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Resistance Identification of Tree Peony Cultivars of Different Flowering Time to Gray Mold Pathogen *Botrytis cinerea*

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***Abstract*. Tree peonies are valuable ornamental plants and are widely cultivated in China and many other countries. Gray mold caused by *Botrytis cinerea* is an increasingly severe disease in Luoyang of China and seriously affects the ornamental value of tree peonies both in the open air and in greenhouses. However, the resistance of different tree peony cultivars to *B. cinerea* remains unknown. In this study, 15 tree peony cultivars belonging to three different flowering times were evaluated for resistance to *B. cinerea* by detached leaf assay measure. Results showed that the resistance of early-flowering peonies was stronger than that of later flowering peonies. Moreover, the correlation between flowering time and resistance of tree peonies was extremely significant (*P* < 0.01). The information obtained in this study can provide theoretical basis both for further exploring the resistance genes of tree peony to *B. cinerea* and for the prevention and controlling of the gray mold.**

Tree peonies belong to section *Moudan* of the genus *Paeonia* in the family Paeoniaceae (Cheng, 2007). They are valuable ornamental and medicinal plants native to China and have been widely used in garden greening and landscaping for a long time due to their gorgeous color, large flower, elegant shape, and rich fragrance (Cheng, 2007). Nowadays, tree peonies have become an internationalized ornamental plant and have been extensively cultivated in many other countries in Asia, America, Europe, and Australia (Yuan et al., 2012).

In recent years, with the growing area of tree peonies and mass propagation of potted tree peonies in greenhouses, the diseases of tree peony have become increasingly serious in China (Yang et al., 2017). At present, more than 20 kinds of fungal diseases of peony have been reported, of which gray mold caused by *Botrytis cinerea* is an increasingly severe disease with a high frequency of occurrence (Yang et al., 2017). The pathogen can cause necrotic leaves, sunken stems, and rotten petals of tree peonies under favorable environmental conditions, thus can seriously affect the ornamental value of tree peonies both in the open air and in greenhouses (Chen, 2011). A recent investigation carried out in Luoyang of Henan province showed that gray mold has become an important limiting factor for tree peony growth and development (Yang et al., 2017).

As the causal agent of gray mold, *B. cinerea* can infect more than 200 plant species and usually cause rot of flowers, leaves, and fruit of vegetables (Liu et al., 2016). In practice, chemical control has long been the main measure to control the disease while we ignored the fact that it can make environmental pollution and induce pathogen resistance (Liu et al., 2016). In recent years, fungicide resistance has been frequently reported in *B. cinerea* for the high genetic variability and short life cycle of the pathogen (Liu et al., 2016). As a consequence, *B. cinerea* is also considered a "high-risk" pathogen in terms of development of fungicide resistance. The long-term practice has proved that cultivating disease-resistant cultivars is the most economic and efficient measure to control gray mold in plants. However, little information has been known on the resistance of different tree peony cultivars.

As one of the main cultivation areas of tree peonies in China, Luoyang is very well known for its cultivation and possesses a wide variety of tree peony cultivars (Cheng, 2007). Zhang (2008) described that tree peony cultivars can be classed into three types according to the initial time of flowering: the early-flowering, the middle-flowering, and the late-flowering. However, the disease resistance of these different flowering tree peony cultivars to *B. cinerea* remains unknown. Therefore, this article reports on the evaluation of resistance of different tree peony cultivars to *B. cinerea*. It is hoped that the results will provide a theoretical basis for tree peony disease resistance breeding.

Materials and Methods

Plant materials. Fifteen tree peony cultivars of different flowering time were tested for resistance to *B. cinerea*. According to the time of initial flowering, the 15 cultivars belong to three types: early flowering, middle flowering, and late flowering (Table 1). The initial flowering time of the early-flowering cultivars is usually Mar. 28 in each year, according to the observation records of more than 10 consecutive years since 2000 (Wang et al., 2017). The initial flowering time of each cultivar type differs by ≈7 days (Wang et al., 2017). In our study, five cultivars were tested in each group. All of the tested cultivars are grown in Sui and Tang Dynasty Town Botanic Gardens, located in Luoyang, Henan province of China.

B. cinerea isolate and culture conditions. The *B. cinerea* isolate (20160401PB) was collected from tree peony flower buds in a plastic-covered greenhouse in Sui and Tang dynasties city ruins botanical garden. The pathogenic fungus was isolated and purified via usual tissue isolation methods (Fang, 1998), and then the isolate was cultured on potato dextrose agar (PDA) plate medium. Re-inoculation and testing were carried out on healthy leaves of the original tree peony. According to Koch's rule, the isolate was identified as the pathogenic fungus of tree peony gray mold.

