# Susceptibility and Activity of Glutathione S-Transferases in Nine Field Populations of Panonychus citri (Acari: Tetranychidae) to Pyridaben and Azocyclotin

Authors: Niu, Jin-Zhi, Liu, Guo-Ying, Dou, Wei, and Wang, Jin-Jun

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# SUSCEPTIBILITY AND ACTIVITY OF GLUTATHIONE *S*-TRANSFERASES IN NINE FIELD POPULATIONS OF *PANONYCHUS CITRI* (ACARI: TETRANYCHIDAE) TO PYRIDABEN AND AZOCYCLOTIN

JIN-ZHI NIU[1], GUO-YING LIU[2], WEI DOU[1] AND JIN-JUN WANG[1]
[1]Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, P.R. China

[2]Sichuan Entry-Exit Inspection and Quarantine Bureau, Chengdu 610041, P.R. China

ABSTRACT

Nine field collected populations of *Panonychus citri* from Chinese citrus orchards were assayed for susceptibility to pyridaben and the alternative acaricide azocyclotin and activity of glutathione *S*-transferases (GSTs). The results showed that populations from Pujiang, Wanzhou, and Pengshan exhibited a low level of sensitivity to pyridaben, but demonstrated a high level of sensitivity to azocyclotin. The correlation coefficient between GSTs activities and the $LC_{50}$ of pyridaben was $r = 0.93$ while the correlation coefficient between GSTs activities and the $LC_{50}$ of azocyclotin was $r = 0.03$. The $V_{max}$ value of CDNB (1-chloro-2, 4-dinitrobenzene) in populations from Beibei, Jintang, Pengshan, Wanzhou, and Zhongxian exhibited a: 2.5-, 11.6-, 7.0-, 5.1-, and 6.4-fold increase in resistance, respectively, relative to the pyridaben susceptible population. In addition, azocyclotin was the most sensitive inhibitor of the GSTs compared with the EA (ethacrynic acid) and pyridaben, based on the values for $I_{50}$. The current study suggested that GSTs might be involved in resistance of *P. citri* to pyridaben and but not azocyclotin in the field.

Key Words: citrus red mite, glutathione *S*-transferases, pyridaben, azocyclotin, resistance

RESUMEN

Nueve poblaciones de *Panonychus citri* recolectadas en huertos de cítricos en China fueron analizadas por su susceptibilidad al piridaben y un acaricida alternativo la azociclotina y la actividad de *S*-transferasa de glutatión (STG). Los resultados mostraron que las poblaciones de *P. citri* en Pujiang, Wanzhou y Pengshan presentaron un bajo nivel de susceptibilidad al piridaben, pero demostró un alto nivel de susceptibilidad a la azociclotina. El coeficiente de correlación entre las actividades de STG y la $CL_{50}$ de piridaben fue de $r = 0.93$, mientras que el coeficiente de correlación entre las actividades de STG y la $CL_{50}$ de azociclotina fue de $r = 0.03$. El valor de $V_{max}$ del CDNB (1-cloro-2, 4-dinitrobenceno) en las poblaciones de *P. citri* en Beibei, Jintang, Pengshan, Wanzhou y Zhongxian exhibió un aumento de: 2.5, 11.6, 7.0, 5.1 y 6.4 veces en resistencia, respectivamente, en relación con la población susceptible al piridaben. Además, azociclotina fue el inhibidor más sensible de la STG en comparación con el EA (ácido etacriníco) y piridaben, basado en los valores de $I_{50}$. El estudio actual sugiere que los STG podrían estar implicados en la resistencia de *P. citri* al piridaben, pero no a la azociclotina en el campo.

The citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), is a major pest worldwide (Gerson 2010). This mite can feed on 111 host plants and very commonly on citrus (Migeon & Dorkeld 2010). In southern China (Feng & Shi 2006) as well as in Japan (Furuhashi 1980), the populations have 2 infestation peaks every year, one in early summer (Jun-Jul), and the other in autumn (Oct-Nov), but maintain low density during late summer and winter. Failure to implement timely pest management adversely affects citrus harvest quantity and quality (weight, sugar content, and appearance) (Wang et al. 1999). Control of this mite depends largely on acaricide applications. Compared with insects, phytophagous mites including this species have biological/ecological traits which favor rapid development of resistance to acaricides, such as a short life cycle, abundant progeny, and arrhenotokous reproduction. For example, *Tetranychus urticae* and *P. ulmi* from the family Tetranychidae are among the 10 arthropod species, which were the first to develop pesticide resistance. Indeed *P. citri* is the third species that developed severe resistance from this family, and the control resistant mites has become exceedingly challenging (Van Leeuwen et al. 2010). According to the Arthropod Pesticide Resistance Database, 48 cases of resistance to acaricides by *P. citri* have been reported around the world, including 22 cases in China, 15 in Japan, 9 in USA, 1 in South Africa, and 1 in Georgia (Whalon et al. 2010). In USA, acaricide resistance of citrus red mite has been reported in California and Florida, and the acaricides include carbophe-

