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ORIGINAL ARTICLE

Frequencies and mechanisms of pesticide resistance in *Tetranychus urticae* field populations in China

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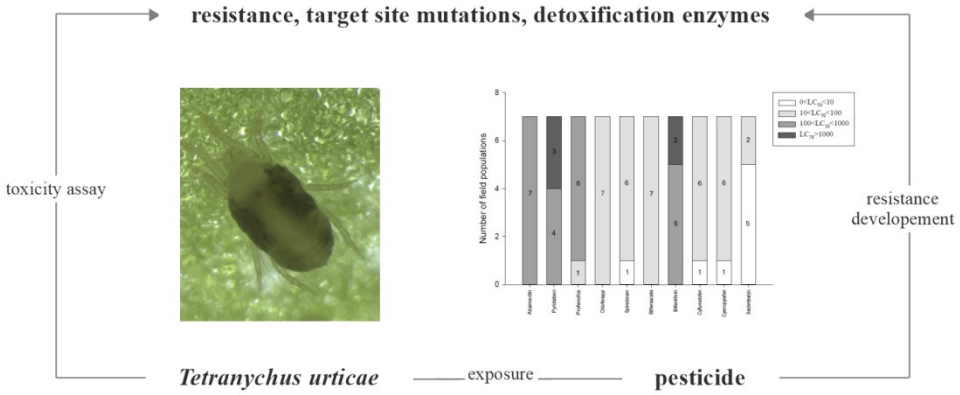
Abstract The two-spotted spider mite, *Tetranychus urticae,* is an important agricultural pest worldwide. It is extremely polyphagous and has developed resistance to many

This is an Accepted Article that has been peer-reviewed and approved for publication in the Insect Science but has yet to undergo copy-editing and proof correction. Please cite this article as doi: 10.1111/1744-7917.12957.

pesticides. Here, we assessed the pesticide resistance of seven field populations of *T. urticae* in China, their target site mutations, and the activities of their detoxification enzymes. The results showed that abamectin and the traditional pesticides pyridaben, profenofos, and bifenthrin had higher resistance or lower toxicity than more recently developed pesticides including chlorfenapyr, spinetoram, cyflumetofen, cyenopyrafen, bifenazate, and B-azolemiteacrylic. The frequency of point mutations related to abamectin resistance, G314D in the glutamate-gated chloride channel 1 (GluCl1) and G326E in GluCl3, ranged from 47% to 70% and from 0 to 97%, respectively. The frequency of point mutations in A1215D and F1538I of the voltage-gated sodium channel gene (VGSC), which may increase resistance to pyrethroids, ranged from 88% to 100% and from 10% to 100%, respectively. For target sites related to organophosphate resistance, mutation frequencies ranged from 25% to 92% for G119S and from 0 to 23% for A201S in the acetycholinesterase gene (Ace). Mutation G126S in the bifenazate resistance-related cytochrome b gene (Cytb) was observed in three of the seven *T. urticae* populations. Higher activities of detoxification enzymes (P450, GST, CarEs, and UGTs) were observed in two *T. urticae* populations, with significant difference in XY-SX population. These results provide useful information on the status of pesticide resistance of *T. urticae* in China and suggest that *T. urticae* field populations may have multiple resistance mechanisms.

Graphical Abstract: Previous research has shown that *Tetranychus urticae* has developed resistance to many pesticides. We assessed the pesticide resistance of seven field populations of *T. urticae* in China, their target site mutations, and the activities of their detoxification enzymes. The results suggest that *T. urticae* field populations may have multiple resistance mechanisms.



Key words detoxification enzyme; field populations; pesticide resistance; target site mutations; *Tetranychus urticae*

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, is a global agricultural and horticultural pest that causes substantial economic damage to its host plants. It can harm at least 1100 crops including vegetables, fruit trees, cotton, and corn (Van Leeuwen *et al.*, 2010). Using its piercing and sucking mouthparts, *T. urticae* absorbs the sap of host plant leaves and tender stems, causing leaf abscission when the damage is serious (Agut *et al.*, 2018). Control of *T. urticae* currently depends mainly on the application of pesticides as

evidenced by the increasing demand and market value of acaricides (Van Leeuwen *et al.*, 2015; Jeschke, 2021), although biological control by predatory mites has been used in some areas (Vacacela *et al.*, 2019). Because of its short life cycle, high reproductive potential, arrhenotokous parthenogenesis, and frequent exposure to pesticides, *T. urticae* has evolved resistance to most kinds of pesticides (van Leeuwen *et al.*, 2010). In China and other regions, *T. urticae* has developed resistance to organophosphates (OPs), carbamates, pyrethroids, and even some recently developed compounds (Xu *et al.*, 2018b; Liu *et al.*, 2020; Namin *et al.*, 2020; Papapostolou *et al.*, 2020; Simma *et al.*, 2020; Solmaz *et al.*, 2020). Failures of chemical control resulting from resistance have frequently occurred in the field (Van Leeuwen *et al.*, 2010; Funayama, 2015).

