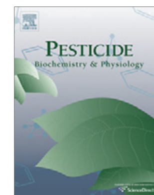
Pesticide Biochemistry and Physiology 97 (2010) 209–213

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest



Expression responses of nine cytochrome P450 genes to xenobiotics in the cotton bollworm *Helicoverpa armigera*

Xiaojie Zhou [a,b], Chengfa Sheng [a], Mei Li [a], Hua Wan [a,b], Dong Liu [a,b], Xinghui Qiu [a,*]

[a] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[b] Graduate School, Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Article history:
Received 28 October 2009
Accepted 12 February 2010
Available online 20 February 2010

Keywords:
Helicoverpa armigera
Cytochrome P450
Xenobiotics
Induction
Gossypol
Xanthotoxin
Phenobarbital
Deltamethrin

ABSTRACT

Cytochrome P450s play a crucial role in insect adaptation to their host plants and in insecticide resistance through metabolic detoxification. The efficacy of P450 mediated detoxification depends on the levels of P450 transcript which may change after exposure to xenobiotics. In this study, expression response of nine cytochrome P450 genes in *Helicoverpa armigera*, belonging to *CYP6* and *CYP9* families, were examined by using quantitative real-time PCR method. Results showed that these cytochrome P450 genes were differentially affected by plant chemicals and synthetic compounds, and displayed tissue-, concentration- and inducer-dependent response. Phenobarbital acted as a general inducer for cytochrome P450s, whereas gossypol specifically increased the expression of *CYP6AE14*. Xanthotoxin showed moderate induction effect on several of the nine genes. Deltamethrin displayed significantly both induction and suppression impact on the expression of cytochrome P450s. These findings provide clues for further elucidating the function and regulation mechanism of these cytochrome P450 genes in *H. armigera*, a pest responsible for severe losses in many crops.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

The bollworm, *Helicoverpa armigera* (Hübner), is a polyphagous insect responsible for severe yield losses in many different crops including cotton [1,2]. The persistence of this pest in modern agriculture is mainly attributed to its resistance to commonly applied insecticides and adaptability to a wide array of host plants [3–5]. In insects, enzymatic detoxification of plant toxins and insecticides is well documented to be one of the common and major mechanisms of host plant adaptability and insecticide resistance [2,4,6–8]. Among the insect detoxification systems, NADPH-dependent cytochrome P450 monooxygenases are the most prominent, represented by around 48–164 genes in insect genomes [9,10]. Given the overall importance of cytochrome P450 monooxygenases in insecticide resistance and allelochemical adaptation, structures and functions of cytochrome P450s in this pest have been attracting intensive interest.

Previous studies had been focused on the tissue distribution, developmental expression of P450 monoxygenases as a whole, their interactions with xenobiotic compounds, and the associations with insecticide resistance [6,11,12]. Characterization of individual cytochrome P450s in an insect is difficult to achieve due to the genetic diversity, broad substrate specificity and catalytic versatility of cytochrome P450s. Fortunately, molecular approaches have been allowing investigators to study structure, function and regulation of individual P450s in insects. Until now, eleven cytochrome P450 genes (excluding allelic genes) with a full coding sequence have been identified in *H. armigera* (http://www.ncbi.nlm.nih.gov/sites/entrez). The first P450 gene cloned from *H. armigera* was *CYP6B2* [1]. *CYP6B6* and *CYP6B7* were subsequently isolated from Australian *H. armigera* and the CYP6B7 was suggested as the P450 form responsible for pyrethroid metabolism in *H. armigera* [13]. More recently, Yang et al. [14] isolated *CYP9A12* and *CYP9A14* and Wee et al. [15] reported *CYP337B1* and *CYP4S1* from *H. armigera*. In addition, constitutive over-expression of *CYP9A12*, *CYP9A14*, *CYP337B1* and *CYP4S1* has been documented to be associated with fenvalerate resistance in this pest [14,15]. Mao et al. (2007) identified a cytochrome P450 *CYP6AE14* which is related to gossypol tolerance [16]. Both CYP9A12 and CYP9A14 heterologously expressed in *Saccharomyces cerevisiae* play roles in clearance of esfenvalerate [17]. However, the role and regulation of individual cytochrome P450s in this important pest remains poorly understood.

