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Voltage-gated sodium channel gene mutation and P450 gene expression are associated with the resistance of *Aphis spiraecola* Patch (Hemiptera: Aphididae) to lambda-cyhalothrin

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Research Paper

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Abstract

Aphis spiraecola Patch is one of the most economically important tree fruit pests worldwide. The pyrethroid insecticide lambda-cyhalothrin is commonly used to control *A. spiraecola*. In this 2-year study, we quantified the resistance level of *A. spiraecola* to lambda-cyhalothrin in different regions of the Shaanxi province, China. The results showed that *A. spiraecola* had reached extremely high resistance levels with a 174-fold resistance ratio (RR) found in the Xunyi region. In addition, we compared the enzymatic activity and expression level of P450 genes among eight *A. spiraecola* populations. The P450 activity of *A. spiraecola* was significantly increased in five regions (Xunyi, Liquan, Fengxiang, Luochuan, and Xinping) compared to susceptible strain (SS). The expression levels of *CYP6CY7*, *CYP6CY14*, *CYP6CY22, P4504C1-like*, *P4506a13*, *CYP4CZ1*, *CYP380C47,* and *CYP4CJ2* genes were significantly increased under lambda-cyhalothrin treatment and in the resistant field populations. A L1014F mutation in the sodium channel gene was found and the mutation rate was positively correlated with the LC_{50} of lambda-cyhalothrin. In conclusion, the levels of lambda-cyhalothrin resistance of *A. spiraecola* field populations were associated with P450s and L1014F mutations. Our combined findings provide evidence on the resistance mechanism of *A. spiraecola* to lambda-cyhalothrin and give a theoretical basis for rational and effective control of this pest species.

Introduction

Aphis spiraecola Patch (Hemiptera: Aphididae), which was mistakenly synonymized with *Aphis citricola* (van der Goot) (Eastop and Blackman, 1988), is a polyphagous aphid species that is distributed among temperate and tropical regions of the globe. In the last few decades, *A. spiraecola* has become a widely distributed pest of apple and citrus orchards (CABI, 2022). This invasive aphid causes the curling of young leaves, reduces the growth of infested shoots and excretes honeydew on leaves and fruit thus favouring the development of the fungal disease sooty mould, leading to economic losses (Mu *et al.*, 2002).

Currently, insecticides are commonly used for managing *A. spiraecola*. However, the extensive use of insecticides has led to the resistance of insects to multiple insecticide classes, including organophosphates, carbamates, and neonicotinoids (Bass *et al.*, 2014; Chen *et al.*, 2016; Margaritopoulos *et al.*, 2021). Lambda-cyhalothrin is a broad-spectrum, fast-acting type-II pyrethroid insecticide, which is widely used due to its relatively low toxicity to humans and high effectiveness in controlling insects (Soderlund and Bloomquist, 1989; Nasuti *et al.*, 2003). Resistance to pyrethroid insecticides has been found in some aphid species. For instance, *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover have been reported to have developed resistance to *β*-cypermethrin (Tang *et al.*, 2017; Wang *et al.*, 2021). On the other hand, *Rhopalosiphum padi* (L.) has been reported to possess resistance to lambda-cyhalothrin (Zuo *et al.*, 2016b; Wang *et al.*, 2018, 2020). The resistance levels of *A. spiraecola* to lambda-cyhalothrin have not been documented.

There are several mechanisms underlying the development of insect resistance to insecticides, and some species may exhibit more than one of these mechanisms simultaneously. Insect resistance to pesticides can be attributed to two major mechanisms: metabolic resistance and target site insensitivity (Liu, 2015; Yang and Zhang, 2015; Barres *et al.*, 2016). In many insect populations that have developed resistance to insecticides, the constitutive expression

levels of detoxification enzyme (e.g. Glutathione S-transferases, GSTs; carboxylesterases, CarEs; and Cytochrome P450 monooxygenases, P450s) genes were significantly increased. Hu *et al.* (2022b) found that the overexpression of the *CYP4CJ6* gene conferred resistance to imidacloprid and thiamethoxam in the Indian grain aphid, *Sitobion miscanthi* (Takahashi). Some P450 gene expression were significantly increased and associated with lambda-cyhalothrin resistance in *R. padi* (Wang *et al.*, 2020, 2022). In the chlorpyrifos-resistant strain of Laodelphax striatellus (Fallen), the expression of *LsCarE1* was found to be up-regulated by 32.06-fold (Zhang *et al.*, 2012). GSTs can mediate insecticide resistance through various mechanisms (Pavlidi *et al.*, 2018). In the codling moth, *Cydia pomonella* (L.), it was found that *GSTs* were overexpressed in the lambda-cyhalothrin resistant strain, and it was confirmed that the GSTs contribute to the resistance through the sequestration mechanism (Hu *et al.*, 2022a).

The α subunit of the insect sodium channel is composed of four structurally similar domains (I–IV), each containing six hydrophobic transmembrane helices (S1–S6) and a P-loop. The voltage sensing module is formed by segments S1–S4, while segments S5 and S6, along with the P-loop, form the sodium ion passage pore module (Dong *et al.*, 2014). Mutations associated with knockdown resistance (*kdr*) mostly occur in the S5 and S6 transmembrane helices of domain II in the sodium channel. For instance, one mutation involves the substitution of phenylalanine with leucine at position 1014 in the transmembrane segment IIS6 (*kdr*, L1014F, Martinez-Torres *et al.*, 1999). Another mutation involves the substitution of leucine with methionine at position 918 in the transmembrane segment IIS4-S5 (super-*kdr*, M918L, Eleftherianos *et al.*, 2008). There were multiple leucine substitutions at position 918 and other site-specific mutations may also be present in the sodium channel (Dong *et al.*, 2014). Mutations in the target site of the voltage-gated sodium channel (VGSC) gene are involved in aphid resistance to pyrethroids (Guillemaud *et al.*, 2003; Bass *et al.*, 2014; Scott, 2019). For instance, the M918 T mutation in the sodium channel of the English grain aphid, *Sitobion avenae* (Fabricius), played a significant role in conferring resistance to pyrethroids (Foster *et al.*, 2014). Similarly, the M918 T/L/V and L1014F mutations contributed to pyrethroid resistance in various insects such as *M. persicae*, *A. gossypii*, and *R. padi*. (Eleftherianos *et al.*, 2008; Chen *et al.*, 2017; Wang *et al.*, 2020; Munkhbayar *et al.*, 2021). However, the underlying mechanisms involved in pesticide resistance of *A. spiraecola* are still unclear.

