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Resistant inheritance and cross-resistance of cyflumetofen in *Tetranychus cinnabarinus* (Boisduval)

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ABSTRACT

As a new acaricide, cyflumetofen can effectively control *Tetranychus*, *Panonychus*, as well as other phytophagous mites. But its risk and the way of genetic and resistant inheritance in mites are not clear. In this study, two cyflumetofen-resistant strains (CyR and YN-CyR) were selected for 104 and 12 generations, and developed 104.7-fold and 25.6-fold resistance, respectively. Three crossing groups (CyR_80 × SS, CyR_104 × SS, YN-CyR × SS) were conducted to explore the resistant inheritance of cyflumetofen in *T. cinnabarinus* changed along with resistant level or not. The results of reciprocal crosses and backcrosses revealed that the incomplete recessive and multiple genes trait involved in two resistant strains. The different stage of resistance also has a same genetic trait. A cross-resistance study revealed that there was no cross-resistance between cyflumetofen and other four acaricides including avermectin, fenpropathrin, propargite and bifenazate respectively, but the cross-resistance to pyridaben reached a high level with 63.8-fold, which indicates an underlying mechanism that can both mediate cyflumetofen- and pyridaben-resistance in *T. cinnabarinus*.

1. Introduction

Tetranychus cinnabarinus (carmine spider mite) is one of the most important agriculture mite pests in China, which feed on > 100 kinds of crops, such as beans, eggplant, pepper, tomato and so on [1,2]. This mite shares many characteristics in morphological, biological, and molecular aspects with *T. urticae*, and is also considered as red form of two spotted spider mite [3]. However its unique characters make some researchers classify it into a different but very close species with *T. urticae* [4,5]. Currently, its control has been based on the use of insecticides and acaricides [6]. However, because of the frequent application of acaricides and insecticides, and strong reproductive ability, short generation cycle, arrhenotokous parthenogenesis, highly close relative mating rate of *T. cinnabarinus*, the mites could develop resistance quickly [1,7].

As a novel benzoyl acetonitrile acaricide, cyflumetofen was developed by Otsuka AgriTechno Co., Ltd., and widely used in many countries since 2007 [8]. Cyflumetofen works as the inhibitor of complex II in the mitochondrial electron transport chain [8,9]. It shows striking activity on kinds of pest mites, such as *Tetranychus urticae* (two-spotted spider mite), *Tetranychus kanzawai* (Kanzawa spider mite) and *Panonychus citri* (citrus red mite), during the whole life span from egg to adult. Besides, cyflumetofen is also effective to a series of mite strains which have developed resistance to other acaricides [9]. Another advantage of this acaricide is its security to mammals, aquatic organisms, beneficial organisms, natural enemies and other non-target organisms. With none occurrence of phytotoxicity to plants, cyflumetofen can be rapidly metabolized in the soil and water [10–12]. At present, many studies focus on chemical synthesis and environmental degradation of cyflumetofen [13–16]. Attentions are also paid to its resistant mechanism in mites, such as overexpression of a GST gene *TuGSTd05* enhanced the ability of detoxification against cyflumetofen in *T. urticae* [17]. However, the resistant inheritance of cyflumetofen in mites has not been fully elucidated. Understanding of the resistant inheritance of insecticide/acaricide is necessary for the sustainability of pest control [18,19]. The inheritance modes of fenpyroximate, pyridaben and abamectin resistance in *T. urticae* revealed different genetics responses [20,21]. Insecticide/acaricide resistance in natural populations may be monogenic or multiple genes with a major phenotypic effect. Therefore, study on inheritance of mites involved in insecticide/acaricide resistance will aid in our understanding on the development of resistance.

In this study, we demonstrated the same inheritance pattern of cyflumetofen-resistance in both two resistance-selecting strains, as well as its cross-resistance with other five acaricides in *T. cinnabarinus*. The current study can provide important information for further understanding of cyflumetofen-resistance development in *T. cinnabarinus* and

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the reasonable usage of this acaricide.

