Bulletin of Entomological Research

cambridge.org/ber

Research Paper

*These authors contributed equally to this work.

Cite this article: Hu G-L *et al* (2022). *CYP4CJ6*-mediated resistance to two neonicotinoid insecticides in *Sitobion miscanthi* (Takahashi). *Bulletin of Entomological Research* **112**, 646–655. https://doi.org/10.1017/S0007485322000037

Received: 7 August 2021
Revised: 6 November 2021
Accepted: 25 January 2022
First published online: 17 February 2022

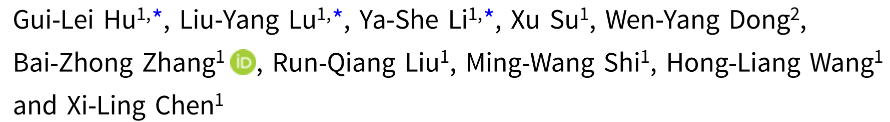
Keywords:
CYP4CJ6; insecticides; miR-316; RNAi; *Sitobion miscanthi*

Author for correspondence:
Bai-Zhong Zhang,
Email: baizhongok@163.com

© The Author(s), 2022. Published by Cambridge University Press

CAMBRIDGE UNIVERSITY PRESS

CYP4CJ6-mediated resistance to two neonicotinoid insecticides in *Sitobion miscanthi* (Takahashi)



Gui-Lei Hu[1,*], Liu-Yang Lu[1,*], Ya-She Li[1,*], Xu Su[1], Wen-Yang Dong[2], Bai-Zhong Zhang[1], Run-Qiang Liu[1], Ming-Wang Shi[1], Hong-Liang Wang[1] and Xi-Ling Chen[1]

[1]College of Resources and Environment, Henan Engineering Research Center of Biological Pesticide & Fertilizer Development and Synergistic Application, Henan Institute of Science and Technology, Xinxiang 453003, P.R. China and [2]Department of Entomology, China Agricultural University, Beijing 100193, P.R. China

Abstract

The wheat aphid *Sitobion miscanthi* (CWA) is an important harmful pest in wheat fields. Insecticide application is the main method to effectively control wheat aphids. However, CWA has developed resistance to some insecticides due to its extensive application, and understanding resistance mechanisms is crucial for the management of CWA. In our study, a new P450 gene, *CYP4CJ6*, was identified from CWA and showed a positive response to imidacloprid and thiamethoxam. Transcription of *CYP4CJ6* was significantly induced by both imidacloprid and thiamethoxam, and overexpression of *CYP4CJ6* in the imidacloprid-resistant strain was also observed. The sensitivity of CWA to these two insecticides was increased after the knockdown of *CYP4CJ6*. These results indicated that *CYP4CJ6* could be associated with CWA resistance to imidacloprid and thiamethoxam. Subsequently, the posttranscriptional regulatory mechanism was assessed, and miR-316 was confirmed to participate in the posttranscriptional regulation of *CYP4CJ6*. These results are crucial for clarifying the roles of P450 in the resistance of CWA to insecticides.

Introduction

Wheat aphids are serious pests in wheat-growing areas and have caused major losses to wheat production in China (Wang *et al*., 2006). They mainly damage crops by feeding on plant juice and spreading the yellow dwarf virus, which causes wheat yellow dwarf disease, like *Schizaphis graminum* (Rondani) (George and Gair, 2010). The aphids and combined diseases can cause premature plant decline, seed weight reduction, reduced yield and grain quality, and even the death of whole plants (Zhou *et al*., 2013). It is critical to manage wheat aphids, particularly in Northern China. There are 32 species of aphids that endanger wheat crops worldwide, mainly *Sitobion miscanthi* (Takahashi), *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (Linnae), *S. graminum* and *Acyrthosiphon dirhodum* (Walker) (Wang *et al*., 2011; Hu *et al*., 2015; Duan *et al*., 2016).

The Chinese wheat aphid (CWA), *S. miscanthi* (Hemiptera: Aphididae) was incorrectly called *Sitobion avenae* Fabricius in China (Jiang *et al*., 2019), which is one of the main wheat pests worldwide and causes economic losses through direct feeding and virus transmission (Li *et al*., 2016; Zhang *et al*., 2020).

The control of wheat aphids mainly relies on synthetic insecticides, i.g. organophosphates, carbamates, and pyrethroids. So far, neonicotinoid insecticides like imidacloprid and thiamethoxam have been widely applied in the prevention of aphids and other stinging or sucking pests, not only because of their high efficacy, but also low toxicity to humans and effective duration (Devine *et al*., 1996; Lu and Gao, 2009; Cui *et al*., 2012; Kim *et al*., 2015). However, with the frequent and long-term application of neonicotinoids, neonicotinoid-resistant pests have appeared (Liu *et al*., 2003), such as wheat aphids (Cui *et al*., 2012; Tang *et al*., 2017; Wang *et al*., 2018; Zhang *et al*., 2020). The most common causes of insecticide resistance include enhanced metabolic detoxification and reduced sensitivity of target sites. R81T resistance mutation has been previously found in aphids (*Myzus persicae* and *Aphis gossypii*) to be associated with neonicotinoid resistance phenotype (Hirata *et al*., 2015, 2017; Wang *et al*., 2016; Mezei *et al*., 2020; Sial *et al*., 2020). The increased detoxification mediated by the overexpression of P450s is a common mechanism of insecticide resistance (Li *et al*., 2007; Puinean *et al*., 2010; Schuler, 2011, 2012; Mohammed *et al*., 2014; Liu *et al*., 2015; Zhang *et al*., 2020). It has been reported that resistance to neonicotinoids is mediated in part by *CYP6CM1* in *Bemisia tabaci* (Jones *et al*., 2011). For example, overexpression of *CYP6CM1* is associated with imidacloprid resistance in *B. tabaci* (Karunker *et al*., 2008). Another common characteristic of P450

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

is inducibility (Brandt *et al.*, 2002); the expression level of P450 in pests can be significantly induced by insecticides (Zhang *et al.*, 2016*a*, 2016*b*, 2019).

The expression of P450 genes can be regulated by both transcriptional and posttranscriptional regulation. At the posttranscriptional level, microRNAs (miRNAs) with a length of approximately 22 nucleotides can affect the posttranscriptional regulation of P450 genes by binding to coding sequences, 3′ UTRs, or 5′ UTRs (Tamasi *et al.*, 2011; Pritchard *et al.*, 2012; Asgari, 2013). It has been reported that posttranscriptional regulation of P450 genes occurs when some miRNAs bind to their 3′UTRs or coding sequences (Hong *et al.*, 2014; Peng *et al.*, 2016; Ma *et al.*, 2017, 2019*a*, 2019*b*). For instance, the expression of *CYP325BG3* was regulated by miR-71 (Hong *et al.*, 2014), and the expression of *CYP6AG11* was regulated by miR-278-3p in *Culex pipiens* (Lei *et al.*, 2015).

Although P450s are critical in the metabolism of insects to insecticides, the molecular mechanism of P450 in *S. miscanthi* in response to insecticides is still obscure. In the current study, we found that *CYP4CJ6* could be involved in insecticide resistance. To better understand the molecular mechanism of the inducible expression of *CYP4CJ6*, posttranscriptional regulation of *CYP4CJ6* was explored. It is essential to clarify the molecular genetic mechanism of P450s in the resistance of CWA to insecticides.

