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Baseline sensitivity and resistance risk assessment of *Valsa mali* to pyraclostrobin

Hao Feng†, Shuai Wang†, Zhaoyang Liu, Jianqiang Miao, Mingxia Zhou and Lili Huang*

Abstract

Pyraclostrobin, a quinone outside inhibitor (QoI) fungicide, has been registered to control apple tree Valsa canker (AVC) caused by *Valsa mali* in China. However, there is no data available regarding the resistance risk of *V. mali* to pyraclostrobin. In this study, the sensitivities of 120 *V. mali* isolates to pyraclostrobin were detected. The isolates were collected from apple orchards with no application of pyraclostrob at six provinces in China during 2013–2015, and showed similar sensitivity to pyraclostrobin. The EC_{50} values of these 120 *V. mali* isolates to pyraclostrobin ranged from 0.0014 to 0.0240 μg/mL, indicating an excellent inhibitory efficacy of pyraclostrobin to the pathogen. The EC_{50} values were distributed as a unimodal curve with a mean value of 0.0091 μg/mL, and the mean EC_{50} displayed correlation with geographic location. Meanwhile, three pyraclostrobin-resistant mutants (PR mutants) of *V. mali* were obtained using fungicide adaption method, with a resistance factor (RF) of 41.0, 56.8 and 22.0, respectively. The mutants showed a stable resistance to pyraclostrobin after 10 transfers on pyraclostrobin-free medium. Comparing with the corresponding parental isolates, the hyphal growth, mycelial dry weight and pathogenicity of PR mutants were significantly reduced, but the number of propagules showed no significant difference. More importantly, no cross-resistance of PR mutants to pyraclostrobin, tebuconazole, difenoconazole, imazalil and thiophanate-methyl was detected. In conclusion, *V. mali* showed a moderate risk to pyraclostrobin, and pyraclostrobin could be used as an alternative fungicide to control AVC in the field in China.

Keywords: *Valsa Mali*, Pyraclostrobin, Baseline sensitivity, Resistant risk

Background

The apple tree Valsa canker (AVC), caused by *Valsa mali*, is the most serious disease of apple tree in Asia, especially in China (Abe et al. 2007; Suzaki 2008; Wang et al. 2011, 2014; Li et al. 2013). Shaving diseased bark tissues and spraying fungicide on the wound were commonly used to control AVC. However, it is very difficult to cure the canker, because the pathogen could penetrate deep into the xylem (Ke et al. 2013). Meanwhile, this method also leads to the weakening of trees, making the tree more susceptible to the disease. Thus, how to inhibit the penetration of pathogen spores is the key for disease prevention. In 2013, annual dissemination of the pathogen in the field was studied systematically, and the peak period of pathogen dissemination was found to be from bud stage to young fruit stage of apple tree (Du et al. 2013). Once pathogen spores colonize on the bark of apple tree, they then penetrate into bark tissues through lenticels, cracks, injuries, etc. (Ke et al. 2013). There is a latent period from pathogen colonizing in superficial tissues to entering into interior tissues, and the length of this latent period depends on the tree vigor (Meng et al. 2019). According to the annual dissemination and main penetration pathways of the pathogen, a prevention technology was developed and confirmed to be effective to prevent AVC by spraying fungicides or biological agents onto the bark surface for two times during June to August (Li et al. 2016). Thus, it is very important to select the appropriate and effective agents. Based on the information from China Pesticide Information Network (http://www.chinapesticide.org.cn/ywb/index.jhtml), more than 20 kinds of chemical agents

* Correspondence: huanglili@nwsuaf.edu.cn
†Hao Feng and Shuai Wang contributed equally to this work.
State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, China

were registered to control AVC. Among them, the benzimidazole fungicide thiophanate-methyl was widely used in the past few years, which was effective in preventing infection of *V. mali* but showed little capacity in inhibiting the expansion of lesions (Tamura and Saito 1982). In recent years, the demethylation inhibitors, tebuconazole and difenoconazole, have been demonstrated to be very effective against *V. mali* and have become alternative fungicides to control AVC (Chen et al. 2009; Ma et al. 2012; Guo et al. 2015; Gao et al. 2017). Long-term use of single fungicide increased the risk of fungicide resistance. Although no fungicide-resistant strain of *V. mali* was found, the sensitivity of *V. mali* to difenoconazole has declined (Liu et al. 2019). Thus, it is urgent to find more fungicides with high efficiency in the control of AVC.