In this study, we quantified the toxicity of lambda-cyhalothrin to field populations of *A. spiraecola* from different regions of the Shaanxi province, China. The changes in detoxification enzyme activities and gene transcription levels of *A. spiraecola* in response to sublethal doses of lambda-cyhalothrin were quantified. Furthermore, we characterized the mutation target sites of VSGC and examined the mutation rates of L1014F. Our main objective was to investigate the initial resistance mechanisms of *A. spiraecola* to lambda-cyhalothrin and to establish a theoretical foundation for effective pest resistance management programmes of the aphid.

Materials and methods

Insects

The *A. spiraecola* laboratory susceptible strain (SS) was collected from apple orchards in Yangling (Shaanxi Province) in 2020. The SS strain was reared on apple seedlings without exposure to any insecticides. The *A. spiraecola* field populations were collected from apple trees in eight regions of Shaanxi province, one of the most important apple-growing areas in China (table 1). All aphids were reared on apple seedlings at 25 ± 1°C, 60 ± 10% relative humidity, and a 16:8 h (L:D) photoperiod in the laboratory.

Susceptibility of A. spiraecola to lambda-cyhalothrin

Lambda-cyhalothrin, [cyano- (3-phenoxyphenyl) methyl] 3-(2-chloro-33,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate (95% purity, Ningbo Sanjiang Yinong Chemical Co., Ltd., China) was used in this study. A leaf dip method modified by Zuo *et al.* (2016b) was used for the bioassays. Five to six serial concentrations of lambda-cyhalothrin were prepared using 0.1% tween-100 as solvent. Leaves with 30 apterous adult aphids were dipped in the insecticide dilutions for 10 s. Then, the leaves were removed from the solution, and residual droplets on the leaves were adsorbed with clean, dry filter paper. One replicate consisted of 30 aphids exposed to serial concentrations of insecticide. There were three replicates of each concentration. The treated aphids were kept at 25 ± 1°C, 60 ± 10% RH, and 16 h / 8 h dark-light cycle. After 24 h, mortality was assessed with a microscope. Aphids were considered dead if they did not move after gentle prodding with a fine brush.

Assay of detoxification enzyme activity in A. spiraecola

Protein content determination

For this assay, we used ten adults of *A. spiraecola* from each region. Three detoxification enzymes (P450, CarE, and GST) were extracted with 1 mL of PBS (phosphate-buffered saline) buffer. The detoxification enzymes were homogenized with PBS of different pH (pH8.0 for GST, pH7.8 for P450, and pH7.0 for CarE). The homogenate was centrifuged under 12,000 RPM at 4 °C for 30 min. The method of Bradford (1976) was used to measure the protein content and detected by a BCA protein assay kit (Beijing Solar Science and Technology Co., Ltd., Beijing, China).

Detoxification enzyme activity

The activity of the three detoxifying enzymes (P450, GST, and CarE) was measured following the method of Su *et al.* (2021). For GST activity, CDNB (1-chloro-2,4-dinitrobenzene) and GSH (reduced glutathione) were used as the substrate for the reaction with enzyme solution, and the changed absorbance was

Table 1. Sampling locations for *A. spiraecola* in apple orchards of Shaanxi

City	Place	Abbreviation	Latitude and longitude
Baoji	Fufeng	FF	34°38'N 107°91'E
Baoji	Fengxiang	FX	34°30'N 107°28'E
Weinan	Luochuan	LC	35°47'N 109°29'E
Xianyang	Liquan	LQ	34°48'N 108°43'E
Xianyang	Qianxian	QX	34°53'N 108°25'E
Xianyang	Xingping	XP	34°32'N 108°39'E
Xianyang	Xunyi	XY	35°71'N 108°17'E
Yan'an	Baishui	BS	35°13'N 109°38'E

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measured at 340 nm for 5 min. In contrast, P450 was measured at 400 nm after the p-nitroanisole and NADPH reacted with the enzyme solution at 30 °C for 2 h and was calculated with PBS buffer as a control. Chromogenic agents and α-naphthyl acetate were used for CarE. Each of them was mixed with the enzyme solution and reacted at 30 °C for 10 min, and the absorbance at 600 nm was measured, which also used PBS buffer as a control. Chromogenic agents consisted of 5% sodium dodecyl sulphonate and 1% fast blue B salt solution with a volume ratio of 5:2. All works were replicated three times.

RNA extraction and cDNA synthesis

Ten *A. spiraecola* apterous adult aphids were placed in 1.5 mL RNase-free centrifuge tubes (30 adults per treatment) and stored in a refrigerator at −80°C for RNA extraction. Total RNA was extracted by TRIGene® Reagent (TRIGene® Biotech (Beijing) Co., Ltd., Beijing, China). DNase I (Takara, Kyoto, Japan) for DNA decontamination in total RNA was performed. HiScript® II Q RT SuperMix Kit (Vazyme Biotech Co., Ltd, Nanjing, China) was used to reverse transcribe first-strand cDNA with RNA as a template. The system was 1 μl total RNA (1 μg), 4 μl 4 × gDNA Wiper Mix, and 11 μl RNase-free water, reacted at 42°C for 2 min. 4 μl 5 × HiScript II qRT SuperMix II was added to the previous system at 50 °C for 15 min and 85 °C for 15 s. The first-strand cDNA was used as a template for quantitative real-time PCR (hereafter referred to as qPCR).

Quantitative real-time PCR (qPCR)

For analyses of P450 gene expression in aphids from field populations and SS strain, 10 aphids were randomly taken for RNA extraction and cDNA synthesis for each of the three replicates. The baseline toxicity of SS was determined by the above bioassay method, and P450 gene expression was examined by treating SS of *A. spiraecola* with the λ-cyhalothrin LC_{50} concentration. Twenty-five apterous adult aphids were treated with LC_{50} concentrations of λ-cyhalothrin. The carrier (ddH_2O with 0.01% (v/v) Triton X-100) was used as a control. Ten surviving aphids were collected from each of the three replicates 24 h after treatment for RNA extraction and cDNA synthesis.

