

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

Resistance to pyrethroid and oxadiazine insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) populations in Brazil

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Thesis presented to obtain the degree of Doctor in
Science. Area: Entomology

**Piracicaba
2018**

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Resistance to pyrethroid and oxadiazine insecticides in *Helicoverpa armigera*
(Lepidoptera: Noctuidae) populations in Brazil
versão revisada de acordo com a resolução CoPGr 6018 de 2011

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RESUMO

Resistência de *Helicoverpa armigera* (Lepidoptera: Noctuidae) a inseticidas dos grupos piretroides e oxadiazinias no Brasil

Helicoverpa armigera (Hübner) foi reportada oficialmente no Brasil em 2013, ano em que causou grandes perdas em lavouras de soja e algodão no país. Devido ao ataque severo de *H. armigera* e por ser mais tolerante do que as demais pragas que ocorriam no Brasil, houve um aumento significativo da pressão de seleção com inseticidas no campo. Inúmeros casos de resistência desta praga a inseticidas do grupo dos piretroides já havia sido reportado em alguns países do Velho Mundo. Dentro desse contexto o objetivo desse trabalho foi caracterizar a suscetibilidade e investigar possíveis mecanismos de resistência a piretroides bem como indoxacarb no Brasil. A mortalidade das populações de *H. armigera* foi menor do que 50 % quando tratadas com a dose máxima de 10 µg i.a./lagarta de 3º instar para fenvalerato e deltametrina. As populações de campo de *H. armigera* monitoradas entre os anos de 2013 a 2016 na dose diagnóstica de 10 µg i.a./lagarta de 3º instar apresentaram mortalidade de 10 a 40%. A frequência do gene P450 *CYP337B3* foi maior do que 0,95 em 33 populações testada. Além disso, as bases genéticas da resistência de *H. armigera* a piretroides foram investigadas e a razão de resistência com a linhagem suscetível foi de 780 vezes. O grau de dominância variou de 0,66 a 0,92, incompletamente e completamente dominante e a resistência foi caracterizada como autossômica e poligênica. Adicionalmente investigou-se a presença de possíveis mutações no canal de sódio bem como a expressão de outros genes P450 em uma linhagem resistente a piretroides. Foi possível detectar duas mutações não-sinonímias V937G, e Q960H no canal de sódio e os genes *CYP6AB10*, *CYP301A*, *CYP4S13* e *CYP321A5* foram super expressos na linhagem resistente. A suscetibilidade de populações de *H. armigera* para o inseticida indoxacarb foi caracterizada a partir de bioensaios de ingestão com lagartas de 3º instar. Os valores de CL₅₀ variaram de 0,22 (0,16 – 0,28) µg i.a./cm² até 0,57 (0,41 – 0,82) µg i.a./cm² variando em 2,6 vezes. As populações foram monitoradas ao longo das safras agrícolas entre 2013 e 2017 com a concentração diagnóstica de 6,1 µg i.a./cm² e observou-se uma diminuição na suscetibilidade da praga a indoxacarb. Uma linhagem resistente a indoxacarb foi selecionada em laboratório e comparada com uma linhagem suscetível de referência, apresentando uma razão de resistência de 297,5 vezes. Os resultados obtidos são extremamente importantes e poderão contribuir na tomada de decisões bem como na implementação de programas de manejo da resistência de insetos (MRI) no Brasil.

Palavras-chave: Manejo da resistência de insetos; *Helicoverpa armigera*; Inseticidas que atuam no canal de sódio; Mecanismos de resistência; Indoxacarb; Fenvalerato; Deltametrina

ABSTRACT

Resistance to pyrethroid and oxadiazine insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) populations in Brazil

Helicoverpa armigera (Hübner) was officially reported in Brazil in 2013 causing serious damage to several crops, especially soybean and cotton crops. Because of this severe damage and also because *H. armigera* is more tolerant to insecticides in compare to other lepidopteran pests in Brazil, there was a significant increase of selection pressure with insecticides in the field. Many cases of insecticide resistance, especially to pyrethroids, have been reported in some countries of the Old World. The main objective of the present study was to characterize the susceptibility of *H. armigera* and to investigate the mechanisms of its resistance to pyrethroids and indoxacarb in Brazilian populations. Mortality of *H. armigera* populations was less than 50% when treated with the highest dose of 10 µg a.i./3rd-instar larva of fenvalerate and deltamethrin. Field populations of *H. armigera* monitored from 2013 to 2016 growing seasons showed mean mortalities of 10 to 40% at the diagnostic dose of 10 µg a.i./3rd-instar larva. The resistance ratio to pyrethroid was 780-fold. The frequency of the chimeric P450 *CYP337B3* gene was above 0.95 in all 33 populations screened. The genetic basis of *H. armigera* resistance to pyrethroids was also investigated. The dominance degree varied from 0.66 to 0.92, i.e., incompletely to completely dominant, and resistance was characterized as autosomal and polygenic. Possible mutations in the sodium channel were investigated, as well as the expression of other P450 genes via RT-qPCR. Two non-synonymous mutations, V937G and Q960H were found, and the genes *CYP6AB10*, *CYP301A*, *CYP4S13* and *CYP321A5* were up-regulated in the Brazilian pyrethroid-resistant strain compared to the susceptible strain. The susceptibility of *H. armigera* populations to indoxacarb was characterized with a diet overlay bioassay in 3rd-instar larvae. LC₅₀ values ranged from 0.22 (0.16–0.28) µg a.i./cm² to 0.57 (0.41–0.82) µg a.i./cm², varying 2.6-fold. The populations were monitored through the 2013–2017 growing seasons, with the diagnostic dose of 6.1 µg a.i./cm²; during the period, the susceptibility to indoxacarb decreased. An indoxacarb-resistant strain was selected under laboratory conditions and showed a resistance ratio of 297.5-fold. These results will contribute to decision-making and implementation of insect resistance-management (IRM) programs in Brazil and other recently invaded countries in Brazil.

Keywords: Resistance management; *Helicoverpa armigera*; Sodium channel; Mechanisms of resistance; Indoxacarb; Fenvalerate; Deltamethrin

1. INTRODUCTION

Insecticide resistance is one of the main problems for the success of pest control worldwide (Sparks and Nauen, 2015; Sparks and Lorsbach, 2017). Insect resistance was first documented in the United States in 1914 for a strain of San José scale, where sulphur, an inorganic insecticide, was no longer controlling this insect (Melander, 1914). From this first documentation until 2017, more than 15,000 cases of insecticide resistance have been reported for different pest species all over the world (Sparks and Lorsbach, 2017).

Resistance is defined as “the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species” (World Health Organization, 1957). The IRAC committee defined resistance as “a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species” (Insecticide Resistance Action Committee, 2018).

Resistance is a heritable genetic characteristic that can enable an insect and its descendants to tolerate a higher dose of insecticide than others in a population. The evolution of resistance in a population starts with the genetic variability among individuals, where some can be more adaptable to changes than others (Georghiou, 1972; Georghiou and Taylor, 1977a). This means that under high selection pressure from a specific insecticide, the individuals able to tolerate the high amount will survive. Furthermore, these pre-adapted individuals have some mechanisms which allow them to tolerate and survive the exposure (Georghiou, 1969; Georghiou, 1972; Roush and McKenzie, 1987). If their descendants possess this same characteristic, it can be termed resistance.

Selection pressure is a key factor in the rate of resistance evolution, which is an external factor that most of the time is determined by farmers at the time of insecticide spraying on their crops (Georghiou, 1972; Roush and McKenzie, 1987). Spraying insecticides with the same mode of action (MoA), with a high dose or for a long period, can create a situation where the pre-adapted individuals will have advantages over non-adapted individuals, and as a consequence the frequency of resistant individuals may increase (Roush and McKenzie, 1987; Tabashnik et al., 2003).

