Baseline sensitivity and biochemical responses of *Valsa mali* to propamidine

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ABSTRACT

In the current study, baseline sensitivity of *Valsa mali* to propamidine was determined using 80 strains collected from apple orchards in Shaanxi Province, China. The median effective concentration (EC_{50}) values for propamidine inhibiting mycelial growth ranged from 0.086 to 0.852 μg/mL, with a mean of 0.405 ± 0.137 μg/mL. After treated with propamidine, mycelia were contorted with an increased number of branches, loss of fruiting body production, and decreased cell membrane permeability. Moreover, the enzyme activities of the complexes I, II, IV and ATPase in the mitochondrial respiratory chain were increased significantly, while the enzyme activities of complexes III decreased. Importantly, both on detached leaves and branches of apple trees, propamidine applied at 100 μg/mL exhibited over 75% protective and curative efficacies, which were even better than the efficacies obtained by carbendazim at the same concentration. These results indicated that propamidine could be used as an alternative compound in controlling Valsa canker and mitochondrial respiratory chains might be correlated with the action mode of propamidine. This study encourages further investigation for the action mechanism of propamidine against plant pathogens and the information could be valuable for synthesis of new antifungal drugs with novel modes of action.

1. Introduction

Apple Valsa canker, caused by the filamentous ascomycete fungus *Valsa mali* Miyabe & Yamada, is a devastating fungal disease with a worldwide distribution, especially in eastern Asia, such as Japan, Korea and China [1–3]. Trunks, main branches and apple fruits at different developmental phases can be infected, especially when frost, sunscald, nutritional deficiencies, mechanical damage, or other pest occurs [4,5]. The disease caused by *V. mali* can result in serious losses of yield and quality of apple production [6].

Due to the limit of breeding apples for resistance to Valsa canker, in practice, fungicide applications are still the fast and main method for the control of Valsa canker, such as benzimidazole fungicide carbendazim and strobilurin fungicides [6,7]. However, the fungus is highly destructive and capable of infecting into the phloem and xylem of apple, therefore, fungicides with strong curative activity are urgently needed [8]. Carbendazim has been used in China for more than 30 years to control fungal diseases, and plant pathogens resistant to carbendazim has been reported widely [9,10]. In addition, resistance to strobilurin fungicides has also been reported in several plant pathogens [11–13]. To delay *V. mali* resistance to these fungicides, alternative fungicides with different modes of action are needed for controlling Apple Valsa canker.

Propamidine is an aromatic diamidine compound. It has long been used for the treatment of human parasites such as those causing *African trypanosomiasis*, *Acanthamoeba keratitis*, and other protozoan diseases [14–16]. The activity of propamidine against agricultural fungi was first documented in the control of gray mold caused by *Botrytis cinerea* Pers: Fr [17]. Subsequently, propamidine analogues such as betamidine, butamidine, and pentamidine were synthesized and found to have both antibacterial and antifungal activities [18,19]. Popamidine has only been registered for the control of the gray mold caused by *B. cinerea* on tomato and cucumber and has not been used for the control of Valsa canker in China. In addition, the action mechanism of propamidine is still unclear. This current situation provides an opportunity to study the antifungal activity and the action mode of propamidine against *V. mali* prior to its registration and legal use [17].

The aims of the present research were to: (a) establish the baseline sensitivity of *V. mali* to propamidine, (b) evaluate the potential value of propamidine against *V. mali* both on detached apple leaves and branches, (c) study the biochemical responses of *V. mali* treated with propamidine, and (d) further explore the action mode of propamidine.

2. Materials and methods

2.1. Fungicides and strains

Technical grade carbendazim (98.0%) was purchased from Aladdin

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Biotechnology Company (Shanghai, China) and was dissolved in 0.1 mol/L hydrochloric acid (HCl) at 10 mg/mL and stored at 4 °C. Technical grade propamidine (97.0%) was stored in our laboratory.

Twenty-two *V. mali* strains were kindly provided by the State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A & F University. Fifty-eight strains were collected from apple orchards in Yangling and Dali in Shaanxi province of China. These strains were isolated and identified according to Zang et al. [8]. All these eighty *V. mali* strains were retransferred on potato-dextrose-agar (PDA) plates at 25 °C before sensitivity testing.

2.2. Baseline sensitivity of V. mali to propamidine

The sensitivity of *V. mali* to propamidine was determined *in vitro* by inhibition of mycelial growth according to Wang et al. [10]. Briefly, inverted mycelia plugs (5 mm in diameter) cut from the edge of 3-day-old colony were transferred to PDA plates (9 cm in diameter) amended with propamidine at the concentrations of 0, 0.078125, 0.15625, 0.3125, 0.625, 1.25, or 2.5 μg/mL. The colony diameters were measured after incubation at 25 °C for 3 days in darkness. The EC_{50} values for each treatment were calculated by regressing the percentage growth inhibition against the log-transformed fungicide concentration [10].