Experimental procedures

Insects and cell culture

CWA, the susceptible strain (SA-S) originated from approximately 1000 wingless aphids of parthenogenic lineages from the infested plant leaves of the wheat field in Xihua (Coordinates, N23.16 E113.23) of Henan Province of China in May 2003 and retained in a greenhouse for more than 10 years with no exposure to any insecticides. The culture condition was mentioned previously (Lu and Gao, 2009). The aphid-dipping method was used to screen for resistance in each generation (Chen *et al.*, 2013). The susceptibility of the strain to imidacloprid (SA-S) was determined, which had an LC_{50} value of 0.62 μg ml^{-1}. The imidacloprid-resistant strain (SA-R) was obtained from the SA-S strain by continual selection with gradually increased concentrations of imidacloprid based on the LC_{50} values from the bioassay of their parent generations for 25 generations in the laboratory. Approximately 5000 apterous aphids in each generation were selected by the leaf-dipping method, and mortality was maintained at 40–80%. Finally, an SA-R strain of CWA (LC_{50} = 20.76 μg ml^{-1} after 24 h) was obtained by continuous selection of imidacloprid with 33.48-fold resistance compared with the SA-S strain. Both of the strains were fed on wheat seedlings at 23 °C, 60–70% relative humidity, and a photoperiod of 16:8 h (light:dark) under standard conditions (Lu and Gao, 2009). The human embryonic kidney cell line 293T was cultured in DMEM (Servicebio, Wuhan, China) supplemented with 10% fetal bovine serum (Servicebio, Wuhan, China), 1% penicillin, and 1% streptomycin. 293T cells were cultured at 37 °C with 5% CO_2.

Bioassays of a leaf with aphid-dipping

Insecticide toxicity to CWA was determined by using the aphid-dipping method with a slight modification (Chen *et al.*, 2013). Insecticides were dissolved in acetone, and then diluted to serial concentrations with 0.05% (v/v) Triton X-100 in water. Wheat leaves were cut into 20 mm long pieces. The leaves with aphids were dipped into 0.05% (v/v) Triton X-100 water with insecticides for 10 s, or dipped into 0.05% (v/v) Triton X-100 water without insecticides as a control. The leaves with treated aphids were put into a glass tube (6 cm in length, 2 cm diameter) with the open end covered with cotton to prevent insect escape. Each concentration had three biological repeats, each with 20 healthy apterous aphids. Mortality was recorded after insecticide exposure at 24 h.

Rapid amplification of cDNA ends (RACE)

The 3′ and 5′ RACE first-strand cDNAs were synthesized, and the PCR system was constructed according to the instructions of the Smart™ Race cDNA Amplification Kit (Clontech). Based on the sequence fragment of *CYP4C1*-like, 4 gene-specific primers (GSPs) were designed to amplify the full-length cDNAs. The designed primers are presented in table 1. The thermal cycling was performed using touchdown PCR as follows: 5 cycles at 94 °C for 30 s and 72 °C for 3 min; 5 cycles at 94 °C for 30 s, 70 °C for 30 s, and 72 °C for 3 min; and 27 cycles at 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min. The products of the 3′ and 5′ RACE were cloned into the pGEM-T Easy Vector and sequenced.

Quantitative real-time PCR and data analysis

MagZol™ Reagent (TRIzol Reagent) (Magen, Guangzhou, China) was used to extract total RNA from 10 live aphids, and cDNA was synthesized with Honor™ II 1st Strand cDNA Synthesis SuperMix for qPCR with gDNA digester (Novogene, Tianjin, China). qPCR was performed on the ABI7500 platform (Applied Biosystems) with Unique Aptamer™ qPCR SYBR® Green Master Mix (Novogene, Tianjin, China). Each qPCR was performed with three biological replicates and two technical replicates. The $2^{-\Delta\Delta Ct}$ method of relative quantification was used to process the above data (Pfaffl, 2001). *Actin* (Xu *et al.*, 2014) and *18S* rRNA (Broackes-Carter *et al.*, 2002) were used as housekeeping genes for CWA. The primers used are presented in table 1.

Insecticides and dsRNA feeding assays

We used glass tubes with openings at both ends for feeding assays. One end was sealed with double-layer parafilm, and the solution containing either 100 μl of 0.5 mol l^{-1} sterile sucrose (artificial diet) with 10 μg ml^{-1} imidacloprid (95.3%) or thiamethoxam (5 μg ml^{-1}, 97.0%) was sandwiched between the two parafilm layers. These concentrations were used based on our preliminary experiments of mortality between about 20 and 40%. A brush was used to gently place fifty healthy apterous aphids of the imidacloprid-resistant strain into the glass tube, and then the other end of the glass tube was closed with gauze. The aphids were fed an artificial diet containing insecticides for 24 h. The control was the same treatment without the insecticides. The experiments were performed in three independent biological replicates, and at least 40 live aphids were collected for RNA extraction.

Primers containing the T7 polymerase promoter sequence were used to amplify the sequences of the target genes and the green fluorescent protein (*GFP*) gene (table 1). PCR products were used as templates to amplify dsRNA using the MEGA script RNAi kit (Ambion, USA).

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

Table 1. List of primers used for qPCR and dsRNA synthesis

Application	Gene name	Sequence (5′–3′)	Product length (bp)
cDNA isolation (RT-PCR)	*CYP4C1*-like (*CYP4CJ6)*	F:GCGATGATGCAACCCAAAGATA	1253
		R:GGATTCAAACTTCGCATTCCGTAA	
5′ and 3′ cDNA end isolation (rapid amplification of cDNA ends)	*CYP4C1*-like (*CYP4CJ6)*	3′GPS1:TGCCATGAAATATGTAGAAGCC	–
		3′GPS2:TGGATACTTACCGTTCAGCGC	–
		5′GPS1:GTCTTGAAGTGAAACGTGTTGG	–
		5′GPS2:GTTAAAATGAACTACTGGACCG	–
dsRNA synthesis	ds*CYP4CJ6*	taatacgactcactatagggAGATCTCGTCTCACGTGCCT	391
		taatacgactcactatagggTCCACCAATATGCGTGAGTG	
	ds*GFP*	taatacgactcactatagggTGACCACCCTGACCTAC	288
		taatacgactcactatagggTTGATGCCGTTCTTCTGC	
qRT-PCR of miRNA	mir-316	F: TTTGGTGTCTTTTTCCGCTTTG	69
		R: GTGCAGGGTCCGAGGT	
	U6	F: CAGCACATATACTAAAATTGGAACG	76
		R: ACGAATTTGCGTGTCATCC	
qRT-PCR	*CYP4CJ6*	F: CATGGAAACTTGCGATGATG	109
		R: ACGTATGATCGGCCTGAAAG	
	18S rRNA	F: CGGCTACCACATCCAAGGAA	128
		R: GCTGGAATTACCGCGGCT	
	Actin	F: CCGAAAAGCTGTCATAATGAAGACCGAAGACC	231
		R: GGTGAAACCTTGTCTACTGTTACATCTTG	
CYP4CJ6-UTR	3′ *UTR*	F: TCTAGACGGCACTGTAGAGGGGCACATCATT	150
		R: CTCGAGGCCCATTATTGTTGTCTTTTATTAC	

Note: F: forward primer, R: reverse primer; the sequence of the T7 promotor is represented by small letters; the restriction sites are represented by red letters.

To process the dsRNA feeding experiments, the rearing method and the artificial diet used were mentioned previously (Gong *et al*., 2014; Zhang *et al*., 2019) with minor modifications. ds*CYP4CJ6* was added to 0.5 mol l^{-1} sterile sucrose solution (the artificial diet) at a final concentration of 50 ng μl^{-1}. ds*GFP* was also mixed into 0.5 mol l^{-1} sterile sucrose solution (the artificial diet) at a final concentration of 50 ng μl^{-1} as the control. The experiments were performed in three independent biological replicates. To investigate the silencing efficiency of *CYP4CJ6*, aphids were collected at 24, 48, and 72 h postfeeding for qPCR.

The susceptibility of aphids to insecticides was investigated after silencing *CYP4CJ6*. For the ds*RNA* feeding assays, fifty healthy apterous aphids of the imidacloprid-resistant strain fed ds*CYP4CJ6* with the artificial diet for 24 h were transferred to the artificial diet containing imidacloprid (10 $\mu g\ ml^{-1}$) or thiamethoxam (5 $\mu g\ ml^{-1}$). ds*GFP* was added to the artificial diet as the control. Aphid mortality was recorded after insecticide treatments for 24 h. The experiments were performed in three independent biological replicates.

miRNA target studies of CYP4CJ6

A 150-bp fragment of the 3′ UTR of *CYP4CJ6* containing a predicted target site of miR-316 was inserted into the pmirGLO vector (Promega, Madison, USA) downstream of the luciferase gene with Xba I and Xho I restriction sites, generating the pmirGLO-*CYP4CJ6*-UTR target construct. The mutated miR-316 target DNA sequence was synthesized by GenePharm Co., Ltd. (Shanghai, China) and inserted into the pmirGLO vector to generate the pmir GLO-*CYP4CJ6*-Mut target construct.