nothion, chlorfenson, demeton, dicofol, dioxathion, ethion, parathion, and tetradifon. In Japan, citrus red mite populations have developed resistance amitraz, benzoximate, binapacryl, chlorfenson, DDT, dicofol, dimethoate, fluoroacetate, oxydeprofos, phenkapton, and quinomethionate (Whalon et al. 2010). In addition, the citrus red mite has developed resistance to the recently developed acaricide, bifenzate, in Belgium, Japan, and Spain (Van Leeuwen et al. 2011). In China, the resistance status of citrus red mite is more severe compared to other counties, because Chinese citrus growers prefer to use acaricide sprays as the main means to control this mite (Lu et al. 2009). To control the mite population below the economic injury levels of 3-5 mites per leaf, weekly sprays are common during the peaks of heavy infestations (Ho 2000). Although acaricide spraying was an effective control measure in the past, its continued use in citrus orchards has disrupted natural biological systems and led to dramatic resurgences in mite populations. Resurgences are often a result of undesirable effects of acaricides on non-target organisms and the development of acaricide resistance (Zhao 2000). Increasing resistance levels to the most commonly used acaricides have led to multiple treatments, including overdoses; thereby raising serious environmental and human health concerns. Since acaricide resistance was first reported in 1979 in China, this mite has developed resistance to dicofol, pyrethroids, organotin miticide, hexythiazox, spirotetramat, amitraz, propargite, diafenthiuron, abamectin, and mitochondrial electron transport inhibitor (METI) acaricides (Hu et al. 2010; Huang 1979; Ran et al. 2009). These problems have highlighted the need to establish an efficient resistance management strategy based on all available information concerning the extent and nature of resistance.

The METI acaricides (i.e., tebufenpyrad, fenpyroximate, pyridaben, and fenazaquin), which are now widely used globally, were developed in the 1990s and inhibit complex I (NADH: ubiquinone oxidoreductase) of the mitochondrial respiratory pathway (Hollingworth & Ahammadsahib 1995). Pyridaben (2-*tert*-butyl-5-(4-*tert*-butyl-benzoylthio)-4-chloropyridazin-3(2H)-one) is a pyridazinone-derived acaricide that functions by binding its active site to a crucial co-enzyme in mitochondria (complex I at coenzyme site Q), and thus inhibits electron transport in phytophagous mites and insects (Denholm et al. 1998; Hirata et al. 1995). However, azocyclotin is an organotin miticide whose mode of action is to disrupt ATP formation by inhibiting oxidative phosphorylation (Van Leeuwen et al. 2010).

Pyridaben was introduced in China in 1992 and has been widely used in ornamentals and orchards to control mite pests resistant to conventional acaricides (Shi & Feng 2006). According to the Institute of the Control of Agrochemicals, Ministry of Agriculture, P. R. China (ICAMA), 346 commercial products of pyridaben were registered for use against *P. citri*. Compared to pyridaben, commercial products of other METI acaricides registered in China between 2008 and 2010 were 2 products containing fenazaquin and 44 products containing fenpyroximate, (ICAMA 2010). However, several field populations of *P. citri* have already developed high levels of pyridaben resistance in spite of its short term use. Recent investigation has demonstrated that the populations collected from the Chongqing municipality, Pinghe in Fujian Province, Linhai in Zhejiang province, and Yidu in Hubei province have expressed 163.3-, 266.5-, 417.9-, and 601.5-fold resistance to pyridaben, respectively (Hu et al. 2010). Judging by the present status, effort needs to be placed on resistance management to ensure future effective pest control. In addition, better understanding of mechanisms of resistance to pyridaben in *P. citri* is needed for logical selection of alternate acaricides for resistance management.

Glutathione *S*-transferases (GSTs, EC 2.5.1.18) belong to a supergene family of enzymes that are involved in phase II detoxification of xenobiotics, protection from oxidative damage, and intracellular transport of hormones, endogenous metabolites, and exogenous chemicals (Freitas et al. 2007; Huang et al. 1998). In insects, GSTs have been implicated as a major detoxification mechanism for several classes of insecticides i.e., organophosphates, pyrethroids, carbamates, and chlorinated hydrocarbons such as DDT (Lumjuan et al. 2005; Willoughby et al. 2007; Zhu et al. 2007). However, elevated levels of GSTs activity have been shown recently to be associated with the resistance of spider mite to acaricides, particularly in abamectin resistant populations of *T. urticae* (Konanz & Nauen 2004). Furthermore, experiments with synergists suggest that GSTs have a major role in pyridaben resistance in *P. citri* (Liu et al. 2010; Meng et al. 2000).

