Journal of Agricultural Science; Vol. 14, No. 4; 2022
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Geographic Origin and Host Dependent Metabolic Responses Affect *Spodoptera frugiperda* Susceptibility to Insecticides

Indyra F. Carvalho[1], Larissa L. Machado[1], Camila G. Neitzke[1], Larissa L. Erdmann[1], Lauren T. Oliveira[1], Ivan R. Carvalho[1], Moises J. Zotti[1] & Ana Paula S. Afonso da Rosa[2]

[1] Department of Crop Protection, Federal University of Pelotas (UFPel), Capão do Leão, Rio Grande do Sul, Brazil

[2] Brazilian Agricultural Research Corporation (EMBRAPA), Capão do Leão, Rio Grande do Sul, Brazil

Correspondence: Indyra F. Carvalho, Department of Crop Protection, Federal University of Pelotas (UFPel), Eliseu Maciel AVE, Capão do Leão, RS 96050-500, Brazil. E-mail: indyrafaria@gmail.com

Received: August 13, 2021 Accepted: September 30, 2021 Online Published: March 15, 2022

doi:10.5539/jas.v14n4p177 URL: https://doi.org/10.5539/jas.v14n4p177

Abstract

Spodoptera frugiperda is one of the most harmful pest threatening crops in tropical regions. It is particularly difficult to control due to its high polyphagia, mobility and the evolution of resistance. One of the main strategies enabling this insect to extend its geographic range, is the expression of a wide repertoire of detoxification enzymes, that neutralizes diverse environmental xenobiotics as well as insecticides. Hence adaptation to particular ecological niches can lead to different enzyme expression which consequently may affect its control. Even though the molecular basis of adaption is well known, there is not much applied studies regarding the effect of the geographic region in *S. frugiperda* physiology and management. Thus, the objective of this work was to determine the activity of detoxification enzymes, as well as the susceptibility to insecticides of *S. frugiperda* from distinct geographic origins in local hosts plants. Larvae from Pelotas, a region characterized by crop succession with oats and maize and larvae from Cascavel, which is characterized by highly intensive maize systems thought the year were used in the experiments. Our study showed that only larvae from Pelotas had increased enzyme activity after feeding on oat plants. In most insecticide treatments, mortality increased on population Cascavel after feeding on oats, the same was not observed on larvae from Pelotas. Our results are in accordance with previous studies that pointed distinct geographic conditions could lead to genetic variability and different responses to control. Information on *S. frugiperda* dynamics is essential to develop local management programs as well to better understand the complex nature of plant-insect interactions.

Keywords: detoxification, fall armyworm, genetic variability, resistance, local host, populations

1. Introduction

The Fall Armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) is a major pest of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and rice (*Oryza sativa* L.) crops (Pogue, 2002). It has a high dispersion capacity and can feed on more than 80 plant species, including weeds and winter crops, which favors its adaption thought the year in all tropical and subtropical regions of the American continent and more recently, also in African and Asian countries (Nagoshi et al., 2008; Goergen et al., 2016; EPPO, 2018). Its main form of control is through synthetic insecticides and transgenic crops expressing *Bacillus thuringiensis* Berliner (*Bt*) proteins. However, there are already several reports of control failures attributed to the evolution of resistance (Yu et al., 2003; Monnerat et al., 2015; Nascimento et al., 2016; Yang et al., 2018; Gutiérrez-Moreno et al., 2019).

Resistance to chemical insecticides is mainly caused by enhanced enzymatic detoxification, that sequestrate and metabolize xenobiotic molecules into non or less toxic forms (Yu et al., 2003; Després et al., 2007). In fact, enzymatic detoxification is one of the most common defense mechanisms of insects and plants (Després et al., 2007). This process relies on large superfamilies of enzymes, such as carboxylesterases (CarE), Glutathione-S-transferases (GSTs) and cytochrome P450 monooxygenases, with varied substrate specificities that enables insects to catalyze a plurality of different reactions. For that reason, they are pointed as the main mechanism to mediate resistance to insecticides as well as to plant allelochemicals (Yu, 1991; Després et al., 2007; Nitao et al., 2003; Hartmann et al., 2005).

Moreover, the expression of theses enzymes can be influenced by various factors other than exposure to xenobiotics, such as plant species and diversity in regional zones, developmental stages of insects, and amount and chemical composition of host plants (Després et al., 2007). This can guide populations to increase the level of adaptation, and therefore, enhance greater genetic similarity between populations that evolved in the same host plant or same landscape (Petersen et al., 2001). Hence, the selection pressure over specific detoxification, digestion and chemosensory genes, can result in genetic variability, especially in highly mobile and polyphagous insects such as *S. frugiperda* (Pashley, 1988; Nosil et al., 2002; Martinelli et al., 2007). Being that, it is expected that FAW from different geographic origins will have different detoxification enzyme expression that can lead to changes in susceptibility to synthetic insecticides, since these enzymes are also involved in resistance to these molecules (Wood et al., 1981; Xue et al., 2010).

There are several molecular studies evidencing the role hosts and the geographic distance have on genetic variability in *S. frugiperda* populations (Busato et al., 2004; Martinelli et al., 2007; Souza et al., 2015; Gouin et al., 2017). These authors also report that this could have major impact in economical entomology, as it may lead to different responses to strategies of control. However, there is not much applied studies regarding how phenotypic plasticity of FAW populations from different geographic zones can affect this pest physiology and management. Therefore, the present study, aimed to determine the activity of detoxification enzymes and the susceptibility to insecticide of *S. frugiperda* from distinct geographic origins, under different host regimens. This information is essential to develop more effective strategies for managing this pest and to understand the plant-insect interactions in any particular region.

2. Material and Methods

2.1 Insect Colonies

Spodoptera frugiperda specimens (n = 300) were collected from the city of Pelotas, Rio Grande do Sul-BR (Pelotas population) (31°46′19″ S, 52°20′34″ W) and from the city of Cascavel, Paraná-BR (Cascavel population) (24°36′ S, 51°23′ W) from leaves and stems of maize plants. They were sent to the Brazilian Agricultural Research Corporation (Embrapa) in the county of Capão do Leão, Rio Grande do Sul-BR, where they were individualized and reared on artificial diet by Greene, Leppla, and Dickerson (1976), according to the methods of Parra (2001) (25±2 °C, 70±10% RH and 14h10 [L:D] photoperiod).