Pesticide resistance in *T. urticae* has been associated with multiple resistance mechanisms, including increased metabolic detoxification activity (van Leeuwen & Tirry, 2007) and overexpression of metabolic proteins including cytochrome-P450-monooxoygenases (P450), glutathione S-transferases (GST), carboxylesterase (CarEs), and uridine diphosphate-glycosyltransferases (UGTs) (Riga *et al.*, 2014; Khalighi *et al.*, 2016; Wei *et al.*, 2019; Wang *et al.*, 2020; Xu *et al.*, 2021). Target-site mutations in resistance genes have been determined to be the main resistance mechanism (Ilias *et al.*, 2014). For example, *T. urticae* resistance to the widely used pesticide abamectin involves mutations in the abamectin target, the glutamate-gated chloride channel gene (GluCl) (Kwon *et al.*, 2010a; Dermauw *et al.*, 2012; Xue *et al.*, 2021). Similarly, resistance of

the mite to pyrethroids and organophosphates (OPs) results from mutations in target sites of the genes that regulate the voltage-gated sodium channel (VGSC) and that encode acetycholinesterase (Ace), respectively (Kwon *et al.*, 2009; Tsagkarakou *et al.*, 2009; Khajehali *et al.*, 2010). In the case of the recently developed pesticide bifenazate, *T. urticae* resistance has been linked to several point mutations in cytochrome b (Cytb) (Van Leeuwen *et al.*, 2008; Fotoukkiaii *et al.*, 2020); although no related mutations have been found in field populations of *T. urticae* in China (Gong *et al.*, 2014; Xu *et al.*, 2018).

A few studies have assessed the insecticide resistance of *T. urticae* populations and the related frequencies of point mutations in resistance genes in some areas in China (Tang *et al.*, 2014; Xu *et al.*, 2018b). However, these studies involved quite limited areas of the country, and Xu *et al.* (2018b) failed to find a positive correlation between mutation frequencies and resistance level, suggesting the existence of an unknown resistance mechanism(s). It follows that research is needed to assess the resistance status and potential resistance mechanisms of additional *T. urticae* field populations in China.

In this study, we assessed the resistance of seven *T. urticae* field populations to 10 pesticides in China. We also assessed mutations in resistance genes related to abamectin, OPs, pyrethroids, and bifenazate, and the activities of the detoxifying enzymes P450, GST, CarEs, and UGTs. The data obtained will help guide the selection of acaricides for the management of *T. urticae* in China.

Materials and methods

Mites

One laboratory strain (IPP-SS) and seven field populations of *T. urticae* were assessed in this study. The laboratory strain was provided by Dr. Xuenong Xu of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, and has never been selected for more than 10 years. The seven *T. urticae* field populations were collected from six provinces in China from 2019 to 2020 (Table 1). For each population, three subsamples, each with >1 000 mites, were collected from *T. urticae*-infested leaves. The three subsamples were combined to yield one sample for each of the seven field populations. The collected mites were reared on discs of young leaves of kidney bean (*Phaseolus vulgrais* Linn, cv. Bifeng) in a growth chamber at 26 ± 1 °C and with 60% ± 5% relative humidity (RH) and a 16 h : 8 h (L : D) photoperiod.

Acaricides

All of the tested chemicals were commercial formulations and were provided by chemical companies as follows: abamectin 1.8% EC (Chang-sheng, Veyong Biochemical Hebei, China), pyridaben 15% EC (Jin-guo-yuan, Yongnong Biotechnology Co., Ltd., Zhejiang,

China), bifenthrin 25 g/L EC (Quzhou Chemical Co., Ltd., Zhejiang, China), profenofos 40% EC (Curacron, Yongnong Biological Science Co., Ltd), chlorfenapyr 240 g/L SC (Palit, BASF Chemical, Shanghai, China), bifenazate 43% SC (Acramite, Macdermid Chemical Co., Ltd., Shanghai, China), spinetoram 6% SC (Exalt, Dow Agrosciences, Beijing, China), 20% cyflumetofen SC (Jin-man-zhi, FMC Plant Protection, Jingsu, China), cyenopyrafen 30% SC (Nissan Chemical Industry Co., Ltd., Shanghai, China), and 30% B-azolemiteacrylic SC (Bao-zhuo, Zhonghua Pesticide Chemical R&D Co., Ltd., Shenyang, China).

Toxicity bioassays

Toxicity bioassays were conducted using a leaf dip method as previously described (Xu *et al.*, 2018b). Based on a preliminary experiment, each pesticide was serially diluted with distilled water to generate a series of 6 to 7 concentrations; each concentration was represented by three replicates, and distilled water was used as the control. Leaf disks (2 cm in diameter) were immerged in the diluted solutions for 10 s, air-dried in a fume hood, and then placed with the reverse side up on 0.2% agar in plastic Petri dishes (3.5 cm diameter, one disk per dish). Active female adult mites were then placed in each dish (25–30 per dish), and the dishes were covered with a lid with small holes to avoid water vapor accumulation. The dishes were kept in an incubator under the same conditions described earlier. After 24 h, the mites were assessed with the aid of a dissecting microscope; mites that did not move when gently touched with the tip of a brush and were recorded as dead. The mortality of

the control treatment did not exceed 20%. LC_{50} values were calculated as described in the Statistical analysis section.

Detection of resistance-associated gene mutations

Total genomic DNA was extracted from individual female mites using the KAPA Express Extract DNA Extraction Kit (Pukeri Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions. Individual mites were homogenized with a plastic pestle and mortar containing 30 μL of extraction solution (0.5 μL of 1 U/μL Rapid Extraction Enzyme, 3.0 μL of 1 U/μL Rapid Extraction Buffer, and 26.5 μL of ddH_2O.) Extractions were conducted at 75°C for 10 min and 95°C for 5 min. DNA extracts were stored at −20°C.

For abamectin resistance, the G314D mutation in GluCl1 was detected by PCR amplification of specific alleles as previously described (Wang *et al.*, 2016), and the G326E in GluCl3 was detected by cleaved amplified polymorphic sequences (Xu *et al.*, 2018a). The 20 μL PCR reaction contained 1.0 μL of genomic DNA, 1.0 μL of each primer (10 μmol/L) of 5′-GAT CCA AAT GCT ATT CCT GCC-3′ (forward primer) and 5′-GAG GGA AAC CCA TAC CAC CAC-3′ (reverse primer), 10.0 μL of *Taq* E/μL 2×*Es* Mix, and 7.0 μL of ddH_2O. PCR amplification was performed in a S1000 thermal cycler (Bio-Rad); and the cycling conditions were an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s, 72°C for 1 min, and a final extension at 72°C for 2 min. Then, the PCR products were

purified and digested with restriction endonuclease *Hinf* I (Promega, USA) and the reaction was incubate at 37° for 15 min (Xu *et al.*, 2018a).