Twenty-five P450 genes of *A. spiraecola* were identified from the transcriptome sequence. Primer Premier 5.0 (Tsingke Biological Technology Co., Ltd., Beijing, China) was used to design primers, as shown in table S1. The qPCR analysis was performed with ChamQ SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China) in Rotor Q thermocycler (Qiagen, Hilden, Germany). For the system and conditions of the reaction referred to Su *et al.* (2021). Lambda-actin gene and elongation factor 1-alpha (*EF1-α*) were regarded as internal reference genes (Wang *et al.*, 2018; Fan *et al.*, 2019; Li *et al.*, 2021). Primers for qPCR amplification efficiency were determined by a 5-fold serial dilution with a cDNA template. Each sample was subjected to three biological replicates and three mechanical replicates. The LightCycler 480 system (Roche) was adapted for qPCR detection and then the relative gene expression level was analysed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Mutation detection

Mutation sites in the IIS4-IIS6 region of VGSC had been reported to be linked with pyrethroid resistance in many pests, in particular M918L and L1014F (Foster *et al.*, 2014; Chen *et al.*, 2017; Wang *et al.*, 2020). Based on the sequence of the sodium channel α subunit, we genotyped for the mutation of the VGSC gene from individuals of *A. spiraecola* field and laboratory SSs. The EZNA® Tissue DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) was used to extract genomic DNA (gDNA) from each individual aphid according to the recommended protocol. The gDNA was used as a template and the primer pairs of *AsVGSC* are shown in table S1. The PCR reaction system was 25 μl, including 2.5 μl 2 × SuperStar HiFi PCR Mix (GenStar® Biotech (Beijing) Co., Ltd., Beijing, China), 1 μl 10 μM upstream/downstream primers, and 1 μl genomic DNA, and the rest was supplemented with RNase-free water to 25 μl. The reaction conditions are as follows: 94°C for 3 min, then 35 cycles of 94°C for 30 s, 60–65°C for 30 s, 72°C for 1 min, and finally 72°C for 5 min. PCR products were analysed on 1% agarose gel (40 ml 1 × TAE buffer with 0.4 g agarose) and coloured under a DL2000 DNA marker (Dining Biotech Co., Ltd., Beijing, China). Finally, it was sent to Shanghai Sangon Biotech Co., Ltd., (Shanghai, China) for sequencing.

Statistical analysis

LC_{50} values were calculated and compared against the control. The two compared values were considered significantly different if their respective 95% CIs did not overlap (Litchfield and Wilcoxon, 1949; Wolfe and Hanley, 2002). The activities of three detoxification enzymes (P450, GST, and CarE) and the relative expression levels of P450 genes were compared statistically using SPSS 25.0 (SPSS, Chicago, IL) with $\alpha = 0.05$. Resistance ratios (RR) were used to determine levels of resistance of *A. spiraecola* to lambda-cyhalothrin. The resistance levels used were based on Shen and Wu (1995): susceptible (RR < 3-fold), decreased susceptibility (3- < RR ≤ 5-fold), low resistance (RR = 5- to 10-fold), moderate resistance (10- < RR ≤ 40-fold), high resistance (RR = 40-160-fold), and extremely high resistance (RR > 160-fold). Linear regression analyses were used to establish the relationship between the median lethal concentration of lambda-cyhalothrin and the mutation rates of sodium channel L1014F. All bioassay analyses were conducted using DPS software (Zhejiang University, Hangzhou, China).

Results

Susceptibility of field populations of A. spiraecola to lambda-cyhalothrin

The susceptibility of *A. spiraecola* to lambda-cyhalothrin was found to differ from one region to another and the LC_{50} values varied between years in the same area (table 2). The LC_{50} values recorded in four regions - BS, QX, FF, and LC - showed an increasing trend from 2021 to 2022, while such an increase of LC_{50} values was not found in the other regions. The LC_{50} of the SS in the laboratory was 2.07 (1.686–2.742) mg l^{-1}. The RR values of *A. spiraecola* to lambda-cyhalothrin were highest in the XY and XP areas in 2021 (174.230 and 123.240, respectively). However, for both locations, the RR values decreased in 2022 to 111.082 in XY and to 103.197 in XP. The lowest RR values were recorded in the BS and FF locations.

Metabolic enzymes activity of A. spiraecola

In 2021, Cytochrome P450 activity in field populations was significantly higher (*t*-test; $P < 0.01$) in five locations (XY, LQ, FX,

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Table 2. Susceptibility of eight *A. spiraecola* field populations to lambda-cyhalothrin

Location (year)	Slope ± SEM	χ^2	LC_{50} (mg L^{-1})	(95% CL)	RR[a]
SS	2.325 ± 0.355	3.744	2.071	1.686–2.742	1
FF (2021)	1.697 ± 0.357	0.459	88.824	53.545–115.721	42.889
FF (2022)	3.194 ± 0.454	2.025	102.695	81. 036–120.796	49.587
FX (2021)	1.390 ± 0.263	2.531	142.546	108.808–201.048	68.829
FX (2022)	3.691 ± 0.461	1.028	120.030	101.763–136.489	57.956
LC (2021)	1.568 ± 0.395	2.656	108.380	63.753–143.322	52.332
LC (2022)	2.568 ± 0.396	1.864	145.879	122.746–172.404	70.439
LQ (2021)	2.919 ± 0.643	1.494	192.109	155.516–218.178	92.762
LQ (2022)	2.978 ± 0.414	2.594	149.677	128.122–172.996	72.273
QX (2021)	2.592 ± 0.399	1.567	86.307	62.308–105.628	41.674
QX (2022)	2.546 ± 0.391	0.812	124.057	100.058–146.217	59.902
XP (2021)	3.138 ± 0.420	0.600	255.230	214.766–293.333	123.240
XP (2022)	2.482 ± 0.400	0.201	213.720	180.275–270.127	103.197
XY (2021)	2.272 ± 0.447	1.104	360.831	293.569–462.443	174.230
XY (2022)	2.752 ± 0.461	0.720	230.051	193.268–298.993	111.082
BS (2021)	1.228 ± 0.226	0.429	76.207	51.634–99.961	36.797
BS (2022)	2.840 ± 0.404	0.490	120.776	100.282–140.239	58.318

SEM, Standard error of the mean; $\chi 2$, Chi-square value; CL, 95% confidence limits; SS, Susceptible strain.
[a]RR: Resistance ratios = resistant strain LC_{50} of lambda-cyhalothrin/ LC_{50} of susceptible strain.

