Journal of Pest Science
https://doi.org/10.1007/s10340-021-01452-6

ORIGINAL PAPER

Transgenic overexpression of P450 genes confers deltamethrin resistance in the fall armyworm, *Spodoptera frugiperda*

Xien Chen[1] · **Subba Reddy Palli**[1]

Received: 26 May 2021 / Revised: 25 August 2021 / Accepted: 17 October 2021

Abstract

The fall armyworm (FAW), *Spodoptera frugiperda*, is a polyphagous insect pest feeding on many host plants, including some major crops such as corn and rice. This pest has also developed resistance to many insecticides. Recent genome and transcriptome sequencing efforts identified 117–425 P450 genes in the FAW, but their function in detoxifying plant toxins and insecticides is largely unknown. This study found that a P450 gene, *SfCYP321A8*, is upregulated in the first instar FAW larvae fed on deltamethrin. A transgenic FAW overexpressing *SfCYP321A8* was produced to investigate its function in deltamethrin resistance. Transgenic FAW expressing the gene coding for a P450 known to metabolize deltamethrin in *Tribolium castaneum*, *TcCYP6BQ9*, was also produced. P450 genes are highly expressed in different tissues of transgenic larvae. The P450 activity in the midgut and fat body of both transgenic FAW lines is significantly higher than in wild-type larvae. Deltamethrin bioassays showed that the transgenic larvae expressing *SfCYP321A8* or *TcCYP6BQ9* are 10.3- or 15.3-fold more tolerant, respectively, than the wild-type larvae. These studies report on the production of FAW transgenic lines expressing P450 genes and show that *SfCYP321A8* contributes to deltamethrin resistance in FAW. The transformation methods developed could be used in functional genomics studies in FAW and other lepidopteran pests.

Keywords Transgenesis · *PiggyBac* · P450 · *TcCYP6BQ9* · *SfCYP321A8* · Deltamethrin · *Spodoptera frugiperda*

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a native species of tropical and subtropical regions of the Americas. Due to the strong migration ability of FAW adults, this important agricultural pest has rapidly spread from the Americas to over 100 countries worldwide (Gui et al. 2020; Nagoshi et al. 2017). FAW is a highly polyphagous pest with a broad range feeding on > 300 species of host plants, including some major food crops, such as rice and corn/maize (Montezano et al. 2018). This pest is a major threat to international food security. Because severe damage inflicted by FAW on crop plants leads to significant economic losses, the farmers often use massive amounts of chemical insecticides to control this pest. As a result of repeated pesticide applications, the FAW developed resistance to many insecticides (Carvalho et al. 2013; Diez-Rodriguez and Omoto 2001). Recently, FAW resistance to transgenic *Bt* maize was reported (Farias et al. 2014; Huang et al. 2014; Omoto et al. 2016). Understanding the insecticide resistance mechanisms is essential for the successful management of this notorious agricultural pest.

The major mechanisms of insecticide resistance in insect pests include increased expression of metabolic enzymes sequestering and/or detoxifying insecticide and mutation in the target protein, making it less sensitive to the insecticide (Ffrench-Constant 2013; Hemingway et al. 2002; Li et al. 2007). Insect P450s play important roles in detoxifying insecticides and plant toxins (Feyereisen 2012). The overexpression of metabolic enzymes has been reported to be associated with resistance to different insecticides in many insect species (Bass and Field 2011; Liu et al. 2015). Recent genome and transcriptome sequences estimated the number of P50 genes in FAW between 117 and 425 (Gouin et al. 2017; Gui et al. 2020; Liu et al. 2019; Rane et al. 2019). The products of some of these genes may contribute to its

Communicated by Guy Smagghe.

✉ Subba Reddy Palli
rpalli@uky.edu

[1] Department of Entomology, College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY 40546, USA

Published online: 29 October 2021

adaption to a wide range of host plants and resistance to multiple insecticides. However, the relationship between FAW P450s and insecticide resistance is still not well understood.

CRISPR/Cas9-mediated genome editing methods have been used to study insecticide resistance by introducing mutations in target proteins of insecticides (Douris et al. 2017; Wang et al. 2017; Wang et al. 2020). However, employing CRISPR/Cas9 technology to enhance the in vivo expression of metabolic enzymes, such as P450s, is challenging in lepidopteran insects. Transgenic manipulation of gene expression in these insects provides an alternate method to overexpress P450 genes in vivo. Taking advantage of efficient transformation systems in the fruit fly, *Drosophila melanogaster*, several P450 genes from other insects have been ectopically expressed in vivo and evaluated their function in insecticide resistance (Mallott et al. 2019; Pang et al. 2016; Zhu et al. 2010). However, transformation systems are not well developed in lepidopteran pest insects. We recently established a *piggyBac*-based transformation system in the FAW (Chen et al. 2020b). We wanted to test if this transformation system could be used to determine the function of P450 genes in FAW. Gimenez et al. reported that copy number variation of detoxification genes might play an important role in insecticide resistance of fall armyworm (Gimenez et al. 2020). Recent studies on *Spodoptera exigua*, a close relative of FAW, reported overexpression of *SeCYP321A8* conferred resistance to deltamethrin (Hu et al. 2021). The function of CYP9A cluster in pyrethroid resistance has been reported in *Helicoverpa armigera* (Shi et al. 2021; Tian et al. 2021). In Sf9 cells, insecticides and allelochemicals induce P450 genes, but deltamethrin did not induce the overexpression of *SfCYP321A8* (Giraudo et al. 2015). However, whether *SfCYP321A8* is involved in deltamethrin resistance in vivo is unknown. In this work, we found that the expression of *SfCYP321A8* in FAW larvae was upregulated by deltamethrin. The transgenic FAW lines expressing *SfCYP321A8*, and a known deltamethrin resistance conforming P450 gene from *Tribolium castaneum*, *TcCYP6BQ9*, were produced. The P450 genes are overexpressed in both lines and P450s produced increased tolerance of transgenic FAW to deltamethrin.

Materials and methods

Insect rearing

The laboratory strain of FAW purchased from Benzon Research Inc. (Pennsylvania, USA) has been reared for multiple generations without exposure to pesticides. Artificial diet purchased from Southland Product Inc. (Arkansas, USA) was used for larval rearing. Adults were fed on 10% sucrose solution and maintained at 23 ± 1 °C, 75 ± 5% relative humidity and 16:8 (light: dark) cycle.

