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Abstract:

The genus *Spodoptera* (Lepidoptera: Noctuidae) comprises some of the most polyphagous and destructive agricultural pests worldwide. The success of many species of this genus is due to their striking abilities to adapt to a broad range of host plants. Superfamilies of detoxification genes play a crucial role in the adaption to overcome plant defense mechanisms mediated by numerous secondary metabolites and toxins. Over the past decade, a substantial amount of expression data in *Spodoptera* larvae was produced for those genes in response to xenobiotics such as plant secondary metabolites, but also insecticide exposure. However, this information is scattered throughout the literature and in most cases does not allow to clearly identify candidate genes involved in host-plant adaptation and insecticide resistance. In the present review, we analyzed and compiled information on close to 600 pairs of inducers (xenobiotics) and induced genes from four main *Spodoptera* species: *S. exigua*, *S. frugiperda*, *S. littoralis* and *S. litura*. The cytochrome P450 monooxygenases (P450s; encoded by *CYP* genes) were the most upregulated detoxification genes across the literature for all four species. Most of the data was provided from studies on *S. litura,* followed by *S. exigua*, *S. frugiperda* and *S. littoralis*. We examined whether these detoxification genes were reported for larval survival under xenobiotic challenge in forward and reverse genetic studies. We further analyzed whether biochemical assays were carried out showing the ability of corresponding enzymes and transporters to breakdown and excrete xenobiotics, respectively. This revealed a clear disparity between species and the lack of genetic and biochemical information in *S. frugiperda*. Finally, we

discussed the biological importance of detoxification genes for this genus and propose a workflow to study the involvement of these enzymes in an ecological and agricultural context.

Keywords: *Spodoptera*, cytochrome P450, glutathione S-transferases, carboxylesterases, UDP-glycosyl transferases, ABC transporters

1. Introduction

An immense number of complex trophic interactions were known between plants and arthropods, two groups encompassing about half of all macroscopic organisms (Strong, 1988). During the course of their co-evolution, plants and herbivorous arthropods have engaged in an arms race for survival (Ehrlich and Raven, 1964). On the one hand, plants have evolved a myriad of specialized repellent and toxic metabolites directed towards phytophagous arthropods to defend themselves from herbivory (Li et al., 2020a). On the other hand, insects have found ways to bypass these chemicals to exploit plants as a food source (Vogel et al., 2014). The ability to metabolize, sequester and detoxify plant toxins is known as one of the central evolutionary solution that arthropods have developed to feed on plants (Despres et al., 2007; Heidel-Fischer and Vogel, 2015). The detoxification pathway in insects allows the processing of toxicants present in their diet, including insecticides (Despres et al., 2007; Heckel, 2014). It is conventionally split into three phases that convert lipophilic substrates into hydrophilic products more easily excretable from the insect's body (Berenbaum and Johnson, 2015; Despres et al., 2007). In phase I (functionalization) cytochrome P450 monooxygenases (P450s) and carboxylesterases (CEs) render non-polar molecules "functional", *i.e.* with an active center by appending reactive groups suitable for subsequent conjugation. These intermediary metabolites

may then fuel into phase II (conjugation) where glutathione S-transferases (GSTs) and (UDP)-glycosyl transferases (UGTs) catalyze the conjugation of target molecules, including phase I products, and facilitate their excretion. At last, phase III (transport) involves ATP-binding cassette (ABC) transporters that mediate efflux of the resulting products (Amezian et al., 2021).

Among insect herbivores, the order Lepidoptera comprises several species among the most important crop pests in the world. Some of these species belong to the genus *Spodoptera* (Guenée). This genus, also known as the armyworms, is a group of 31 species of noctuid moths present on 6 continents (Kergoat et al., 2021; Pogue, 2002). With fifteen species out of 31 considered as agricultural pests *Spodoptera* is unquestionably one of the most destructive crop genus among Noctuidae, jeopardizing agricultural production and causing billions of dollars of yield losses annually (Pogue, 2002). A few characteristics shared by *Spodoptera* moths have elevated some members to a global pest status. These include high fecundity, a short life cycle under tropical and sub-tropical conditions, a rather long adult life span of *ca.* 10 days allowing females to lay over 1000 eggs on average and resulting in year-round population outbreaks (Ferry et al., 2004; Montezano et al., 2018; Narvekar et al., 2018; Silva et al., 2017a; Silva et al., 2017b). More importantly, most species in this group are highly polyphagous and can feed on more than 100 host-plants (Montezano et al., 2018; Pogue, 2002; Robinson et al., 2010; CABI, 2020, https://www.cabi.org/cpc/) including numerous crops.

The beet armyworm, *S. exigua* (Hübner), the fall armyworm, *S. frugiperda* (J. E. Smith), the Egyptian cotton leafworm, *S. littoralis* (Boisduval) and the tobacco cutworm, *S. litura* (Fabricius), are four *Spodoptera* species widely distributed across the globe and are destructive to virtually all economically important crops including maize, rice, cotton, soybean,

vegetables and ornamentals (Bin-Cheng, 1994; Ellis, 2004; Pogue, 2002; Wyckhuys and O'Neil, 2006). *S. exigua* originates from East Asia, and it has spread across the Americas during the nineteenth century before becoming a serious pest worldwide (Capinera, 1999). *S. frugiperda* has recently made its way outside of its native range, the American continent and has gone global at a lightning-speed hardly ever witnessed before for a pest species (Richardson et al., 2020; Stokstad, 2017). It was detected in West Africa for the first time in early 2016 (Nagoshi et al., 2017) and across the whole continent (44 countries) after only two years (CABI, 2020; Stokstad, 2017). In 2018, it reached the Asian continent through India and has been found in virtually every country of East and South-East Asia, as well as Australia (Chormule et al., 2019; Li et al., 2020b; Ma et al., 2019; Sempurna et al., 2020; Wu et al., 2019). *S. littoralis* ranges from Southern Europe to Africa and is found in the Middle East as well as in some islands of the Pacific Ocean. *S. litura* is present throughout the Middle East and Asia including India, China, and Japan, but also extends southward to Australia and the Pacific Ocean.

Over the years, due to an intensive selection pressure, it has become more difficult to control members of the *Spodoptera* genus as many developed resistance to a wide variety of synthetic insecticides. For instance, high levels of resistance were reported to 43, 40, 31 and 42 insecticides in *S. exigua*, *S. frugiperda, S. littoralis* and *S. litura* respectively including *Bacillus thuringiensis* (Bt) toxins (Mota-Sanchez and Wise, 2020). The emergence of resistance to synthetic insecticides is presumed to reflect arthropods host-plant adaptation and their reliance on responses to similar chemical structures found among plant defense compounds (Gordon, 1961; McKenzie and Batterham, 1994). Approx. 400MY of plant-insect interactions has worked as an evolutionary driving force for diversification and sophistication of gene superfamilies such

as those involved in detoxification (Feyereisen, 2011; Harari et al., 2020; Sezutsu et al., 2013). The increasing number of sequenced genomes has uncovered a genetic basis of resistance mechanisms that are thought to structurally and functionally overlap with host-plant adaptation (Dermauw et al., 2013; Despres et al., 2007; Grbić et al., 2011; Rane et al., 2019). The genomes of *S. litura* and *S. frugiperda* are available since 2017 and revealed large expansions in most detoxification gene families as compared to the specialist *Bombyx mori* (Table S1) (Cheng et al., 2017; Gouin et al., 2017). The genome of *S. exigua* was published last year (Zhang et al., 2020) and others are in progress, such as that of *S. littoralis.* The investigation of their detoxification gene families will help to understand to what extent these mechanisms allow species such as *Spodoptera* moths to successfully feed on different host-plants and to develop resistance to insecticides.

The role of the detoxification enzymes in insecticide resistance and host-plant adaptation is now well-acknowledged (Dermauw and Van Leeuwen, 2014; Feyereisen, 2005; Li et al., 2007; Pavlidi et al., 2018). Overexpression, enhanced activity or a broader substrate range of candidate enzymes are indicators pointing towards metabolic resistance. The monitoring of resistant populations of *S. exigua*, *S. frugiperda, S. littoralis* and *S. litura* across the world has allowed to document countless cases of enhanced activities in major detoxification enzyme families. Meanwhile a substantial body of data has accumulated on detoxification genes induced upon xenobiotic exposure or in insecticide resistant populations of *Spodoptera* species, providing many potential candidate enzymes or transporters involved in resistance phenotypes. Yet, very few of them have been biochemically validated to be involved *per se* in the detoxification of xenobiotics.

In this review, we take a comprehensive look at responses of detoxification genes induced in *Spodoptera* species, either from insecticide resistant populations or after exposure to xenobiotics, focusing on data published during the past decade (the data can be found in Supplementary material).

2. Responses of detoxification genes to xenobiotics in the *Spodoptera* genus

We collected data from recently published studies (from 2010 to 2020) investigating the changes in expression of detoxification genes associated with insecticide resistance and host-plant adaptation in *Spodoptera* species (Supplementary material). Data encompasses the nature of up- or down- regulated genes assessed in quantitative RT-PCR, microarray, semi-quantitative assays or RNA-seq when genes were duly annotated. A notice was added for significant validation of genes in genetic-functional approaches as well as from expression-based metabolic techniques in heterologous systems. It is important to note that gene induction is dependent on the dose, *i.e.* the concentration of inducers, and the time of exposure. While aware of these features, including this information was beyond scope of the present work. This makes comparisons between insects and xenobiotics difficult, which will be discussed below. Nevertheless, the data compiled here will provide useful information for those working in the field (see excel sheet in supplementary material). The transcriptomic responses of detoxification genes were obtained from resistant field or laboratory-raised *Spodoptera* populations or after exposure of insects to xenobiotics (in total 51 different compounds, Figure S1), including plant secondary metabolites (PSM, n=22), insecticides (n=21), herbicides (n=2), model inducers (n=2) and heavy metals (n=4). We excluded on purpose studies on Bt resistance mechanisms. Among

149 all *Spodoptera* species described to date only four have been investigated post-2010 for their
150 detoxification capability at gene expression level, namely *S. litura*, *S. frugiperda*, *S. exigua* and *S.*
151 *littoralis*.

152 The number of genes found up- and down-regulated per species are presented in Figure
153 1A. Expression data of detoxification genes in *S. littoralis* is scarce: over the past decade a single
154 gene, UGT46A6, was shown to be upregulated in the antennae after deltamethrin exposure
155 (Bozzolan et al., 2014). Expression data of detoxification genes in *S. litura* exceeds the
156 information available for both *S. frugiperda* and *S. exigua*, however the increasing numbers of
157 studies being published in both species may shortly close this gap. The total and overlapping
158 number of detoxification genes found upregulated in the three *Spodoptera* species was always
159 higher than that of downregulated genes (Figure 1A). The data altogether reflects the extent of
160 the transcriptomic response generated by these four generalists *Spodoptera* species under
161 xenobiotic challenges or intense insecticide pressure. This is also illustrated in Figure 1B by the
162 relatively high share of overlapping genes between species for the two major xenobiotics types
163 (PSM and insecticides). This revealed two findings: i) induction mechanisms of detoxification
164 enzymes are overlapping with respect to inducing xenobiotics, ii) detoxification enzymes might
165 have broad substrate specificities, for example encompassing both phytochemicals and
166 synthetic pesticides.

167 This being said and as pointed out below, the majority of up- and down- regulated
168 detoxification genes from the cited literature in *Spodoptera* lack respective validation studies;
169 therefore, it is premature to assume that the up-regulated genes are involved in the
170 detoxification of the xenobiotics. Indeed, detoxification enzymes may convert xenobiotics into

more toxic metabolites. In that case, tolerance to insecticides or plant secondary metabolites can be conferred by downregulating these detoxification genes as it was shown in *Varroa destructor*. The suppression of CYP4EP4 expression increased the tolerance of mites to coumaphos (Vlogiannitis et al., 2021). In addition, exposure to xenobiotics tend to induce a large number of genes and most enzymes that are upregulated by the presence of a putative toxin are not directly involved in the metabolization of that toxin. Host plant generalists in particular may induce a variety of defense mechanisms that eventually succeed in allowing the insect to feed on the plant. It has been suggested that both the overlapping spectrum and induction plasticity observed in detoxification enzymes of generalist species is a result of their feeding strategy (Vogel et al., 2014). It is possible that due to the diversity of toxic plant chemicals encountered in their diet, generalists are able to exhibit a larger inducible palette of enzymes. In that respect, the number of overlapping genes induced between PSM and insecticides presented in Figure 1B might be underestimated. For instance, although the pyrethroid insecticide deltamethrin was the only xenobiotic exposed to all four species surveyed (Figure S2A), only 12 genes were reported to be upregulated and none of them was shared between species. Similarly, λ-cyhalothrin, chlorpyrifos and xanthotoxin were the sole xenobiotics used on three out of four species and yet, the lack of genes surveyed in *S. frugiperda* and *S. exigua* make conclusions difficult (Figure S2B,C,D). Extending both the number of detoxification genes assayed and compounds used in future studies would be of great interest to identify evolutionary conserved detoxification responses among close *Spodoptera* species.

