18.1)*
ANP	279	5	2.14 ± 0.21	0.145 (0.118–0.178)	1.78 (1.14–3.39)	4.41	21.7 (21.3–22.1)*	7.9 (6.5–9.3)*
GBN	265	5	1.85 ± 0.25	0.168 (0.118–0.224)	3.03 (1.72–7.68)	1.25	25.2 (24.7–25.7)*	13.5 (11.5–15.4)*
VDN	317	6	1.47 ± 0.16	0.305 (0.214–0.409)	11.56 (6.36–28.38)	2.92	45.7 (45.1–46.3)*	51.5 (49.6–53.5)*
IRQ	264	5	1.85 ± 0.23	0.410 (0.301–0.525)	7.37 (4.36–17.05)	1.51	60.4 (60.6–62.1)*	32.8 (30.7–34.9)*
SMR	276	5	2.44 ± 0.29	0.626 (0.490–0.776)	5.60 (3.71–10.56)	1.58	93.8 (93.0–94.6)*	24.9 (22.9–26.9)*

[a] Total number of insects

[b] Degree of Freedom

[c] Standard Error

[d] Milligrams of active ingredient per Liter of water

[e] Chi square ($P > 0.05$)

[f] Resistance ratio: ratio of the LC_{50} and LC_{99} estimates between the resistant populations and the most susceptible population, determined through the Robertson and Preisler (1992) method and the ratios confidential intervals at 95 %

* Resistance ratio significant because the confidence interval did not bracket the value 1.0

Table 3 Relative toxicity of spinetoram to 2nd-instar larvae of *Tuta absoluta*

Population	n^a	DF[b]	Slope ± SE[c]	LC_{50} (CI95 %)[d]	LC_{99} (CI95 %)[d]	χ^{2e}	RR_{50} (CI95 %)[f]	RR_{99} (CI95 %)[f]
PLT	290	6	2.18 ± 0.40	0.047 (0.030–0.063)	0.554 (0.317–1.694)	4.04	1.00 (0.32–1.68)	1.00 (0.34–2.90)
PLN	317	6	2.98 ± 0.21	0.048 (0.036–0.061)	0.724 (0.454–1.440)	2.41	1.02 (0.46–1.58)	1.30 (0.33–2.28)
TNG	283	5	1.28 ± 0.18	0.077 (0.060–0.096)	0.698 (0.462–1.318)	2.54	1.62 (1.10–2.15)*	1.25 (0.23–2.28)
GBN	283	5	1.35 ± 0.16	0.085 (0.065–0.106)	0.881 (0.571–1.717)	3.13	1.78 (0.71–2.85)	1.59 (0.74–3.10)
ANP	281	5	2.27 ± 0.27	0.103 (0.079–0.131)	1.089 (0.703–2.129)	2.85	2.13 (1.65–2.62)*	1.96 (0.87–3.05)
VDN	274	5	2.28 ± 0.27	0.117 (0.090–0.147)	1.223 (0.795–2.354)	2.19	2.46 (2.00–2.93)*	2.20 (1.10–3.30)*
IRQ	276	5	1.72 ± 0.18	0.298 (0.234–0.379)	6.712 (3.821–15.323)	2.29	6.28 (6.01–6.54)*	12.11 (10.84–13.38)*
SMR	280	5	1.83 ± 0.18	0.308 (0.246–0.389)	5.794 (3.409–12.464)	0.89	6.51 (6.23–6.78)*	10.45 (9.23–11.69)*
Pooled	2,199	23	1.52 ± 0.07	0.097 (0.061–0.139)	3.323 (1.643–11.169)	196.25	2.01 (1.53–2.50)*	6.01 (5.10–6.91)*

[a] Total number of insects

[b] Degree of Freedom

[c] Standard Error

[d] Milligrams of active ingredient per Liter of water

[e] Chi square ($P > 0.05$)

[f] Resistance ratio: ratio of the LC_{50} and LC_{99} estimates between the resistant populations and the most susceptible population, determined through the Robertson and Preisler (1992) method and the ratios confidential intervals at 95 %