Deltamethrin bioassays

Bioassays were conducted using the newly first instar larvae and leaf disk method (Yang et al. 2017). Serial dilutions of the active ingredient of deltamethrin (EMD Millipore Crop., USA) were prepared in 0.1% Triton X-100. Brussels sprout leaves were washed and dried and cut into small, ~ 1 × 0.5 cm pieces. The leaf disks were dipped in the insecticide solution for 10 s and air-dried on food wrap film at room temperature for 20–30 min. Leaf disks dipped in sterile distilled water containing 0.1% Triton X-100 were used as controls. The treated leaf disks were transferred to 2.0-ml Eppendorf tubes. Fifteen newly molted first instar larvae were transferred into each tube. The tubes were sealed with cotton and kept at 27 ± 2 °C, 65 ± 5% relative humidity, and a 16:8 h (light: dark) cycle. Each treatment contained three replicates. Mortality was recorded at 48 h after exposure to deltamethrin. Larvae were considered dead if they failed to move when prodded with a brush. The survived neonates treated with LC_{50} of deltamethrin or 0.1% Triton X-100 at different time points post-treatment were collected and frozen at -80 °C for RNA extraction.

RNA isolation, cDNA preparation and RT-qPCR

Total RNA was isolated using TRI reagent (Molecular Research Center Inc., Cincinnati, OH), and converted to cDNA using the M-MLV Reverse Transcriptase (Invitrogen, USA). RT-qPCR reactions were conducted in Applied Biosystems Step One Plus™ Real-Time PCR System. Each RT-qPCR reaction (10 µL final volume) contained 5 µL of 2xSYBR Mix (BioRad, USA), 0.8 µL of 20-fold diluted cDNA, 3.4 µL of nuclease-free water, and 0.4 µL each of 10 µM forward and reverse gene-specific primers. An initial incubation of 95 °C for 3 min followed by 40 cycles of 95 °C for 5 s and 60 °C for 60 s settings was used. The expression of FAW *28S rRNA*, which is more stably expressed in the tested samples in this study (Figure S1), was used for normalizing mRNA levels. Each treatment contains three biological replicates. Primers used for RT-qPCR reactions are listed in Table 1.

Construction of transformation vectors

A *piggyBac* vector, pBac:hr5ie1-EGFP-SV40:ie1-Cas9-SV40, was digested with AatII and ApaI to remove the Cas9 gene cassette. The ORFs (open reading frame) of *SfCYP321A8* (GenBank: KC789751.1) and *TcCYP6BQ*9 (GenBank: KC686852.1) were amplified using Prime STAR GXL DNA Polymerase (TaKaRa, Japan). The

Table 1 Primers used in this study

Primer name	Sequence (5′-3′)
SfCYP321A8-qF	GCGCAATGAGAGCTACTGGA
SfCYP321A8-qR	GAGCGCAGGTGTTAGGACTT
TcCYP6BQ9-qF	TGCAATTCTCGCAACACTTC
TcCYP6BQ9-qR	GTCCCAAGAAAACTCCACCA
28S rRNA-qF	CTGCTTACAGAGACGAGGTTAAG
28S rRNA-qR	GGGTAGTAGTCCAGACCAGAAT
SfCYP321A8-F	GACACTGGCGGCGACAAGAGACGTCATGTTGTTTCTACCTTTGAGTTTG
SfCYP321A8-R	CTGATTATGATCTAGAGTCGCTATTTGATATTTCTCGGAATTAGTTGG
TcCYP6BQ9-F	GACACTGGCGGCGACAAGAGACGTCATGACTCTAATAACAAACAACC
TcCYP6BQ9-R	CTGATTATGATCTAGAGTCGCTAGTCCAGCTTTTCTACATCCAGC
Pub/P2009-F	TAAATGACAAGCAATGAGATCTCTGAACTTAGGCAGGATTTCCTG
Pub/P2009-Rsf	ACTCTTTGTTATTAGAGTCATTTGTCTGTAATAGAAGAAAATTG
Pub/P2009-Rtc	CTCAAAGGTAGAAACAACATTTGTCTGTAATAGAAGAAAATTG
Pub/P2009-tF	TGTGGTATATTGCACAGTGAAGTG
SfCYP321A8-tR	GAGCGCAGGTGTTAGGACTTGACC
TcCYP6BQ9-tR	AAGAGATAGTCTGTAGCGTCTCC

forward primers contained a 20 bp homologous arm on the 5' end of AatII site and the reverse primers contained a 20 bp homologous arm on the 5' end of ApaI site of pBac hr5ie1-EGFP-SV40 ie1-Cas9-SV40 vector. The amplified DNA fragments were cloned into the AatII/AapI digested pBac:hr5ie1-EGFP-SV40:ie1-Cas9-SV40 vector using the Gibson assembly master mix (NEB, USA). Two intermediate vectors, pBac:hr5ie1-EGFP-SV40:ie1-*SfCYP321A*8-SV40 and pBac:hr5ie1-EGFP-SV40:ie1-*TcCYP6BQ9*-SV40, were produced. These two vectors were then digested with BglII and AatII to remove the ie1 promoter. A ubiquitously active FAW promoter, *Sfpub/P2009* (Chen et al. 2020a), was amplified from the genomic DNA using the Prime STAR GXL DNA Polymerase (TaKaRa, Japan) and primers containing 20 bp homologous arms on either end of BglII and AatII sites of the two intermediate vectors. The amplified PCR products were inserted into the BglII/AatII sites of these vectors to produce the final transformation vectors, pBac:hr5ie1-EGFP-SV40:SfPub/P2009-*SfCYP321A8*-SV40 and pBac:hr5ie1-EGFP-SV40:SfPub/P2009-*TcCYP6BQ9*-SV40. The constructs were transformed into *E. coli* 10G competent cells. Positive transformants were selected and plasmid DNAs were prepared for microinjection. Primers used for preparing vector constructs are listed in Table 1.

Transformation of FAW

Hyperactive *piggyBac* transposase mRNA was prepared with mMESSAGE mMACHINE kit (Invitrogen, USA) and stored in -80 ℃. A mixture of hyperactive transposase mRNA (400 ng/μL) and each final vector (300 ng/μL) was injected into fresh FAW embryos (less than 4 h old) as previously described (Chen et al. 2020b). The larvae hatched (G0) were reared under conditions as described above. Collection and screening of newly hatched first instar larvae (G1) were performed as described previously (Chen et al. 2020b). All transgenic larvae and adults were reared under normal conditions.