2.1. Phase I detoxification: functionalization of xenobiotics

193 *2.1.1. P450 enzymes encoded by the CYP genes*

194 P450 enzymes are heme thiolate proteins that catalyze a wide spectrum of reactions
195 including oxidations involving C–C or C=N bond cleavage, hydrolysis, dehydration,
196 dehydrogenation, dehalogenation and most notably monooxygenation of a variety of substrates
197 (Mansuy, 1998) altogether encompassing 60 different types of chemical reactions (Feyereisen,
198 2011, 2012). They are known to play an important role in the interactions of insects with plants.
199 They are usually considered as the first line of enzymatic defense against xenobiotics and have
200 been studied in great detail (Dermauw et al., 2020; Nauen et al., 2022; Feyereisen, 2005, 2012).
201 Beyond their major role in detoxification, P450s are also involved in pheromone, hormone
202 biosynthesis and in cuticular hydrocarbon production (Petryk et al., 2003; Qiu et al., 2012; Reed
203 et al., 1994; Rewitz et al., 2006; Warren et al., 2002). Insect P450s are classified into six clans:
204 the mito (mitochondrial) clan, clan CYP2, clan CYP3, clan CYP4, clan CYP16 and clan CYP20
205 (Dermauw et al., 2020; Nelson, 1998). P450 sharing 40% sequence identity belong to the same
206 family while subfamilies are defined by a 55% sequence identity cutoff (Figure 2A). *S. frugiperda*
207 and *S. litura* have large CYPomes (Table S1). The number of manually curated *CYP* genes mounts
208 to 138 in *S. litura* and 136 in *S. frugiperda* (Cheng et al., 2017; Gouin et al., 2017). The size and
209 structure of clans in these two species are quite different due to P450 blooms in clans CYP3 and
210 CYP4 (Figure 2A). The number of genes in clans CYP3 and CYP4 are four to six times higher than
211 that in the two other clans and are unequally distributed in *CYP* subfamilies. For instance,
212 subfamilies in the mitochondrial and CYP2 clan encompass no more than one or two genes
213 whereas some CYP3 and CYP4 subfamilies have up to nine genes such as the *CYP6AEs* (9 genes
214 in *S. litura* and 11 genes in *S. frugiperda*). This distribution however follows a power-law pattern

of many *CYP* families with few genes and few families with many genes widespread in arthropod CYPomes (Dermauw et al., 2020). P450 from clan CYP3 and CYP4 are commonly associated with detoxification, and this is reflected in the expression data collected in *Spodoptera* species: 67% of all *CYPs* induced belonged to clan CYP3 while 22% belonged to clan 4 (Figure 2B).

2.1.1.1. Mitochondrial clan and CYP2 clan

P450 genes from the mitochondrial clan accounted for 6% of all upregulated P450 genes across the literature (Figure 2B). In line with what is known of the biological function of orthologous genes in other species, there were very few reports of mitochondrial and CYP2 P450s induced in *Spodoptera* within the limits outlined in this review (induced by xenobiotics or in resistant populations). The four ecdysteroidogenic genes *CYP302, CYP314, CYP315, CYP306*, involved in the biosynthesis of molting hormones (Dermauw et al., 2020; Rewitz et al., 2006), were mostly absent from the list of differentially expressed genes (Figure S3). A few other P450 genes from the mitochondrial clan including *CYP339, CYP428* and *CYP49* were not found differentially regulated in response to xenobiotics and there is very limited evidence as to the role they play in other species. In *S. frugiperda* the most induced mitochondrial P450s belonged to the *CYP333* family, representing approx. 80% of all induced P450s from this clan (Figure 2C). *CYP333B4* was induced by 7 out of 11 different treatments applied to larvae and Sf9 cells, and fipronil was the sole treatment that induced the expression of *CYP333B4* in both larval midgut and Sf9 cells (Giraudo et al., 2015). This makes *CYP333B4* the most frequently induced P450 in *S. frugiperda*. In *S. litura CYP333B3* was induced by four different chemicals including xanthotoxin, imidacloprid, fluralaner and indoxacarb (Cheng et al., 2017; Jia et al., 2020; Shi et al., 2019). The

mitochondrial CYP333 gene family was analyzed in *B. mori* by looking at its cluster organization and intron-exon relationships with P450s from other insect species and it was found that *CYP333B1* and *CYP333B2* formed an orthologous group in the phylogenetic tree with the mitochondrial P450 *Dm*CYP12 suggesting functional conservation (Ai et al., 2011). CYP12 genes have been associated with insecticide resistance in *Drosophila* and *Anopheles* (Feyereisen, 2005) and *CYP12A1* is phenobarbital-inducible in the housefly *Musca domestica* (Guzov et al., 1998). This is corroborated by the fact that *BmCYP333A2* (BMIBMGA005356) was earlier found induced 2.3-, 3.5- and 3.3-fold in response to diazinon, imidacloprid and permethrin, respectively (Yamamoto et al., 2010). In this respect, upregulation of *CYP333B3* and *CYP333B4* in both *S. frugiperda* and *S. litura* to PSM and insecticide exposure is consistent with what was found for this P450s family in other insect species before and points toward a general role in xenobiotic metabolism. Biochemical and genetic characterizations are still lacking and should be performed in order to functionally validate the role of these two CYPs in *Spodoptera* species.

2.1.1.2. CYP3 clan

CYP321 genes

CYP321 family members accounted for ca. 20% of all CYP3 P450s upregulated in *S. exigua*, *S. litura* and *S. frugiperda* (Figure 2C) (Carvalho et al., 2013; Cheng et al., 2017; Giraudo et al., 2015; Hu et al., 2019c; Jia et al., 2020; Liu et al., 2019; Nascimento et al., 2015; Shi et al., 2019; Wang et al., 2017a; Wang et al., 2017b). In particular *CYP321A7*, *CYP321A8* and *CYP321A9* were among those most often upregulated from this family (Cheng et al., 2017; Giraudo et al., 2015; Hu et al., 2019c; Jia et al., 2020; Wang et al., 2017a). In *S. litura* the role of several CYP321

259 genes were confirmed in insecticide resistance and plant toxin metabolism. Indeed, *CYP321A7*
260 was significantly overexpressed after imidacloprid, xanthotoxin and ricin exposure (Cheng et al.,
261 2017). Subsequent RNAi-mediated knock-down substantially affected imidacloprid-challenged
262 5th instar larvae compared to GFP control. *CYP321A7* was by far the most often overexpressed
263 P450 in *S. litura*. Transcript levels were induced by a wide array of plant allelochemicals
264 including coumarin, flavone, isoasatone A, jasmonic acid, methyl jasmonate, methyl salicylate,
265 ricin, salicylic acid and xanthotoxin (Cheng et al., 2017; Wang et al., 2017a). Hu and coworkers
266 (2019c) assessed the expression patterns of 68 CYP genes in response to five different
267 insecticides in *S. exigua* fat body cells. Among them *CYP321A16* and *CYP332A1* were found up-
268 regulated suggesting their potential involvement in a xenobiotic response. In a follow-up study,
269 it was shown that transgenic *D. melanogaster* flies expressing *SeCYP321A16* and *SeCYP332A1*
270 were significantly more tolerant to chlorpyrifos treatments than wildtype flies (Hu et al., 2020).
271 Recombinant *Se*CYP321A16 and *Se*CYP332A1 expressed in Sf9 cells were shown to metabolize
272 chlorpyrifos *in vitro* demonstrating that these P450s likely contribute to the resistance of *S.*
273 *exigua* to this insecticide. In another study, *CYP321B1* was upregulated after chlorpyrifos and *β*-
274 cypermethrin exposure in *S. litura* larvae and RNAi-mediated silencing of *CYP321B1* significantly
275 increased mortality of larvae under the same treatment conditions (Wang et al., 2017b). It is
276 also noteworthy to mention the recent discovery that the overexpression of *S. exigua*
277 CYP321A8, which is responsible for resistance to chlorpyrifos, cypermethrin, and deltamethrin
278 in a strain from China, is due to two distinct mechanisms: the overexpression of transcription
279 factors CncC and Maf as well as a mutation in the promoter region resulting in a new predicted

cis-acting element that putatively facilitates the binding of the nuclear receptor Knirps (Hu et al., 2021).

CYP6 genes

In *S. litura* and *S. exigua* CYP6 family genes accounted for approx. 50% and 40% of induced CYP3 clan P450s, respectively (Figure 2C). In *Spodoptera* this family is divided into six subfamilies including CYP6AB, CYP6B and CYP6AE, which are the most represented and both quantitatively and qualitatively involved in the detoxification of xenobiotics. Others include CYP6AN, CYP6AW and CYP6CT genes of which very little is known to date (Figure 2A).

CYP6AB genes were reported to be induced in nine different studies (Carvalho et al., 2013; Cheng et al., 2017; Hafeez et al., 2019a; Hu et al., 2019c; Jia et al., 2020; Lu et al., 2020; Lu et al., 2019b; Sun et al., 2019; Wang et al., 2015c), and *CYP6AB12*, *CYP6AB60* and *CYP6AB31* are among those most often upregulated (Cheng et al., 2017; Hu et al., 2019c; Jia et al., 2020; Lu et al., 2020; Lu et al., 2019b; Sun et al., 2019). *CYP6AB12* was induced in *S. litura* only and overexpressed upon imidacloprid and ricin exposure in an RNA-seq study, but was downregulated by xanthotoxin (Cheng et al., 2017). *CYP6AB12* transcript levels were also significantly higher after fluralaner exposure, an isoxazoline insecticide (Jia et al., 2020, gene4502). *CYP6AB12* expression pattern was shown to be midgut-specific and implicated in *S. litura* pyrethroid insecticide resistance (Lu et al., 2020; Lu et al., 2019b). Lu et al. (2020) nicely linked increased levels of *CYP6AB12* transcripts to ROS (reactive oxygen species) bursts triggered by pyrethroid insecticide exposure and mediated by the Cap'n'collar isoform C (CncC)/Muscle aponeurosis fibromatosis (Maf) transcription pathway. The roles of two

302 additional *Sl*CYP6AB genes (*CYP6AB14* and *CYP6AB60*) were functionally confirmed in insecticide
303 tolerance and upon exposure to various phytochemicals including coumarin, flavone, tomatine
304 and xanthotoxin and by increased larval sensitivity to these toxins after RNAi-mediated silencing
305 of respective P450 genes (Sun et al., 2019; Wang et al., 2015c). Noteworthy, the deltamethrin-
306 inducible *SeCYP6AB14* was similarly validated in an RNAi-mediated silencing assay which
307 resulted in enhanced deltamethrin sensitivity of exposed larvae (Hafeez et al., 2019a).
308 Altogether these results strongly advocate for the role of the CYP6AB subfamily in metabolic
309 resistance to plant allelochemicals and pyrethroid insecticides. CYP6AB P450s were shown to be
310 involved in xenobiotic metabolism in other species *i.e., CYP6AB56* of cabbage moth *Mamestra*
311 *brassicae* was also induced by deltamethrin and shown to be important by an RNAi-mediated
312 knock-down assay using the same insecticide (Zhou et al., 2017).