Resistance is governed by three major factors, genetic, biological and operational (Georghiou and Taylor, 1977a; Georghiou and Taylor, 1977b). Genetic factors are associated with intrinsic characteristics, which involves the frequency of the resistant allele (R) in a

population as well as the number of R alleles, inheritance of resistance such as the degree of dominance, past selection by other chemicals, and the combination of R with other fitness factors in the genome (Georghiou and Taylor, 1977a). Biological factors involve biotic characteristics such as generation turnover; offspring per generation; reproductive type (e.g., sexual, parthenogenetic); behavioral characteristics such as migration, dispersal or isolation; and monophagy or polyphagy. Operational factors are those associated with external factors, which can be manipulated by humans, involving the characteristics of the chemical, such as the mode of action, persistence, and formulation, as well as the characteristics of application in the field (Georghiou and Taylor, 1977a; Georghiou and Taylor, 1977b).

Resistance is always associated with mechanisms that enable an individual to tolerate more chemicals than the others (Roush and McKenzie, 1987). These mechanisms can be i) metabolic, associated with the detoxification of the toxic compound by enzymes such as P450, esterase, or glutathione-S-transferase, or the excretion rate due to ABC transporters; ii) target-site mutations, based on changes in the structure of the site or the number of sites where the pesticide binds to cause toxicity to the insect; iii) penetration, when the insecticide takes more time or cannot cross the cuticle of the insect; and iv) behavioral, when the insect is able to evade contact with the toxic compound. Metabolic and target-site mechanisms are considered to be the most important and have been associated with many cases of resistance in the literature (Joußen and Heckel, 2016).

Only the operational factor can be managed and changed by farmers, and also recommended by the companies who offer the technologies. The evolution of insect resistance to insecticides can force companies and growers to spend large sums once a pest-control tactic becomes inefficient and it becomes necessary to develop or adopt a new technology to control pests in the field (Sparks and Lorschbach, 2017). Furthermore, the development of a new technology is costly in time and money. Currently it is necessary to test around 159 574 compounds for each product developed, and on average, 10 years of research with a total cost of U\$286 million are needed to find and develop a new agrochemical (Sparks and Lorschbach, 2017).

Thus, when an insecticide becomes ineffective because of resistance, most of the time and money invested during this discovery process is lost. In the United States, estimated crop losses in 2005 due to pesticide resistance were about U\$1.5 billion year⁻¹ (Pimentel, 2005). In 1990 and 1991, Australia suffered losses of around A\$150 million due to *Helicoverpa armigera* management in cotton fields after this pest evolved resistance to several insecticides, including pyrethroids (Fitt, 1994).

In the 1940s, the first class of synthetic organic insecticides, such as DDT, was introduced. However, a case of resistance in houseflies was reported only a few years later (Keiding and Van Deurs, 1949). Nowadays it is possible to find different groups of insecticides with different modes of action, including pyrethroids, oxadiazines, neonicotinoids, carbamates, spinosyns, growth regulators, and the recently introduced diamides. Although many different classes of insecticides are currently available on the market, many of them target the nervous and muscular systems, and some of the compounds affect different sites in the same target (Sparks and Nauen, 2015). The classical example is the sodium channel, a transmembrane protein with four domains, each one containing six subunits (Dong, 2007). The sodium channel is the target of pyrethroids, a sodium-channel modulator, and oxadiazines such as indoxacarb and metaflumizone, which are sodium-channel blockers (Lapied et al., 2001; Dong, 2007). Cases of resistance for most of the classes of insecticides, especially pyrethroids, have been reported for different pest species worldwide (Dong, 2007).

Members of the order Lepidoptera are responsible for 4,425 cases of resistance reported among all the insect orders; *Plutella xylostela* has the most, 862 cases, followed by the cotton bollworm *Helicoverpa armigera* with 856 cases (Arthropod Pesticide Resistance Database, 2018). The cotton bollworm is a generalist and worldwide-distributed pest, and causes serious damage to many economically important crops around the world (Fitt, 1989). In many countries of Oceania, Asia, Africa and Europe, this is a major pest attacking cotton, chickpea, tomato and sorghum (Anderson et al., 2016).

In 2013 *H. armigera* was detected for the first time in the Americas, causing serious damage to soybean and cotton crops in Bahia and Goiás states in Brazil (Czepak et al., 2013; Specht et al., 2013). However, some records confirm that this pest has been present in Brazil since 2008 (Sosa-Gómez et al., 2016). After that, this pest spread rapidly in a short period of time, invading other countries in South and Central America including Argentina, Paraguay, Uruguay and Puerto Rico (Leite et al., 2014; Mastrangelo et al., 2014; Murúa et al., 2014; Kriticós et al., 2015; Arnemann et al., 2016; Pearce et al., 2017). In 2015 a single moth was detected in a tomato plantation in Florida, causing a pest alert in the USA (Hayden and Bambila, 2015).

Brazilian crops are attacked by several economically important lepidopteran pests, e.g. *Spodoptera frugiperda*, *Helicoverpa zea*, *Heliothis virescens* and *Chrysodeixis includens*. However, *H. armigera* has become one of the most destructive and difficult pests to control, mainly because most of the insecticides recommended by Brazilian government agencies for emergency use against this pest have cases of resistance reported in the countries where this

species originated. Furthermore, the cotton bollworm has proved to be more tolerant to conventional insecticides than the other lepidopteran pests in Brazil (Durigan et al., 2017).

H. armigera populations in Brazil arrived in multiple invasions from Pakistan, India, China and some European countries, and in these countries, resistance evolved rapidly to most of the insecticides recommended to control *H. armigera* in Brazil (Tay et al., 2013; Leite et al., 2014; Anderson et al., 2016; Tay et al., 2017; Pearce et al., 2017). Resistance of *H. armigera* to pyrethroids has been reported in Australia, China, India and Pakistan, and recently in Brazil (Gunning et al., 1984; Forrester, 1990; Pittendrigh, 1997; McCaffery, 1998; Martin et al., 2002; Grubor and Heckel, 2007; Djihinto et al., 2009; Joußen et al., 2012; Rasool et al., 2014; Qayyum et al., 2015; Bird, 2015; Anderson et al., 2016; Durigan et al., 2017; Bird, 2018). A total of 18 cases of *H. armigera* resistance to indoxacarb have been reported in the Old World, in Australia, China and Pakistan (Aheer et al., 2009; Qayyum et al., 2015; Bird, 2015; Bird, 2017; Wang et al., 2017).

Considering that *H. armigera* is an invasive, polyphagous and destructive pest and also has a background indicating a high capacity to evolve resistance to many insecticides, becoming difficult to control, the main objectives of this study were to characterize and monitor the susceptibility to pyrethroids and indoxacarb in populations of *H. armigera* in Brazil, in addition to investigating and elucidating possible mechanisms of resistance associated with pyrethroid resistance, as well as inheritance patterns. The results provided enough information to manage this pest, to implement an insect resistance-management (IRM) program, and also to prevent the evolution of *H. armigera* resistance to insecticides in Brazil.

