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Full Length Research Paper

Laboratory selection for spirodiclofen resistance and cross-resistance in *Panonychus citri*

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Selection for spirodiclofen resistance was done in the laboratory with a susceptible strain of the citrus red mite, *Panonychus citri* (McGregor). Successive selections for spirodiclofen resistance through 42 generations resulted in a high level of resistance and the resistance ratio at LC_{50} was 103 compared with that of the original susceptible strain. In the selected strain, the level of resistance to spirodiclofen was high at all developmental stages except eggs and *P. citri* adult infertility caused by spirodiclofen was decreased. Cross-resistance to spirotetramat was detected but not to 10 other acaricides. Synergist experiments indicated that, piperonyl butoxide (PBO), *S*,*S*,*S*-tributyl-phosphorotrithioate (DEF) and diethyl maleate (DEM) synergized the toxicity of spirodiclofen in the selected strain by 3.3, 2.3 and 1.6 fold and the resistance ratio decreased from 103 to 38, 45 and 65, respectively, but no synergism was found in the susceptible strain.

Key words: Spirodiclofen, laboratory selection, cross-resistance, synergism, *Panonychus citri.*

INTRODUCTION

Spirodiclofen belongs to the acaricidal group of spiroyclic tetronic, which are the acid derivatives newly discovered and developed by Bayer CropScience. Spirodiclofen has excellent efficacy against all developmental stages of mites, including agriculturally important pests such as *Tetranychus urticae*, *Panonychus ulmi*, *Panonychus citri*, *Aculus schlechtendali*, *Phyllocoptruta oleivora* and *Brevipalpus phoenicis* (Bretschneider et al., 2007). Additionally, spirodiclofen reduces the fecundity of adult female mites; the number of eggs laid is greatly reduced and those laid by females that had been exposed to sublethal doses of spirodiclofen are infertile. Furthermore, spirodiclofen does not show cross-resistance to conventional acaricides (Rauch and Nauen, 2002) and has been used worldwide to control phytophagous mites, including various pesticide-resistant mites, in citrus fruit, pome fruit, stone fruit, grapes and nuts under an integrated pest management system.

The citrus red mite, *P. citri* (McGregor) (Acari: Tetranychidae), is an important pest of citrus trees(Zhang et al., 1994). Similar to other phytophagous mites (Van Leeuwen et al., 2010), the citrus red mite has biological/ ecological traits that favor rapid development of resistance to acaricides, including (1) high reproductive potential, (2) arrhenotokous reproduction and (3) low mobility (Yamamoto et al., 1995; Xia, 2003). The citrus red mite has shown field-resistance to organophosphates, dicofol, pyrethroids, organotin miticide, hexythiazox, abamectin, METI (mitochondrial electron transport inhibitor) acaricides and recently, bifenazate (Huang 1979; Huang et al., 1994; Chen et al., 1996; Huang et al., 1999; Chen et al., 2001; Hu et al., 2010; NATESC, 2003; Van Leeuwen et al., 2011). Resistance mechanism in Acari have shown to be similar to those reported from insects, including target-site resistance and enhanced detoxification and more recently, the elucidation of the molecular basis of resistance in mites has started (Van Leeuwen et al., 2010).The development and use of new chemical pesticides and their correct use in resistance management programs remains one of the best approaches to overcoming the problem of acaricide-resistance in mites (Sato et al., 2005). Since spirodiclofen entered Chinese market, it has become one of the most popular acaricides against citrus red mites. However, the possibility of the citrus red mite resistance development to spirodiclofen has become a matter of concern. The deve-

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lopment of spirodiclofen resistance has been found in citrus red mites collected from two citrus orchards in China (Hu et al., 2010). A highly resistant strain of another phytophagous mite, *T. urticae* (Koch), has been achieved in the laboratory (Van Pottelberge et al., 2009a, b).