In early 2000s, a new fungicide, pyraclostrobin, belonging to quinone outside inhibitor (QoI) class, was developed by BASF Corporation, which was found to be preventive, curative, and eradicative against many plant diseases by inhibiting spore germination, mycelial growth, and sporulation of the target pathogenic fungi (Bartlett et al. 2002; Liang et al. 2015). However, QoIs are classified as high risk fungicides for triggering fungal resistance by Fungicide Resistance Action Committee (FRAC). In the past years, some populations of *Botrytis cinerea* have been found to be resistant to pyraclostrobin (Kim and Xiao 2011; Fernández-Ortuño et al. 2012). Meanwhile, isolates of *Alternaria alternata* have also showed resistance to pyraclostrobin (Avenot and Michailides 2015; Fan et al. 2015).

Pyraclostrobin has been confirmed to be effective against *V. mali* in laboratory test (Ma et al. 2012; Guo et al. 2015). However, the resistance risk of *V. mali* to pyraclostrobin is still largely unknown. In this study, the baseline sensitivity to pyraclostrobin of 120 *V. mali* stains from China was determined. Furthermore, pyraclostrobin-resistant mutants (PR mutants) of *V. mali* were obtained by fungicide adaption. Fitness of these resistant mutants in vitro and their cross-resistance to pyraclostrobin and other fungicides were subsequently investigated. The results will provide important guidance for scientific and rational use of pyraclostrobin in AVC control.

Results

Baseline sensitivity of *V. mali* to pyraclostrobin

SHAM was often used to inhibit the alternative oxidase (AOX) in determination of the sensitivity of a pathogen to QoI fungicide. Thus, we firstly evaluated its effects on the mycelial growth of eight randomly selected isolates with different genetic background. The results showed that SHAM at 100 μg/mL had no effect on the EC_{50} value of *V. mali* to pyraclostrobinin on AEA medium, whereas had significant inhibitory activity against the mycelial growth of *V. mali* (Additional file 1: Table S1). Thus, AEA medium without exogenous SHAM was used to detect the baseline sensitivity of *V. mali* to pyraclostrobin. The EC_{50} values of the corresponding isolates to pyraclostrobin ranged from 0.0014 to 0.0240 μg/mL, with a mean value of 0.0091 μg/ mL, and the ratio of the maximum to the minimum EC_{50} values was 17.5 (Additional file 2: Table S2). The frequency distribution of the EC_{50} values of these *V. mali* isolates showed a unimodal curve with a positive skew (Fig. 1). Therefore, no resistance was observed in the field isolates of *V. mali* that we collected. However, the mean EC_{50} values of *V. mali* strains collected from different geographic locations were significantly different. For example, the mean EC_{50} values of strains from Liaoning were highest (0.0128 ± 0.0061 μg/mL), while from Xinjiang were lowest (0.0040 ± 0.0015 μg/ mL) (Table 1).

Generation of pyraclostrobin-resistant (PR) mutants of *V. mali* and the resistance stability of mutants

Two PR mutants (XJVM001R1 and XJVM001R2) were obtained from the parental isolate XJVM001; one PR mutant (972R) was obtained from the parental isolate 972. These three resistant mutants exhibited resistance factor (RF) values of 41.0, 56.8 and 22.0, respectively (Table 2). After 10 transfers on pyraclostrobin-free PDA, XJVM001R1 and XJVM001R2 still kept a moderate resistance to pyraclostrobin, with RF values of 33.9 and 52.5, respectively. However, the RF value of 972R declined from 22.0 to 11.7. Thus, the resistances of XJVM001R1 and XJVM001R2 to pyraclostrobin were more stable than that of 972R.

Characterization of PR mutants and their parental isolates

Compared to their parental isolates, all PR mutants had a smaller colony diameter and a reduced mycelial dry weight. There were no significant differences in propagule number between PR mutants and their parental isolates. However, the virulence of PR mutants was lower than that of their parental isolates (Fig. 2 and Table 3). The EC_{50} values of PR mutants to pyraclostrobin were significantly higher than that of their parental isolates. More importantly, the sensitivity of PR mutants and their parental isolates to pyraclostrobin was not correlated with the sensitivity to tebuconazole, difenoconazole, imazalil, and thiophanate-methyl (Fig. 3 and Table 4). In other words, no cross-resistance in PR mutants to pyraclostrobin, tebuconazole, difenoconazole, imazalil and thiophanate-methyl was detected.