Pathogen inoculation and resistance determination. A detached leaf assay was used to assess the resistance level of the tree peony cultivars to *B. cinerea*. Before inoculation, *B. cinerea* isolate was cultured on PDA medium for 5 d at 25 °C in the dark. Mycelial plugs (5-mm diameter) were prepared for inoculation by cutting from the growing edge. The healthy foliage of different cultivars of tree peonies on squaring stage was used for the inoculation test. The third to fifth healthy leaflets from the top of the branches were cut and placed abaxial face up in plastic trays with filter paper in the bottom saturated with distilled water. Each leaflet was inoculated on the abaxial (lower) side with one mycelial plug. A single leaflet was used as an experimental unit, and four to six replications were conducted per test. The experiment was performed twice. Inoculated leaflets were incubated in a growth chamber at 25 °C in the dark for 12 to 24 h. The leaflets were then placed face up in the plastic trays, covered with plastic wrap, and incubated

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in a climate chamber at 25 °C with a 14-h light/10-h dark photoperiod. Disease symptoms were scored 3 d after inoculation while being observed for 4 consecutive days. The method was modified from Flier et al. (2007).

Determining standard of resistance level. The interaction was considered compatible when conidia were clearly visible on at least half of the replicate leaflets (Flier et al., 2007). Four days after inoculation, the intelligent leaf area meter (YMJ-C) was used to calculate the leaf area and lesion area, respectively. Disease severity was evaluated using a 0 to 5 disease rating scale based on the percentage of relative lesion area and was broadly categorized as resistant (0, 1), moderately resistant (2, 3), and susceptible (4, 5) (Table 2).

Data analysis.

$$\text{Disease index (\%)} = \sum (\text{Number of diseased leaves of each grade} \times \text{disease level value of each grade}) / (\text{Total number of leaves surveyed} \times \text{the maximum level value})$$

$$\text{Relative lesion area (\%)} = [\text{Lesion area of diseased leave (mm}^2)] / [\text{Total area of the leave (mm}^2)] \times 100\%$$

Microsoft Excel (Redmond, WA) was used to work out the averages and SD of disease scores and to prepare the graphs. The correlation between flowering time and resistance of tree peony was analyzed by Pearson correlation using SPSS v19.0 (IBM SPSS Statistics, IBM Corp., Chicago, IL). $P < 0.05$ means the difference is significant, and $P < 0.01$ means the difference is extremely significant.

Results

Reactions of tree peony cultivars to B. cinerea isolate were different. The inoculation results showed that the symptoms of disease resistance and susceptibility are clearly distinguishable on different tree peony cultivars. The lesion area of the susceptible cultivar usually accounts for more than 50% of the entire leaf area (Fig. 1). However, most resistant or moderately resistant cultivars have no or very small lesions, usually no more than 25% of the total leaf area (Fig. 1).

Based on disease scoring, the 15 tree peony cultivars were categorized into resistant, moderately resistant, and susceptible (Table 3). The results showed that 10 of the 15 cultivars were resistant or moderately resistant to *B. cinerea*, whereas the other 5 cultivars were susceptible (Table 3).

The resistance of different flowering tree peony cultivars to B. cinerea was obviously different. The results showed that all of the five early-flowering cultivars were resistant to *B. cinerea* isolate, whereas all of the five late-flowering cultivars were susceptible. In addition, there were differences in resistance among the five middle-flowering cultivars. The results revealed that three of the five middle-flowering cultivars were resistant to *B. cinerea* isolate, whereas the other two were moderately resistant (Table 3). Moreover, the correlation between flowering time and resistance of tree peonies was extremely significant (0.896, $P < 0.01$). To be specific, the resistance of early-flowering peonies to *B. cinerea* isolate was much higher than that of late-flowering ones (Fig. 2, Table 3).

Table 1. Cultivar name, flowering type, flower color, and type of the 15 tree peony cultivars inoculated with *B. cinerea*.

Cultivars	Flowering type	Flower color	Flower type
Five Continents	Early	Blue	Single Dahlia
Xiangyang Red	Early	Red	Thousand layer–shaped
Luoyang Red	Early	Purple red	Rose-shaped
Sheng Dai	Early	Faint red	Thousand layer–shaped
Golden Island	Early	White	Single Dahlia
Two Qiaos	Middle	Complex	Chrysanthemum-shaped
The Eighteenth	Middle	Red	Thousand layer–shaped
Hu Red	Middle	Red	Crown-shaped
Hai Huang	Middle	Yellow	Chrysanthemum-shaped
Hua You	Middle	Red	Rose-shaped
White Lion King	Late	White	Hydrangea-shaped
Purple peony	Late	Purple	Crown-shaped
Changshou Le	Late	Light yellow	Crown-shaped
Wan-Hua Wei	Late	Light yellow	Crown-shaped
Fu-Shou Si	Late	White	Hydrangea-shaped

Table 2. Standard for in vitro resistance identification of tree peonies to gray mold pathogen *B. cinerea*.