Well characterized laboratory-selected strains that exhibit high levels of resistance to a toxin are especially useful in validating and improving resistance management strategies (Shelton et al., 2000; Zhao et al., 2003). In this study, a spirodiclofen-resistant strain was selected from a susceptible strain of *P. citri* under laboratory conditions, as part of the risk assessment of spirodiclofen resistance in this species. This study investigated the levels of resistance at each developmental stage of the mite and the spectrum of cross-resistance to other acaricides in the spirodiclofen-resistant strain. The possible effect of synergists known to inhibit important detoxification enzymes was investigated to gain insight into spirodiclofen detoxification in the resistant strain. Piperonyl butoxide (PBO), *S*,*S*,*S*-tributyl-phosphorotrithioate (DEF) and diethyl maleate (DEM) were used to inhibit cytochrome P450 monooxygenases, esterases and glutathione-*S*-transferases, respectively.

MATERIALS AND METHODS

Acaricides and other chemicals

Spirodiclofen (Envidor®, 240 g [AI]/liter SC) and spirotetramat (Movento®, 240 g [AI]/liter SC) were supplied by Bayer CropScience (Hangzhou, China), abamectin (Hisun®, 1.8% EC) by Zhejian Hisun Chemical Co. (Taizhou, China), fenpropathrin (Meothrin®, 20% EC) by Sumitomo Chemical (Japan), hexythiazox (Nissorun®, 5% EC) by Nippon Soda Co. (Japan), pyridaben (Saomanjing®, 15% EC) by Kesheng Group Co. (Nanjing, China), fenbutation oxide (Torque®, 50% WP) and chlorfenapyr (Chujin®, 100 g [AI]/liter SC) by BASF Co. (China), fenpyroximate (Bamanling®, 50 g [AI]/liter SC) by Nihon Nohyaku Co., clofentezine (Apollo®, 200 g [AI]/liter SC) by Aventis Co. and amitra (Bailing®, 20% EC) by Bailing Agrochemical Co. All of the other chemicals used were obtained from Sigma-Aldrich Co., USA.

Citrus red mite

A susceptible strain of *P. citri* (LS-FJ) that has been maintained in the laboratory without exposure to pesticides since 1998 was used as a control in bioassays. All mites were cultured by the detached leaf method (Hu et al., 2010) at 25 ± 1°C and 70 ± 5% relative humidity with a 16 h light/8 h dark regime. To prevent the detached citrus leaf from drying, it was placed onto a 3 mm layer of water-saturated sponge held in a glass Petri dish. Twenty females were allowed to oviposit on the leaf for 1 day, and then removed. Under these conditions, the mites developed successfully from egg to adult on the detached leaf.

Laboratory selection for resistance

Strain LS-FJ was exposed to successive applications of a gradually increasing concentration of spirodiclofen. About 5000 larvae were used to start the selection experiment. Larvae were used for resistance selection because the morphological intoxication symptoms of spirodiclfofen and spirotetramat are less pronounced in adults (Rauch and Nauen 2002). Twenty females were allowed to oviposit as described earlier. The larvae that hatched after 7 days were used for the selection procedure. The concentration of spirodiclofen used for selection was changed every three generations to approximately the LC_{90} of spirodiclofen, for the last selected generation, by leaf-dip bioassay described further. The spirdiclofen-resistant strain established by this selection procedure was designated SR-FZ.

Bioassays

The toxicity of various acaricides was measured using a detached citrus leaf dip bioassay method similar to that described by Yamamoto et al. (1995) and Hu et al. (2010). Fully expanded *Citrus reticulata* leaves were collected at random from trees that had not been exposed to pesticide, growing in orchards at the fruit research institute of the Fujian Academy of Agricultural Science. Leaves were washed thoroughly and dried before use in the bioassay. The detached leaves were dipped for 8 s into solutions of the test acaricides, which were prepared at 5 to 8 different concentrations using distilled water containing 1 g/L of the nonionic surfactant Triton X-100, and then allowed to air dry. Leaves treated with distilled water containing Triton X-100 alone were used as control. When the leaves had dried, discs of approximately 3 cm diameter were cut from the dry leaf and placed onto a 3 mm layer of water-saturated sponge held in a glass Petri dish (9 cm in diameter).

