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Metabolic Resistance in the Fall Armyworm: An Overview

Indyra F. Carvalho[1], Larissa L. Erdmann[1], Larissa L. Machado[1], Ana Paula S. A. Rosa[2], Moisés J. Zotti[1] & Camila G. Neitzke[1]

[1] Federal University of Pelotas, Rio Grande do Sul, Brazil

[2] Embrapa Clima Temperado, Rio Grande do Sul, Brazil

Correspondence: Indyra F. Carvalho, Biology Institute, Federal University of Pelotas, County of Capão do Leão, RS, 96010-900, Brazil. Tel: 55-539-8485-3194. E-mail: indyrafaria@gmail.com

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Abstract

The Fall Armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), is one of the most important pests in the American continent and has recently become an invasive species in Africa. It's main form of control is through the use of insecticides, however during the last 40 years, due to continuous spraying and high doses used along with FAW's high adaptative capacity, developed resistance to different classes of chemical insecticides. One of the main mechanisms enabling resistance in the FAW is by detoxification enzymes or so-called metabolic resistance. P450s, Carboxylesterases and Glutathione-S-Transferases are the main families of enzymes believed to mediate the detoxification process. These enzymes in the FAW, although widely studied, have been difficult to generalize into patterns. This happens mainly because FAW populations can have high genetic variability within the species, as they have different biotypes meaning that they can be morphologically identical but physiologically different and consequently, enzymatic responses to toxic compounds can also differ. There are also differences due to the diversity of biomes in which *S. frugiperda* is found, which due to adaptations to different host plants and other abiotic factors, it's hard to predict enzymatic responses in insecticide resistance. In this context we aimed to review the literature regarding these three main enzymes families involved in metabolic resistance in *S. frugiperda*, by cataloguing, analysing and summarizing these studies.

Keywords: carboxylesterases, detoxification, enzymes, GST, P450s

1. Introduction

The Fall Armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), is one of the most important pests in the American continent and has recently become an invasive species in Africa, reaching out to about 12 countries in a year, 7 of them in two months (Goergen et al., 2016). Its adults, have high dispersal capacity and can fly up to 100Km per night, allowing its dispersion in several plant species in different regions (Fao, 2017).

Despite the preference for plants of the Poacea family (*e.g.* maize, rice, sorghum), the FAW can feed on more than 80 species and it frequently reaches the economic threshold, achieving the pest status in several crops of economic importance, such as in cotton and soybean crops (Nagoshi & Meagher, 2004). It is estimated that Brazil alone, spends U$ 600 million a year to control FAW's infestations (Wild, 2017).

This happens because in tropical countries, high temperatures and humidity throughout the year enable crops to be cultivated all year long and therefore FAW populations usually have to be supressed in higher frequencies. It's main form of control is through the use of chemical insecticides, however, during the last 40 years, due to continuous spraying and high doses used along with FAW's high adaptative capacity, *S. frugiperda* developed resistance to various classes of chemical insecticides and more recently, to transgenic crops expressing Bt proteins (Yu, 1982; Giraudo et al., 2015; Flagel et al., 2018).

The first-time insecticide resistance was reported in *S. frugiperda* was in 1979, in the city of Tifton, Georgia, where populations collected in maize, were reported to have different behaviour and physiology from susceptible populations, especially regarding decreased efficiency of carbamate insecticides (Young & Mcmillian, 1979). Currently in Brazil there are 185 products registered to control *S. frugiperda* infestations (Agrofit, 2018). However, about 92 of them are pyrethroids and organophosphates, chemical groups in which the FAW has being

commonly described as resistant. Resistance to synthetic insecticides in the FAW is believed to be mediated mainly by target site insensibility and metabolic detoxification enzymes (metabolic resistance) (Yu et al., 2003).

Metabolic resistance is one of the most common defense mechanisms in herbivorous insects due to the coevolution of insects and plants, the metabolization by enzymes is a defense to xenobiotics present in the environment. Mainly, metabolic resistance relies on enzymatic systems that can detoxify and/or sequester toxic molecules interrupting or decreasing its harmful effect. These enzymes can convert the toxic compound in a non-toxic form and/or convert it to a more easily excretable form in the insect's body (Després et al., 2007).