In the present study, 9 field collected populations of *P. citri* from China were investigated. Preliminary susceptibility screening of the commercially important acaricide pyridaben and a potential alternate acaricide, azocyclotin, was conducted. In addition, comparison analyses of the GSTs from the 9 populations were performed (including the activities, the kinetics, and an *in vitro* assay), in order to better understand possible mechanisms of pyridaben resistance in field populations of *P. citri*.

## Materials and Methods

### Mites

In 2010, *P. citri* were collected from citrus orchards in 9 locations in Sichuan Province and Chongqing municipality, China (Table 1). In each

TABLE 1. LOCATIONS, ORIGIN AND YEAR OF SAMPLING OF CHINESE *P. CITRI* POPULATIONS.

| Population | Location | Origin | Collection date |
|---|---|---|---|
| Beibei | Chongqing municipality | *Citrus reticulate* Banco | 4-16-2010 |
| Jiangjin | Chongqing municipality | *Citrus reticulate* Banco | 5-03-2010 |
| Wanzhou | Chongqing municipality | *Citrus reticulate* Banco | 5-11-2010 |
| Zhongxian | Chongqing municipality | *Citrus reticulate* Banco | 5-19-2010 |
| Jintang | Sichuan Province | *Citrus reticulate* Banco | 5-21-2010 |
| Jianyang | Sichuan Province | *Citrus reticulate* Banco | 5-14-2010 |
| Pengshan | Sichuan Province | *Citrus reticulate* Banco | 5-16-2010 |
| Pujiang | Sichuan Province | *Citrus reticulate* Banco | 5-29-2010 |
| Meishan | Sichuan Province | *Citrus reticulate* Banco | 4-11-2010 |

sample location, more than 2,000 mites with associated citrus leaves were collected from more than 10 citrus trees (Table 1). Fresh leaves, collected at random from *Citrus reticulata* Blanco mandarin orange trees with no prior pesticide exposure from orchards at the Citrus Research Institute, Chinese Academy of Agricultural Sciences, were used to rear the collected mites in the laboratory during the experiments. The leaves were replaced every 3 d. Sites were selected based on their importance as citrus production areas, and they received various levels of acaricide applications for the management of *P. citri* (Liu et al. 2010). The identity of *P. citri* was confirmed by J. J. Wang. Voucher specimens (50 for each population) were deposited in the insect collection of Southwest University, Chongqing, China.

### Chemicals

Formulated acaricides used in this study were pyridaben 150 g $L^{-1}$ EC (Saomanjing®) and azocyclotin 200 g $L^{-1}$ SC (Sanzuoxi®), which were purchased from Jiangsu Kesheng Group Co., Jiangsu, China, and Jiangxi Huxing Chemical Co., Jiangxi, China, respectively. Bovine serum albumin (BSA), coomassie brilliant blue G-250, and 1-chloro-2, 4-dinitrobenzene (CDNB) were supplied by Shanghai Chem. Ltd., Shanghai, China. Reduced glutathione (GSH) and Ethacrynic acid (EA) were purchased from Sigma (St. Louis, Missouri, USA).

### Bioassays

In general the bioassay procedures recommended by the Food and Agriculture Organization of the United Nations were followed (FAO 1980). Each field population was assayed by a slide-dip method with 7 treatment concentrations of each acaricide. Each acaricide was diluted with double-distilled water ($ddH_2O$) and various concentrations were tested until a satisfactory range (10-90% mortality) was ascertained. The control group was treated with $ddH_2O$ alone. Each slide, containing 30-40 adult female individuals that were 3-5 d old, was dipped into the pesticide solution for 5 s. The slides were placed in an incubator at 28 ± 1°C, 75-80% relative humidity, with a photoperiod of light: dark, 14:10 h. Mortality was assessed after 24 h. Mites that did not move after stimulation by a camel hair brush were scored as dead. Each treatment (comprising 3 slides) was replicated 3 times on 3 different days. Mortality data was corrected by Abbott's Formula (Abbott 1925) and analyzed by probit analysis to determine the lethal concentrations ($LC_{50}$) (Raymond 1985).

### GSTs Preparation

One hundred female adults from each population were homogenized in 4 mL ice-cold sodium phosphate buffer (0.02M; pH 7.3) and centrifuged at 5,000 g for 5 min at 4°C in a CF16RX refrigerated centrifuge (Hitachi Ltd, Tokyo, Japan). The pellets were discarded and the supernatant was again centrifuged at 4°C for 15 min at 17,500*g*. Finally, the supernatant was used as the enzyme source for GST activity assays. The protein contents of enzyme homogenates were determined according to the Bradford method with BSA as a standard (Bradford 1976). The measurement was performed with the A-5002 thermomax kinetic microplate reader (Tecan Ltd., Salzburg, Austria) at 595 nm.