Target genes related to OP, bifenthrin, and bifenazate resistance were detected by sequencing the PCR products from specific primers (Xu *et al.*, 2018b; Table S1). For each mutation in each population, at least 30 female adults were randomly chosen and assessed. The PCR reaction contained 2.0 μL of genomic DNA, 1.25 μL of the primers, 0.5 μL of dNTPs, 0.5 μL of 1× Q5 Reaction Buffer, 5 μL of 1× Q5 High GC Enhancer, and 0.25 μL of Q5 High-Fidelity DNA Polymerase. PCR amplifications were carried out in a S1000 thermal cycler (Bio-Rad) as follows: initial denaturing for 30 s at 98°C; followed by 35 cycles of 10 s at 98°C, 20 s at 53.7–58.0°C, and 20 s at 72°C; and a final extension of 2 min at 72 °C. The PCR products were purified and sequenced at Sangon Biotech (Shanghai, China).

Determination of detoxification enzyme activities

To assess the activities P450, GST, CarE, and UGT enzymes, and approximately 2000 adult females were homogenized in sodium phosphate buffer (450 μL, 0.1 mol/L, pH 7.4).

The total amount of protein in the homogenate was quantified with the Enhanced BCA Protein Assay Kit (P0010, Beyotime, China). Standard serum albumin solutions were prepared according to the manufacturer's protocol. Equal volumes (10 μL) of the standards and samples were then individually incubated with the kit solutions for 30 min at 37 °C with

orbital shaking (60 r/min) in the dark. Finally, the absorbance at 562 nm of each reaction mixture was recorded using an automatic plate reader (SoftMax Pro GxP Software; Molecular Devices, LLC. U.S.A.).

Enzyme activities of *T. urticae* adults were determined by an enzyme-linked immunosorbent assay (ELISA; Meimian, Nanjing, China), previously described in other literatures (Cui *et al.*, 2018; Gao *et al.*, 2020). All procedures were performed according to the manufacturer's instructions（http://www.mmbio.cn/）. Briefly, the enzyme source was added to ELISA microelisa stripplate with specific solid-phase antibodies of P450, GST, CarEs, UGTs, respectively, which were incubated at 37°C for 30 min. Then the antibody complexes were labeled by horseradish peroxidase (HRP) and detected by the chromogenic assay, through the introduction of 3,3',5,5'-tetramethylbenzidine (TMB) for 10 min at 37°C. The reaction was terminated by adding stop solution and the color-changed solution was measured by optical density at 450 nm using a microplate reader (SoftMax Pro GxP Software; Molecular Devices, LLC., USA). The concentrations were calculated by comparing the ODs of the samples on the standard curve. The final enzyme activities are presented as means ± SE of three replicates.

Statistical analysis

For the bioassay data, the slope ± SE, LC_{50} values and 95% fiducial limits, Chi-square value, and degrees of freedom (df) were calculated by probit analysis using Polo Plus 2.0 software (Probit or Logit analyses, LeOra software, Berkeley, CA, USA). The resistance fold (RF) values were calculated by dividing the LC_{50} of field populations by the LC_{50} of the IPP-SS strain. Resistance was divided into five levels: susceptible ($1 \leq RF \leq 3$), low resistance ($3 < RF \leq 10$), moderate resistance ($10 < RF \leq 100$), high resistance ($100 < RF \leq 1\,000$), and extremely high resistance ($RF > 1\,000$).The frequencies of resistance alleles were calculated according to the following formula: F (R allele) = [(the number of resistance homozygous mites/total number of detected mites) + (the number of resistance heterozygous mites/total number of detected mites/2)]. One-way analysis of variance followed by Tukey-Kramer test in SPSS (version 19.0) was adopted to determine differences in the enzyme activities of *T. urticae* populations.

Results

Resistance of T. urticae field populations to pesticides

The LC_{50} value of the unselected laboratory strain of *T. urticae* (IPP-SS) significantly differed among the 10 pesticides (Table 2). Strain IPP-SS was most sensitive to abamectin, B-azolemiteacrylic, cyenopyrafen, and cyflumetofen, and was least sensitive to pyridaben and profenofos (Table 2).

For the field populations, detailed pesticide resistance data are presented in Table 2. Among the seven *T. urticae* field populations, pesticide resistance differed depending on the population and the pesticide. Compared to the IPP-SS strain, all seven field populations showed high or extremely high resistance to abamectin, with RF values ranging from 3643 (for population JZ-HB) to 20 039 (for population CP-BJ).

With respect to the traditional acaricides, populations CP-BJ, HD-BJ, and YC-NX were susceptible or had low resistance to pyridaben, and the other four populations had medium resistance. Population HD-BJ was susceptible, population YC-NX had low resistance, and the other five populations had moderate resistance to profenofos. All seven populations had high resistance to bifenthrin.

With respect to the new acaricides, all seven field populations were susceptible or had low resistance to spinetoram, and had susceptible to moderate resistance to cyenopyrafen, B-azolemiteacrylic, and chlorfenapyr. For bifenazate, 6 among 7 populations were low resistance and 1 among 7 were moderate resistance; whereas 6 populations were moderate resistance for cyflumetofen.