Understanding the detoxification process in *S. frugiperda* though, is a tough task, especially if you consider the species' complexities. There is a large genetic variability within FAW populations, as the same species can have different biotypes, which means that they can be morphologically identical but physiologically different and consequently, enzymatic responses to insecticides can also differ (Nagoshi & Meagher, 2004). There are also differences due to the diversity of biomes in which *S. frugiperda* is found, which due to adaptations to different host plants and other abiotic factors, it's hard to predict enzymatic responses to insecticide exposure. For example, FAW fed with specific plants, can become more tolerant to insecticides and vice versa (Yu & Ing, 1984; Adamczyk et al., 1997; Silva-Brandão et al., 2017).

Hence metabolic resistance in FAW populations is often associated with phenotypic plasticity, since the production of detoxification enzymes can be induced or supressed in the presence of xenobiotics in their diet or/ and be a result of biotypes and/or specific mutations in genes that transcribe these enzymes, increasing their catalytic capacity in relation to the toxic compound (Després et al., 2007; Silva-Brandão et al., 2017).

This processes typically involves 3 major families of enzymes: Monooxygenases (P450s), Glutathione-S-Transferases (GSTs) and Carboxylesterases (CarEs) (Kranthi, 2005). Their detoxification roles metabolizing insecticides and/or allelochemicals is widely studied in the FAW (Table 3). It is possible to find a fair amount of isolated studies correlating insecticide metabolization to these enzymes' increased activity in resistant populations. These studies usually confirm the correlation, by restoring insecticide activity through the use of the enzyme's inhibitor. However, they also tend to ignore important biotic and abiotic factors, such as the biotype, host plant adaptation and/or geographic isolation. Therefore, we aimed to review the literature available regarding these enzymes particularities, patterns of induction and supression and other parameters in the FAW that can affect its response to chemical control. Summarizing this information, from an agricultural perspective may serve as a tool to prevent and predict insecticide susceptibility in a regional level and can also serve as an intelligent tactic to FAW's integrated pest management.

2. Methods

The information used to write this paper was collected from the Web of Science and Scopus database, the search was done by using the terms "fall armyworm", "resistance", "metabolic", "esterases", "GST" and "P450" from 1979 to June 2017. All studies evaluating monooxygenases (P450s), Glutathione-S-Transferases (GSTs) and Carboxylesterases (CarEs) in *S. frugiperda* were included, those evaluating other enzymes families and/ or different species were excluded. We summarized the literature found in Table 3.

3. Metabolic Detoxification

Metabolic detoxification can be divided into phases. Phase I, usually consists in hydrolysis or/ and oxidation processes and phase II, conjugates products from phase I with endogenous compounds, until the subsequent excretion of the xenobiotic from the insect's body (Berbaum & Johnson, 2015). For example, in the detoxification of apolar insecticides, their molecules are converted to less lipophilic substances or into polar metabolites by oxidation, reduction and/or hydrolyses processes, typical reactions of phase I. The insertion of hydrophilic functional groups increases water solubility, converting the xenobiotic into a more easily excretable compound, P450s and Carboxylesterases (CarEs) are usually described as Phase I enzymes. Subsequently in Phase II, the resulting metabolites of phase I are conjugated with endogenous intermediates, which is usually carbohydrates, proteins or compounds with a sulphate component to be excreted, this process is usually mediated by Glutathione-S-Transferase (GST) enzymes (Kranthi, 2005). It is important to notice that P450s, CarEs and GSTs represent large super families of enzymes, with different substrate specificities which means that they can catalyse a plurality of different reactions, in this overview we limited them to the most common reactions related to insecticide detoxification.

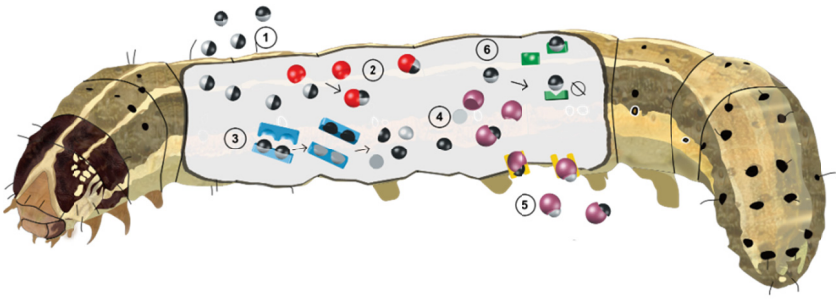

Figure 1. Schematic illustration of detoxification processes in *S. frugiperda*: 1. Insecticide penetration; 2. Sequestration; 3. Phase I; 4. Phase II; 5. Excretion; 6. Target site insensitivity. Figure by Indyra F. Carvalho