2.3. Effect of propamidine on mycelial morphology and fruit body production of V. mali

Mycelia plug (5 mm in diameter) cut from the edge of 3-day-old colony of DL-8, YL-16, or YL-21 (randomly selected) was transferred to PDA plates containing 0 or their EC_{50} values of propamidine. After 2 days cultured at 25 °C, the top area of the colony (10 mm × 10 mm) was cut and put onto a slide glass. The mycelial morphology of *V. mali* was observed by SEM (JSM-6360LV, JEOL, Japan) [10].

Three strains above were used to test the effect of propamidine on the production of fruit body of *V. mali*. Mycelia plugs (5 mm in diameter) cultured as above were transferred to PDA plates containing 0 or their EC_{50} values of propamidine and then cultured at 25 °C in darkness for one week. Then the PDA plates were transferred to light and incubated at 25 °C for another six weeks. The colony was determined and photographed. There were three replications for each treatment and the experiment was repeated twice.

2.4. Efficacy of propamidine on cell membrane permeability

Ten mycelia plugs (5 mm in diameter) cut from 3-day-old colony of DL-8, YL-16, YL-21 were transferred to 250-mL flasks containing 100 mL of PDB (PDA without agar), respectively. After incubation at 25 °C with 175 rpm for 48 h, partial flasks were treated with propamidine at their EC_{50} values. After the flasks were shaken for additional 1 day, fresh mycelia per sample were collected. For cell membrane permeability test, 0.5 g of mycelia were suspended in a 50 mL centrifuge tube containing 20 mL double distilled water. The conductivity of the sample was measured at 0, 1, 2, 3, 4, 5, 6 and 7 h with a conductivity meter (CON510 Eutech/Oakton, Singapore). After 7 h, the mycelia in the centrifuge tube were boiled for 5 min, and the final conductivity was measured. The relative conductivity was calculated according to Duan et al. [9].

2.5. Effect of propamidine on the activities of complexes I, II, III, IV and ATP in mitochondria

Mycelia were collected and prepared as described in Section 2.4. A commercial kit (Innova Bioscience, USA) was used to test the ATP activity according to the manufacturer's instructions. The activities of the complexes I, II, III, and IV in mitochondria were determined spectrophotometrically at 340, 600, 550 and 550 nm according to previous studies, respectively [20,21]. The experiment was conducted three

times with three replicates per treatment.

2.6. Protective and curative activity of propamidine on detached leaves and branches of apple

Leaves and branches (10 cm per segment) cut from apple trees (*Malus domestica* 'Fuji', 5-year-old) with similar growing stages were rinsed with 1% sodium hypochlorite, 75% alcohol, and distilled water in turn. After air-dried, apple branches were wounded with a hole puncher (5 mm in diameter) to remove the bark. For protective activity, leaves or wounded branches were sprayed or daubed with water (negative control), carbendazim at 100 μg/mL (positive control), or propamidine at 50, 100, or 150 μg/mL, respectively. After 24 h, the treated leaves and branches were inoculated with mycelia plugs cut from the margin of the actively growing strain YL-16 (randomly selected). For curative activity, the leaves and branches were inoculated with mycelia plugs. After 24 h, the leaves or branches were sprayed or daubed with water, carbendazim at 100 μg/mL, or propamidine at 50, 100, and 150 μg/mL, respectively. In this test, leaves were wounded with a needle (avoiding the major vein, positive inoculation) before inoculation. Then the inoculated leaves and branches were kept on wet filter paper. After incubation at 25 °C for 3 days, the diameters of each disease lesion in two perpendicular directions on leaves or branches were measured and the lesion area was calculated [6]. Five leaves or branches per concentration were used and the experiment was performed three times.

Lesion area on leaves (cm^2)

$$= \prod \times \text{(the mean value of diameters in two perpendicular directions)}^2$$

$$\text{Lesion area on branches (cm}^2) = 1/4 \times \prod \times \text{length of long lesion}$$
$$\times \text{length of short lesion}$$

2.7. Data analysis

Sigmastat statistical software package (SPSS 19.0) was used to analyze the data obtained in the current research. The analysis of variance (ANOVA) procedure and Fisher's least significant difference (LSD, $p = 0.05$) were used to analyze the significant differences on the EC_{50} values and other biochemical characteristics data.

3. Results

3.1. Baseline sensitivity of V. mali to propamidine

The EC_{50} values of 80 *V. mali* strains for propamidine ranged from 0.086 to 0.852 μg/mL with a mean EC_{50} value of 0.405 ± 0.137 μg/mL. The frequency distribution of EC_{50} values for propamidine was described by a unimodal curve over a narrow range (Fig. 1). The range of variation factor was 9.907, indicating the absence of propamidine-
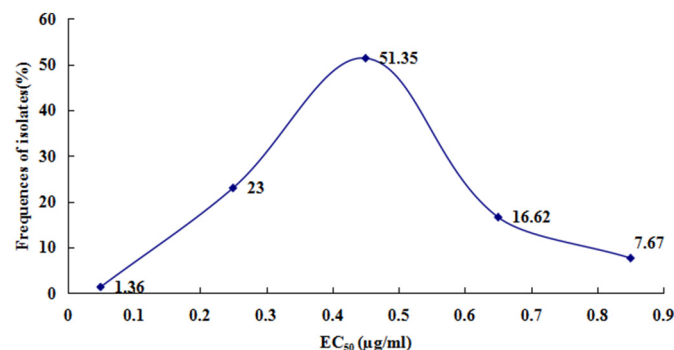