2.2 Hosts

For this study, oat plants (*Avena strigosa*) (BRS139 Neblina cultivar) were established as an alternative host, since in southern Brazil, especially in the Pelotas region, it is a commonly cultivated crop, adopted in rotation and/or succession with soybeans (*Glycine max* L.) and maize in the winter (IPARDES, 2018). Oats seeds were sown in plastic trays (30.0 × 20.0 × 6.00 cm), kept in a refrigerated room at 18 °C, 70% humidity and photoperiod of 10 hours and watered daily. When leaves were about 10-20 cm height they were used in the experiments. Maize plants (*Zea mays*) (AG 9045 cultivar) were stablished as the main host. They were sown in 20 L vases, filled with soil and kept in a greenhouse until stage V3 (40 days after emergence) when they were collected and used in the experiments.

2.3 Host Plant Regimen

First instar larvae from each population (n = 200) were individually transferred from the artificial diet to 16-well plastic trays (2.8 × 4.1 × 1.6 cm) (B16-Biossuply) containing maize leaves (5 × 3 cm) to establish the maize-F_0 population. After two generations feeding on maize (F_1 and F_2), F_2 neonates were transferred individually to trays containing oat leaves, in which they were kept until pupation for two generations (F_3 and F_4). Subsequently, F_4 neonates were transferred to maize, in which they were conducted for the last two cycles (F_5 and F_6). These insects were maintained in a controlled environment (25±2 °C, 70±10% RH and 14h10 [L:D] photoperiod) until the end of the experiments. Plant leaves were replaced on a daily basis to avoid excessive water loss.

Bioassays were performed using third instar larvae (~0.9 cm long) collected after two generations in each tested host (F_2-maize, F_4-oat and F_6-maize, respectively). Assays were conducted this way, to simulate Brazilian field conditions, in which *S. frugiperda* can go through two or three generations (~105 days) in each host from sowing to harvesting (Figure 1). Larvae from the artificial were used as a control.

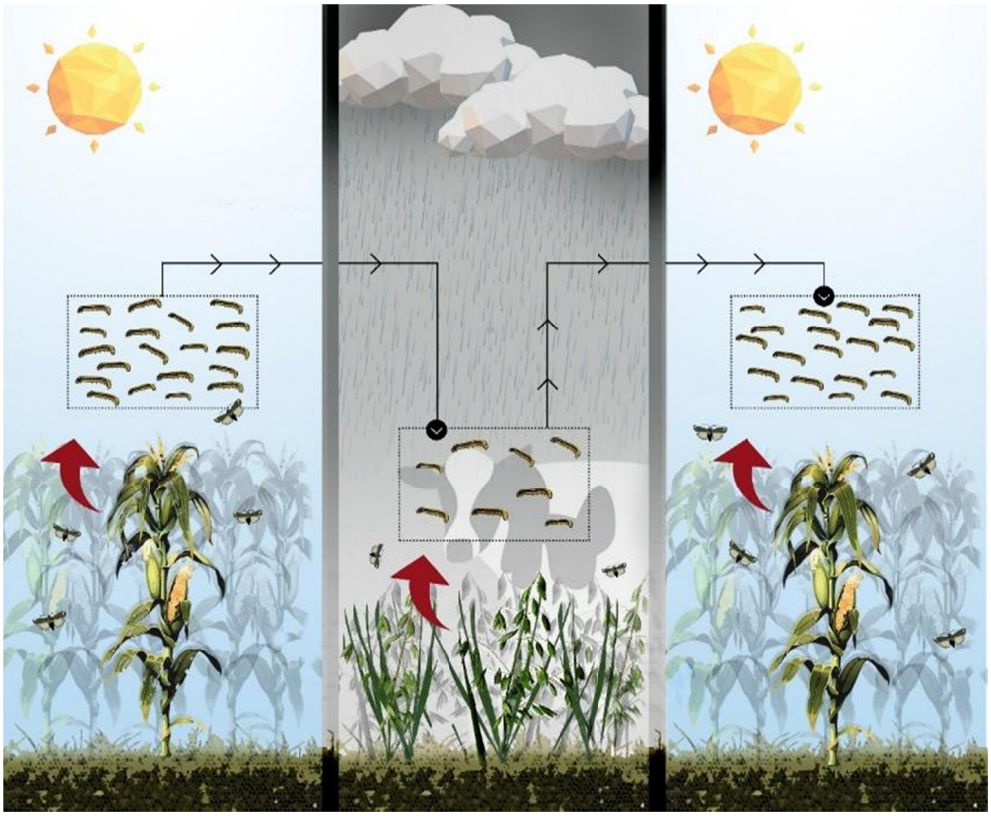

Figure 1. Illustrative scheme of *S. frugiperda* in an successive/crop rotation in a maize-oats regimen in southern Brazil. Photo by Indyra Carvalho, 2019

2.4 Biochemical Assay-Enzyme Activity

2.4.1 Tissue Preparation

Tissue homogenates from whole larvae were used as enzyme sources. Third instar larvae (~0.9 cm long) of each population (n = 24), from the natural hosts (F_2-maize, F_4-oat, and F_6-maize) and from the artificial diet were placed in groups of 3 (~40 mg) in Eppendorfs tubes, homogenized in 400 µl of 0.1 M phosphate buffer, pH 7.0 and then centrifuged at 12,000 g at 4 °C for 15 min. The resulting supernatants were diluted in 400 µL of buffer pH 7.0 (0.1 M), held in ice to reduce proteolysis and used in enzyme assays within 30 min of preparation. Each reaction was run in triplicate (technical replicate) with eight independent biological replicates. Protein concentrations were measured by the method of Bradford (1976) with bovine serum albumin (BSA; Sigma Aldrich, St. Louis, MO) as the standard.