4. Cytochrome P450 Enzymes

P450s or CYPs for genes are a large superfamily of enzymes, that can catalyse at least 60 different chemical reactions, but are widely known for their mixed function oxidase activity, that's why they are usually named cytochrome P450 monooxygenases (MO) (Scott & Wen, 2001). Due to the large number of enzymes from this family and their substrate specificities, MOs are believed to mediate resistance to pyrethroids, mimics of the juvenile hormone, carbamates and organophosphates in the FAW (Bullangpoti et al., 2001; Giraudo et al., 2015). Even though their most studied function in *S. frugiperda* is the metabolism or/and detoxification of xenobiotics, they are involved in important endogenous metabolism, such as biosynthesis of hormones, catalysis of pheromones and metabolism of lipids, among others (Brown et al., 2005; Liu et al., 2015).

P450s enzymes are usually identified by the heme-binding domain in the N-terminal region. The heme group forms a series of complexes in which the cytochrome b5 is the terminal oxidase system, transferring an oxygen atom into the substrate and then reducing the second atom into water (Scott & Wen, 2001). This oxidase complex are Phase I reactions and these enzymes are usually located in the microsomes of the smooth endoplasmic reticulum that usually requires NADPH as cofactors. In the FAW they can be abundantly found in the fat body, in Malpighi tubules and in the midgut (Giraudo et al., 2005).

Each CYP gene, codes for a different P450 enzyme and the complexity of induction, suppression and how the interaction with allelochemicals in the diet influence insecticide susceptibility is hard to generalize. To start, there is striking differences in FAW maize biotype (BM) and rice biotype (BR) genome, for example in the maize biotype, 117 CYP genes were reported to encode for P450s versus 131 CYPs found in the rice biotype, 14 CYPS more than BM. From these genes, CYP6AE86 and CYP3406 were reported to be specific to the rice strain (Gouin et al., 2017). These differences are a result of the adaptive race generalist insects have to face to allelochemicals from host plants and probably reproductive isolation (Adamczyk et al., 1997).

Associated to the biotypes differences is the host plant direct influence. The CYP promoter region in these enzymes contains specific regulatory elements (Res) as an element to the xenobiotic response, for example, in studies in which *S. frugiperda* was fed on diets containing allelochemicals such as monoterpenes, flavonoids, indols, menthol and the widely studied xanthotoxin (a metabolite commonly found in *S. frugiperda*'s diet) P450s activity was induced. These studies reported that this induction enhanced tolerance to insecticide exposure (Yu, 1982; Yu & Ing, 1984; Brown et al., 2005). However, induction does not necessarily mean resistance or the full capacity of metabolization of insecticides. There are also cases on which plants compounds can act as inhibitors, such as *Jatropha gossypifolia* L. (Euphorbiacea) and *Piper aduncum* L. (Piperaceae) extracts, in these cases the allelochemical functioned as a synergist to the synthetic insecticide by inhibiting P450 activity (Bullangpoti et al., 2001; Fazolin et al., 2015). A summarized list of studies regarding the influence of allelochemicals in the enzyme activity and insecticide susceptibility is available for more details in Table 1.

Even though very similar, differences between detoxification of plant metabolites in the diet and synthetic pesticides is common, for example it is believed that polyphagous insects such as the FAW, due to the constant interaction with a diversity of allelochemicals, induces CYPs with low subtract specificity such as the CYP3 and CYP6 subfamilies, in which both are known to be induced to a variety of plant allelochemicals in the FAW (Giraudo et al., 2015). Their orthologous genes in *Helicoverpa armigera* (Lepidoptera: Noctuidae), an also highly polyphagous insect, have low specificity to substracts due to its enzyme structure, in which the P450s

expressed have wider enzyme cavities than others, enabling them to metabolize a higher spectrum of compounds (Rupasinghe et al., 2007).