Disease degrees	Lesion area/leaf area (%)	Disease level value
0	0	0
1	0–5	1
2	6–25	2
3	26–50	3
4	50–75	4
5	>75	5

Note: Lesion area is the area of the diseased part of the leaf; leaf area is the total area of the leaf.

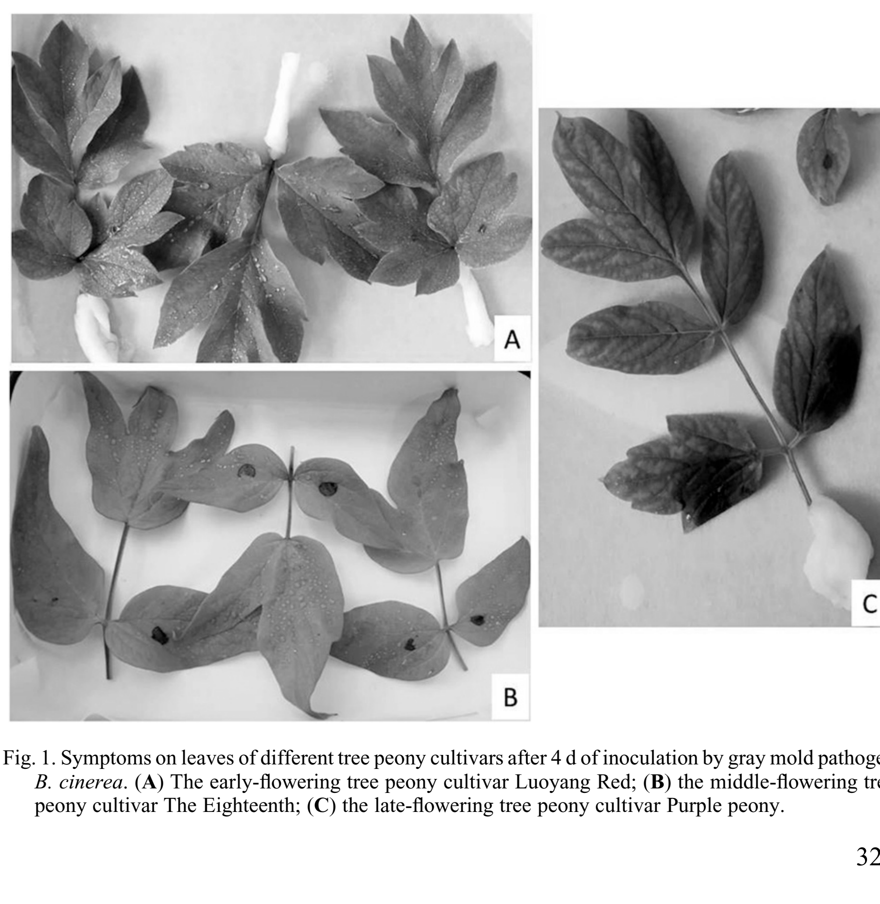

Fig. 1. Symptoms on leaves of different tree peony cultivars after 4 d of inoculation by gray mold pathogen *B. cinerea*. (**A**) The early-flowering tree peony cultivar Luoyang Red; (**B**) the middle-flowering tree peony cultivar The Eighteenth; (**C**) the late-flowering tree peony cultivar Purple peony.

Discussion

Gray mold caused by *B. cinerea* has become more and more serious both on greenhouse and field-cultivated tree peonies in China (Yang et al., 2017); however, the resistance of different tree peony cultivars to *B. cinerea* has been unknown. In this study, a detached leaf assay method was used to assess the resistance level of the tree peony cultivars to *B. cinerea*. Results showed that the resistance of different tree peony cultivars was different. Moreover, the resistance of early-flowering peonies was stronger than that of later flowering ones, and nearly all cultivars of late-flowering peonies are susceptible to *B. cinerea*. In this study, results indicate a significant correlation between resistance and flowering time of tree peonies.