Discussion

It is of great importance to establish baseline sensitivity data of a phytopathogenic fungus to a fungicide, as such

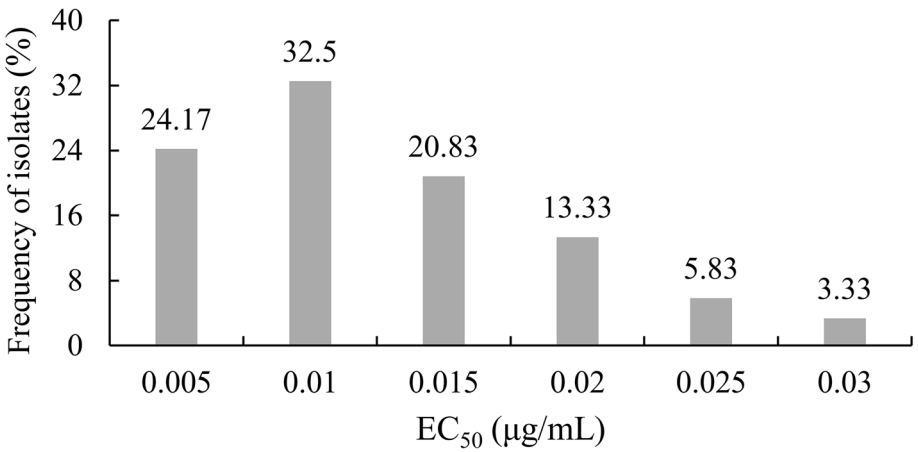

Fig. 1 Frequency distribution of the effective concentrations of pyraclostrobin for 50% inhibition of mycelial growth (EC_{50}) of *Valsa mali*. In total, 120 *V. mali* isolates were collected from apple orchards with no history of pyraclostrob application in China. Values on the bar show frequencies of isolates with corresponding EC_{50} values

data are useful to evaluate the risk of resistance development in sensitive populations of the fungi, and meanwhile provide evidence to suggest future methods for control of fungicide-resistant mutants (Zhang et al. 2015). In this study, the baseline sensitivity and resistance risk of *V. mali* populations in China to pyraclostrobin were reported.

The alternative oxidase (AOX) provides an alternative route for respiration and it will usually be activated after QoIs inhibit mitochondrial respiration in fungi by binding to the Qo site of the cytochrome bc1 complex and blocking electron transfer (Piccirillo et al. 2018). SHAM, an AOX inhibitor, is commonly added into culture media to suppress alternative respiration (Mizutani et al. 1995). In previous study, SHAM was confirmed to possess different effects on the sensitivity of pathogens to QoI fungicide (Di et al. 2016). Thus, it is necessary to firstly detect the effect of SHAM to the mycelial growth of *V. mali* in this study. According to the results we obtained, SHAM (100 μg/mL) has significant inhibitory activity against the mycelial growth of *V. mali*, but does not affect the EC_{50} value of *V. mali* to pyraclostrobin. So, we determined the baseline sensitivity of *V. mali* populations to pyraclostrobin by not amending the medium with SHAM. The results showed that no pyraclostrobin-resistant *V. mali* isolate was detected. This sensitivity baseline could be used for monitoring any future sensitivity shifts to pyraclostrobin in the field populations of *V. mali*. Meanwhile, the mean EC_{50} values of *V. mali* isolates to pyraclostrobin were different. Similar result was also observed when the sensitivity of *Rhizoctonia cerealis* isolates collected from different geographic location to trifluzamide was detected (Qi et al. 2014). It may be related to the natural differences of the strains in different regions, the physiological differences in the strains themselves, as well as the population structure of the strains of *V. mali*.