### Determination of GSTs Activities and Kinetics

GSTs activity was determined with CDNB and GSH as substrates in 96-well microplates (Habig et al. 1974). The total reaction volume per well of a 96-well microplate was 300 µL. This consisted of 100 µL supernatant, 100 µL CDNB (containing 2% [v/v] ethanol) and 100 µL GSH in 0.05 M, pH 7.5 Tris-HCl, with a final concentration of 0.6 mM of CDNB and 6.0 mM and GSH. The non-enzymatic reaction of CDNB with GSH measured without homogenate served as the control. The change in absorbance was measured continuously for 5 min at 340 nm and 37°C in an A-5002 thermomax kinetic microplate reader

(Tecan Ltd., Salzburg, Austria). Changes in absorbance per minute were converted into nmol CDNB conjugated/min/mg protein based on the extinction coefficient of the resulting 2, 4-dinitrophenyl-glutathione ($\varepsilon_{340nm}$ = 9.6 $mM^{-1}$ $cm^{-1}$) (Habig et al. 1974).

The values of $V_{max}$ and $K_m$ of total GSTs from *P. citri* were determined for CDNB and GSH. The activity was recorded as a range of concentrations (0.02-0.6 mM for CDNB or 0.3-6.0 mM for GSH), while the concentrations of the other substrates were kept constant at 6.0 mM GSH or 0.6 mM CDNB. $V_{max}$ and $K_m$ values were calculated by the Michaelis-Menten equation in SPSS 10.0 for Windows (Rauch & Nauen 2004).

### *In vitro* Assay

To evaluate the sensitivity of GSTs to different inhibitors, 3 chemicals (pyridaben, azocyclotin, and EA) were selected to assess *in vitro* inhibition against GSTs. Stock solutions of the inhibitors were diluted with 0.05 M, pH 8.0, Tris-HCl buffer. Twenty-five µL of the enzyme source and 25 µL inhibitor solutions, with an appropriate concentration range (i.e., 1.0-1,000 mg/L for EA, 0.48-384.70 mg/L for pyridaben, and 0.86-1,713.00 mg/L for azocyclotin (which were ascertained from various concentrations of each inhibitor to test the inhibition rate until concentrations causing 10-90% inhibition were delimited), were incubated for 5 min at 37°C and added to the CDNB/GSH substrate mixture as described above. Reactions without the inhibitor were included as controls. The median inhibition concentration ($I_{50}$) for each inhibitor was determined based on the log-concentration vs. probit (% inhibition) regression analysis. Three replications were conducted for each treatment above.

### Statistical Analysis

All the data from the 9 field populations of *P. citri* were analyzed by analysis of variance (ANOVA), the means were separated by Duncan's Multiple Range Test or LSD Test for significance ($P$ = 0.05) with SPSS 10.0 for Windows (SPSS 1999). Regression analysis was performed to calculate the GST kinetic parameters, $LC_{50}$ values of different acaricides, and $I_{50}$ values of different inhibitors. The significant level of resistance rate (RR) was ascertained by the confidence interval overlap of the $LC_{50}$ values. The Chi-square goodness-of-fit test was used to determine the difference between the theoretic and the measured values of acaricide toxicity results. Correlation analyses were conducted to find the relationship between GSTs activity and $LC_{50}$ values of each acaricide against the 9 populations of *P. citri*.

## Results

### Bioassays

Compared with the reference dose of each acaricide, the Jiangjin and Meishan populations were susceptible to pyridaben and azocyclotin, respectively. In addition, the population sampled from Jiangjin expressed the greatest sensitivity to pyridaben and thus was regarded as relatively susceptible to pyridaben compared to the other 8 populations. Comparison among resistance ratios (RR) with the relatively pyridaben-susceptible population indicated that all other 8 field collected populations had significant levels of resistance. Resistance ratios ranged from 2 (Jianyang population) to 140 (Pujiang population) (Table 2).

The Meishan population had the lowest $LC_{50}$ value when treated with azocyclotin and was used as the relatively susceptible population to azocyclotin for comparison to the other 8 populations. Comparison of RR values to the relatively azocyclotin susceptible population suggested that only the Beibei, Jiangjin, Jintang, Wanzhou, and Zhongxian populations had significant levels of resistance. Resistance ratios ranged from 2 (Jianyang, Pengshan, Pujiang, and Wanzhou populations) to 36 (Beibei population) (Table 2).