For the egg and larval bioassays, 20 adult females were transferred to untreated leaf discs and allowed to oviposit for 1 day. Leaves with eggs attached were dipped into an acaricide solution for 8 s and hatched larvae were counted after 7 days. For the larval bioassay, leaves with larvae were treated with acaricides as described earlier and mortality was assessed after 2 days. For adult protonymph and deutonymph assays, 15 to 20 adult female protonymph and deutonymph were transferred to each treated leaf using a camel hair brush. Sterilizing activity against females was also determined following the method by Yamamoto et al. (1995). After inoculation for 1 day on detached leaves treated with spirodiclofen, 10 females were transferred to untreated leaves in order to lay eggs for one day. Unhatched eggs on the untreated leaves were counted 8 days after oviposition. Mortality was assessed after 2 days; mites were prodded with the brush and scored alive if they could walk normally and dead if they could not.

Susceptibility to spirodiclofen at each stage of development

The susceptibility of citrus red mites to spirodiclofen at each developmental stage of the LS-FJ and SR-FZ strains was determined by the bioassay described earlier. Mortality of eggs and the other stages were recorded at 7 days and at 2 days after treatment, respectively. Sterilizing activity against females was also investigated. After feeding for 1 day on leaves treated with spirodiclofen, 20 females were transferred to untreated leaves and allowed to oviposit for 1 day. The number of unhatched eggs on each untreated leaf was counted 7 days after oviposition.

Cross-resistance and synergism studies

The LS-FJ and SR-FZ strains were tested for cross-resistance to 11 other acaricides. Larvae were used for the bioassay of spirodiclfofen, spirotetramat, hexythiazox and clofentezine, and adult females were used for other acaricides.

In order to check for metabolic resistance through the use of synergists, newly hatched larvae were treated with PBO, DEF or DEM, 3 h before treatment with spirodiclofen, as described earlier.

On the basis of preliminary tests, the doses of PBO, DEF and DEM was chosen as the highest dose that caused maximum 5% mortality (500 mg L^{-1} PBO, 10 mg L^{-1} DEF and 200 mg L^{-1} DEM). Mites treated with synergist only served as control.

Data analysis

Abbott's formula was used to calculate percentage mortality after correction for control mortality (Abbott, 1925). All control mortalities were <5%. The LC_{50} values, slopes and 95% confidence limits were calculated by probit analysis (POLO, LeOra Software, Berkeley, CA). Resistance ratios (RRs) were calculated by dividing the LC_{50} value of the resistant strain by the LC_{50} value of the susceptible strain. The RR values of ≤10, 10 to 40, 40 to 160 and ≥160 indicate low, moderate, high and very high resistance, respectively (Fukami et al., 1983). Synergism ratios (SRs) were calculated using the formula and statistics of dose ratios (Robertson and Preisler, 1992).

RESULTS

Selection for spirodiclofen resistance

The development of spirodiclofen resistance in a susceptible strain of the citrus red mite was shown in Figure 1. The successive selection for spirodiclofen resistance through 42 generations resulted in a high level of resistance (the SR-FZ strain) and RR at LC_{50} was 103 compared with the LS-FJ strain. The resistance level increased gradually from the 1st to the 27th generation and rapidly after the 30th selection.

Susceptibility of developmental stages

Susceptibility to spirodiclofen at each developmental stage in the SR-FZ and LS-FJ strains is summarized in Table 1. In the LS-FJ strain, spirodiclofen was active against eggs, larvae, protonymphs and deutonymphs and LC_{50} values were 2.20, 4.60, 2.70 and 7.57 mg (a.i.)/L, respectively. Hatching inhibition by spirodiclofen was observed in eggs laid by treated LS-FJ females (LC_{50}, 40.78 mg (a.i.)/L). High resistance to spirodiclofen was detected in larvae, protonymphs and deutonymphs in the SR-FZ strain. The RR values of spirodiclofen for the larva, protonymph and deutonymph stages of the SR-FZ strain were 103, 157 and 51.2, respectively. However, the eggs of SR-FZ strain was only 4.6-fold more tolerant to spirodiclofen than the eggs of LS-FJ, which showed low resistance in eggs between the two strains. On the other hand, hatching inhibition by spirodiclofen was not found at 1000 mg (a.i.)/L in the SR-FZ strain, but the LC_{50} of sterile against female in LS-FJ was 8.43 mg (a.i.)/L.