Table 1. Studies evaluating allelochemical influence on enzyme activity and insecticide susceptibility

Reference	Allelochemichals	Enzyme	Insecticide Chemical Group	Results
Yu (1982)	Inducers: Monoterpenes	P450	Carbamate, organophospate, pyrethroids	Enhanced tolerance to insecticide
Yu and Ing (1984)	Inducers: Monoterpenes, indoles and flavones	P450	Carbamates and organochlorines	Monoterpenes and flavones induce enzyme activity; insecticides inhibited enzyme substrate activity
Bullangpoti et al. (2001)	Inhibitors: *J. gossypifolia* and *M. azedarach*	P450 and AChE	Pyrethroids	Antifeedants effect, inhibitor activity against P450 and AChE, synergism to pyrethroids
Fazolin et al. (2015)	Inhibitor: *Piper aduncum* L.	P450	Pyrethroids	Synergism effect of *Piper aduncum* to pyrethroid
Giraudo et al. (2015)	Inducers: xanthotoxin, tridecanone, indoles	P450	Pyrethroid, phenylpyrazole, juvenile analogue hormone, diacylhydrazine	The pattern of induction| supression of each gene was specific for each chemical compound

The same is not expected to occur in the metabolization of synthetic insecticides which is believed to induce just a few specific CYPs. The high majority of these, are believed to be expressed in low levels and only when exposed to insecticides, probably because the decoupling of these enzymes produces reactive oxygen in the cell (Giraudo et al., 2015), suggesting that the genes involved in synthetic insecticides metabolization are expressed in different forms, intensity and number, and that some P450s are probably more sensitive to selection pression then CYPs induced by plant allelochemicals (Yu, 1991; Li et al., 2007). For example, P450s expressed in sf9 cell models from tissues involved in the process of detoxification in the FAW, when exposed to insecticides, had just a few genes expressed, most of them from the CYP9 and CYP6 families (Giraudo et al., 2015).

The confirmation that just a few CYP genes are selected in the insecticide resistance is usually demonstrated by comparing susceptible and resistant populations of *S. frugiperda*. It is believed that resistant FAW populations have biochemical and immunological properties different from susceptible ones. In a study comparing 18.506 transcripts in resistant and susceptible *S. frugiperda* to benzoylurea, it was found that 840 transcripts were differently expressed. The analyses, showed that the majority of these transcripts (61.3%) were overexpressed and 38.7% were supressed in the resistant population. The high expression levels of some CYP genes, even without insecticide exposure, showed that gene expression can be constitutive (Diez & Omoto, 2001; Nascimento et al., 2015; Silva-Brandão et al., 2017).

The high selection pressure that FAW can be exposed to, the constant use of high insecticidal doses, indiscriminate use of pesticides and deficient chemical group rotation, accelerates the development of resistance to these chemicals, very different to what happens to plant metabolites, in which high exposure to large amounts of toxic metabolites are usually rare and goes through long processes of biotic and abiotic adaptions through evolutionary years (Després et al., 2015).

Though generalizing and specifying the CYPs involved in each case is not ideal, especially because the same P450 involved in plant allelochemical can also be involved in synthetic pesticide metabolization. There are a few studies demonstrating that, for example, in a study evaluating sequences coding for P450s in the FAW exposed to plant allelochemicals, it was found 42 sequences encoding P450s, distributed among the 14 families. In this study, the majority of these were represented by members of the CYP3, CYP9 and CYP4 families (Giraudo et al., 2015). Very similar results were also observed in FAW resistant to Lufenuron (Benzoylurea), in which CYP3 and CYP4 families were also positively expressed, along of with the members of the CYP9 and CYP6 families (Nascimento et al., 2015).

5. Carboxylesterases

Carboxylesterases (CarEs) form a large group of metabolic enzymes from Phase 1 that belongs to the hydrolases class. CarEs are enzymes that catalyse the hydrolysis of ester bonds on several substrates containing carboxylic esters, in which the target molecule is broken in two or smaller ones by the addition of water and subsequently converting it into its corresponding components of alcohol and acid (Satoh & Hosokawa, 2006).

CarEs are involved in several endogenous and exogenous processes in insects, such as the metabolism of xenobiotics, development regulation, degradation of pheromones and neurogenesis, their function vary according to the species, body region and developmental stage (Biswa et al., 2010; Durand et al., 2010). α-esterases,

β-esterases, juvenile hormone esterases, gliotactins, acetylcholinesterases, neurotactins, neuroligins are enzymes responsible for most of the catalytically active reactions of CarEs (Ranson et al., 2002).

In *S. frugiperda*, they are a known for mediating resistance to pyrethroid insecticides, carbamates and mainly organophosphates (Yu et al., 2003; Li et al., 2007; Carvalho et al., 2013). Basically, because these insecticides have ester, amide and phosphate bonds in their structures.