Table 1 Sensitivity to pyraclostrobin of *Valsa mali* field isolates from different locations in China

Sampling site	Number of isolates	EC_{50} (μg/mL)	
		Range	Mean
Liaoning	10	0.0055–0.0234	0.0128±0.0061 a
Shandong	17	0.0041–0.0240	0.0110±0.0058 ab
Gansu	15	0.0040–0.0184	0.0105±0.0044 ab
Shanxi	36	0.0014–0.0227	0.0094±0.0057 ab
Shaanxi	31	0.0024–0.0232	0.0076±0.0050 bc
Xinjiang	11	0.0022–0.0070	0.0040±0.0015 c

EC_{50} = effective concentration for 50% inhibition of mycelial growth; Multiple range test was used to compare mean EC_{50} values using Fisher's least significant difference test. Values followed by different letters are significantly different at $P < 0.05$

Fungal pathogens may develop resistance to different fungicides under certain selection pressures or under conditions of adversity. Resistance to QoI fungicides has been reported in a broad range of plant pathogenic fungi such as *Plasmopara viticola* (Wong and Wilcox 2000), *Venturia inaequalis* (Steinfeld et al. 2001) and *Magnaporthe grisea* (Avila-Adame and Koller 2003). In this study, 14 wild parental isolates of *V. mali* were randomly selected to produce PR mutants by repeated exposure to pyraclostrobin, and three resistant mutants were obtained. The resistance levels of two PR mutants were still stable over ten generations. The failure in using other parental isolates to generate stable resistant mutants might be related to the genetic variation among different strains (Pang et al. 2013).

Changes in biological traits could affect the competitiveness of resistant mutants and sensitive strains in

Table 2 Level and stability of pyraclostrobin resistance in PR mutants and their parental isolates of *Valsa mali*

Isolate	Origin	EC_{50} (μg/mL)		RF	
		1st generation	10th generation	1st generation	10th generation
XJVM001	Parent	0.0033 d	0.0030 d	–	–
XJVM001R1	PR mutant	0.1350 b	0.1020 b	41.0	33.9
XJVM001R2	PR mutant	0.1870 a	0.1580 a	56.8	52.5
972	Parent	0.0051 d	0.0049 d	–	–
972R	PR mutant	0.1120 c	0.0575 c	22.0	11.7

EC_{50} = effective concentration for 50% inhibition of mycelial growth; RF (resistance factor) = EC_{50} for the resistant mutant/EC_{50} for the sensitive parental isolate; PR mutants were obtained by growing parental isolates on pyraclostrobin-amended medium. PR mutants are listed below their respective parents. Multiple range test was used to compare EC_{50} values using Fisher's least significant difference test. Values followed by different letters are significantly different at $P < 0.05$

nature (Ziogas et al. 2003). Thus, biological fitness parameters are considered to be important factors that affect the formation of a fungicide-resistant population of fungal pathogens in the field (Zhang et al. 2014). The pyraclostrobin-resistant strains of *B. cinerea* showed decreased sporulation rate, spore germination rate and sclerotial production (Markoglou et al. 2006). Meanwhile, the resistance change of *A. alternate* to pyraclostrobin did not affect the mycelial growth, conidial germination, and conidial production, but the resistant strains showed similar or increased virulence compared to that of sensitive strains (Vega and Dewdney 2014). In this study, the mycelial growth, mycelial dry weight and pathogenicity of resistant mutants were reduced compared with those of their sensitive parental strains, but there was no significant difference in the propagules number. The reduced pathogenicity of resistant mutants might be related to their decreased growth.

Clarifying cross-resistance of a pathogen to different fungicides will help to provide a theoretical basis for scientific application of fungicides on the control of the pathogen. Based on our results, no cross-resistance was detected among pyraclostrobin, tebuconazole, difenoconazole, imazalil and thiophanate-methyl in PR mutants. Due to the low fitness of PR mutants and lack of cross-resistance with other fungicides, we concluded that the resistant risk of *V. mali* to pyraclostrobin was at a low-to-moderate level. To avoid the generation of