Cross-resistance and synergism tests

With the laboratory susceptible LS-FJ strain as control, the resistant strain was tested for cross-resistance to different acaricides, and the results were given in Table 2. Based on overlapping 95% CI of LC_{50} values, the SR-FZ strain exhibited no cross-resistance to hexythiazox, fenprpathrin, fenbutatin oxide or γ-cyhalothrin. In the case of abamectin, pyridaben, fenpyroximate, clofen-tezine, chlorfenapyr, bifenthrin and amitraz, the SR-FZ strain had a slightly higher LC_{50}, indicating that this possibly associated resistance is of only minor impor-tance. The highest cross-resistance (RR = 29.5) was observed with the tetronic acid derivate spiromesifen.

The effect on the toxicity of spirodiclofen after treatment with PBO, DEF and DEM was given in Table 3. PBO, DEF and DEM did not significantly synergize spirodiclofen activity in the LS-FJ strain; LC_{50} confidence limits overlapped for mites with and without synergism. PBO, DEF and DEM synergized activities of spirodiclofen in the SR-FZ strain by 3.3-, 2.3- and 1.6-fold and decreased the resistance ratio from 103 to 38, 45 and 65, respectively,.

DISCUSSION

P. citri was shown to develop resistance more slowly to spirodiclofen than other acaricides when selected continuously with increasing concentrations under laboratory conditions. After selection for 12 generations in the laboratory, *P. citri* showed only 3.7-fold resistance to spirodiclofen, but the mite developed 17.1-, 35.0-, 7.3- and >23,000-fold resistance to fenpropathrin, pyridaben, abamectin (Meng et al., 2000, 2002) and hexythiazox (Yamamoto et al., 1995) respectively, under similar conditions. Another phytophagous mite, *T. urticae* (Acari, Tetranychidae), also showed slow development of spirodiclofen resistance. Rauch and Nauen (2002) reported that, laboratory selection of a *T. urticae* strain collected in the field in Italy resulted in a slow increase of resistance over 21 months and 37 selection cycles with spirodiclofen and the selected strain exhibited only a 13-fold resistance. Van Pottelberge et al. (2009b) thought that an accumulation of resistance factors with minor effect during selection in *T. urticae* could be the reason for the slow development of resistance to spirodiclofen although 274-fold high resistance to spirodiclofen was achieved in *T. urticae* selected at last. A high level of resistance to spirodiclofen has been detected in populations of *P. citri* in the field at Fuzhou and Pinghe of Fujian province in China (Hu et al., 2010). All these findings warn that it is inevitable that mites will develop resistance to spirodiclofen and resistance management must be in place before the introduction of the acaricide.

In this study, spirodiclofen was active against all developmental stages and had a sterilizing effect on females of the LS-FJ strain. However, the level of spirodiclofen resistance in the SR-FZ strain was variable between eggs and other developmental stages in which high spirodiclofen resistance was detected. The egg stage showed low resistance to spirodiclofen in the LS-FJ strains, which was previously documented by Van Pottelberge et al. (2009b) in T. urticae. On the other hand