2.4.2 Esterase Activity

Esterase activity was obtained by measuring α-naphthol-Fast Blue B conjugation, according to the methods of Gomori (1953), and Harold and Ottea (2000). The substrate solution was prepared by adding 600 µL of 1-α Naphthyl acetate (Sigma Aldrich, St. Louis, MO) (0,113 M, previously dissolved in 50% acetone in water) to a solution of Fast Blue B salt (90%, Sigma Aldrich, St. Louis, MO) (18 mg in 30 mL of 0.1 M phosphate buffer, pH 7.0), and then filtered using Whatman no. 3 filter paper on ice. The reaction mixtures were placed in individual wells of a microtiter plate (n = 8) in triplicates containing 240 µL of substrate solution and 10 µL of larval homogenate, they were incubated at 30 °C, and the rate of change in absorbance during the initial 10 min was measured at 595 nm (Endpoint) using a microplate reader (Versa-Max, da Molecular Devices) Data were corrected for non-enzymatic activity using incubations without protein as the control. Changes in OD were converted to nmol min^{-1} using the α-Naphthyl extinction coefficient (0.075 mM) and corrected by the total protein content.

2.4.3 Glutathione-S-Transferase Activity

Glutathione-S-transferase (GST) activity was assessed by measuring GST conjugative activity with reduced glutathione (GSH) using 1-chloro2,4-dinitrobenzene (CDNB) as substrate, according to the method of Hemingway (1998). The working solution was prepared by adding 1 mL of CDNB (21 mM, previously dissolved

in 1 mL of Methanol) (99%, Sigma Aldrich, St. Louis, MO) to 20mL of GSH solution (98%, Sigma Aldrich, St. Louis, MO) (61.5 mg in 20 mL of 100 mM phosphate buffer, pH 6.5).

The reaction mixtures were placed in individual wells of a microtiter plate (n = 8) in triplicates, containing 195 mL of the CDNB/GSH solution and 15 µL of larval homogenate, they were incubated at 25 °C and the rate of change in absorbance during the initial 20 min was measured at 340 nm (Endpoint) using a microplate reader (Versa-Max, da Molecular Devices) Data was corrected for non-enzymatic activity using incubations without protein as the control. The absorbance values were transformed to nmol min^{-1} using the extinction coefficient of CDNB (4.39) and the spectrophotometer path length (0.6 cm) than corrected by the total protein content.

2.5 Insecticide Efficiency

These biological assays evaluated the susceptibility of *S. frugiperda* populations from each host (F_2-maize, F_4-oat and F_6-maize) and artificial diet to ethofenproxy (Safety®) (300 g i.a. L^{-1}, pyrethroid), thiamethoxam (141 g i.a. L^{-1}, neonicotinoid) + lambda cyhalothrin (Engeo Pleno®) (106 g i.a. L^{-1}, pyrethroid), spinosad (Tracer®) (480 g i.a. L^{-1}, spinosyns), methomyl (Upmyl®)(215 g i.a. L^{-1}, oxime methylcarbamate). Insecticides with distinct mode of action were chosen since rotating mode of actions is a key component in resistance management and integrated pest management (IPM) strategies. All products and doses used are registered by the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA, 2019).

Third-instar larvae (~0.9 cm long) of both populations, from each host were conditioned in Petri dishes (9.0 cm in diameter) (n = 25), for each insecticide treatment (n = 5). This bioassay used the direct contact method, in which the application of 1mL of the insecticide solution was sprayed in the petri dishes under a Potter Spraying Tower (Burkard Scientific, Uxbridge, UK) in constant pressure of 10 bar, corresponding to an average deposition of 2.499±0.305 mg cm^{-2}. Distilled water was used as control. The order of applications was randomized and the Potter's tower was washed with 95% alcohol and dried with disposable paper towels from one treatment to another.

After spraying, larvae were individualized in 100 mL polystyrene disposable cups containing each respective host and were maintained in controlled environment conditions (25±2 °C, 70±10% RH and 14h10 [L:D] photoperiod), food was replaced every other day. The number of alive and dead larvae was evaluated daily until 120 hours after treatment (HAT). The absence of coordinated movements when touched with a fine brush was used as a criterion of death. Mortality was calculated and corrected in reference to natural mortality in the control using Abbott's (1925) formula. According to Abbott (1925), if mortality is greater than 80%, an insecticide can be considered efficient.

2.6 Data Analysis

Esterases and GST activity data were subjected for analysis of variance (ANOVA) followed by Tukey's multiple comparison test at $\alpha = 0.05$. The percentage data for mortality was subjected to ANOVA assumptions for normality and homoscedasticity (PROC UNIVARIATE) as well as the goodness-of-fit of the models using Chi-square tests. Then, data were submitted to analysis of variance at 5% of probability by the F test to identify the interaction between hosts and origin (PROC GLM). Treatment means were separated by Tukey's test at 5% probability. Statistical analyses were conducted using the software Statistical Analysis System (SAS) version 9.3.

3. Results

3.1 Esterase Activity

Esterase activity was higher in larvae from Pelotas than in the ones from Cascavel, regardless of the food source (Figure 2). Larvae fed on maize from Pelotas (F_2 and F_6) showed significantly lower esterase activity (451.72 nmol min^{-1} mg $protein^{-1}$, *std* = 24.26 and 387.85 nmol min^{-1} mg $protein^{-1}$, *std* = 36.15 respectively) when compared to larvae fed on oat leaves (F_4) (787.44 nmol min^{-1} mg $protein^{-1}$, *std* = 90.46) and artificial diet (565.34 nmol min^{-1} mg $protein^{-1}$, *std* = 44.34) ($P < 0.001$). On the other hand, larvae from Cascavel had higher esterase activity when fed on maize (F_2) (307.42 nmol min^{-1} mg $protein^{-1}$, *std* = 36.92 and artificial diet (489.48 nmol min^{-1} mg $protein^{-1}$, *std* = 120.95) and lowest activity in the last generation on maize (F_6) (154.81 nmol min^{-1} mg $protein^{-1}$, *std* = 21.94) ($P < 0.001$).