Genotype and allelic frequency of target site mutation

The mutation frequencies of GluCls, VGSC, Ace, and Cytb genes from seven field populations of *T. urticae* are indicated in Figure 1. No G314D or G326E mutations related to

abamectin resistance were detected in the IPP-SS strain, indicating that the IPP-SS strain is homozygous susceptible. In field populations, the frequency of G314D and G326E mutations ranged from 47 to 70% and from 0 to 97%, respectively. The frequency of the G326E mutation was the highest in population CP-BJ (Fig. 1A-1). In populations CP-BJ, HD-BJ, and JY-HN, G314D and G326E mutations were higher in 2019–2020 (the current study) than in 2017 (as reported by Xu *et al.*, 2018b) (Fig. 1A-2 and Fig. 1A-3 with "a" indicating data from 2017 and "b" indicating data from 2019–2020).

In the IPP-SS strain, the frequency of mutation was 50% for G119S and 0% for A201S. For field populations, the frequencies ranged from 0 to 33% and from 23 to 92%, respectively (Fig. 1B-1). G119S mutation frequency was highest in the SG-SD population, and A201S mutation frequency was highest in the XY-SX population. G119S mutation frequencies were lower in 2019–2020 than the CP-BJ and HD-BJ populations in 2017, but was higher in 2019–2020 than in 2017 in the JY-HN population.The A201S mutation frequency was lower in 2019–2020 than in 2017 for three populations (Fig. 1B-2 and Fig. 1B-3). In the IPP-SS strain, the frequency of mutation was 3% for A1215D and 0% F1538I (Fig. 1C-1). For the field populations, A1215D mutation frequencies ranged from 97 to 100%, and F1538I mutations ranged from 10% in the JY-HN population to 100% in the HD-BJ and SG-SD populations (Fig. 1C-1). The A1215D mutation frequencies varied only slightly between 2017 and 2019–2020 (Fig. 1C-2), but F11538I mutation frequencies in the JY-HN population were lower in 2019–2020 than in 2017 (Fig. 1C-3).

In the IPP-SS strain, the frequency of mutations in genes related to bifenazate resistance, including G126S, G132A, I136T, S141F, D161G, and P262T, was 0% (data not shown). The frequency of G126S mutations was 67% in the HD-BJ population, 10% in the JZ-HB population, and 50% in the XY-SX population (Fig. 2D-1). In 2017, however, this mutation was not found in HD-BJ, CP-BJ, or JY-HN populations (Fig. 2D-2).

Detoxification enzyme activities

The specific activities of P450, GST, CarEs, and UGTs in the IPP-SS strain and in the seven field populations are shown in Table 3. Expressed as a ratio relative to the activities in the IPP-SS strain, the activities of P450, GST, CarEs, and UGTs in the field populations ranged from 0.78 to 1.64, 1.04 to 2.50, 0.82 to 1.31, and 1.09 to 2.45, respectively. Compared with IPP-SS strain, P450 activity was remarkably higher in XY-SX and lower in HD-BJ (F = 38.88, df= 7,23, P < 0.001). Likewise, a significantly increased GST activity was also detected in XY-SX and JY-HN compared to IPP-SS (F = 16.48, df = 7,31, P < 0.001). CarEs activity was highest in the XY-SX, followed by JY-HN, CP-BJ and SG-SD populations, which were significantly higher than that of IPP-SS (F = 40.51, df = 7,23, P < 0.001). UGTs activity was higher in all of the field populations except HD-BJ and YC-NX than that of the IPP-SS strain and was highest in the JY-HN population (F = 35.65, df = 7,31, P < 0.001). Among all the field populations, the

activities of the tested four detoxification enzymes were significantly higher in Xianyang, Shaanxi (XY-SX) than the IPP-SS strain.

Discussion

In this study, we assessed the resistance (as of 2019–2020) of *T. urticae* field populations in China to both traditional and newly developed acaricides. Abamectin has a long history for controlling Lepidoptera, Hemiptera, and Arachnoidea because of its broad and high toxicity. We found all the tested *T. urticae* field populations evolved extremely high resistance to abamectin with more than 3500-folds. High levels of *T. urticae* resistance to abamectin have also been reported from many other countries (Brown *et al.*, 2017; Xu *et al.*, 2018b; İnak *et al.*, 2019; Namin *et al.*, 2020; Papapostolou *et al.*, 2020; Simma *et al.*, 2020; Xue *et al.*, 2020). The comparison of the three *T. urticae* populations (HD-BJ, CP-BJ, and JY-HN) that were sampled in 2017 (Xu *et al.*, 2018b) and sampled again in 2019–2020 in the current study indicated that the abamectin LC_{50} values increased by 2-folds in CP-BJ and decreased in the other two populations. Based on the high resistance to abamectin of *T. urticae* field populations, abamectin application will often fail to control this mite in most areas in China.

Point mutations G314D in GluCl 1 and G326E in GluCl 3 have often been associated with abamectin resistance in *T. urticae* (Kwon *et al.*, 2010a; Dermauw *et al.*, 2012; Mermans