Inducibility is a general characteristic of cytochrome P450 [18]. Induction of cytochrome P450 genes by chemicals such as allelochemical, and in some cases insecticides, has been well documented [9]. The link between exogenous compounds acting as

* Corresponding author. Address: State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences. 1 Beichen West Road, Chaoyang District, Beijing 100101, China. Fax: +86 10 64807099.
E-mail address: qiuxh@ioz.ac.cn (X. Qiu).

0048-3575/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2010.02.003

induction agents and the induced enzymes metabolizing them has been established in both mammalian and insect detoxification systems [19]. For example, *CYP6B8* and *CYP321A1* are induced by and capable of metabolizing xanthotoxin in *Helicoverpa zea* [20]. Therefore, induction of P450s has been taken as an indication of their roles in the metabolism of xenobiotics [21]. Studying the induction profile of insect P450 enzymes may not only facilitate understanding how insects adapt to their environment, but also provide clue for determining substrate specificity and revealing the mechanism of P450 expression regulation. In the present study, we attempted to investigate the response of cytochrome P450 genes in the cotton bollworm to various xenobiotics. In the absence of a full genome sequence, we conducted this study with nine genes. The nine P450 genes we selected are the identified full length genes available up to date, belonging to CYP6 and CYP9 families. *CYP6* and *CYP9* families have been well documented to be involved in detoxification in insects. Chemicals used included naturally occurring phytochemicals (gossypol and xanthotoxin) and synthetic compound (deltamethrin and phenobarbital).

2. Materials and methods

2.1. Insects

The laboratory strain of *H. armigera*, originally collected from cotton field in Shandong Province of China in 2005, was maintained in an insectary without exposure to any insecticide. This population showed moderate resistance to fenvalerate (300-fold) and marginal resistance to phoxim (1.8-fold) and thiodicarb (4.66-fold) in 2005. The larvae were individually reared on semi-artificial diet [2] and maintained at 25 ± 1 °C and 70% relative humidity (RH) with a photoperiod of 16:8 h (L:D). Adults were kept under the same temperature and light conditions, and provided with a 10% honey solution.

2.2. Induction treatment

Newly molted caterpillars of 6th instar were selected for all treatments. These synchronous larvae were provided with semi-artificial diets containing different chemicals. Four different chemicals belonging to various chemical classes were used as xenobiotics in this study: the sodium phenobarbital (PB, purity 99%, Sigma, St. Louis, MO, USA), gossypol acetic acid (GP, ⩾95%, China Cotton UNIS, Beijing), xanthotoxin (XAN, Sigma, St. Louis, MO, USA) and deltamethrin (DM, 99%, Bayer CropScience, Germany). PB is a commonly used cytochrome P450 inducer. Gossypol is one of the main phytochemicals in the cotton which is a main host plant of *H. armigera*. Gossypol acetic acid, which is a relatively stable crystalline complex of equimolar quantities of gossypol and acetic acid, was used in this study. Xanthotoxin (XAN) is another plant toxin encountered rarely by *H. armigera*. Deltamethrin is a representative of pyrethroid insecticide, which has been extensively used for control of the cotton bollworm. Two concentrations were used for each chemical as follows: 1 and 10 mg g^{-1} (w:w) for PB, 0.05 and 0.5 mg g^{-1} for DM, 0.2 and 2 mg g^{-1} for GP, 0.2 and 1 mg g^{-1} for XAN. The concentrations of xenobiotic were set based on our result in preliminary experiments. At the lower concentration, no significant effect on development of the cotton bollworm was observed, whereas a decrease of body weight gain, but no obvious toxic symptom, was observed in the treatments with the higher dose of DM, GP and XAN. After 48 h, both midgut and fat body were dissected for RNA extraction. Ten synchronous individuals were used for each treatment and three biological replicates were performed for all treatments.

2.3. RNA isolation and cDNA synthesis

Total RNA was extracted using TRIzol (Invitrogen, CA, USA) according to the manufacture's instructions. The quality and concentration of RNA samples were examined by agarose gel electrophoresis and spectrophotometer analysis. RNA was digested by DNase I (Takara, Japan) in order to eliminate the genomic DNA contamination. cDNA was synthesized by reverse transcription in 20 μL reactions containing 1 μg of total RNA, 200U PrimeScript™ reverse transcriptase (Takara, Japan), 20 U RNase inhibitor, 1 μL dNTP mixture (10 mM each) and 1 μL oligo $(dT)_{18}$ primer (50 μM) at 42 °C for 1 h. Three independent RNA preparations representing three biological replicates were used for cDNA synthesis.