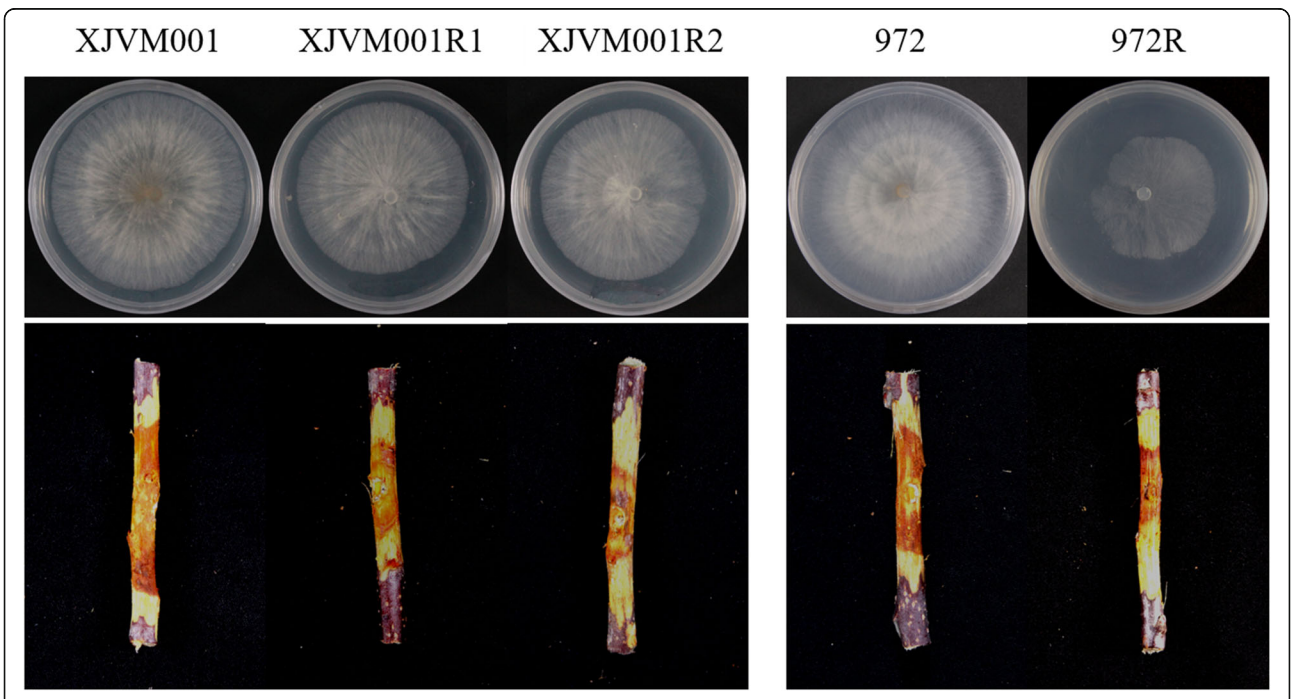

Fig. 2 Mycelial growth and pathogenicity of pyraclostrobin-resistant mutants of *Valsa mali* and their parent isolates. Colony diameters of PR mutants (XJVM001R1, XJVM001R2, 972R) and their parental isolates (XJVM001, 972) were measured after 60 h of incubation at 25 °C on PDA. Pathogenicity was evaluated by measuring the lesion size at 5 days post inoculation at 25 °C

Table 3 Comparison of mycelial growth, number of propagules, dry weight of mycelia and pathogenicity between PR mutants and their parental isolates

Isolate	Colony diameter (mm)	Number of propagules per plate	Dry weight of mycelia (g /flask)	Lesion length on twigs (mm)
XJVM001	72.2 b	267.8 b	0.3071 b	40.3 a
XJVM001R1	62.9 c	263.6 b	0.2878 c	29.2 b
XJVM001R2	61.8 c	265.3 b	0.2882 c	28.3 b
972	78.6 a	311.1 a	0.3129 a	36.5 a
972R	47.6 d	314.2 a	0.2792 d	25.6 b

Multiple range test was used to compare colony diameter, propagule number, dry weight and lesion length using Fisher's least significant difference test. Values followed by different letters are significantly different at $P < 0.05$

pyraclostrobin resistance in *V. mali*, we suggest that fungicides with different mechanisms should be used alternately. Otherwise, applying pyraclostrobin in mixtures with multisite or non-cross-resistant fungicides may be adopted for control of AVC and fungicide resistance management of *V. mali*.

Conclusions

In this study, baseline sensitivity of *V. mali* to pyraclostrobin was established and the efficiency of pyraclostrobin against *V. mali* was subsequently evaluated. All the 120 *V. mali* isolates showed similar and high sensitivity to pyraclostrobin. Meanwhile, the resistance risk of these *V. mali* isolates to pyraclostrobin was evaluated, and no cross-resistance between pyraclostrobin and other fungicides including tebuconazole, difenoconazole, imazalil and thiophanate-methyl was detected in PR mutants. Thus, pyraclostrobin could be used as an alternative fungicide to control AVC in China. Meanwhile, *V. mali* showed a moderated risk to pyraclostrobin, and the corresponding resistance management strategies should also be considered. These results will lay a foundation for the scientific application of pyraclostrobin in the field.