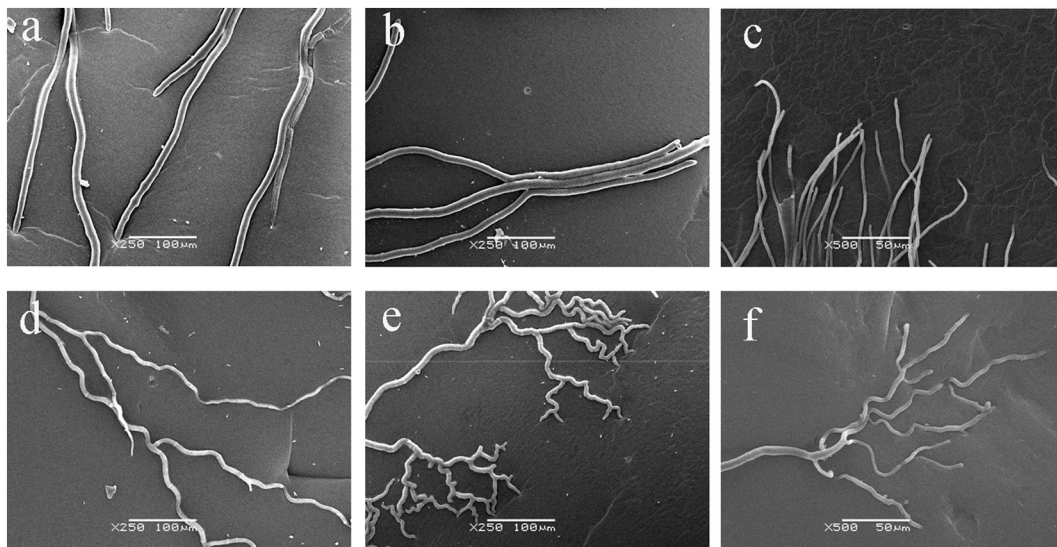**Fig. 1.** Sensitivity distribution of 80 strains of *V. mali* to propamidine.

Fig. 2. Comparison on mycelial morphology of *V. mali* treated with propamidine or not. a, b and c were untreated control; d, e and f were treated with propamidine at their EC$_{50}$ values.

resistant subpopulations among these strains.

3.2. Effect of propamidine on hyphal morphology and fruit body production

The mycelial morphology altered significantly. After treated with propamidine, mycelia were severely distorted and the number of top branches increased (Fig. 2). After incubation at 25 °C for another 6 weeks, a large number of fruit bodies were generated on the colony of three wild-type *V. mali* strains DL-8, YL-16, and YL-21untreated with propamidine, while few fruit body production were observed on the colony treated with propamidine at their EC$_{50}$ values. Moreover, the edge of the treated colony was sparse and thin compared with control (Fig. 3).

3.3. Cell membrane permeability

With propamidine treatment or not, relative conductivity of the three wild-type *V. mali* strains DL-8, YL-16, and YL-21 increased over time. When treated with propamidine at their EC$_{50}$ values, relative conductivity of the three wild-type *V. mali* strains were always lower than the untreated control, indicating the decrease of cell membrane permeability (Fig. 4).

3.4. Enzyme activity in mitochondria

When treated with propamidine at their EC$_{50}$ concentrations, enzyme activities of the three strains differed from each other. The activities of complexes I and II in DL-8 and YL-16 were increased, while these activities did not differ in YL-21. Interestingly, complex III
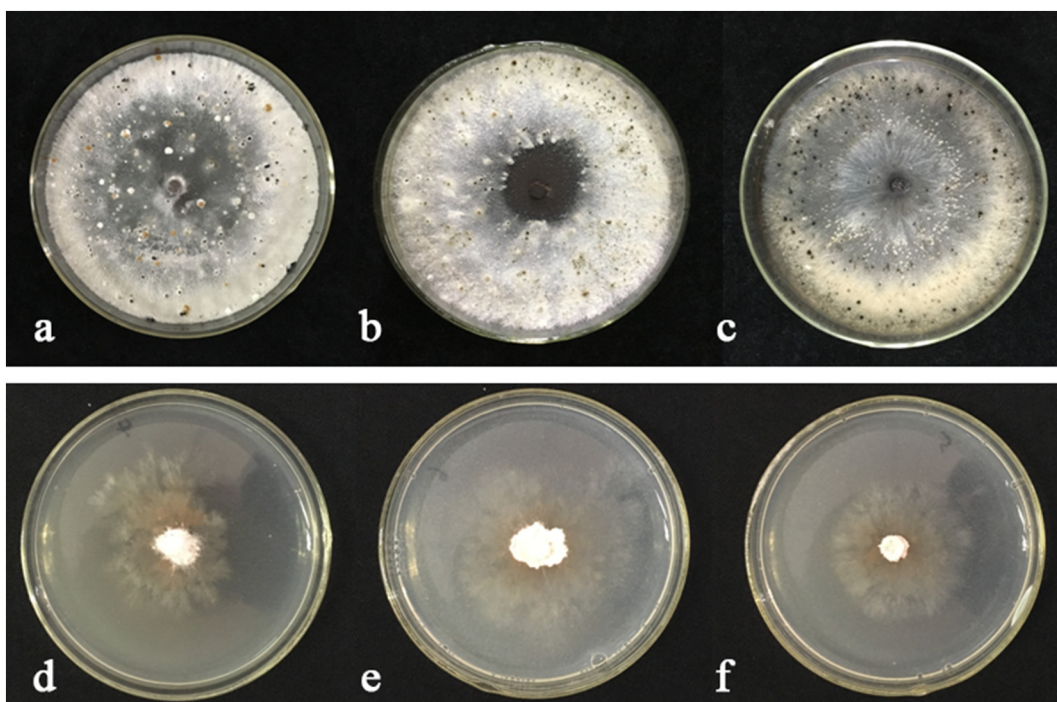