et al., 2017). In this study, the frequencies of G314D and G326E mutations in Chinese *T. urticae* field populations and resistance levels varied among populations. From 2014 (Tang *et al.*, 2014) to 2019–2020 (the current study), the G314D and G326E mutation frequencies were relatively stable even though growers were advised not to apply abamectin during that period. Among the sampled mites in JY-HN population in the current study, 63% had G314D mutation, 0% had G326E mutation, and the abamectin resistance was extremely high (RF = 7 561). This suggests that G326E may not be relevant with abamectin resistance in JY-HN. Similar results were also reported in Terkey and USA, where *T. urticae* populations with high levels of abamectin resistance lacked the G326E mutation (Piraneo *et al.*, 2015; Çağatay *et al.*, 2018). In addition, 4 of 12 Ethiopian *T. urticae* populations having G326E mutation, were not resistant to abamectin (Simma *et al.*, 2020). In current study, the LC_{50} values for the *T. urticae* collected in JY-HN was 3-folds lower in 2019–2020 than in 2017 with high G314D mutation frequencies (Xu *et al.*, 2018b). Given that the positive correlation between G314D and abamectin resistance (Xu *et al.*, 2019) , it seems possible that this population has other resistance mechanisms besides the G314D mutation, such as increased activities of GST, CarEs, and UGTs (Table 3) and gene over-expressions (Çağatay *et al.*, 2018; Riga *et al.*, 2014; Xu *et al.*, 2021). A new point mutation, I321T (ATT→ACT), in GluCl 3 was recently detected in four abamectin-resistant field populations of the red color morph of *T. urticae* in Europe (Simma *et al.*, 2020; Xue *et al.*, 2021). The I321T mutation was not detected in *T. urticae* populations with green color morph in China (data unpublished).

In addition to target mutations, the over-expression of genes that encode detoxification enzymes, including P450, GST, and UGTs (Riga *et al.*, 2014; Pavlidi *et al.*, 2015; Wang *et al.*, 2020; Xu *et al.*, 2021), might be involved in abamectin resistance of mites. Consistent with that possibility, the high abamectin resistance of XY-SX population in this study was predicted to be associated with increased activities of detoxification enzymes. In addition, overexpression of P450, GST, CarEs, and UGTs have been suggested to contribute to abamectin resistance in *T. urticae* (Ahn *et al.*, 2014 ; Choi *et al.*, 2020; Dermauw *et al.*, 2020; Riga *et al.*, 2020; Wang *et al.*, 2020). The P450 genes CYP389 and CYP392 were previously associated with the development of abamectin resistance in spider mites (Riga *et al.*, 2014; Xu *et al.*, 2021; Xu *et al.*, 2021), besides the GST (*TuGSTd10*, *TuGSTd14*, and *TuGSTm09)* and *UGT201D3* (Dermauw *et al.*, 2013; Riga *et al.*, 2015; Wang *et al.*, 2020). In the current study, the activities of the four detoxification enzymes were higher in XY-SX population with abamectin RF values (13 364-folds) than YC-NX (RF = 3643) and HD-BJ (RF = 4411). Determining whether these enzymes are responsible for abamectin resistance in *T. urticae* will require additional studies.

Pyrethroids, OPs, and pyridaben (PDB) have been used for many years to control *T. urticae*, and resistance to these traditional pesticides has been reported (Ilias *et al.*, 2012; Riga *et al.*, 2017). In the current study, seven *T. urticae* field populations had high resistance to the pyrethroid bifenthrin, with RF values 306–1538. The *T. urticae* field populations developed low to middle resistance against pyridaben and profenofos, based on the

resistance fold. However, from the LC_{50} values, it is obvious that these two insecticides have low toxicity activities to IPP-SS. Pyrethroids are regulators of VGSC, and the A1215D and F1538I mutations in VGSC have been associated with bifenthrin resistance (Kwon *et al.*, 2009; Ding *et al.*, 2015). Except for XY-SX population, the *T. urticae* populations with high frequencies of A1215D and F1538I mutations had bifenthrin in current study, which is in agreement with other studies in *Panonychus citri* (Ding *et al.*, 2015) and in *T. urticae* (Riga *et al.*, 2017). In additionally, the *T. urticae* field populations with high resistance to bifenthrin had increased activities of detoxifying enzymes in this study (Table 3). The G119S and A201S mutations were proved to be important for OP resistance (Khajehali *et al.*, 2010), and the *T. urticae* populations with high OP resistance in current study also had high frequencies of G119S mutation. In this present study, low to medium levels of pyridaben resistance were found in field *T. urticae* populations; and the JY-HN population with relatively high pyridaben resistance had high activities of CarEs and UGTs. However, two previous studies indicated that P450 and CarEs were involved in the pyridaben resistance of mites (van Pottelberge *et al.*, 2009; Namin *et al.*, 2020).

Except for CP-BJ population, the *T. urticae* field populations in China showed low bifenazate resistance. In 2014, five populations of *T. urticae* collected in Beijing, China, showed no or low resistance to bifenazate (Gong *et al.*, 2014). The bifenazate LC_{50} of CP-BJ population increased from 6 mg/L by slide-dip method in 2014 (Gong *et al.*, 2014) to 29 mg/L by leaf dip method in 2017 (Xu *et al.*, 2018) and to 97 mg/L in 2019–2020 (the current

study). Although this suggests *T. urticae* resistance to bifenazate in Beijing is gradually increasing, bifenazate is currently considered a highly effective acaricide for mite control. Among the target sites related to bifenazate resistance in cytochrome b gene (van Leeuwen *et al.*, 2008; van Nieuwenhuyse *et al.*, 2009; Ilias *et al.*, 2014; Shi *et al.*, 2019; Fotoukkiaii *et al.*, 2020), we observed the G126S mutation with frequencies ranging from 10% to 67% in JZ-HB, XY-SX, and HD-BJ populations. However, we found the resistance of the corresponding field populations was not high, indicating this mutation might not confer high resistance to *T. urticae* (van Nieuwenhuyse *et al.*, 2009). This is consistent with the results in previous studies, which found the G126S mutation alone was not associated with high resistance to bifenazate unless it occurred along with I126T or S141F mutation (Van Leeuwen *et al.*, 2008; Gong *et al.*, 2014). In addition, only 1 of 288 *T. urticae* mites in Huairou, Beijing had G126S mutation in 2013, and no mutations were found in other districts in Beijing in 2017 (Gong *et al.*, 2014; Xu *et al.*, 2018). A recent study, however, found the G126S mutation in 100% of mites sampled in four areas in Beijing and in 72% of the mites sampled in Chengdu, Sichuan (Shi *et al.*, 2019). Therefore, this mutation increased gradually with bifenazate application, and the resistance risk in China is increasing. On the other hand, a newly discovered mutation in cytochrome b, G132A, was related to bifenazate resistance (Fotoukkiaii *et al.*, 2020) but was not detected in current study. Given its current RF values and the point mutations detected, bifenazate should be considered carefully for *T. urticae* control in China.

