Basis and Practice of Apple *Marssonina* leaf blotch management in China

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Abstract

Diplocarpon mali *is the causal agent of Marssonina leaf blotch of apple, which causes severe early defoliation. Little is known about the pathogen biological characteristic, limiting the development of prevention and control technology. In these years, Isolation and culture methods in vitro were established successfully in our previous study, and the pure culture of D. mali was obtained. Based on this, biological characteristics of the pathogen were further investigated. Through histology and cytology techniques, it was found that the pathogen had an obligate parasitic stage and then turned into necrotrophic stage during its infection progress. Furthermore, the periods after blooming are explicit to be important for primary infection and are critical for disease control according to spores transmission and infection rules and orchard temperature and humidity. We practiced and demonstrated this spray schedule in Shaanxi apple production areas and showed very good control efficacy of Marssonina leaf blotch.*

Keywords: *Diplocarpon mali*, biological characteristics, infection process, disease control

Introduction

Diplocarpon mali Harada & Sawamura (anamorph *Marssonina coronaria* [Ell. & J. J. Davis] J. J. Davis) is the causal agent of *Marssonina* leaf blotch of apple, which results in severe premature defoliation during growing season of apple tree (Harada *et al.*, 1974). The disease has been recorded in many countries of Asia and Europe (Wöhner & Emeriewen, 2018). Especially in China, the disease has become a significant disease problem in recent two decades (Zhou *et al*., 2008; Zhao, 2012; Feng *et al*., 2019). Here we summarized some information based on our previous studies on *Marssonina* leaf blotch of apple in China, including the pathogen biology, infection progress, and disease control practice.

Material and Methods

Isolation and cultivation of *D. mali* followed Zhao *et al*. (2009 and 2010)'s the instructions. The procedure for inoculation, histology and cytology study were described by Zhao *et al*. (2013). The domesticated apple (*Malus* × *domestica* Borkh.) Fuji (*M. domestica*) and Shandingzi (*M. baccata*) inoculated with *D. mali* were used for comparative transcriptome analysis (Feng *et al*., 2019). And orchards with 10 years Fuji apple trees threatened by *Marssonina* leaf blotch in Pucheng Shaanxi province were selected for control study. (Zhou *et al*., 2008).

Results

No effective in vitro culture system and limited information associated with biology of *D. mali*, resulted in the control difficulty to *Marssonina* leaf blotch. Thus, we first established the system of pathogen isolation and cultivation, followed by acquiring the information related

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to its epidemiology and getting insight into the interaction between *D. mali* and apple, and achieving to find the key point of the disease control. The results are as follows.

Isolation and cultivation *D. mali* was successfully isolated by single spore isolation (Zhao *et al*., 2009 a). PCDB (potato and carrot dextrose broth) and PCSB (potato and carrot sucrose broth) incubated at 25℃ for 14 days are recommended for mycelial growth and conidial production (Zhao *et al*., 2010). This information is very useful for host-pathogen interaction research and high effective fungicides assessment *in vitro* (Zhao *et al*., 2009 b).

Virulence differentiation Xinjiang crabapple, which showed high susceptibility to *D. mali*, was selected to evaluate strain virulence and to reveal virulence composition of its population in Shaanxi province. *D. mali* strains exhibited obvious virulence differentiation, but the differentiation was unrelated to their geographical origins (Zhao, 2012).

Infection process During the infection progress, *D. mali* could form a typical haustorial structure in host cells with extension of subcuticular and intercellular hyphae, similar to those observed in obligate biotrophic fungi (figure 1). Meanwhile, necrotrophic intracellular hyphae and peculiar subcuticular hyphal strands (SHS) could also be observed during the progress (Zhao *et al*., 2013). When the necrotrophic intracellular hyphae and peculiar SHS were formed, the pathogen began to proliferate and reproduce quickly (figure 2, Zhao *et al*., 2013). These results suggest that *D. mali* may behave like a hemibiotroph and can use both biotrophic and necrotrophic strategies to infect leaf tissues.

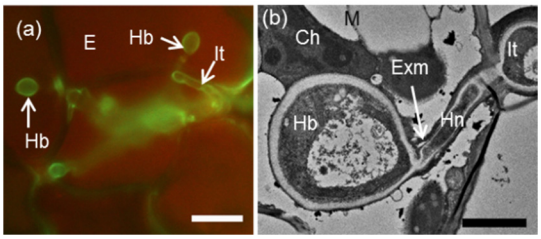

Figure 1. The haustoria of *D. mali* on apple leaves. (a) *D. mali* extends underneath of the cuticle, and colonizes in epidermal cells by forming haustoria; Bar = 10 μm. (b) a haustorium in a spongy cell with a slender haustorial neck and a nearly spherical haustorium body; note, chloroplasts in the cell are disorganized; Bar=1 μm. Ch: chloroplasts E: epidermal cell, Exm: extrahaustorial membrane, Hb: haustorium body, Hn: haustorium neck, It: intercellular hypha, M: mesophyll cell.

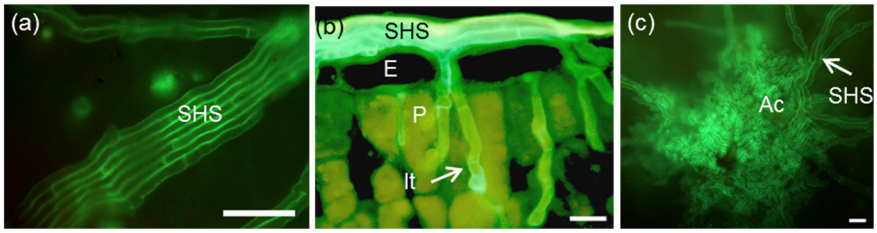

Figure 2. The subcuticular hyphal strands (SHS) of *D. mali*. (a) the SHS consist of many parallel subcuticular hyphae; Bar=10 μm. (b) SHS generates intercellular hyphae extending through the interspace of epidermal cells into the palisade tissue; Bar=10 μm. (c) an immature acervulus with SHS on apple leaves; Bar=10 μm. Ac: acervulus, E: epidermal cell, It: intercellular hypha, P: palisade cell.