### Activity of GSTs

The Pujiang population exhibited 4.7-fold increased GST activity in comparison with the pyridaben susceptible population (Jiangjin population), and GST activity in the Beibei, Jintang, Meishan, Pengshan, Wanzhou, and Zhongxian populations was increased 2.3-,1.9-, 1.8-, 2.1-, 2.2-, and 2.7-fold, respectively, in comparison with the Jiangjin population. GST activity in the Jianyang population was 0.9-fold lower than in the Jiangjin population (Fig. 1). In addition, the correlation coefficient between GST activity and $LC_{50}$ of pyridaben was $r$ = 0.93, while the correlation coefficient between GST activity and $LC_{50}$ of azocyclotin was $r$ = 0.03 (Fig. 2).

### Kinetic Parameters of GSTs

The kinetic parameters of GSTs in *P. citri* were determined from Lineweaver-Burk plots and presented in Table 3. For the catalytic activity of GSTs toward GSH and CDNB as expressed by the $V_{max}$ value, the Pujiang population had the highest $V_{max}$ value and exhibited a 276.0-fold increased catalytic activity of GSH in comparison with the susceptible Jiangjin population. The catalytic activity of GSH in Jintang, Pengshan, and Zhongxian was increased (8.8-, 2.5-, and 19.0-fold, respectively) compared with the susceptible Jiangjin population. The catalytic activity of GSH in the Beibei, Jianyang, Meishan, and Wanzhou

TABLE 2. SUSCEPTIBILITIES OF 9 FIELD-COLLECTED POPULATIONS OF *P. CITRI* TO PYRIDABEN AND AZOCYCLOTIN.

| Acaricide | Population | *n* | $\chi^{2a}$ | Slope (±SE) | $LC_{50}$ [95%CI] (mg liter$^{-1}$) | RR [95%CI] |
|---|---|---|---|---|---|---|
| Pyridaben | Jiangjin | 616 | 1.33 | 1.12 (±0.17) | 0.2 [0.2; 0.3] | — |
| | Beibei | 710 | 2.11 | 0.52 (±0.11) | 2.0 [1.1; 3.1] | 9 [6; 11] |
| | Jianyang | 687 | 4.68 | 0.89 (±0.13) | 0.5 [0.3; 0.6] | 2 [2; 2] |
| | Jintang | 485 | 3.71 | 0.61 (±0.13) | 1.7 [1.0; 3.0] | 8 [6; 10] |
| | Meishan | 575 | 2.18 | 0.63 (±0.12) | 1.1 [0.7; 1.4] | 5 [4; 5] |
| | Pengshan | 473 | 0.23 | 0.93 (±0.18) | 2.6 [1.6; 4.0] | 12 [9; 14] |
| | Pujiang | 527 | 2.00 | 1.57 (±0.33) | 30.9 [23.0; 55.1] | 140[134; 192] |
| | Wanzhou | 519 | 0.99 | 0.75 (±0.16) | 5.3 [3.8; 7.8] | 24 [22; 27] |
| | Zhongxian | 670 | 2.08 | 1.22 (±0.17) | 14.9 [11.7; 20.6] | 68 [68; 71] |
| Azocyclotin | Meishan | 470 | 5.48 | 0.31 (±0.08) | 40 [21; 70] | — |
| | Beibei | 566 | 2.06 | 0.63 (±0.10) | 1463 [1063; 1656] | 36 [30; 37] |
| | Jiangjin | 515 | 5.29 | 1.00 (±0.15) | 128 [72; 206] | 3 [2; 5] |
| | Jintang | 688 | 4.03 | 0.45 (±0.15) | 304 [239; 337] | 8 [7; 8] |
| | Jianyang | 639 | 5.86 | 0.61 (±0.12) | 88 [(50; 139) | 2 [1; 3] |
| | Pengshan | 507 | 5.50 | 0.71 (±0.12) | 87 [53; 154] | 2 [1; 3] |
| | Pujiang | 519 | 3.76 | 0.80 (±0.13) | 60 [32; 93] | 2 [1; 2] |
| | Wanzhou | 498 | 4.07 | 0.70 (±0.09) | 104 [71; 157] | 2 [2; 4] |
| | Zhongxian | 621 | 3.39 | 0.46 (±0.10) | 734 [683; 1115] | 18 [17;25] |

Note: *n* = number of mites; RR = resistance ratio; CI = confidence interval.
[a]Chi-squared goodness-of-fit test.