313 The CYP6B subfamily is one of the major groups of CYP6s involved in PSM and insecticide
314 metabolism and has been extensively studied (reviewed in Heckel, 2014; Li et al., 2007).
315 Members of this subfamily were overexpressed to a high extent in *Spodoptera* (Carvalho et al.,
316 2013; Cheng et al., 2017; Giraudo et al., 2015; Jia et al., 2020; Li et al., 2019; Liu et al., 2018; Liu
317 et al., 2019; Lu et al., 2019a; Shi et al., 2019; Wang et al., 2015a; Wang et al., 2018c; Wang et al.,
318 2016; Zhou et al., 2012a; Zhou et al., 2012b). In *S. litura* larvae, *CYP6B48* (Cheng et al., 2017; Liu
319 et al., 2018; Liu et al., 2019; Wang et al., 2015a), *CYP6B58* (Cheng et al., 2017; Liu et al., 2018;
320 Wang et al., 2015a) and *CYP6B47* (Cheng et al., 2017; Liu et al., 2018; Zhou et al., 2012a; Zhou et
321 al., 2012b) were highly responsive to xenobiotic challenges and the most recurrent genes
322 induced. Inducers were as diverse as flavones, ricin, imidacloprid, fenvalerate and α-
323 cypermethrin. Most notably, the furanocoumarin xanthotoxin significantly enhanced the

transcripts of all three *S. litura* CYP genes as well as those of *SfCYP6B39* (Giraudo et al., 2015). *SfCYP6B39* was moreover recently found overexpressed 257-fold in a Brazilian population showing resistance to deltamethrin and chlorpyrifos (Boaventura et al., 2020a). Insect CYP6B enzymes are known for their ability to detoxify furanocoumarins in specialists of the *Papilio* genus feeding on plants producing these toxic metabolites (Cohen et al., 1992; Hung et al., 1995; Li et al., 2002; Ma et al., 1994; Petersen et al., 2001). Investigating the metabolizing activity of CYP6Bs belonging to generalist Lepidoptera species including *Papilio glaucus* and *Helicoverpa zea* has shown that CYP6B enzymes were also capable of metabolizing furanocoumarins, but with less efficiency. Subsequent metabolic assays showed that the generalist *Hz*CYP6B8 exhibited substantial catalytic activity against other plant allelochemicals (quercetin, flavone, chlorogenic acid, indole-3-carbinol, rutin, etc.) as well as insecticides (cypermethrin, aldrin and diazinon) but with lower efficiency (Rupasinghe et al., 2007). In *H. armigera CYP6B6* was recombinantly expressed in *E. coli* and the recombinant protein was able to transform esfenvalerate into 4'-hydroxy fenvalerate, as well as capsaicin into several metabolites (Tian et al., 2017; Tian et al., 2019). It would be of interest to know whether CYP6Bs orthologous genes in species with a similar host plant range such as *Spodoptera*, are able (similar to *H. zea* or *H. armigera*) to metabolize phytochemicals and insecticides upon induction. To date, *SlCYP6B41* is the only member of this family confirmed by RNAi-mediated knock-down to be involved in imidacloprid tolerance in *Spodoptera* (Cheng et al., 2017).

CYP6AE is a third CYP6 subfamily with an increasing body of evidence for its involvement in the metabolism of plant phytochemicals in Lepidoptera as most notably documented in *H. armigera* (Celorio-Mancera Mde et al., 2011; Krempl et al., 2016a; Krempl et al., 2016b; Liu et

al., 2015; Shi et al., 2018; Tao et al., 2012; Wang et al., 2018a; Zhou et al., 2010). In *Spodoptera* the accumulation of CYP6AE transcripts was reported in several studies (Carvalho et al., 2013; Cheng et al., 2017; Cui et al., 2020; Hafeez et al., 2020; Hu et al., 2019c; Shi et al., 2019). Hafeez et al. (2020) investigated the effect of quercetin exposure on tolerance of *S. exigua* larvae to λ-cyhalothrin. They showed in RT-qPCR assays that exposing larvae to quercetin, λ-cyhalothrin and their combination, resulted in higher transcript levels of *CYP6AE10*. RNAi to silence this P450 in larvae led to increased mortality suggesting that CYP6AE10 might take part in the detoxification of these xenobiotics. *CYP6AE10* and *CYP6AE47*, were found highly upregulated in *S. exigua* after larvae were exposed to various insecticides such as λ-cyhalothrin, chlorantraniliprole, metaflumizone and indoxacarb (Hu et al., 2019c) confirming the results of Hafeez et al. (2020). In another study, RNAi-based silencing of *SlCYP6AE71* led to an increase in sensitivity in treated larvae suggesting that this P450 is involved in *S. litura* tolerance to imidacloprid (Cheng et al., 2017). In *S. frugiperda*, *CYP6AE44* was upregulated in two different studies. Carvalho et al. (2013) used EST sequences from SPODOBASE (Negre et al., 2006) to analyze the gene expression in two *S. frugiperda* populations, resistant to organophosphates (OP) and pyrethroids, in a microarray-based study. Identification of the EST sequences by BLAST searches against the reference genome (LepidoDB, www.genouest.fr) revealed the overexpression of, among others, *CYP6AE44* in the OP resistant strain (Carvalho et al., 2013). In a more recent study, *CYP6AE44* was also found upregulated in Sf9 cells previously challenged with the alkaloid harmine, a monoamine oxidase inhibitor (Cui et al., 2020).

CYP9 genes

In *S. litura* CYP9s are subdivided into four subfamilies: CYP9A with 15 genes and CYP9AJ, CYP9BS and CYP9G encompassing one gene each. *S. frugiperda* has three CYP9 subfamilies: CYP9A with 14 genes, CYP9G and CYP9AJ with a single gene each (Figure 2A). Of these subfamilies, CYP9As have undergone recent CYP blooms and are organized in clusters on chromosome 29 and chromosome 6 in *S. litura* and *S. frugiperda,* respectively (Cheng et al., 2017; Xiao et al., 2020) (Figure 3). As noted earlier, *Spodoptera* species did not have genes reported yet commonly responding to deltamethrin exposure in the literature (Figure S2). However, most of the genes found upregulated by deltamethrin belonged to the CYP9A subfamily. Similarly, a closer look at genes that were induced by PSM and insecticides shows that three out the four found in both *S. litura* and *S. frugiperda* overlaps are CYP9As (Figure S4) *CYP9A40* was upregulated in *S. litura* (Wang et al., 2015b), *CYP9A30*, *CYP9A31*, *CYP9A32* and *CYP9A59* were upregulated in *S. frugiperda* (Boaventura et al., 2020a; Giraudo et al., 2015), while *CYP9A105*, *CYP9A12*, *CYP9A98* were upregulated in *S. exigua* (Hafeez et al., 2019a; Hu et al., 2019c; Wang et al., 2018b). These observations in *Spodoptera* are somewhat consistent with additional reports on the CYP9As associated with pyrethroid resistance in other insect pests such as *H. armigera* and *Locusta migratoria* (Brun-Barale et al., 2010; Guo et al., 2015; Zhu et al., 2016).

Although organized in clusters (Figure 3) and sharing high sequence homology – only three pairs of clustered CYP9As can be considered as 1:1 orthologues, while *CYP9A28-31* share 76-90% amino acid sequence identity (Sezutsu et al., 2013) – the expression patterns of CYP9As in *Spodoptera* are quite diverse in response to PSMs and insecticides (Carvalho et al., 2013; Cheng et al., 2017; Giraudo et al., 2015; Hafeez et al., 2019a; Hafeez et al., 2019b; Hu et al.,

2019c; Nascimento et al., 2015; Wang et al., 2018b; Wang et al., 2015b; Wang et al., 2018c; Wang et al., 2016; Zhou et al., 2012a) suggesting mechanisms of differential regulation of gene expression. This is exemplified in *S. litura* by an RNA-seq study showing complex expression profiles of the CYP9A clustered genes in the midgut, Malpighian tubules and fat bodies of larvae exposed to two PSMs (xanthotoxin and ricin) and imidacloprid (Cheng et al., 2017). Altogether, CYP9 genes accounted for roughly 50% of all upregulated CYPs from clan CYP3 in *S. frugiperda* (Figure 2C). The share of upregulated genes belonging to the CYP9 family was also high in *S. exigua* (ca. 40%) and less so in *S. litura* (*ca.* 20%) (Figure 2C). Although somewhat biased by the methodology used to assess transcript levels (RNA-seq *vs* RT-qPCR and microarrays) the data highlights the frequency at which *CYP9A30*, *CYP9A31* and *CYP9A32* are upregulated in *Spodoptera* (*e.g.* in 4, 7 and 5 different conditions respectively in *S. frugiperda* mainly reported by Giraudo et al. (2015)). Recently, Boaventura et al. (2020a) also reported overexpression of *CYP9A59* and a CYP9A-like gene (by 267-fold) in a deltamethrin and chlorpyrifos resistant *S. frugiperda* strain from Brazil. Currently, only two CYP9A genes from *S. litura* have been confirmed to play a role in detoxification of xenobiotics by RNAi-based silencing: *dsCYP9A40* injection into larvae resulted in increased susceptibility to quercetin, cinnamic acid, deltamethrin and methoxyfenozide (Wang et al., 2015b) and *dsCYP9A31* injections were associated with increased mortality of larvae to imidacloprid (Cheng et al., 2017). In *S. exigua*, four CYP9As were linked to metabolic detoxification of xenobiotics in similar RNAi experiments: *SeCYP9A10* to α-cypermethrin (Hafeez et al., 2019b), *SeCYP9A21v3* in a chlorantraniliprole-resistant field population from China (Wang et al., 2018c), *Se*C*YP9A105* in α-cypermethrin,

deltamethrin and fenvalerate treated larvae (Wang et al., 2018b) and *SeCYP9A98*, in tolerance of larvae to deltamethrin exposure (Hafeez et al., 2019a).

CYP9As stand out for being metabolizing enzymes of xenobiotics and are notorious for their alleged and sometimes confirmed role in insecticide resistance phenotypes. For instance, *CYP9A12* and *CYP9A14* were associated with resistance to pyrethroids in *H. armigera*, elevated transcript levels of these two CYPs were reported in a fenvalerate-selected resistant laboratory strain (Yang et al., 2008) and subsequent functional expression of recombinant proteins in *Saccharomyces cerevisiae* showed clearance activity against the pyrethroid esfenvalerate, providing strong evidence that enhanced expression of pyrethroid-detoxifying enzymes can confer a resistance phenotype (Yang et al., 2008). It is only very recently that the first functional study involving a CYP9A in *Spodoptera* provided evidence that CYP9A186 conferred emamectin benzoate (EB) and abamectin resistance in *S. exigua* (Zuo et al., 2021a). The use of CRISPR/Cas9 to knockout *CYP9A186* fully restored the susceptibility of the resistant strains to EB and abamectin. Although *CYP9A186* was also found 10-fold overexpressed in the EB-resistant population, the heterologous expression of CYP9A186 from both susceptible and resistant insects combined with *in vitro* metabolic bioassays showed that a single substitution (F116V) in the P450 substrate recognition site 1 (SRS1) enabled enhanced metabolism of EB and abamectin and conferred resistance in *S. exigua*.

2.1.1.3. CYP4 clan

P450s from clan CYP4 accounted for 22% of all CYPs upregulated in the *Spodoptera* literature compiled in this review (Figure 2B). Clan CYP4 comprises 52 P450 genes in *S. litura* and

38 in *S. frugiperda* (Figure 2A). They were found upregulated in *S. litura* (32 genes), *S. frugiperda* (5) and *S. exigua* (10) (Figure 2C). The CYP4 family accounted for approximately 70% of clan CYP4 P450s upregulated in *S. frugiperda*, 65% in *S. exigua* and 75% in *S. litura*, suggesting that they might have a crucial role in xenobiotic response (Cheng et al., 2017; Cui et al., 2020; Giraudo et al., 2015; Hu et al., 2019c; Jia et al., 2020; Li et al., 2019; Liu et al., 2018; Shi et al., 2019; Wang et al., 2018c; Wang et al., 2016; Yi et al., 2018).

CYP4G75 was found upregulated under several conditions in *S. litura*, and hence the one most often upregulated in this species (Cheng et al., 2017; Li et al., 2019). It was moderately upregulated by exposure to imidacloprid and ricin in the midgut and Malpighian tubules of larvae but repressed in xanthotoxin treatments as well as in fat bodies of all treatments considered (Cheng et al., 2017). In another study where larvae were challenged with tomatine, *CYP4G75* was also induced in the midgut and repressed in fat bodies (Li et al., 2019). The remainder CYP4G genes induced in *Spodoptera* were limited to *SlCYP4G106*, *SlCYP4G109*, *SlCYP4G74* and *SeCYP4G37* genes (Wang et al., 2016). The CYP4G family is well-described for its involvement in cuticular hydrocarbon synthesis in several insect species (Balabanidou et al., 2016; Feyereisen, 2020; Kefi et al., 2019; Qiu et al., 2012; Wang et al., 2019). *S. litura* and *S. frugiperda* CYP4G family encompasses 4 genes, but whether these genes are involved in cuticular hydrocarbon synthesis and capable of providing a tolerance phenotype to sustained insecticide exposure is still uncertain and needs to be investigated yet.

Some CYP4S and CYP4L genes were found upregulated a few times across the literature. *CYP4S8* (Cheng et al., 2017; Hu et al., 2019c; Jia et al., 2020; Shi et al., 2019) and *CYP4S9* (Cheng et al., 2017; Hu et al., 2019c; Jia et al., 2020; Yi et al., 2018) for instance were induced in *S.*

exigua and *S. litura*, respectively, by fluralaner, abamectin, λ-cyhalothrin, chlorantraniliprole, metaflumizone, and indoxacarb. *CYP4S9* in particular was strongly induced after abamectin exposure in fat body cells of *S. exigua* (Hu et al., 2019c). *CYP4L12* (Cheng et al., 2017; Jia et al., 2020), and *CYP4L9* (Cheng et al., 2017) were strongly induced in the midgut of *S. litura* after xanthotoxin and ricin exposure, and to a lesser extent after imidacloprid treatment in fat bodies and Malpighian tubules (Cheng et al., 2017). The CYP4L family was first established as a new subfamily in *M. sexta* and has repeatedly been associated with sex pheromone recognition and clearance of receptors in sensory organs such as antennae, pheromone glands and the base of sensilla trichodea (Feng et al., 2017; Maïbeche-Coisne et al., 2002; Maïbeche-Coisne et al., 2005; Rong et al., 2019; Snyder et al., 1995).

