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RESEARCH ARTICLE

Occurrence and pathogens of fruit shrink disease in *Ziziphus jujuba* Mill.

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Abstract **The occurrence of fruit shrink disease in *Ziziphus jujuba* Mill. 'Linyilizao' was investigated from 2006 to 2009 in Cangxian of Hebei Province, Taiyuan, and Taigu of Shanxi Province, and the pathogens were isolated from the diseased fruits sampled from both Cangxian and Taiyuan in 2006 and 2007. The pathogens in leaf, fruit, and shoot collected from Cangxian were isolated from June to October in 2008 at 30-day intervals. The incidence of the disease varied significantly with location and year, and the highest incidence was observed in Cangxian during 2006. Three kinds of key pathogens were isolated from the fruits from July to September, i.e., *Alternaria alternata* Keissler (mainly in July), *Phoma destructiva* Plowr. (mainly in September), and *Fusicoccum* sp., of which *A. alternata* was the most dominant. In leaves, both *A. alternata* (much more dominant) and *P. destructiva* could be isolated throughout June to October, but no *Fusicoccum* sp. was found. At the white-ripening stage of fruit, the isolation rate of *A. alternata* was the highest in leaves (36.67%), followed by extension shoot (6.67%) and fruit (1.11%) while that of *P. destructiva* was the highest in extension shoot (31.11%), followed by fruit (13.33%) and leaf (12.22%), with *Fusicoccum* sp. isolated only in extension shoot (2.22%). The frequency of isolating two or three kinds of above three pathogens together was very low, with the highest rate (19.30%) found in *A. alternata* and *P. destructiva* from leaves in July.**

Keywords *Ziziphus jujuba* Mill., jujube fruit shrink disease, pathogen, occurrence

Introduction

Ziziphus jujuba Mill. 'Linyilizao', originated in Linyi county (in the southern part of Shanxi Province of China), has been cultivated for more than 3000 years (Qu and Wang, 1993; Liu and Wang, 2009). After Beijing Asian Games, the growing area of 'Linyilizao' has been increased sharply due to its wide adaptability, early bearing, large fruit, high yield, and rich in nutrition. In 2006, its growing area reached around 1.5 million hm^2 and ranked the second fresh-eating cultivar of Chinese jujube (Liu, 2008). It is widely distributed in vast areas of Shanxi, Shaanxi, Henan, Hebei, Beijing, and Chongqing provinces or municipal cities (Liu, 2008). With the expansion of growing area, the jujube fruit shrink disease (JFSD) becomes more severe, usually causing over 30% yield losses and lowering the market value of jujube fruit greatly due to bitter pulp and predrop (Zhang et al., 2008). The pathogens of JFSD of several cultivars of *Z. jujuba* such as 'Pozao', 'Huizao', 'Zanhuangdazao', and 'Hupingzao' have been studied by Chen et al. (1989), Qu et al. (1992), Xu et al. (1995, 2009), Zheng et al. (1996), Kang et al. (1998), and Kuang (2007). However, the pathogens of the disease published varied with cultivars, locations, and researchers, and nine kinds of fungi and two kinds of bacteria alone or mixed have been reported. The commonly isolated causal agents are *Alaternaria alternata*, *Phoma destructive*, and *Fusicoccum* sp., infecting Chinese jujube trees singly or jointly.

The symptoms can be divided into the brown cortex and the fruit shrink (Kang et al., 1998), or three groups, the head shrink, the middle shrink, and the base shrink (Zheng et al.,

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1998). The symptoms of infected fruits do not appear until the white-ripening stage (usually in the middle of August). The development of symptom of this disease can be divided into five stages, namely, halo, watermarks, precoloring, shrinking, and shedding (Zhang et al., 2008).

The incidence of the disease is influenced by many factors, such as climate, topography, soil type, cultivation condition, location of orchard, intercropping, cultivar, age and vigor of tree, mature stage of fruit, and so on (Zhang et al., 2008). Among these factors, location of orchard, mature stage of fruit, and climate are the three key ones.

The infection time of JFSD (Zheng et al., 1995; Kang et al., 1997) and the infection order of its pathogen (Luo et al., 2009) have been reported. The aims of this study were to determine the occurrence of JFSD at different location in different time and to determine the infection time and infection peak of the pathogens in different time and organs.