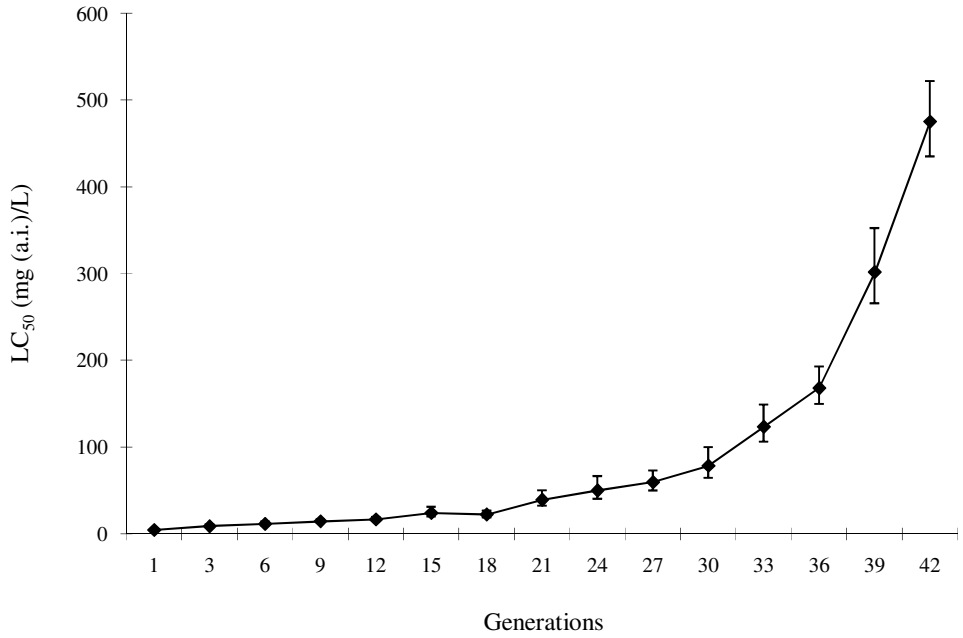

Figure 1. Resistance selection of spirodiclofen in *p. citri.*

Table 1. Susceptibility to spirodiclofen at each developmental stage of the LS-FJ and SR-FZ strains of the citrus red mite.

Developmental stage	LS-FJ		SR-FZ		RR[a]
	LC_{50} (95%CI) (mg (a.i.)/L)	Slope (±SE)	LC_{50} (95%CI) (mg (a.i.)/L)	Slope (±SE)	
Egg	2.20(1.70-2.82)	1.4 (0.1)	10.08 (8.08-12.62)	1.9 (0.2)	4.6
Larva	4.60 (3.78-5.53)	2.2 (0.3)	475.06(434.96-521.81)	3.2 (0.3)	103
Protonymph	2.70 (2.02-3.48)	1.3 (0.2)	422.15 (381.15-461.48)	3.6 (0.4)	157
Deutonymph	7.57 (6.01-9.49)	1.5 (0.2)	387.46 (349.23-422.34)	3.9 (0. 5)	51.2
Sterilization	8.43(6.84-10.11)	2.3 (0.3)	>1000	-	-

[a] The resistance ratio (RR) is (LC_{50} of SR-FZ)/(LC_{50} of LS-FJ).

Table 2. Cross-resistance spectrum of the SR-FZ strain of the citrus red mite.

Acaricide	LS-FJ		SR-FZ		RR[a]
	LC_{50}(95%CI) (mg (a.i.)/L) (±SE)	Slope	LC_{50}(95%CI) (mg (a.i.)/L) (±SE)	Slope	
Spirodiclofen	4.60 (3.78-5.53)	2.2 (0.3)	475.06 (434.96-521.81)	3.2 (0.3)	103
Spirotetramat	1.20 (0.98-1.44)	1.9 (0.2)	35.33 (31.01-40.41)	2.3 (0.3)	29.5
Abamectin	0.02 (0.01-0.03)	1.4 (0.2)	0.053 (0.041-0.069)	1.4 (0.1)	2.3
Hexythiazox	5.52 (4.22-6.98)	1.5 (0.2)	6.49 (5.13-8.17)	1.4 (0.2)	1.2
Pyridaben	1.01 (0.76-1.31)	1.5 (0.2)	3.75 (2.86-4.88)	1.4 (0.1)	3.7
Fenpropathrin	4.76 (3.75-6.02)	1.8 (0.2)	7.51 (5.92-9.45)	1.7 (0.2)	1.6
Fenbutatin oxide	31.90 (24.33-40.50)	2.8 (0.3)	29.63 (25.97-33.50)	3.0 (0.3)	0.9
Fenpyroximate	2.48 (1.99-3.05)	1.9 (0.2)	11.63 (9.27-14.47)	1.7 (0.2)	4.7
Clofentezine	15.29 (12.47-18.63)	1.8 (0.2)	28.27(22.82-36.49)	1.4 (0.2)	1.8
Chlorfenapyr	2.69 (1.93-3.65)	1.2 (0.2)	5.67 (4.32-7.24)	1.4 (0.1)	2.1
Bifenthrin	7.28 (5.42-9.31)	1.4 (0.2)	12.96 (10.29-16.26)	1.5 (0.2)	1.8
Amitraz	16.49 (12.59-20.35)	1.6 (0.2)	44.39 (34.68-56.46)	1.3 (0.1)	2.7