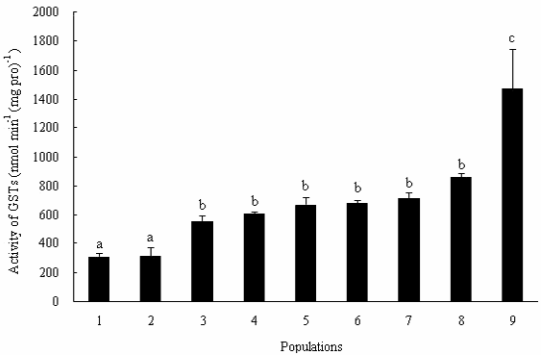


Fig. 1. Glutathione *S*-transferases activity of various populations of *P. citri*. 1, 2, 3, 4, 5, 6, 7, 8, 9 corresponding to Jianyang, Jiangjin, Meishan, Jintang, Pengshan, Wanzhou, Beibei, Zhongxian, Pujiang, respectively. Each value represents the mean of three determinations (*n* = 3). Error bars represent SE. Different letters indicate significant differences in ANOVA (LSD, $P < 0.05$).

populations decreased compared with the susceptible Jiangjin population. In addition, the catalytic activities of CDNB in the Beibei, Jintang, Pengshan, Wanzhou, and Zhongxian populations were significantly higher than the pyridaben susceptible population, and exhibited 2.5-, 11.6-, 7.0-, 5.1-, and 6.4-fold increases, respectively. However, the catalytic activity of CDNB in the Jianyang, Meishan, and Pujiang populations was 0.7-, 0.1- and 0.3-fold lower, respectively, than that of the pyridaben susceptible population; but no significant difference of catalytic activity of CDNB was detected among these populations ($P < 0.05$) (Table 3).

All field collected populations had higher $K_m$ values of GSH compared to the reference $K_m$ value in the pyridaben susceptible population. Among these, the Pengshan population exhibited a 4.1-fold increase in $K_m$ value. In addition, the $K_m$ value of CDNB in the Jintang, Meishan, Pengshan, Wanzhou, and Zhongxian populations suggested 2.3-, 1.3-, 10.2-, 5.4-, and 2.9-fold increases, respectively, compared with the pyridaben susceptible population. However, the Pujiang population was determined as having the smallest $K_m$ value toward CDNB, while the Pengshan population was determined as having the largest $K_m$ value toward CDNB among all the populations (Table 3).

*In vitro* assay

The median inhibition concentrations ($I_{50}$s) of the 3 inhibitors (EA, pyridaben, and azocyclotin) were calculated for GSTs from the 9 field collected populations of *P. citri* (Table 4). The statistical analysis indicated that azocyclotin was the most sensitive inhibitor of the GSTs ($I_{50}$ values ranging from 0.00 to 0.52 mg liter$^{-1}$) compared with the $I_{50}$ values of EA and pyridaben, which ranged between 0.03 to 3.60 and 0.03 to 28.94 mg liter$^{-1}$, respectively.

## DISCUSSION

*Panonychus citri* presents a challenge to pest managers due to its inherent ability to develop re-

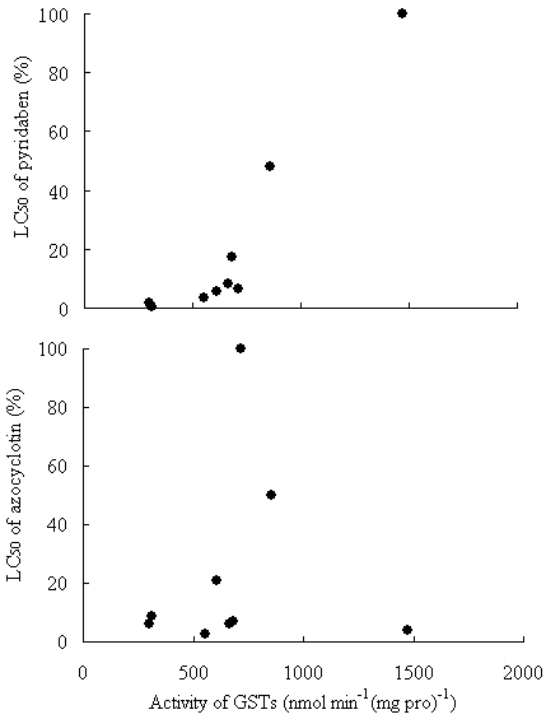


Fig. 2. Correlation analyses between glutathione *S*-transferases activity and $LC_{50}$ of acaricides on various populations of *P. citri*. Percentage of $LC_{50}$ of pyridaben and azocyclotin was calculated based on the highest $LC_{50}$ value of pyridaben (Pujiang population) and $LC_{50}$ value of azocyclotin (Beibei population), respectively.