Materials and methods

Materials

The sample 'Linyilizao' trees were located in three germplasm repository of Chinese jujube including Cangxian (Hebei Province), Taiyuan (Shanxi Province), and Taigu (Shanxi Province). Three typical adult trees were selected in each site for investigation.

Investigation on disease severity

About 30 to 40 fruits were picked randomly from the east, west, south, and north part of each sample tree from late August to middle September during 2006–2009. The percentage of diseased fruit (PDF) and disease index (DI) were calculated referring to Kang's method (1997).

Isolation and identification of pathogens

Diseased fruits were taken from sample trees. Pieces (5 mm × 2 mm × 1 mm) of infected tissues cut from the scab margins were surface-sterilized with 0.5% NaClO for 3 min and then placed on potato dextrose agar (PDA) in Petri dishes and incubated at 25ºC (Singh and Sumbali, 1998; Ann et al., 1999) for seven days. Then, the kinds of the pathogens were investigated, and the fungus cultured from the sample tissues was transferred to 2% water agar for single hyphal tip isolation. Single hyphal tip isolates were cultured on PDA for further studies. There were 5- to 10-day-old cultures on PDA that were cut into pieces (approx. 10 mm× 5 mm× 3 mm) and then transferred into test tubes containing sterile distilled water and kept at −20ºC.

Meanwhile, the fungi in each Petri dish were investigated by using PDA to study the cultural and morphological characteristics of the fungus. All tested isolates were grown on PDA in Petri dishes at 24ºC under 2000 lx (12 h/day) for 20 days. Colonial patterns were recorded daily and described. Identification of the isolated fungi was determined according to the descriptions by Wei (1982), Lu (2001), Zhang (2003), Roberts (2005), and Sun and Zhang (2008).

Infection time of pathogens

In terms of pathogen invasion period, the organs of jujube tree were collected from Cangxian at 30-day intervals from June to October. Ten samples of fruit, extension shoot, bearing shoot, and leaf were collected on each tested tree. The isolation and investigation methods were the same as that mentioned before.

Data statistical analysis

All of the comparisons of means were subjected to analysis of variance and the significant differences among treatments were determined with a least significant difference separation test using SPSS 16.0 for Windows.

Results

Occurrence of JFSD

The investigation of disease severity of JFSD was conducted from 2006 to 2009 in Cangxian County. Figure 1 indicated that the highest percentage of diseased fruit (PDF) was 48.42% in 2006, and the lowest was 17.34% in 2009, with a significant difference between them. The disease index (DI) was the highest in 2007 (33.75%) and the lowest in 2009 (6.43%), and there was also a significant difference between the two years.

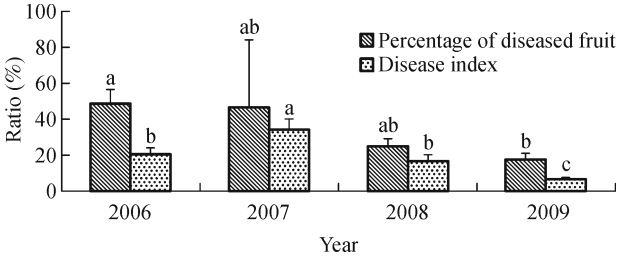

Figure 1 The incidences of JFSD of 'Linyilizao' in different years (Cangxian). In the different group at the same row, the histograms with different letters in the superscripts show significantly different at 0.05 level.

The disease severities in different locations were compared in 2006. The results showed that there was a significant difference in PDF between provinces, while there was no significant difference between the two locations (Taiyuan and Taigu) within Shanxi Province (Fig. 2). The DI disease tended

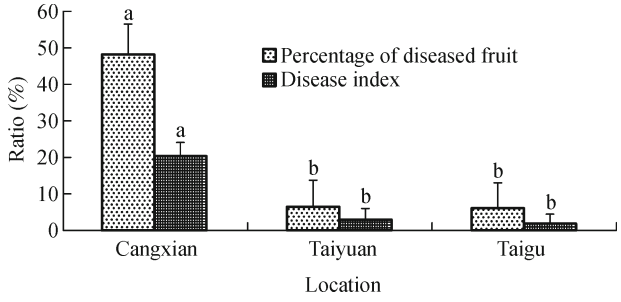

Figure 2 The incidences of JFSD of 'Linyilizao' in different regions (2006). In the different group at the same row, the histograms with different letters in the superscripts show significantly different at 0.05 level.

to be higher in the area with relatively warmer climate, such as in Cangxian.