Fig. 3. Comparison in fruit body production of *V. mali* incubated at 25 °C for 50 days with propamidine treatment or not. a, b, and c: control; d, e, and f: propamidine at their EC$_{50}$ concentrations.
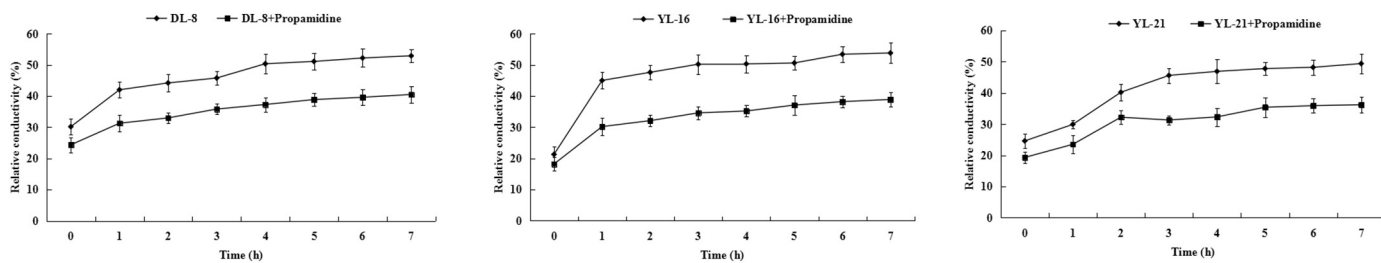
Fig. 4. Relative conductivity of mycelia of three wild-type strains DL-8, YL-16, and YL-21 with or without propamidine treatment. Bars indicate standard deviation of three experiments.
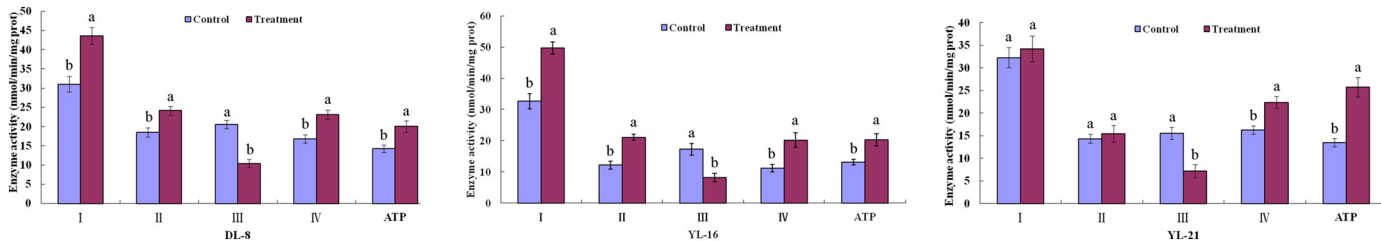

Fig. 5. Enzyme activities in mitochondria of three wild-type strains DL-8, YL-16, and YL-21 with or without propamidine treatment. Bars indicate standard deviation of three experiments.

activities were significantly decreased while complex IV and ATP activities increased in all three strains (Fig. 5).

3.5. Protective and curative activity of propamidine on detached leaves and branches of apple

As expected, wild-type strain YL-16 exhibited strong pathogenicity both on detached apple leaves and branches when treated with water (Figs. 6, 7). On detached apple leaves, 85.68% protective and 90.92% curative efficacies were obtained when treated with propamidine at 100 μg/mL. On detached apple branches, 75.58% protective and 84.55% curative efficacies were obtained when treated with propamidine at 100 μg/mL. These values were significantly higher than that obtained by carbendazim at 100 μg/mL, respectively. Importantly, when treated with propamidine at 150 μg/mL, 90.46% protective and 95.83% curative efficacies on detached apple leaves and 83.86% protective and 90.61% curative efficacies on detached apple branches were

obtained, respectively. Moreover, propamidine at 50 μg/mL also obtained over 55% protective and curative efficacies both on detached apple leaves and branches (Table 1). These data indicated that propamidine exhibited both protective and curative activity against apple Valsa canker.