In *S. frugiperda* CarEs' mediated resistance may occur through quantitative changes due to the overproduction of esterases by gene amplification and/or positive regulation of one or two CarEs genes (which may result in inhibition or increase of the number of esterases in the FAW) or by qualitative changes resulting from structural mutations of the enzyme. Due to this functional plurality, resistance mediated by CarEs is usually demonstrated as a result of a combination with other resistance mechanisms, and is therefore commonly related to multiple and/or cross resistance (McCord & Yu, 1987; Satoh & Hosokawa, 2006).

In cases of cross-resistance for example, several studies demonstrate the increase in CarEs activity in association with target site insensibility to the insecticide. In *S. frugiperda* resistant to carbamates and organophosphates for example, CarEs activity is significantly higher in resistant individuals and the enzyme acetylcholinesterase (AChE) in these populations is less sensitive to inhibition (McCord & Yu, 1987; Bullangpoti et al., 2001; Yu et al., 2003).

AchE is by far the most studied CarE, mainly because of its vital importance in the FAW's nervous system and for that it is also one of the main targets of many insecticides. There for, insecticide metabolization in generally correlated to AChE insensitivity. For example, in *S. frugiperda* resistant to organophosphates, isoforms of AChE are detected conferring resistance to most organophosphates tested. In this same study the EST9555 gene, was also over expressed, and by annotation it was observed that the *S. frugiperda*'s sequence was very similar to the *Myzus persicae* (Hemiptera: Aphididae) sequence encoding for the carboxylase E4 enzyme (Carvalho et al., 2013). In *M. persicae*, the CarE E4 is known to confer resistance to many organophosphates and carbamates. It's recombinant form when expressed in *Escherichia coli* exposed to a carbamate, was responsible to hydrolyse 64% of it in 2.5 hours and an organophosphate in 1.25 hours (Lan et al., 2005).

Even though widely studied there are currently few functional data on purified and/or recombinant CarEs and their specific physiological role in *S. frugiperda*. Much of the studies in the FAW are demonstrative studies correlating the increase of esterases and esterases activity with the decrease of the insecticidal effect which is generally confirmed by its inhibition using synergists such as S, S, S-tributyl phosphonothioate (DEF) (Usmani & Konwles, 2001). There is also the fact that most current information is inferred from genomes of already sequenced model organisms such as *Drosophila melanogaster* (Diptera: Drosophilidae) and *Bombyx mori* (Lepidoptera: Bombycidae), this comparison between different species though, may not be appropriate mainly due to the variation of CarEs expressed in each organism.

Though in recent years, due to technological advances such as interference RNA technology and genome sequencing, it is being possible to investigate FAW's CarEs specificity and functions (Mao et al., 2007; Shi et al., 2016; Gouin et al., 2017). In 2017, when *S. frugiperda* genome was sequenced, it was possible to demonstrate that, although similar to other species, there are significant differences in the expression of genes related to the metabolism of xenobiotics. It revealed that the *S. frugiperda* genome have about 24 more genes encoding for esterases than its closest organism *B. mori*, this is very important mainly because most studies on CarEs in the FAW are deducted from the *B. mori* genome.

It was also noted that the esterases genes varied according to the FAW's biotype, as some of them are exclusively related to its race. For example, the rice biotype presented 6 more genes expressing CarEs than the maize biotype, their expression and their role in insecticide metabolization though is still uncertain (Gouin et al., 2017).

6. Glutathione-S-Transferase

Glutathione-S-transferases (GSTs) are a large multifunctional group of enzymes present in mammals, insects, bacteria, protozoa and fungi (Krathi, 2005). They are involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress (Ketterman et al., 2011). Due to a wide range of substrates, they play an important role in the resistance to different classes of insecticides, including organophosphates and pyrethroids. The DDT-dehydrocholinesterase, is the most famous and studied GST, due its direct association to DDT resistance in house flies and mosquitoes (Enayalti et al., 2005).

There are two groups of GSTs, classified according to their location in the cell: the microsomal and the cytosolic. Although both catalyse similar reactions, microsomal GSTs are not commonly described in the metabolism of

insecticides. Cytosolic GSTs form 6 large classes of enzymes identified by Greek letters in the literature, they are the Delta (Δ), Epsilon (ε), Zeta (ζ), Sigma (σ), Omega (Ω) and Theta (θ) classes (Ranson et al., 2002).

GSTs catalyse the conjugation of a tripeptide, the reduced glutathione (GSH) (Glu-Cys-Gly) to a variety of endogenous and xenobiotic substrates that have electrophilic centres, converting these reactive molecules into less toxic conjugates and/or more hydrophilic compounds (Kirby & Ottea, 1994). They can also metabolize insecticides indirectly by removing free radicals and reactive oxygen produced in the process of degradation of insecticides (Hayes & Pulford, 1995). Although GSTs may be involved in the sequestration of substrates, they are enzymes that generally act after processes of Phase 1 (Krathi, 2005).