Field surveys from 2006 to 2009 showed that JFSD appeared in early August (stone hardening stage), which developed rapidly in early September (white ripening stage) and fully developed in late September (crisp full-red stage). Figure 3 indicated that the disease was more serious at white ripening stage (with PDF and DI of 48.42% and 20.34%, respectively) than at crisp full-red stage (25.00% and 7.44%). This is because most of the diseased fruits were dropped at crisp full-red stage, and the symptoms developed at crisp full-red stage later than at white ripening stage.

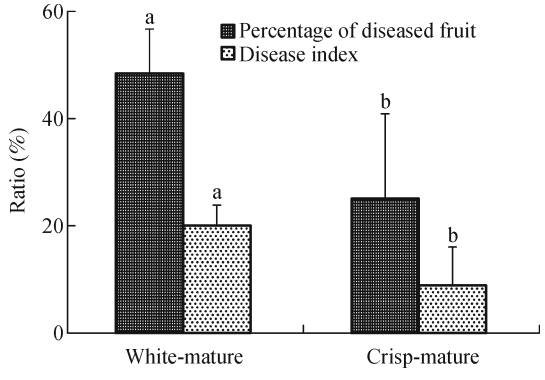

Figure 3 The incidences of JFSD of 'Linyilizao' at different ripening stages in 2006. In the different group at the same row, the histograms with different letters in the superscripts show significantly different at 0.05 level.

The kinds of pathogens

Five fungal species were isolated from typical diseased fruits sampled in 2006 and 2007. Among these fungi, *Phoma destructiva* Plowr. was the most predominant, followed by *Alternaria alternata* Keissler and *Fusicoccum* species, which agrees with Kang's (1998) result in cultivar 'Pozao', 'Banzao', and 'Huizao' of Chinese jujube. Other two kinds of fungi isolated as components of the mycoflora including *Aspergillus* sp. and *Penicillium* sp. showed no pathogenicity. No bacteria were isolated.

Table 1 showed that the highest isolation rate was from *A. alternata*, followed by *P. destructiva* and *Fusicoccum* sp. The isolates varied with years and locations. Moreover, the highest frequency of *P. destructiva* occurred in Cangxian in 2006 (75%), followed by *A. alternata* in Taiyuan in 2006 (50%), and *Fusicoccum* sp. in Cangxian in 2006 (32.5%).

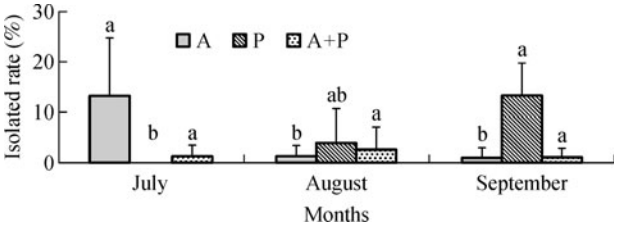

Figure 4 Isolated rates of pathogen from diseased fruit in 2008. In the different group at the same row, the histograms with different letters in the superscripts show significantly different at 0.05 level.

Infection time of JFSD

A. alternata and *P. destructiva* were alone or mixed isolated from fruits in July, August, and September in 2008, but no *Fusicoccum* sp. was found (Fig. 4). The highest isolation frequency of *A. alternata* and *P. destructiva* was observed in July and in September, respectively. These two fungi were isolated together in August.

Figure 5 showed that the pathogen with the highest frequency in leaves from June to October in 2008 was *A. alternata*, followed by *P. destructiva*, and no *Fusicoccum* sp. was isolated. There were two peaks of the isolation frequency of *A. alternata*, i.e., June (79.02%) and August (85.33%); the

Table 1 The isolation results of diseased fruits from different sites and years

Year	Location	Number of isolated fruit	Number of isolated tissue	Percentage of isolation		
				A (%)	P (%)	F (%)
2006	Cangxian	20	40	25.00c	75.00a	32.50a
	Taiyuan	50	120	50.00a	37.50b	0.00c
2007	Cangxian	20	60	8.33d	0.00c	25.00b
	Taiyuan	25	60	41.67b	0.00c	0.00c

A = *Alternaria alternata* Keissler, P = *Phoma destructiva* Plowr., F = *Fusicoccum* sp. (the same for the following figures.). In the same column, the numbers followed with different letters show significantly different at 0.05 level.