Methods

Pathogen isolates, culture media and fungicides

A total of 120 isolates of *V. mali* were tested in this study (Additional file 3: Table S3), which were collected

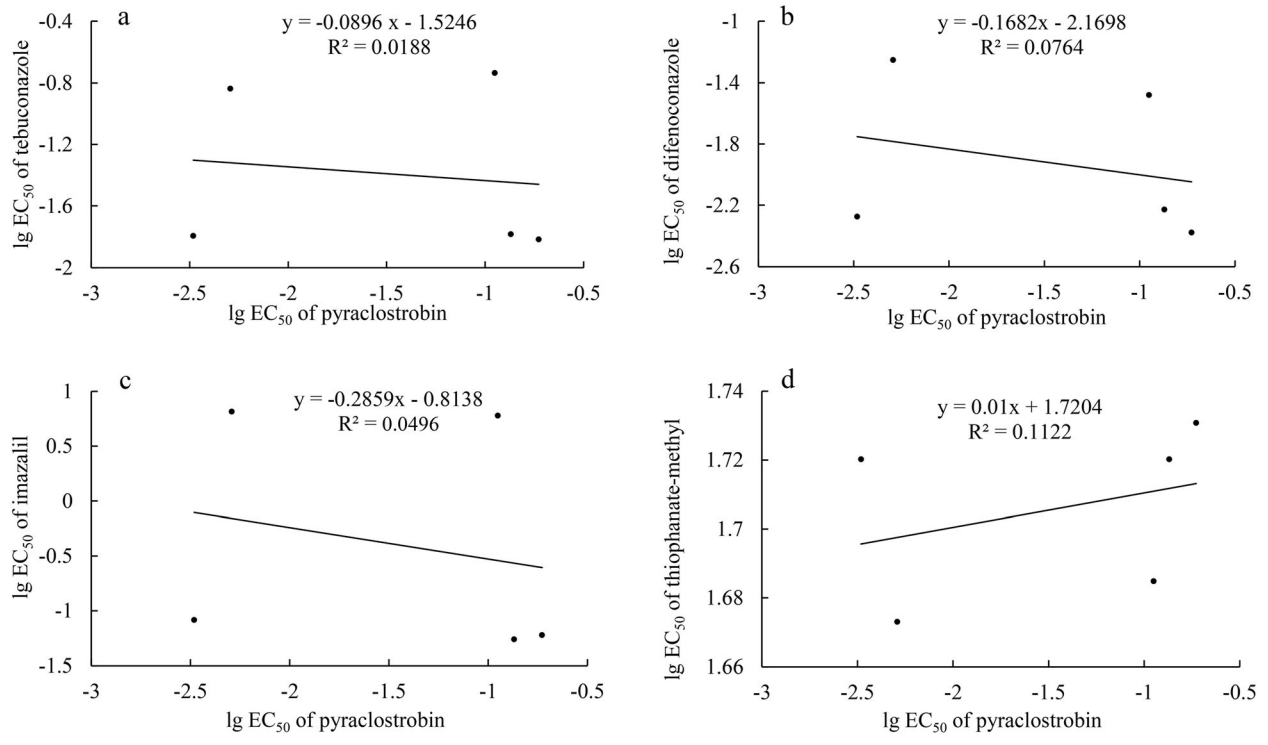

Fig. 3 Cross-resistance assays between pyraclostrobin and other four fungicides in *Valsa mali*. **a** Cross-resistance assay between pyraclostrobin and tebuconazole. **b** Cross-resistance assay between pyraclostrobin and difenoconazole. **c** Cross-resistance assay between pyraclostrobin and imazalil. **d** cross-resistance between pyraclostrobin and thiophanate-methyl

Table 4 Sensitivities of pyraclostrobin-sensitive and pyraclostrobin-resistant mutants of *Valsa mali* to different fungicides