Very few expression data were available for clan CYP4 P450s in *S. frugiperda* (Supplementary material). Only five genes out of 38 were reported upregulated in the literature, most of them were found induced in one single situation except for *CYP4M14* which was moderately upregulated by exposure to xanthotoxin in larval midguts and by 2-tridecanone and methoprene in Sf9 cells (Giraudo et al., 2015).

2.1.2. Carboxylesterases

The carboxylesterase (CE) gene superfamily is the second group of enzymes to participate in the functionalization of lipophilic exo- and endogenous compounds. It encompasses enzymes hydrolyzing diverse carboxylic, thio-, phospho-, and other ester substrates into their alcohol and acid components by relying on a catalytic triad of amino acid residues including a reactive serine nucleophile (Oakeshott et al., 2005). Similar to P450s, CEs

476 are widespread in prokaryotes and eukaryotes (Oakeshott et al., 2005). The CE gene family
477 classification is based on phylogenetic analyses and substrate specificities resulting in 3 classes
478 and 33 clades (Ranson et al., 2002; Teese et al., 2010). The first class contains proteins
479 considered to be non-catalytically active (with the exception of acetylcholinesterases) and
480 involved in neuro/developmental functions (Biswas et al., 2010). The second class encompasses
481 catalytically active, excreted enzymes involved in insect hormone and pheromone processing,
482 found mostly expressed in the antennae and insect olfactory organs (Vogt et al., 1985). The
483 third class contains active enzymes usually expressed in the midgut with intracellular
484 localization to microsomes, cytosol and mitochondria and are predicted to have digestion or
485 detoxification functions based on their expression in the midgut (Oakeshott et al., 2005; Small
486 and Hemingway, 2000; Teese et al., 2010). Some esterases were shown to be involved in
487 insecticide resistance and most of these are linked to the third class, with also a few belonging
488 to the second class (Claudianos et al., 2006; Cui et al., 2011; Teese et al., 2010).

489 The CE gene family is, just as P450s, very consistent in *Spodoptera* genomes. The
490 genome of *S. litura* contains 110 CE genes (Cheng et al., 2017) (Table S1). Over the past decade,
491 70 different CEs were reported upregulated *in S. litura* upon xenobiotics exposures or in
492 insecticide resistant populations (Supplementary material). Although *S. frugiperda* possesses 93
493 CE genes (Gouin et al., 2017), little information on their expression is available in the literature -
494 only seven genes were reported upregulated to date (see below for more details). In *S. exigua*,
495 only one predicted CE gene was found upregulated in a chlorantraniliprole-selected resistant
496 laboratory strain (Unigene0045545, orthologous to carboxylesterase ae17 [*B. mori*]) as revealed
497 by RNA sequencing (Wang et al., 2018c). However, the amount of expression data gathered in *S.*

frugiperda and *S. exigua* over the past decade is limited, which is mostly due to the lack of RNA-seq datasets (Figure 1A). The lack of a reference genome for *S. littoralis* makes it difficult to thoroughly analyze the CE gene family in this species. However, a recent transcriptome assembly provided a well-curated set of annotated gene transcripts of 56 CE genes from different chemosensory and non-chemosensory organs (Walker et al., 2019), including the 30 previously described genes (Durand et al., 2010; Durand et al., 2012; Merlin et al., 2007).

CEs are found under various denominations across the literature (e.g. CXE, COE or CarE) which makes comparisons between studies difficult. Nonetheless, we below refer to the nomenclature used by the authors in the cited literature. CEs were associated with the detoxification of xenobiotics in *S. litura* larvae, as shown by their inducibility after ricin, xanthotoxin and imidacloprid treatments in an RNA-seq study (Cheng et al., 2017). The involvement of these CEs in metabolic resistance was further exemplified when *dsCOE057* and *dsCOE058*-injected larvae showed increased susceptibility compared to the control after imidacloprid exposure. This RNA-seq study revealed that *COE050* was primarily induced in the midgut by all three treatments, in Malpighian tubules after imidacloprid and ricin exposure as well as in fat bodies by xanthotoxin. In another study, high levels of *COE50* transcripts were detected in two indoxacarb resistant laboratory and field populations (Shi et al., 2019). Besides *COE050*, three additional CEs were shown to be overexpressed multiple times in the presence of a xenobiotic: *COE024*, *COE030* and *COE037* were similarly induced by ricin, imidacloprid and xanthotoxin (Cheng et al., 2017) and *COE030* (gene5053) was also shown to be induced by fluralaner (Jia et al., 2020).

519 In *S. frugiperda*, the five CEs differentially expressed in a lufenuron-resistant population
520 when compared to a susceptible population were all upregulated (Nascimento et al., 2015). The
521 microarray analysis carried out by Carvalho et al. (2013) revealed that *CXE13* and *CXE001c* were
522 overexpressed 21-fold and 3-fold in chlorpyrifos and λ-cyhalothrin resistant *S. frugiperda*
523 populations, respectively, when compared to a susceptible population. *CXE13* was characterized
524 in *S. litura* and *S. exigua* for its ability to metabolize plant volatiles and sex pheromones (He et
525 al., 2014). *Se*CXE13 and *Sl*CXE13 were functionally expressed in High Five cells and purified.
526 Recombinant enzymes were used in enzyme activity and kinetic studies with 20 different sex
527 pheromones and other acetates. The two homologous esterases displayed a broad substrate
528 spectrum and a highly similar hydrolysis pattern. Among the 20 acetate derivatives tested, 18
529 were hydrolyzed to different degrees. However, forward genetic-based functional studies are
530 still necessary to confirm the ability of these enzymes to metabolize insecticides.
531 CEs are one of the three major types of proteins commonly accepted to be involved in
532 arthropods olfaction process (Vogt, 2005). Several CEs are odorant-degrading enzymes and are
533 hence often excreted into the cellular interspace or in cuticular wax layers to clear olfactory and
534 gustatory receptors from environmental cues (Ferkovich et al., 1982; Vogt and Riddiford, 1986).
535 *SlCXE10* (sic) was found highly expressed in adults antennae and shown to hydrolyze a green
536 leaf ester (Z3-6:Ac) produced by host-plants with high efficiency in kinetic studies combined
537 with GC-MS analyses (Durand et al., 2010). The sequence analysis of *SlCXE10* predicted it to
538 belong to the third class of CEs, known to be implicated in detoxification. Similarly, a recent
539 study identified and amplified the cuticular *SeCXE11*. Its purified recombinant protein showed

high hydrolytic activity towards two plant volatiles, *i.e.* (Z)-3-hexenyl caproate and pentyl acetate with >50% degradation (He et al., 2020).

2.2. Phase II detoxification: conjugation of xenobiotics or metabolites

2.2.1. Glutathione S-transferases

Although detoxification mediated by glutathione S-transferases (GSTs) can principally fall into phase I (Ranson et al., 2001), they are best known for conjugating the thiol group of glutathione (GSH) to molecules possessing an electrophilic center (Enayati et al., 2005). The target molecules, endogenous metabolites or reactive products formed by phase I P450s or CEs, are thus rendered more water soluble which facilitates their elimination from the insect body (Enayati et al., 2005). The role of GSTs in protecting insects from adverse effects of plant chemicals is well-known, but most studies have focused on their involvement in insecticide resistance (Pavlidi et al., 2018). GSTs have been classified into two major groups according to their location within the cells, *i.e.* cytosolic or microsomal. The cytosolic GSTs are subdivided into six different classes: sigma (s), zeta (z), theta (t) and omega (o) classes are found ubiquitously across taxa and are believed to play roles in conserved endogenous functions, while two additional classes restricted to insects form multigene families and are involved in xenobiotic detoxification: epsilon (e) and delta (d) (Chelvanayagam et al., 2001; Ranson et al., 2002). Some GSTs were not assigned to any existing class and were hence designated as "unclassified".

The genome of *S. litura* contains 47 GST genes (Table S1) out of which the epsilon class counts 20 members of two clusters of recently duplicated genes on chromosome 9 and 14

562 (Cheng et al., 2017). The theta, sigma, delta, zeta and omega classes encompass 1, 7, 5, 2 and 3
563 genes, respectively. The remaining ones are split between five microsomal and four
564 "unclassified" GSTs. The GST repertoire of *S. frugiperda* includes 46 genes, as reported in the
565 manually annotated reference genome (Gouin et al., 2017). These numbers are quite similar to
566 those found in the genomes of *H. armigera* (42) and *H. zea* (40) (Pearce et al., 2017).

567 GSTs were ubiquitously overexpressed in *Spodoptera* in response to all kinds of
568 xenobiotics and stressors, with the notable exception of clofibrate and phenobarbital, two
569 model inducers (Supplementary material). In *S. litura*, a total of 31 GSTs were reported
570 upregulated, 19 of those belonged to the epsilon class, some being relatively frequently
571 overexpressed such as *GSTe2* (Deng et al., 2009; Li et al., 2019; Liu et al., 2019), *GSTe3* (Deng et
572 al., 2009; Huang et al., 2011; Liu et al., 2019; Zhang et al., 2016) and *GSTe6* (Cheng et al., 2017).
573 In *S. exigua*, 21 GST genes were found induced, from which nine, four, four and three belonged
574 to the GSTe, GSTo, GSTs and GSTd classes, respectively (Hu et al., 2019a; Hu et al., 2019b; Wang
575 et al., 2018c; Xu et al., 2016).

576 *GSTe1* was one of the most upregulated GSTs in *S. exigua* (Hu et al., 2019a; Hu et al.,
577 2019b) and by far the most frequently upregulated in *S. litura*, but not in *S. frugiperda* (Chen et
578 al., 2018; Huang et al., 2011; Ling et al., 2019; Liu et al., 2018; Xu et al., 2015; Zhang et al., 2016;
579 Zou et al., 2016). *SlGSTe1* was, among other GSTs including *GSTe3*, *GTSe4* and *GSTe5*,
580 upregulated between 3-fold and 5-fold at 48h, 72h and 96h after treatment of sublethal doses
581 of fluralaner (Liu et al., 2018). However, synergist assays using dimethyl maleate (DEM) showed
582 no difference in susceptibility of fluralaner treated insects. A total of four independent studies
583 reported the induction of *SlGSTe1* after chlorpyrifos exposure or in a chlorpyrifos resistance

584 phenotype (Chen et al., 2018; Huang et al., 2011; Xu et al., 2015; Zhang et al., 2016). *SlGSTe1*
585 mRNA transcript levels were 5.51-fold increased after chlorpyrifos exposure in a chlorpyrifos-
586 selected population compared to a susceptible population (Zhang et al., 2016). Other GSTs were
587 also highly inducible in this laboratory-selected population including *SlGSTe3*, *SlGSTe10*,
588 *SlGSTe15*, *SlGSTt1*, *SlGSTo2*, *SlGSTs5*, *SlMGST1-2* and *SlMGST1-3*. Interestingly, *SlGSTe13*,
589 *SlMGST1-1*, *SlGSTt1* and *SlGSTz1* were specifically upregulated in the selected population, but
590 not inducible when larvae were exposed to chlorpyrifos. Xu et al. (2015) analyzed the
591 detoxification activity of *SlGSTe1* in *S. litura* for several insecticides and heavy metals. They
592 showed that *Sl*GSTe1 protein level was upregulated in the gut of insects after feeding on
593 chlorpyrifos and cadmium. Although *Sl*GSTe1 was demonstrated to bind to some heavy metals
594 with high affinity, further research is necessary to determine whether GSTs are able to detoxify
595 toxic metals by directly sequestering them. Recombinantly expressed GSTe1 enzymes exerted
596 high activity towards 1-chloro-2,4-dinitrobenzene (CDNB), a GST model substrate (Deng et al.,
597 2009). *SlGSTe1* expression was modulated by additional PSMs such as asatone, isoasatone A,
598 allyl-isothiocyanate (AITC) and indole-3-carbinol (I3C) (Ling et al., 2019; Zou et al., 2016). It was
599 overexpressed at the mRNA and protein level in the midgut of *S. litura* larvae fed on *Brassica*
600 *juncea* or on I3C-/AITC-supplemented diets (Zou et al., 2016). In the same study, a two-
601 dimensional electrophoresis revealed that *Sl*GSTe1 was the only detoxification enzyme
602 overexpressed in the midgut in a dose-dependent manner. The enzyme was shown to catalyze
603 the conjugation of I3C and xanthotoxin in the presence of reduced glutathione with high
604 efficiency. The authors further functionally validated the role of *SlGSTe1 in vivo* by RNAi-based
605 silencing of the gene, inhibiting larval growth and feeding rates. Additional *S. litura* GSTs were