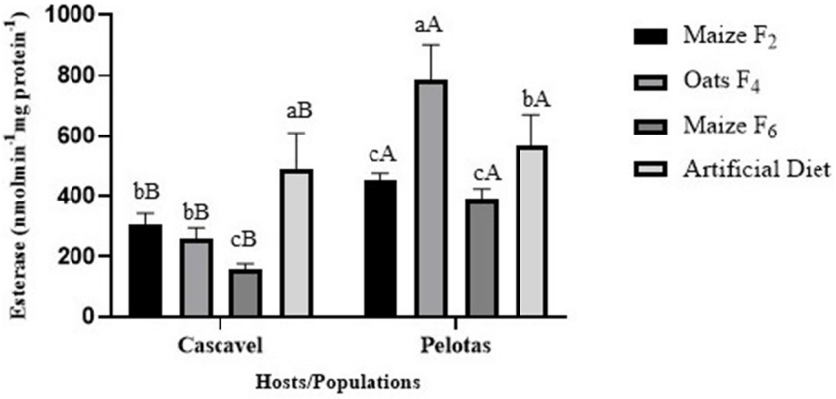

Figure 2. Esterase activity (nmol min^{-1} mg $protein^{-1}$) (means±SEM) in *Spodoptera frugiperda* populations fed on maize (F_2), oats (F_4), maize (F_6) and artificial diet. Means followed by distinct upper letters differ between populations and lower distinct letters differ between hosts in the same population by Tukey test ($P > 0.05$)

3.2 Glutathione-S-Transferase Activity

GST was also more active in larvae from Pelotas than the ones from Cascavel, regardless of the food source (Figure 3). However, larvae fed on oats (F_4) from Pelotas showed significantly higher GST activity (22.00 nmol min^{-1} mg $protein^{-1}$, *std* = 4.63) than the ones fed on maize (F_2 and F_6) (14.01 nmol min^{-1} mg $protein^{-1}$, *std* = 5.14 and 7.09 nmol min^{-1} mg $protein^{-1}$, *std* = 0.54) ($P < 0.001$). Similar results were also obtained from Cascavel larvae, in which the lowest GST activity was also in the last generation in maize (F_6) (3.62 nmol min^{-1} mg $protein^{-1}$, *std* = 0.22), when compared to the other food sources ($P < 0.001$). Cascavel population, however, had no significant difference in GST activity when fed on maize (F_2) (09.07 nmol min^{-1} mg $protein^{-1}$, *std* = 1.122), oat leaves (F_4) (10.14 nmol min^{-1} mg $protein^{-1}$, *std* = 3.38) or artificial diet (11.60 nmol min^{-1} mg $protein^{-1}$, *std* = 1.89) (P > 0.578).

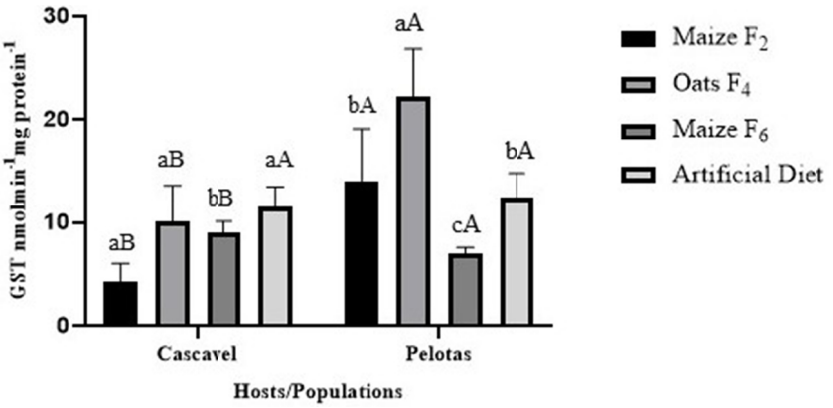

Figure 3. GST activity (nmol min^{-1} mg $protein^{-1}$) (means±SEM) in *Spodoptera frugiperda* populations fed on maize (F_2), oats (F_4), maize (F_6) and artificial diet. Means followed by distinct upper letters differ among populations and lower distinct letters differ between hosts in the same population by Tukey test ($P > 0.05$)

3.3 Insecticide Susceptibility

The susceptibility of the Cascavel and Pelotas populations to insecticides in response to the diet is shown in Table 1. Mortality between populations varied according to the insecticide tested and the diet. Mortality for methomyl was high for both populations, especially for insects from the population of Pelotas fed on maize (F_2) (100.0%). However, there is a tendency for the population of Cascavel to be more sensitive to the use of this insecticide. For the treatment with spinosad, populations fed maize (F_2) showed low mortality, for the other food sources the mortality was higher than 80%, especially for the food maize (F_6), which, regardless of the population, was observed 100% mortality. For the tested insecticides, etofenproxi had the least effect on the mortality of *S. frugiperda*. For the treatment with mortality, the highest mortality was observed for the population of Pelotas, and

only for insects fed on maize (F_2) the efficiency observed was lower than 80%. For the Cascavel population, the highest mortality was for oat-fed insects (F_4) (72%), but still below recommended (80%).

Table 1. Corrected mortality (%) of *Spodoptera frugiperda* populations 120 HAT, fed on maize (F_2), oats (F_4), maize (F_6) and artificial diet

	Cascavel	Pelotas
	---------- Mortality (%±SE) ----------	
Methomyl		
Maize (F_2)	84.00±0.07 bcB	100.00±0.20 aA
Oats (F_4)	96.00±0.04 aA	88.00±0.06 bB
Maize (F_6)	92.00±0.05 abA	70.00±0.09 cB
Artificial Diet	76.00±0.08 cA	80.00±0.08 bA
Espinosad		
Maize (F_2)	12.00±0.06 cA	23.00±0.09 dA
Oats (F_4)	92.00±0.05 bA	72.00±0.09 bA
Maize (F_6)	100.00±0.00 aA	100.00±0.00 aA
Artificial Diet	72.00±0.19 bA	48.00±0.10 cB
Etofenproxi		
Maize (F_2)	8.00±0.05 cA	00.00±0.00 cB
Oats (F_4)	16.00±0.07 bB	36.00±0.09 aA
Maize (F_6)	64.00±0.09 aA	35.00±0.10 aB
Artificial Diet	16.00 ±0.07 bA	8.00±0.05 bB
Lambda-cyhalothrin + Thiamethoxam		
Maize (F_2)	52.00±0.19 bA	55.00±0.10 cA
Oats (F_4)	72.00±0.09 aA	84.00±0.07 abA
Maize (F_6)	68.00±0.09 aB	91.00±0.05 aA
Artificial Diet	60.00± 0.10 abB	72.00±0.09 bA

Note. [a] Mortality (%) values within a line followed by the same upper letter are not significantly different (F test, $P > 0.05$).

