Identification of Two *Botryosphaeria dothidea* Strains Infecting Leaves of Yellow Peach (*Amygdalus persica*)

Siqing YI[1], Guangning PENG[1], Kaifa GUO[1], Jianhong YAN[2], Yanhong LEI[1], Shiyu CAO[1], Xianzhi NI[1], Chenzhong JIN[1], Hongyan LIU[1]*

1. Key Laboratory of Green Prevention and Control for Pests, Hunan University of Humanities Science and Technology, Loudi 417000, China; 2. Loudi Bureau of Agriculture and Rural Affairs, Loudi 417000, China

Abstract [**Objectives**] This study was conducted to clarify the species composition of pathogens causing leaf spot disease in yellow peach in Loudi area of Hunan Province and to screen effective control agents. [**Methods**] Twenty leaf spot samples were collected from yellow peach planting bases in Lengshuijiang and Shuangjiang, Loudi City. Two isolates were obtained through tissue isolation and purification. Identification was performed based on morphological characteristics combined with rDNA-ITS and Bt-2a gene sequence analysis and pathogenicity assay. [**Results**] The pathogen causing yellow peach leaf spot in this area was *Botryosphaeria dothidea*. [**Conclusions**] This study provides a reference basis for the prevention and control of yellow peach leaf spot in Loudi area.

Key words Loudi; Yellow peach; Leaf spot disease; *Botryosphaeria dothidea*

DOI:10.19759/j.cnki.2164-4993.2026.01.011

Yellow peach (*Amygdalus persica*), belongs to *Amygdalus* L. in the Rosaceae family. It is characterized by golden-yellow skin, tender and juicy flesh, and a relatively high content of soluble solids. The fruit is known for its multiple health benefits, including lowering blood lipids, promoting bowel movement, enhancing immunity, and removing spots. With its excellent edible quality, economic value, and strong tolerance to cold and drought, yellow peach is widely cultivated in regions such as Hunan, Zhejiang, Anhui, Shandong, and Shanghai. It is cultivated in hilly and mountainous areas of Hunan Province at altitudes below 1 400 m. The distinctive yellow peach industry has become a pillar industry for rural revitalization in the region[1]. In recent years, cities such as Lianyuan and Lengshuijiang in Hunan Province have vigorously promoted the high-quality development of the yellow peach industry. Its cultivation area has reached nearly 0.26×10^4 hm^2, and the Shuiyunfeng Yellow Peach of Lengshuijiang City has been certified as a National Geographical Indication Agricultural Product[2]. *Botryosphaeria dothidea* is the most common species in the genus *Botryosphaeria*. It has a broad host range and can infect more than 50 plant species in 34 genera of 20 families. Various fruit trees, including apple, pear, peach, grape, and kiwifruit, are frequently infected by *B. dothidea*, leading to diseases such as ring rot in apples and pears[3], infectious gummosis in peach[4], grapevine canker[5], and ripe rot in kiwifruit fruit[6]. In the study, two *Botryosphaeria* strains were isolated from the leaves of the Shuiyunfeng Yellow Peach orchard in Lengshuijiang City, and subjected to observations of cultural characteristics and morphological features, as well as multi-gene sequence analysis. Additionally, their pathogenicity was assessed. The study aimed not only to clarify the taxonomic classification of these strains, but also to demonstrate that *Botryosphaeria* can infect not only the fruits and branches, but also the leaves of yellow peach trees.

Materials and Methods

Experimental materials

In July 2024, a total of 20 typical leaf disease samples of yellow peach were collected from Huangtao (yellow peach) planting bases in Shuangjiang Township, Loudi City, and Lengshuijiang City. The diseased leaves of yellow peach exhibited circular or elliptical lesions measuring 2 – 4 mm in diameter. The lesion margins appeared purplish-brown to reddish-brown, accompanied by faint concentric rings. In the later stages of infection, a grayish-brown mold-like substance formed on the surface of the lesions. The central part of the lesions dried out and eventually developed perforations, and a clear boundary could be observed between diseased and healthy tissues. The collected samples were promptly transported to the laboratory for subsequent isolation and culture.