Microconidia Microconidia, which are commonly associated with acervuli of *D. mali* on diseased apple leaves in autumn, have been proved belongs to *D. mali*. However, it could not cause the disease on apple leaves (unpublished data). Considering its generation condition and low level of metabolic activity, microconidia should not be considered as the primary inoculum and probably acts an important role in sexual recombination stage, which is unknown until now.

Control study Prevention of *Marssonina* leaf blotch of apple mainly relies on resistant varieties, chemical and agricultural control measures. Our studies demonstrated that

Shandingzi is much more resistant to *D. mali* than Fuji based on both cytology (Wang *et al*., 2012) and comparative transcriptome analysis (Feng *et al*., 2019), which could be used as a resistant germplasm for breeding. Additionally, disease control in the field was practiced for several years considering spores transmission and infection rules and orchard temperature and humidity. We found that periods after blooming until young fruit stage are the key period for primary infection of *D. mali*, which are critical for disease control (figure 3). According to the frequency and duration of rain in this period, the specific spray time could be adjusted. The non-systemic protective fungicide propineb exhibited 90% control efficacy if it was sprayed during this period (figure 3, Zhou *et al*., 2008).

(a) Control efficiency of propineb on *Marssonina* leaf blotch with different application time

Year	Treat -ment	Application time of fungicide	Disease index	Control efficacy
2005	T51	4-29	0.19b	97.04a
	T54	5-10	0.41b	93.68a
	T55	5-19	2.06b	68.54b
	CK		6.54a	
2006	T61	4-29	35.84b	52.00b
	T62	4-29, 5-23	15.37cd	79.41ba
	T63	4-29,5-23,6-12	5.19ed	93.05a
	T64	4-29,5-23,6-12,7-03	1.71ed	97.71a
	T65	4-29,5-23,6-12,7-03,7-20	0.47e	99.37a
	T66	4-29,5-23,6-12,7-03,7-20,8-09	0.08e	99.90a
	T67	5-23,6-12,7-03,7-20,8-09	6.6ed	91.16a
	T68	6-12, 7-03,7-20,8-09	29.76cb	60.14b
	T69	7-03,7-20,8-09	34.06b	54.38b
	T610	7-20,8-09	33.49b	55.15b
	T611	8-09	31.43b	57.91b
	CK		76.87a	

(b) Regression equation and EC_{50} of two fungicides against *D. mali* in different growing period

Fungicides	Pathogen developmental stages	Regression equation	EC_{50}/ μg·ml^{-1}
Propineb	Conidia germination	*Y*=4.86937+4.18252*X*	1.07
	Mycelium growth	*Y*=4.17174+0.99983*X*	6.67
	Acervulus formation	*Y*=3.62126+5.88636*X*	1.70
Difenoconazole	Conidia germination	*Y*=1.97282+1.20186*X*	331.131
	Mycelium growth	*Y*=10.38982+2.68130*X*	0.009
	Acervulus formation	*Y*=8.37419+2.08920*X*	0.024

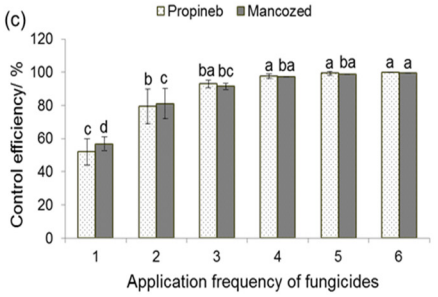

Figure 3. Control efficacy of propineb and mancozed. (a) Control efficiency of propineb on *Marssonina* leaf blotch with different application time. The survey time was on August 31st in 2005 and August 18th in 2006. The lowercase showed significantly difference among treatments based on Fisher's least significant difference (*P* =0. 05) for disease index, and Duncan's multiple range test (*P* =0. 05) for control efficiency. (b) Regression equation and EC_{50} of propineb and difenoconazole against *D. mali* in different growing period. *X* Showed concentration logarithm, *Y* showed inhibition probability. (c) Effect of application frequency of fungicides on controlling *Marssonina* leaf blotch on apple. The application time of fungicides started from April 29th 2006 until August 9th. Different lowercase showed significantly difference between treatments based on Duncan's multiple range test (*P* =0. 05).

Discussion

In the past decade, we have made lots of effort to reveal the biology characteristics of *D. mali*, the interaction between it and the host, and the disease control stagey. According to the green islands on the diseased apple leaves and slow growth rate on the artificial media in the optimum environment, *D. mali* was speculated to have a strong parasitic property (Zhao *et al*., 2009). Haustoria and SHS of *D. mali* as infection strategies suggested that it can use both biotrophic and necrotrophic strategies to establish infections on apple leaves (Zhao *et al*., 2013). What more, conidia were isolated and confirmed to play important roles in the life cycle of *D. mali*. However, no ascospore was isolated in Shaanxi province until now. Although miroconidia were also isolated, the role of miroconidia together with sexual reproduction in the disease cycle is still unclear. Thus, only conidia represent pathogenicity,

and are thought to be primary inoculums responsible for disease epidemiology. The primary infection usually occurred right after blossom which is the key period for disease control.

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