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2. HIGH FREQUENCY OF *CYP337B3* GENE ASSOCIATED WITH CONTROL FAILURES OF *Helicoverpa armigera* WITH PYRETHROID INSECTICIDES IN BRAZIL

Abstract

Control failures with the use of pyrethroid insecticides have been reported frequently for populations of *Helicoverpa armigera* (Hübner) in Brazil, since its detection in 2013. Here, we confirmed and investigated the metabolic mechanisms of pyrethroid resistance in *H. armigera* populations from Brazil. Mortality of *H. armigera* populations was lower than 50% at the highest dose (10 µg a.i./3rd instar larva) of the pyrethroids deltamethrin and fenvalerate in dose-response bioassays. Very low mortality (10 to 40%) was obtained at a diagnostic dose of 10 µg a.i./larva for each pyrethroid in *H. armigera* populations collected from different agricultural regions in Brazil, from 2013 to 2016. In synergist bioassays, when larvae were treated with PBO synergist, the mortality of all populations tested was 100%. The frequency of the cytochrome P450 *CYP337B3* gene was above 0.95 in all populations of *H. armigera*. We found only fourteen heterozygous *H. armigera* out of 497 individuals tested for this gene subfamily. Our results indicated that *H. armigera* populations from Brazil have different degrees of susceptibility to deltamethrin and fenvalerate, but all populations can be considered tolerant to pyrethroid insecticides. The chimeric P450 *CYP337B3* enzyme is one of the main mechanisms of pyrethroid resistance in Brazilian *H. armigera* populations.

Keywords: Cotton bollworm; Insect resistance management; Metabolic detoxification; Pyrethroid resistance

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2.1. Introduction

The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) was officially detected for the first time in the Americas in 2013, when larvae were collected under soybean and cotton plants in central Brazil (Czepak et al., 2013; Tay et al., 2013). *H. armigera* has become widely distributed across South America and was recently detected in Puerto Rico and in Florida, USA (Leite et al., 2014; Mastrangelo et al., 2014; Murúa et al., 2014; Hayden, J.E.; Bambila, 2015; Kriticos et al., 2015; Arnemann et al., 2016). Even before *H. armigera* invaded the Americas, it was frequently reported as a serious agricultural pest with a wide host range in Oceania and other Old World countries (Fitt, 1989; Tay et al., 2017). *H. armigera* individuals from Brazil share their genetic material with individuals from Asia, Europe, and Africa, suggesting multiple invasive events in Brazil (Leite et al., 2014; Anderson et al., 2016;

Tay et al., 2017).

H. armigera has become a primary pest of soybean and cotton crops, and its larvae have also caused damage in bean, maize, sorghum and vegetable crops in Brazil (Czepak et al., 2013; Specht et al., 2013; Murúa et al., 2016). This species is more widely polyphagous and tolerant to insecticides than the native lepidopteran pests (Carvalho et al., 2013; Stanley et al., 2009). Therefore, since *H. armigera* was detected, the frequency and dosages of insecticide sprayings and the adoption of Bt genetically modified crops have increased in Brazil.

Pesticide resistance has been reported in *H. armigera* populations around the world (Sparks and Nauen, 2015). The Arthropod Pesticide Resistance Database (2017) (Arthropod Pesticide Resistance Database, 2017) records 763 cases of resistance to 49 different active ingredients, especially pyrethroid insecticides. The multiple selection events of resistance to different insecticides in *H. armigera* populations are associated with intrinsic characteristics of this species, such as its polyphagous feeding habit, short development cycle, high dispersal capacity, and high genetic variability (Fitt, 1989; McCaffery, 1998; Feng et al., 2005).

Resistance to pyrethroid insecticides has been frequently reported in *H. armigera* populations since the 1970s (Forrester, 1990; Pittendrigh, 1997; McCaffery, 1998; Grubor & Heckel, 2007; Djihinto et al., 2009; Brun-barale et al., 2010; Joußen et al., 2012; Rasool et al., 2014; Bird, 2015; Qayyum et al., 2015; Anderson et al., 2016). The main mechanisms of pyrethroid resistance in insects are linked to reduced target-site sensitivity and enzymatic detoxification of insecticide molecules (Gunning et al., 1984; Gunning et al., 1991; Yang et al., 2004; Joußen et al., 2012). Specifically in *H. armigera*, pyrethroid resistance has been associated with the increase of insecticide detoxification by cytochrome P450 monooxygenase enzymes in different regions of the world (Pittendrigh, 1997; Grubor & Heckel, 2007; Feyereisen, 2012; Joußen et al., 2012; Heckel, 2012; David et al., 2013; Liu et al., 2014; Rasool et al., 2014; Han et al., 2015; Xu et al., 2016).

The high selection pressure imposed by the frequent insecticide applications in the field, and the resistance history of *H. armigera* populations in their native geographic areas presage a rapid evolution of pyrethroid resistance in Brazilian agricultural crop systems. In fact, growers and companies have frequently reported insecticide control failures for *H. armigera* in central and northeastern Brazil. It is crucial to understand *H. armigera* pyrethroid susceptibility in order to contribute to insect resistance management (IRM) programs.

For these reasons, we evaluated the susceptibility of *H. armigera* populations from Brazil to two pyrethroid insecticides, deltamethrin and fenvalerate. Then, we monitored the susceptibility to these insecticides in *H. armigera* populations collected in central and

northeastern Brazil from 2013 to 2016. We also investigated the mechanisms involved in *H. armigera* pyrethroid survival. We carried out bioassays with insecticide synergists, and estimated the frequency of the chimeric P450 enzyme *CYP337B3* gene, previously reported as the main genetic mechanism of pyrethroid resistance in *H. armigera* populations in the Old World and Australia (Joußen et al., 2012; Rasool et al., 2014; Daly & Fisk, 1992).

2.2 Material and Methods

2.2.1. Insect sampling

H. armigera larvae were sampled in the main growing regions from 2013 to early 2016, in cotton, soybean, bean, millet and maize. Each population consisted of \approx 500 to 1000 larvae. For each field-collected sample, we conducted a molecular identification to distinguish *H. armigera* from the native *H. zea*, through the PCR-RFLP technique described by Behere et al. (2008). Field-collected larvae were reared for at least one generation under controlled laboratory conditions of 25 ± 1 °C, $70 \pm 10\%$ RH, and 14 h of photophase. Pupae were pretreated with a copper sulfate solution (10%) and were maintained in cylindrical cages made out of PVC tubes (30 cm high \times 25 cm diameter) covered with a tulle type material until the emergence of adults. Afterwards, moths were also maintained in the same type of cages with 40 couples each. Eggs were collected from the tulle material, where the females laid their eggs. Neonate larvae were placed in plastic containers containing an artificial diet adapted from Greene et al. (1976). Larvae were kept in the plastic containers containing artificial diet until they reached the third instar, when they were used in the toxicity bioassays.

2.2.2 Dose-mortality response bioassays with pyrethroids

Dose-mortality response bioassays were performed using a topical bioassay with third-instar larvae. For each *H. armigera* population (Table 1: BA33, BA43, BA44, BA45, BA49, GO02, GO03, MS05 and MT11), six to eight doses (1 to 10 μ g a.i./larva) of deltamethrin (purity: 98.5%) and fenvalerate (purity: 98.6%) dissolved in 1 μ L of acetone were tested. After insecticide application, larvae were individually placed in a 12-well acrylic plate (Costar®) containing artificial diet. Forty-eight larvae were tested for each insecticide concentration. The plates were kept under controlled conditions in a climate-controlled chamber at 25 ± 1 °C, $60 \pm 10\%$ RH, and a photoperiod of 14:10 h. Mortality was assessed after 48 h and data were subjected to a Probit analysis (Finney, 1971) using SAS Software (SAS, 2000) in order to

determine the regression lines and slopes, and also to estimate the median and 90th percentile lethal doses (LD_{50} and LD_{90}).

Table 1. Population code, year of collection, crop and location of *H. armigera* populations from major Brazilian producer regions.