Experimental methods

Isolation and purification of pathogen Pathogenic fungi were isolated from peach leaves showing typical symptoms using the tissue isolation method[7]. In specific, diseased peach leaves were surface-sterilized with 75% ethanol and air-dried. Tissue pieces measuring 5 mm × 5 mm were excised from the junction between diseased and healthy areas. On a clean bench, the tissue pieces were treated with 1% sodium hypochlorite for 60 s, rinsed three times with sterile water, and dried with sterilized filter paper. The tissue pieces were inoculated onto PDA plates, with four pieces

---

Received: September 7, 2025 Accepted: November 9, 2025

Supported by Natural Science Foundation of Hunan Province (2024JJ7257; 2025JJ70341); Research and Innovation Project of Hunan University of Humanities, Science and Technology (ZSCX2024Y11); Undergraduate Innovation and Entrepreneurship Training Program (S202410553043).

Siqing YI (1986 –), female, P. R. China, master, devoted to research about fungal diseases.

* Corresponding author. Hongyan LIU (1981 –), female, lecturer, master, devoted to research about fungal diseases and their control.

per plate, and incubated at 25 ℃ for 72 h to allow growth. Once the colonies had grown to a suitable size, mycelia from the colony edges was selected for purification. Some strains were further purified by single-spore isolation using WA medium. The purified strains were preserved on PDA slants and stored at 4 ℃ for later use.

Observation of cultural characteristics and morphological features of the pathogen The isolated strains were cultured on PDA medium at a constant temperature of 25 ℃, and their colony morphology, color, and other characteristics were observed. To induce the formation of fruiting bodies, the fungi were inoculated onto PDA medium and incubated in a cultivation chamber at 24 ℃ under ultraviolet light for 5 d. The morphological features of the conidiomata and conidia of the isolates were examined under an optical microscope.

Molecular identification DNA extraction of the pathogen: Based on the morphological identification data, representative strains were selected and cultured on PDA medium for 5 d. Fresh mycelia were then collected, placed in a sterilized mortar, and ground with liquid nitrogen. DNA was extracted from the pathogen using a DNA extraction kit. Subsequently, PCR amplification was performed using primer pairs ITS1/ITS4, EF1-986r/EF1-728f, and BT-2a/BT-2b.

The PCR reaction system was prepared as follows: upstream primer ITS1/EF1-986r/BT-2a 2 μl, downstream primer ITS4/EF1-728F/BT-2b 2 μl, 2×Easy Taq PCR SuperMix (+ dye) 25 μl, DNA template 1 μl, and ddH$_2$O added to a total volume of 50 μl.

The PCR amplification protocol consisted of following steps: initial denaturation at 94 ℃ for 4 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s, annealing at 55 ℃/48 ℃/58 ℃ (ITS/EF1-986r/Bt-2a) for 30 s and extension at 72 ℃ for 40 s, and a final extension at 72 ℃ for 10 min after cycling. After the reaction, the PCR products were detected by 1% agarose gel electrophoresis. The PCR products amplified with different primers were then sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing.

Sequence alignment and phylogenetic tree construction: After sequencing, the obtained gene sequences were corrected and subjected to BLAST analysis via the GenBank database to retrieve and compare homologous sequences. Relevant sequences were downloaded to compile a dataset containing genetically related sequence resources. Using bioinformatics methods, phylogenetic comparison was conducted between the experimental strains and representative species recorded in the database. A phylogenetic tree was constructed using MEGA 7.0 software. Based on the degree of sequence homology and the phylogenetic relationships depicted in the tree, the taxonomic status of the strains was determined, enabling molecular identification of the isolates.