293T cells (a human renal epithelial cell line transfected with the adenovirus E1A gene) presented by the Institute of Microbiology Chinese Academy of Sciences were cultured in a 96-well plate and transfected with target plasmids and an miRNA agomir (a dsRNA formed with the miRNA and its complimentary sequence) of each miRNA or agomir-NC using the Calcium Phosphate Cell Transfection Kit (Beyotime, Nanjing, China) according to the manufacturer's instructions. Each well contained 0.5 mg of the plasmid with a final concentration of miRNA agomir of 150 nmol l^{-1}. Luciferase assays (performed in the same manner as the promoter assays) were conducted at 24 h posttransfection. The normalized firefly luciferase activity (firefly luciferase activity/Renilla luciferase activity) was compared to that of the control pmirGLO vector. The mean of the relative luciferase expression ratio (firefly luciferase/Renilla luciferase) of the control was set to 1.0. For each transfection, the luciferase activity was averaged from three replicates.

miRNA feeding and subsequent impact on the expression of CYP4CJ6

An antagomir (inhibitor) and an agomir (mimics) of miR-316 synthesized by GenePharma (Shanghai, China) were used to feed aphids of imidacloprid-resistant strains. The antagomir and

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

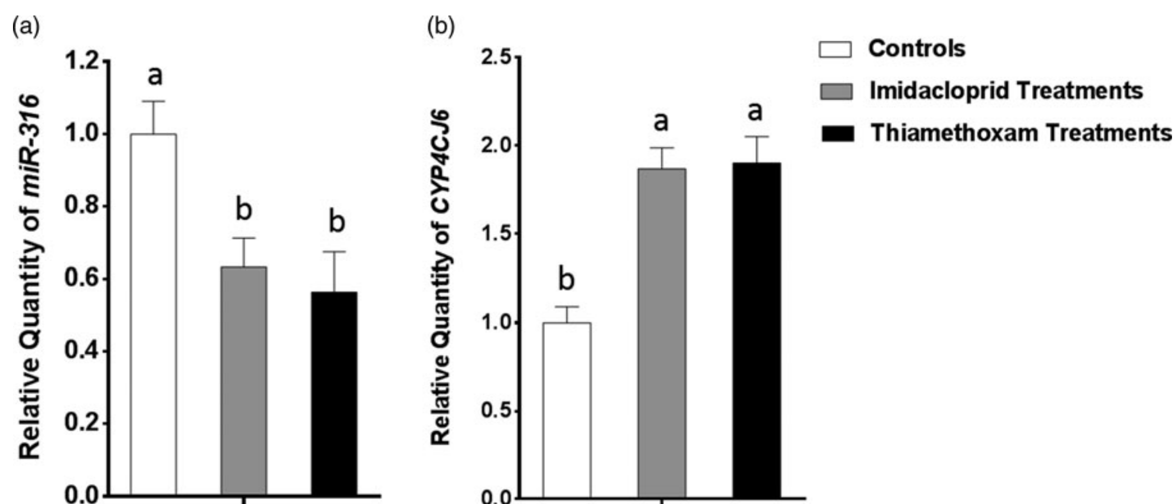


Figure 1. Relative expression of miR-316 and *CYP4CJ6* in CWA fed imidacloprid and thiamethoxam. (A) Relative expression of *miR-316* in CWA fed 10 μg ml^{-1} imidacloprid and 5 μg ml^{-1} thiamethoxam for 24 h. (B) Relative expression of *CYP4CJ6* in *CWA* when fed 10 μg ml^{-1} imidacloprid and 5 μg ml^{-1} thiamethoxam for 24 h. The bars represented by different letters (a, b) indicate significant differences between the treatments and the controls ($P < 0.05$).

agomir of miR-316 were added to the artificial diet at a final concentration of 2.5 mmol l^{-1}. For the control, an artificial diet containing a negative control (NC antagomir or NC agomir) was adopted in the experiments. The rearing method and artificial diet were the same as described above. Fifty healthy apterous aphids of the imidacloprid-resistant strain were fed the artificial diet for 24 h. The experiments required three independent repetitions. Then, the aphids were collected to extract their RNA. The expression of *CYP4CJ6* was investigated by qPCR.

Statistical analyses

The data were analyzed by an unpaired t-test using the GraphPad InStat 3.0 software (GraphPad Software, San Diego, CA, USA).

Results

Cloning, sequence analysis and induction expression of CYP4CJ6 *by imidacloprid and thiamethoxam*

Transcriptome analysis and differential gene expression (DGE) profiling of CWA (imidacloprid-exposed library and unexposed library) were performed under imidacloprid exposure, the transcriptome clean reads and computationally assembled sequences were submitted to the NCBI/SRA database, under accession number: SRX374716. And the results showed that the expression of *CYP4C1*-like genes (renamed *CYP4CJ6*) could be induced by imidacloprid, which could be related to the resistance of CWA to imidacloprid. In addition, all reads from the 2 sRNA libraries (imidacloprid-exposed library and unexposed library) were submitted to the NCBI SRA database (Accessions No: SRP309979 or PRJNA708304). The significantly downregulated expression of miR-316 under imidacloprid exposure indicated that miR-316 could be involved in imidacloprid resistance.

The hypothetical miRNA target sequences within the UTRs of transcripts from *S. miscanthi* including the targets of the 77 differentially expressed miRNAs between controls and imidacloprid treatments were predicted (Zhang *et al.*, 2021), which was conducted by the Miranda, RNAhybrid, and Target Scan programs (Enright *et al.*, 2003; Rehmsmeier *et al.*, 2004; Betel *et al.*, 2008). The results indicated that miR-316 could post-transcriptionally regulate expression of *CYP4CJ6*, and negative regulation of miR-316 and *CYP4CJ6* was also observed under imidacloprid exposure.

To further explore the function of this P450 gene, the full-length sequence was obtained by the 3′/5′-RACE technique and named *CYP4CJ6*. It consists of a 327-bp 5′ UTR, a 1533-bp open reading frame (ORF) coding for 511 amino acid residues and a 283-bp 3′ UTR. The nucleotide sequence of *CYP4CJ6* in CWA has been submitted to NCBI, and the accession number is MT975435.

To further understand the effects of insecticides on the expression of miR-316 and *CYP4CJ6*, aphids from the SA-R strain were exposed to both imidacloprid (10 μg ml^{-1}) and thiamethoxam (5 μg ml^{-1}) for 24 h. The qRT-PCR results showed that the expression of miR-316 was significantly decreased after insecticide exposure (fig. 1A), whereas the expression of *CYP4CJ6* was significantly increased (fig. 1B). These data suggested that the expression levels of *CYP4CJ6* and miR-316 were negatively correlated under exposure to both imidacloprid and thiamethoxam for 24 h.

Increased susceptibility to insecticides after silencing CYP4CJ6 *in the aphids*

To examine the efficiency of *CYP4CJ6* knockdown, qRT-PCR analysis revealed the time-dependent suppression of RNAi (ds*CYP4CJ6*), as shown in fig. 2a. The expression of *CYP4CJ6* was significantly reduced in ds*CYP4CJ6*-fed aphids by 52.5, 45.3, and 51.5% compared with controls (ds*GFP*-fed aphids) at 24, 48, and 72 h after feeding ds*RNA*. The efficiency of *CYP4CJ6* knockdown was relatively stable from 24 to 72 h. The actual mortality was significantly higher in ds*CYP4CJ6*-fed aphids (59.7 and 62.8%, respectively) than in controls (ds*GFP*-fed aphids) (23.5 and 30.6%, respectively) after RNAi for 24 h, and then aphids were exposed to imidacloprid (10 μg ml^{-1}) or thiamethoxam (5 μg ml^{-1}) for 24 h, as shown in fig. 2b.

