

Quantification of enzymes related to insecticide resistance in *Bemisia tabaci* from the state of Sinaloa

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Abstract

The whitefly *Bemisia tabaci* (Gennadius) is one of the most invasive pests and causes direct damage to crop by feeding on the sap and indirect damage by being a vector of more than 100 phytopathogenic viruses. At present its control is based on the use of chemical insecticides, because the populations have been constantly subjected to a high selection pressure. An alternative that contributes to understanding the origin of resistance in a population are biochemical tests, which show the parameter of the detoxifying enzyme present. The objective of the present work was the quantification of these enzymes in *B. tabaci* in the three main solanaceae producing areas of the north (Guasave, Sinaloa de Leyva, Mochis), center (Culiacán, Navolato, Elota) and south (Concordia, Rosario, Esquinapa) of the state of Sinaloa. Whitefly adults were collected at these sites and the enzymatic levels of α and β esterases, glutathione S-transferases, acetylcholinesterases and oxidases were determined, additionally a susceptible laboratory line was used as a reference. The enzymes with the highest presence were α -esterases, β -esterases and oxidases, followed by glutathione S-transferases and acetylcholinesterase. Therefore, it is concluded that resistance to insecticides in *B. tabaci* in the state of Sinaloa is due to the high content of α - β -esterases and oxidases, while acetylcholinesterase is not a relevant mechanism in the populations of this producing region.

Keywords: *Bemisia tabaci*, insecticides, resistance.

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Introduction

The state of Sinaloa is the main national producer of solanaceae such as tomato, pepper and eggplant, whose combined production value exceeds 18 billion pesos (SIAP, 2019). The trade in these vegetables is highly dependent on the United States market since Sinaloa serves as the main producer and exporter during the winter to the United States of America (FAS-USDA, 2018).

The whitefly *Bemisia tabaci* is one of the most destructive and invasive pests in the world (GISD, 2020), it has a host range that amounts to more than 1 000 cultivated and wild plants (Oliveira *et al.*, 2001; Simmons *et al.*, 2008; Abd-Rabou and Simmons, 2010) and causes direct damage to crops by feeding on plant sap and excreting sugary substances on leaves and fruits that damage their quality and promote the development of fungi such as *Fumago* spp., likewise, causes indirect damage by being a vector of more than 100 phytopathogenic viruses (Horowitz and Ishaaya, 2014).

Excessive populations of *B. tabaci* can reduce crop yields by up to 50% (Raveesh, 2018), therefore, continuous applications of chemical insecticides are carried out for their control, which caused the development of resistance (Palumbo *et al.*, 2001; Ahmad *et al.*, 2010).

World registries on *B. tabaci* mention resistance to 64 active ingredients of toxicological groups such as avermectins, neonicotinoids, buprofezin, organophosphates, pyrethroids, carbamates, phenylpyrazoles, chlorinated cyclodiene, butenolids, pyridine azomethine, acaricides and insecticides METI and pyriproxyfen (APRD, 2020).

This is due to a diverse set of resistance mechanisms, which were corroborated in countries that lead the world production of Solanaceae, China (Wang *et al.*, 2018), India (Naveen *et al.*, 2017), Turkey (Erdogan *et al.*, 2008), Egypt (Farghaley *et al.*, 2016) and the United States of America (Longhurst *et al.*, 2014). In Mexico, Aguilar-Medel *et al.* (2007) evaluated the susceptibility of populations from the states of Baja California and Sinaloa to the insecticides acetamiprid, cypermethrin, imidacloprid, pymetrozine and thiamethoxam, being the population from Sinaloa the most resistant, in another study Gutiérrez-Olivares (2007) reports tolerance in populations of San Luis Potosi to imidacloprid and Servin-Villegas *et al.* (2006) to thiamethoxam and endosulfan in Baja California.

Resistance to insecticides involves mutations in the sites of action, on gene expression, less cuticular penetration, resistance to knockdown, greater storage and excretion, in addition to the production of detoxifying enzymes (Vais *et al.*, 1997; Ahmad *et al.*, 2006; Bass and Field, 2011).

The latter are the main mechanism of resistance, particularly the production of esterases, oxidases and glutathione S-transferases (GST) (Flores *et al.*, 2006). The objective of the present work was the quantification of detoxifying enzymes in *B. tabaci* in the three main solanaceae producing areas of the state of Sinaloa.

Materials and methods

To carry out this study, three populations of *B. tabaci* were collected in the producing areas of the north (Guasave, Sinaloa de Leyva, Mochis), center (Culiacán, Navolato, Elota) and south (Concordia, Rosario, Esquinapa) of Solanaceae crops in the open field in the state of Sinaloa, Mexico, in the 2018-2019 agricultural cycle.