2.4. Real-time RT-PCR analysis

The expression levels of cytochrome P450 genes were quantified by quantitative real-time PCR (qRT-PCR) using a Mx3000P qPCR System (Stratagene, La Jolla, CA, USA) and RealMasterMix SYBR Green PCR kit (TianGen, Beijing, China). The elongation factor-1α (EF-1α, EF-F/EF-R primer set) was used as a reference gene to normalize the target gene expression levels among samples [14,22]. qRT-PCR of each cDNA sample and template-free was performed in triplicate. All the primer sets used in this study were listed in Table 1. Specificity of the PCR amplification was checked by a melt curve analysis (MxPro 4.0 program, Stratagene) and by sequencing the PCR products. qTR-PCR was run in a 25-μL reaction containing 11.25 μL RealMasterMix/SYBR solution, 0.5 μL each of forward and reverse primer (10 μM), 3.5 μL cDNA template using the following cycling parameters: 95 °C for 5 min, followed by 45 cycles of 95 °C for 30 s, 55–60 °C for 30 s and 68 °C for 40 s. The melting curves of amplicons were measured by taking continuous fluorescence reading whilst increasing temperature from 58 to 95 °C with 0.5 °C increments for 10 s. For each gene, a serial dilution from 10- to 1000-fold of each cDNA templates was performed in order to assess efficiency of PCR. The relative expression levels of target genes were calculated by the comparative C_T method as described by Livak and Schmittgen [23]. Results were expressed as mean expression ratio (±S.E.) of three biological replicates between xenobiotic treatments and controls. One-way analysis of variance (ANOVA) and the Fisher's least significant difference were performed to determine the statistical difference between means (SPSS, version 13). A p-value of <0.05 was considered significant.

3. Results

To investigate the expression of cytochrome P450 genes in response to allelochemicals present in their natural host plants (GP, XAN), and two synthetic chemicals that may (DM) or may not (PB) exist in their environment, RNA samples from the final instar larvae fed for 48 h on diets incorporated with the chemicals were quantitatively analyzed by real-time PCR. The results showed that the expression of the nine cytochrome P450 genes was differentially affected by the xenobiotics tested (Figs. 1–4).

3.1. Gossypol

Gossypol is a main plant toxin in cotton, a preferred host plant for the cotton bollworm. Most cotton cultivars store gossypol in their pigmented glands. qRT-PCR result showed that GP increased the expression of *CYP6AE14* in both fatbody and midgut at both tested concentrations. However, GP at 2 mg g^{-1} decreased the expression of *CYP9A14* in the midgut and *CYP6B6* in the fat body (Fig. 1).

Table 1
PCR primers for qRT-PCR used in this study.

Gene	Primers	Sequence (from 5′ to 3′)	Fragment length(bp)	Annealing temperature	GenBank Accession No.or reference
EF-1α	EF-F EF-R	GACAAACGTACCATCGAGAAG GATACCAGCCTCGAACTCAC	279	58	U20129/[14]
CYP6AE12	q6AE12-F q6AE12-R	GAAGGTGCTGCGTCAGAATCTG CAGTCCATAGTGAAGCCAGCCA	171	56	DQ256407
CYP6AE14	q6AE14-F q6AE14-R	TGTGCATTTGGCGTTGAA TCCGAGATGTGGGCGTAT	241	56	DQ986461
CYP6B2	q6B2-F q6B2-R	CGCCTGGTTAGACTGGTTTC TGTCGCAACTCGTTTATGCT	292	60	U18085/[29]
CYP6B6	q6B6-F q6B6-R	TTGAAGAAAGGCGTATGAAA ACACGCAAGATACACAAAGG	232	60	U64800/[29]
CYP6B7	q6B7-F q6B7-R	TGGACCGAAAGGAGGAATAC TTAACAAGAGGGATCTCCATAAG	85	55	AF031468
CYP9A12	q9A12-F q9A12-R	ATCACCTCATAGAAGATATCC CATGTCTTTCCATTCTTGACC	233	55	AY371318/[14]
CYP9A14	q9A14-F q9A14-R	ACCCTGAGGTACAGGAGA TAGACCACACCGGGATCA	258	58	AY487948/[14]
CYP9A17	q9A17-F q9A17-R	ATCACTTCGTAGACGATACCA CATGTCTTTCCATTCTTGACC	233	56	AY753201
CYP9A18	q9A18-F q9A18-R	AGGAGCGATTGGCACAGGAG GGAAGGCATAGACTGGGATTTGG	249	58	DQ256408