Isolate	EC_{50} (μg/mL)				
	Pyraclostrobin	Tebuconazole	Difenoconazole	Imazalil	Thiophanate-methyl
XJVM001	0.00329	0.0161	0.00531	0.0825	52.5
XJVM001R1	0.13500	0.0165	0.00586	0.0548	52.5
XJVM001R2	0.18700	0.0153	0.00418	0.0599	53.8
972	0.00508	0.1450	0.05570	6.5200	47.1
972R	0.11200	0.1830	0.03300	5.9900	48.4

from infected apple trees in orchards with no application of pyraclostrobin at different geographic regions of China (Liaoning, Shandong, Gansu, Shanxi, Shaanxi, and Xinjiang) during 2013–2015. All the isolates were maintained on potato dextrose agar (PDA) medium at 4 °C. Alkyl ester agar (AEA) medium was used to detect the sensitivity of *V. mali* to pyraclostrobin. PDA medium was used to detect the sensitivity of the pathogen to other fungicides. PDB medium was used to culture strains of *V. mali*. All fungicides used were technical-grade products, including pyraclostrobin (97.5% a.i.; Xi'an Hytech Agrochemicals Co., Ltd., China), tebuconazole (97% a.i.; Jiangsu Flag Chemical Industry Co., Ltd., China), difenoconazole (95% a.i.; Xi'an Hytech Agrochemicals Co., Ltd.), imazalil (98% a.i.; Anhui Guangxin Agrochemical Co., Ltd., China), and thiophanate-methyl (97% a.i.; Anhui Huaxing Chemical Industry Co., Ltd., China). Pyraclostrobin, tebuconazole, difenoconazole and imazalil were respectively dissolved in acetone to produce a stock solution containing 10 mg/mL of the active ingredient. Tiophanate-methyl was dissolved in acetone and then diluted with 0.1% Tween-20 to produce a stock solution containing 3.2 mg/mL of the active ingredient. Fungicide stock solution was then stored in the dark at 4 °C until used.

Baseline sensitivity of *V. mali* isolates to pyraclostrobin

The sensitivities of 120 isolates of *V. mali* to pyraclostrobin were determined by measuring mycelial growth on agar plates. Mycelial plugs (5 mm in diameter) from the edge of a 3-day-old colony were transferred to a series of AEA plates amended with increasing concentrations of pyraclostrobin (0, 0.000625, 0.0025, 0.01, 0.025, 0.04, 0.16, 0.64, and 2.56 μg/mL), and the final concentration of acetone in the medium was standardized at 0.1% (v/v). Plates without pyraclostrobin were used as control. The plates were incubated at 25 °C for three days in a growth chamber. For each plate, the EC_{50} value was calculated by measuring the average colony diameter and regressing the percentage growth inhibition against the log of fungicide concentration. The experiment was repeated twice with three replicates for each treatment.

Generation of PR mutants of *V. mali*

Fourteen isolates (03–8, XJVM001, XJVM006, 878, 881, 889, 891, 893, 902, 910, 972, 980, 982 and 985) of *V. mali* were randomly selected to generate PR mutants. Fresh mycelial plugs from colony margins were transferred to AEA plates (seven plugs per plate) containing pyraclostrobin of 80 μg/mL. After incubation at 25 °C for two weeks, any fast-growing sectors from the otherwise restricted colonies were transferred to a series of AEA plates amended with increasing concentrations of pyraclostrobin (100, 200 and 500 μg/mL) (Wang et al. 2015).

Resistance level and stability of PR mutants

To determine the resistance level of PR mutants to pyraclostrobin, mycelial plugs of the mutants were placed on AEA plates amended with 0, 0.002, 0.02, 0.2, 2, 20, and 200 μg/mL of pyraclostrobin. The EC_{50} values were calculated by regressing the percentage growth inhibition against the log of fungicide concentration. The level of fungicide resistance, termed as resistance factor (RF), was equal to the EC_{50} value

Table 5 Fungicides and concentrations used to analyze cross-resistance between pyraclostrob and other fungicides in pyraclostrobin-sensitive and pyraclostrobin-resistant *Valsa mali* isolates