4. Discussion

In the current research, baseline sensitivity of 80 *V. mali* wild-type strains to propamidine was established by inhibition of mycelial growth. The EC_{50} values ranged from 0.086 to 0.852 μg/mL, with a mean of 0.405 ± 0.137 μg/mL. All these strains were not exposed to propamidine. Therefore, the baseline sensitivity could be used in resistance monitoring and assessment of resistance risk programs after it's legal use in future. The frequency distribution curve was unimodal with a narrow range, which might imply a low potential for resistance risk of *V. mali* to propamidine. Although resistance to propamidine had been
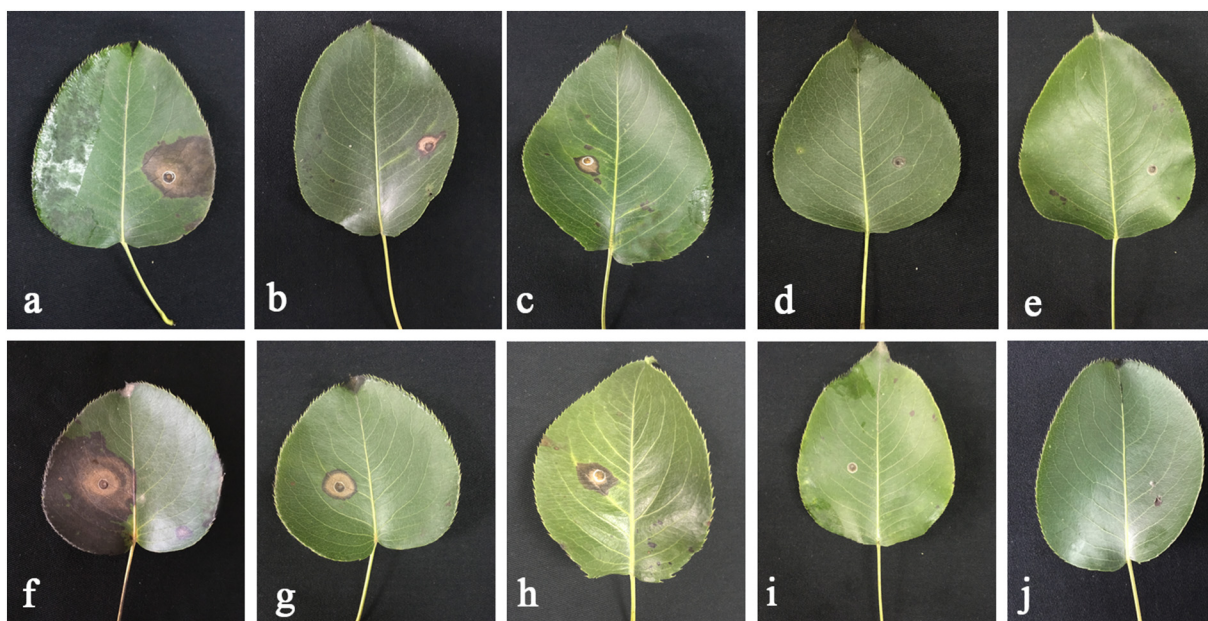

Fig. 6. Protective (a–e) and curative (f–j) activity of propamidine against *V. mali* YL-16 on detached apple leaves. a, f: water control; b, g: carbendazim at 100 μg/mL; c, h: propamidine at 50 μg/mL; d, i: propamidine at 100 μg/mL; e, j: propamidine at 150 μg/mL.
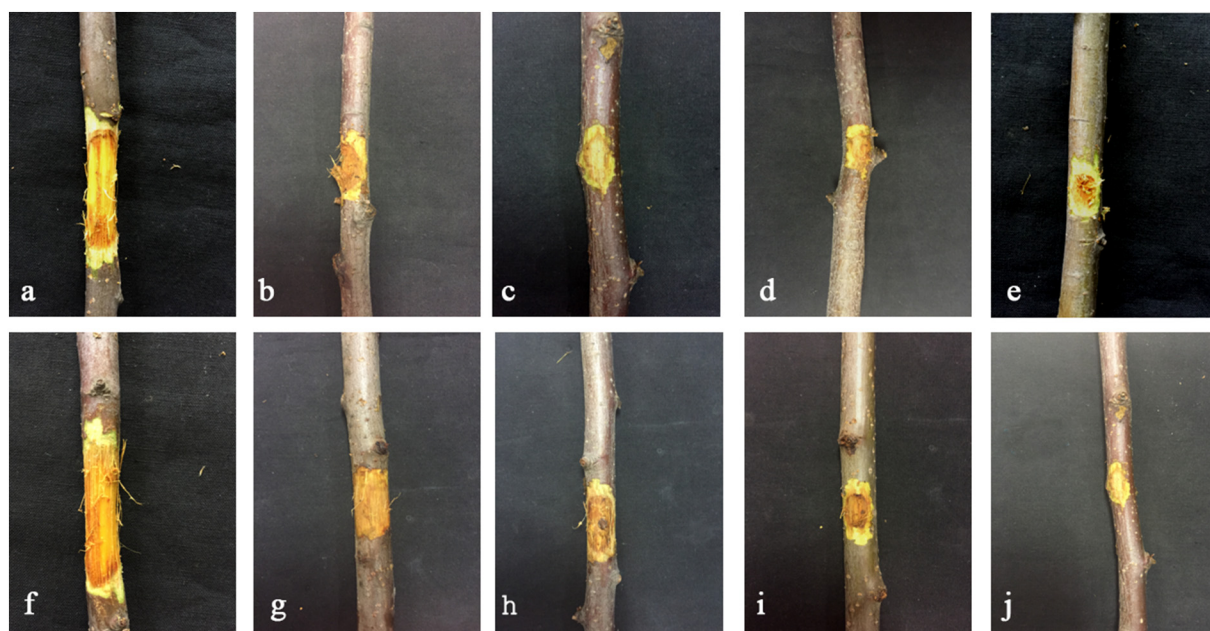
Fig. 7. Protective (a–e) and curative (f–j) activity of propamidine against *V. mali* YL-16 on detached apple branches. a, f: water control; b, g: carbendazim at 100 μg/mL; c, h: propamidine at 50 μg/mL; d, i: propamidine at 100 μg/mL; e, j: propamidine at 150 μg/mL.