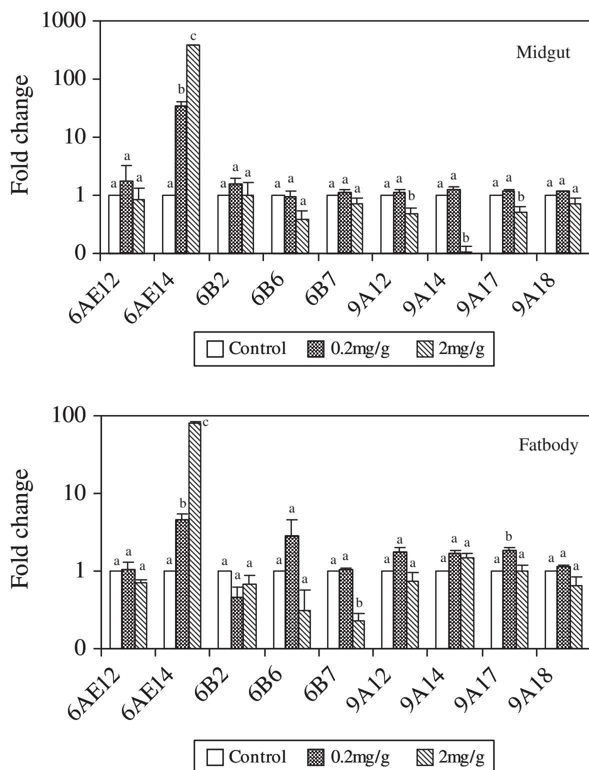


Fig. 1. Effects of gossypol on CYP mRNA levels in the midgut (top) and fatbody (bottom) of final instar larvae of *Helicoverpa armigera* determined by qRT-PCR. Data presented are the ratio of transcript level of each gene versus control expressed as fold change for each treatment. Bars sharing the same letter for each gene are not significantly different at $p < 0.05$. Data of CYP9A12 and CYP9A17 are from Zhou et al. [30].

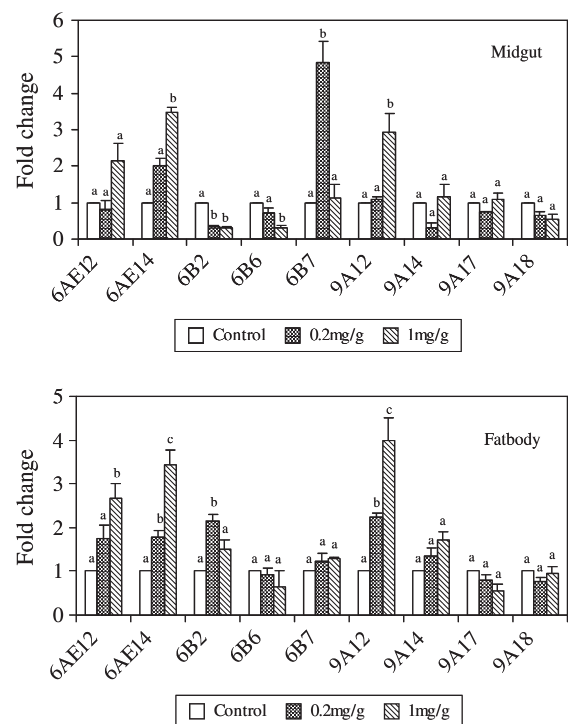


Fig. 2. Effects of xanthotoxin on CYP mRNA levels in the midgut (top) and fatbody (bottom) of final instar larvae of *Helicoverpa armigera* determined by qRT-PCR. Data presented are the ratio of transcript level of each gene versus control expressed as fold change for each treatment. Bars sharing the same letter for each gene are not significantly different at $p < 0.05$.