In addition to bifenazate, five other newly registered pesticides (chlorfenapyr, spinetoram, cyflumetofen, cyenopyrafen, and B-azolemiteacrylic) were also highly toxic to the seven populations of *T. urticae* in the current study, which is consistent with results obtained in 2017 with *T. urticae* in China (Xu *et al.*, 2018b). Our results showed that the LC_{50} values of cyflumetofen, cyenopyrafen, and B-azolemiteacrylic, which function as respiratory chain complex inhibitors, ranged from 1 to 52 mg/L among the seven field populations. Cyflumetofen is a semi-esterase-dependent precursor acaricide, which acts as a complex II inhibitor in the mitochondrial electron transport chain; previous research revealed that when esterase activity is inhibited, the toxicity of cyflumetofen is greatly reduced (Liu *et al.*, 2020). In the current study, the HD-BJ population had the lowest resistance to cyflumetofen and cyenopyrafen among the the tested field populations; the HD-BJ population also had the lowest levels of activity of P450 and CarEs. This is consistent with previous reports that P450 and CarEs are jointly involved in resistance to cyflumetofen (Riga *et al.*, 2015; Liu *et al.*, 2020). Spinetoram, which was registered as an insecticide rather than an acaricide, had LC_{50} values in current study that ranged from 5 to 80 mg/L, indicating that it is toxic to adult females of *T. urticae*. This is consistent with previous report (Ismail *et al.*, 2007). Because sublethal levels of spinetoram may cause *T. urticae* outbreaks (Wang *et al.*, 2016), additional research is needed to determine the dose of spinetoram required to properly control the pest.

In conclusion, our findings exhibited the current insecticide resistance status of *T. urticae* in China to 10 frequently used and newly developed insecticides. The results showed that the *T. urticae* populations developed high or extremely high resistance to abamectin and bifenthrin, compared to the other tested insecticides. The frequencies of some certain point mutations related to abamectin, pyrethroids, and organophosphate resistance almost came close to 100%. The newly emerging point mutation related to bifenazate resistance should be paid attention. Additionally, increased activities of detoxification enzymes were existed, suggesting that *T. urticae* field populations may have multiple resistance mechanisms.

Acknowledgments

This research was supported by the National Key R&D Program of China (2016YFD0200500), the National Natural Science Foundation of China (32072458), China Agriculture Research System of MOF and MARA (CARS-25), the Beijing Key Laboratory for Pest Control and Sustainable Cultivation of Vegetables, and the Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-ASTIP-IVFCAAS). The granting agencies had no role in study design, data collection, and analysis, decision to publish, or manuscript preparation.

Disclosure

Experiment design ：YZ, SLW. Experiment perform: YZ. Data analysis: YZ. Project administration: SLW. Resources: SLW, YJZ. Supervision: QJW, WX, ZJG. Validation: YZ, DDX. Writing & editing: YZ, SLW.

All authors contributed to the article and approved the submitted version.

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Manuscript received March 17, 2021

Final version received June 9, 2021

Accepted July 14, 2021

Supporting Information

Table S1 Primers and amplified PCR products used to detect mutations in target sites associated with the pesticide resistance of *Tetranychus urticae.*

Table 1 Background information on the *Tetranychus urticae* field populations collected in this study.

Population	Collection location	Collection site	Collection date	Host plant
HD-BJ	Haidian, Beijing	E116º30' N39º95'	April, 2020	Cole
CP-BJ	Changping, Beijing	E116º23' N40º22'	August, 2019	Chili
YC-NX	Yinchuan, Ningxia	E106º27' N38º47'	September, 2019	Watermelon
XY-SX	Xianyang, Shaanxi	E108º72' N34º36'	April, 2019	Watermelon
JZ-HB	Jingzhou, Hubei	E112º18' N30º35'	August, 2019	Eggplant
SG-SD	Shouguang, Shandong	E118º73' N36º86'	July, 2020	Chili
JY-HN	Jiyang, Hainan	E109º49' N18º21'	May, 2020	Eggplant

Table 2 Responses of seven field populations of *Tetranychus urticae* to 10 pesticides.