606 confirmed to play a role in xenobiotics detoxification in RNAi-based knock-down experiments,
607 such as *SlGSTs1* to tomatine (Li et al., 2019), *SlGSTe20* and *SlGSTe07* to imidacloprid (Cheng et
608 al., 2017). The confirmation of catalytic activity of candidate GSTs against phytochemicals and
609 insecticides is limited in *S. exigua* and only *Se*GSTe6, *Se*GSTd3, *Se*GSTo2 were investigated for
610 their ability to clear chlorpyrifos and cypermethrin insecticides in the presence of GSH using
611 HPLC analysis (Hu et al., 2019b).
612
613 *2.2.2. (UDP)-glycosyl transferases*
614 UDP-glycosyltransferases (UGTs) constitute an enzyme superfamily found in all kingdoms
615 of life and responsible for conjugating lipophilic endo- and xenobiotic substrates into more
616 water-soluble glycosylated compounds (Bock, 2016). UGTs are membrane-bound proteins
617 divided into two main domains: the N-terminal domain binds to aglycone substrates while the
618 C-terminal domain is responsible for binding the sugar donor and anchoring the protein to lipid
619 membranes. The C-terminal domain encompasses a signature motif of 44 amino-acids highly
620 conserved across all organisms that catalyzes the linking of activated UDP-glucose moieties to
621 specific substrates (Ahn et al., 2012; Krempl et al., 2016b). UGTs are named and classified in
622 accordance with the nomenclature guidelines of the UGT Nomenclature Committee (Mackenzie
623 et al., 1997), which groups them into families designated by a number including sequences that
624 share ∼45% or more amino acid sequence identity; subfamilies are designated by a capital
625 letter and group sequences with more than ∼60% amino acid sequence identity. In this
626 international nomenclature, families numbered 31 to 50 and 301 to 351 have been assigned to
627 arthropods. UDP-glycosyltransferases are given multiple roles in insects such as olfaction, cuticle

628 formation, endobiotic modulation, sequestration and detoxification of xenobiotics (Ahn et al.,
629 2012; Despres et al., 2007; Heidel-Fischer and Vogel, 2015; Hopkins and Kramer, 1992; Wang et
630 al., 1999).

631 The genome of the specialist silkworm *B. mori* contains 44 UGT genes, in comparison the
632 generalists *H. armigera* and *H. zea* possess 46 and 42 UGT genes, respectively (Table S1). In the
633 *Spodoptera* genus, the reference genome of *S. frugiperda* contains 47 UGTs (Gouin et al., 2017).
634 No information is available on the exact number of UGT genes in *S. litura* and *S. exigua* although
635 a few selected genes have been investigated (Hu et al., 2019c; Li et al., 2019; Shi et al., 2019).
636 However, *SlittUGT46A6* (*S. littoralis* UGT46A6) was reported to be induced after topical
637 deltamethrin application onto antennae, suggesting a role in clearance of xenobiotics and
638 involvement in olfaction (Bozzolan et al., 2014). In contrast, 17 UGTs were reported upregulated
639 in *S. litura* by two different studies (Supplementary material). The first study demonstrated in a
640 *S. litura* population resistant to the oxadiazine indoxacarb that 10 UGT genes were significantly
641 over-expressed as compared to a susceptible strain, however functional expression studies
642 confirming their involvement in resistance were lacking (Shi et al., 2019). The second study
643 revealed that exposing larvae to tomatine-supplemented artificial diet induced the expression
644 of seven UGT genes mostly belonging to UGT33 and UGT40 families (Li et al., 2019). UGT33 was
645 by far the most represented family throughout the literature of all surveyed *Spodoptera* species,
646 documented by a total of 16 independent experimental proofs in *S. litura* (at least four UGT
647 genes out of 17), *S. exigua* (four UGT genes out of nine) and *S. frugiperda* (two UGT genes out of
648 five). In *S. exigua* most notably, multiple UGTs were shown to respond in a very similar manner
649 to λ-cyhalothrin, chlorantraniliprole, metaflumizone and indoxacarb, but not abamectin (Hu et

al., 2019c). Indeed, out of 32 UGTs tested, only two were significantly upregulated by abamectin (13-fold for *UGT40D5* and 7-fold for *UGT33T3*) whereas most of the remaining UGTs were significantly downregulated. In contrast, λ-cyhalothrin, chlorantraniliprole, metaflumizone and indoxacarb treatments induced most UGTs in similar expression profiles. For example, *UGT33J3* was found upregulated *ca.* 10-fold after treatment with all aforementioned insecticides. Additional members of UGT40 family were reported to be induced including *SfUGT40D5* (Cui et al., 2020), *SlUGT40Q1* (Li et al., 2019) *SfUGT40-07* (Carvalho et al., 2013), and *SeUGT40R4* (Cui et al., 2020; Hu et al., 2019c).

Benzoxazinoids (BXDs) are known defensive components of grasses such as maize and rye. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) the main BXD in maize and is stored as the inert glucosides (*2R*)-DIMBOA-Glc. When plant tissues are ingested by chewing insects, (*2R*)-DIMBOA-Glc is hydrolyzed by plant specific β-glucosidases, hence releasing the toxic aglycone DIMBOA (Wouters et al., 2014). It was demonstrated that *Spodoptera* species use stereoselective re-glycosylation of activated DIMBOA in their midgut as a detoxification strategy (Wouters et al., 2014). Analyses of larval frass using LC-MS/MS and NMR showed that the main BDXs found in feces was (*2S*)-DIMBOA-Glc, the non-toxic enantiomer of the naturally occurring (*2R*)-DIMBOA-Glc (Glauser et al., 2011; Vassao et al., 2018; Wouters et al., 2014). The molecular work carried out by Israni et al. (2020) recently identified the genes responsible for DIMBOA detoxification in *S. frugiperda*. BDX-metabolizing *SfUGT33F28* and *SfUGT40L8* were highly expressed in the midgut and fat bodies, respectively, and *SfUGT33F28* was inducible when larvae were transferred from bean-based artificial diet to maize plants. Gene silencing *in vivo* of *SfUGT33F28* was strongly correlated with the reduction of (*2S*)-DIMBOA-Glc accumulation in

672 frass, gut DIMBOA-UGT activity and larval growth rate. In addition, *N*-glucosylation of 6-
673 methoxy-2-benzoxazolinone (MBOA), a toxic breakdown product of DIMBOA, was also reported
674 in *S. frugiperda* and *S. littoralis* (Maag et al., 2014).
675
676 *2.3. Phase III: transport and excretion*
677
678 The ATP-binding cassette (ABC) transporter superfamily is the largest membrane
679 transporter family across all kingdoms of life; however, they are still poorly described in insects,
680 although a recent review analyzed ABCs from more than 150 arthropod species and highlighted
681 specific expansions of ABC transporter families which suggest evolutionary adaptation (Denecke
682 et al., 2021). ABC transporters are subdivided into eight subfamilies indicated by the letters A-H.
683 They have been linked to insecticide resistance to at least 27 different chemistries by facilitating
684 efflux of insecticides and acaricides [for a comprehensive review see Dermauw and Van
685 Leeuwen (2014)]. Several recently published papers demonstrate an increasing interest on
686 studying the significance of this gene family in xenobiotic resistant phenotypes (He et al., 2019a;
687 He et al., 2019b; Jin et al., 2019; Li et al., 2020c; Meng et al., 2020; Rosner and Merzendorfer,
688 2020), particularly since an ABC transporter was identified as a crucial receptor for Bt Cry1 toxin
689 binding (Heckel, 2012; Jurat-Fuentes et al., 2021). With the extension of powerful genetic
690 methods and high-throughput sequencing, a clearer picture of gene numbers, sequences and
691 expression profiles of members belonging to this gene superfamily is starting to emerge.
692 Despite the known role of ABCs in detoxification, the information gathered thus far in
693 *Spodoptera* is limited. Elevated transcript levels of ABC transporters in the presence of PSMs or
694 insecticides are only documented in *S. litura*: 38 out of 54 ABC annotated genes were reported

upregulated by three different studies in response to xanthotoxin, tomatine, ricin, imidacloprid as well as in an indoxacarb resistant field strain (Cheng et al., 2017; Li et al., 2019; Shi et al., 2019; Supplementary material). ABC transporters were highly induced in larval midgut by ricin treatments (Cheng et al., 2017), and in another study *ABCG1*, *ABCC4*, *ABCG4* were found strongly expressed (>9-fold) in the midgut after larvae fed on tomatine, while *ABCF4*, *ABCA2*, *ABCB6* were only moderately induced (>2-fold) (Li et al., 2019). In addition, ABC genes might be associated with indoxacarb resistance in an indoxacarb resistant strain of *S. litura*, as nine of them were differentially expressed in a resistant population. Most reports on upregulated ABC genes in *S. litura* were individual findings based on a single condition or tissue, however, *ABCC3*, *ABCB3-1*, *ABCB3-2* and *ABCH1* were independently reported to be induced 4, 3, 3 and 3 times (Cheng et al., 2017; Shi et al., 2019). In accordance with what is known about ABC subfamilies involved in transport of metabolites and conjugates, genes of ABCC and ABCG were most represented among those upregulated by PSMs or insecticides. More precisely, 10 and 11 different ABCCs and ABCGs were found induced in different studies (Cheng et al., 2017; Li et al., 2019; Shi et al., 2019; Supplementary material). Zuo et al. (2017) used CRISPR/Cas9 technology to introduce a four-nucleotide deletion in *S. exigua* P-glycoprotein (*ABCB1*) generating a truncated peptide in a *Se*P-gp (-/-) knockout strain. The susceptibility of mutated larvae to 12 insecticides were tested and showed that deletion of P-gp increased insecticide susceptibility against emamectin benzoate (EB) and abamectin, but not spinosad, chlorfenapyr, beta-cypermethrin, carbosulfan indoxacarb, chlorpyrifos, phoxim, diafenthiuron, chlorfluazuron and chlorantraniliprole, suggesting that P-gp might contribute to abamectin and EB excretion in *S. exigua*.

717

718 **3. Gene expansion and detoxification capacity of *Spodoptera* species**

719 It has been proposed that generalist herbivores exposed to a wide diversity of
720 phytochemicals have expanded their palette of detoxification enzymes as an evolutionary
721 requirement, allowing them to tolerate novel xenobiotics when expanding to newly colonized
722 ecosystems (Heidel-Fischer and Vogel, 2015). This genomic plasticity is in most cases, if not
723 always, embedded in the structural organizations of detoxification genes as many were shown
724 to have expanded and originated from recent tandem duplications forming gene clusters.
725 Although the underlying mechanisms triggering such blooms are still obscure, transposable
726 elements (TE) have been shown to often arise in close proximity of clustered genes (Le Goff and
727 Hilliou, 2017; Rostant et al., 2012) and evidence accumulates suggesting that increasing
728 episodes of TE activity could have been an important source for gene duplication in hexapods
729 (Roelofs et al., 2020).