Population code	Collection year	Crop	City, State ^a	Latitude (S)	Longitude (W)
BA 27	Feb-2013	Maize	Barreiras - BA	12°09'10"	44°59'24"
^b BA 33	Jun-2013	Bean	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
BA 34	Jan-2014	Soybean	São Desidério - BA	12°21'48"	44°58'24"
^b BA 43	Jan-2014	Soybean	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
^b BA 44	Feb-2014	Cotton	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
^b BA 49	May-2014	Maize	Correntina - BA	13°20'36"	44°38'12"
BA 52	Nov-2014	Soybean	São Desidério - BA	12°21'48"	44°58'24"
BA 61	Apr-2015	Millet	São Desidério - BA	12°21'48"	44°58'24"
BA 64	Jun-2015	Cotton	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
BA 66	Oct-2015	Bean	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
BA 69	Jan-2016	Soybean	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
BA 70	Mar-2016	Soybean	Luis Eduardo Magalhães - BA	12°05'58"	45°47'54"
GO 02	Mar-2014	Soybean	Mineiros - GO	17°34'10"	52°33'04"
^b GO 03	Mar-2014	Soybean	Cristalina - GO	16°46'07"	47°36'49"
GO 04	Jan-2015	Soybean	Montividiu - GO	17°26'39"	51°10'29"
GO 05	Feb-2015	Soybean	Santo Antonio do Rio Verde - GO	18°09'57"	47°56'47"
GO 06	Mar-2016	Soybean	Mineiros - GO	17°34'10"	52°33'04"
MA 04	Jan-2013	Soybean	Balsas - MA	07°31'57"	46°02'08"
MS 03	May-2013	Cotton	Chapadão do Sul - MS	18°47'39"	52°37'22"
^b MS 05	Oct-2013	Cotton	Costa Rica - MS	18°32'38"	53°07'45"
MS 08	Dec-2016	Soybean	Chapadão do Sul - MS	18°47'39"	52°37'22"
MT 06	Nov-2012	Soybean	Rondonópolis - MT	16°28'15"	54°38'08"
^b MT 11	Oct-2014	Soybean	Primavera do Leste - MT	15°33'32"	54°17'46"
MT 15	May-2015	Cotton	Campo Verde - MT	15°32'48"	55°10'08"
MT 16	May-2015	Cotton	Sapezal -MT	12°59'22"	58°45'51"
MT 17	Jan-2016	Soybean	Sapezal -MT	12°59'22"	58°45'51"
MT 19	Mar-2016	Soybean	Primavera do Leste - MT	15°33'32"	54°17'46"
MT 23	May-2016	Millet	Primavera do Leste - MT	15°33'32"	54°17'46"
MT 27	Oct-2016	Soybean	Nova Mutum - MT	13°49'44"	56°04'56"
PR 05	Feb-2015	Soybean	Londrina - PR	23°18'37"	51°09'46"
RS 02	Mar-2014	Soybean	Itaara - RS	29°36'31"	53°45'55"
SP 15	Jan-2015	Soybean	Viradouro - SP	20°52'23"	48°17'49"
SP 19	Apr-2016	Bean	Limeira - SP	22°33'53"	47°24'06"

^a State abbreviations: BA, Bahia; GO, Goiás; MA, Maranhão; MS, Mato Grosso do Sul; MT, Mato Grosso; PR, Paraná; RS, Rio Grande do Sul; SP, São Paulo. ^b Populations used in the Baseline Susceptibility bioassays.

2.2.3. Monitoring the susceptibility of *H. armigera* to pyrethroids over growing seasons

The dose of 10 µg a.i./larva of each insecticide was used as a diagnostic dose for monitoring the susceptibility to pyrethroids in *H. armigera* populations collected from different Brazilian agricultural regions. This dose is five times higher than the LC₅₀ of 1.864 µg a.i./larva found by Joußen et al. (2012) in a fenvalerate-resistant strain of *H. armigera* from Australia. In each of 15 populations, 240 larvae were monitored through the growing seasons from 2013 to 2016 (Table 1). Larval survival obtained from monitoring was subjected to a multiple comparison analysis, using the many-to-one comparison procedure of Dunnett (1955) using SAS Software (SAS, 2000). All the populations tested were compared with the same control in order to evaluate the evolution of the susceptibility since the detection of *H. armigera* in Brazil. Population BA33, the population collected in Bahia State at the time of the official report of *H. armigera* in Brazil in 2013, was used as the control. This BA33 population is our reference for population susceptibility to insecticides.

2.2.4. Synergist bioassays

Third-instar larvae were treated with the synergists piperonyl butoxide (PBO, Sigma Aldrich, 90%), diethyl maleate (DEM, Sigma Aldrich, 97%) and S,S,S-tributyl phosphorotrithiotate (DEF, Chem Service, 97.2%). The synergists were diluted in pure acetone at a dose of 1 µg a.i./larva each and were applied topically (1 µL on the dorsum of the larva) 2 h before the insecticide application. We tested four treatments for each synergist: (a) Control: acetone; (b) synergists (PBO, DEM and DEF at 1 µg a.i./larva); (c) diagnostic dose of insecticide (10 µg a.i./larva) (d) synergists + diagnostic dose of insecticide (10 µg a.i./larva). Thirty-six larvae each of three different populations (BA33, BA43, and BA52) were tested in each treatment, and mortality was assessed 48 h after application. The results were subjected to the analysis of variance (ANOVA) and means were compared using a Tukey test ($p = 0.05$; SAS, 2000).

2.2.5. Screening of the P450 *CYP337B* gene subfamily

2.2.5.1. Insect sampling and DNA extraction

Screening for the different members of the P450 *CYP337B* gene subfamily was carried out in 33 *H. armigera* populations collected from 2012 to 2016, resulting in 497 individuals

from different crops, years and regions (Table 2). The insects were frozen at $-20\text{ }^{\circ}\text{C}$ and genomic DNA was extracted from thoracic tissue of moths using the CTAB method adapted from Clark et al. (2001). DNA concentrations were estimated and diluted to concentration of $10\text{ ng}/\mu\text{L}$.

2.2.5.2. PCR for detection of the P450 *CYP337B* gene subfamily

Screening for the *CYP337B1*, *CYP337B2* and *CYP337B3* gene subfamilies frequency followed the methodology and three specific primers described by Joußen et al. (2012). PCR amplification was performed with 20 ng of total DNA, 37.5 mM MgCl_2 , 2.5 mM dNTPs, 20 pmol of each primer, 1U Taq DNA Polymerase (Life Technologies, Carlsbad, CA, USA), and 10% $10\times$ Taq Buffer and MiliQ water in a total volume of 25 μL . The PCR cycles followed an initial denaturation of $94\text{ }^{\circ}\text{C}$ during 5 min followed by 30 cycles of 30 s at $94\text{ }^{\circ}\text{C}$, 30 s with the annealing at $55\text{ }^{\circ}\text{C}$, elongation at $72\text{ }^{\circ}\text{C}$ for 1.5 min, with a final extension step at $72\text{ }^{\circ}\text{C}$ for 10 min. PCR products (2 μL) were observed in an agarose gel (1.5% w/v) stained with SYBR Safe. Afterwards, the presence or absence of each of the three genes (*CYP337B1*, *CYP337B2* or *CYP337B3*) was used to calculate gene frequencies in each population.