3.2. Xanthotoxin

Xanthotoxin is a furanocoumarin encountered rarely by *H. armigera*. This chemical showed moderate induction effect on several of the nine genes. Xanthotoxin induced (3- to 5- fold) the expression of *CYP6AE14*, *CYP6B7* and *CYP9A12* in the midgut. 2- to 4- fold induction of *CYP6AE12*, *CYP6AE14*, *CYP6B2* and *CYP9A12* was observed in the fat body in response to xanthotoxin (Fig. 2).

3.3. Deltamethrin

Deltamethrin showed significantly both induction and suppression impact on the expression of cytochrome P450s (Fig. 3). In the

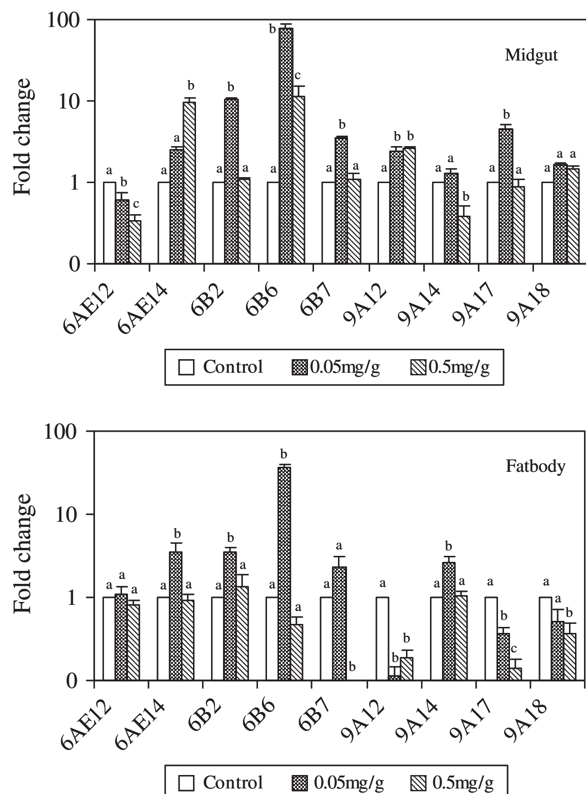


Fig. 3. Effects of deltamethrin on CYP mRNA levels in the midgut (top) and fatbody (bottom) of final instar larvae of *Helicoverpa armigera* determined by qRT-PCR. Data presented are the ratio of transcript level of each gene versus control expressed as fold change for each treatment. Bars sharing the same letter for each gene are not significantly different at $p < 0.05$. Data of CYP9A12 and CYP9A17 are from Zhou et al. [30].

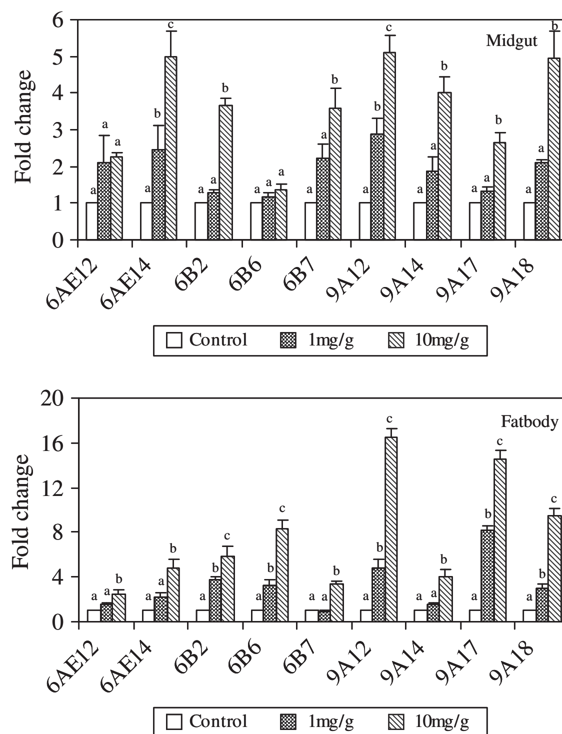


Fig. 4. Effects of phenobarbital on CYP mRNA levels in the midgut (top) and fatbody (bottom) of final instar larvae of *Helicoverpa armigera* determined by qRT-PCR. Data presented are the ratio of transcript level of each gene versus control expressed as fold change for each treatment. Bars sharing the same letter for each gene are not significantly different at $p < 0.05$. Data of CYP9A12 and CYP9A17 are from Zhou et al. [30].

midgut, the expression of *CYP6AE14*, *CYP6B2*, *CYP6B6*, *CYP6B7*, *CYP9A12*, and *CYP9A17* were induced, whereas *CYP6AE12*, *CYP9A14* were suppressed. Notably, high induction ratios were obtained for *CYP6B6* (78.8-fold at 0.05 mg g^{-1}). In the fat body, deltamethrin at lower concentration increased the expression of *CYP6AE14*, *CYP6B2*, *CYP6B6*, *CYP9A14*, whereas DM at the higher dose decreased the levels of *CYP6B7*, *CYP9A12*, *CYP9A17* and *CYP9A18*.