Pesticide	Population	N[†]	Slope ± SE	LC50 (mg/L) 95%FL (mg/L)[‡]	χ^2 (df)[§]	RF[¶]
Abamectin	IPP-SS	533	2.39 ± 0.18	0.05 (0.04–0.05)	1.62 (4)	1.00
	HD-BJ	567	2.05 ± 0.17	202.90 (177.30–235.99)	1.45 (4)	4410.89
	CP-BJ	581	0.91 ± 0.11	921.78 (613.37–1678.79)	0.76 (4)	20038.61
	YC-NX	547	1.31 ± 0.14	167.57 (132.27–228.96)	0.58 (3)	3642.85
	XY-SX	524	2.35 ± 0.24	614.76 (534.65–731.67)	1.86 (3)	13364.24
	JZ-HB	546	2.16 ± 0.17	162.70 (141.75–188.52)	0.23 (4)	3536.76
	SG-SD	537	1.56 ± 0.12	578.12 (467.78–732.72)	3.59 (4)	12567.83
	JY-HN	586	1.32 ± 0.12	347.82 (284.73–436.71)	3.83 (4)	7561.28
Pyridaben	IPP-SS	597	1.04 ± 0.10	66.74 (51.36–91.72)	1.10 (5)	1.00
	HD-BJ	540	0.95 ± 0.09	230.06 (174.55–305.21)	2.11 (4)	3.45
	CP-BJ	541	2.33 ± 0.21	113.50 (72.61–151.54)	3.28 (3)	1.70
	YC-NX	523	1.18 ± 0.10	370.39 (293.60–476.77)	2.59 (4)	5.55
	XY-SX	525	1.30 ± 0.12	797.88 (637.77–983.38)	0.68 (3)	11.96
	JZ-HB	552	2.10 ± 0.15	2085.41 (1745.17–2535.54)	2.48 (4)	31.25
	SG-SD	527	1.10 ± 0.11	1662.83 (1272.95–2298.88)	1.73 (4)	24.92
	JY-HN	549	1.11 ± 0.14	2900.37 (2024.90–4966.94)	0.39 (3)	43.46
Profenofos	IPP-SS	536	0.89 ± 0.11	18.87 (13.91–27.60)	0.60 (4)	1.00
	HD-BJ	524	1.66 ± 0.15	40.94 (33.97–50.86)	2.93 (3)	2.17
	CP-BJ	513	1.77 ± 0.17	377.31 (319.14–446.59)	1.39 (4)	20.00

	YC-NX	511	1.97 ± 0.16	123.56 (104.10–144.32)	1.64 (4)	6.55
	XY-SX	576	1.58 ± 0.14	258.81 (215.01–332.37)	1.88 (4)	13.72
	JZ-HB	518	1.31 ± 0.11	446.83 (342.62–616.68)	1.49 (4)	23.68
	SG-SD	623	1.50 ± 0.11	250.02 (206.30–310.53)	0.85 (5)	13.25
	JY-HN	615	1.38 ± 0.12	408.79 (323.69–545.62)	4.76 (5)	21.67
Chlorfenapyr	IPP-SS	517	2.35 ± 0.17	4.26 (3.66–4.93)	1.04 (4)	1.00
	HD-BJ	601	1.13 ± 0.11	24.66 (19.81–31.11)	0.80 (5)	5.78
	CP-BJ	510	2.16 ± 0.17	62.57 (53.01–75.93)	1.80 (3)	14.67
	YC-NX	601	2.06 ± 0.18	77.66 (68.16–89.54）	2.70 (4)	18.21
	XY-SX	552	1.82 ± 0.13	60.93 (51.23–71.81)	2.00 (3)	14.29
	JZ-HB	539	0.91 ± 0.13	14.16 (9.46–19.01)	0.41 (3)	3.32
	SG-SD	554	1.25 ± 0.12	64.63 (51.05–79.91)	0.80 (3)	15.16
	JY-HN	520	1.82 ± 0.14	26.48 (22.41–31.07)	2.40 (4)	6.21
Spinetoram	IPP-SS	478	1.14 ± 0.12	8.47 (6.39–11.99)	1.71 (3)	1.00
	HD-BJ	553	1.18 ± 0.14	5.21 (3.92–7.74)	0.88 (4)	0.62
	CP-BJ	597	2.00 ± 0.19	22.92 (20.08–26.03)	2.85 (3)	2.71
	YC-NX	549	1.55 ± 0.14	46.19 (37.49–55.27)	1.75 (3)	5.45
	XY-SX	534	2.12 ± 0.15	14.35 (12.48–16.69)	0.35 (3)	1.70
	JZ-HB	560	1.30 ± 0.14	79.73 (60.36–116.33)	1.23 (4)	9.41
	SG-SD	570	1.35 ± 0.12	11.09(9.01–13.48)	1.45 (4)	1.31
	JY-HN	531	1.51 ± 0.13	59.52(48.44–75.76)	1.76 (4)	7.03
Bifenazate	IPP-SS	603	1.08 ± 0.11	7.49(5.12–10.07)	1.32 (6)	1.00

	HD-BJ	634	1.17 ± 0.12	46.85(37.54–61.30)	0.97 (5)	6.26
	CP-BJ	583	1.26 ± 0.13	97.37(71.91–147.12)	1.24 (5)	13.00
	YC-NX	610	1.70 ± 0.18	32.96(24.20–52.31)	4.99 (3)	4.40
	XY-SX	570	1.83 ± 0.15	26.90(23.16–31.17)	1.47 (4)	3.59
	JZ-HB	561	1.95 ± 0.13	38.61(32.87–45.69）	0.69 (4)	5.16
	SG-SD	611	1.69 ± 0.12	28.57(24.31–33.73)	3.44 (5)	3.81
	JY-HN	577	1.69 ± 0.13	45.95(37.89–57.67)	2.78 (4)	6.14
Bifenthrin	IPP-SS	508	1.82 ± 0.15	1.61(1.27–1.96)	2.02 (3)	1.00
	HD-BJ	585	1.03 ± 0.12	491.84(331.08–875.73)	1.94 (5)	306.25
	CP-BJ	569	1.81 ± 0.15	918.48(783.46–1097.60)	0.69 (4)	571.90
	YC-NX	565	1.28 ± 0.13	840.62(597.01–1335.76)	1.26 (3)	523.42
	XY-SX	599	2.07 ± 0.15	1791.45(1553.35–2076.74)	1.36 (5)	1115.47
	JZ-HB	561	2.29 ± 0.18	772.05(673.80–895.38)	3.47 (4)	480.73
	SG-SD	544	1.16 ± 0.11	2470.79(1831.35–3557.76)	1.15 (4)	1538.48
	JY-HN	542	1.72 ± 0.16	947.90(803.73–1133.10)	1.52 (3)	590.23
Cyflumetofen	IPP-SS	496	1.93 ± 0.20	0.63(0.53–0.77)	0.97 (3)	1.00
	HD-BJ	524	0.33 ± 0.10	1.45(0.52–2.72)	2.73 (4)	2.30
	CP-BJ	608	1.50 ± 0.14	32.90(27.47–40.70)	2.79 (5)	52.31
	YC-NX	508	1.71 ± 0.19	39.21(30.49–55.53)	1.49 (4)	62.34
	XY-SX	620	1.75 ± 0.14	48.37(40.84–58.86)	0.70 (5)	76.90
	JZ-HB	520	1.51 ± 0.12	52.00(42.28–65.75)	2.53 (4)	82.68
	SG-SD	587	1.74 ± 0.11	13.73(11.50–16.28)	2.05 (5)	21.83