Detection of P450 insertion in transgenic insects

Three EGFP-positive and -negative larvae from G2 generation were randomly selected. Genomic DNA from each individual was extracted using DNeasy Blood & Tissue Kits (Qiagen, USA). A forward primer located in Sfpub/P2009 promoter and a reverse primer located in *SfCYP321A8* or *TcCYP6BQ9* were used in PCR reactions to amplify DNA fragments located between the primers using FAW genomic DNA as a template. The amplified DNA fragments were run on agarose gels. A fragment of *28S rRNA* was amplified from genomic DNA as an internal control.

Transgenic overexpression of *SfCYP321A8* and *TcCYP6BQ9*

The EGFP-positive first instar larvae from G2 transgenic *SfCYP321A8* and *TcCYP6BQ9* lines were collected at 0 h, 24 h, and 48 h after hatching. Five tissues, head, midgut, fat body, Malpighian tubules, and epidermis, were dissected from EGFP-positive and -negative G2 sixth instar larvae. Total RNAs were extracted and converted into cDNAs for qRT-PCR analysis as described above.

P450 activity assay in tissues of transgenic insects

Midgut and fat body were dissected from the wild-type and transgenic FAW sixth instar larvae and homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM PMSF, 0.1 mM DTT, and 1 mM PTU in 1.7-ml tubes. The homogenates were centrifuged at 1000 × *g* for 5 min at 4 ℃. Bradford assay was sued to determine protein concentration in the supernatant. The supernatant was diluted with sodium phosphate buffer to a final protein concentration of 1 mg/ml. Commercial P450-Glo substrate, Luciferin-Be (Promega, Madison, WI, USA), was used to measure P450 activity using white 96-well plates and methods described previously (Inceoglu et al. 2009). To initiate P450 activity assay, 50 µM of Luciferin-Be substrate was added to each well containing 40 µL of protein and 0.1 M sodium phosphate buffer was added to each reaction to make a final volume of 100 µL. The luminescent reactions were incubated at room temperature for 1 h. Then, 25 µL of the reaction mixture was transferred to a new well. After adding 25 µL of luciferin detection reagent, the reactions were incubated at 27 °C for an additional 20 min. The luminescence was quantified using SpectraMax i3x (San Jose, CA, USA). The average relative luminescence unit (RLU) values in wells containing all assay components except the protein were used as a blank. Three biological replicates were performed for each treatment.

Statistical analysis

LC_{50} (lethal concentration that kills 50% of the individuals) values were calculated by simple logistic regression in GraphPad Prism 8. IBM SPSS Statistic 25 was used for statistical analysis. All data are shown as mean ± SD (standard deviation). The significant difference between the two groups was analyzed using the independent sample *t* test; $p < 0.05$ was considered statistically significant.

Results

Deltamethrin induces *SfCYP321A8* expression

The leaf-disk assays were performed to determine deltamethrin toxicity in wild-type FAW larvae. The neonate FAW larvae were fed on leaf disks treated with different concentrations of deltamethrin. The mortality was recorded at 48 h post-treatment. The LC_{50} value of deltamethrin for wild-type FAW was determined as 0.34 mg/L (Fig. 1A). To test if *SfCYP321A8* gene is induced by deltamethrin treatment, the neonate larvae were fed on leaf disks treated with LC_{50} concentration of deltamethrin, and the surviving larvae were collected at 6, 12, 24, and 48 h post-treatment. The

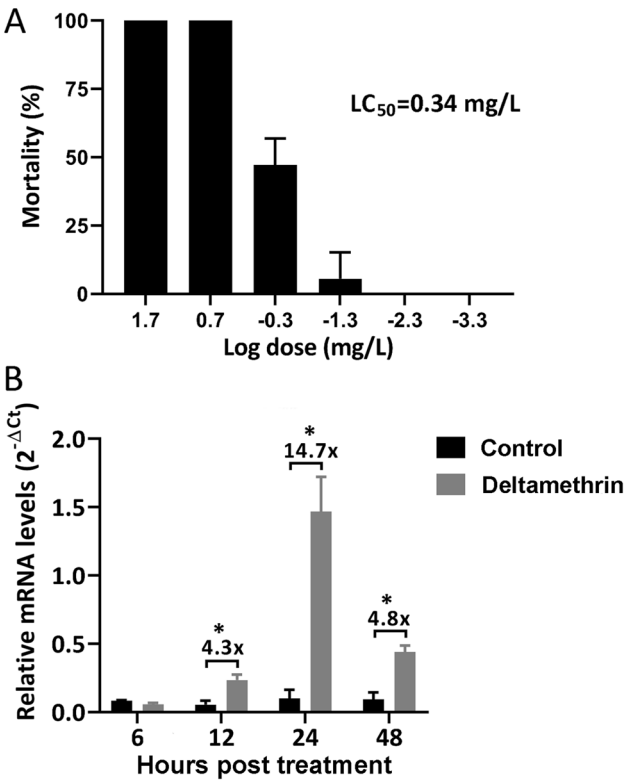

Fig. 1 Deltamethrin bioassays in FAW neonate larvae **A** and induction of *SfCYP321A8* gene by deltamethrin **B**. FAW neonate larvae were fed on leaf disks treated with different concentrations of deltamethrin; the mortality was recorded at 48 h after treatment. The neonate larvae were fed on leaf disks treated with LC_{50} concentration of deltamethrin and the larvae were collected at 6–48 h after feeding. Total RNA was isolated and used in RT-qPCR used to determine SfCYP321A8 mRNA levels. *28S rRNA* was used as a reference gene. Mean ± SD ($n = 3$) are shown. Data were analyzed using student's t-test. *, $p < 0.05$

SfCYP321A8 mRNA levels were determined by RT-qPCR. As shown in Fig. 1B, a significant increase in *SfCYP321A8* mRNA levels was detected in deltamethrin-treated larvae at 12 h (4.3-fold) and 24 h (14.7-fold) after treatment when compared to its levels in control larvae. Then, the *SfCYP321A8* mRNA levels decreased to 4.8-fold by 48 h after treatment. These data suggest that *SfCYP321A8* expression is induced by deltamethrin.