[b] Mortality (%) values within a column followed by the same lower letter are not significantly different (Tukey's test, $P > 0.05$).

4. Discussion

Oat plants produce important secondary metabolites in its vacuoles, such as flavonoids and saponisins, which are known to be strong inducers of detoxification enzymes in *S. frugiperda*, even at low concentration levels (Maizel et al., 1964; Wheeler et al., 1993; Bahraminejad et al., 2017). In addition to that, previous biological studies indicated that oats were suitable for *S. frugiperda* development, with survival rates, pupal weight, larval and pupae biomass very similar to the ones observed in maize and artificial diet (Dias et al., 2016; Silva et al., 2017). Therefore, it is consequently expected that this food source could decrease susceptibility to insecticide by activating or stimulating its main detoxification mechanism as well as being chemically balanced to favor resilience to stressful conditions in this species.

However, our data showed that enzymatic response to the host plant and insecticide susceptibility varied greatly in larvae from distinct geographic origins. We observed increased enzyme activity in population Pelotas when fed on oats, which is in accordance with other studies in which flavones (abundant secondary metabolite in oat leaves) induced GSTs and Esterases (Wheeler et al., 1993; Giraudo et al., 2015). In contrast, enzyme activity in population from Cascavel had no significant increase when fed on maize (F_2) or oats (F_4). Therefore, if allelochemicals were responsible for enzyme induction, it was triggered by different mechanisms and/or had different sensitivity levels in each population.

This result can be attributed to prior feeding experience of Pelotas larvae on regional host plants as well as the effect of environmental conditions inherent by it, since comparable selection pressure can occur across crops, increasing overall selection pressure for resistance (Roush, 1989; Golikhajeh et al., 2017). Pelotas-RS, is located in

the extreme southern Brazil, in a region known as lowlands, these areas have a very particular climate, with high pluviometry rates and low temperatures in the winter, that makes its cropping systems very different from the rest of the country. It is also more geographically closer to Argentina and Uruguay than to most Brazilian states, which may limit the genetic flux among other Brazilian populations.

In Lowlands rice crops are predominant in the landscape and maize is used only as an alternative to crop rotating/succession systems aiming to control weeds and diseases. Another common practice is the adoption of cover crops in the winter, such as oats (*Avena strigosa*) birdsfoot trefoil (*Lotus maizeiculatus* L.) and rye-grass (*Lolium multiflorum* L.) (ASRGC, 2016). In contrast, maize crops from Cascavel, PR, are characterized by highly mechanized systems throughout the year, with cover crops used in rotation only by small and medium scale farmers (IPARDES, 2018). *S. frugiperda* from this region, are also located next to other production landscapes from Brazil, with more favorable climate conditions, which can favor its migration and the genetic flow to these regions.

S. frugiperda from Pelotas have to metabolize a wider set of hosts under a very specific climate condition, which may have led to greater fitness on regional host plants where they were collected (Percy & John, 2007, Singer & McBride, 2009). Local adaptation is an evolutionary process that facilitates an organism's survival in a particular environment (Golikhajeh et al., 2017). Insects can up or down regulate detoxification enzymes when they encounter unfavorable conditions, as well as other important physiological pathways, like digestion, by overexpressing proteases and α-amylase in the larval midgut, for example, that may enable the insect to overcome the stressful scenario (Glendinning, 2002; Golikhajeh et al., 2017)

Hence, a good fitness of larvae in a local host, may be one of the factors influencing resilience to toxic compounds, such as synthetic insecticides. As observed in this study, mortality is significantly associated with the insecticide used, the population of Cascavel was more susceptible to the insecticide metomyl, while the population of Pelotas was more susceptible to the insecticides etofemproxy and lambda-cialotrian+tiamethoxam. These differences may be associated with the resistance of populations resulting from the selection pressure of each environment. Significant differences in susceptibility among Pelotas and Rattlesnake were observed when larvae were reared on the artificial diet, indicating that the host plant could have influenced insecticide performance.

Therefore, these high levels of enzymes in Pelotas larvae when fed on oats may be part of an overall fitness and adaption to its landscape, which can contribute to the restructuring of high densities of this pest in the field in the absence of maize, even when control measures are applied. The same effect is not expected in population Cascavel, since oats seemed to have had a detrimental or no effect on the insect resilience to insecticide, in spinosad, etofenproxi, and methomyl treatments, mortality increased over 80.0% after feeding on oat plants, that also influenced insecticide performance on the subsequent generations on maize (F_6).

Managment of this pest in different agrosystem is another important aspect that can have influenced our results. Over-expression of esterases, GST and P450s genes as well as mutations are common in insecticide resistant populations (Li, Schuler, & Berenbaum, 2007). Considering the different origins of the larvae in this study, the low insecticide efficiency and the increased enzyme activity in population Pelotas could be due to the evolution of resistance to these products, as a result of the selection pressure in its local agrosystem. An interesting point, is that if resistance is evolving in these populations, it can be probably host dependent since insecticide efficiency varied in great extension when host plants were altered, this is one great strategy to investigate for resistance management.