2.2.5.3. Sequence analysis

Positive PCR products of each gene in the *CYP337B* subfamily (*CYP337B1*, *CYP337B2* and *CYP337B3*) were sequenced to confirm the PCR specificity (GenBank accession number MF435019–MF435037). The samples were purified with the ExoSap enzymes (Invitrogen). The reactions of purification were incubated for 30 min at $37\text{ }^{\circ}\text{C}$ and then 15 min at $80\text{ }^{\circ}\text{C}$ to denature the enzymes. The sequencing of the samples was performed by the Animal Biotechnology Laboratory from ESALQ/ USP (Piracicaba, SP, Brazil) by the Sanger Sequencing method. The sequencing data were edited and aligned manually with Sequencher 4.8 software (Genes Code Corporation, Ann Arbor, MI, USA) and compared with subfamilies sequences available from GenBank NCBI using the BLASTn tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Afterwards, Bayesian phylogenetic analysis was carried to compare Brazilian *CYP337B3* subfamily alleles of *H. armigera* with others one originated from Australia (JQ284029) (Joußen et al., 2012), China (KM675664, KM675665 and, KM675666) (Han et al., 2015) and Pakistan (KJ636466) (Rasool et al., 2014). All sequences were aligned and interrupted at 328 bp to phylogenetic analysis. We used the

software PAUP* 4.0b10 (Swofford D., 2002) and modeltest 2 (Nylander, 2004) to selected the substitution model using the Akaike information criteria (Akaike, 1974). The Bayesian phylogenies was estimated using Mrbayes, version 3.2 (Ronquist and Huelsenbeck, 2003) with 10million generations using two independent runs with one cold and three heated chains. The *CYP337B2* subfamily allele JQ284028 was defined as outgroup. The first 25% and the last 25% of tree were discarded as burn-ins; the remaining 50% trees were used to construct a consensus tree with Bayesian posterior probabilities observed in the software Figtree version 1.3.1 (Rambaut, 2009).

2.3. Results

2.3.1. Dose-mortality response bioassays with pyrethroids

For all nine *H. armigera* populations tested, the maximum dose tested (10 µg a.i./larva) resulted in mortality lower than 50% of the individuals. Therefore, it was not possible to estimate the LD₅₀ or LD₉₀. The maximum mortality caused at 10 µg a.i./larva was 40% for both insecticides.

2.3.2. Susceptibility monitoring through growing seasons

In the monitoring of *H. armigera* susceptibility to deltamethrin, all 15 populations tested showed high survival, above 40% (Fig. 1). The highest survival was observed in population BA69 (93% ± 0.44%) collected during the 2015–2016 growing season. According to Dunnett's test, populations BA43 (50% ± 1.96%) and BA66 (40.4% ± 2.3%) differed from the control sampled in 2013 (BA33, 75% ± 1.83%), showing lower survival for deltamethrin than the control population.

For fenvalerate, population BA43, sampled in 2014, was the most susceptible (40% ± 1.4%) and differed from the control (BA33, 70% ± 1.37%). Populations MT11 (90% ± 1.83%), GO06 (92% ± 2.47%) and MS08 (92% ± 0.81%) also differed from BA33, but showed higher survival than the control. For both insecticides, a decrease was observed of the susceptibility throughout the cropping years.

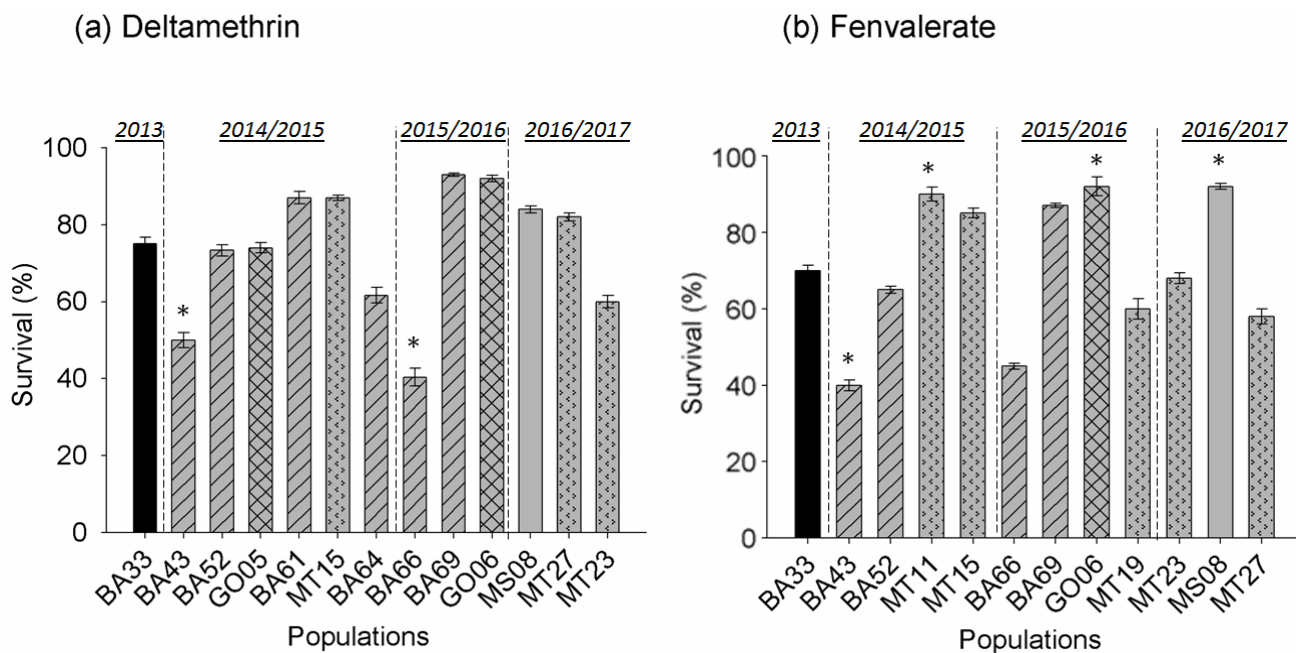


Fig. 1. Survival of *H. armigera* larvae monitored through the crop years with the diagnostic dose of 10 µg a.i./larva of deltamethrin (a) and fenvalerate (b) using a topical bioassay.

■ Population used as a control, collected in 2013; ▨ Populations collected in Bahia State; ▩ Populations collected in Goiás State; ▪ Populations collected in Mato Grosso State; ▫ Populations collected in Mato Grosso do Sul State. * Populations that differ from the control (BA33), by Dunnett's test. $n=240$ larvae.

2.3.3. Synergist bioassays

For the insecticide deltamethrin all the populations had 100% mortality when the larvae were treated with PBO (Fig. 2a, b and c). The treatment with DEF also showed a synergism with deltamethrin presenting mortality values ranging from 79% \pm 0.17% to 100%. DEM applied before deltamethrin resulted on an intermediate mortality going from 42.42% \pm 0.20% to 65% \pm 0.36%. Tukey's test showed that mortality obtained from the treatment with the diagnostic dose + PBO differed from the treatment that used only the diagnostic dose (Fig. 2a, b and c). No significant differences were observed between the treatments with deltamethrin (10 µg a.i./larva) and pretreatment with DEM.

For fenvalerate when only the diagnostic dose (10 µg a.i./larva) was applied the mortality ranged from 36.11% \pm 0.63% to 47.22% \pm 0.09%. In the treatment with the diagnostic dose of fenvalerate + PBO the populations showed a mortality between 97.22% \pm 0.10% and 100%. Mortality at the diagnostic dose of fenvalerate + DEF treatment was between 47% \pm 0.09% to 83.33% \pm 0.17%, while the treatment with diagnostic dose of fenvalerate + DEM showed a mortality from 37.14% \pm 0.26% to 50% \pm 0.60% (Fig. 2d, e and f). No mortality was observed when we applied only the synergists. Based on Tukey's test, for all populations the treatment with the diagnostic dose of fenvalerate + PBO showed higher mortality than only the

diagnostic dose of fenvalerate treatment, however the application with DEM had no effect on the insects mortality. Therefore, for both insecticides the toxicity increased when PBO synergist was used as pretreatment followed by the DEF pretreatment.