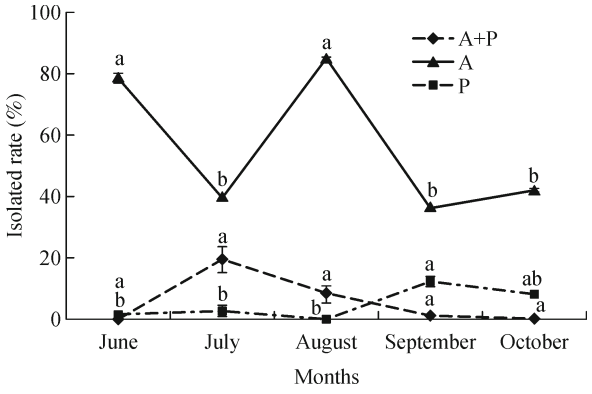

Figure 5 The isolation result of pathogen from jujube leaf (2008). In the different group at the same row, the line graphs with different letters in the superscripts show significantly different at 0.05 level.

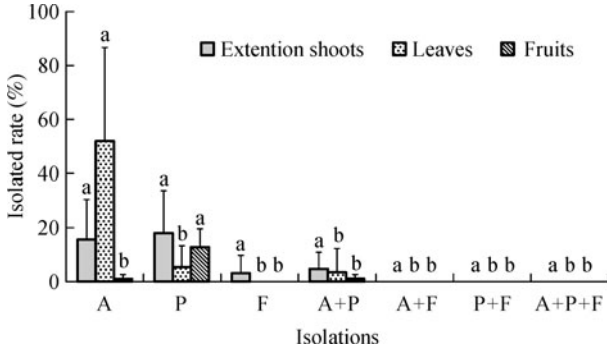

Figure 6 The isolation results of pathogen from different organs (September 6, 2008). In the different group at the same row, the histograms with different letters in the superscripts show significantly different at 0.05 level.

only one peak for *P. destructiva* was observed in September (12.22%); the rate of *A. alternata* and *P. destructiva* isolated together was the highest in July (19.30%), followed by August (8.00%), and very low in June, September, and October.

Different organs including extension shoot, fruit, and leaf were sampled on September 6, 2008, in Cangxian. Figure 6 showed that the isolation rate of *A. alternata* was the highest in leaves (36.67%), followed by extension shoot (6.67%) and fruit (1.11%), with that of *P. destructiva* being extension shoot (31.11%), fruit (13.33%), and leaves (12.22%). *F.* sp. was only isolated in extension shoot (2.22%). *A. alternata* and *P. destructiva* were hardly isolated simultaneously, with isolation rate of 5.00%, 3.91% and 1.11% in extension shoot, leaves, and fruits, respectively.

Discussion

Disputes on the pathogen of JFSD have lasted for about 30 years (Zhang et al., 2008). Up to now, nine fungi and two bacteria have been reported. This experiment showed that the most dominant pathogens in *Ziziphus jujuba* 'Linyilizao' were *A. alternata*, *P. destructiva*, and *Fusicoccum* sp., which consist of that with Kang's result (1998) in *Ziziphus jujuba* 'Pozao' but disaccords with Qu et al. (1992) and Zheng et al. (1995, 1996). More researches should be done to further clarify the pathogen of JFSD.

The JFSD pathogen could infect jujube tree from flowering season to harvest time (Kang et al., 1997; Zheng et al., 1998). Luo et al. (2009) reported that the infection order of the main JFSD pathogens was *A. tenuissima*, *A. alternata*, and *Coniothyrium olivaceum*, without indicating the peaks of infection. Our study showed that the infection of pathogen had obvious peaks, i.e., *A. alternata* in July and *P. destructive* in September. Thus, for proper control of JFSD, appropriate fungicide should be sprayed after flowering.

It is difficult to evaluate the resistance of different germplasm to JFSD in the field as the disease severity of JFSD varied with location, year, and ripening stage of fruit (Kang et al., 1997). This study showed that 'Linyilizao' could be used as an excellent control cultivar for screening resistant germplasm, because it is very susceptible to JFSD and could show typical symptom of JFSD in almost all locations, years, and ripening stages.

Acknowledgements

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