Results and Analysis

Typical field symptoms of yellow peach leaf spot disease

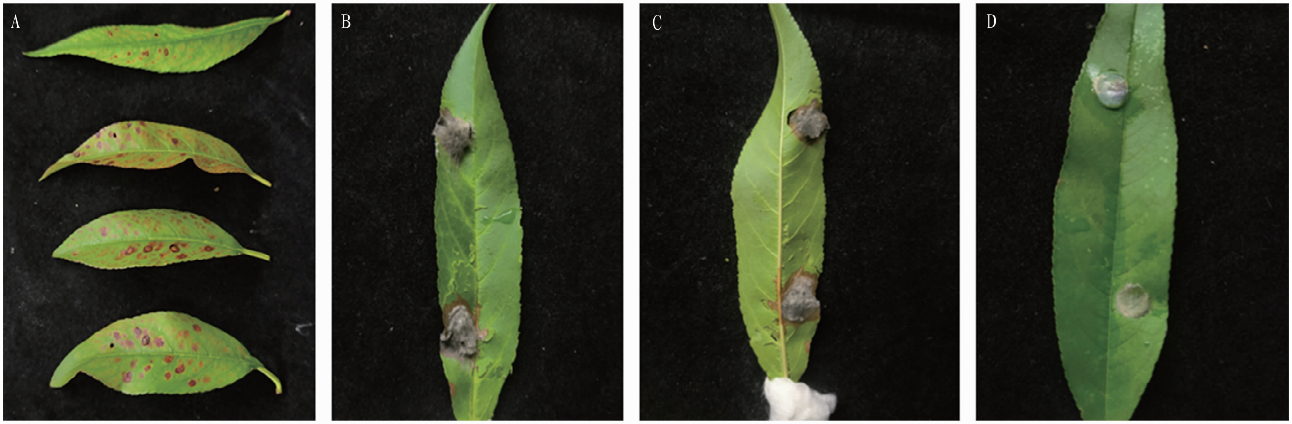
In Loudi area, yellow peach leaf spot disease can occur from the sprouting stage to the maturity stage. In the early stage of infection, circular or nearly circular lesions appear on the leaves, with diameters ranging from approximately 1 to 4 mm. The lesions exhibit faint concentric rings and purplish-brown margins. As the disease progresses, grayish-brown mold-like substances develop on the lesions. The central parts of the lesions dry out and fall off, while the surrounding leaf tissue turns yellow, and the lesions gradually enlarge. When adjacent lesions merge and the necrotic tissue detaches, perforation symptoms appear on the leaves. The edges of the perforations are neat, and when numerous perforations occur, the leaves may subsequently abscise (Fig. 1). Yellow peach brown spot perforation disease primarily affects peach tree leaves, but can also damage new shoots and fruits.

Colony and morphological identification of the isolated strains

Two strains of *B. dothidea* were isolated from 20 diseased peach leaf samples. On PDA plates, both test strains formed circular colonies that were initially white. After 5 d of culture, the colonies began to turn grayish-green, with dark green coloration on the reverse side. The colonies exhibited a growth rate of 34.5 mm/d and produced abundant aerial hyphae (Fig. 2A). When induced under ultraviolet light on pine needle PDA medium, conidiomata were produced (Fig. 2B). The conidiomata were spherical, with an average size of approximately 221.2 (214.2 – 244.8) μm × 225.9 (204 – 255) μm (Fig. 2C – D). Conidia developed inside the conidiomata. Observations revealed long fusiform, colorless, single-celled, and thin-walled structures, featuring a smooth outer wall that occasionally contained 1 – 2 septa. Based on these morphological characteristics, the isolates were preliminarily identified as belonging to the genus *Botryosphaeria*.

Molecular identification of the strains

Polymerase chain reaction (PCR) amplification targeting the ITS gene sequence was conducted for two pathogenic strains. Detection via agarose gel electrophoresis successfully yielded specific bands approximately 500 bp in length. The obtained sequences were subsequently uploaded to the National Center for Biotechnology Information (NCBI) database, and homology analysis was performed using the Basic Local Alignment Search Tool (BLAST). The 18S rRNA ITS gene sequences of the two pathogenic strains showed over 99.75% similarity with the previously deposited strains *B. dothidea* (GenBank accession no. MN918795.1), *Fusarium oxysporum* (GenBank accession no. MN959996.1), and *Diaporthe perseae* (GenBank accession no. MG745373.1). Based on the analysis of pathogenicity test results, only the strain with the strongest pathogenicity was selected for phylogenetic analysis using MEGA7. Branch support rates were calculated through the bootstrap method with 1 000 repetitions. The phylogenetic tree analysis further confirmed that this pathogen clustered within the same branch as *B. dothidea*, with a support rate of 100% (Fig. 3). Finally, integrating morphological characteristics and molecular biological identification results, the pathogen was conclusively identified as *B. dothidea*.