V. mali strains	Fungicides & Concentrations (μg/mL)				
	Pyraclostrobin	Tebuconazole	Difenoconazole	Imazalil	Thiophanate-methyl
XJVM001	0.000625, 0.0025, 0.01, 0.04, 0.16	0.005, 0.01, 0.02, 0.04, 0.08	0.002, 0.004, 0.008, 0.016, 0.032	0.025, 0.05, 0.1, 0.2, 0.4	40, 60, 80, 120, 240
XJVM001R1	0.002, 0.02, 0.2, 2, 20	0.005, 0.01, 0.02, 0.04, 0.08	0.002, 0.004, 0.008, 0.016, 0.032	0.025, 0.05, 0.1, 0.2, 0.4	40, 60, 80, 120, 240
XJVM001R2	0.002, 0.02, 0.2, 2, 20	0.005, 0.01, 0.02, 0.04, 0.08	0.002, 0.004, 0.008, 0.016, 0.032	0.025, 0.05, 0.1, 0.2, 0.4	40, 60, 80, 120, 240
972	0.000625, 0.0025, 0.01, 0.04, 0.16	0.05, 0.1, 0.2, 0.4, 0.8	0.015, 0.03, 0.06, 0.12, 0.24	2, 4, 8, 16, 32	40, 60, 80, 120, 240
972R	0.002, 0.02, 0.2, 2, 20	0.05, 0.1, 0.2, 0.4, 0.8	0.015, 0.03, 0.06, 0.12, 0.24	2, 4, 8, 16, 32	40, 60, 80, 120, 240

of the resistant mutant divided by the EC_{50} value of the parental isolate (Wang et al. 2015). To evaluate the stability of the resistant phenotypes, the PR mutants were subjected to ten successive transfers on new fungicide-free PDA plates. The RFs at the 1st and 10th generations were determined as described by Wang et al. (2015).

Hyphal growth, dry weight, propagule number, and pathogenicity of the PR mutants and their parental isolates

Hyphal growth and propagule number of PR mutants and their parental isolates were determined on fungicide-free PDA plates. Colony diameters were measured perpendicularly after incubation at 25 °C for 60 h. The number of propagules was subjectively evaluated when the mutants were cultured for 25 d under natural light at room temperature. Moreover, five mycelial plugs taken from the colony edge of each PR mutant and parental isolate were transferred to a flask containing 100 mL of PDB. After 3 d cultivation at 25 °C with shaking at 100 rpm, the mycelia were collected, dried (56 °C for 12 h), and weighed. There were three replicate plates and three replicate flasks for each isolate, and the experiment was repeated three times. The virulence of PR mutants and parental isolates was determined by a scald wounding method, which was carried out by inoculating detached twigs of *Malus domestica* Borkh. cv. 'Fuji' with a 5 mm mycelial plug derived from the margin of a young colony (Zang et al. 2007; Wei et al. 2010). After 5 d incubation at 25 °C, the length of each necrotic lesion was measured. Three twigs for each isolate were inoculated, and the experiment was performed three times.

Cross-resistance analysis

Pyraclostrobin-resistant and pyraclostrobin-sensitive isolates were used to assess their sensitivity to five fungicides, including pyraclostrobin, tebuconazole, difenoconazole, imazalil, and thiophanate-methyl. It was proceeded by using the same method of mycelial growth inhibition described above with fungicides at various final concentrations (Table 5). The individual EC_{50} values for these fungicides in all isolates were calculated. The experiment was repeated three times.

Statistical analysis

The data from different repetitions were used for statistical analysis. All statistical analysis was performed using SPSS 16.0 (Statistical Package for the Social Science, SPSS Inc., Chicago, IL). A regression equation was derived by correlating the log10 of inhibitor concentration of pyraclostrobin with the probit of inhibition percentage of average radial mycelial growth of *V. mali*, and effective concentration for 50% inhibition (EC_{50}) of *V. mali* was calculated from the regression equation. The ANOVA procedure of SPSS ($P < 0.05$) was used to determine significant differences among the EC_{50} values.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42483-020-00072-9.

Additional file 1: Table S1. Sensitivity assay of *Valsa mali* to pyraclostrobin in AEA medium with or without SHAM.

Additional file 2: Table S2. Sensitivity of 120 *Valsa mali* isolates to pyraclostrobin.

Additional file 3: Table S3. Details of 120 *Valsa mali* isolates used in this study.

Abbreviations

AEA: Alkyl ester agar; ANOVA: Analysis of variance; AVC: Apple Valsa canker; EC_{50}: Effective concentration for 50% inhibition of mycelial growth; FRAC: Fungicide Resistance Action Committee; PDA: Potato dextrose agar; PDB: Potato dextrose broth; PR: Pyraclostrobin-resistant; QoI: Quinone outside inhibitor; RF: Resistance factor; SHAM: Salicylhydroxamic acid; *V. mali*: *Valsa mali*

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Authors' contributions

LH and HF designed the research. SW, ZL and MZ performed the experiments. HF, SW and JM analyzed the data. HF and SW wrote the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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