	JY-HN	550	1.36 ± 0.13	30.81(25.11–37.31)	0.59 (4)	48.97
Cyenopyrafen	IPP-SS	529	1.62 ± 0.13	0.50(0.42–0.60)	1.65 (4)	1.00
	HD-BJ	602	1.68 ± 0.18	1.95(1.29–2.59)	2.04 (3)	3.88
	CP-BJ	538	1.19 ± 0.13	47.61(35.28-71.63)	0.82 (3)	94.84
	YC-NX	541	1.73 ± 0.17	13.92(9.78–18.46)	3.84 (3)	27.73
	XY-SX	624	1.43 ± 0.11	29.26(24.13–36.41)	0.95 (5)	58.27
	JZ-HB	584	1.27 ± 0.10	11.59(9.29–14.25)	2.28 (4)	23.01
	SG-SD	602	1.42 ± 0.13	34.07(26.46–46.89)	0.48 (3)	67.87
	JY-HN	548	1.53 ± 0.13	25.69(21.52–30.81)	1.83 (4）	51.16
B-azolemiteacrylic	IPP-SS	517	1.68 ± 0.13	0.48(0.40–0.58)	0.97 (4)	1.00
	HD-BJ	514	2.08 ± 0.28	18.25(15.59–20.79)	1.38 (3)	37.71
	CP-BJ	556	1.39 ± 0.15	6.23(4.91–8.62)	1.03 (3)	12.87
	YC-NX	598	1.68 ± 0.20	0.96(0.73–1.16)	0.94 (3)	1.98
	XY-SX	602	1.92 ± 0.16	1.30(1.140–1.48)	0.05 (3)	2.68
	JZ-HB	564	1.05 ± 0.11	1.59(1.23–2.12)	2.69 (4)	3.29
	SG-SD	636	1.37 ± 0.10	19.13(15.87–23.01)	2.27 (5)	39.50
	JY-HN	577	1.48 ± 0.14	2.24(1.84–2.82)	1.51 (3)	4.62

[†]Number of adult mites assayed, including controls.

[‡]FL = fiducial limit.

[§]Chi-square value and degrees of freedom.

[¶]RF (resistance fold) = LC_{50} value of a field population/LC50 value of the IPP-SS strain.

Table 3 Activities (means ± SE) of detoxification enzymes in the laboratory strain IPP-SS and in the seven field populations of *Tetranychus urticae*.

Population	P450		GST		CarEs		UGTs	
	nmol·min^{-1}·mg pro^{-1}	Ratio	nmol·min^{-1}·mg pro^{-1}	Ratio	nmol·min^{-1}·mg pro^{-1}	Ratio	nmol·min^{-1}·mg pro^{-1}	Ratio
IPP-SS	1.08 ± 0.05 bc	–	0.46 ± 0.01 c	–	4.00 ± 0.11 cd	–	9.05 ± 0.73d	–
HD-BJ	0.84 ± 0.03 d	0.78	0.48 ± 0.02 bc	1.04	3.26 ± 0.07 e	0.82	11.68 ± 0.97cd	1.29
CP-BJ	1.20 ± 0.06 b	1.11	0.61 ± 0.03 bc	1.33	4.77 ± 0.10 ab	1.19	14.22 ± 0.66c	1.57
YC-NX	0.96 ± 0.01 cd	0.89	0.64 ± 0.07 bc	1.38	3.59 ± 0.01 de	0.90	9.83 ± 0.19d	1.09
XY-SX	1.77 ± 0.17 a	1.64	1.15 ± 0.11 a	2.50	5.25 ± 0.08 a	1.31	19.40 ± 1.23b	2.15
JZ-HB	1.22 ± 0.07 b	1.13	0.60 ± 0.05 bc	1.30	4.34 ± 0.24 bc	1.08	12.96 ± 0.58c	1.43
SG-SD	1.10 ± 0.03 bc	1.01	0.58 ± 0.02 bc	1.26	4.81 ± 0.27 ab	1.20	14.03 ± 0.84c	1.55
JY-HN	1.19 ± 0.00 b	1.10	0.71 ± 0.07 b	1.55	5.20 ± 0.04 a	1.30	22.13 ± 0.78a	2.45

Means in each column followed by different letters show statistical differences according to Tukey-Kramer test.

Figure legends

Fig. 1 Frequencies of mutations in resistance alleles in seven field populations and in the laboratory strain IPP-SS of *Tetranychus urticae* in 2019–2020 in the current study (A-1, B-1, C-1, and D-1); for each pair of genes in A-1, B-1, and C-1, the first and second column refer to the first and the second gene, respectively, listed at the top. Also presented are comparisons of the mutation frequencies in three field populations in 2017 (Xu *et al.*, 2018b) and in 2019–2020 (the current study) (A-2, A-3, etc., with "a" and "b" along the bottom axis indicating data from 2017 and 2019–2020, respectively). SS, RS, and RR indicate homozygous susceptible, heterozygous, and homozygous resistant, respectively.