Modulation of miRNA impacts on the susceptibility of S. miscanthi *to imidacloprid*

The expression of miR-316 was significantly lower in *S. miscanthi* adults fed an artificial diet containing the corresponding miR-316

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

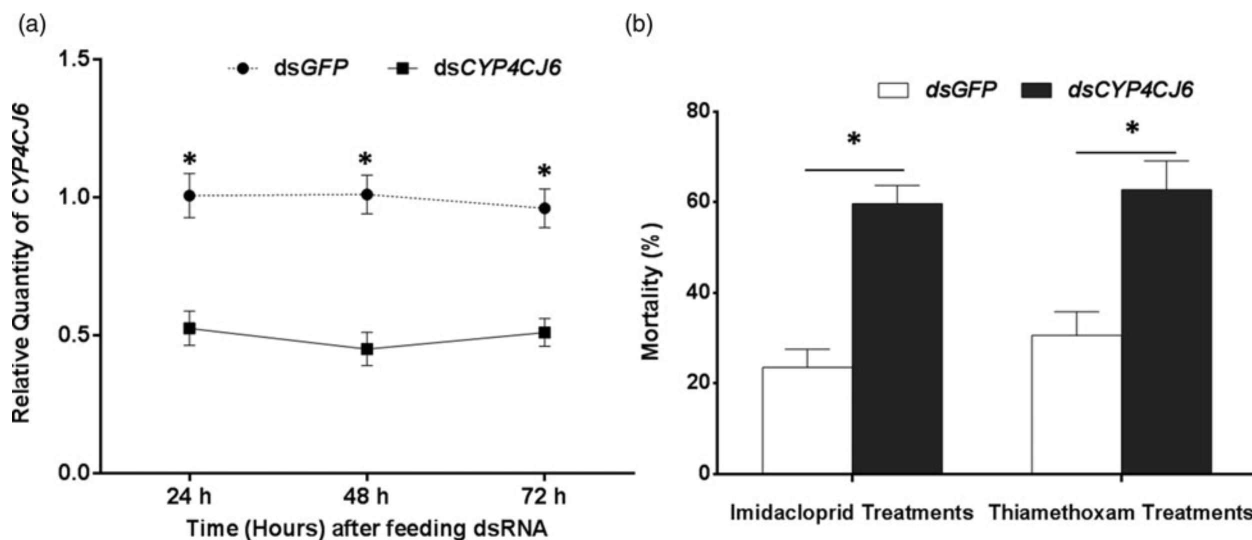


Figure 2. Relative expression of *CYP4CJ6* and the mortality rate of CWA fed 10 μg ml^{-1} imidacloprid and 5 μg ml^{-1} thiamethoxam *in vivo*. (a) Relative expression of *CYP4CJ6*. (b) Mortality rate (%) of CWA fed 10 μg ml^{-1} imidacloprid and 5 μg ml^{-1} thiamethoxam. The results are shown as the means ± SE for three independent biological replicates. The significant differences between the treatment and control are represented by asterisks (*). (Student's *t*-test, $P < 0.05$).

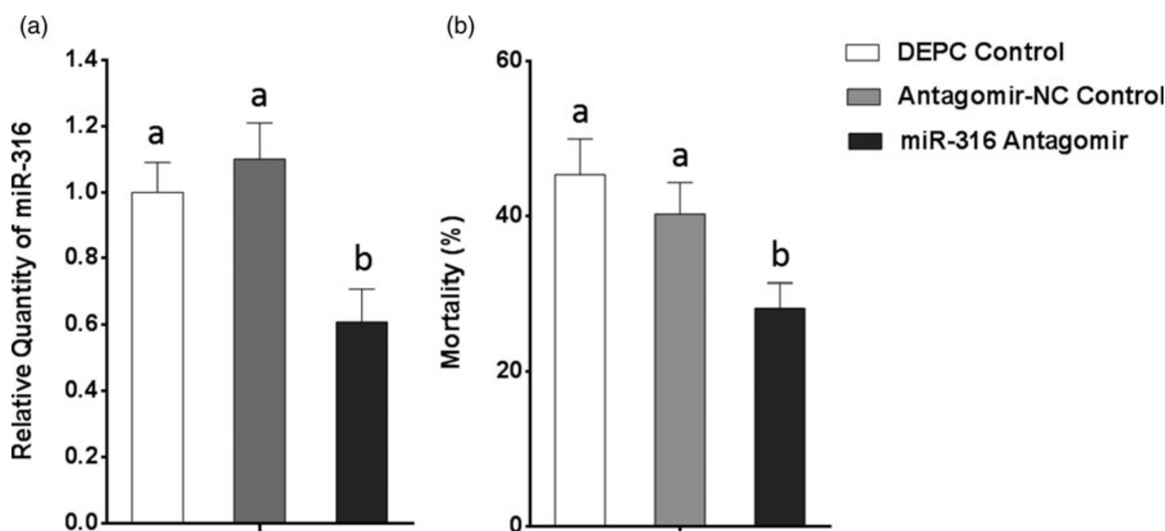


Figure 3. Effects of miR-316 modulation on imidacloprid susceptibility. (A) Orally mediated inhibition of miR-316. (B) The effects of modulating the levels of miR-316 using a feeding antagomir on imidacloprid tolerance in *S. miscanthi*. Mean mortality ± SE. Error bars indicate 95% confidence intervals. Different letters on the bars of the histogram indicate significant differences based on ANOVA followed by Tukey's HSD multiple comparison test ($P < 0.05$).

antagomir than in adults fed a DEPC water control or the NC control, and the depression efficiency of miR-316 reached 39.1% (fig. 3A). Under imidacloprid exposure, the mortality decreased by 21.73% in the aphids fed the miR-316 antagomir compared with those in the controls (fig. 3B).

Expression levels of miR-316 and CYP4CJ6 *in the two strains of CWA*

To further identify the participation of miR-316 and *CYP4CJ6* in imidacloprid resistance, the expression of *CYP4CJ6* in the SA-R and SA-S strains was investigated by qPCR. The data showed that the expression level of miR-316 was significantly decreased in the SA-R strain compared to the SA-S strain (with a percentage of 69.8%), while the expression level of *CYP4CJ6* was significantly increased in the SA-R strain compared to the SA-S strain (with a percentage of 323.0%) (fig. 4). Thus, the expression of *CYP4CJ6* and miR-316 was significantly negatively correlated.

miR-316 regulates the expression of CYP4CJ6

With 3 software programs, miRanda (Betel *et al.*, 2008) and RNAhybrid (Rehmsmeier *et al.*, 2004), and TargetScan (Enright *et al.*, 2003) a binding site of miR-316 was predicted in the 3′ UTR of *CYP4CJ6* (fig. 5A). To determine whether miR-316 could regulate the expression of *CYP4CJ6*, the 3′ UTRs of *CYP4CJ6* carrying the binding site of miR-316 were cloned into a pmirGLO vector to obtain the pmirGLO-miR-316-Target. Under cotransfection of the pmirGLO-miR-316-target with miR-316 agomir, the luciferase activities were significantly lower