The great diversity of GST enzymes in generalist insects such as the FAW reflects the ability of such herbivores to adapt to a wide range of allelochemicals. Like P450s, GSTs are differentially regulated in response to various allelochemical inducers, different stages of development and in specific tissues (Yu & Abo-Elghar, 2000). The complexity of suppression and induction patterns and GST's substrates specificities in *S. frugiperda* has represented a great difficulty in understanding its functions and roles in insecticide resistance. In the FAW, just like the other enzymes, the exposure to a particular allelochemical in the diet can suppress or stimulate GSTs, how this expression influences the of insecticide metabolization is still a gap to be fulfilled. For example, there are many studies evaluating various plant allelochemical such as phenolic compounds that can inhibit GSTs in the FAW and curiously when facing insecticide exposure, some of these compounds increased insecticides toxicity (Yu & Abo-Elghar, 2000). In contrast, *S. frugiperda* fed on chickpeas, for example, a potent stimulator of GSTs, were twice as tolerant to organophosphate insecticides as those fed with soybean grains (Yu & Ing, 1984). A summarized list of GST response to xenobiotic exposure can be found in Table 2.

In most cases individual GST enzymes involved in resistance have not been identified and their action has been implicated by association with other enzymes. In cases where resistance has been studied in more detail, resistance has been attributed to increases in one or more GST enzymes, as a result of gene amplification or more commonly by upregulation, than by qualitative changes in individual enzymes, differently from what happens with the CarEs enzymes for example (Enayalti et al., 2005).

Table 2. Studies evaluating GST response to plant allelochemicals

Reference	Enzyme	Results	Xenobiotic exposure
Wheeler et al. (1993)	P450, general esterases and GST	Flavone induces enzyme activity	Inducer: Flavonoids
Yu and Abo-Elghar (2000)	GST	The pattern of induction| supression of each gene was specific for each chemical compound;	Inhibitors: Flavonoids, Phenols and a,b-Unsaturated carbonyl compounds, Isothiocyanates and Organotins
Yu (2002)	GST (Microsomal and cytosolic)	Both GSTs had an antioxidant nature; Cytosolic GSTs showed a broader substrate specificity and was less sensitive to inhibition. Microsomal GST was not induced by xanthotoxin and indole 3-acetonitrile.	Inhibitors: Flavonoids, Phenols, a,b-Unsaturated carbonyl, Organotin and Halogenated compound

In a study evaluating *S. frugiperda* gene expression in resistant populations to organophosphate 19 different GST genes were identified, in this population most GST genes were upregulated, depending on its correlation with other detoxification enzymes and interestingly with geographic location. In the same study populations resistant to *Bacillus thuringiensis* Cry1 toxins (Cry1F and Cry1Ac) and organophosphate from Puerto Rico, had high GTS expression, much more then CarEs and P450s enzymes. This study indicated the potential association of GSTs with multiple resistance/cross-resistance of Bt and organophosphate (Zhu et al., 2015).

In other insects, GTS is commonly described as a phase 2 process in the organophosphate metabolism and it can play a significant role in FAW resistance to these compounds. The conjugation of GSH to organophosphates results in its detoxification in two main pathways: O-dealkylation, where glutathione is conjugated to an alkyl portion of the insecticide and O-alimentation, where the GSH reacts with the group that left.

More recent studies, are also reinforcing GST's involvement in organophosphates metabolization. A study evaluating Expressed Sequence Tags (ESTs) in *S. frugiperda* resistant to OPs demonstrated that of the 27 ESTs evaluated, 10 were equivalent to GSTs enzymes. Among the significantly overexpressed ESTs, sequences from the epsilon family and from the sigma family were specially overexpressed (Carvalho et al., 2013). The study pointed that these sequences have a 50% similarity to the GST3 encoders of the epsilon family in *Plutella xylostella*, an insect in which the overexpression of the PXGSTE1 enzyme in resistant strains is able to metabolize organophosphate insecticides (Huang et al., 1998).