2. Materials and methods

2.1. Strains

2.1.1. Susceptible strain (SS)

Carmine spider mite was collected in the field of Beibei District, Chongqing, China, and feed on fresh cowpea seedlings in the indoor illumination constant temperature incubator for > 15 years without any pesticide exposure. This population was considered as the relatively susceptible strain (SS) and used for the selection of cyflumetofen resistance. The rearing conditions were as follows: 26 ± 1 °C temperature, 35%–55% relative humidity (RH), and a photoperiod of 14:10 h (L:D).

2.1.2. Cyflumetofen-resistant strain (CyR and YN-CyR)

The cyflumetofen-resistant strain (CyR) was from Wang's study [22], in which the CyR had been selected for 43 generations, and continued to be selected for 61 generations (CyR_104) in this study. The resistance-selecting method "spraying leaf" was replaced with "spraying leaf-disc" from generation 50. The "spraying leaf-disc" method referred to Van Leeuwen [23,24]. Briefly, the mites were placed on bean leaf discs, and sprayed cyflumetofen (20% SC) with 70% of mortality. 24 h later, the survivals were transferred to new bean leaf discs and moved away after 1–2 days' oviposition. The next selecting cycle was conducted after the strain increased. The concentration of cyflumetofen should be increased after several generations of treatment to keep a constant selection pressure. Bioassay was carried out every 4–6 generations to calculate the LC_{50} and observe the resistance development (the bioassay was paused from 50 to 68 generations due to the change of resistance-selecting method).

In order to demonstrate the genetic trait of cyflumetofen-resistance in *T. cinnabarinus* more reasonable, another resistance-selecting strain of *T. cinnabarinus* (YN-CyR) was collected from field (Yunnan, China), and selected for 12 generations in laboratory. Also, the resistant trait was estimated twice (19.4-fold in CyR_80 and 104.7-fold in CyR_104, respectively) in CyR for revealing the resistant inheritance of cyflumetofen in *T. cinnabarinus* changed along with resistant level or not.

2.2. Bioassay

Bioassay was carried out with the modified residual coated vial (RCV) method [25]. Briefly, acaricides were dissolved and diluted with acetone to several concentrations. Thirty three-days-old adult female mites were transferred into acaricide-coated centrifuge tube. After 24 h, these mites were checked under anatomical microscope. Mites showing immobility or only 1–2 pairs of legs in a slight shiver were considered dead. The bioassay for cross-resistance was conducted when the strain had been selected for 80 generations (CyR_80). Each dose of treatment and control were repeated three times. Statistical analysis of LC_{50}-values, slopes and 95% confidence limits were calculated by probit analysis [26].

2.2.1. Chemicals

The 95.5% cyflumetofen TC and 20% cyflumetofen SC were obtained from Jiangsu Food Machinery Corporation Chemical Technology Co., Ltd.; 92.9% fenpropathrin and 96% avermectin were obtained from Hebei Veyong animal's pharmaceutcal co., Ltd.; 90% propargite was obtained from Qingdao Hansencn Biotechnology Co., Ltd.; 95% pyridaben was obtained from Shandong SINO-AGRI United Biotechnology Co., Ltd.; 97% bifenazate was obtained from Zhejiang Yinbang Chemicals Co., Ltd.

Table 1
The selection of resistance to cyflumetofen in *T. cinnabarinus*.