In fact, resistance is commonly pointed as being a result of elevated levels of enzymes to model subtracts and/or constitutive overexpression of these genes in resistant strains. Since most pesticides in use are esters of substituted phosphoric, carbamic or cyclopropane carboxylic acids, they can consequently be subject to degradation by P450s, Esterases and GSTs (Devonshire, 1991). However, demonstrating a direct link between them, is not that simple. *S. frugiperda* genome has about 117 CYP (encoding for P450s enzymes), 46 GST and 96 EST genes, each of them encodes for a different enzyme, that can catalyze a very specific reaction or a number of them, depending on subtract specifity, isoforms and its relation to other enzyme pathways (Feyereisen, 1999; Yu, 2002; Gouin et al., 2017).

There are cases in which the defense mechanism to metabolites from a pest to their host plant is also used in defense to other toxic chemicals. For example, the cotton bollworm (*Helicoverpa armigera*) tolerant to gossypol has high midgut P450 enzyme activities that enhance tolerance to deltamethrin (Tao, Xue, Huang, Chen, & Mao, 2012). *S. littoralis* and *S. exigua* induces the same CYP genes with plant compounds (quercetin, cinnamid acid, tannin) and synthetic insecticides (deltamethrine, methoxyfenozide) (Wang et al., 2018). Hence, in the present study we are cautious to make a direct link of resistance and increased enzyme activity.

Furthermore, there is a number of studies evidencing molecular variation among geographic populations, mostly linked to host plant adaptions, however how this impact applied studies, regarding control and management has been very little explored (Johnson, 1987; Busato et al., 2004; Martinelli et al., 2007; Souza et al., 2015). Souza et al. (2015) particularly interested us, they demonstrated a significant effect of the geographic isolation in the genetic variability among six populations of *S. frugiperda* from Brazilian regions, among them were larvae from Pelotas and Cascavel. By clustering and population structure analyses using SSR markers, they identified two major distinctive groups, one of them composed by Cascavel and four other populations, and the other represented only by larvae from Pelotas. They pointed that the genetic distance observed in the dendrogram for this two major group was significantly associated with the geographic distance, probably due to growing conditions and distinguished biome. It was also suggested that *S. frugiperda* from Pelotas may be affected by a putative geographical isolation and for that, gene flow is limited. This corroborates with our data and may indicate that specific control tools to the FAW especially for Pelotas population, may be needed.

Additionally, even though the focus of the present work was not to qualify or quantify allelochemicals in local hosts, it would be interesting to explore this in future research. Especially because these compounds can be used in local pest management strategies or even in plant breeding programs. There are a number of studies exploring plant allelochemicals such as phenols and α, β-unsaturated carbonyl compounds that can inhibit GSTs and CarEs in the FAW and increase insecticide toxicity (Yu & Abo-Elghar, 2000; Bullangpoti et al., 2012). There are also a number of studies demonstrating the opposite effect, in which allelochemicals present in the diet, such as monoterpenes and flavonoids stimulated these enzymes, enabling *S. frugiperda* to be more tolerant to insecticides (Yu & Ing, 1984; Caballero et al., 2008; Ofosuhene et al., 2009; Giraudo et al., 2015). In either way, this information has exploitation potential in local programs.

To conclude, our study showed that susceptibility of FAW larvae to insecticides was influenced to a greater extent by the host plant. The pattern and intensity of this influence in enzyme activity, though seemed highly dependent on this pest life history in the region where they were collected. That being the case, we suggest that the variation of detoxifying enzyme's activity and susceptibility to insecticides among different geographical populations were a result of past environmental interactions. Nevertheless, to obtain more knowledge regarding the geographic effect on this pest's physiology, more studies on genetic variation and biological parameters on local hosts are needed. This information can contribute to our knowledge of the highly complex nature of plant-herbivore interactions and can support the development of local and regionally adapted control programs.

Acknowledgements

We gratefully acknowledge the financial support for this research by the Brazilian Agricultural Research Corporation (EMBRAPA), Lowland Station, Pelotas-RS.

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*(2), 265-267. https://doi.org/10.1093/jee/18.2.265a

ASRGC. (2016). *Atlas Socioeconômico do Rio Grande do Sul*. Retrieved from https://atlassocioeconomico.rs.gov.br/milho

Bahraminejad, S., Asenstorfer, R. E., Riley, I. T., & Schultz, C. J. (2007). Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *Journal of Phytopathology, 0*(0), 071003002748001. https://doi.org/10.1111/j.1439-0434.2007.01309.x

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Bullangpoti, V., Wajnberg, E., Audant, P., & Feyereisen, R. (2012). Antifeedant activity of Jatropha gossypifolia and Melia azedarach senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergists. *Pest Management Science, 68*(9), 1255-1264. https://doi.org/10.1002/ps.3291

Busato, G. R., Grützmacher, A. D., Oliveira, A. C. de, Vieira, E. A., Zimmer, P. D., Kopp, M. M., ... Magalhães, T. R. (2004). Análise da estrutura e diversidade molecular de populações de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) associadas às culturas de milho e arroz no Rio Grande do Sul. *Neotropical Entomology, 33*(6), 709-716. https://doi.org/10.1590/S1519-566X2004000600008

Caballero, C., López-Olguín, J., Ruiz, M., Ortego, F., & Castañera, P. (2008). Antifeedant activity and effects of terpenoids on detoxication enzymes of the beet armyworm, *Spodoptera exigua* (Hübner). *Spanish Journal of Agricultural Research, 6*(S1), 177. https://doi.org/10.5424/sjar/200806S1-386

Després, L., David, J.-P., & Gallet, C. (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology & Evolution, 22*(6), 298-307. https://doi.org/10.1016/j.tree.2007.02.010

Devonshire, A. L. (1991). Role of esterases in resistance of insects to insecticides. *Biochemical Society Transactions, 19*(3), 755-759. https://doi.org/10.1042/bst0190755

Dias, A. S., Marucci, R. C., Mendes, S. M., Moreira, S. G., Araújo, O. G., Santos, C. A. dos, & Barbosa, T. A. (2016). Bioecology of *Spodoptera frugiperda* (J.E. Smith, 1757) in different cover crops. *Bioscience Journal*, 337-345. https://doi.org/10.14393/BJ-v32n2a2016-29759