The same happen in FAW resistant to pyrethroids, in which all three overexpressed ESTs were GSTs and the sigma family were highly overexpressed (Carvalho et al., 2013). However, even though pyrethroid inducing GSTs is commonly reported in *S. frugiperda*, GSTs have not yet been detected in its direct metabolism. It is believed that unlike organophosphates, pyrethroids are not metabolized directly by GSTs but they contribute to resistance by protecting the insect from the peroxidation products and the oxidative stress that happens when the FAW is exposed to a xenobiotic (Yu, 2002), it's also believed that GSTs can sequester these molecules until they are metabolized by other detoxification enzymes (Kostaropoulos et al., 2001).

As noted above, GST activity and its involvement in insecticide resistance is still very little understood in detail, even though it's correlation to the process has been demonstrated in several FAW studies (Wheeler et al., 1993; Abo-Elghar & Yu, 2000; Yu, 2002). The recent release of *S. frugiperda* genome, showed that there is still a lot to look for in the GST superfamily. For example, in the *S. frugiperda* genome they identified 46 GST genes. When comparing this result to other lepidopterans, they noticed that a recent divergence of the delta and epsilon classes has interestingly expanded in comparison to the other six classes of GST in lepidopterans. They also highlighted differences in the biotypes, in which the rice biotype retained all GST genes from maize, with the exception of GST8 (Gouin et al., 2017).

7. Final Considerations

Determining the identity of these three large groups of enzymes involved in insecticide metabolism has been difficult due to the lack of knowledge of the complexity of these families in the FAW and the difficulties of identifying truly orthologous genes among different model organisms (Ranson et al., 2002).

Much of what is known today is inferred from other species and more recent studies have shown considerable differences in these enzymes expression, for example there are 15 members of the CYP9 family in *S. frugiperda* versus 4 members in *B. mori* and none in *P. xylostella*, these insects are the closest lepidoptera members used for annotation in most FAW studies, and relevant differences are also reported to happen to GSTs and CarEs families (Giraudo et al., 2015; Gouin et al., 2017). Although these results can be disheartening and generate uncertainties about what we believed to happen in the FAW so far, there is an optimistic perspective about what these news in enzyme detoxification can bring, and a more detailed comprehension of this complexities, can help elucidate their function in the FAW and other insects of economic importance and perhaps this knowledge can also be explored as new targets in the insecticide industry.

What we noticed so far, is that there is a big amount of isolated studies on the FAW's physiology and genomics, and difficulties of finding specificities in *S. frugiperda* is mainly because its enzyme expression and its related processes are dependent on several other factors, such as environment, host plants interaction and the selection pressure in which *S. frugiperda* is submitted. There is still a lot to learn about these patterns of repression and induction, as much as these enzyme particularities in highly polyphagous pests such as the FAW.

The importance of this information extrapolates the academic level as it can be a tool to optimize integrated pest management tactics. As noticed in this overview the host plant interaction with tolerance and/or resistance to insecticides should be taken in consideration when planning crops rotation and/or picking a proper insecticide in the market. Rationally choosing a host plant that disfavours the FAW establishment in the field and/or survivorship to spraying can help reduce the costs of control, especially for smallholders in which access to cut edge technology is not always available and the optimization of control measures can bring significant differences in the final production costs.

Since selecting a control method nowadays, commonly ignores the fact that there are differences in *S. frugiperda* susceptibility to insecticides when fed on different host plants, cultivars and even the same species collected in different continents (Yu, 1982; Hull-Sanders et al., 2007) we strongly propose a regional level analyses as a tool for organizations to guide an intelligent FAW management. Starting by a proper identification of FAW biotypes as well as flora of the region, since changes in enzymatic activity in response to external factors may affect resistance and tolerance to adverse conditions. Unfavourable conditions to the FAW should be explored regionally, especially due to the diversity of biomes in which FAW is present. These factors can also be taken in account, in future investigations, even though restrictions in enzymes studies and the technology available is still very limiting making it almost impossible to consider different variables at the same time.

In this overview we constantly commented on the endogenous roles these enzymes have in the FAW besides xenobiotic metabolization, but a more detailed analyses is needed to find a correlation between these processes. Studies of how and if metabolic enzymes affect other metabolic pathways is also an interesting area to explore. We know that resistance is a complete biological phenomenon that enables the insect to maintain its vital processes and survive in adverse environmental conditions. As so, the metabolic pathways adjacent to the

classical detoxification route, may be closely related to a fitness cost and may represent an additional mechanism contributing to resistance. For example, in one of the studies, it was reported that in *S. frugiperda* resistant to benzoylurea, the transcripts for ubiquinol-cytokine and the reductase complex were also overexpressed. This is particularly interesting because the cytochrome c ubiquinol is not directly linked to insecticide resistance, since its main function is associated with electrons transport in the cellular respiration process (Carvalho et al., 2013).