* Resistance ratio significant because the confidence interval did not bracket the value 1.0

acetate, the specific activity values ranged from 0.79 (Pelotas—RS) to 2.11 µmol α-naphthol/min/mg of protein (Iraquara—BA). For substrate β-naphthyl acetate, the specific activity values ranged from 0.79 (Paulínia—SP) to 1.58 µmol β-naphthol/min/mg of protein (Sumaré-SP). The *T. absoluta* populations showed higher enzymatic activities against β-naphthyl acetate than against α-naphthyl acetate. Conjugation by glutathione *S*-transferases showed statistically significant differences among certain populations of *T. absoluta* (Fig. 1b), with specific activity values ranging from 1.70 nmol of GS-DNB/min/mg of protein (Venda Nova do Imigrante—ES) to 4.26 nmol of GS-DNB/min/mg of protein (Iraquara–BA). However, the activities found were similar in most populations (Fig. 1b). The activity of cytochrome P450-dependent monooxygenases showed statistically significant differences among *T. absoluta* populations (Fig. 1c), with specific activity values ranging from 2.94 (Guaraciaba do Norte—CE) to 18.73 nmol 4-nitrophenol/min/mg of protein (Venda Nova do Imigrante—ES). The activity of these enzymes showed a high level of variability among the populations (Fig. 1c).

Correlations

A strong association ($r^2 = 0.87$, $P < 0.001$, $n = 8$) between the LC_{50}s of spinosad and the LC_{50}s of spinetoram was observed for the *T. absoluta* populations (Fig. 2). No significant correlations were found for spinosad ($r = 0.19$, $P = 0.103$, $n = 72$) or spinetoram ($r = 0.22$, $P = 0.052$, $n = 72$) among the logarithms of the LC_{50} values for *T. absoluta* populations or their esterase activities toward the α-naphthyl acetate substrate. However, significant correlations were observed for spinosad ($r = 0.50$, $P < 0.001$, $n = 72$) and spinetoram ($r = 0.43$, $P < 0.001$, $n = 72$) for the esterase activities of *T. absoluta* populations against the β-naphthyl substrate. Very weak but significant correlations were also observed between the LC_{50} logarithms for spinetoram and glutathione *S*-transferase ($r = 0.26$, $P = 0.025$, $n = 72$) as well as between the cytochrome P450-dependent monooxygenase ($r = 0.25$, $P = 0.034$, $n = 72$) activities in the *T. absoluta* populations. However, no statistically significant correlation was observed for the toxicity of spinosad and glutathione *S*-transferase ($r = 0.05$, $P = 0.63$, $n = 72$) or for the cytochrome P450-dependent monooxygenase ($r = 0.13$, $P = 0.282$, $n = 72$) activities in the *T. absoluta* populations.

Discussion

The overuse of insecticides has increasingly selected for insecticide resistance, leading to the rapid replacement of existing agents by products with new modes of action, such as the spinosad registered in early 2000 to control *T. absoluta* in Brazil. However, with the loss of efficacy of the previously used molecules (Silva et al. 2011), the use of spinosad has increased in recent years, thereby compromising its efficacy. A moderate to high resistance of *T. absoluta* to spinosad was detected after little more than a decade of spinosad use (this study). Relatively high levels of resistance (>50) to spinosad suggest a continuing use of this insecticide in the control of *T. absoluta* or other tomato