Table 1
Protective and curative activity of propamidine against *V. mali* YL-16 on detached apple leaves and branches.

Treatment	Protective activity on leaves		Curative activity on leaves		Protective activity on branches		Curative activity on branches	
	Lesion area (cm^2)[a]	Efficacy (%)[b]	Lesion area (cm^2)	Efficacy (%)	Lesion area (cm^2)	Efficacy (%)	Lesion area (cm^2)	Efficacy (%)
Propamidine (50 μg/mL)	1.65 b	64.21 d	2.92 b	68.80 c	2.04 b	56.69 c	2.21 b	64.81 c
Propamidine (100 μg/mL)	0.66 c	85.68 ab	0.85 c	90.92 a	1.15 bc	75.58 b	0.97 c	84.55 b
Propamidine (150 μg/mL)	0.44 c	90.46 a	0.39 c	95.83 a	0.76 c	83.86 a	0.59 c	90.61 a
Carbendazim (100 μg/mL)	1.30 b	71.80 c	2.35 b	78.38 b	1.93 b	59.02 c	2.19 b	65.13 c
Water	4.61 a	–	9.36 a	–	4.71 a	–	6.28 a	–

[a] Values followed by the same letter within the same column were not significantly different in LSD (least significant difference) tests at $P = 0.05$.
[b] Control efficacy = [(Lesion area of control − Lesion area of treatment)/(Lesion area of control)] × 100%.

reported in *B. cinerea* in laboratory, there was no cross-resistance between propamidine and other traditional fungicides such as benzimidazole fungicide carbendazim, dicarboximide fungicide iprodione, and anilino-pyrimidine fungicide pyrimethanil, indicating the action mode of propamidine differed from traditional fungicides [17]. Moreover, the risk of *B. cinerea* resistance to propamidine was low [17]. In this study, our efforts to induce *V. mali* propamidine-resistant strains was unsuccessful. Taken together, the risk of *V. mali* resistance to propamidine might be low.

Cell membranes have selective permeability and able to regulate the intracellular balance by controlling materials that enter and exit [22]. They participate in a series of cellular processes, such as cell signalling, cell adhesion, and ion conductivity [23,24]. After treated with propamidine, mycelial morphology altered significantly. More importantly, cell membrane permeability decreased, indicating that the concentration of electrolytes in solution decreased, which was consistent with the previous study that against *Sclerotinia sclerotiorum* [10]. These suggested that propamidine might destroy the function of cell membrane and disrupt the balance between intracellular and extracellular fluids.

For *V. mali*, fruit body are essential for long-term survival and propagation in their life-cycle. Under suitable conditions, conidia germinate and then easily penetrate into wounded host phloem and xylem [25,26]. With hyphal growth, pycnidia and asexual fruit body formation, including a large number of conidiophores and conidia [27,28]. In this research, the strains treated with propamidine lost the ability of fruit body production, which was consistent with the previous study

that the ability of sclerotial production decreased when *S. sclerotiorum* strains treated with propamidine [10]. These data suggested that propamidine could interrupt the reinfection of *V. mali* in their life cycle.

Previous studies demonstrated that propamidine might be a small molecular DNA binding drug. Propamidine and its homologues, such as betamidine, butamidine, and pentamidine, could bind the minor groove of AT-rich sequences in DNA [29–31]. These compounds contained the structure of the aromatic rings and their structures were free to rotate. The study on the action mechanism of pentamidine against *Saccharomyces cerevisiae* suggested that pentamidine might target on the mitochondrial respiratory chain [32]. Other studies have found that pentamidine and their homologues could inhibit protein biosynthesis in mouse hepatocytes and the bioactivity of introns in 26SrRNA against *Pneumocystis carinii* [33,34]. In the present study, complex III activities were significantly decreased while complex IV and ATP activities increased one day after propamidine treatment, which was consistent with our previous study that propamidine could inhibit the mitochondrial complex III activity in *B. cinerea* [19]. These results indicated that mitochondrial respiratory chain might be conferred with the molecular action target of propamidine. Although numerous studies have been conducted, the action mode of propamidine is still unclear. The work to further explore the action mode of propamidine is underway in our laboratory.