3.4. Phenobarbital

Phenobarbital is a synthetic compound not encountered naturally. Our result showed that it acted as a general inducer for cytochrome P450s in the cotton bollworm, increasing the level of P450 transcripts in tissue- and concentration-specific manners (Fig. 4). In the midguts, seven of the nine genes tested were induced by 2- to 5-fold. In the fat body, stronger induction (2- to 16-fold) of all the nine genes was observed than in the midguts.

4. Discussion

One feature of P450 genes is that the transcription rate of many of them is induced by xenobiotic compounds such as insecticides or plant allelochemicals [9]. The same species exposing to different chemicals will induce a different, sometimes overlapping subset of P450 genes [21]. In the cotton bollworm, the P450s are regulated by multiple chemicals and different cytochrome P450s are not uniformly responsive to xenobiotics in terms of the number of genes and fold induction, reflecting the complexity of induction response. PB, a synthetic compound, is a general and moderately effective inducer of cytochrome P450 genes in *H. armigera*. Seven of the nine cytochrome P450 genes in the midgut and all the nine genes tested in the fat body are induced after exposure to phenobarbital. In contrast, gossypol, a polyphenolic aldehyde that the cotton bollworms commonly encounter in their host plants, is highly selective in the number of cytochrome P450 genes that it induces, only *CYP6AE14* in both fatbody and midgut tissue was induced, and notably to a high degree. The high selectivity of gossypol was also found in another polyphagous insect, *H. zea* [6]. Overall, our data did not display obvious pattern demonstrating that the natural plant toxins are superior to or more effective than synthetic compounds as inducers, which is in keeping with the observation in *H. zea* [24].

The fact that *CYP6AE14* is highly induced in response to gossypol strongly suggests CYP6AE14 a major protein involving in gossypol metabolism. This suggestion is consistent with the result of Mao et al., who have illustrated that CYP6AE14 confers the ability to tolerate gossypol for the cotton bollworm [16]. *CYP6AE14* is also induced by the other three chemicals tested to lesser extent (Figs. 1–4), indicating that this protein may participate in the metabolism of chemicals except gossypol. Similarly, *CYP6AE14* was found to be weakly induced by other phenolic compounds [16].

It has been suggested that xenobiotic inducible genes may be more likely to be involved in resistance [25]. Several of cytochrome P450 genes such as *Cyp6a2*, *Cyp6g1* and *Cyp6a8* in *Drosophila* induced by PB have been associated with DDT resistance phenotype [25,26]. *CYP9A12* are induced by multiple xenobiotics (PB, XAN and DM) in the midgut in this study, and this gene has been proved to be involved in pyrethroid resistance in the cotton bollworm [14]. Similar phenomenon has been also documented for *CYP6A1* and *CYP6D1* in the *Musca domestica* (Review in Feyereisen, 2005) [9]. These results are in support of the hypothesis that xenobiotic

inducibility of a detoxification enzyme may represent a risk factor for resistance, because if mutations change the normal regulatory network involved in the induction of a P450 gene, this may result in higher constitutive expression of the gene [25].

Due to their toxicity, insecticides generally are unlikely to cause induction [27]. A study in *Drosophila melanogaster* showed that six chemically distinct insecticides did not induce the expression of P450s with exception of DDT, and even DDT exposure only led to weak induction of a single P450 (*Cyp12d1*, 3-fold) [19]. Another study using microarray showed that permethrin exposure of larvae of the mosquito (*Aedes aegypti*) resulted in a significant overexpression of three CYP genes (*CYP9M8*, *CYP9M9* and *CYP314A1*), whereas temephos exposure did not induce any detoxification gene [28]. In our work, deltamethrin induction was observed in six CYP genes in the midgut and four genes in the fat body. Discrepancies in insecticide induction among these studies may result from the differences in insecticides, exposure regime, and the species and status of animal used. Notably, *CYP6B6* transcription is highly inducible by deltamethrin at a low dose in both midgut and fat body. This observation makes us suggest that CYP6B6 may contribute to deltamethrin metabolism in this pest, although it is unlikely to be responsible for fenvalerate resistance in ANO2 strain of *H. armigera* [29].