sistance in a few generations. In recent years, some conventional control options for citrus red mite have become restricted, because the use of more toxic compounds, such as dicofol, has been strictly prohibited. Some novel acaricides, such as METIs and spirodiclofen, have been introduced recently. However, after several years of effective use in citrus, resistance problems have arisen. In some citrus growing areas, growers use these novel acaricides more than 5 times per growing season while the label guidelines specify only 2 applications per year (Hu et al. 2010). Development of resistance in citrus red mite can increase grower costs by more than two-fold and theses can mount further as resistance levels escalate. In the current study, the susceptibilities of 9 field collected populations of *P. citri* to the acaricides, pyridaben, and azocyclotin, were determined. Mites from the Pengshan, Pujiang, Wanzhou, and Zhongxian populations exhibited >10-fold resistance to pyridaben compared with the pyridaben-susceptible population collected in Jiangjin. However, with the exception of the Zhongxian population, the preceding populations also had the lowest resistance ratio (<5) for azocyclotin compared with the azocyclotin-susceptible population collected in Meishan. Therefore, azocyclotin may be an effective alternative to manage resistance to pyridaben. Previous studies with a field-collected population of *P. citri* selected with pyridaben for 12 generations showed that it had developed 35-fold resistance, while it still exhibited a low level of cross-resistance (4-fold) to azocyclotin (Meng et al. 2000). A similar low level of cross resistance to azocyclotin also was found in *T. urticae*. A pyridaben-resistant colony, maintained in the laboratory, was established by treatment with pyridaben for 20 generations (PR-20 population); and it was extremely resistant to pyridaben (resistance ratio = 240), but showed low levels of resistance (resistance ratio = 3.8) to azocyclotin (Kim et al. 2006). Pyridaben belongs to the METI-acaricides, and cross-resistance between METIs has been reported both in laboratory-selected populations and in field-collected populations of *T. urticae* (Kim et al. 2006; Stumpf & Nauen 2001). The responses of $F_2$ females from the reciprocal crosses of resistant and susceptible individuals suggest that resistance to pyridaben is under monogenic control (Van Pottelberge et al. 2009b). Monogenic resistance, which is considered more likely to spread within populations than polygenic resistance, tends to be more stable and is less easily managed (Roush & McKenzie 1987). Based on the different modes of action between pyridaben and azocyclotin, azocyclotin may be a good rotation acaricide for the management of pyridaben resistance.

The mechanisms of resistance to acaricides in mite species are reduced penetration, enhanced metabolism, and target site insensitivity. Of these factors, enhanced metabolic detoxification by GSTs, mixed function oxidases (MFOs), and/or esterases (ESTs) has been considered as a main mechanism of acaricide resistance of mites (Van Nieuwenhuyse et al. 2009; Van Pottelberge et al. 2009a). Experiments with synergists (DEM, PBO, and TPP inhibitors against GSTs, MFO, and EST, respectively) suggest that GSTs play a major role in pyridaben resistance in field collected pyridaben-resistant *P. citri* populations (Liu et al. 2010), while the same result was also reported in a laboratory pyridaben-selected population of *P. citri* (Meng et al. 2000). However, an experiment with synergists involving the PR-20 population of *T. urticae* (a field collected population that was further selected with pyridaben for 20 generations) revealed that MFO plays a major role in pyridaben resistance in this population (Kim et al. 2006). We suggest that the different results from the synergist experiments between *P. citri* and *T. urticae* might be caused by differences in interspecific and non-specialized synergistic activity (Young et al. 2005). In the present study, the activity of GSTs from 9 field collected citrus red mites increased as the pyridaben resistance lev-

TABLE 3. APPARENT KINETIC PARAMETERS OF GLUTATHIONE *S*-TRANSFERASES TOWARDS GSH AND CDNB IN THE 9 FIELD-COLLECTED POPULATIONS OF *P. CITRI*.

| | GSH | | CDNB | |
|---|---|---|---|---|
| Population | $V_{max}$ | $K_m$ | $V_{max}$ | $K_m$ |
| Jiangjin | 311 ± 11 a | 87 ± 11 a | 210 ± 10 a | 58 ± 14 bc |
| Jianyang | 264 ± 64 a | 87 ± 2 a | 153 ± 25 a | 12 ± 2 a |
| Meishan | 11 ± 0 a | 328 ± 12 cd | 16 ± 1 a | 73 ± 10 c |
| Jintang | 2744 ± 178 a | 306 ± 15 c | 2427 ± 36 f | 134 ± 18 d |
| Beibei | 194 ± 53 a | 190 ± 59 b | 527 ± 37 b | 33 ± 2 ab |
| Pengshan | 776 ± 148 a | 359 ± 14 d | 1484 ± 393 d | 593 ± 56 f |
| Wanzhou | 11 ± 1 a | 343 ± 25 cd | 1088 ± 26 c | 316 ± 14 e |
| Zhongxian | 5907 ± 276 a | 318 ± 25 cd | 1334 ± 280 cd | 167 ± 11 d |
| Pujiang | 85841 ± 10084 b | 120 ± 7 a | 68 ± 4 a | 5 ± 0 a |