EPPO Global Database. (2018). *First report of Spodoptera frugiperda in India* (EPPO Reporting Service No. 08). Retrieved from https://gd.eppo.int/reporting/article-6348

Feyereisen, R. (1999). Insect p450 enzymes. *Annual Review of Entomology, 44*(1), 507-533. https://doi.org/10.1146/annurev.ento.44.1.507

Giraudo, M., Hilliou, F., Fricaux, T., Audant, P., Feyereisen, R., & Le Goff, G. (2015). Cytochrome P450s from the fall armyworm (*Spodoptera frugiperda*): Responses to plant allelochemicals and pesticides: P450 induction in fall armyworm. *Insect Molecular Biology, 24*(1), 115-128. https://doi.org/10.1111/imb.12140

Glendinning, J. I. (2002). How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata, 104*(1), 15-25. https://doi.org/10.1046/j.1570-7458.2002.00986.x

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, noctuidae), a new alien invasive pest in west and central africa. *PLoS ONE, 11*(10), e0165632. https://doi.org/10.1371/journal.pone.0165632

Golikhajeh, N., Naseri, B., & Razmjou, J. (2017). Geographic origin and host cultivar influence on digestive physiology of *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae. *Journal of Insect Science, 17*(1), 12. https://doi.org/10.1093/jisesa/iew104

Gomori, G. (1953). Human esterases. *J Lab Clin Med*, 445-53.

Gouin, A., Bretaudeau, A., Nam, K., Gimenez, S., Aury, J.-M., Duvic, B., … Fournier, P. (2017). Two genomes of highly polyphagous lepidopteran pests (*Spodoptera frugiperda*, Noctuidae) with different host-plant ranges. *Scientific Reports, 7*(1), 11816. https://doi.org/10.1038/s41598-017-10461-4

Greene, G. L., Leppla, N. C., & Dickerson, W. A. (1976). Velvetbean caterpillar: A rearing procedure and artificial medium123. *Journal of Economic Entomology, 69*(4), 487-488. https://doi.org/10.1093/jee/69.4.487

Gutiérrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Terán-Santofimio, H., Rodriguez-Maciel, J. C., & DiFonzo, C. (2019). Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puertorico and Mexico. *Journal of Economic Entomology, 112*(2), 792-802. https://doi.org/10.1093/jee/toy372

Harold, J. A., & Ottea, J. A. (2000). Characterization of esterases associated with profenofos resistance in the tobacco budworm, *Heliothis virescens* (F.). *Arch Insect Biochem Physiol, 45*(2), 47-59. https://doi.org/10.1002/1520-6327(200010)45:2<47::AID-ARCH1>3.0.CO;2-H

Hartmann, T., Theuring, C., Beuerle, T., Klewer, N., Schulz, S., Singer, M. S., & Bernays, E. A. (2005). Specific recognition, detoxification and metabolism of pyrrolizidine alkaloids by the polyphagous arctiid Estigmene acrea. *Insect Biochemistry and Molecular Biology, 35*(5), 391-411. https://doi.org/10.1016/j.ibmb.2004.12.010

Hemingway, K. (1998). Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Medical and Veterinary Entomology, 12*(1), 1-12. https://doi.org/10.1046/j.1365-2915.1998.00082.x

IPARDES (Instituto Paranaense de Desenvolvimento Econômico e Social). (2018). *Paraná detém maior índice de produtividade agrícola*. Retrieved June 14, 2018, from http://www.ipardes.gov.br/index.php?pg_conteudo=1&cod_noticia=943

Johnson, S. J. (1987). Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the western hemisphere. *International Journal of Tropical Insect Science, 8*(4-5-6), 543-549. https://doi.org/10.1017/S1742758400022591

Li, X., Schuler, M. A., & Berenbaum, M. R. (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology, 52*(1), 231-253. https://doi.org/10.1146/annurev.ento.51.110104.151104

Maizel, J. V., Burkhardt, H. J., & Mitchell, H. K. (1964). Avenacin, an antimicrobial substance isolated from avena sativa. I. Isolation and antimicrobial activity. *Biochemistry, 3*(3), 424-426. https://doi.org/10.1021/bi00891a020

MAPA (Ministério da Agricultura, Pecuária e Abastecimento). (2019). Retrieved September 29, 2019, from http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons

Martinelli, S., Clark, P. L., Zucchi, M. I., Silva-Filho, M. C., Foster, J. E., & Omoto, C. (2007). Genetic structure and molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in maize and cotton fields in Brazil. *Bulletin of Entomological Research, 97*(3), 225-231. https://doi.org/10.1017/S0007485307004944

Monnerat, R., Martins, E., Macedo, C., Queiroz, P., Praça, L., Soares, C. M., ... Bravo, A. (2015). Evidence of field-evolved resistance of *Spodoptera frugiperda* to Bt maize expressing cry1f in Brazil that is still sensitive to modified bt toxins. *PLoS ONE, 10*(4), e0119544. https://doi.org/10.1371/journal.pone.0119544

Nagoshi, R., & Meagher, R. (2008). Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. *Florida Entomologist, 91*(4), 546-554. https://doi.org/10.1653/0015-4040-91.4.546

Nascimento, A. R. B. do, Farias, J. R., Bernardi, D., Horikoshi, R. J., & Omoto, C. (2016). Genetic basis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to the chitin synthesis inhibitor lufenuron: Inheritance of lufenuron resistance in *Spodoptera frugiperda*. *Pest Management Science, 72*(4), 810-815. https://doi.org/10.1002/ps.4057

Nitao, J. K., Berhow, M., Duval, S. M., Weisleder, D., Vaughn, S. F., Zangerl, A., & Berenbaum, M. R. (2003). Characterization of Furanocoumarin Metabolites in Parsnip Webworm, *Depressaria pastinacella*. *Journal of Chemical Ecology, 29*(3), 671-682. https://doi.org/10.1023/A:1022872704016

Nosil, P., Crespi, B. J., & Sandoval, C. P. (2002). Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature, 417*(6887), 440-443. https://doi.org/10.1038/417440a

Ofosuhene Sintim, H., Tashiro, T., & Motoyama, N. (2009). Response of the cutworm *Spodoptera litura* to sesame leaves or crude extracts in diet. *Journal of Insect Science, 9*(52), 1-13. https://doi.org/10.1673/031.009.5201

Parra, J. R. P. (2001). *Técnicas de criação de insetos para programas de controle biologico*. ESALQ/FEALQ.