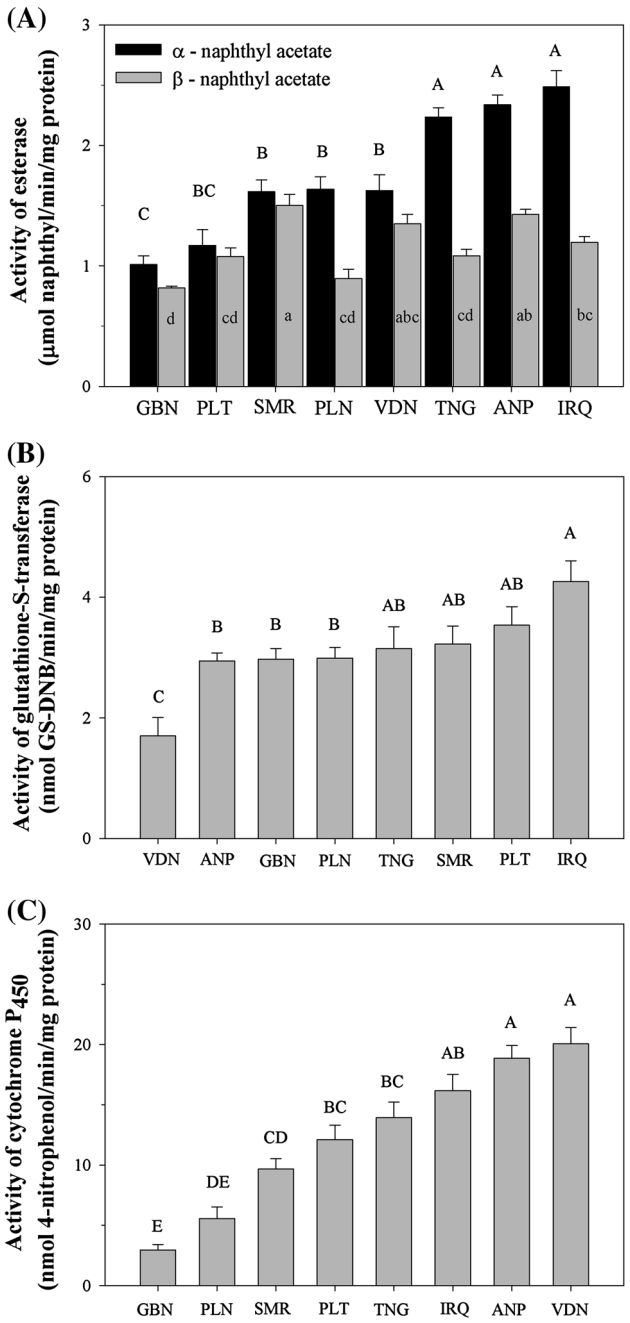

Fig. 1 Esterase activity (*black bar* α-naphtil acetate and *gray bar* β-naphthyl acetate) (**a**), glutathione *S*-transferase activity (**b**), and cytochrome P450-dependent monooxygenase activity (**c**) of eight populations of *Tuta absoluta*. Means followed by the same letter in the bar of the same color do not differ by Tukey test ($P < 0.05$)

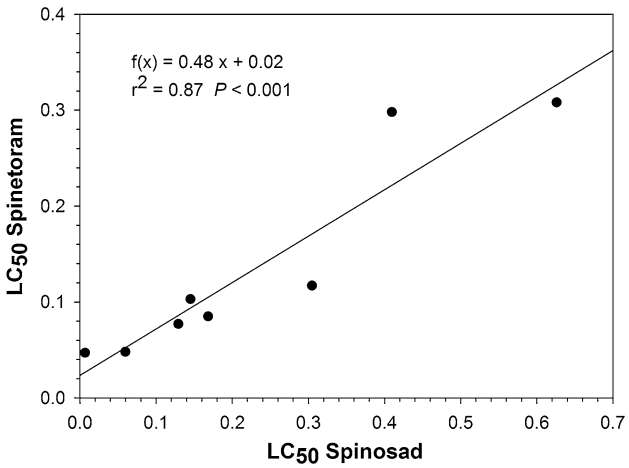

Fig. 2 Regression line between susceptibility of *Tuta absoluta* populations to spinosad and spinetoram

pests. These levels of resistance have not yet produced control failures in the field (spinosad field rate 60 mg/L), but monitoring of populations over the short term to avoid possible failures to control *T. absoluta* by the insecticide is necessary. Resistance to spinosad has previously been reported in the laboratory and the field a few years after the introduction of spinosad to control Lepidoptera (Moulton et al. 2000; Zhao et al. 2002; Shono and Scott 2003). Apparently, the resistance of *T. absoluta* to spinosad in Brazil is a recent event (<3 years) because a low level of variation (<5 times) of population responses to spinosad has recently been observed (Silva et al. 2011), suggesting that resistance to spinosad may develop slowly in the field. Populations of *T. absoluta* showed low variation in the response to spinetoram, but the expected strong correlation was found between the susceptibility of *T. absoluta* to spinetoram and that to spinosad. Such a pattern of cross-resistance between spinosyns has been associated with target site alteration (Salgado and Sparks 2005). Other studies have also observed cross-resistance between spinosyns, e.g., in *Plutella xylostella* (Linneaus), *Drosophila melanogaster* (Meigen), and *Choristoneura rosaceana* (Harris), under exposure to spinosad (Sial and Brunner 2010; Watson et al. 2010; Sparks et al. 2012).