LC, and XP) compared to the control (SS, fig. 1A). Compared with the SS, the field population did not show a significant increase in the GST enzyme activity, except in the case of the LC and FF populations in 2021 (fig. 1B). Similarly, the CarE enzyme activity was not significantly increased (fig. 1C). The activity levels of P450 differed significantly between field strains and SS in 2022 (fig. 1a). GST and CarE activity were significantly lower in the samples from different regions in 2022

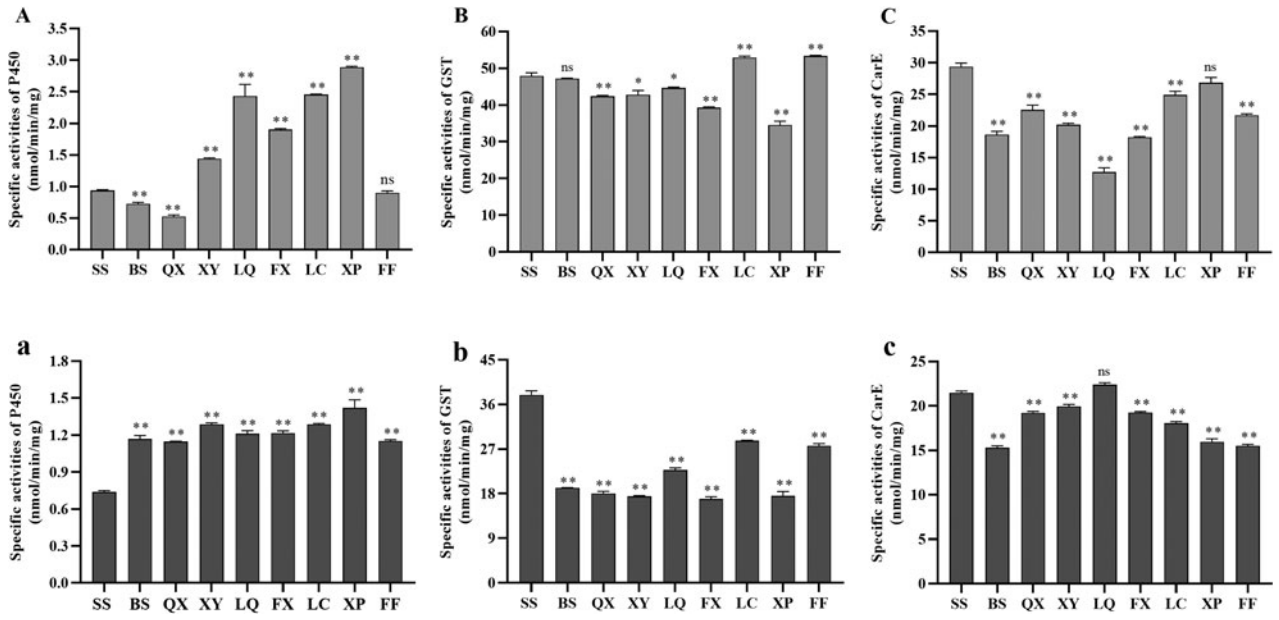

Figure 1. Specific activities of detoxifying enzymes of *A. spiraecola* in field populations relative to the susceptible laboratory strain (SS). (A)-(C) The specific activity of three detoxification enzymes in samples collected in 2021. (A) The specific activity of P450; (B) The specific activity of GST; (C) The specific activity of CarE. (a)–(c) The specific activity of three detoxification enzymes in samples collected in 2022. (a) The specific activity of P450; (b) The specific activity of GST; (c) The specific activity of CarE. Data are shown as mean ± SEM (standard error of mean). The asterisks above bars denote significant differences between treatments and SS according to the *t*-test. ns = not significant; *, $P < 0.05$; **, $P < 0.01$.

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compared with SS, except the CarE enzyme in LQ population (fig. 1b and 1c).

The expression levels of P450 genes in A. spiraecola

The relative expression levels of 25 P450 genes (table S1) identified from the transcriptome are shown in figs 2 and 3. The results indicated that compared with the control (SS strain), the expression levels of 14 P450 genes in XY were significantly up-regulated, while 18 P450 genes in XP were significantly higher than the SS (fig. 2). The qPCR analyses showed that the expression levels of one P450 gene were not significantly affected, while nine P450 genes were significantly down-regulated (fig. 3A) and fifteen P450 genes were significantly up-regulated after treatment with a sublethal concentration of lambda-cyhalothrin (fig. 3B). There were eight P450 genes were expressed with up-regulated levels in both sublethal effects and field populations.

Mutations of sodium channel site in A. spiraecola

A 486 bp with M918 site and a 605 bp with L1014 site of *AsVGSC* were amplified. The L1014F mutation in the VGSC gene was found to be present in *A. spiraecola*, while the M918L was not found (fig. 4). The CTT to TTT appeared in all the sequence chromatogram with a mixed peak, indicating that the L1014F was heterozygous (fig. 4B). The results showed that the mutation frequency of L1014F in the SS strain was 3.3%, while the mutation frequency was 80.0%, 70.6%, and 76.7% in the population from XY, LQ, and XP, respectively (table 3). There was a significant positive linear correlation between the L1014F mutation frequency and LC_{50} of lambda-cyhalothrin in *A. spiraecola* ($r = 0.767$, $P < 0.05$) (fig. 5).