Each value represents the mean (± SE). Mean values within the same column followed by different letters are significantly different in ANOVA (LSD, $P < 0.05$). $V_{max}$: nmolmin$^{-1}$(mg pro)$^{-1}$. $K_m$: µM.

els increased (Pujiang > Zhongxian > Wanzhou > Pengshan > Beibei > Jintang > Meishan > Jianyang > Jiangjin) with the exception of the Beibei and Jianyang populations. The analysis showed that the correlation coefficient between GSTs activities and the $LC_{50}$ of pyridaben was $r = 0.93$, while the correlation between GSTs activities and the $LC_{50}$ of azocyclotin was only $r = 0.03$ (Fig. 2). This could mean that in *P. citri* field populations GSTs were involved in resistance of to pyridaben but not to azocyclotin.

All the kinetic parameters (including $V_{max}$ and $K_m$ for both GSH and CDNB) in the Jintang, Pengshan, and Zhongxian populations increased significantly compared with the pyridaben susceptible population from Jiangjin. In addition, the catalytic activity of CDNB (presented by $V_{max}$) in the Beibei, Jintang, Pengshan, Wanzhou, and Zhongxian populations were found to be significantly greater than the pyridaben susceptible population (2.5-,11.6-, 7.0-, 5.1-, and 6.4-fold increase, respectively), indicating an over-expression (Konanz & Nauen 2004) or structural alteration (Wang et al. 2008) of GSTs in these populations. The $K_m$ value of GSH in all field collected populations increased relative to the $K_m$ value in the pyridaben susceptible population. In addition, the $K_m$ values of CDNB in the Jintang, Meishan, Pengshan, Wanzhou, and Zhongxian populations was 2.3-, 1.3-, 10.2-, 5.4-, and 2.9-fold, respectively, greater than the $K_m$ value of the pyridaben susceptible population.

Ethacrynic acid (EA) always presents a strong inhibitory effect on GSTs in many pests, i.e., *Blattella germanica* (L.) (Blattellidae) (Yu & Huang 2000), *T. urticae* (Konanz & Nauen 2004), and *Liposcelis paeta* (Pearman) (Liposcelididae) (Wu et al. 2009). In our current study, azocyclotin had the greatest inhibitory effect on GSTs compared with EA and pyridaben, indicating that azocyclotin could be used as an effective pesticide synergist for the control of pesticide resistance caused

TABLE 4. $I_{50}$ VALUES OF *IN VITRO* INHIBITION OF GLUTATHIONE S-TRANSFERASES FROM 9 FIELD-COLLECTED POPULATIONS OF *P. CITRI*.

| | $I_{50}$ | | |
|---|---|---|---|
| Population | EA | Pyridaben | Azocyclotin |
| Jiangjin | 0.03 ± 0.00 a | 3.21 ± 0.53 a | 0.03 ± 0.00 a |
| Jianyang | 0.37 ± 0.00 b | 10.26 ± 0.00 b | 0.52 ± 0.18 c |
| Meishan | 0.83 ± 0.20 c | 27.21 ± 2.38 e | 0.32 ± 0.16 b |
| Jintang | 3.60 ± 0.45 e | 12.72 ± 4.41 c | 0.00 ± 0.00 a |
| Beibei | 1.16 ± 0.02 d | 17.55 ± 0.74 cd | 0.08 ± 0.01 a |
| Pengshan | 0.06 ± 0.00 a | 24.21 ± 2.52 de | 0.02 ± 0.00 a |
| Wanzhou | 0.98 ± 0.03 cd | 28.94 ± 9.17 e | 0.49 ± 0.00 c |
| Zhongxian | 0.24 ± 0.00 ab | 18.40 ± 3.28 bcd | 0.01 ± 0.00 a |
| Pujiang | 1.09 ± 0.02 cd | 0.03 ± 0.01 a | 0.00 ± 0.00 a |

Inhibition measured as 50% inhibitory concentration (i.e., $I_{50}$) in mg liter$^{-1}$. Each value represents the mean (± SE). Mean values within the same column followed by different letters are significantly different in ANOVA (LSD, $P < 0.05$).

by GSTs, i.e., azocyclotin could block GST activity due to rapid depletion of GSH (Li et al. 2009). Nevertheless, the effectiveness of azocyclotin as a synergist requires further research.

In summary, the present study has provided some basic information on the toxicity of pyridaben and azocyclotin against *P. citri*, and certain characteristics of GST activity in 9 field populations of *P. citri* were described. In addition, our study illustrated that GSTs could be associated with varying susceptibility levels to pyridaben. This is only the first step in the biochemical differentiation of various *P. citri* field populations. The results of further studies will contribute to the understanding of the mechanisms of acaricide resistance in *P. citri*.

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