In addition, a susceptible line maintained in the laboratory was included, which has been free of selection pressure for a period of more than two years. The captured whitefly adults were collected in plastic containers for their conservation in 70% alcohol and at a temperature of 4 °C in refrigeration. The analyzes were carried out in the Toxicology Laboratory of the Department of Agricultural Parasitology of the Autonomous Agrarian University Antonio Narro.

Protein determination

The protein source was obtained from eight samples, with three repetitions, of 100, 150, 200, 250, 300, 350 and 400 individuals of *B. tabaci*, using the methodology described by Bradford (1976) modified by Brogdon (1984); Brogdon and Barber (1987). In 2 ml Eppendorf tubes, 1 000 µl of buffer solution (KPO₄ 0.05 M, pH 7.2) and the different densities of the insect mentioned above were added, to later crush them and make up to 1 ml.

In a 96-well microplate, 20 µl of homogenate, 80 µl of buffer solution and 200 µl of diluted dye (4:1 dye:water) of Coomassie Brilliant Blue (Bio-Rad Kit II) were placed in triplicate for each repetition. The absorbance readings were taken with a 630 nm filter. The values of µg ml⁻¹ of protein comprised in the range of 80 to 120 µg (homogenate carried out with 300 individuals) were calculated.

Determination of enzyme levels

The enzymatic levels of α-esterases and β-esterases were determined with the methodology of Brogdon and Dickinson (1983). In a transparent 96-well microplate, 100 µl of the homogenate was added with 100 µl of the substrate α-naphthyl acetate for α-esterases and β-naphthyl acetate for the case of β-esterases, it was incubated for 10 min and 100 µl of Fast-Blue dye were added to incubate again for 2 min, the absorbances were taken with a 540 nm filter.

For the determination of glutathione S-transferases, the methodology described by Brogdon and Barber (1990) was used for this, in a microplate 100 µl of homogenate, 100 µl of reduced Glutathione as substrate and 100 µl of 1-chloro-2,4'-dinitrobenzene (CDNB) as a dye. The absorbances were recorded with a 340 nm filter, their readings were at time zero (T₀) and time five (T₅), the difference between the two readings (T₅-T₀), was considered for the result for the analysis.

Acetylcholinesterase levels were determined with the Brogdon (1988) methodology, applying 100 µl of the homogenate to a microplate, 100 µl acetylcholine-iodysed 3 mm as a substrate and 100 µl of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) as a dye. The absorbances were recorded with a 414 nm filter and the readings were taken at time zero (T₀) and ten (T₁₀), the difference considered as a result for the analysis.

Finally, the determination of oxidase levels was obtained with the method of Brogdon *et al.* (1997), adding 100 μl of the homogenate, 200 μl of 3,3',5,5'- Tetramethyl-Benzidine Dihydrochloride (TBMZ) as substrate and 25 μl of 3% H_2O_2 as dye, were incubated for 5 min and they took the readings with a 620 nm filter.

Analysis of results

With the absorbance readings obtained, a frequency distribution was made and a resistance threshold was established based on the highest value (absorbance) of the susceptible line. The percentage of resistance was obtained with the number of means that exceeded the resistance threshold and were classified according to Montella *et al.* (2007) with minor modifications such as: 'unaltered' (0-5%), 'incipiently altered' (6-30%), 'moderately altered' (31-50%), 'altered' (51-75%) and 'very altered' (> 76%). To know the variation in the enzymatic activity between populations, an Anova and Tukey's test ($p= 0.05$) were carried out with the statistical program R version 3.3.1.

Results and discussion

Protein determination

The density of 300 adults reached the required levels of protein (80 to 120 μg), most of their repetitions were located near the threshold, while in the densities of 200 and 400 individuals the absorbance values were distributed in different protein contents. (Figure 1). Bradford (1976) mentions that values outside the range are not reliable for the quantification of enzymatic in tissues; for their part, Dary *et al.* (1990) report that there is a close relationship between sample size and amount of protein, so values outside this range may present differences in the results obtained.