730 Detoxification gene families have undergone prominent blooms over the course of
731 evolution (Feyereisen, 2011; Ranson et al., 2002). The sequencing of both *S. litura* and *S.*
732 *frugiperda* genomes have revealed large families of detoxification genes in comparison to the
733 monophagous Lepidoptera *B. mori*, CYP genes for example are estimated twice as numerous in
734 both *Spodoptera* species (Table S1). Quite remarkable P450 blooms have occurred in this genus
735 for a few families and seem to be restricted to CYP3 and CYP4 clans. Although P450 blooms are
736 not restricted to a particular CYP clan (Dermauw et al., 2020) no P450 expansions were yet seen
737 in the mitochondrial clan and CYP2 clan. In clan CYP3, the CYP6, CYP9, CYP321 and CYP324
738 families have seen expansions in both genomes compared to *B. mori* (Cheng et al., 2017; Gouin

et al., 2017) and the CYP6AE, CYP6B and CYP321 conserve their synteny across noctuid lineages. In the fall armyworm, the family *Sf*CYP340 of clan CYP4 encompasses 39 genes recently reported to be organized in one large cluster on chromosome 14 (Xiao et al., 2020) was also expanded in the genome of *S. litura* (Cheng et al., 2017) and *Helicoverpa* species (Pearce et al., 2017). Xiao et al. (2020) further analyzed cluster organizations of P450s in the fall armyworm and found that a total of 163 P450 genes were mapped to its 23 chromosomes. Gene clusters can be dated by looking at their distribution in extinct species and conservation across closely related species or clades. The CYP6AE cluster is widespread in noctuid moths and has conserved its head to tail organization. The role of CYP6AE genes in detoxification has been extensively studied in *H. armigera*. Most notably, *HaCYP6AE14* was shown to respond to various plant allelochemicals and insecticides such as xanthotoxin, deltamethrin, monoterpenes, tomatine, taxol and nicotine suggesting an involvement in phytotoxin metabolism (Krempl et al., 2016a; Shi et al., 2018; Zhou et al., 2010). A recent study showed that nine out of ten *Ha*CYP6AEs were able to metabolize esfenvalerate, among which CYP6AE11, CYP6AE14 and CYP6AE17 had the highest metabolic efficiency (Shi et al., 2018). Genome editing to knockout the *Ha*CYP6AE cluster resulted in increased susceptibility of insects to both plant toxins and synthetic insecticides (Wang et al., 2018a). Individually expressing CYP6AEs in heterologous systems helped to identify candidate genes involved in xenobiotic metabolism. CYP6AEs showed distinct enzymatic activities towards tested compounds in particular: xanthotoxin was metabolized by CYP6AE19; 2-tridecanone by CYP6AE11, CYP6AE14, CYP6AE19 and indoxacarb by CYP6AE17 and CYP6AE18 (Wang et al., 2018a). As pointed out by Dermauw et al. (2020) there was no pattern between the catalytic activity, the phylogeny and the position on the cluster of these P450s

761 suggesting that “there is a selective advantage to keep clusters as heritable units”. The
762 conserved CYP9A gene cluster in *Spodoptera* has been linked to PSM and insecticide tolerance
763 (Boaventura et al., 2020a; Gimenez et al., 2020; Giraudo et al., 2015). In *S. frugiperda* Giraudo et
764 al. (2015) showed complex induction patterns of those P450s to 11 different xenobiotics
765 implying the involvement of a complex regulation network. Figure 3 presents the number of
766 induction occurrences across the literature from selected gene clusters in *S. litura* and *S.*
767 *frugiperda*. The data collected in the present review suggests that most CYP9A genes are
768 inducible by xenobiotics. Although functional evidence for their role in insecticide and plant
769 toxin metabolism is still scarce, a a recent report demonstrated that CYP9A transcriptomic
770 responses can match their metabolic capacity (Zuo et al., 2021a). Induction patterns of clustered
771 genes highlight the role of detoxification genes in xenobiotic response as heritable units
772 advantageous when selected as functional units. Gimenez et al. (2020) surprisingly found that
773 the whole CYP9A cluster was present in two copies in a resistant Puerto Rico (PR) fall armyworm
774 population, providing enhanced detoxification capability in this specific haplotype.

775 In *S. litura* 23 members from the large CEs gene family are split in two clusters on the
776 chromosome 2 (Figure 3). The genome of *S. frugiperda* was reported to contain 96 CEs, which is
777 24 more than in *B. mori*, with the notable expansion of clade 001, also found clustered (Gouin et
778 al., 2017). *Spodoptera’s* expanded gene families were enriched not only in phase I enzymes but
779 also in phase II and transport systems, such as GSTs, UGTs and ABC transporters (Gouin et al.,
780 2017). Huang et al. (2011) identified that three genes of the highly expanded *Sl*GST epsilon class
781 were intronless, namely GSTe1, GSTe2 and GSTe3, suggesting that these genes have duplicated
782 by retrotransposition. Analysis of exon-intron relationships between interspecific lineages are of

783 importance when it comes to establish gene evolution at specific loci (Gouin et al., 2017). Gouin
784 et al. (2017) found patterns supporting lineage-specific expansions through tandem duplications
785 of *Sf*UGT genes such as those of the UGT33 and UGT40 families. A handful of these expansions
786 were shown to be specific to Lepidoptera (Wang et al., 2014). For the CYP9As, the expansion
787 specificity might even be stronger as it seems to be restricted to the Noctuid lineage only *i.e.,*
788 there are 15 CYP9A genes in *S. frugiperda*, eight in *H. armigera* and *H. zea* compared to four in
789 monophagous *B. mori*, while none were found in the cruciferous specialist *P. xylostella*. The size
790 of the detoxification gene families in that respect has been argued to be linked to polyphagia
791 and the ability of insects to easily develop insecticide resistance, although this is still debated
792 (Dermauw et al., 2018; Feyereisen, 2011; Rane et al., 2019; Rane et al., 2016). The data
793 gathered in this review shows that genes organized in clusters respond, to a great extent,
794 frequently to xenobiotic exposures which may indicate an adaptation of *Spodoptera* species to
795 common ecological and metabolic challenges, with a particular emphasis on their ability to cope
796 with plant metabolites and probably to insecticides as well (Figure 3). In that prospect it would
797 be of great interest to analyze xenobiotic responses of more specialized *Spodoptera* species and
798 sequence their genomes in order to compare detoxification gene family organizations, promoter
799 regions and introns for transcription factor binding sites.
800
801 **4. Conclusion and perspectives**
802 Several species of the genus *Spodoptera* are considered a major threat to cultivated
803 plants worldwide. A few are listed at the top of the quarantined-species lists and closely
804 monitored (Ellis, 2004; EPPO, 2021). Despite the colossal efforts made by intergovernmental

805 plant protection agencies to limit the spread of these insects across borders (Goergen et al.,
806 2016; Nagoshi et al., 2011; Van de Vossenberg and Van der Straten, 2014), some of them such
807 as the fall armyworm have migrated over continents and settled in those places where climate
808 allows. The ability to rapidly overcome the chemical challenges encountered when expanding to
809 new environments and new host-plants is at the basis of their tremendous success. This
810 versatility is argued to find ground in their "DETOXome" on top of broad substrate specificity
811 and transcriptional plasticity. In that respect the comparison of genomes and the biology of
812 generalist *Spodoptera* species to specialist *Spodoptera* species, such as *S. picta* feeding on a few
813 Amaryllidaceae plant species, would be of great interest (number of genes, blooms, cluster
814 conservation and organization, etc.).

815 The amount of information on inducibility and inducers of detoxification genes in
816 *Spodoptera* has greatly advanced our knowledge that metabolism of xenobiotics plays an
817 important role in their ability to adapt to new chemicals. In *S. litura* for example, close to 300
818 detoxification genes have been transcriptionally assessed in the context of xenobiotic response
819 (Figure 4). Out of these, only 15 were investigated at the genetic level while one was validated
820 using biochemical/functional studies. In *S. frugiperda* the acknowledgement of the role of
821 detoxification genes in insecticide metabolism is largely hampered by the complete lack of
822 genetic functional validation studies and heterologously expressed enzymes as highlighted in
823 Figure 4. Since 2021, two additional functional validation studies have demonstrated the
824 involvement of P450s in *S. exigua* (Hu et al., 2021; Zuo et al., 2021a) and more similar work is
825 expected in the years to come for these four *Spodoptera* species. Transcriptomic studies show
826 that a fairly large number of genes are induced when insects are exposed to phytochemicals or

827 insecticides and that a number of them are potentially involved in the metabolism of toxic
828 products. In some cases, it has been possible to show that a drastic suppression of the
829 expression of these genes jeopardized the insects' success to survive on a host-plant or
830 insecticide exposure. In most cases though, there is still no evidence that these up- and down-
831 regulation contributes to xenobiotic metabolism. Down-regulation of detox genes in particular is
832 usually overlooked although it may be an adaptive process when downstream metabolites of a
833 detoxification enzyme are more toxic than the xenobiotic parent (Vlogiannitis et al., 2021).

834 Selection for increased insecticide detoxification in crop pests and subsequent
835 development of resistance is a major threat to sustainable yields and global food safety.
836 Therefore, the detection of insecticide resistance and its major molecular drivers are of utmost
837 importance, and best tackled by a standardized workflow as suggested in Figure 5. Toxicological
838 (synergist) assays followed by transcriptomic approaches are often favored these days over RT-
839 qPCR studies as they allow a holistic view on all the biologically relevant genes in xenobiotic
840 response. In particular RNA-seq studies enable to grasp the complete transcriptional changes
841 that occur when insects are exposed to xenobiotics (Vandenhole et al., 2020). Functional
842 genetic studies using forward and reverse genetics approaches will help to validate the role of
843 identified candidate genes in resistance. RNAi for instance has been used to silence genes in
844 *Spodoptera*, although other studies have indicated very low efficiencies of dsRNA-mediated
845 gene silencing in Lepidoptera species (Cooper et al., 2019; Shukla et al., 2016). The CRISPR/Cas9
846 genome editing system has become a widely used reverse genetic tool to study gene function in
847 insects, including *Spodoptera* (Zuo et al., 2021a; Zuo et al., 2021b). In addition to the genetic
848 validation, biochemical and pharmacokinetic studies utilizing recombinantly expressed enzymes

849 and LC or GC-MS/MS should be carried out to functionally validate xenobiotic detoxification
850 capabilities and identify the resulting metabolites. Indeed, metabolism (of a compound) is not
851 synonymous with detoxification. Detoxification requires proof that the product of the reaction
852 is less toxic than its parent (as demonstrated for 4-hydroxylated metabolites of pyrethroid
853 insecticides (Zimmer et al., 2014; Zimmer and Nauen, 2011) and flupyradifurone metabolites
854 (Haas et al., 2021)). Indeed, such proof is rarely provided. Studies should also focus on
855 identifying the origin of increased enzyme activity such as: i) mutation(s) in the amino-acid
856 sequence resulting in increased metabolism or a change in substrate specificity (Riveron et al.,
857 2014; Zimmer et al., 2018; Zuo et al., 2021a) ii) increased enzyme titer due to gene copy number
858 variation (CNV) or duplication events, iii) changes in *cis*-acting factors and distal regulatory
859 modules such as SNPs and TEs, iv) changes in *trans*-acting factors such as upregulation or
860 activation of transcription factors. This information will further facilitate our understanding of
861 the drivers of xenobiotic-mediated induction phenomena in resistance and hostplant adaptation
862 (Amezian et al., 2021). The increasing availability of genomes should help identifying these
863 regulatory changes in promoters, introns and transcription factor binding sites (TFBS).
864 The identification and characterization of host-plant adaptation and insecticide
865 resistance genetic markers in *Spodoptera* will supplement genome-wide association studies
866 (GWAS), QTL mapping and pooled targeted DNA-seq that have shown to be powerful tools for
867 detecting traits or variants associated with specific phenotypes such as resistance (Battlay et al.,
868 2018; Boaventura et al., 2020b; Cattel et al., 2020; Snoeck et al., 2019).
869

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Figure legends

Figure 1 – Overview of expression levels data collected in the literature for detoxification genes in the genus *Spodoptera*. (A) Venn diagram displaying the number of unique or overlapping up- (left) and down- (right) regulated genes after exposure to xenobiotics or in resistant populations of *S. litura* (green), *S. frugiperda* (yellow) and *S. exigua* (brown). For each species "n =" indicates as follows: the number of total detoxification genes up- (or down-) regulated / number of detoxification genes gathered from the literature / number of detoxification genes manually curated in the reference genome. Similarly, the number of corresponding references and nature of expression data is given underneath as follows: (nb of references / microarrays: qRT-PCR: RNAseq). Expression data from *S. littoralis* was purposely excluded from the diagram for only one UGT was upregulated after deltamethrin exposure (B) Venn diagram displaying the number of unique or overlapping detoxification genes upregulated

after plant secondary metabolite (PSM, green) or insecticides (yellow) exposure. The proportion of detoxification gene types upregulated are given as pie charts in green (after PSM exposure), yellow (after insecticides exposure) or in blue (intersection). Methods for figures are detailed in Supplementary Information.

Figure 2 – Characteristics of P450 response to xenobiotics in *Spodoptera* species. (A) Structure of CYPomes in *S. litura* and *S. frugiperda*. The number of family members is given in parenthesis. CYP3 and CYP4 clans have undergone large expansions and blooms through duplication of specific P450 families. **(B)** The proportion of CYP families accounting for upregulated P450 genes after xenobiotic exposure is depicted for each CYP clan in *S. litura* (top left), *S. frugiperda* (top right) and *S. exigua* (bottom left). On top of each stacked bar is given as follows: "n= 'number of genes from related clan involved' ('total number of genes belonging to related clan')". **(C)** CYP clan (%) origin of upregulated P450 across *Spodoptera* species (n=4). P450 with uncomplete annotation were marked as 'ND'. Methods for figures are detailed in Supplementary Information.

Figure 3 – Expression prevalence of clustered detoxification genes from the literature. The number of upregulation occurrences of genes in selected clusters are shown in *S. litura* (blue) and *S. frugiperda* (grey). Caution should be taken when comparing the inducibility of genes presented above as the data was collected from various studies with different experimental procedures. For example, gene expression in *S. frugiperda* has been assessed by RTqPCR (Giraudo et al., 2015), microarray (Carvalho et al., 2013) and RNA-seq (do Nascimento et al.,

2015) which produces a biased picture of reality. ADH: alcohol dehydrogenase GR: gustative receptor. Methods for figures are detailed in Supplementary Information.