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

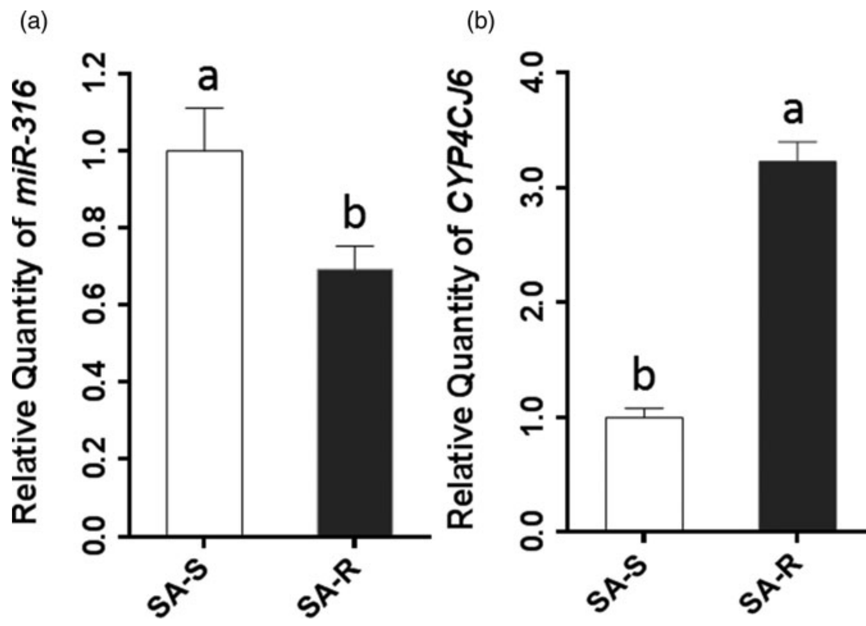
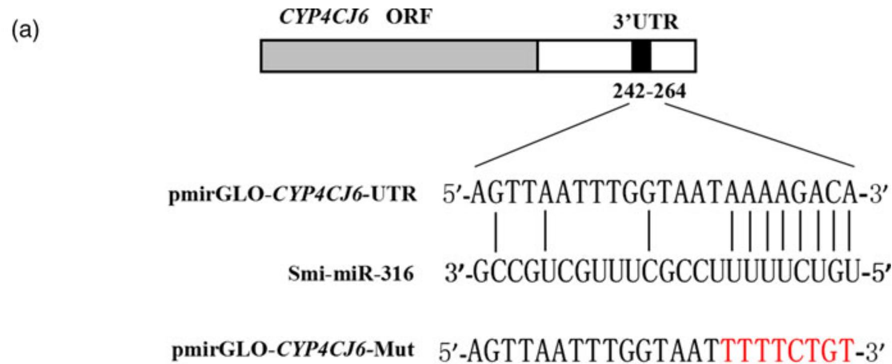


Figure 4. Relative expression of miR-316 and CY*P4CJ6 in vivo* in CWA. The results are shown as means ± SE for three independent biological replicates. (A) miR-316 expression; (B) *CYP4CJ6* expression. The bars represented by different letters indicate significant differences between treatment and control (Student's *t*-test, $P < 0.05$).



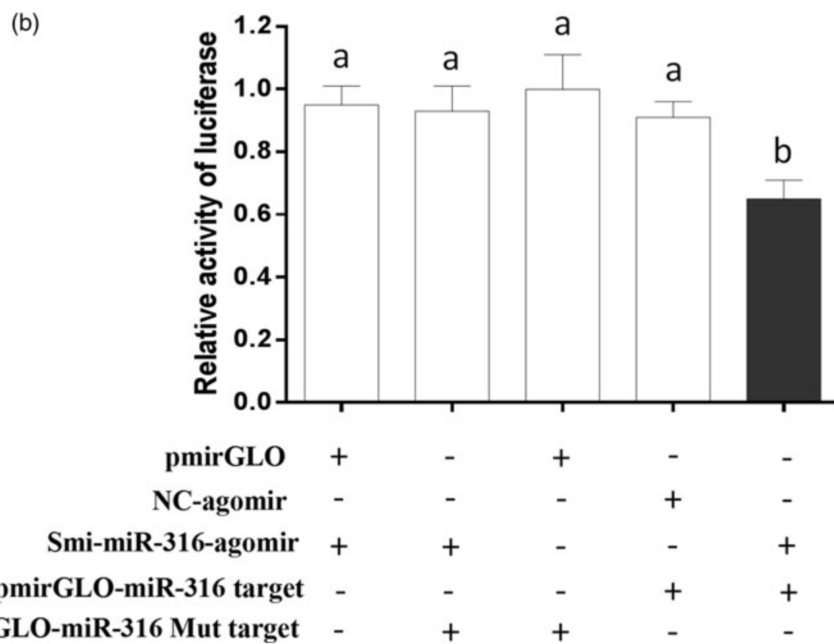


Figure 5. Regulation of *CYP4CJ6* by miR-316 by dual luciferase reporter assay. (A) The binding sites of miR-316 predicted by software in the 3′ UTRs of *CYP4CJ6*. (B) Luciferase reporter assays performed by cotransfection of the miR-316 agomir with a luciferase reporter gene linked to the 3′ UTR of *CYP4CJ6*. The mathematical symbols '+' and '–' are used to indicate that a component was added or not added. The bars represented by different letters indicate significant differences based on ANOVA followed by Tukey's HSD multiple comparison test ($P < 0.05$).

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

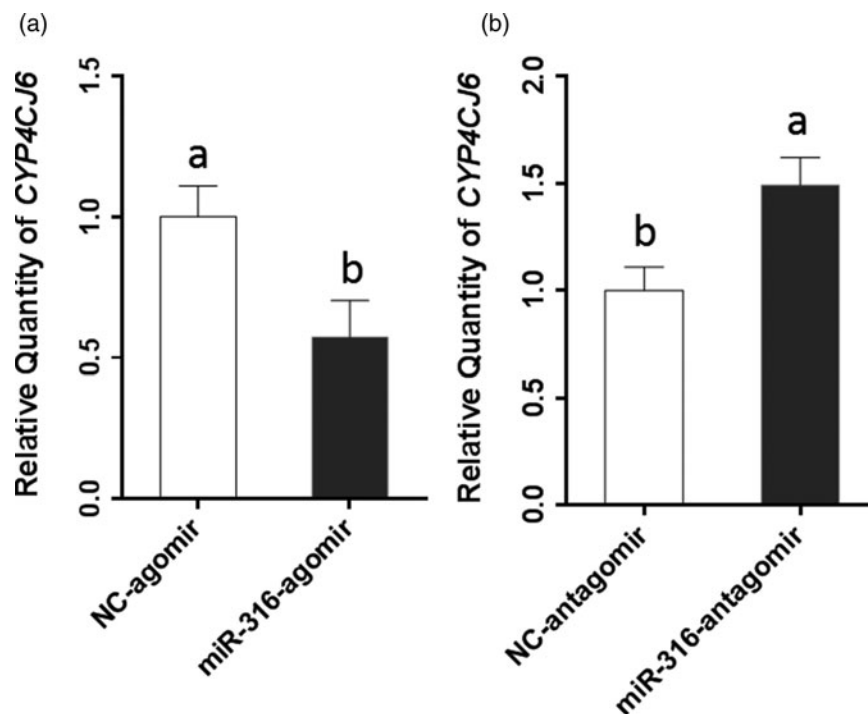


Figure 6. Relative expression of *CYP4CJ6* regulated by miR-316 *in vitro*. The results are presented as the mean ± SD of three biological independent replicates. (A) The effects of the miR-316 agomir on *CYP4CJ6* expression; (B) The effects of the miR-316 antagomir on *CYP4CJ6* expression. The bars represented by different letters (a, b) indicate significant differences between the treatments and the controls (Student's *t*-test, $P < 0.05$).

than those of the negative agomir control. However, cotransfection of the target mutated plasmid (pmirGLO-CYP4CJ6-Mut) with the miR-316 agomir did not significantly reduce the relative luciferase activity (fig. 5B).

To validate whether the expression of *CYP4CJ6* is regulated by miR-316 *in vivo*, *S. miscanthi* were fed an artificial diet containing agomir or antagomir of miR-316 for 24 h. Subsequently, the expression levels of *CYP4CJ6* were examined via qRT-PCR. Compared to that in the control group, the expression of *CYP4CJ6* in the group fed miR-316 agomir decreased by 42.7% (fig. 6A), while the expression of *CYP4CJ6* increased by 48.2% in the group fed miR-316 antagomir (fig. 6B).