Discussion

While pyrethroid insecticides are being increasingly applied in pest control (Bass *et al.*, 2014; Scott *et al.*, 2015; Wang *et al.*, 2020), long-term pyrethroid use and overuse have produced varying levels of resistance in aphid species such as *R. padi*. For instance, this insect pest has been found to possess resistance levels of 18.2, 13.1, and 24.14 to bifenthrin, deltamethrin, and lambda-cyhalothrin, respectively (Zuo *et al.*, 2016b; Wang *et al.*, 2018, 2020). *A. gossypii* had more than 5000-fold resistance to beta-cypermethrin (Chen *et al.*, 2016, 2017). Tang *et al.* (2017)

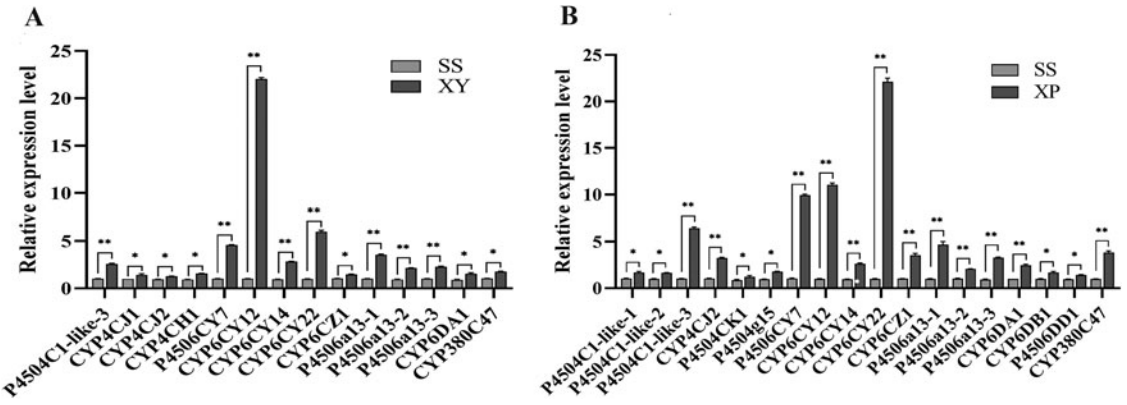

Figure 2. Relative expression levels of P450 genes of *A. spiraecola* and the susceptible laboratory strain (SS) that were up-regulated in two field populations: XY (A) and XP (B). Data are shown as mean ± SEM values. The asterisks above bars denote significant differences between treatments according to the *t*-test. ns = not significant; *, $P < 0.05$; **, $P < 0.01$.

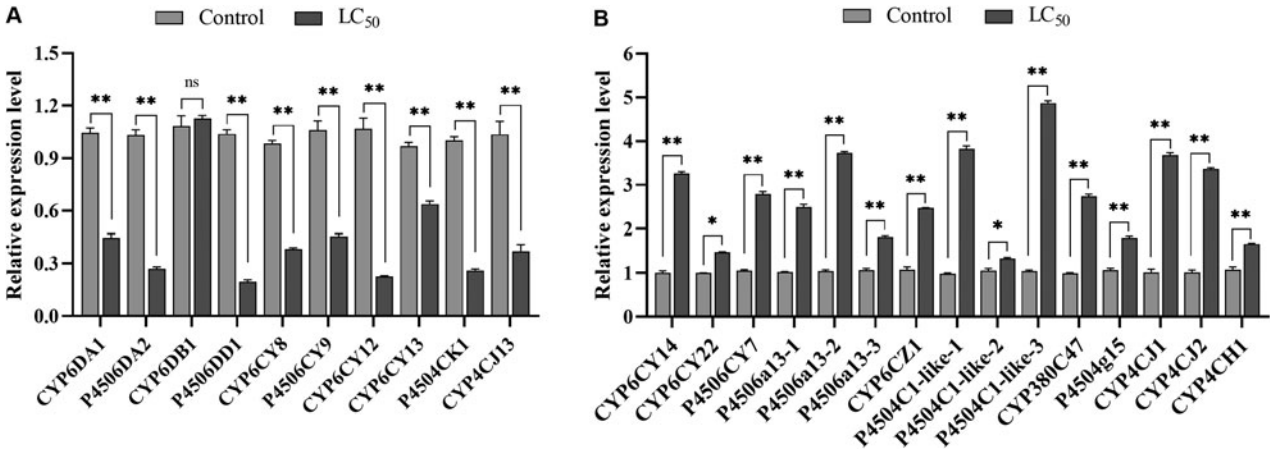

Figure 3. Relative expression levels of P450 genes of *A. spiraecola* and the susceptible laboratory strain (SS) treated with the LC_{50} concentrations of lambda-cyhalothrin. (A) P450 genes were not up-regulated; and (B) P450 genes were up-regulated. Data are shown as mean ± SEM values. Asterisks above bars denote significant differences between treatments according to Student's *t*-test. ns = not significant; *, $P < 0.05$; **, $P < 0.01$.

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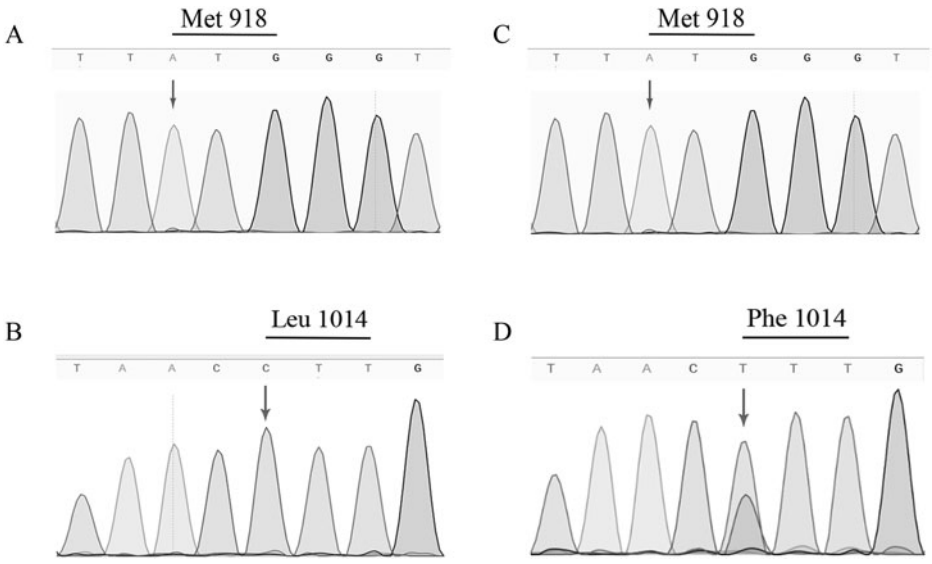

Figure 4. Detection of voltage-gated sodium channel mutation in *A. spiraecola*. (A) and (B) Partial nucleotide sequence chromatograms of the M918 site in *A. spiraecola* voltage-gated sodium channel between Susceptible Strain (A) and field populations (B). No mutation was found in the field population. (C) and (D) Partial sequence of the nucleotide at the L1014F mutation site in the *A. spiraecola* voltage-gated sodium channel in Susceptible Strain (C) and field population (D). The field strains of *A. spiraecola* showed the heterozygous mutation.