Detached apple branches were commonly used for the *V. mali* virulence test. In this study, detached apple leaves were also used for pathogenicity assay due to their advantages of easy obtain of material,

accurate and stable, and easy to operate [35]. Both on detached apple leaves and branches, propamidine exhibited significantly better protective and curative efficacy against *V. mali* that the reference fungicide carbendazim. These results suggested that propamidine exhibited the potential value as an alternative candidate for the management of Apple Valsa canker. Previous studies demonstrated that tissues of bark, phloem and xylem could be invaded extensively by *V. mali* and fungicide application was not always successful [7]. However, chemical control is still the main method at present. Importantly, propamidine exhibited both excellent protective and curative activity, and the curative activity was always better than the protective activity, therefore, propamidine should be applied either as a protective or a curative fungicide. Moreover, propamidine could be used in combination with other fungicides that have different modes of action. In addition, comprehensive prevention measures such as physical, chemical, and biological agents should be designed for the management of Apple Valsa canker. Meanwhile, new strategies including fully understanding the infection mechanism and breeding with genetic resistance against *V. mali* are necessary.

In our knowledge, the current research is the first report on the baseline sensitivity of Chinese *V. mali* populations to propamidine. It demonstrated that both on detached apple leaves and branches, propamidine exhibited both protective and curative activity against *V. mali*. Biochemical responses indicated that propamidine might target on the mitochondrial respiratory chain, which still need further study. The current study encourages further investigation on the action mechanism of propamidine against plant pathogens and the information provided in this study will be useful for the development of alternative antifungal drugs.

Acknowledgements

References

[1] K. Abe, N. Kotoda, H. Kato, J. Soejima, Genetic studies on resistance to Valsa canker in apple: genetic variance and breeding values estimated from intra- and inter-specific hybrid progeny populations, Tree Genet. Genomes 7 (2011) 363–372.

[2] L. Vasilyeva, W.G. Kim, *Valsa mali* Miyabe et Yamada, the causal fungus of apple tree canker in East Asia, Mycobiology 28 (2000) 153–157.

[3] N. Song, Q.Q. Dai, B.T. Zhu, Y.X. Wu, M. Xu, R.T. Voegele, X.N. Gao, Z.S. Kang, L.L. Huang, Gα proteins Gvm2 and Gvm3 regulate vegetative growth, asexual development, and pathogenicityon apple in *Valsa mali*, PLoS One 12 (2017) e0173141.

[4] X.W. Ke, Z.Y. Yin, N. Song, Q.Q. Dai, R.T. Voegele, Y.Y. Liu, H.Y. Wang, X.N. Gao, Z.S. Kang, L.L. Huang, Transcriptome profiling to identify genes involved in pathogenicity of *Valsa mali* on apple tree, Fungal Genet. Biol. 68 (2014) 31–38.

[5] H.X. Peng, X.Y. Wei, Y.X. Xiao, Y. Sun, S.P. Shang, A.R. Biggs, M.L. Gleason, M.Q. Zhu, G.Y. Sun, Management of Valsa Canker on apple with adjustments to potassium nutrition, Plant Dis. 100 (2015) 884–889.

[6] Y. Wang, Y. Sun, L.R. Han, X. Zhang, J.T. Feng, Potential use of cuminic acid as a botanical fungicide against *Valsa mali*, Microb. Pathog. 106 (2017) 9–15.

[7] K. Abe, N. Kotoda, H. Kato, J. Soejima, Resistance sources to Valsa canker (*Valsa ceratosperma*) in a germplasm collection of diverse *Malus* species, Plant Breed. 126 (2007) 449–453.

[8] R. Zang, Z.L. Li, X.W. Ke, X.J. Wang, Z.Y. Yin, Z.S. Kang, L.L. Huang, A nested PCR assay for detecting *Valsa Mali* var. *Mali* in different tissues of apple trees, Plant Dis. 96 (2012) 1645–1652.

[9] Y.B. Duan, C.Y. Ge, S.M. Liu, X.J. Feng, C.J. Chen, M.G. Zhou, Effect of

[10] phenylpyrrole fungicide fludioxonil on morphological and physiological characteristics of *Sclerotinia sclerotiorum*, Pestic. Biochem. Physiol. 106 (2013) 61–67.

[11] Y. Wang, Y. Sun, Y. Zhang, Y.X. Zhang, L.R. Han, X. Zhang, J.T. Feng, Sensitivity and biochemical characteristics of *Sclerotinia sclerotiorum* to propamidine, Pestic. Biochem. Physiol. 135 (2017) 82–88.

[11] B.A. Fraaije, J.A. Lucas, W.S. Clark, F.J. Burnett, QoI resistance development in populations of cereal pathogens in the UK, Proceedings of the BCPC International Congress, 2003 10-12 November, 2, The British Crop Protection Council, Alton, Hampshire, Crop Sci. Tech., UK, 2003, pp. 689–694.

[12] U. Gisi, H. Sierotzki, A. Cook, A. McCaffery, Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides, Pest Manag. Sci. 58 (2002) (2002) 859–867.

[13] S.F.F. Torriani, P.C. Brunner, B.A. McDonald, H. Sierotzki, QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*, Pest Manag. Sci. 65 (2009) 155–162.