Production of transgenic FAW expressing *SfCYP321A8* and *TcCYP6BQ9*

To investigate whether in vivo overexpression of *SfCYP321A8* could make FAW more tolerant to deltamethrin, the *piggyBac* system was employed to produce transgenic FAW. We previously identified a P450 gene *TcCYP6BQ9* in *T. castaneum* that is involved in deltamethrin resistance (Zhu et al. 2010). Knockdown of this gene

reduced deltamethrin resistance in *T. castaneum,* and its overexpression in *D. melanogaster* increased resistance to deltamethrin. To test if *TcCYP6BQ9* expression increases deltamethrin resistance in FAW, a transgenic FAW line expressing this gene was produced. From about 2000 eggs injected with each transformation vector, 153 transgenic *SfCYP321A8* neonate larvae and 118 transgenic *TcCYP6BQ9* neonate larvae were identified based on the EGFP marker gene expression in the G1 generation (Table 2). The adults developed from G1 positive larvae were crossed with wild-type adults to produce G2 generation. The positive transgenic larvae for both genes showed strong EGFP signals in G2 (Fig. 2B), indicating that the transgenic insertions are inheritable.

Three EGFP-positive and -negative larvae from G2 generation were randomly selected for genomic DNA extraction.

Table 2 Germline transformation efficiency of P450 genes

Transgenic plasmid	Injected eggs (n)	G0 Larvae (n)	G0 Pupae (n)	G1 Positive (n)
Sfpub/P2009-SfCYP321A8	~2000	~700	~600	153
Sfpub/P2009-TcCYP6BQ9	~2400	~900	~700	118

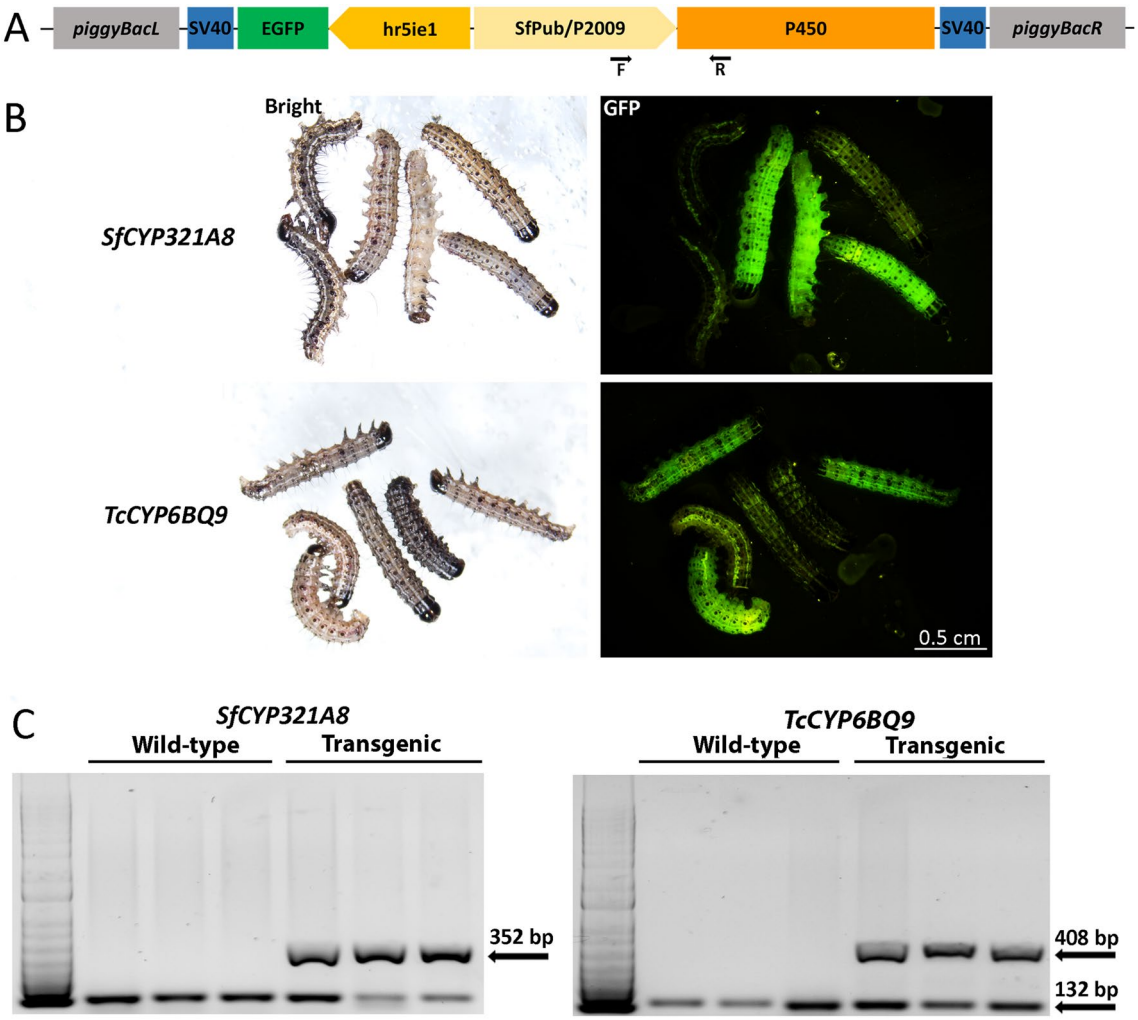

Fig. 2 Establishment and characterization of transgenic P450 FAW. **A** Schematic representation of *piggyBac*-based P450 expression constructs. *SfCYP321A8* or *TcCYP6BQ9* expression is driven by a highly active FAW promoter, *Spud/P2009*, and expression of a marker gene, *EGFP*, is under the control of hr5ie1 promoter. **B** Fluorescence in EGFP-negative and -positive animals. **C** RT-PCR analysis of transgenic P450 integrated in the genome of transgenic animals. Genomic DNA was extracted from three EGFP-negative and three EGFP-positive larvae individually. PCR reactions were performed using a forward primer located in *SfPub/P2009* promoter and a reverse primer located in *SfCYP321A8* or *TcCYP6BQ9*. A fragment of *28S rRNA* was amplified from each genomic DNA was used as an internal control. The PCR products were resolved on 1% agarose gel

A forward primer located in *Spud/P2009* promoter and a reverse primer located in *SfCYP321A8* or *TcCYP6BQ9* were used to PCR amplify the DNA between the primers. As shown in Fig. 2C, expected size DNA fragments were amplified using genomic DNA isolated from EGFP positive larvae but not from EGFP negative, suggesting that the transgenic P450 expression cassettes were successfully integrated into the FAW genome.