The assessment of the mechanisms of resistance may allow the prediction of patterns of cross- and multiple resistances. Although the mechanisms of resistance to spinosyns for other pests have been suggested and/or elucidated (Sparks et al. 2012), they are yet to be detailed for *T. absoluta*. The moderate correlation of *T. absoluta* esterases (particularly β-esterases) with the variation in the susceptibility of the populations to spinosyns suggests that these esterases contribute to the resistance of *T. absoluta* populations to spinosyns to a modest degree. Conversely, glutathione *S*-transferases and cytochrome P450-dependent monooxygenases appear not to be involved with the susceptibility of *T. absoluta* populations to spinosyns. In a recent study, Reyes et al. (2012) found no association of *T. absoluta* susceptibility with detoxifying enzymes. There is strong evidence that insecticide resistance may arise as a result of increased metabolic detoxification (Scott 1989), but most cases of resistance to spinosyns have been

associated with altered target sites. The more homogeneous response of the populations to spinetoram supports this hypothesis and may be related to the structural differences between the spinosyns. Spinetoram bears an ethyl group ($–C_2H_5$) in its major and minor components (spinosyn J and L), whereas spinosad bears a methyl group ($–CH_3$) (spinosyn A and D) (Sparks et al. 2012), and the improved activity of spinetoram compared with spinosad may be associated with enhanced activity at the nACh receptors (Crouse et al. 2012). Additionally, the 3′-*O*-ethyl analog of spinosyn J was found to be more potent against the nicotinic receptor than spinosyn A (Salgado and Sparks 2005).

In most reported cases, spinosad resistance has been associated with changes in nicotinic acetylcholine receptors (nAChR) (Sparks et al. 2012; Puinean et al. 2013). More specifically, studies of the resistance of *Drosophila melanogaster* to spinosad attributed the resistance to a change in the α6 subunit (Dα6) domain of the acetylcholine receptors (Perry et al. 2007; Watson et al. 2010). Despite the modest association of *T. absoluta* β-esterases with resistance observed in this study, target site alteration (e.g., an altered *T. absoluta* Dα6) may also play a role in the resistance of this pest to spinosyns. If such a mechanism is demonstrated, cross-resistance to other insecticides may not occur, and it may be feasible to counteract resistance to spinosyns. Physiological studies have suggested that the action of spinosyns involves a new interaction at nicotinic acetylcholine receptors (Salgado and Saar 2004). This mechanism has decreased the risk of cross-resistance, even with other nAChR-binding insecticides.

The levels of resistance to spinosad observed here are still lower than the dose recommended by the manufacturer. However, we suggest that it is urgent to implement strategies for managing the resistance of *T. absoluta* to spinosyns before control failures occur in the field. Therefore, the approach to insecticide resistance used by the World Health Organization (WHO) is relatively compatible with the context of this susceptibility survey of *T. absoluta* populations to spinosyns in Brazil (Guedes and Siqueira 2012; Gontijo et al. 2013). Monitoring the susceptibility of *T. absoluta* populations to spinosyns in various geographical areas should be established as soon as possible to mitigate the increase in resistance before control failures occur in the field. Care must be taken to prevent potential cross-resistance problems that would decrease the effectiveness of the insecticides, particularly in the case of products that may show relatively low lethal toxicity to non-target insects, as observed with spinosad (Torres et al. 2002; Desneux et al. 2007; Arnó and Gabarra 2011; Campos et al. 2011; Biondi et al. 2012). Shifts in the susceptibility of *T. absoluta* populations to spinosyns may be rapidly detected based on a diagnostic concentration. If a diagnostic concentration is not available, insecticide rotation must be suggested to maintain the effectiveness of spinosyns in the field, particularly with novel products such as diamides and pyrroles that are used to reverse the susceptibility of *T. absoluta* to spinosyns, extending their shelf life. In addition, the selection of *T. absoluta* for resistance to spinosad and the characterization of this resistance may clarify the involvement of detoxifying enzymes and the genetic basis of resistance to spinosyns. This information will assist in detection, monitoring, modeling, and risk assessment (Balasubramani et al. 2008), refining the programs used for the management of resistance to spinosyns.

Author contributions

MRC, TBMS, WMS, and JES: designed research and conducted experiments; MRC, WMS, and HAAS: conceived and designed research, analyzed data, and wrote manuscript. All authors read and approved the manuscript.

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