[14] C. Bailly, D. Perrine, J.C. Lancelot, C. Saturnino, M. Robba, M.J. Waring, Sequence-selective binding to DNA of bis (amidinophenoxy) alkanes related to propamidine and pentamidine, Biochem. J. 323 (1997) 23–31.

[15] A.T. Fuller, Antibacterial action and chemical constitution in long-chain aliphatic bases, Biochem. J. 36 (1942) 548–558.

[16] W.O. Elson, The antibacterial and fungistatic properties of propamidine, J. Infect. Dis. 76 (1945) 193–197.

[17] J. Hou, Y.N. Gao, J.T. Feng, Z.Q. Ma, X. Zhang, Sensitivity of *Botrytis cinerea* to propamidine: in vitro determination of baseline sensitivity and the risk of resistance, Eur. J. Plant Pathol. 128 (2010) 261–267.

[18] S.K. Jones, J.E. Hall, M.A. Allen, S.D. Morrison, K.A. Ohemeng, V.V. Reddy, J.D. Geratz, R.R. Tidwell, Novel pentamidine analogs in the treatment of experimental *Pneumocystis carinii* pneumonia, Antimicrob. Agents Chemother. 34 (1990) 1026–1030.

[19] F.L. Wu, W.B. Jin, J.T. Feng, A.L. Chen, Z.Q. Ma, X. Zhang, Propamidine decrease mitochondrial complex III activity of *Botrytis cinerea*, BMB Rep. 43 (2010) 614–621.

[20] A. Barrientos, In vivo and in organello assessment of OXPHOS activities, Methods 26 (2002) 307–316.

[21] M. Spinazzi, A. Casarin, V. Pertegato, L. Salviati, C. Angelini, Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells, Nat. Protoc. 7 (2012) 1235–1246.

[22] I. Budin, N.K. Devaraj, Membrane assembly driven by a biomimetic coupling reaction, J. Am. Chem. Soc. 134 (2012) 751–753.

[23] S.J. Singer, G.L. Nicolson, The fluid mosaic model of the structure of cell membranes, Science 175 (1972) 720–731.

[24] H.T. Mcmahon, J.L. Gallop, Membrane curvature and mechanisms of dynamic cell membrane remodelling, Nature 438 (2005) 590–596.

[25] O. Tamura, I. Saito, Histopathological changes of apple bark infected by *Valsa ceratosperma* (Tode ex Fr.) Maire during dormant and growing periods, Ann. Phytopathol. Soc. Jpn 48 (1982) 490–498.

[26] K. Sawamura, Y. Harada, K. Yukita, Apple Valsa canker caused by *Valsa ceratosperma* (Tode: Fr.) Maire, Bull. Fac. Agric. Hirosaki Univ. 53 (1990) 10–32.

[27] X.W. Ke, L.L. Huang, Q.M. Han, X.N. Gao, Z.S. Kang, Histological and cytological investigations of the infection and colonization of apple bark by *Valsa Mali* var, *Mali*, Aust. Plant Pathol. 42 (2012) 85–93.

[28] K. Suzaki, Population structure of *Valsa ceratosperma*, causal fungus of Valsa canker, in apple and pear orchards, J. Plant Pathol. 74 (2008) 128–132.

[29] K.E. Miletti, M.J. Leibowitz, Pentamidine inhibition of group I intron splicing in *Candida albicans* correlates with growth inhibition, Antimicrob. Agents Ch. 44 (2000) 958–966.

[30] A. Lansiaux, F. Tanious, Z. Mishal, L. Dassonneville, A. Kumar, C.E. Stephens, Q. Hu, W.D. Wilson, D.W. Boykin, C. Bailly, Distribution of furamidine analogues in tumor cells: targeting of the nucleus or mitochondria depending on the amidine substitution, Cancer Res. 62 (2002) 7219–7229.

[31] B. Nguyen, M.P.H. Lee, D. Hamelberg, A. Joubert, C. Bailly, R. Brun, S. Neidle, W.D. Wilson, Strong binding in the DNA minor groove by an aromatic diamidine with a shape that does not match the curvature of the groove, J. Am. Chem. Soc. 124 (2002) 13680–13681.

[32] Y. Zhang, A. Bell, P.S. Perlman, M.J. Leibowitz, Pentamidine inhibits mitochondrial intron splicing and translation in *Saccharomyces cerevisiae*, RNA 6 (2000) 937–951.

[33] K. Bielawski, A. Galicka, A. Bielawska, K. Sredzinska, Inhibitory effects of pentamidine analogues on protein biosynthesis *in vitro*, Acta Biochim. Pol. 47 (2000) 113–120.

[34] A.B. Montgomery, J.M. Luce, J. Turner, E. Lin, R.J. Debs, K.J. Corkery, E.N. Brunette, P.C. Hopewell, Aerosolised pentamidine as sole therapy for *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome, Lancet 330 (1987) 480–483.

[35] J.L. Wei, L.L. Huang, Z.P. Gao, X.W. Ke, Z.S. Kang, Laboratory evaluation methods of apple Valsa canker disease caused by *Valsa ceratosperma* sensu Kobayashi, Acta Phytopathol. Sin. 40 (2010) 14–20.