Journal of Integrative Agriculture 2017, 16(11): 2496–2503

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RESEARCH ARTICLE

Identification of two *Monilia* species from apricot in China

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Abstract

Monilinia fructicola, *Monilia mumecola* and *Monilia yunnanensis* have been reported as the causal agents of brown rot disease on stone fruits in China. Up to date, these species have been identified from peach and plum, and *M. mumecola* has also been reported on apricot recently. To investigate whether *M. fructicola* and *M. yunnanensis* can cause brown rot disease on apricot in China, 37 isolates were collected from four orchards in Chongqing and Beijing municipalities in 2014. These isolates were divided into two phenotypes according to their distinct colony appearances. Two representative isolates of each phenotype and reference species of *M. mumecola* from apricot were selected for further analysis. Based on the morphological characterization and molecular identification, the two phenotypes of isolates were identified as *M. fructicola* and *M. yunnanensis*, respectively.

Keywords: brown rot, apricot, *Monilinia fructicola*, *Monilia yunnanensis*

1. Introduction

Common apricot (*Prunus armeniaca* L.) has been cultivated in China for over 3 500 years, and China is considered as one of the original centers of apricot in the world (Zhao *et al*. 2005; Zhebentyayeva *et al*. 2012). Up to date, as many as 9 of the total 10 apricot species that have been confirmed in worldwide were grown in China (Zhao *et al*. 2005). China is the top producer of apricot, both for production area and the total yield in the world (Zhao *et al*. 2005; Ren 2007). Apricot is one of the most favorite fruits in China, not only for the unique flavor and blight color, but also the rich nutrition and medical value. Apricot contains abundant carotene, which is about 22.4 times of apple and is the highest concentration in all fruits (Ren 2007). Therefore, production of apricot for fresh or processed fruit is economically important in China (Ren 2007). Furthermore, apricot is also considered as one of the most favorable trees for economical forest in north and northwest areas of China (Zhao *et al*. 2005). However, apricot cultivation is hampered by high sensitivity to diseases such as brown rot caused by *Monilinia* spp. (Zhebentyayeva *et al*. 2012).

Brown rot is one of the most troublesome diseases on stone fruits, including peach, plum, apricot and cherry worldwide, causing considerable losses in inadequately protected orchards (Luo 2017). Its causal agents in China were reported as *Monilinia laxa* (anamorph: *Monilia laxa*) and *Monilinia fructigena* (anamorph: *Monilia fructigena*)

Received 12 October, 2016 Accepted 13 March, 2017
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doi: 10.1016/S2095-3119(17)61734-9

in the early 20th century (Dai *et al*. 1958; Wang *et al*. 1998). But in 2005, *Monilinia fructicola* (anamorph: *Monilia fructicola*), which is widely distributed in the eastern United States, Canada, Australia and New Zealand was reported in China for the first time (Zhu *et al*. 2005), soon it was discovered that the species was actually the dominant species and existed in China for a long time (Hu *et al*. 2011; Zhu *et al*. 2011; Yin *et al*. 2015). Recently, *Monilia mumecola* was also identified as the pathogen of brown rot disease on stone fruits of peach, apricot, cherry and plum in China (Hu *et al*. 2011; Yin *et al*. 2014a, b, 2015), this species was initially isolated from mume in 1982, Japan and then nomenclature was fulfilled in 2004 (Harada *et al*. 1990; Harada *et al*. 2004). *Monilia yunnanensis* is a new species isolated from peach in 2011, China (Hu *et al*. 2011), up to now it was also reported causing brown rot on hawthorn and plum (Zhao *et al*. 2013; Yin *et al*. 2015). The extensive studies carried out to investigate the population of *Monilia* species in China indicated that the *Monilia* species causing brown rot disease on stone fruits in China were actually *M*. *fructicola*, *M*. *mumecola* and *M*. *yunnanensis* (Hu *et al*. 2011; Yin *et al*. 2015). These three species have been reported on peach (Hu *et al*. 2011) and plum (Yin *et al*. 2015). The apricot brown rot caused by *M*. *mumecola* has also been reported recently (Yin *et al*. 2014b), but studies of whether the other two species could cause brown rot diseases on apricot in China have not yet been conducted.

Cultural practices (such as eliminating overwinter inocula) and fungicide application were the main strategies used to control brown rot disease caused by *Monilia* spp. on stone fruits. However, fungicide resistance, especially for isolates of *M*. *fructicola* has been reported in many countries including China (Elmer and Gaunt 1986; Schnabel *et al*. 2004; Luo *et al*. 2008; Luo and Schnabel 2008a, b; Chen *et al*. 2014). In order to control brown rot effectively, information about the species structure would be crucial for developing effective management strategies.

Therefore, in this study, the causal agents of apricot brown rot were identified based on morphological investigation and molecular analysis. This information would provide a solid basis for making the scientific management strategies for apricot brown rot control in China.

2. Materials and methods

2.1. Collection of *Monilia* isolates from apricot

In order to identify the *Monilia* spp. on apricot in China, sampling was carried out in representative apricot production areas of China in 2014. In total, 37 samples with brown rot symptom were collected, and each single spore isolate was obtained from a different sample. Of them, 18 samples were collected from two apricot orchards in Beijing, northern part of China, and 19 samples were collected from two orchards of Chongqing, southwestern part of China. Single spore isolation was conducted as described previously (Luo *et al*. 2002).

2.2. Morphological observation

Isolates were inoculated on potato dextrose agar medium (PDA), 200 mL juice from 200 g potato, 20 g dextrose, and 18 g agar L^{-1}, and incubated at 22°C in darkness. The above mentioned 37 isolates could be divided into two phenotypes (Phenotypes 1 and 2) based on their colony morphologies. Two representative isolates of each phenotype were selected from different places for further study (Table 1). Meanwhile, two *M*. *mumecola* isolates from apricot that were identified previously were chosen as reference isolates (Yin *et al*. 2014b). An agar piece (5-mm in diameter) with mycelia was cut from the edge of the advancing 4-d-old colony and placed upside down onto the center of fresh PDA, then incubated at 22°C in darkness. The diameter of colonies was measured in two perpendicular directions with two replicates, mean growth rate of colonies were calculated. The experiment was conducted twice.

2.3. Pathogenicity test *in vivo*

Pathogenicity of representative isolates was tested on detached apricot fruit. Healthy apricot fruit were washed, surface sterilized with 75% ethanol and rinsed with sterile water. Plugs with actively growing mycelium, cutting from the edge of a 4-d-old colony, were inserted into a small hole that made in the flesh of apricot fruit. PDA plug without fungi was used as control. The inoculated fruit were kept in a porcelain container with wet gauze at the bottom which was covered with cling film, and incubated at 22°C with 12 h/12 h of light-dark rhythm. The cling film was uncovered after 3 d incubation for sporulation. The developed lesions and conidia were investigated after 4 d of inoculation. Two fruits were used for each isolate and the experiments were conducted twice.

Table 1 Representative *Monilinia* spp. isolates from apricot in China

Isolates name	Species	Origin	
		County	Municipality/Province
CCT14-D10	*M. fructicola*	Dianjiang	Chongqing
CCT14-D2-1	*M. fructicola*	Dianjiang	Chongqing
YBT14-Y1	*M. yunnanensis*	Yanqing	Beijing
YBT14-P22	*M. yunnanensis*	Pinggu	Beijing
MHT13-2-1a	*M. mumecola*	Wuhan	Hubei
MHT13-1-2b	*M. mumecola*	Wuhan	Hubei