[a] The resistance ratio (RR) is (LC_{50} of SR-FZ) / (LC_{50} of LS-FJ).

Table 3. Synergism in the LS-FJ and SR-FZ strains of the citrus red mite.

Untreated	LS-FJ			SR-FZ			SR[b]
	LC_{50}(95%CI)(mg(a.i.)/L)	Slope (±SE)	SR[a]	LC_{50}(95%CI)(mg(a.i.)/L)	Slope(±SE)	SR	
	4.60 (3.78-5.53)	2.2 (0.3)	1	475.06 (434.96-521.81)	3.2 (0.3)	1	103
PBO	3.83 (3.33-4.37)	2.2 (0.2)	1.2	145.68 (126.59-165.94)	2.3 (0.2)	3.3	38
DEF	4.50 (4.01-5.07)	2.7 (0.3)	1.0	204.45 (180.63-233.43)	2.5 (0.2)	2.3	45
DEM	4.68 (4.15-5.30)	2.6 (0.2)	1.0	303.67 (271.88-341.82)	2.8 (0.3)	1.6	65

[a] The synergism ratio (SR) is (LC_{50} of spirodiclofen alone) / (LC_{50} of spirodiclofen + synergist); [b] the resistance ratio (RR) is (LC_{50} of SR-FZ)/(LC_{50} of LS-FJ).

hand, high resistance to spirodiclofen was also detected in eggs of *P. citri* collected from fields (unpublished data). These differences may be caused by the different selection environment resulting in different resistance mechanisms. Levels of resistance to dicofol and amitraz in the citrus red mite varied, depending on the developmental stage (Inoue and Saito, 1972; Inoue, 1984), but the level of hexythiazox resistance in the mite was high at all developmental stages (Yamamoto et al., 1995). There was almost no inhibitory effect of spirodiclofen on the fertility of resistant female mites and the loss of *P. citri* adult infertility (as an effect of treatment with spirodiclofen) is a major factor in the development of resistance, which was also reported for *T. urticae* (Van Pottelberge et al., 2009a).