Pashley, D. P. (1988). Quantitative genetics, development and physiological adaptation in host strains of fall armyworm. *Evolution, 42*(1), 93. https://doi.org/10.2307/2409118

Percy, D. M. (2007). John n. Thompson, the geographic mosaic of coevolution. *Systematic Biology, 56*(1), 149-151. https://doi.org/10.1080/10635150601115640

Petersen, R. A., Zangerl, A. R., Berenbaum, M. R., & Schuler, M. A. (2001). Expression of CYP6B1 and CYP6B3 cytochrome P450 monooxygenases and furanocoumarin metabolism in different tissues of *Papilio polyxenes* (Lepidoptera: Papilionidae). *Insect Biochemistry and Molecular Biology, 31*(6-7), 679-690. https://doi.org/10.1016/S0965-1748(00)00174-0

Pogue, M. (2002). *A world revision of the genus Spodoptera guenée (Lepidoptera: Noctuidae)*. Philadelphia: American Entomological Society.

Roush, R. T. (1989). Designing resistance management programs: How can you choose? *Pesticide Science, 26*(4), 423-441. https://doi.org/10.1002/ps.2780260409

Silva, D. M. da, Bueno, A. de F., Andrade, K., Stecca, C. dos S., Neves, P. M. O. J., & Oliveira, M. C. N. de. (2017). Biology and nutrition of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on different food sources. *Scientia Agricola, 74*(1), 18-31. https://doi.org/10.1590/1678-992x-2015-0160

Singer, M. C., & McBride, C. S. (2009). Multitrait, host-associated divergence among sets of butterfly populations: Implications for reproductive isolation and ecological speciation. *Evolution, 64*(4), 921-933. https://doi.org/10.1111/j.1558-5646.2009.00866.x

Souza, I. R. P., Mendes , S. M., Rafael, H. A., Barros, B. de A., Pinto, M. de O., Carneiro, N. P., ... Pastina, M. M. (2015). Population Structure of *Spodoptera frugiperda* Collected in Maize from Different Brazilian Geographic Regions. *Revista Brasileira de Milho e Sorgo, 14*(3), 300-315. https://doi.org/10.18512/1980-6477/rbms.v14n3p300-315

Tao, X.-Y., Xue, X.-Y., Huang, Y.-P., Chen, X.-Y., & Mao, Y.-B. (2012). Gossypol-enhanced P450 gene pool contributes to cotton bollworm tolerance to a pyrethroid insecticide: Gossypol Enhances Insecticide Tolerance. *Molecular Ecology, 21*(17), 4371-4385. https://doi.org/10.1111/j.1365-294X.2012.05548.x

Wang, R.-L., Liu, S.-W., Baerson, S., Qin, Z., Ma, Z.-H., Su, Y.-J., & Zhang, J.-E. (2018). Identification and Functional Analysis of a Novel Cytochrome P450 Gene CYP9A105 Associated with Pyrethroid Detoxification in *Spodoptera exigua* Hübner. *International Journal of Molecular Sciences, 19*(3), 737. https://doi.org/10.3390/ijms19030737

Wheeler, G. S., Slansky, F., & Yu, S. J. (1993). Fall armyworm sensitivity to flavone: Limited role of constitutive and induced detoxifying enzyme activity. *Journal of Chemical Ecology, 19*(4), 645-667. https://doi.org/10.1007/BF00984999

Wood, K. A., Wilson, B. H., & Graves, J. B. (1981). Influence of Host Plant on the Susceptibility of the Fall Armyworm to Insecticides1. *Journal of Economic Entomology, 74*(1), 96-98. https://doi.org/10.1093/jee/74.1.96

Xue, M., Pang, Y.-H., Li, Q.-L., & Liu, T.-X. (2010). Effects of four host plants on susceptibility of *Spodoptera litura* (Lepidoptera: Noctuidae) larvae to five insecticides and activities of detoxification esterases. *Pest Management Science, 66*(12), 1273-1279. https://doi.org/10.1002/ps.2005

Yang, F., Morsello, S., Head, G. P., Sansone, C., Huang, F., Gilreath, R. T., & Kerns, D. L. (2018). F_2 screen, inheritance and cross-resistance of field-derived Vip3A resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected from Louisiana, USA: F_2 screen and resistance to Vip3A in *S. frugiperda*. *Pest Management Science, 74*(8), 1769-1778. https://doi.org/10.1002/ps.4805

Yu, S. J. (1991). Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). *Pesticide Biochemistry and Physiology, 39*(1), 84-91. https://doi.org/10.1016/0048-3575(91)90216-9

Yu, S. J. (2002). Biochemical Characteristics of Microsomal and Cytosolic Glutathione S-Transferases in Larvae of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith). *Pesticide Biochemistry and Physiology, 72*(2), 100-110. https://doi.org/10.1006/pest.2001.2585

Yu, S. J., & Abo-Elghar, G. E. (2000). Allelochemicals as Inhibitors of Glutathione S-Transferases in the Fall Armyworm. *Pesticide Biochemistry and Physiology, 68*(3), 173-183. https://doi.org/10.1006/pest.2000.2514

Yu, S. J., Nguyen, S. N., & Abo-Elghar, G. E. (2003). Biochemical characteristics of insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). *Pesticide Biochemistry and Physiology, 77*(1), 1-11. https://doi.org/10.1016/S0048-3575(03)00079-8

Yu, Simon J., & Ing, R. T. (1984). Microsomal biphenyl hydroxylase of fall armyworm larvae and its induction by allelochemicals and host plants. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 78*(1), 145-152. https://doi.org/10.1016/0742-8413(84)90062-8