Transgenic animals overexpress *SfCYP321A8* and *TcCYP6BQ9*

RT-qPCR analysis was performed to determine *SfCYP321A8* and *TcCYP6BQ9* mRNA levels in the first instar larvae. The *SfCYP321A8* mRNA levels increased by 783-, 389-, and 418-fold in the newly hatched, 24-h-, and 48-h-old first instar larvae transgenic *SfCYP321A8* larvae, respectively, when compared to its levels in the same age wild-type larvae (Fig. 3A). No *TcCYP6BQ9* mRNA was detected in newly hatched, 24-h-, and 48-h-old first instar wild-type larvae, but high levels of *TcCYP6BQ9* mRNA were detected in the same age *TcCYP6BQ9* transgenic larvae (Fig. 3B). The mRNA levels of *TcCYP6BQ9* in *TcCYP6BQ9* in transgenic larvae are similar to the levels of *SfCYP321A8* mRNA in *SfCYP321A8* transgenic larvae. *SfCYP321A8* and *TcCYP6BQ9* mRNA levels were determined in different tissues dissected from transgenic and wild-type last instar larvae of G2. An increase in *SfCYP321A8* mRNA levels by 337–1160-fold was detected in different tissues from *SfCYP321A8* transgenic larvae when compared to its levels in wild-type larvae (Fig. 3C). The tissues dissected from *TcCYP6BQ9* transgenic larvae also showed high levels of *TcCYP6BQ9* mRNA (Fig. 3D).

An increase in P450 activity in tissues from transgenic larvae expressing *SfCYP321A8* and *TcCYP6BQ9*

To test whether overexpressing of *SfCYP321A8* and *TcCYP6BQ9* could lead to an overall increase in total P450 activity in the transgenic animals, major tissues involved in insecticide resistance, midgut, and fat body were tested for P450 activity. The total P450 activity in the midgut dissected from *SfCYP321A8* and *TcCYP6BQ9* transgenic

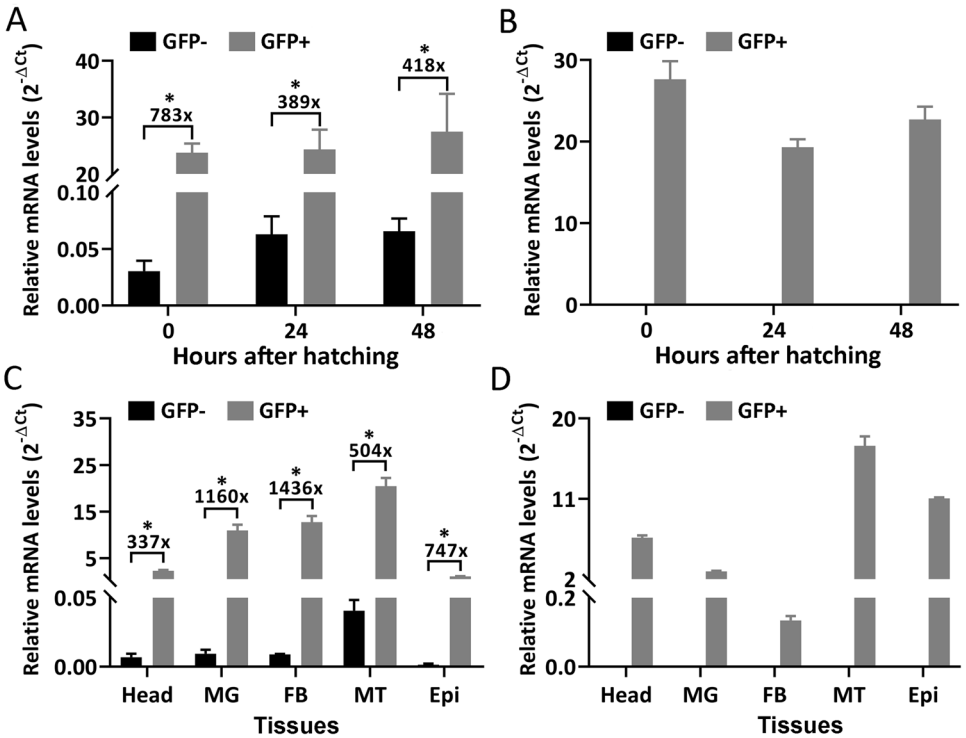

Fig. 3 Overexpression of transgenic P450s in EGFP-positive animals. Expression of *SfCYP321A8* **A** and *TcCYP6BQ9* **B** in first instar larvae. The EGFP-positive first instar larvae from G2 transgenic *SfCYP321A8* line and transgenic *TcCYP6BQ9* line were collected at 0 h, 24 h, and 48 h after hatch. Total RNA was isolated and used for RT-qPCR analysis. Expression of *SfCYP321A8* **C** and *TcCYP6BQ9* **D** in different tissues. Five tissues, head, midgut (MG), fat body (FB), Malpighian tubules (MT), and epidermis (Epi) were dissected from the sixth instar larvae of both EGFP-positive and -negative G2 animals. Total RNA was extracted and converted to cDNA and used for qRT-PCR analysis. *28S rRNA* was used as a reference gene. Mean ± SD ($n = 3$) are shown. Data were analyzed using student's *t*-test. *, $p < 0.05$. GFP-, EGFP-negative animals and GFP+, EGFP-positive animals

larvae increased by 1.92 and 2.02-fold, respectively, when compared to the P450 activity in wild-type larvae (Fig. 4). Similarly, the total P450 activity in the fat body dissected from *SfCYP321A8* and *TcCYP6BQ9* transgenic larvae increased by 2.13- and 2.98-fold, respectively, when compared to the P450 activity in wild-type larvae (Fig. 4).

Transgenic animals expressing *SfCYP321A8* and *TcCYP6BQ9* are resistant to deltamethrin

Leaf-disk assays were performed to evaluate the efficacy of deltamethrin in transgenic larvae expressing *SfCYP321A8* and *TcCYP6BQ9*. The deltamethrin LC_{50} increased by 10.3- and 15.3-fold, in transgenic larvae expressing *SfCYP321A8* and *TcCYP6BQ9*, respectively, when compared to wild-type larvae (Fig. 5). These data suggest that overexpression of *SfCYP321A8* and *TcCYP6BQ9* increased deltamethrin tolerance in FAW larvae.