Besides the almost impossible task to address detoxification enzymes as a unique biological complex in the FAW, we do expect a change in subsequent studies regarding these enzymes as system, especially because of the molecular and genomic advances seen in recent years. Interference RNA, microarrays, free access to SPODOBASE (an EST database from all organs of *S. frugiperda*) are good examples of technologies that are now becoming more accessible for scientist all over the globe (Nègre et al., 2006; Nascimento et al., 2015). Advances such as the full *S. frugiperda* genome released in 2017 is now available for more detailed investigations, but for that, the demonstrative and basic studies we have until now need to be properly comprehended, we believe that these studies will guide scientists and technicians along new challenges (Gouin et al., 2017). Therefore, we hope that overviews such as this one, contribute in some way to future studies in search of more sustainable options regarding insecticide use and for a more detailed comprehension of this insect's biology, physiology and behaviour.

Table 3. List of metabolic resistance studies in *S. frugiperda* used as source of information for this overview, organized by chronological order, subject and country of research

Reference		Country
Young and McMillian (1979)	Differential Feeding by Two Strains of Fall Armyworm Larvae on Carbaryl Treated Surfaces	USA
Wood et al. (1981)	Influence of Host Plant on the Susceptibility of the Fall Armyworm to Insecticides	USA
Yu (1982)	Induction of Microsomal Oxidases by Host Plants in the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith)	USA
Yu and Ing (1984)	Microsomal biphenyl hydroxylase of Fall armyworm larvae and its induction by allelochemicals and host plants.	USA
McCord and Yu (1987)	The Mechanisms of Carbaryl Resistance in the Fall Armyworm *Spodoptera frugiperda* (J. E. Smith)	USA
Yu (1991)	Insecticide Resistance in the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith)	USA
Wheeler et al. (1993)	Fall armyworm sensitivity to flavone: limited role of constitutive and induced detoxifying enzyme activity	USA
Kirby and Ottea (1994)	Multiple mechanisms for enhancement of Glutathione S-Transferase activities in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)	USA
Adamczyk et al. (1997)	Susceptibility of Fall Armyworm Collected from Different Plant Hosts to Selected Insecticides and Transgenic Bt Cotton	USA
Yu and Abo-Elghar (2000)	Allelochemicals as inhibitors of Glutathione S-Transferases in the Fall Armyworm	USA
Bullangpoti et al. (2001)	Antifeedant activity of *Jatropha gossypifolia* and *Melia azedarach* senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergist	THA
Yu (2002)	Biochemical characteristics of Microsomal and Cytosolic Glutathione S-Transferases in larvae of the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith)	USA
Yu et al. (2003)	Biochemical characteristics of insecticide resistance in the Fall armyworm, *Spodoptera frugiperda* (J.E. Smith)	USA
Nègre et al. (2006)	SPODOBASE: an EST database for the lepidopteran crop pest *Spodoptera frugiperda*	FR
Carvalho et al. (2013)	Investigating the Molecular Mechanisms of Organophosphate and Pyrethroid Resistance in the Fall Armyworm *Spodoptera frugiperda*	UK
Nascimento et al. (2015)	Comparative transcriptome analysis of lufenuron-resistant and susceptible strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)	BR
Giraudo et al. (2015)	Cytochrome P450s from the Fall armyworm (*Spodoptera frugiperda*): responses to plant allelochemicals and pesticides	FR
Fazolin et al. (2015)	Sinérgico alternativo para o manejo da resistência da lagarta-do-cartucho do milho a piretróides	BR
Zhu et al. (2015)	Evidence of multiple/cross resistance to Bt and organophosphate insecticides in Puerto Rico population of the Fall armyworm, *Spodoptera frugiperda*	EUA
Silva-Brandão et al. (2017)	Transcript expression plasticity as a response to alternative larval host plants in the speciation process of corn and rice strains of *Spodoptera frugiperda*	BR
Flagel et al. (2017)	Mutational disruption of the ABCC2 gene in fall armyworm, *Spodoptera frugiperda*, confers resistance to the Cry1Fa and Cry1A.105 insecticidal proteins	EUA
Gouin et al. (2017)	Two genomes of highly polyphagous lepidopteran pests (*Spodoptera frugiperda*, Noctuidae) with different host-plant ranges	FR

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