In summary, expression of cytochrome P450 in the cotton bollworm is differentially affected by plant chemicals and/or synthetic compounds. In view of the absence of information on the substrate profiles of these P450s, it is currently difficult to evaluate the biological and ecological significance of each P450. However, our data provide clues for further elucidating the function and regulation mechanism of these cytochrome P450 genes.

Acknowledgments

This work was supported by grants from National Basic Research Program (973 Program, No. 2006CB102003), Key Technologies R&D Program (No. 2006BAD08A03) and National Natural Science Foundation of China (No. 30370946) to X Qiu.

References

[1] X.P. Wang, A.A. Hobbs, Isolation and sequence analysis of a cDNA clone for a pyrethroid inducible cytochrome P450 from *Helicoverpa armigera*, Insect Biochem. Mol. Biol. 25 (1995) 1001–1009.

[2] K. Wu, P. Gong, A new and practical artificial diet for the cotton bollworm, Entomol. Sin. 4 (1997) 277–282.

[3] W. Tan, Y. Guo, Effects of host plant on susceptibility to deltamethrin and detoxification enzyme of *Heliothis armigera* (Lepidoptera: Noctuidea), J. Econ. Entomol. 89 (1996) 11–14.

[4] N. Forrester, M. Cahill, L. Bird, J. Layland, Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia, Bull. Entomol. Res. Suppl. Ser. 1 (1993) 1–132.

[5] X. Qiu, W. Li, Y. Tian, X. Leng, Cytochrome P450 monooxygenases in the cotton bollworm (Lepidoptera: Noctuidae): tissue difference and induction, J. Econ. Entomol. 96 (2003) 1283–1289.

[6] X. Li, M.R. Berenbaum, M.A. Schuler, Plant allelochemicals differentially regulate *Helicoverpa zea* cytochrome P450 genes, Insect Mol. Biol. 11 (2002) 343–351.

[7] Y. Yang, Y. Wu, S. Chen, G.J. Devine, I. Denholm, P. Jewess, G.D. Moores, The involvement of microsomal oxidases in pyrethroid resistance in *Helicoverpa armigera* from Asia, Insect Biochem. Mol. Biol. 34 (2004) 763–773.

[8] S. Chen, Y. Yang, Y. Wu, Correlation between fenvalerate resistance and cytochrome P450-mediated *O*-demethylation activity in *Helicoverpa armigera* (Lepidoptera: Noctuidae), J. Econ. Entomol. 98 (2005) 943–946.

[9] R. Feyereisen, Insect cytochrome P450, in: L.I. Gilbert, K. Iatrou, S.S. Gill (Eds.), Comprehensive Molecular Insect Science, vol. 4, Elsevier Science Publishers, Oxford, 2005, pp. 1–77.

[10] J.G. Scott, Insect cytochrome P450s: thinking beyond detoxification, in: N. Liu (Ed.), Recent Advances in Insect Physiology, Toxicology and Molecular Biology, Research Signpost, Kerala, India, 2008, pp. 117–124.

[11] X. Qiu, X. Leng, The tissue distribution of monooxygenase activities of cotton bollworm, *Helicoverpa armigera*, Acta Ecol. Sin. 20 (2000) 299–303.

[12] X. Qiu, W. Li, X. Leng, Comparison of components and activities of microsomal P450 monooxygenases from the cotton bollworm, *Helicoverpa armigera* during different developmental stages, Acta Entomol. Sin. 44 (2001) 142–147.

[13] C. Ranasinghe, A.A. Hobbs, Isolation and characterization of two cytochrome P450 cDNA clones for CYP6B6 and CYP6B7 from *Helicoverpa armigera* (Hübner): possible involvement of CYP6B7 in pyrethroid resistance, Insect Biochem. Mol. Biol. 28 (1998) 571–580.