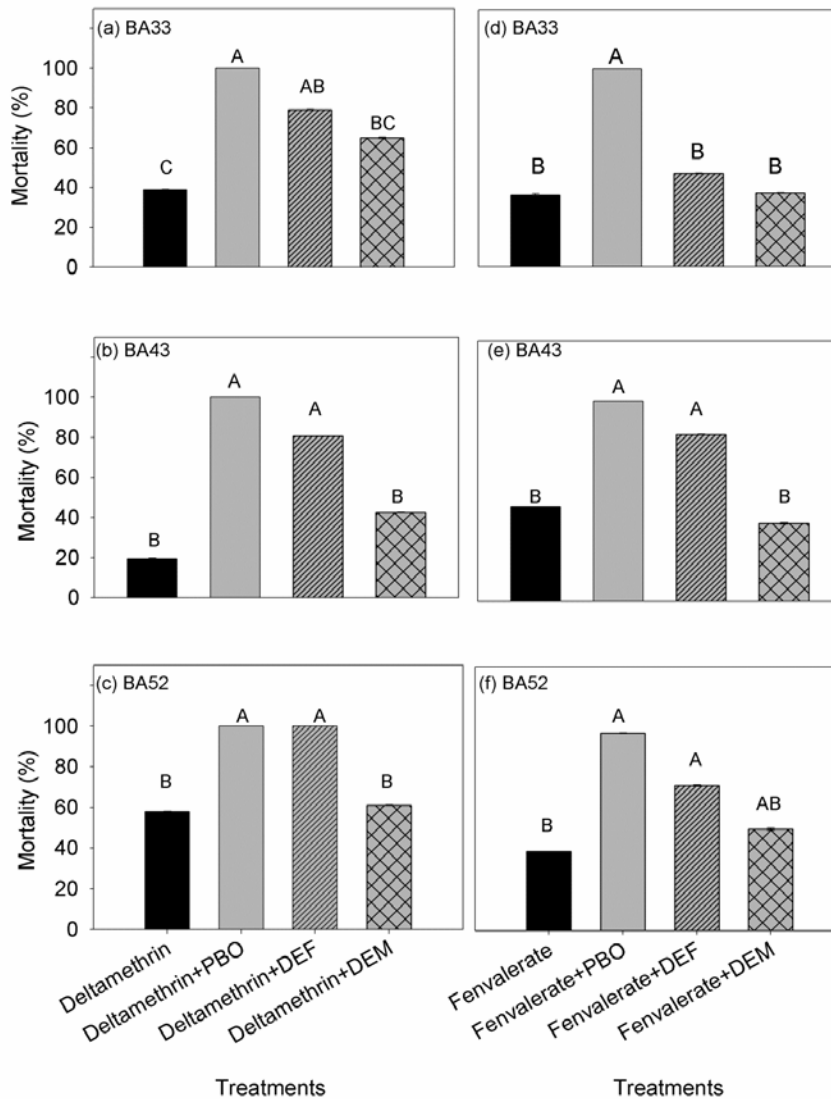


Fig. 2. Effect of synergist (PBO, DEF and DEM) treatment on *H. armigera* (third-instar larvae) mortality to deltamethrin (a, b and c) and fenvalerate (d, e and f). *Bars followed by the same letter do not differ from each other according to Tukey's test ($p < 0.05$).

3.4. *CYP337B* gene subfamily frequencies in Brazilian populations of *H. armigera*

All insects screened were positive for the chimeric P450 *CYP337B3* gene subfamily. Of the total populations screened (33), 21 showed a *CYP337B3* frequency equal to 1.0 (Fig. 3). Of the 994 alleles screened, 980 were positive for *CYP337B3*, eight positive for *CYP337B2* and

six positive for *CYP337B1*. The *CYP337B2* gene was found in the populations collected in Bahia state (BA33, BA 44, BA66, BA69 and BA70: $f = 0.025$) and in Mato Grosso state (MT11: $f = 0.025$; MT23: $f = 0.042$; MT27: $f = 0.071$) which were heterozygous with *CYP337B3*. The *CYP337B1* was positive in populations collected in Bahia state (BA27: $f = 0.028$; BA34: $f = 0.056$ and BA44: $f = 0.025$), Mato Grosso state (MT27: $f = 0.071$) and Mato Grosso do Sul state (MS03: $f = 0.038$; MS05: $f = 0.029$). The individuals who were positive for *CYP337B1* and *CYP337B2* were also positive for *CYP337B3*, i.e. heterozygous. No frequency differences were observed through the years sampled, indicating that the *CYP337B3* frequency has always been high since the introduction of this species into Brazil.

The phylogenetic analysis indicates that Brazilian *CYP337B3* subfamily alleles of *H. armigera* are closer to Chinese and Pakistan when compared to the Australian allele (Fig. 4). The Brazilian *CYP337B3* alleles MF435033, MF435034, MF435035, and MF435036 have 100% of identity to Chinese allele KM675664 and 99% identity to Pakistan and Australia allele when compared for the BLASTn tool. The Brazilian *CYP337B3* allele MF435037 has 99% of identity with Chinese (KM675664 and KM675665) and Pakistan (KJ636466) alleles and 98% of identity with the Australian allele.

Table 2. Population code, year of collection, crop, location, total of insects screened and *CYP337B1*, *CYP337B2* and *CYP337B3* gene frequencies in *H. armigera* populations in Brazil.

Population code	Collection year	Crop	Collection site	Total insects screened	<i>CYP337B1</i> frequency	<i>CYP337B2</i> frequency	<i>CYP337B3</i> frequency
BA 27	Feb-2013	Maize	Barreiras - BA	18	0.028	0.000	0.972
BA 33	Jun-2013	Bean	Luis Eduardo Magalhães - BA	20	0.000	0.025	0.975
BA 34	Jan-2014	Soybean	São Desidério - BA	09	0.056	0.000	0.944
BA 43	Jan-2014	Soybean	Luis Eduardo Magalhães - BA	20	0.000	0.000	1.000
BA 44	Feb-2014	Cotton	Luis Eduardo Magalhães - BA	20	0.025	0.025	0.950
BA 49	May-2014	Maize	Correntina - BA	19	0.000	0.000	1.000
BA 52	Nov-2014	Soybean	São Desidério - BA	20	0.000	0.000	1.000
BA 61	Apr-2015	Millet	São Desidério - BA	04	0.000	0.000	1.000
BA 64	Jun-2015	Cotton	Luis Eduardo Magalhães - BA	15	0.000	0.000	1.000
BA 66	Oct-2015	Bean	Luis Eduardo Magalhães - BA	20	0.000	0.025	0.975
BA 69	Jan-2016	Soybean	Luis Eduardo Magalhães - BA	20	0.000	0.025	0.975
BA 70	Mar-2016	Soybean	Luis Eduardo Magalhães - BA	20	0.000	0.025	0.975
GO 02	Mar-2014	Soybean	Mineiros - GO	03	0.000	0.000	1.000

GO 03	Mar-2014	Soybean	Cristalina - GO	03	0.000	0.000	1.000
GO 04	Jan-2015	Soybean	Montividiu - GO	20	0.000	0.000	1.000
GO 05	Feb-2015	Soybean	Santo Antonio do Rio Verde - GO	08	0.000	0.000	1.000
GO 06	Mar-2016	Soybean	Mineiros - GO	20	0.000	0.000	1.000
MA 04	Jan-2013	Soybean	Balsas - MA	05	0.000	0.000	1.000
MS 03	May-2013	Cotton	Chapadão do Sul - MS	13	0.038	0.000	0.962
MS 05	Oct-2013	Cotton	Costa Rica - MS	17	0.029	0.000	0.971
MS 08	Dec-2016	Soybean	Chapadão do Sul - MS	14	0.000	0.000	1.000
MT 06	Nov-2012	Soybean	Rondonópolis - MT	07	0.000	0.000	1.000
MT 11	Oct-2014	Soybean	Primavera do Leste - MT	20	0.000	0.025	0.975
MT 15	May-2015	Cotton	Campo Verde - MT	20	0.000	0.000	1.000
MT 16	May-2015	Cotton	Sapezal -MT	20	0.000	0.000	1.000
MT 17	Jan-2016	Soybean	Sapezal -MT	20	0.000	0.000	1.000
MT 19	Mar-2016	Soybean	Primavera do Leste - MT	18	0.000	0.000	1.000
MT 23	May-2016	Millet	Primavera do Leste - MT	12	0.000	0.042	0.958
MT 27	Oct-2016	Soybean	Nova Mutum - MT	07	0.071	0.071	0.857
PR 05	Feb-15	Soybean	Londrina - PR	20	0.000	0.000	1.000

RS 02	Mar-14	Soybean	Itaara - RS	12	0.000	0.000	1.000
SP 15	Jan-15	Soybean	Viradouro - SP	15	0.000	0.000	1.000
SP 19	Apr-16	Bean	Limeira - SP	20	0.000	0.000	1.000

State abbreviations: BA, Bahia; GO, Goiás; MA, Maranhão; MS, Mato Grosso do Sul; MT, Mato Grosso; PR, Paraná; RS, Rio Grande do Sul; SP, São Paulo.