Discussion

Imidacloprid and thiamethoxam are neonicotinoid insecticides that can act on insect nicotinic acetylcholine receptors (nAChRs) and have many advantages, such as high efficiency, long duration, broad spectrum, low toxicity to mammals and high control efficacy in planthoppers and aphids (Buckingham *et al.*, 1997; Roat *et al.*, 2020). In addition, it has been also reported that lethal toxicity of imidacloprid and thiamethoxam was observed in *Anagrus nilaparvatae*, *Trichogramma cacoeciae*, *Gonatocerus ashmeadi* (Hymenoptera: Mymanidae), *Apis mellifera* (Hymenoptera: Apidae) and so on (Schuld and Schmuck, 2000; Byrne and Toscano, 2007; Wang *et al.*, 2008; Yang *et al.*, 2012; Tavares *et al.*, 2015). However, the resistance of insects to neonicotinoids is becoming increasingly serious due to the frequency and scope of the application of neonicotinoid insecticides (Liu *et al.*, 2003; Chen *et al.*, 2020).

The substantially increased expression of P450 genes plays a partial role in insecticide resistance (Li *et al.*, 2007; Puinean *et al.*, 2010; Schuler, 2011, 2012; Liu *et al.*, 2015; Zhang *et al.*, 2020). In *Diaphorina citri* (Homoptera: Psyllidae), increased expression of *CYP303A1*, *CYP4C62*, and *CYP6BD5* participated in the detoxification of imidacloprid (Tian *et al.*, 2019). Insecticide resistance can result from the interactions between insecticides and inducible regulatory mechanisms (Goff *et al.*, 2006; Zhang *et al.*, 2016*a*, 2016*b*). Furthermore, another common characteristic of P450s is inducibility, which has been proven in insects (Harrison *et al.*, 2001; Hu *et al.*, 2014; Zhang *et al.*, 2019). In the present study, we also found that expression of *CYP4CJ6* can be significantly enhanced by imidacloprid or thiamethoxam treatments compared to the controls, suggesting that *CYP4CJ6* could be involved in the response of *S. miscanthi* to these two insecticides.

The overexpression of the P450 gene is the key factor in insecticide resistance in insects (Li *et al.*, 2007; Puinean *et al.*, 2010; Liu *et al.*, 2015). To further confirm that *CYP4CJ6* acts on imidacloprid resistance, the expression of *CYP4CJ6* was investigated in imidacloprid-resistant and imidacloprid-susceptible strains (apterous aphids). Our qPCR data indicated that *CYP4CJ6* was significantly overexpressed in the imidacloprid-resistant strain, and the expression of *CYP4CJ6* could be induced significantly by both imidacloprid and thiamethoxam. Similarly, the overexpression of 11 P450 genes was found in a laboratory-selected imidacloprid-resistant strain of CWA and was induced significantly by imidacloprid (Zhang *et al.*, 2020). Further RNAi experiments by feeding assays indicated that silencing the expression of *CYP4CJ6* could significantly increase the susceptibility to imidacloprid and thiamethoxam in the resistant aphids of CWA; the RNAi method in aphids has also been studied in other reports (Peng *et al.*, 2016; Ma *et al.*, 2019*a*, 2019*b*; Zhang *et al.*, 2020, 2021). So, *CYP4CJ6* in CWA could be significantly induced by imidacloprid and thiamethoxam, and the overexpression of *CYP4CJ6* in the imidacloprid-resistant strain played a crucial role in the resistance of imidacloprid and thiamethoxam in our current study. The regulation of P450 genes involves transcriptional and posttranscriptional regulation mechanisms, such as regulation by insecticide DNA methylation and posttranscriptional

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

suppression by miRNAs (Tamasi *et al.*, 2011). However, previous studies have tended to explore the transcriptional mechanism of P450 genes, while few have simultaneously clarified their findings at the posttranscriptional level.

To further explore the molecular genetic mechanism of *CYP4CJ6*, upregulated expression of *CYP4CJ6* by miR-316 was investigated at the posttranscriptional level. miRNAs, as small noncoding RNAs, can act on the expression of target genes at the posttranscriptional level (Pritchard *et al.*, 2012; Asgari, 2013). It has been reported that miRNAs are involved in the regulation of P450 gene expression. The expression of *CYP1A1* in humans was observed to be suppressed by miR-892a (Choi *et al.*, 2012). In *Aphis gossypii*, *CYP4CJ1* could be regulated by miR-4133-3p (Ma *et al.*, 2019*a*, 2019*b*). Moreover, in *C. pipiens*, miRNAs could participate in insecticide resistance by regulating the gene expression of P450 (Hong *et al.*, 2014; Lei *et al.*, 2015).

Similarly, in our previous study on the differential expression of miRNAs under imidacloprid exposure for 24 h of *Sitobion miscanthi*, we found that 77 miRNAs were significantly differentially expressed under imidacloprid exposure based on high-throughput sequencing of short RNA libraries, suggesting that differentially expressed miRNAs (including miR-316) may participate in the resistance mechanism of *S. miscanthi* to insecticides (Zhang *et al.*, 2021). At the same time, the expression of *CYP4CJ6* could be regulated by miR-316 acting on the 3′ UTRs by feeding miR-316 antagomir/agomir *in vitro* and dual luciferase reporter assay methods. These observations indicated that miR-316 could play a role in the resistance of CWA by regulating the expression of *CYP4CJ6* to insecticides. A similar report showed that one cytochrome P450 and two glutathione S-transferase genes could be recognized by mse-miR-316 in *Manduca sexta* (Zhang *et al.*, 2015). There were other reports that regulation of the Wnt pathway by miR-316 is involved in caste differentiation within social insect groups (Shi *et al.*, 2015). Furthermore, miR-316 was also identified from winged and wingless morphs of *S. avenae*, and dietary uptake of miR-316 agomirs and antagomirs led to significantly higher mortality.

In conclusion, *CYP4CJ6* overexpression was related to imidacloprid resistance, as confirmed by RNAi feeding experiments. Enhancement of *CYP4CJ6* expression was observed when the aphids were exposed to different insecticides, suggesting the hypothesis that it could be involved in the metabolism of the insecticides. Furthermore, *CYP4CJ6* was verified to be regulated posttranscriptionally by miR-316.

Financial support. This work is supported by the Key Science and Technology Program of Henan (Agriculture) (212102110441), the Key Scientific Projects of Institutions of Henan (21A210008), the Project of Plant Protection Key Discipline of Henan Province (1070202190011005), and the Graduate Education Innovation Training Base Project of Henan Province in 2021 (107020221005).

Author contributions.

X. C. and B. Z. conceived and designed the experiments. L. L., and G. H. performed the experiments. L. L., W. D, Y. L., and B. Z. analyzed the data and wrote the manuscript. X. S., and G. H. participated in the data analysis. All authors reviewed the manuscript.

Conflict of interest. The authors declare that they have no competing interests.

References

Asgari S (2013) MicroRNA functions in insects. *Insect Biochemistry and Molecular Biology* **43**, 388–397.

Betel D, Wilson M, Gabow A, Marks DS and Sander C (2008) The microRNA.org resource: targets and expression. *Nucleic Acids Research* **36**, 149–153.

Brandt A, Scharf M, Pedra JHF, Holmes G, Dean A, Kreitman M and Pittendrigh BR (2002) Differential expression and induction of two Drosophila cytochrome P450 genes near the Rst (2) DDT locus. *Insect Molecular Biology* **11**, 337–341.

Broackes-Carter FC, Nathalie M, Deborah G, Stephen H, John B and Ann H (2002) Temporal regulation of CFTR expression during ovine lung development: implications for cf gene therapy. *Human Molecular Genetics* **2**, 125.

Buckingham S, Lapied B, Corronc H and Sattelle F (1997) Imidacloprid actions on insect neuronal acetylcholine receptors. *Journal of Experimental Biology* **200**, 2685–2692.