Figure 4 – State of play of detoxification gene studies in *Spodoptera* species. Donutplot displaying for each species surveyed (x-axis) the number of detoxification genes for which data is available in three main categories of detoxification gene studies (y-axis): Expression levels, measurements of transcripts levels after xenobiotic exposure or in insecticide resistant populations (*i.e.*, data obtained through microarray, RTqPCR or RNAseq assays); Genetic validation, in vitro or in vivo functional genetic characterization of detoxification genes using molecular tools such as RNAi, CRISPR/Cas9; Biochemical studies, functional expression of recombinant detoxification enzymes and study of the interaction with xenobiotics. The donut rings show the corresponding share of CYP (brown), CCE (yellow), GST (green), UGT (magenta), ABC (purple). Methods for figures are detailed in Supplementary Information.

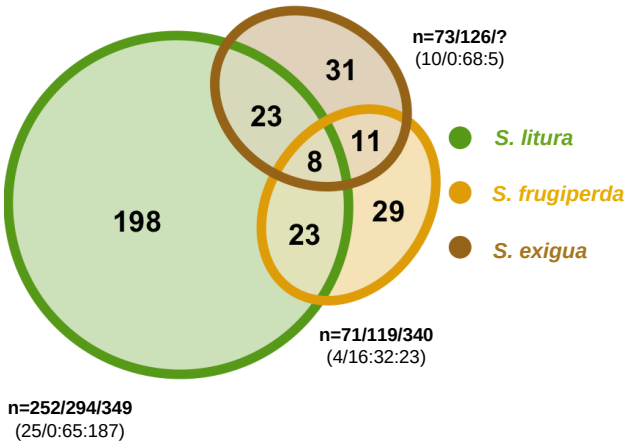
Figure 5 – Workflow to investigate detoxification mechanisms involved in host plant adaptation and insecticide resistance.

The identification of detoxification (blue box) as being the main plant-adaptation or resistance mechanism is typically achieved when insect P450, GST, CE enzymatic activities are probed against model substrates after xenobiotic challenge or feeding on specific plant material. Monitoring of insecticide metabolic resistance is commonly done by performing viability assays using synergists of specific detoxification enzyme families (*e.g.* piperonyl butoxide for P450s). Once enhanced xenobiotic metabolism and transport are suspected comes the identification of

the genes involved (red box). Genetic characterization usually starts with assessing the gene transcripts level by RT-qPCR or RNA-seq. Validation of candidate genes in the observed phenotype is then achieved using forward and reverse genetic approaches. At last, the functional expression of putative detoxification enzymes followed by a biochemical description of the detoxification mechanism (orange box) bring ultimate proof for the metabolism at play. Along to this three-step process, it remains crucial to identify the underlying genomic cause leading to a change of detoxification ability (mutation).

A

Number of UP-regulated genes per species – all xenobiotics considered

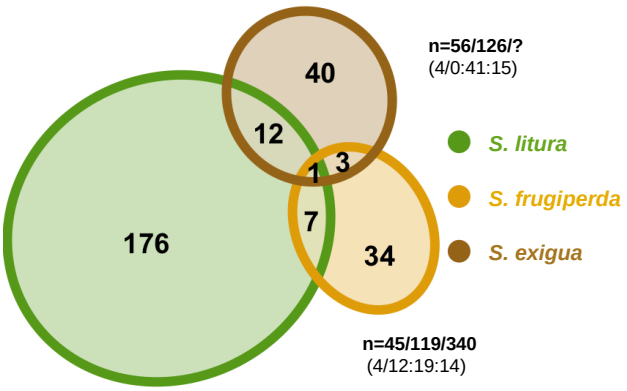


n=73/126/?
(10/0:68:5)

n=71/119/340
(4/16:32:23)

n=252/294/349
(25/0:65:187)

Number of DOWN-regulated genes per species - all xenobiotics considered

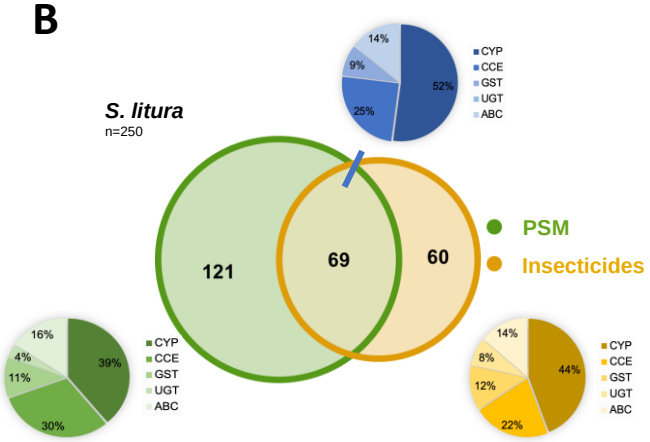


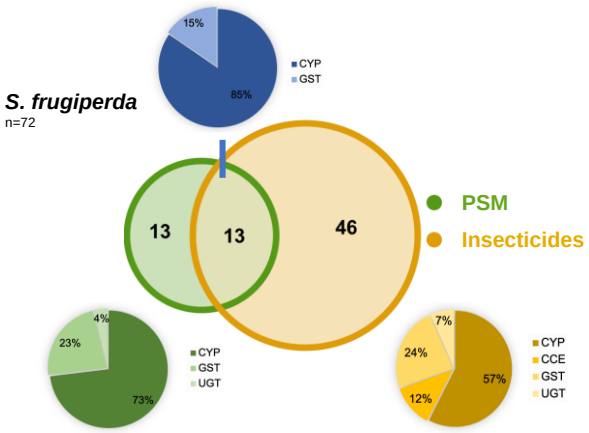
n=56/126/?
(4/0:41:15)

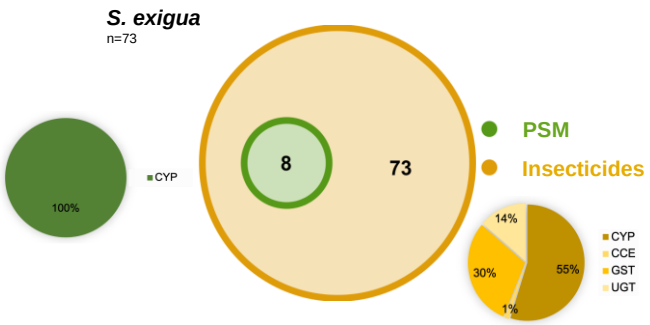
n=45/119/340
(4/12:19:14)

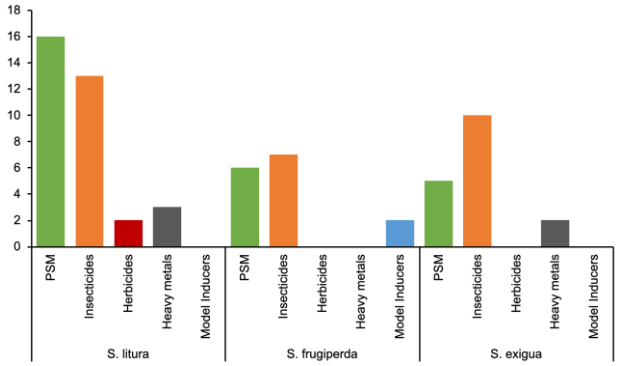
n=196/294/349
(6/0:14:182)