Table 3. Mutation frequencies of L1014F in the sodium channel of *A. spiraecola*

Region	Sample tested	Individuals with mutation	Mutation rate (%)
SS	30	1	3.3
FF	30	18	60.0
FX	30	21	70.0
LC	30	20	66.7
LQ	30	22	70.6
QX	30	19	63.3
XP	30	23	76.7
XY	30	24	80.0
BS	30	18	60.0

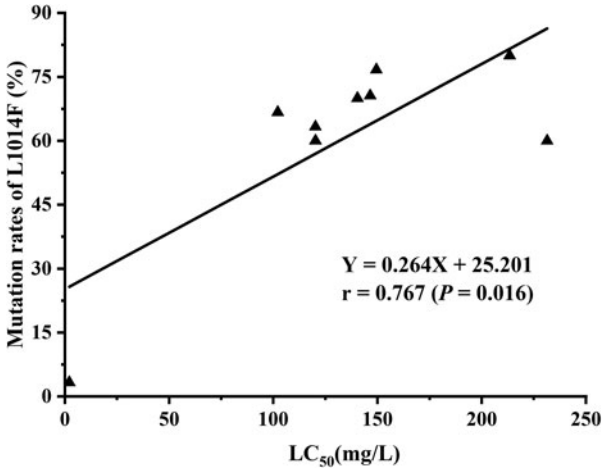

Figure 5. Linear regression analysis of the LC_{50} of lambda-cyhalothrin and the mutation frequencies of L1014F in the sodium channel of *A. spiraecola*.

found that the resistant strain of *M. persicae* had 260.80- and 123.15-fold resistance levels to *β*-cypermethrin and cypermethrin, respectively, relative to the SS. In this study, we found that the resistance of *A. spiraecola* to lambda-cyhalothrin ranged from moderate to extremely high levels.

In recent years, apple trees have been severely damaged by *A. spiraecola*, leading to extensive use of lambda-cyhalothrin spray in the regions. The frequent applications of insecticides had caused the rapid increasing in insecticide resistance among *A. spiraecola* populations. One mechanism underlying insect resistance is metabolic resistance, manifested by the variation of activity of detoxifying enzymes and the related gene expression. In the present study, the activity level of P450 detoxification enzyme was significantly increased in XY, LQ, FX, LC, and XP populations of *A. spiraecola* when compared to the SS in 2021, but was not up-regulated in the three population (BS, QX, and FF) with lower resistant to lambda-cyhalothrin. However, the resistant level of BS, QX, and FF populations increased in 2022, and the activity of P450 enzymes was significantly higher than SS in all regions in this year, indicating the P450 may contribute to the resistance. It has been widely reported that the upregulation of P450 gene can lead to the insect resistance to pyrethroids (Xi *et al.*, 2015; Wang *et al.*, 2019, 2020). The elevated P450 activity was the primary mechanism of lambda-cyhalothrin resistance in *Helicoverpa armigera* (Wang *et al.* (2019). The increased activity of the P450s leads to the development of lambda-cyhalothrin resistance in *Aphis glycines* (Xi *et al.*, 2015). Notably, the P450 enzyme activity in the XY population of *A. spiraecola* was lower than that in XP population. However, the LC_{50} of XY population for lambda-cyhalothrin was the highest, which could potentially be attributed to the L1014F mutation of the sodium channel gene in XY population. The mutation rates of the sodium channel were closely associated with the RRs of *A. spiraecola* population to lambda-cyhalothrin. The mutation rate of the L1014F in the sodium channel in the XY population was highest among all regions.

Eight P450 genes (*CYP6CY7*, *CYP6CY14*, *CYP6CY22*, *P4504C1-like*, *P4506a13*, *CYP4CZ1*, *CYP380C47*, and *CYP4CJ2*) were up-regulated in both *A. spiraecola* field populations and under exposure to LC_{50} concentration of lambda-cyhalothrin in SS. Five P450 genes (*P4504C1-like*, *P4506a13*, *CYP6CY14*, *CYP4CJ1,* and *CYP4CJ2*) were highly expressed in *A. spiraecola* under exposure

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to LC_{50} concentration of lambda-cyhalothrin. Overexpression of detoxification enzyme genes can contribute to insecticide resistance, as found in *A. glycines* (Xi *et al.*, 2015). Similarly, overexpression of *CYP6A2* was related to pyrethroid resistance in *A. gossypii* (Peng *et al.*, 2016). Wang *et al.* (2020) showed that the overexpression *CYP6DC1* and *CYP380C47* played important roles in the pyrethroid resistance of *R. padi*. Our results revealed that the P450 genes were related to lambda-cyhalothrin resistance in *A. spiraecola*. The documented high levels of resistance in the FF and LC populations may also be associated with GST enzyme activities. The effect of GST enzyme activities on pyrethroid resistance in insects has been documented in various species of insects (Pavlidi *et al.*, 2018). GSTs contributed to resistance of *C. pomonella* to pyrethroid (Wei *et al.*, 2020; Hu *et al.*, 2022a). The enhanced GST enzyme activity had been found to provide protection to tissues from oxidative damage and increase resistance to permethrin and lambda-cyhalothrin in *Nilaparvata lugens* (Vontas *et al.*, 2001). Further research is needed to investigate the role of GSTs in the lambda-cyhalothrin resistance of *A. spiraecola*.

It is known that target-site insensitivity can contribute to pesticide resistance in insects. The L1014F and M918L mutations in sodium channel reduced insect susceptibility to pyrethroid (Rinkevich *et al.*, 2013; Foster *et al.*, 2014; Field *et al.*, 2017; Mingeot *et al.*, 2021). In the case of *M. persicae*, for example, mutations such as L1014F and M918 T/L have been associated with resistance to pyrethroids. (Martinez-Torres *et al.*, 1999; Eleftherianos *et al.*, 2008; Fontaine *et al.*, 2011). The mutation M918L in *R. padi* (Zuo *et al.*, 2016a; Wang *et al.*, 2020) and *A. gossypii* (Chen *et al.*, 2017) has been observed to result in significant resistance to beta-cypermethrin and deltamethrin, respectively. In this study, the mutation rates of L1014F were above 60% in *A. spiraecol* from all regions. The documented levels of resistance in *A. spiraecola* observed in these regions were associated with L1014F mutation at the sodium channel, which is comparable to the high levels of resistance to pyrethroids contributed by sodium channel mutations in *M. persicae*, *R. padi*, and *A. gossypii*. Therefore, the resistance of *A. spiraecola* to lambda-cyhalothrin is linked to the L1014F mutation in the sodium channel.