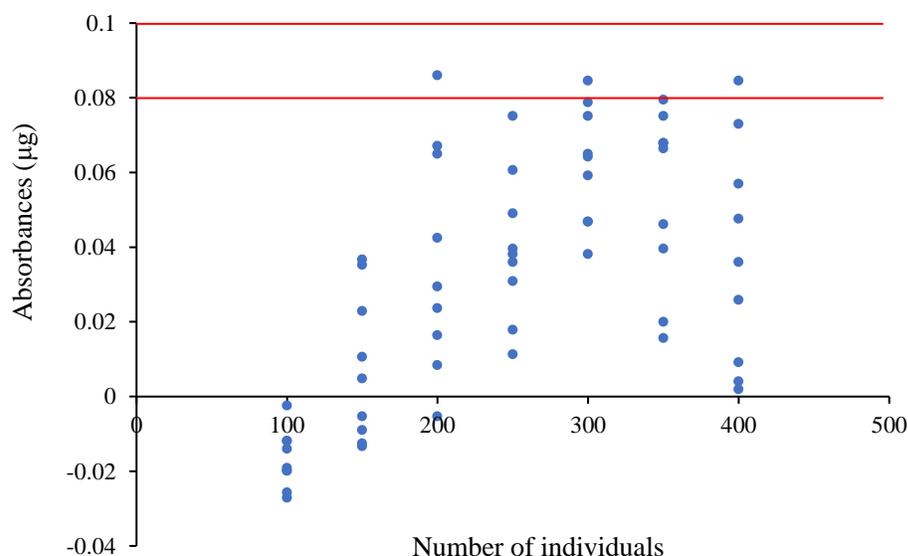


Figure 1. Protein absorbances in the different densities of *B. tabaci* homogenate in buffer solution (KPO_4 0.05 M, pH 7.2).

Enzyme levels

In relation to α -esterases and β -esterases, the three field populations behaved homogeneously (Table 1) with means of 3.453, 3.489 and 3.513, for the northern, central and southern populations, respectively; however, they were different from the susceptible line with the lowest mean of 1.488.

Table 1. Means and absorbances of the enzymes α -esterases, β -esterases, from the different solanaceae producing areas in the state of Sinaloa.

Zone	α -esterases		β -esterases	
	Mean \pm SD ¹		Mean \pm SD ¹	
LS ²	1.488 \pm 0.027	b	1.592 \pm 0.011	b
North	3.453 \pm 0.049	a	3.539 \pm 0.09	a
Center	3.489 \pm 0.093	a	3.539 \pm 0.119	a
South	3.513 \pm 0.093	a	3.496 \pm 0.063	a

Means with different letters present a significant difference ($p=0.05$). ¹= standard deviation; ²= susceptible line.

The α -esterases and β -esterases can occur separately or together in insects (Bisset, 2002), depending on the toxic interaction, detoxification can be carried out by two mechanisms: catalytic hydrolysis that are not inhibited by organophosphates and are not catalytic as it is inhibited by organophosphates (Costa, 2006).

Previous studies reported that these enzymes confer resistance to insecticides such as pyrethroids (Yang *et al.*, 2001; Flores *et al.*, 2006; Landeros *et al.*, 2010), organochlorines (Bisset *et al.*, 2001), neonicotinoids (Dávila-Medina, 2012), organophosphates and carbamates (Cerna *et al.*, 2013), the high activity of esterases in populations is directly related to resistance to these insecticides, coinciding with previous studies in whiteflies (Byrne and Devonshire, 1993; Kang *et al.*, 2006; Roditakis *et al.*, 2006; Liang *et al.*, 2007; Alon *et al.*, 2008). The oxidases were the enzymes with the highest values in the central zone with a mean of 1.216, followed by the north zone with 1.073 and south with 1.068 (Table 2), the means of the three zones were significantly different with respect to the susceptible line (p -value 0.002), which presented the lowest absorbance value with 0.971.

Table 2. Means and absorbances of the oxidase enzymes and glutathione S-transferases, of the different solanaceae producing areas in the state of Sinaloa.

Zone	Oxidase		Glutathione S-transferases	
	Mean \pm SD ¹		Mean \pm SD ¹	
LS ²	0.971 \pm 0.007	c	0.04 \pm 0.068	a
North	1.073 \pm 0.04	b	0 \pm 0	a
Center	1.216 \pm 0.04	a	0.152 \pm 0.155	a
South	1.068 \pm 0.006	b	0.034 \pm 0.084	a

Means with different letter present significant difference (Tukey > 0.05). ¹= standard deviation; ²= susceptible line.

Oxidases act by oxidizing the insecticidal molecule allowing them to enter other enzymatic systems and be expelled (Pimentel *et al.*, 2008), in *B. tabaci* their activity has been correlated with resistance to neonicotinoids and avermectins (Rauch and Nauen, 2003; Wang and Wu, 2007; Roditakis *et al.*, 2010). In the case of glutathione S-transferases, the susceptible line presented an average of 0.040, when taken as resistance threshold, the highest average was presented in the center population with 0.152, the southern population that showed an average of 0.034, the highest glutathione S-transferases content (Table 2).