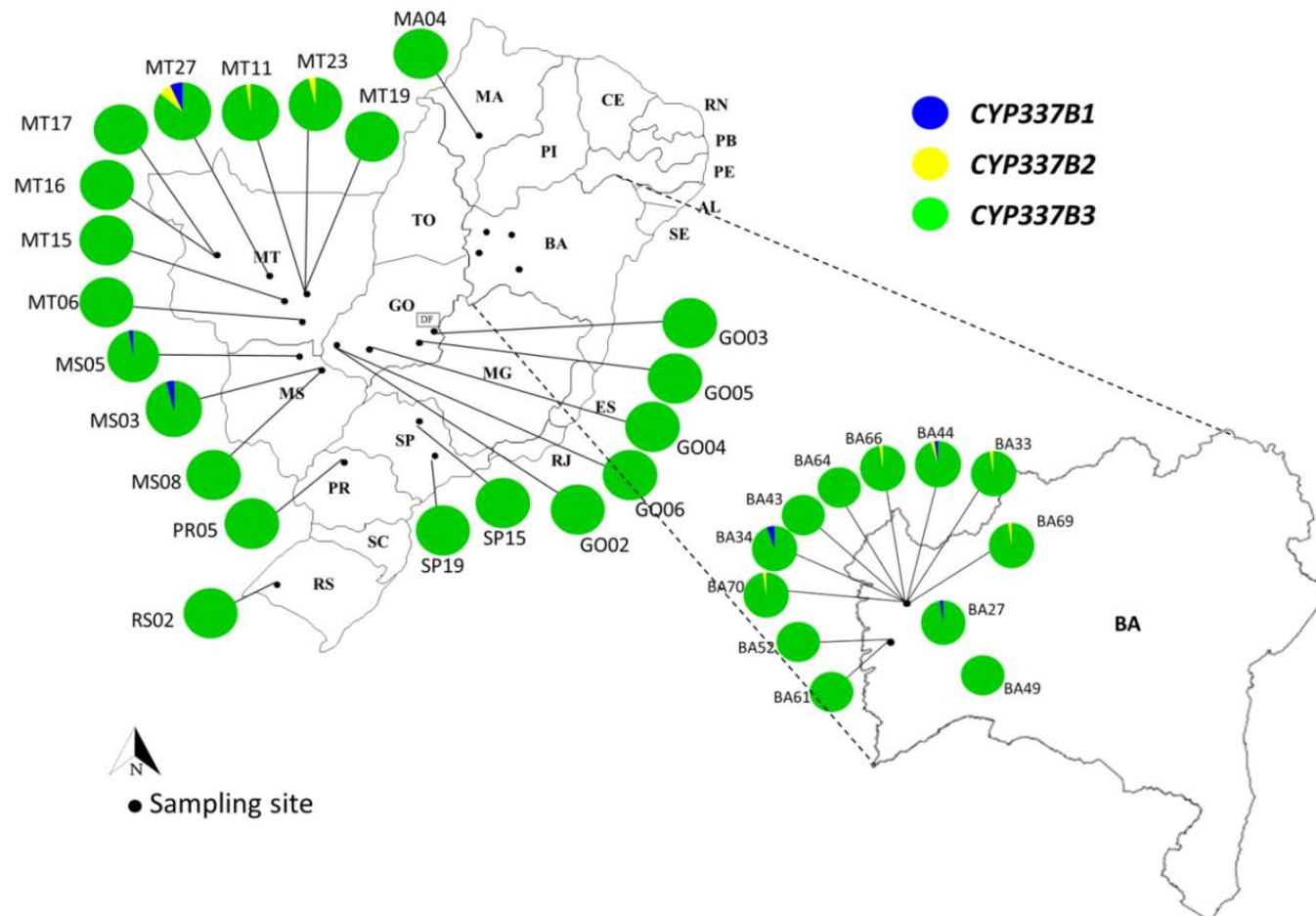


Fig. 3. Geographic distribution and frequency of *CYP337B1*, *CYP337B2* and *CYP337B3* gene subfamilies in *Helicoverpa armigera* (Lepidoptera: Noctuidae) populations in Brazil. State abbreviations: BA, Bahia; GO, Goiás; MA, Maranhão; MS, Mato Grosso do Sul; MT, Mato Grosso; PR, Paraná; RS, Rio Grande do Sul; SP, São Paulo.

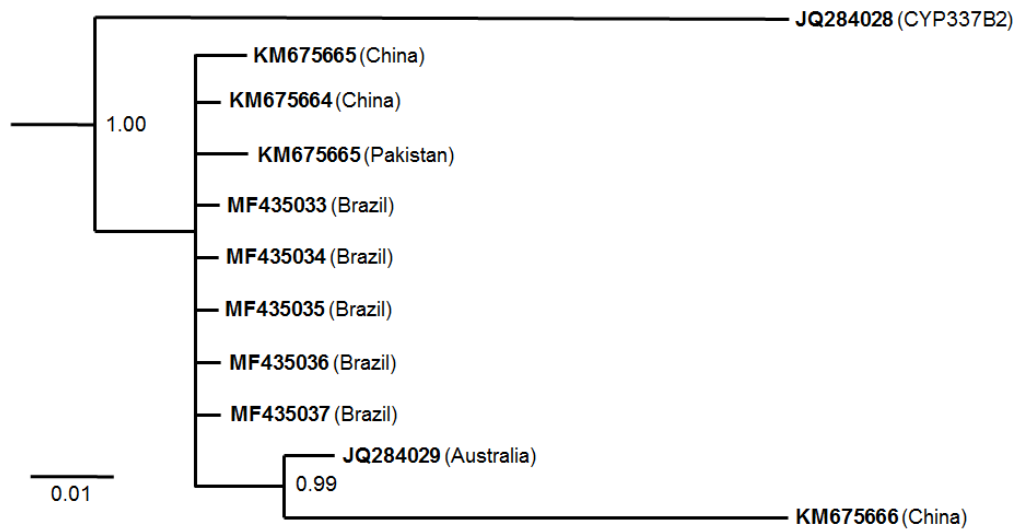


Fig. 4. Bayesian phylogeny of *CYP337B3* subfamily alleles (328 bp) of *H. armigera*. Nodes number shows Bayesian posterior probabilities supports values. Nodes below 50% (bootstrap) or 0.50 (posteriori probability) were not recorded in the tree.

2.4 Discussion

Pesticide resistance and tolerance are commonly reported traits in invasive agricultural pests (Haddi et al., 2012; Wang et al., 2013). Tolerance is considered as the natural ability of a population to resist the toxic effect of an insecticide. It can be developed within a single generation, because of physiological adaptation, as a detoxification process by enzyme induction. The resistance level may decrease when the insects cease to be exposed to the insecticide. Resistance is a genetic change in response to selection pressure by the toxic compound, where a structural genetic change occurs and is also heritable (Yu, 2008). Here, we confirmed the resistance of Brazilian *H. armigera* populations to pyrethroid insecticides. This result was expected based on the many cases of pyrethroid resistance reported in Old World countries that are possible origins of the Brazilian *H. armigera* populations (Martin et al., 2002; Mironidis et al., 2013; Yang et al., 2013; Rasool et al., 2014; Han et al., 2015; Qayyum et al., 2015; Anderson et al., 2016; Xu et al., 2016; Tay et al., 2017).