In conclusion, *A. spiraecola* has developed resistance to lambda-cyhalothrin in apple orchards in the Shaanxi province. Resistance was associated with increased activity of P450 detoxification enzymes and the overexpression of P450 genes. The L1014F mutation in the sodium channel was detected and played a role in the resistance of *A. spiraecola* to lambda-cyhalothrin. We postulate that a variety of resistance mechanisms may be involved in the occurrence of resistance of *A. spiraecola* to lambda-cyhalothrin. This study provides a theoretical basis for the scientific management of this global pest and an initial investigation into the resistance mechanism of the aphid to pyrethroids.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485323000603.

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Competing interests. None.

References

Barres B, Micoud A, Corio-Costet MF, Debieu D, Fillinger-David S, Walker AS, Délye C, Grosman J and Siegwart M (2016) Trends and challenges in pesticide resistance detection. *Trends in Plant Science* **21**, 834–853.

Bass C, Puinean AM, Zimmer CT, Denholm I, Field LM, Foster SP, Gutbrod O, Nauen R, Slater R and Williamson MS (2014) The evolution of pesticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology* **51**, 41–51.

Bradford MM (1976) A rapid and sensitive method for the quantitation of micro-gram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

CABI (2022) '*Aphis spiraecola* (Spirea aphid)', CABI Compendium. CABI International. https://doi.org/10.1079/cabicompendium.6221.

Chen XW, Li F, Chen AQ, Ma KS, Liang P, Liu Y, Song DL and Gao XW (2016) Both point mutations and low expression levels of the nicotinic acetylcholine receptor β1 subunit are associated with imidacloprid resistance in an *Aphis gossypii*. (Glover) population from a Bt cotton field in China. *Pesticide Biochemistry and Physiology* **141**, 1–8.

Chen XW, Tie MY, Chen AQ, Ma KS, Li F, Liang PZ, Liu Y, Song DL and Gao XW (2017) Pyrethroid resistance associated with M918L mutation and detoxifying metabolism in *Aphis gossypii* from Bt cotton growing regions of China. *Pest Management Science* **73**, 2353–2359.

Dong K, Du Y, Rinkevich F, Nomura Y, Xu P, Wang L, Silver K and Zhorov BS (2014) Molecular biology of insect sodium channels and pyrethroid resistance. *Insect Biochemistry and Molecular Biology* **50**, 1–17.

Eastop VF and Blackman RL (1988) The identity of *Aphis citricola* van der Goot. *Systematic Entomology* **13**, 157–160.

Eleftherianos I, Foster SP, Williamson MS and Denholm I (2008) Characterization of the M918T sodium channel gene mutation associated with strong resistance to pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer). *Bulletin of Entomological Research* **98**, 183–191.

Fan JQ, Han PF, Gao Y, Liu ZF, Zhang PJ, Yang J and Fan RJ (2019) Expression of the cytochrome P450 gene *AcCYP6CY14* and its role in imidacloprid resistance in *Aphis citricola*. *Chinese Journal of Applied Entomology* **56**, 298–306.

Field LM, Davies TE, O'Reilly AO, Williamson MS and Wallace BA (2017) Voltage-gated sodium channels as targets for pyrethroid insecticides. *European Biophysics Journal* **46**, 675–679.

Fontaine S, Caddoux L, Brazier C, Bertho C, Bertolla P, Micoud A and Roy L (2011) Uncommon associations in target resistance among French populations of *Myzus persicae* from oilseed rape crops. *Pest Management Science* **67**, 881–885.

Foster SP, Paul VL, Slater R, Warren A, Denholm I, Field LM and Williamson MS (2014) A mutation (L1014F) in the voltage-gated sodium channel of the grain aphid, *Sitobion avenae*, is associated with resistance to pyrethroid insecticides. *Pest Management Science* **70**, 1249–1253.

Guillemaud T, Brun A, Anthony N, Sauge MH, Boll R, Delorme R, Fournier D, Lapchin L and Vanlerberghe-Masutti F (2003) Incidence of insecticide resistance alleles in sexually-reproducing populations of the peach-potato aphid *Myzus persicae* (Hemiptera: Aphididae) from southern France. *Bulletin of Entomological Research* **93**, 289–297.

Hu C, Liu JY, Wang W, Mota-Sanchez D, He S, Shi Y and Yang XQ (2022a) Glutathione *S*-Transferase genes are involved in lambda-cyhalothrin resistance in *Cydia pomonella* via sequestration. *Journal of Agricultural and Food Chemistry* **70**, 2265–2279.

Hu GL, Lu LY, Li YS, Su X, Dong WY, Zhang BZ, Liu RQ, Shi MW, Wang HL and Chen XL (2022b) *CYP4CJ6*-mediated resistance to two neonicotinoid insecticides in *Sitobion miscanthi* (Takahashi). *Bulletin of Entomological Research* **112**, 646–655.

Li MY, Li XA, Wang C, Li QC, Zhu SG, Zhang YH, Li XR, Yang FS and Zhu X (2021) Selection and validation of reference genes for qRT-PCR analysis of *Rhopalosiphum padi* (Hemiptera: Aphididae). *Frontiers in Physiology* **12**, 663338.

Litchfield J and Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. *Journal of Pharmacology and Experimental Therapeutics* **96**, 99–113.

Liu N (2015) Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annual Review of Entomology* **60**, 537–559.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ methods. *Methods (San Diego, Calif.)* **25**, 402–408.

Margaritopoulos JT, Kati AN, Voudouris CC, Skouras PJ and Tsitsipis JA (2021) Long-term studies on the evolution of resistance of *Myzus persicae* (Hemiptera: Aphididae) to insecticides in Greece. *Bulletin of Entomological Research* **111**, 1–16.