In *B. tabaci*, it disables the action of neonicotinoids (Yang *et al.*, 2016), organophosphates and pyrethroids (Ortelli *et al.*, 2003; Hu *et al.*, 2014) pyriproxyfen (Ma *et al.*, 2010) and diafenthiuron (Zhang *et al.*, 2015). These enzymes have been reported to act by conjugating the glutathione thiol group (GSH; \(\gamma\)-L-glutamyl-L-cysteinyl-glycine) to compounds that have an electrophilic center, thereby eliminating substrates from a cell by increasing its solubility in water (Low *et al.*, 2010).

Finally, the presence of acetylcholinesterases in the populations of Sinaloa and in the laboratory susceptible line did not present significant differences (Table 3). Other studies mention that the resistance of *B. tabaci* to insecticides (such as methamidophos, chlorpyrifos, phoxim, fenvalerate, avermectin, emamectin benzoate, spinosad, fipronil and imidacloprid) is related to the insensitivity or absence of acetylcholinesterase Kang *et al.* (2006) since it is considered as non-metabolic resistance which is associated with a mutation in the Acetylcholinesterase site of action (Ramya *et al.*, 2016).

Table 3. Means and absorbances of the acetylcholinesterase enzyme in the different solanaceae producing areas in the state of Sinaloa.

Zone	Acetylcholinesterase	
	Mean \pm SD	
LS ²	0.006 \pm 0.011	a
North	0 \pm 0	a
Center	0.006 \pm 0.003	a
South	0.005 \pm 0.003	a

Means with different letter present significant difference (Tukey > 0.05). ¹= standard deviation; ²= susceptible line.

The main detoxifying mechanisms in the state of Sinaloa were α -esterases, β -esterases and oxidases, presenting a resistance ratio of 100% and being categorized as highly 'altered' for the three areas under study. In the case of glutathione S-transferases, only in the central zone it was presented as an important detoxification mechanism by reporting a resistance factor of 66% and a classification of 'altered', while in the North and South zones, it is not considered as an important mechanism of resistance to insecticides by showing a null proportion of resistance and being classified as 'unchanged'.

As regards acetylcholinesterase, it is considered as a mechanism of little relevance for the development of resistance to insecticides in *B. tabaci* in the state of Sinaloa, as it is not registered in the physical-chemical tests (Table 4).

Table 4. Proportion of resistance (%) in the North, Central and South zones of Sinaloa, in comparison.

Zone	α -esterases	β -esterases	Glutathione S-transferases	Acetylcholinesterase	Oxidases
North	100 ^e	100 ^e	0 ^a	0 ^a	100 ^d
Center	100 ^e	100 ^e	66 ^d	0 ^a	100 ^d
South	100 ^e	100 ^e	0 ^a	0 ^a	100 ^d

Classification according to Montella *et al.* (2007). a= 'unaltered'; b= 'incipiently altered'; c= 'moderately altered'; d= 'altered'; e= 'very altered'.

Understanding these resistance mechanisms is the most important aspect for managing resistance in insect pests (Guo *et al.*, 2014; Zhang *et al.*, 2016; Horowitz, *et al.*, 2020). Kang *et al.* (2006) point out that the presence or absence of detoxifying enzymes and the difference in resistance to insecticides could be associated with different antecedents of chemical application in the field; the State of Sinaloa, was documented by Cortinas (2000) as one of the areas with the highest consumption of pesticides in the country, representing 30% of national consumption, while 70% is represented by the set of other producing areas (Jalisco-Nayarit-Colima, Sonora-Baja California, Tamaulipas, Michoacán, Chiapas, Veracruz, Tabasco, State of Mexico, Puebla-Oaxaca).

The most frequently used pesticides in Northwest Mexico are dithiocarbamates, bipyridyls, organophosphates, organochlorines, carbamates, pyrethroids and inorganic compounds (Leyva-Morales *et al.*, 2014), which coincides with the high levels of resistance and detoxification mechanisms reported in the present work.

Conclusions

Through the analysis of the enzymatic levels of three populations of *B. tabaci* from the state of Sinaloa, it was possible to attribute the resistance to insecticides of the North Central and South populations of the state to the enzyme systems of α -esterases, β -esterases and Oxidases in addition to Glutathione S-transferases for the population of the center. These resistance mechanisms coincide with the chemical inputs used for the control of *B. tabaci* in the state, such as: pyrethroids, organochlorines, neonicotinoids, organophosphates and carbamates. Due to the above, the detection of the origin of resistance and its understanding allows us to take the correct measures and actions to manage physiological resistance and restore sensitivity in populations. Which would reduce the high costs in the control, the losses in the harvests, and the contamination of the environment.

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