Byrne FJ and Toscano NC (2007) Lethal toxicity of systemic residues of imidacloprid against Homalodisca vitripennis (Homoptera: Cicadellidae) eggs and its parasitoid *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae). *Biological Control* **43**, 130–135.

Chen X, Xia X, Wang H, Qiao K and Wang K (2013) Cross–resistance to clothianidin and acetamiprid in the imidacloprid resistant strain of *Aphis gossypii* (Hemiptera: Aphididae) and the related enzyme mechanisms. *Acta Entomologica Sinica* **56**, 1143–1151.

Chen XD, Ebert TA, Pelz-Stelinski KS and Stelinski LL (2020) Fitness costs associated with thiamethoxam and imidacloprid resistance in three field populations of *Diaphorina citri* (Hemiptera: Liviidae) from Florida. *Bulletin of Entomological Research* **110**, 512–520.

Choi YM, An S, Lee EM, Kim K, Choi SJ, Kim JS, Jang HH, An IS and Bae S (2012) *CYP1A1* is a target of miR-892a-mediated post-transcriptional repression. *International Journal of Oncology* **41**, 331–336.

Cui L, Sun L, Yang D, Yan X and Yuan H (2012) Effects of cycloxaprid, a novel cis–nitromethylene neonicotinoid insecticide, on the feeding behaviour of *Sitobion avenae*. *Pest Management Science* **68**, 1484–1491.

Devine GJ, Harling ZK, Scarr AW and Devonshire AL (1996) Lethal and sublethal effects of imidacloprid on nicotine–tolerant *Myzus nicotianae* and *Myzus persicae*. *Pesticide Science* **48**, 57–62.

Duan X, Peng X, Qiao X and Chen M (2016) Life cycle and population genetics of bird cherry–oat aphids *Rhopalosiphum padi* in China: an important pest on wheat crops. *Journal of Pest Science* **90**, 1–14.

Enright AJ, John B, Gaul U, Tuschl T, Sander C and Marks DS (2003) MicroRNA targets in Drosophila. *Genome Biology* **5**, R1.

George KS and Gair R (2010) Crop loss assessment on winter wheat attacked by the grain aphid, *Sitobion avenae* (F.). *Plant Pathology* **28**, 143–149.

Goff GL, Hilliou F, Siegfried BD, Boundy S, Wajnberg E, Sofer L, Audant P, ffrench-Constant RH and Feyereisen R (2006) Xenobiotic response in *Drosophila melanogaster*: sex dependence of P450 and GST gene induction. *Insect Biochemistry and Molecular Biology* **36**, 674–682.

Gong YH, Yu XR, Shang QL, Shi XY and Gao XW (2014) Oral delivery mediated RNA interference of a carboxylesterase gene results in reduced resistance to organophosphorus insecticides in the cotton aphid, *Aphis gossypii* glover. *PLoS One* **9**, e102823.

Harrison TL, Zangerl AR, Schuler MA and Berenbaum MR (2001) Developmental variation in cytochrome P450 expression in *Papilio polyxenes* in response to xanthotoxin, a hostplant allelochemical. *Archives of Insect Biochemistry and Physiology* **48**, 179–189.

Hirata K, Kiyota R, Matsuura A, Toda S, Yamamoto A and Iwasa T (2015) Association between the R81T mutation in the nicotinic acetylcholine receptor β1 subunit of *Aphis gossypii* and the differential resistance to acetamiprid and imidacloprid. *Journal of Pesticide Science* **41**, 25–31.

Hirata K, Jouraku A, Kuwazaki S, Kanazawa J and Iwasa T (2017) The R81T mutation in the nicotinic acetylcholine receptor of *Aphis gossypii* is associated with neonicotinoid insecticide resistance with differential effects for cyano-and nitro-substituted neonicotinoids. *Pesticide Biochemistry and Physiology* **143**, 57–65.

Hong S, Guo Q, Wang W, Hu S, Fang F, Lv Y, Yu J, Zou F, Lei Z, Ma K, Ma L, Zhou D, Sun Y, Zhang D, Shen B and Zhu C (2014) Identification of differentially expressed microRNAs in *Culex pipiens* and their potential roles in pyrethroid resistance. *Insect Biochemistry and Molecular Biology* **55**, 39–50.

Hu Z, Lin Q, Chen H, Li Z, Yin F and Feng X (2014) Identification of a novel cytochrome P450 gene, *CYP*321*E*1 from the diamondback moth, *Plutella xylostella* (L.) and RNA interference to evaluate its role in chlorantraniliprole resistance. *Bulletin of Entomological Research* **104**, 716–723.

Hu XS, Liu XF, Thieme T, Zhang GS, Liu TX and Zhao HY (2015) Testing the fecundity advantage hypothesis with *Sitobion avenae*, *Rhopalosiphum padi*, and *Schizaphis graminum* (hemiptera: aphididae) feeding on ten wheat accessions. *Scientific Report* **5**, 18549.

Jiang X, Zhang Q, Qin Y, Yin H, Zhang S, Li Q, Zhang Y, Fan J and Chen J (2019) A chromosome-level draft genome of the grain aphid *Sitobion miscanthi*. *Gigascience* **8**, giz101.

Jones CM, Daniels M, Andrews M, Slater R, Lind RJ, Gorman K and Denholm I (2011) Age-specific expression of a P450 monooxygenase (*CYP*6*CM*1) correlates with neonicotinoid resistance in *Bemisia tabaci*. *Pesticide Biochemistry and Physiology* **101**, 53–58.

Karunker I, Benting J, Lueke B, Ponge T, Nauen R, Roditakis E and Morin S (2008) Over-expression of cytochrome P450 *CYP6CM*1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera:Aleyrodidae). *Insect Biochemistry and Molecular Biology* **38**, 634–644.

Kim JI, Kwon M, Ki GH, Kim SY and Lee SH (2015) Two mutations in nAChR beta subunit is associated with imidacloprid resistance in the *Aphis gossypii*. *Journal of Asia–Pacific Entomology* **18**, 291–296.

Lei Z, Lv Y, Wang W, Guo Q, Zou F, Hu S, Fang F, Tian M, Liu B, Liu X, Ma K, Ma L, Zhou D, Zhang D, Sun Y, Shen B and Zhu C (2015) MiR-278-3p regulates pyrethroid resistance in *Culex pipiens* pallens. *Parasitology Research* **114**, 699–706.

Li XC, Schuler MA and Berenbaum MR (2007) Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology* **52**, 231–253.

Li Y, Xu Z, Shi L, Shen G and He L (2016) Insecticide resistance monitoring and metabolic mechanism study of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in Chongqing, China. *Pesticide Biochemistry and Physiology* **132**, 21–28.

Liu ZW, Han ZJ, Wang Y, Zhang L, Zhang H and Liu C (2003) Selection for imidacloprid resistance in *Nilaparvata lugens*: cross–resistance patterns and possible mechanisms. *Pest Management Science* **59**, 770–775.

Liu N, Li M, Gong Y, Liu F and Li T (2015) Cytochrome P450s-their expression, regulation, and role in insecticide resistance. *Pesticide Biochemistry and Physiology* **120**, 77–81.

Lu Y and Gao X (2009) Multiple mechanisms responsible for differential susceptibilities of *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) to pirimicarb. *Bulletin of Entomological Research* **99**, 611–617.

Ma K, Li F, Liu Y, Liang P, Chen X and Gao X (2017) Identification of microRNAs and their response to the stress of plant allelochemicals in *Aphis gossypii* (Hemiptera: Aphididae). *BMC Molecular Biology* **18**, 5.

Ma K, Li F, Tang Q, Liang P, Liu Y, Zhang B and Gao X (2019*a*) *CYP4CJ1*-mediated gossypol and tannic acid tolerance in *Aphis gossypii* glover. *Chemosphere* **219**, 961–970.

Ma K, Tang Q, Zhang B, Liang P, Wang B and Gao X (2019*b*) Overexpression of multiple cytochrome P450 genes associated with sulfoxaflor resistance in *Aphis gossypii* Glover. *Pesticide Biochemistry and Physiology* **157**, 204–210.