Martinez-Torres C, Foster SP, Field LM, Devonshire AL and Williamson MS (1999) A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Insect Molecular Biology* **8**, 339–346.

Mingeot D, Hautier L and Jansen JP (2021) Structuration of multilocus genotypes associated with insecticide resistance of the peach potato aphid, *Myzus persicae* (Sulzer), in potato fields in southern Belgium. *Pest Management Science* **77**, 482–491.

Mu SM, Liu ZD, Kong FH, Gao XW, Shi AJ and Qiu YQ (2002) Studies on apple yield loss rate and economic threshold caused by *Aphis citricola*. *Journal of Shandong Agricultural University (Natural Science)* **33**, 87–88 +100.

Munkhbayar O, Liu N, Li M and Qiu XH (2021) First report of voltage-gated sodium channel M918V and molecular diagnostics of nicotinic acetylcholine receptor R81T in the cotton aphid. *Journal of Applied Entomology* **145**, 261–269.

Nasuti C, Cantalamessa F, Falcioni G and Gabbianelli R (2003) Different effects of type I and type II pyrethroids on erythrocyte plasma membrane properties and enzymatic activity in rats. *Toxicology* **191**, 233–244.

Pavlidi N, Vontas J and Van Leeuwen T (2018) The role of glutathione *S*-transferases (GSTs) in insecticide resistance in crop pests and disease vectors. *Current Opinion in Insect Science* **27**, 97–102.

Peng TF, Pan YO, Yang C, Gao XW, Xi JH, Wu YQ, Huang X, Zhu E, Xin XC, Zhan C and Shang QL (2016) Over-expression of *CYP6A2* is associated with spirotetramat resistance and cross-resistance in the resistant strain of *Aphis gossypii* Glover. *Pesticide Biochemistry and Physiology* **126**, 64–69.

Rinkevich FD, Du YZ and Dong K (2013) Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. *Pesticide Biochemistry and Physiology* **106**, 93–100.

Scott JG (2019) Life and death at the voltage-sensitive sodium channel: evolution in response to insecticide use. *Annual Review of Entomology* **64**, 243–257.

Scott JG, Yoshimizu MH and Kasai S (2015) Pyrethroid resistance in *Culex pipiens* mosquitoes. *Pesticide Biochemistry and Physiology* **120**, 68–76.

Shen JL and Wu YD (1995) *Insecticide Resistance in Cotton Bollworm and its Management*. Beijing, China: China Agricultural Press, pp. 259–280.

Soderlund DM and Bloomquist JR (1989) Neurotoxic actions of pyrethroid insecticides. *Annual Review of Entomology* **34**, 77–96.

Su S, Jian CZ, Zhang XH, Fang SS, Peng X, Piñero JC and Chen MH (2021) Sublethal effects of abamectin on the development, reproduction, detoxification enzyme activity, and related gene expression of the oriental fruit moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology* **114**, 2430–2438.

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.

Vontas JG, Small GJ and Hemingway J (2001) Glutathione *S*-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochemical Journal* **357**, 65–72.

Wang K, Huang YN, Li XY and Chen MH (2018) Functional analysis of a carboxylesterase gene associated with sporocarp and cyhalothrin resistance in *Rhopalosiphum padi* (L.). *Frontiers in Physiology* **9**, 992.

Wang ZG, Jiang SS, Mota-Sanchez D, Wang W, Li XR, Gao YL, Lu XP and Yang XQ (2019) Cytochrome P450-mediated λ-cyhalothrin-resistance in a field strain of *Helicoverpa armigera* from northeast China. *Journal of Agricultural and Food Chemistry* **67**, 3546–3553.

Wang K, Bai JY, Zhao JN, Su S, Liu L, Han ZJ and Chen MH (2020) *Super-kdr* mutation M918L and multiple cytochrome P450s associated with the resistance of *Rhopalosiphum padi* to pyrethroid. *Pest Management Science* **76**, 2809–2817.

Wang ZJ, Liang CR, Shang ZY, Yu QT and Xue CB (2021) Insecticide resistance and resistance mechanisms in the melon aphid, *Aphis gossypii*, in Shandong, China. *Pesticide Biochemistry and Physiology* **172**, 104768.

Wang K, Zhao JN, Han ZJ and Chen MH (2022) Comparative transcriptome and RNA interference reveal *CYP6DC*1 and *CYP*380*C*47 related to lambda-cyhalothrin resistance in *Rhopalosiphum padi*. *Pesticide Biochemistry and Physiology* **183**, 105088.

Wei ZH, Liu M, Hu C and Yang XQ (2020) Overexpression of glutathione S-transferase genes in field lambda-cyhalothrin-resistant population of *Cydia pomonella*: reference gene selection and expression analysis. *Journal of Agricultural and Food Chemistry* **68**, 5825–5834.

Wolfe R and Hanley J (2002) If we're so different why do we keep overlapping? When 1 plus 1 doesn't make 2. *Canadian Medical Association Journal* **166**, 65–66.

Xi JH, Pan YO, Bi R, Gao XW, Chen XW, Peng TF, Zhang M, Zhang H, Hu XY and Shang QL (2015) Elevated expression of esterase and cytochrome P450 are related with lambda-cyhalothrin resistance and lead to cross resistance in *Aphis glycines* Matsumura. *Pesticide Biochemistry and Physiology* **118**, 77–81.

Yang XQ and Zhang YL (2015) Investigation of insecticide-resistance status of *Cydia pomonella* in Chinese populations. *Bulletin of Entomological Research* **105**, 316–325.

Zhang YL, Wang LH, Guo HF, Li GQ, Zhang ZC, Xie L and Fang JC (2012) A transcriptome-based screen of carboxylesterase-like genes that are involved in chlorpyrifos resistance in *Laodelphax striatellus* (fallén). *Pesticide Biochemistry and Physiology* **104**, 224–228.

Zuo YY, Peng X, Wang K, Lin FF, Li YT and Chen MH (2016a) Expression patterns, mutation detection, and RNA interference of *Rhopalosiphum padi* voltage-gated sodium channel genes. *Scientific Reports* **6**, 30166.

Zuo YY, Wang K, Zhang M, Peng X, Piñero JC and Chen MH (2016b) Regional susceptibilities of *Rhopalosiphum padi* (Hemiptera: Aphididae) to ten insecticides. *Florida Entomologist* **99**, 269–275.

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