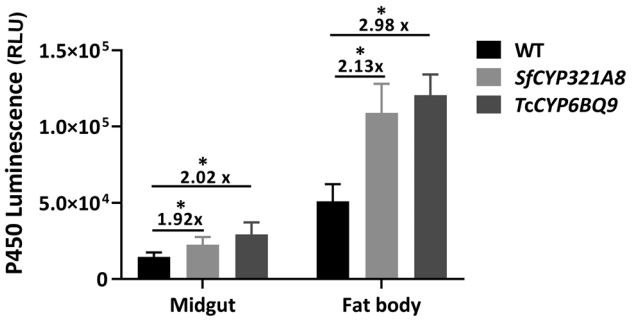

Fig. 4 P450-Glo assay of proteins extracted from the midgut and fat body from transgenic animals expressing *SfCYP321A8* and *TcCYP6BQ9*. Midgut and fat body were dissected from the wild-type and transgenic FAW sixth instar larvae, and homogenates were prepared and assayed using Luciferin-Be as substrate. Mean ± SD ($n = 3$). Data were analyzed using student's t-test. *, $p < 0.05$

Discussion

P450 enzymes metabolize insecticides and plant toxins (Dermauw et al. 2020; Feyereisen 1999). Overexpression of P450s has been widely reported to be responsible for insecticide resistance (Bass and Field 2011; Liu et al. 2015). Since FAW feeds on many host plants, it is tempting to speculate that P450s may be involved in FAW adaption to a wide range of host plants and its resistance to many insecticides. However, the function of FAW P450s, especially their roles in insecticide resistance and detoxification of plant toxins, is largely unknown.

FAW larvae showed higher sensitivity to chlorantraniliprole after silencing P450 genes, *SfCYP321A8*, *SfCYP321A9*, and *SfCYP321B1* (Bai-Zhong et al. 2020). More than 10 FAW P450 genes, including *SfCYP321A8*, were upregulated in larvae treated with sublethal doses of three insecticides, chlorantraniliprole, emamectin benzoate, and *Bacillus thuringiensis* (Zhang et al. 2020). These data suggest that *SfCYP321A8* may be involved in the detoxification of multiple classes of insecticides. In *Spodoptera exigua*, a close relative of FAW, overexpression of *SeCYP321A8* conferred resistance to deltamethrin (Hu et al. 2021). Here, we found that *SfCYP321A8* is upregulated after deltamethrin treatment suggesting a role for it in the detoxification of deltamethrin. However, it has been reported that *SfCYP321A8* was not induced by deltamethrin treatment in Sf9 cells (Giraudo et al. 2015). Sf9 cells likely lack the transcription factors that mediate deltamethrin induction of *SfCYP321A8*.

To study functions of P450s in insecticide resistance, reverse genetics methods such as the RNAi-mediated gene knockdown and CRISPR/Cas9-mediated gene knockout technologies have been successfully used in suppressing the expression of P450 genes or abolishing the function of P450s in many insect species (Amezian et al. 2021; Dulbecco et al. 2021; Kariyanna et al. 2020; Wang et al. 2018). To date, P450 overexpression in vivo is only achieved in the model insect *D. melanogaster* by transgenesis (Daborn

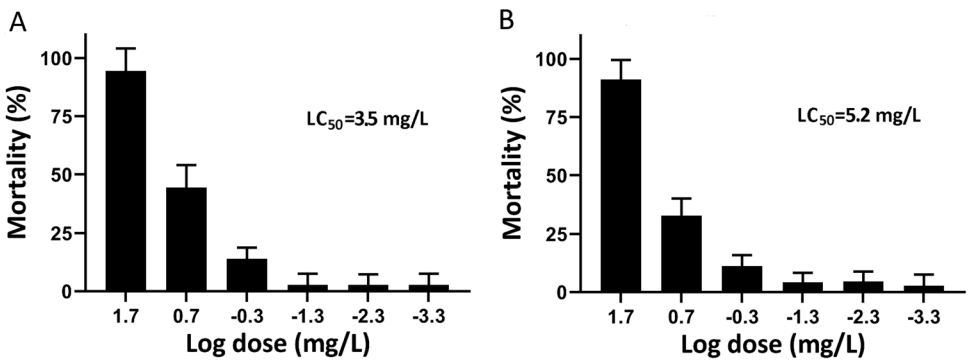

Fig. 5 Bioassays of deltamethrin in transgenic *SfCYP321A8* **A** and *TcCYP6BQ9* **B** in neonate larvae. The EGFP-positive neonates were collected under the fluorescent microscope and fed on leaf disks treated with different concentrations of deltamethrin. Mortality was recorded at 48 h post-treatment. Mean ± SD ($n = 3$) are shown

et al. 2007). The transgenic *D. melanogaster* is also used for overexpression of P450 genes from other insects, such as *T. castaneum* (Zhu et al. 2010), *Plutella xylostella* (Mallott et al. 2019), and *Nilaparvata lugens* (Pang et al. 2016). Due to the phylogenetic differences of P450 genes between *D. melanogaster* and other insect species and differences in P450 signaling pathways between *D. melanogaster* and other insects, it is desirable to overexpress P450 genes in the insect species of origin or its close relative. However, it is still challenging to generate transgenic strains in non-model insects. The difficulty of delivering transgenic components into fresh embryos, and the low rate of transposition resulting in the low transformation efficiency in non-model insects, especially in lepidopteran insects, are preventing successful transformation in these insects. We recently developed methods for the transformation of FAW by using the mRNA of a hyperactive version of *piggyBac* transposase to replace the commonly used helper plasmid expressing transposase (Chen et al. 2020b). Following these methods, we produced transgenic FAW expressing *SfCYP321A8* and *TcCYP6BQ9*. The mRNA levels of *SfCYP321A8* and *TcCYP6BQ9,* and P450 activity were significantly increased in the transgenic P450 larvae. The transgenic insects expressing *SfCYP321A8* and *TcCYP6BQ9* showed increased tolerance to the deltamethrin. We also found a discrepancy between the levels of overexpression of *SfCYP321A8* (> 400-fold), the level of increase in total P450 activity (> two-fold), and the deltamethrin tolerance (> ten-fold). These results suggest that the contribution of SfCYP321A8 to overall P450 activity in FAW is not very high and multiple P450s may be involved in deltamethrin resistance in this insect.