[14] Y. Yang, S. Chen, S. Wu, L. Yue, Y. Wu, Constitutive overexpression of multiple cytochrome P450 genes associated with pyrethroid resistance in *Helicoverpa armigera*, J. Econ. Entomol. 99 (2006) 1784–1789.

[15] C.W. Wee, S.F. Lee, C. Robin, D.G. Heckel, Identification of candidate genes for fenvalerate resistance in *Helicoverpa armigera* using cDNA-AFLP, Insect Mol. Biol. 17 (2008) 351–360.

[16] Y.B. Mao, W.J. Cai, J.W. Wang, G.J. Hong, X.Y. Tao, L.J. Wang, Y.P. Huang, X.Y. Chen, Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol, Nat. Biotechnol. 25 (2007) 1307–1313.

[17] Y. Yang, L. Yue, S. Chen, Y. Wu, Functional expression of *Helicoverpa armigera CYP9A12* and *CYP9A14*, Pestic. Biochem. Physiol. 92 (2008) 101–105.

[18] T.L. Harrison, A.R. Zangerl, M.A. Schuler, M.R. Berenbaum, Developmental variation in cytochrome P450 expression in *Papilio polyxenes* in response to xanthotoxin, a hostplant allelochemicals, Arch. Insect Biochem. Physiol. 48 (2001) 179–189.

[19] L. Willoughby, H. Chung, C. Lumb, C. Robin, P. Batterham, P.J. Daborn, A comparison of *Drosophila melanogaster* detoxification gene induction responses for six insecticides, caffeine and phenobarbital, Insect Biochem. Mol. Biol. 36 (2006) 934–942.

[20] X. Li, J. Baudry, M.R. Berenbaum, M.A. Schuler, Structural and functional divergence of insect CYP6B proteins: from specialist to generalist cytochrome P450, Proc. Natl. Acad. Sci. USA 101 (2004) 2939–2944.

[21] J.L. Stevens, M.J. Snyder, J.F. Koener, R. Feyereisen, Inducible P450s of the CYP9 family from larval *Manduca sexta* midgut, Insect Biochem. Mol. Biol. 30 (2000) 559–568.

[22] X. Li, M.A. Schuler, M.R. Berenbaum, Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes, Nature 419 (2002) 712–715.

[23] K.G. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (2001) 402–408.

[24] Z. Wen, R. Zeng, G. Niu, M.R. Berenbaum, M.A. Schuler, Ecological significance of induction of broad-substrate cytochrome P450s by natural and synthetic inducers in *Helicoverpa zea*, J. Chem. Ecol. 35 (2009) 183–189.

[25] G.L. Goff, F. Hilliou, B.D. Siegfried, S.D. Boudy, E. Wajnberg, L. Sofer, P. Audant, H.C. Richard, R. Feyereisen, Xenobiotic response in *Drosophila melanogaster*: sex dependence of P450 and GST gene induction, Insect Biochem. Mol. Biol. 36 (2006) 674–682.

[26] W. Sun, V.M. Margam, L. Sun, G. Buczkowski, G.W. Bennett, B. Schemerhorn, W.M. Muir, B.R. Pittendrigh, Genome-wide analysis of phenobarbital-inducible genes in *Drosophila melanogaster*, Insect Mol. Biol. 15 (2006) 455–464.

[27] C. Ranasinghe, M. Headlam, A.A. Hobbs, Induction of the mRNA for *CYP6B2*, a pyrethroid inducible cytochrome P450, in *Helicoverpa armigera* (Hübner) by dietary monoterpenes, Arch. Insect Biochem. Physiol. 34 (1997) 99–109.

[28] R. Poupardin, S. Reynaud, C. Strode, H. Ranson, J. Vontas, J.P. David, Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: impact on larval tolerance to chemical insecticides, Insect Biochem. Mol. Biol. 38 (2008) 540–551.

[29] V.D. Grubor, D.G. Heckel, Evaluation of the role of CYP6B cytochrome P450s in pyrethroid resistant Australian *Helicoverpa armigera*, Insect Mol. Biol. 16 (2007) 15–23.

[30] X. Zhou, C. Ma, M. Li, C. Sheng, H. Liu, X. Qiu, CYP9A12 and CYP9A17 in the cotton bollworm, *Helicoverpa armigera*: sequence similarity, expression profile and xenobiotic response, Pest Manag. Sci. 66 (2010) 65–73.