Mezei I, Bielza P, Siebert MW, Torne M, Gomez LE, Valverde-Garcia P, Belando A, Moreno I, Grávalos C, Cifuentes D and Sparks TC (2020) Sulfoxaflor efficacy in the laboratory against imidacloprid-resistant and susceptible populations of the green peach aphid, *Myzus persicae*: impact of the R81T mutation in the nicotinic acetylcholine receptor. *Pesticide Biochemistry and Physiology* **166**, 104582.

Mohammed BR, Wilding CS, Collier PJ and Deeni YY (2014) Cloning of *Anopheles gambiae CYP6M*2 gene promoter and construction of its luciferase reporter system. *International Journal of Energy Research* **3**, 259–264.

Peng TF, Pan YO, Gao XW, Xi JH, Zhang L, Ma KS, Wu Y, Zhang J and Shang QL (2016) Reduced abundance of the CYP6CY3-targeting let-7 and miR-100 miRNAs accounts for host adaptation of *Myzus persicae* nicotianae. *Insect Biochemistry and Molecular Biology* **75**, 89–97.

Pfaffl MW (2001) A new mathematical model for relative quantification in real–time RT–PCR. *Nucleic Acids Research* **29**, 45–45.

Pritchard CC, Cheng HH and Tewari M (2012) MicroRNA profifiling: approaches and considerations. *Nature Reviews Genetics* **13**, 358–369.

Puinean AM, Foster SP, Oliphant L, Denholm I, Field LM, Millar NS, Williamson MS and Bass C (2010) Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the *aphid Myzus persicae*. *PLoS Genetics* **6**, 1000999.

Rehmsmeier M, Steffen P, Hochsmann M and Giegerich R (2004) Fast and effective prediction of microRNA/target duplexes. *RNA* **10**, 1507–1517.

Roat TC, Santos-Pinto JRAP, Miotelo L, de Souza CL, Palma MS and Malaspina O (2020) Using a toxicoproteomic approach to investigate the effects of thiamethoxam into the brain of *Apis mellifera*. *Chemosphere* **258**, 127362.

Schuld M and Schmuck R (2000) Effects of thiacloprid, a new chloronicotinyl insecticide, on the egg parasitoid *Trichogramma cacoeciae*. *Ecotoxicology* **9**, 197–205.

Schuler MA (2011) P450s in plant-insect interactions. *BBA-Proteins Proteom* **1814**, 36–45.

Schuler MA (2012) Insect P450s: mounted for battle in their war against toxins. *Molecular Ecology* **21**, 4157–4159.

Shi YY, Zheng HJ, Pan QZ, Wang ZL and Zeng ZJ (2015) Differentially expressed microRNAs between queen and worker larvae of the honey bee (*Apis mellifera*). *Apidologi* **46**, 35–45.

Sial MU, Zhao Z, Zhang L, Zhang Y, Mao L and Jiang H (2020) Loop-mediated isothermal amplification for the detection of R81T mutation in nAChR with crude genomic DNA extracted from individual *Myzus persicae*. *Journal of Pest Science* **93**, 531–541.

Tamasi V, Monostory K, Prough RA and Falus A (2011) Role of xenobiotic metabolism in cancer: involvement of transcriptional and miRNA regulation of P450s. *Cellular and Molecular Life Sciences* **68**, 1131–1146.

Tang QL, Ma KS, Hou YM and Gao XW (2017) Monitoring insecticide resistance and diagnostics of resistance mechanisms in the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in China. *Pesticide Biochemistry and Physiology* **143**, 39–47.

Tavares DA, Roat TC, Carvalho SM, Silva-Zacarin ECM and Malaspina O (2015) In vitro effects of thiamethoxam on larvae of Africanized honey bee Apis mellifera (Hymenoptera: Apidae). *Chemosphere* **135**, 370–378.

Tian F, Li C, Wang Z, Liu J and Zeng X (2019) Identification of detoxification genes in imidacloprid-resistant Asian *Citrus psyllid* (Hemiptera: Lividae) and their expression patterns under stress of eight insecticides. *Pest Management Science* **75**, 1400–1410.

Wang MF, Yuan GH, Chen JL, Lei Z and Wu Z (2006) Research advances of occurrence pattern damage characteristics of wheat aphid and resistance identification of wheat. *Journal of Hennan Agricultural Sciences* **7**, 58–60.

Wang HY, Yang Y, Su JY, Shen JL, Gao CF and Zhu YC (2008) Assessment of the impact of insecticides on *Anagrus nilaparvatae* (Pang et Wang) (Hymenoptera: Mymanidae), an egg parasitoid of the rice planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). *Crop Protection* **27**, 514–522.

Wang G, Cui LL, Jie D, Francis F, Yong L and Tooker J (2011) Combining intercropping with semiochemical releases: optimization of alternative control of *Sitobion avenae* in wheat crops in China. *Entomologia Experimentalis Et Applicata* **140**, 189–195.

Wang NX, Watson GB, Loso MR and Sparks TC (2016) Molecular modeling of sulfoxaflor and neonicotinoid binding in insect nicotinic acetylcholine receptors: impact of the Myzus β1 R81T mutation. *Pest Management Science* **72**, 1467–1474.

Wang K, Zhang M, Huang Y, Yang Z, Su S and Chen M (2018) Characterisation of imidacloprid resistance in the bird cherry–oat aphid, *Rhopalosiphum padi*, a serious pest on wheat crops. *Pest Management Science* **74**, 1457–1465.

Xu L, Wu M and Han ZJ (2014) Biochemical and molecular characterisation and cross–resistance in field and laboratory chlorpyrifos–resistant strains of *Laodelphax striatellus* (Hemiptera: Delphacidae) from eastern China. *Pest Management Science* **70**, 1118–1129.

Yang EC, Chang HC, Wu WY and Chen YW (2012) Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. *PLoS One* **7**, e49472.

Zhang X, Zheng Y, Cao X, Ren R, Yu X and Jiang H (2015) Identification and profiling of *Manduca sexta* microRNAs and their possible roles in regulating specific transcripts in fat body, hemocytes, and midgut. *Insect Biochemistry and Molecular Biology* **62**, 11–22.

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press

Zhang BZ, Kong FC, Cui RK and Zeng XN (2016*a*) Gene expression of detoxification enzymes in insecticide-resistant and insecticide-susceptible *Bemisia tabaci* strains after diafenthiuron exposure. *Journal of Agricultural Science* **154**, 742–753.

Zhang B, Kong F, Wang H, Gao X, Zeng X and Shi X (2016*b*) Insecticide induction of o-demethylase activity and expression of cytochrome P450 genes in the red imported fire ant (*Solenopsis invicta* Buren). *Journal of Integrative Agriculture* **15**, 135–144.

Zhang BZ, Ma KS, Liu JJ, Lu LY, Chen XL, Zhang SP and Gao XW (2019) Differential expression of genes in greenbug (*Schizaphis graminum* Rondani) treated by imidacloprid and RNA interference. *Pest Management Science* **75**, 1726–1733.

Zhang B, Su X, Xie L, Zhen C, Hu G, Jiang K, Huang ZY, Liu R, Gao Y, Chen X and Gao X (2020) Multiple detoxification genes confer imidacloprid resistance to *Sitobion avenae* Fabricius. *Crop Protection* **128**, 105014.

Zhang B, Hu G, Lu L, Hu S, Li Y, Su X, Dong W, Zhen C, Liu R, Kong F, Shi M and Chen X (2021) Identification of differentially expressed micro-rnas under imidacloprid exposure in sitobion miscanthi. *Pesticide Biochemistry and Physiology* **177**, 104885.

Zhou H, Chen J, Liu Y, Frédéric F, Eric H, Claude B, Sun JR and Cheng D (2013) Influence of garlic intercropping or active emitted volatiles in releasers on aphid and related beneficial in wheat fields in China. *Journal of Integrative Agriculture* **12**, 467–473.

https://doi.org/10.1017/S0007485322000037 Published online by Cambridge University Press