Number of generation selected	Slop	χ^2	LC_{50} mg/L	95% CL	RR[a]	RR[b]
CyS[c]	1.78	1.45	0.79	0.65–0.93	–	1
SS(CyR_0)	2.33	0.29	2.19	2.00–2.38	1	2.8
CyR_4[c]	3.13	6.39	3.09	2.91–3.27	1.4	3.9
CyR_8[c]	2.22	0.94	3.21	2.87–3.55	1.5	4.1
CyR_12[c]	2.77	5.94	4.33	4.07–4.59	2.0	5.5
CyR_16[c]	3.55	1.19	4.49	4.27–4.71	2.1	5.7
CyR_20[c]	3.67	1.19	5.07	4.78–5.36	2.3	6.4
CyR_24[c]	3.98	1.83	5.45	5.22–5.68	2.5	6.9
CyR_28[c]	3.69	1.24	6.59	6.42–6.76	3.0	8.3
CyR_33[c]	3.94	2.96	10.02	8.90–11.61	4.6	12.7
CyR_38[c]	2.74	1.09	12.86	11.01–16.94	5.9	16.3
CyR_43[c]	5.19	1.80	16.85	15.26–20.17	7.7	21. 3
CyR_45	7.58	6.78	23.42	21.24–24.89	10.7	29.7
CyR_47	8.57	9.01	24.41	23.19–26.30	11.2	30.9
CyR_49	5.90	0.61	24.64	22.82–28.93	11.3	31.2
CyR_69	5.15	2.10	26.13	15.01–31.26	11.9	33.1
CyR_75	4.36	2.48	31.52	28.94–34.10	14.4	39.9
CyR_80	4.45	1.89	42.47	38.48–46.15	19.4	53.8
CyR_85	3.11	1.57	47.34	42.11–52.74	21.6	59.9
CyR_88	3.16	0.62	48.66	43.40–53.62	22.2	61.6
CyR_93	2.20	0.18	56.89	47.90–71.45	26.0	72.0
CyR_97	3.86	1.29	58.80	52.69–64.20	26.9	74.4
CyR_100	5.57	0.40	114.39	107.87–124.02	49.3	114.8
CyR_104	9.79	1.16	229.31	222.45–236.44	104.7	290.3
YN-CyR_0	2.35	0.68	8.39	7.35–9.76	3.8	10.6
YN-CyR_4	2.07	1.13	18.79	16.14–22.10	8.6	23.8
YN-CyR_8	3.27	0.87	37.27	33.72–41.05	17.0	47.2
YN-CyR_{12}	5.54	0.38	56.09	52.82–59.33	25.6	71.0

[a] Resistance ratio; LC_{50} of the selected generation/LC_{50} of the SS (CyR_0) strain.
[b] Resistance ratio; LC_{50} of the selected generation/LC_{50} of the CyS strain.
[c] Meant the data comes from Wang et al. [22].

Table 2
Bioassay of 5 insecticides or acaricides to the SS and CyR_80.

Chemical	Strains	χ^2 (*P*-value)	Slope ± SE	LC_{50} (95%CI) mg/L	RR[a]
Avermectin	SS	4.3 (0.12)	1.69 ± 0.3	0.9 (0.6–1.2)	–
	CyR	3.1 (0.22)	1.44 ± 0.2	0.8 (0.6–1.1)	0.9
Fenpropathrin	SS	0.4 (0.94)	2.85 ± 0.4	700.6 (620.6–782.4)	–
	CyR	0.2 (0.69)	2.02 ± 0.5	514.4 (396.4–638.3)	0.7
Propargite	SS	2.8 (0.25)	3.66 ± 0.4	205.7 (183.4–226.9)	–
	CyR	1.1 (0.57)	2.80 ± 0.4	219.4 (190.0–253.4)	1.1
Bifenazate	SS	1.0 (0.60)	1.70 ± 0.5	61.8 (46.0–77.1)	–
	CyR	0.5 (0.78)	2.63 ± 0.5	68.2 (59.3–81.1)	1.1
Pyridaben	SS	1.3 (0.54)	1.39 ± 0.3	114.6 (78.6–155.3)	–
	CyR	0.6 (0.73)	2.61 ± 0.5	7308.0 (6431.0–8565.0)	63.8

[a] Resistance ratio; LC_{50} of CyR/LC_{50} of SS.

2.3. Crossing experiments

In attempt to estimate the inheritace mode of cyflumetofen resistance in *T. cinnabarinus*, three crossing groups (CyR_80 × SS, CyR_104 × SS, YN-CyR × SS) were established in this study. The individuals of susceptible (SS; genotype SS) and resistant (CyR_80, CyR_104 or YN-CyR; genotype RR) strains were reciprocally crossed to produce hybrid F_1 females (F_1RS and F_1SR). Briefly, 10 teleiochrysalid females of one strain and 20 adult males of another strain were transferred to fresh leaf discs. Each crossing groups were repeated thirty times. After teleiochrysalid females moulting, the diploid females were fertilised by the haploid males. Two days later, all the haploid males were moved away and leaving females to lay eggs for 2 days, then fostered the F_1 progenies to maturation. The three-days-old female adults of F_1 were collected (about 600 individuals) for bioassay with

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