Brazilian growers have frequently reported control failures for pyrethroid insecticides in the field. This was confirmed by the high larval survival of *H. armigera* populations in our susceptibility-monitoring bioassays (40 to 90% survival for both deltamethrin and fenvalerate). Pyrethroids are also used in Brazil to control other pest species that occur in the same crop field as *H. armigera*, and most of the pyrethroids are sprayed in mixtures with other compounds such as diamide insecticides, contributing to the high selection pressure in the field and the increase in the survival of this species during the growing season.

The high pyrethroid resistance and survival in monitoring tests of *H. armigera* populations in Brazil are higher than reported in its native regions (Rasool et al., 2014; Han et al., 2015; Rossiter, 2008). This could be explained by the high frequency of resistance alleles in *H. armigera* founder individuals and the high selection pressure applied in Brazil. As soon as *H. armigera* was detected, the government agencies recommended pyrethroids as emergency insecticides to control this pest (Embrapa, 2013).

The susceptibility of the *H. armigera* populations assessed here changed over time. Populations collected in summer 2013–2014 (BA33 and BA43) were more susceptible than populations tested in later years (BA69 and GO06). Currently, pyrethroid insecticides have been replaced by diamide, spinosyn or avermectin.

The screening for P450 enzyme suppression with PBO and PCR suggested that the chimeric *CYP337B3* P450 enzyme is an important mechanism of pyrethroid resistance in Brazilian *H. armigera* populations (Joußen et al., 2012; Rasool et al., 2014). The enzyme-suppression bioassays indicate suppression of P450 and esterase enzymes in the three populations tested, which showed almost 100% mortality to both pyrethroid insecticides when exposed to PBO. Furthermore, PCR screening of the chimeric P450 enzyme *CYP337B3* gene showed a frequency higher than 95% in all 33 tested populations from different Brazilian regions. The chimeric P450 enzyme *CYP337B3* had been described as promoting cross-resistance to fenvalerate and cypermethrin, and our results suggest cross-resistance to deltamethrin (Rasool et al., 2014).

The chimeric *CYP337B3* is derived from an unequal crossover from two other P450 gene subfamilies, *CYP337B1* and *CYP337B2*, resulting in an enzyme with higher substrate specificity and capacity to detoxify pyrethroid molecules (Joußen et al., 2012). Brazilian *CYP337B3* alleles are more similar to the Chinese alleles and Pakistan allele when compared to the Australian allele. This fact reinforces the hypothesis of Asiatic origin of Brazilian *H. armigera* individuals (Leite et al., 2014; Anderson et al., 2016; Tay et al., 2017).

We detected only 14 susceptible genes (*CYP337B1* or *CYP337B2*), and found no homozygous susceptible individuals. Synergist bioassays indicate esterase enzymes as a secondary pyrethroid resistance mechanism in Brazilian *H. armigera* populations, since treatment with the synergist DEF, an inhibitor of esterase enzymes, increased the mortality of *H. armigera* individuals when applied together with deltamethrin and fenvalerate. The mortality varied among populations and insecticides, with higher mortality when DEF was applied before deltamethrin. Thus, we suggest that a secondary pyrethroid resistance mechanism confers differing pyrethroid susceptibilities among *H. armigera* populations in Brazil. Enhanced

esterase enzyme production was also reported to cause resistance in Australian and Old World *H. armigera* populations (Gunning, 1996; Gunning et al., 1999; Kranthi et al., 2001; Young et al., 2005; Achaleke et al., 2009). However, the esterase enzymes have been associated with a high fitness cost, while the chimeric P450 *CYP337B3* enzyme has not so far been shown to be associated with a fitness cost (Gunning, 1996; Gunning et al., 1999; Gunning et al., 2005; Gunning et al., 2007; Gunning & Moores, 2010).

In summary, Brazilian *H. armigera* populations showed high tolerance to pyrethroids, and our results suggest that the chimeric P450 enzyme *CYP337B3* is an important mechanism of pyrethroid resistance. However, since our diagnostic dose of 10 µg a.i./larva resulted in a lower mortality than the LC₅₀ of 2 µg a.i./larva in the Australian population homozygous for *CYP337B3*, it is likely that additional P450s and perhaps esterases also contribute to pyrethroid resistance in Brazilian populations of *H. armigera*.

These important results may stimulate Brazilian growers, companies and government agencies to implement a resistance-management program using the successful example of the Australian Insecticide Resistance Management strategy for pyrethroids. Through this program, since the application of pyrethroids was strictly limited to once a year, *CYP337B3*-mediated pyrethroid resistance increased only slowly over a 10-year period (Forrester, 1990; Forrester et al., 1993). This demonstrates the importance and effectiveness of insecticide resistance-management strategies.

The high frequency of the chimeric P450 enzyme *CYP337B3* gene in *H. armigera* populations indicates that pyrethroid insecticides should not be recommended in Brazil or in the rest of the Americas, due to the high dispersal capacity and gene flow among *H. armigera* populations (Feng et al., 2005; Jones et al., 2015; Anderson et al., 2016; Leite et al., 2016). Resistance-management programs for chemical insecticides and *Bt* are crucial and must be implemented with urgency to avoid the loss of commercial products that will make it more difficult to manage this invasive species in the field.

3. Conclusions

- ✓ *H. armigera* populations in Brazil are highly resistant to pyrethroids;
- ✓ *CYP337B3* alleles found in Brazil are the same found in China and Pakistan confirming its pest origin;
- ✓ Pyrethroids should not be recommended to control this pest.

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6. FINAL CONSIDERATIONS

The results found in the current study confirm the hypothesis that *H. armigera* individuals who invaded Brazil were already resistant to pyrethroids. Here we prove that one of the main mechanisms of pyrethroid resistance, the chimeric P450 *CYP337B3*, is present in every insect of *H. armigera* in Brazil, in a frequency of > 95% in most of the populations sampled from 2013 to 2017. We also prove that the inheritance of this resistance has a dominant characteristic being also autosomal, two factors that contribute for the rapid spread of the resistant allele increasing resistance evolution rate in the field. Furthermore, our findings suggest that pyrethroid resistance in *H. armigera* populations in Brazil is due not by a unique gene but there are probably other mechanisms conferring the high resistance found. The results show that the susceptibility of *H. armigera* to indoxacarb decreased throughout the cropping years and if it may become a problem in the future and as the laboratory selected strain also possesses the *CYP337B3* gene, it is possible the existence of a multiple resistance case in Brazil. The two non-synonymous mutations found in the sodium channel and also the high expression of some other P450s must be more explored to confirm their linkage with pyrethroid or indoxacarb resistance.

Based on these findings we highlight the importance to know and monitor the pest in the field in order to choose the best control methods based in the concept of integrated pest management (IPM), integrating different tactics such as biological, chemical, cultural, biotechnology control. In the case of an invasive pest, it is very important to know and understand the resistance background in its countries of origin and recommend a program for emergency control based on this background. In the case of *H. armigera* in Brazil, the authorities recommended pyrethroids in emergency use however the resistance frequency in the countries of its pest origin was extremely high. Finally, our results suggest that the implementation of a resistance management program for *H. armigera* in Brazil is urgent and essential if we want to keep our fields productivity and sustainability. The implementation of an IRM program in Brazil is crucial to preserve the insecticide molecules and also Bt toxins available and effective for a longer period of time in the field.