P450-mediated insecticide resistance is conferred through overexpression of multiple P450 genes (Liu et al. 2015). Several transcription factors, including a nuclear receptor *FTZ-F1*, and heterodimer of zip transcription factors, *CLC* and *Mad*, and GPCRs, have been found to mediate constitutive overexpression of multiple P450 genes in insecticide-resistant insects (Amezian et al. 2021; Bo et al. 2020; Gaddelapati et al. 2018; Kalsi and Palli 2015; Li et al. 2019; Liu et al. 2021; Palli 2020). The expression of multiple P450 genes could be manipulated by transgenic overexpression or knockdown/knockout of these transcription factors. In this work, we used a constitutive promoter, *Sfpub/P2009*, which allowed expressing high levels of P450 genes in the whole body. The conditional expression of transgenic P450 genes could be achieved by employing tissue-, stage-specific, or inducible promoters, which is likely to help in P450 functional studies. Moreover, not only the P450 genes but also many other detoxifying enzyme genes from FAW and other insects could be overexpressed in vivo using the transformation methods reported in this paper.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01452-6.

Acknowledgements We thank Dr. Anjiang Tan of Shanghai institute of plant physiology and ecology, China, for the gift of *piggyBac* vector, and Jeff Howell from the University of Kentucky for help with FAW rearing. This material is based on the work supported by the National Science Foundation I/UCRC, the Center for Arthropod Management Technologies, under grant no. IIP-1821936, and by industry partners.

References

Amezian D, Nauen R, Le Goff G (2021) Transcriptional regulation of xenobiotic detoxification genes in insects - An overview. Pestic Biochem Physiol 174:104822. https://doi.org/10.1016/j.pestbp.2021.104822

Bai-Zhong Z et al (2020) Silencing of Cytochrome P450 in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) by RNA Interference Enhances Susceptibility to Chlorantraniliprole. J Insect Sci 20:12. https://doi.org/10.1093/jisesa/ieaa047

Bass C, Field LM (2011) Gene amplification and insecticide resistance. Pest Manag Sci 67:886–890. https://doi.org/10.1002/ps.2189

Bo H et al (2020) Xenobiotic transcription factors CncC and maf regulate expression of CYP321A16 and CYP332A1 that mediate chlorpyrifos resistance in Spodoptera exigua. J Hazard Mater 398:122971. https://doi.org/10.1016/j.jhazmat.2020.122971

Carvalho RA, Omoto C, Field LM, Williamson MS, Bass C (2013) Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLoS ONE 8:e62268. https://doi.org/10.1371/journal.pone.0062268

Chen X, Chereddy S, Gurusamy D, Palli SR (2020a) Identification and characterization of highly active promoters from the fall armyworm *Spodoptera Frugiperda*. Insect Biochem Mol Biol 126:103455. https://doi.org/10.1016/j.ibmb.2020.103455

Chen X, Koo J, Gurusamy D, Mogilicherla K, Reddy Palli S (2020b) *Caenorhabditis elegans* systemic RNA interference defective protein 1 enhances RNAi efficiency in a lepidopteran insect, the fall armyworm, in a tissue-specific manner. RNA Biol. https://doi.org/10.1080/15476286.2020.1842632

Daborn PJ, Lumb C, Boey A, Wong W, Ffrench-Constant RH, Batterham P (2007) Evaluating the insecticide resistance potential of eight *Drosophila melanogaster* cytochrome P450 genes by transgenic over-expression. Insect Biochem Mol Biol 37:512–519. https://doi.org/10.1016/j.ibmb.2007.02.008

Dermauw W, Van Leeuwen T, Feyereisen R (2020) Diversity and evolution of the P450 family in arthropods. Insect Biochem Mol Biol 127:103490. https://doi.org/10.1016/j.ibmb.2020.103490

Diez-Rodriguez GI, Omoto C (2001) Inheritance of lambda-cyhalothrin resistance in *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae). Neotrop Entomol 30:311–316. https://doi.org/10.1590/S1519-566X2001000200016

Douris V et al (2017) Investigation of the contribution of RyR target-site mutations in diamide resistance by CRISPR/Cas9 genome modification in *Drosophila*. Insect Biochem Mol Biol 87:127–135. https://doi.org/10.1016/j.ibmb.2017.06.013

Dulbecco AB, Moriconi DE, Pedrini N (2021) Knockdown of CYP4PR1, a cytochrome P450 gene highly expressed in the integument tissue of Triatoma infestans, increases susceptibility to deltamethrin in pyrethroid-resistant insects. Pestic Biochem Physiol 173:104781. https://doi.org/10.1016/j.pestbp.2021.104781

Farias JR, Andow DA, Horikoshi RJ, Sorgatto RJ, Fresia P, dos Santos AC, Omoto C (2014) Field-evolved resistance to Cry1F maize by