B





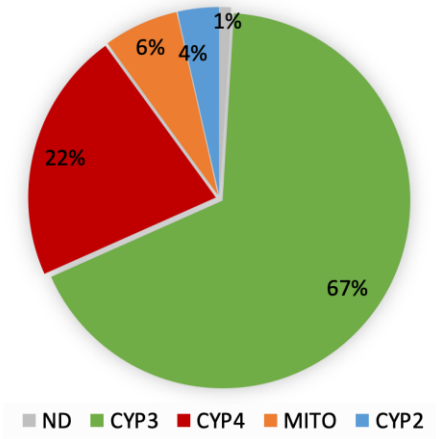




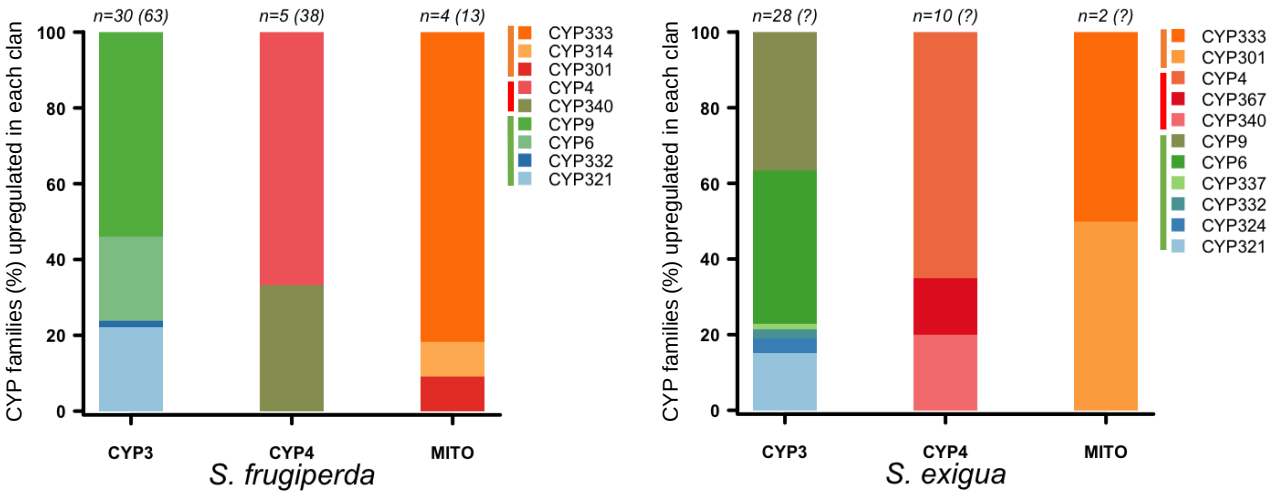
A

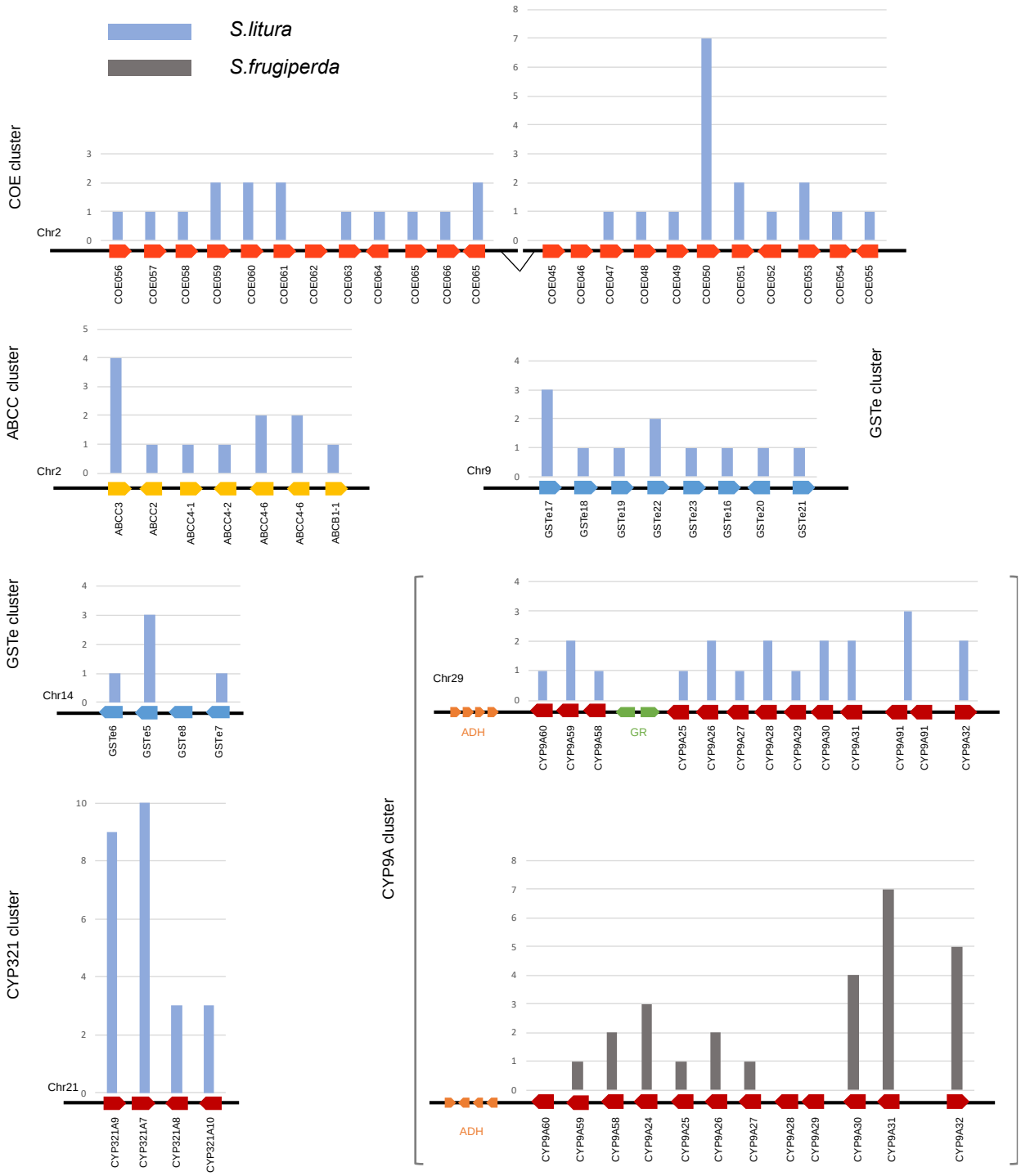
	Clan	Families and subfamilies	Total
S.litura	MITO	CYP49A(1), CYP301A(1), CYP301B(1), CYP302A(1), CYP314A(1), CYP315A(1), CYP333A(1), CYP333B(2), CYP339A(1), CYP428(1)	11
	CYP2	CYP15C(1), CYP18A(1), CYP18B(1), CYP303A(1), CYP305B(1), CYP306A(1), CYP307A(1)	7
	CYP3	CYP6AB(5), CYP6AE(9), CYP6AN(1), CYP6AW(1), CYP6B(6), CYP6CT(1), CYP9A(15), CYP9AJ(1), CYP9BS(1), CYP9G(1), CYP321A(5), CYP321B(5), CYP324A(3), CYP332A(2), CYP337B(1), CYP338A(1), CYP354A(1), CYP365A(1), CYP3097A(1)	60
	CYP4	CYP4AU(2), CYP4CG(2), CYP4G(4), CYP4L(3), CYP4M(4), CYP4S(2), CYP340AA(1), CYP340AB(1), CYP340AD(2), CYP340AQ(1), CYP340AX(5), CYP340G(1), CYP340K(1), CYP340L(5), CYP340Q(1), CYP341A(1), CYP341B(7), CYP366A(1), CYP367A(4), CYP367B(1), CYP421B(3)	52
S.frugiperda	MITO	CYP49A(1), CYP301A(1), CYP301B(1), CYP302A(1), CYP314A(1), CYP315A(2), CYP333A(1) CYP333B(3), CYP339A(1), CYP428A(1)	13
	CYP2	CYP15C(1), CYP18A(1), CYP18B(1), CYP303A(1), CYP304F(1), CYP305B(1), CYP306A(1), CYP307A(1)	8
	CYP3	CYP6AB(5), CYP6AE(11), CYP6AN(3), CYP6AW(1), CYP6B(7), CYP6CT(1), CYP9A(14), CYP9AJ(1), CYP9G(1), CYP321A(5), CYP321B(3), CYP324A(5), CYP332A(1), CYP337B(1), CYP338A(1), CYP354A(1), CYP365A(1), CYP3097A(1)	63
	CYP4	CYP4AU(3), CYP4CG(2), CYP4G(4), CYP4L(3), CYP4M(4), CYP4S(2), CYP340AD(1), CYP340K(1), CYP340L(9), CYP341A(1), CYP341B(4), CYP366A(1), CYP367A(1), CYP367B(1), CYP421B(1)	38

B

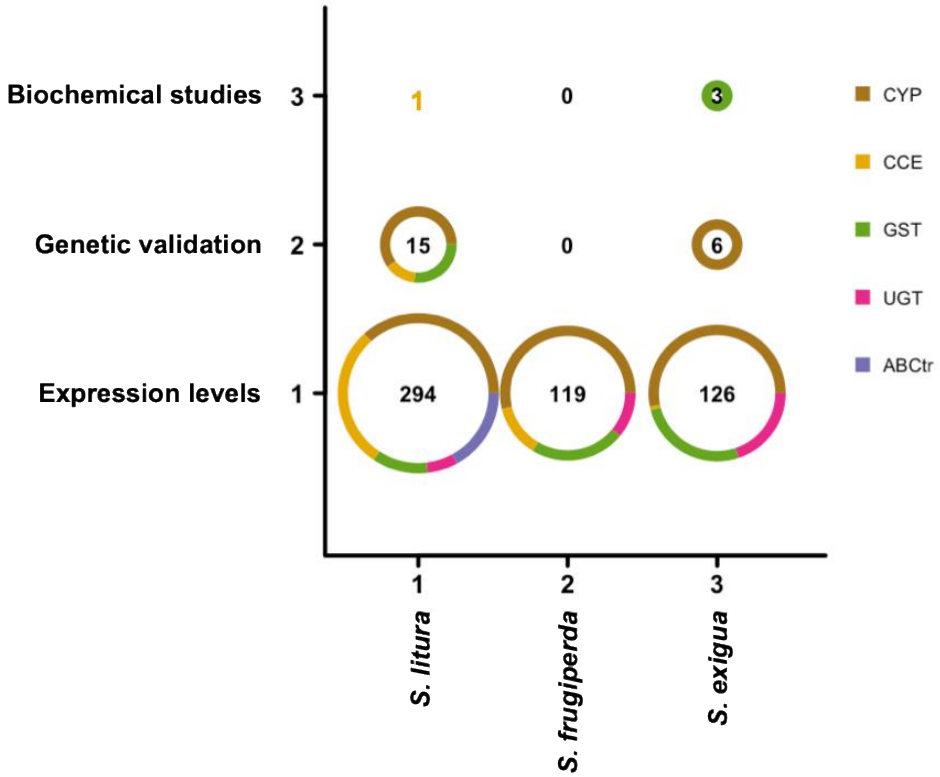


C

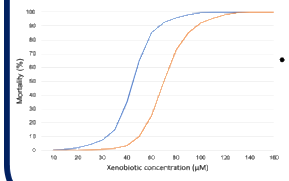



S.litura
S.frugiperda
COE cluster
Chr2
COE056
COE057
COE058
COE059
COE060
COE061
COE062
COE063
COE064
COE065
COE066
COE065
COE045
COE046
COE047
COE048
COE049
COE050
COE051
COE052
COE053
COE054
COE055
ABCC cluster
Chr2
ABCC3
ABCC2
ABCC4-1
ABCC4-2
ABCC4-6
ABCC4-6
ABCB1-1
GSTe cluster
Chr9
GSTe17
GSTe18
GSTe19
GSTe22
GSTe23
GSTe16
GSTe20
GSTe21
GSTe cluster
Chr14
GSTe6
GSTe5
GSTe8
GSTe7
CYP9A cluster
Chr29
ADH
CYP9A60
CYP9A59
CYP9A58
GR
CYP9A25
CYP9A26
CYP9A27
CYP9A28
CYP9A29
CYP9A30
CYP9A31
CYP9A91
CYP9A91
CYP9A32
ADH
CYP9A60
CYP9A59
CYP9A58
CYP9A24
CYP9A25
CYP9A26
CYP9A27
CYP9A28
CYP9A29
CYP9A30
CYP9A31
CYP9A32
CYP321 cluster
Chr21
CYP321A9
CYP321A7
CYP321A8
CYP321A10

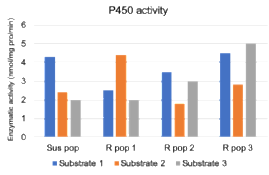
Number of detoxification genes for which data is available in three main categories of detoxification genes studies



Involvement of detoxification mechanisms

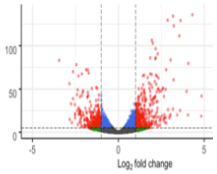


- **Toxicological tests with synergists**
  - *e.g.* PBO, TCPPE for P450
  - *e.g.* DEF, TPP for CE
  - *e.g.* DEM for GST

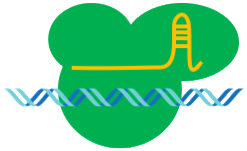


- **Activity measurements with model substrates**
  - *e.g.* ECOD, EROD, PNOD for P450
  - *e.g.* pNPA, α/β-NA for CE
  - *e.g.* CDNB for GST

Genetic characterization of detoxification genes

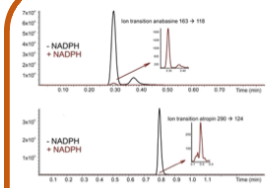


- **Gene expression level assessment**
  - Microarray
  - RTqPCR
  - RNA-seq

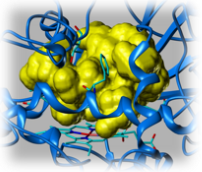


- **Genetic functional studies**
  - Knock-Down (RNAi...)
  - Knock-Out (CRISPR/Cas9...)
  - Overexpression (transfection, HDR...)
  - Mutation

Proof of detoxification mechanism at the biochemical level



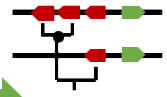
- **Functional expression-based metabolism**
  - Heterologous expression (cells, yeasts, bacteria)
  - LC and GC-MS/MS analysis
  - NMR



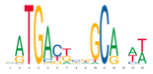
- **3D structure**
  - Modelling and docking
  - Crystallography

Identification of mechanisms underlying the change in detoxification ability

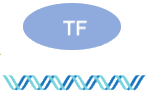
- *Qualitative* change due to mutation/polymorphism in detoxification enzyme nucleotide sequence



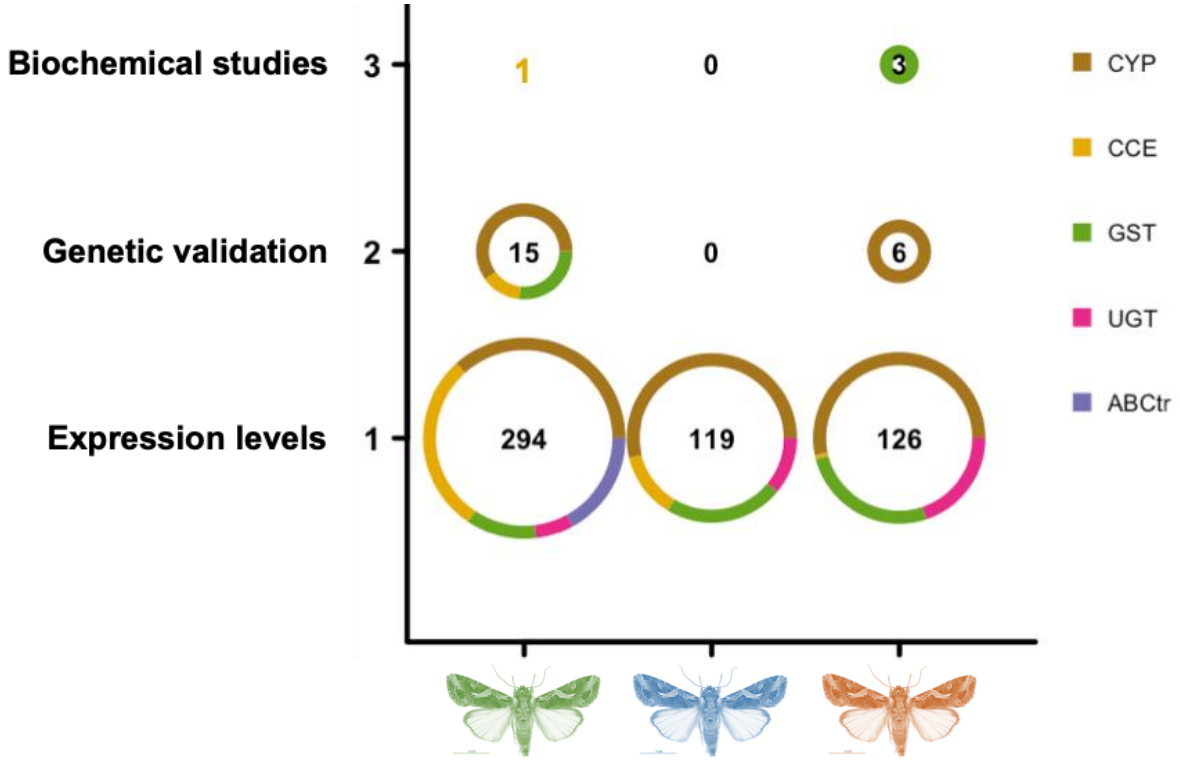
- *Quantitative* change due to duplication events and amplification of detoxification gene families



- *Quantitative* change due to *cis*-acting factors (SNPs, TEs, polymorphism...)



- *Quantitative* change due to *trans*-acting factors (TF upregulation, activation...)



S. litura *S. frugiperda* *S. exigua*