The SR-FZ strain showed considerable cross-resistance to spirotetramat, although the resistance factor was lower than that for spirodiclofen. According to the insecticide resistance action committee (IRAC) classification, spirotetramat is in the same group as spirodiclofen and spiromesifen. Cross-resistance between spirodiclofen and spirotetramat has been found for spirodiclofen-resistant citrus red mite in the field (Hu et al., 2010). Van Pottelberge et al. (2009b) reported cross-resistance between spirodiclofen and spiromesifen in a laboratory-selected spirodiclofen-resistant strain of *T. urticae*. The existence of cross-resistance among tetronic acid derivative acaricides potentially limits their use and development. There was low or no cross-resistance to 11 other acaricides that have modes of action distinctly different from that of spirodiclofen, and consequently no problem is anticipated for using spirodiclofen in alteration with these acaricides. Spirodiclofen was formerly found to be fully effective against several strains of *T. urticae* showing resistance to the organophosphates hexythiazox, dicofol, clofentezine, pyridaben, fenpyroximate, abamectin and others (Nauen et al., 2001). Spirodiclofen-resistant *T. urticae* also showed no cross-resistance towards abamectin, acequinocyl, bifenazate, etoxazole, pyridaben or tebufenpyrad. Similar results were obtained with *Panonychus ulmi* (Koch) strains resistant to dicofol, organotin and clofentezine (Nauen et al., 2001; Pree et al., 2005). Spirodiclofen was even more toxic to a field-collected multiresistant strain of *P. citri* exhibiting high resistance to fenpropathrin and pyridaben than it was to the susceptible strain (Hu et al., 2010). The three synergists tested in this study did not change the toxicity of spirodiclofen to the larvae of the LS-FJ strain, but could increase the spirodiclofen toxicity in larvae of the SR-FZ strain. Van Pottelberge et al. (2009b) reported that, PBO and DEF could synergize the reproductive effect in females of an unselected susceptible strain. In contrast to the LS-FJ strain, PBO, DEF and DEM could synergize spirodiclofen toxicity in the SR-FZ strain, which suggests that P450 monooxygenases, esterase and glutathione *S*-transferases might be involved in the metabolism of spirodiclofen. However, P450 monooxygenases might play the most important role in detoxification of the compound in the SR-FZ strain because the highest synergism ratio was found for PBO. In this study, DEM showed slight synergism on spirodiclofen toxicity in the SR-FZ strain, which conflicts with the reports by Van Pottelberge et al. (2009b) and by Rauch and Nauen. (2002). However, enzyme assays done by Van Pottelberge et al. (2009b) indicated that, glutathione-*S*-transferases could be involved in the metabolic detoxification of spirodiclofen.

The following conclusions can be drawn from this study: (1) The potential for development of resistance to spirodiclofen exists in *P. citri* and resistance management will be necessary; (2) levels of resistance would be high at each developmental stage, except eggs of this mite species, and loss of *P. citri* adult infertility is a major factor in the development of resistance; (3) spiroztetramat showed cross-resistance to spirodiclofen, but no obvious cross-resistance to other 10 acaricides tested; (4) P450 monooxygenases, esterase and glutathione *S*-transferases might all be involved in an enhanced spirodiclofen metabolism in the spirodiclofen-resistant strain of *P. citri*. Further studies, including the stability of spirodiclofen resistance, genetic analysis and sensitivity of the target site Acetyl-CoA carboxylase (ACCase), must be undertaken in order to select the proper tactics for managing resistance to spirodiclofen.

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REFERENCES

Abbott SW (1925). A method of computing the effectiveness of an insecticide. J. Econ. Ent. 18: 265-267.

Bretschneider T, Fischer R, Nauen R (2007). Inhibitors of Lipid Synthesis (Acetyl-CoA-carboxylase Inhibitors), in Modern Crop Protection Compounds, ed. by Kramer W and Schirmer U. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp: 909-925.

Chen GH, Wang AZ (2001). Test on the resistance of Panonychus citri to Dicofol and pyridaben. Hunan Agri. Sci. 4: 48.

Chen NC, Huang HZ, Peng YL (1996). Monitoring of pesticide-resistance and preliminary studies on main resistance mechanism of citric spider mite (*Panonychus citri* McGregor). Pestic. 35: 15-18.

Fukami J, Uesugi Y, Ishizuka K (1983). Pest resistance to pesticides. Soft Science Inc., Tokyo, Japan.

Hu JF, Wang CF, Wang J, You Y, Chen F (2010). Monitoring of resistance to spirodiclofen and five other acaricides in *Panonychus citri* collected from Chinese citrus orchards. Pest Manag. Sci. 66: 1025–1030.

Huang GY, Xu ZH, Fang ZG (1999). Studies on pesticide resistance of *Panonychus citri*. J. Zhejiang Fore. College 6: 252-259.

Huang HJ, Huang YY, Mo YS, Chen BX (1994). Studies on the Resistance of *Panonychus citri* to Several Acaricides. Guangdong Agric. Sci. 8: 38-40.

Huang MD (1979). Integrated management of citrus red mite. Chinese Science Press, Beijing. pp: 361-369.