Spodoptera frugiperda (Lepidoptera: Noctuidae) in Brazil. Crop Prot 64:150–158. https://doi.org/10.1016/j.cropro.2014.06.019
Feyereisen R (1999) Insect P450 enzymes. Ann Rev Entomol 44:507–533. https://doi.org/10.1146/annurev.ento.44.1.507
Feyereisen R (2012) Insect CYP genes and P450 enzymes. In: Insect molecular biology and biochemistry. pp 236–316. Elsevier, Amsterdam https://doi.org/10.1016/B978-0-12-384747-8.10008-X
Ffrench-Constant RH (2013) The molecular genetics of insecticide resistance. Genetics 194:807–815. https://doi.org/10.1534/genetics.112.141895
Gaddelapati SC, Kalsi M, Roy A, Palli SR (2018) Cap "n" collar C regulates genes responsible for imidacloprid resistance in the Colorado potato beetle *Leptinotarsa Decemlineata*. Insect Biochem Mol Biol 99:54–62. https://doi.org/10.1016/j.ibmb.2018.05.006
Gimenez S et al (2020) Adaptation by copy number variation increases insecticide resistance in the fall armyworm. Commun Biol 3:664. https://doi.org/10.1038/s42003-020-01382-6
Giraudo M, Hilliou F, Fricaux T, Audant P, Feyereisen R, Le Goff G (2015) Cytochrome P450s from the fall armyworm (*Spodoptera frugiperda*): responses to plant allelochemicals and pesticides. Insect Mol Biol 24:115–128. https://doi.org/10.1111/imb.12140
Gouin A et al (2017) Two genomes of highly polyphagous lepidopteran pests *(Spodoptera frugiperda*, Noctuidae) with different host-plant ranges. Sci Rep 7:11816. https://doi.org/10.1038/s41598-017-10461-4
Gui F et al (2020) Genomic and transcriptomic analysis unveils population evolution and development of pesticide resistance in fall armyworm *Spodoptera frugiperda* Protein. Cell. https://doi.org/10.1007/s13238-020-00795-7
Hemingway J, Field L, Vontas J (2002) An overview of insecticide resistance. Science 298:96–97. https://doi.org/10.1126/science.1078052
Hu B et al (2021) Changes in both trans- and cis-regulatory elements mediate insecticide resistance in a lepidopteron pest. Spodoptera Exigua PLoS Genet 17:e1009403. https://doi.org/10.1371/journal.pgen.1009403
Huang F et al (2014) Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. PLoS ONE 9:e112958. https://doi.org/10.1371/journal.pone.0112958
Inceoglu AB, Waite TD, Christiansen JA, McAbee RD, Kamita SG, Hammock BD, Cornel AJ (2009) A rapid luminescent assay for measuring cytochrome P450 activity in individual larval *Culex* pipiens complex mosquitoes (Diptera: Culicidae). J Med Entomol 46:83–92. https://doi.org/10.1603/033.046.0111
Kalsi M, Palli SR (2015) Transcription factors, CncC and Maf, regulate expression of CYP6BQ genes responsible for deltamethrin resistance in *Tribolium castaneum*. Insect Biochem Mol Biol 65:47–56. https://doi.org/10.1016/j.ibmb.2015.08.002
Kariyanna B, Prabhuraj A, Asokan R, Ramkumar G, Venkatesan T, Gracy RG, Mohan M (2020) Genome mining and functional analysis of cytochrome P450 genes involved in insecticide resistance in *Leucinodes orbonalis* (Lepidoptera: Crambidae). Biotechnol Appl Biochem. https://doi.org/10.1002/bab.1997
Li X, Schuler MA, Berenbaum MR (2007) Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu Rev Entomol 52:231–253. https://doi.org/10.1146/annurev.ento.51.110104.151104
Li X, Shan C, Li F, Liang P, Smagghe G, Gao X (2019) Pest Manag Sci 75:1172–1180. https://doi.org/10.1002/ps.5279
Liu H et al. (2019) Chromosome level draft genomes of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), an alien invasive pest in China https://doi.org/10.1101/671560
Liu N, Li M, Gong Y, Liu F, Li T (2015) Cytochrome P450s–Their expression, regulation, and role in insecticide resistance. Pestic Biochem Physiol 120:77–81. https://doi.org/10.1016/j.pestbp.2015.01.006
Liu N, Wang Y, Li T, Feng X (2021) G-protein coupled receptors (GPCRs): signaling pathways, characterization, and functions in insect physiology and toxicology. Int J Mol Sci. https://doi.org/10.3390/ijms22105260
Mallott M et al (2019) A flavin-dependent monooxgenase confers resistance to chlorantraniliprole in the diamondback moth *Plutella Xylostella*. Insect Biochem Mol Biol 115:103247. https://doi.org/10.1016/j.ibmb.2019.103247
Montezano DG et al (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. Afr Entomol 26:286–300. https://doi.org/10.4001/003.026.0286
Nagoshi RN et al (2017) Fall armyworm migration across the Lesser Antilles and the potential for genetic exchanges between North and South American populations. PLoS ONE 12:e0171743. https://doi.org/10.1371/journal.pone.0171743
Omoto C et al (2016) Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag Sci 72:1727–1736. https://doi.org/10.1002/ps.4201
Palli SR (2020) CncC/Maf-mediated xenobiotic response pathway in insects. Arch Insect Biochem Physiol 104:e21674. https://doi.org/10.1002/arch.21674
Pang R, Chen M, Liang Z, Yue X, Ge H, Zhang W (2016) Functional analysis of CYP6ER1, a P450 gene associated with imidacloprid resistance in *Nilaparvata lugens*. Sci Rep 6:34992. https://doi.org/10.1038/srep34992
Rane RV, Ghodke AB, Hoffmann AA, Edwards OR, Walsh TK, Oakeshott JG (2019) Detoxifying enzyme complements and host use phenotypes in 160 insect species. Curr Opin Insect Sci 31:131–138. https://doi.org/10.1016/j.cois.2018.12.008
Shi Y, Jiang Q, Yang Y, Feyereisen R, Wu Y (2021) Pyrethroid metabolism by eleven Helicoverpa armigera P450s from the CYP6B and CYP9A subfamilies. Insect Biochem Mol Biol 135:103597. https://doi.org/10.1016/j.ibmb.2021.103597
Tian K, Feng J, Zhu J, Cheng J, Li M, Qiu X (2021) Pyrethrin-resembling pyrethroids are metabolized more readily than heavily modified ones by CYP9As from Helicoverpa armigera. Pestic Biochem Physiol 176:104871. https://doi.org/10.1016/j.pestbp.2021.104871
Wang H et al (2018) CYP6AE gene cluster knockout in *Helicoverpa armigera* reveals role in detoxification of phytochemicals and insecticides. Nat Commun 9:4820. https://doi.org/10.1038/s41467-018-07226-6
Wang J et al (2017) CRISPR/Cas9 mediated genome editing of *Helicoverpa armigera* with mutations of an ABC transporter gene HaABCA2 confers resistance to Bacillus thuringiensis Cry2A toxins. Insect Biochem Mol Biol 87:147–153. https://doi.org/10.1016/j.ibmb.2017.07.002
Wang X, Cao X, Jiang D, Yang Y, Wu Y (2020) CRISPR/Cas9 mediated ryanodine receptor I4790M knockin confers unequal resistance to diamides in *Plutella xylostella*. Insect Biochem Mol Biol 125:103453. https://doi.org/10.1016/j.ibmb.2020.103453
Yang F, Kerns DL, Head GP, Price P, Huang F (2017) Cross-resistance to purified Bt proteins, Bt corn and Bt cotton in a Cry2Ab2-corn resistant strain of *Spodoptera frugiperda*. Pest Manag Sci 73:2495–2503. https://doi.org/10.1002/ps.4644
Zhang B et al (2020) Effects of three insecticides at the sublethal dose on the expression of cytochrome P450 genes in Spodoptera frugiperda (*Lepidoptera: Noctuidae*). Acta Entomol Sin 63:565–573
Zhu F et al (2010) A brain-specific cytochrome P450 responsible for the majority of deltamethrin resistance in the QTC279 strain of *Tribolium castaneum*. Proc Natl Acad Sci USA 107:8557–8562. https://doi.org/10.1073/pnas.1000059107