Previous studies have shown that the tree peony cultivars of different flowering times differ in other aspects (Hou et al., 2018; Zhang, 2008). A study conducted on the biological characteristics of nearly 600 different flowering cultivars in Central Plains show that there are obvious differences between tree peony cultivars of different flowering times (Zhang, 2008). Zhang (2008) found that the growth potential of early-flowering cultivars was much stronger than that of middle- or late-flowering cultivars. Moreover, the late-flowering cultivars even showed a tendency of growth weakening (Zhang, 2008). Hou et al. (2018) further demonstrated that the flowering time of the tree peony cultivar is determined by a set of candidate functional genes. Consequently, we can infer from our results that the resistance of different flowering cultivars to *B. cinerea* is probably also controlled by different genes; however, this needs further confirmation at the molecular level.

Table 3. Reactions of the 15 tree peony cultivars to gray mold pathogen *B. cinerea*.

Cultivars	Resistance	Flowering type
Five Continents	R	Early
Xiangyang Red	R	
Luoyang Red	R	
Sheng Dai	R	
Golden Island	R	
Two Qiaos	MR	Middle
The Eighteenth	R	
Hu Red	R	
Hai Huang	MR	
Hua You	R	
White Lion King	S	Late
Purple peony	S	
Changshou Le	S	
Wan-Hua Wei	S	
Fu-Shou Si	S	

R = resistant; MR = moderately resistant; S = susceptible.

In recent years, exploring and making use of excellent genes with disease resistance in germplasm resources show good potential for disease resistance breeding due to the rich resistance resources in plants (Vleeshouwers et al., 2011). The germplasm resources of tree peonies are abundant and diverse (Cheng, 2007). According to the record, ≈2000 wild and cultivated species of tree peonies are grown throughout the world (Yuan et al., 2012). Thus, it can provide valuable resistance resources for breeding disease-resistant cultivars. In particular, with the completion of the sequencing of the peony genome, a solid foundation was laid for the realization of molecular breeding for disease resistance of tree peonies in the future (Bai et al., 2018). Meanwhile, through genomic technology, it is expected to rapidly develop a series of excellent cultivars, such as ornamental peony, oil peony, cut flower peony, and medicinal peony.

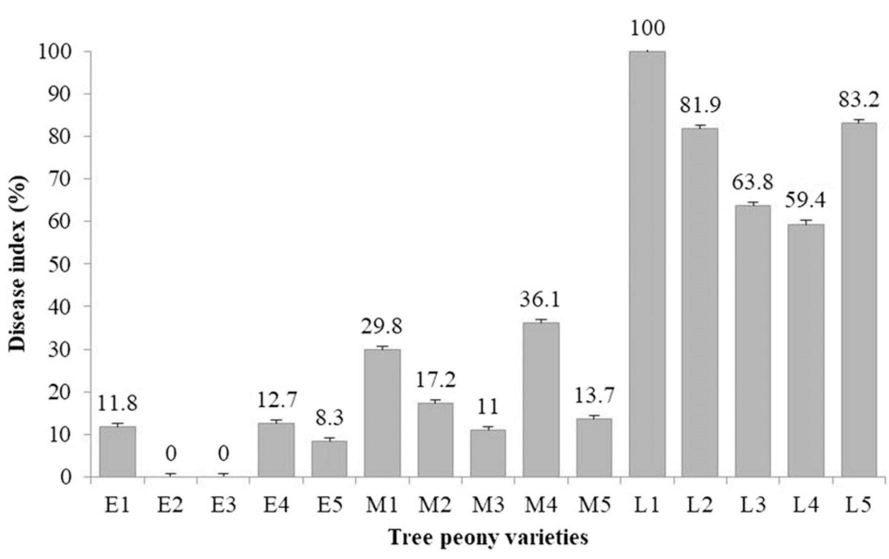

Fig. 2. Disease index of the 15 tree peony cultivars under detached leaf assay. E1 to E5 are five tree peony cultivars of early-flowering time (Five Continents, Xiangyang Red, Luoyang Red, Sheng Dai, Golden Island); M1 to M5 are five tree peony cultivars of middle-flowering time (Two Qiaos, The Eighteenth, Hu Red, Hai Huang, Hua You); L1 to L5 are five tree peony cultivars of late-flowering time (White Lion King, Purple peony, Changshou Le, Wan-Hua Wei, Fu-Shou Si).

Overall, the present study reveals that different cultivars of tree peony have different resistance to the pathogen of gray mold. Based on the current findings and the available information on the germplasm resources of tree peonies, it is proposed that the resistant and moderately resistant cultivars identified in the present study can be used directly as donor parents in peony breeding, as well as for identification of broad-spectrum sources of resistance against *B. cinerea*. However, it should be noted that this study has examined only a few cultivars of tree peony. More cultivars of tree peony need to be tested for disease resistance to better assist molecular resistance breeding in the future.

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