Inoue K (1984). Resistance to Amitraz in the Citrus Red Mite, *Panonychus citri* (McGregor) in Relation to Population Genetics. Jpn. J. Appl. Entomol. Zool. 28: 260-268.

Inoue T, Saito T (1972). The susceptibilities of various stages of dicofol resistant and susceptible citrus red mite, *Panonychus citri* (McGregor), against dicofol. Jpn. J. Appl. Entomol. Zool. 16: 152-154.

Meng HS, Wang KY, Jiang XY, Yi MQ (2000). Selection and Biochemical Mechanism of the Resistance to Pyridaben in *Panonychus citri*. Chinese J. Pestic. Sci. 2(3): 30-34.

Meng HS, Wang KY, Jiang XY, Yi MQ (2002). Studies on resistance selection by abamectin and fenpropathrin and activity change of detoxicant enzymes in *Panonychus citri*. Acta Entomol. Sin. 45(1): 58-62.

NATESC (2003). Pesticides resistance monitoring of Chinese agricultural pest (No. 1). Pestic. Express 18: 23-24.

Nauen R, Stumpf N, Elbert A, Zebitz CPW, Kraus W (2001). Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest Manag. Sci. 57: 253-261.

Pree DJ, Whitty KJ, Van Driel L (2005). Baseline susceptibility and cross resistance of some new acaricides in the European red mite, *Panonychus ulmi*. Exp. Appl. Acarol. 37 (3-4):165-171.

Rauch N, Nauen R (2002) Spirodiclofen resistance risk assessment in *Tetranychus urticae* (Acari: Tetranychidae): a biochemical approach. Pest. Biochem. Physiol. 74(2): 91-101.

Robertson JL, Preisler HK (1992). Binary response: design, fit, and other problems, in Pesticide Bioassays with Arthropods, ed. by Robertson JL and Preisler HK. Chapman and Hall,NewYork, NY, pp. 35-50.

Sato ME, da Silva MZ, Raga A, de Souza Filho MF (2005). Abamectin resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae): selection, cross-resistance and stability of resistance. Neotr. Entomol. 34: 991-998.

Shelton AM, Tang JD, Roush RT., Metz TD, Earle ED (2000). Field tests on managing resistance to Bt-engineered plants. Nat. Biotechnol. 18: 339-342.

Van Leeuwen T, Van Nieuwenhuyse P, Vanholme B, Dermauw W, Nauen R, Tirry L (2011). Parallel evolution of cytochrome b mediated bifenazate resistance in the citrus red mite *Panonychus citri*. Insect Mol. Biol. 20(1): 135-140

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010) Acaricide resistance mechanisms in the two spotted spider mite Tetranychus urticae and other important Acari: A review. Insect Biochem. Mol. Biol. 40: 563-572.

Van Pottelberge S, Khajehali J, Van Leeuwen T, Tirry L (2009a) Effects of spirodiclofen on reproduction in a susceptible and resistant strain of *Tetranychus urticae* (Acari:Tetranychidae). Exp. Appl. Acarol. 47:301-309

Van Pottelberge S, Van Leeuwen T, Khajehali, J, Tirry L (2009b). Genetic and biochemical analysis of a laboratory-selected spirodiclofen-resistant strain of *Tetranychus urticae* Koch (Acari: Tetranychidae). Pest Manag. Sci. 65: 358-366.

Xia JY (2003). Pesticides resistance risk assessment and management progress of Chinese agricultural pest. Plant Prot. Techn. Exten. 23 : 35-38.

Yamamoto A, Yoneda H, Hatano R, Asada M (1995). Laboratory selections of populations in the citrus red mite, *Panonychus citri* (McGregor), with hexythiazox and their cross resistance spectrum. J. Pestic. Sci. 20: 493-501.

Zhang YY, Zhao ZM, Wu SC (1994). IPM of citrus mites. Plant Prot. Technol. Exten. 14: 23-24.

Zhao JZ, Cao J, Li YX, Collins HL, Roush RT, Earle ED, Shelton AM (2003), Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. Nat. Biotechnol. 21: 1493-1497.