A. Field symptoms; B, C. Pathogenicity assay of isolates; D. Control.

Fig. 1 Field symptoms of yellow peach leaf spot disease and pathogenicity assay

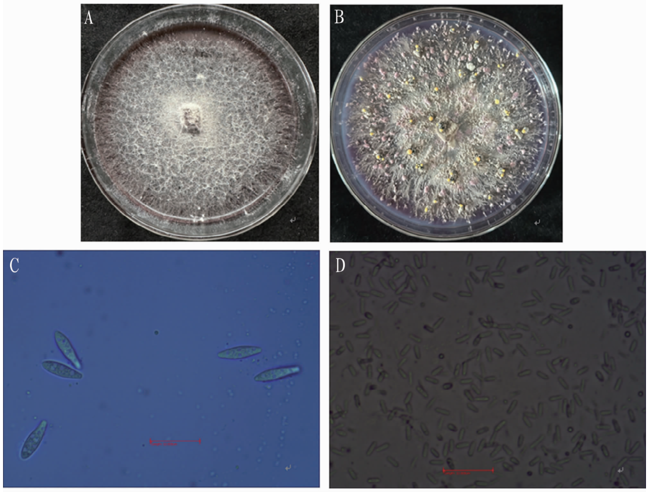


Fig. 2 Colony and morphological characteristics of the isolates

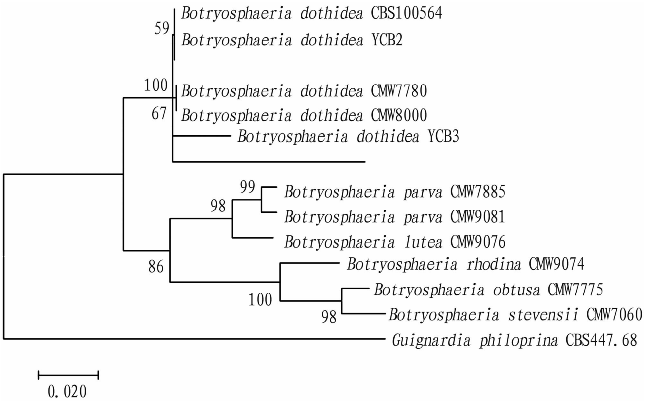


Fig. 3 Phylogenetic tree constructed based on ITS and Bt-2a gene sequences of representative strain

Conclusions and Discussion

The yellow peach industry has become a prominent emblem of the development philosophy that "lucid waters and lush mountains are invaluable assets" in Loudi area. It serves as a pivotal force in poverty alleviation, enriching residents and strengthening the city, and provides solid support for the effective linkage between targeted poverty alleviation and rural revitalization. In recent years, the cultivation area of yellow peach in Loudi area has increased annually, accompanied by increasingly prominent issues of pests and diseases[8]. Yellow peach trees may be affected by diseases at various stages of growth and development, among which fungal diseases are particularly diverse and can damage all parts of the tree. Different types of diseases primarily target different parts of the peach tree. The most common diseases include leaf curl, shot hole disease, gummosis, brown rot, anthracnose, soft rot, and leaf spot disease. Leaf spot disease is one of the most prevalent and severe diseases affecting cherry trees. It directly impairs leaf photosynthesis, weakens tree vigor, and leads to reduced yield and quality of yellow peaches, resulting in significant economic losses.

Fungi of the genus *Botryosphaeria* are widely distributed in the world. Species within this genus and their anamorphic stages act as common pathogens, saprophytes, or endophytes in many monocotyledonous, dicotyledonous, and gymnosperm plants[9]. They cause symptoms such as leaf spots, shoot blight and necrosis, stem cankers, and fruit or seed rot[10-11]. Fungi of the genus *Botryosphaeria* can cause diseases in many fruit trees, leading to weakened tree vigor, reduced fruit yield and quality, and in severe cases, entire tree death, resulting in significant harm and economic losses. Existing literature reports that *B. dothidea* infects and damages plants of the Rosaceae family, causing diseases such as peach branch gummosis[12], cherry leaf spot[13], and kiwifruit soft rot[14-15].

In this study, based on morphological characteristics and analysis of the 28S rRNA ITS and Bt-2a gene sequences, combined with phylogenetic tree results and pathogenicity assays, *B. dothidea* was conclusively identified as the pathogen responsible for leaf spot disease in yellow peach trees in Lengshuijiang and Shuangjiang, Loudi City, Hunan Province. Furthermore, Yu *et al.*[16] isolated a *B. dothidea* strain from 'Jinxiu' yellow peach fruit. Whether infection of yellow peach leaves subsequently leads to transmission and infection of the fruit requires further investigation in subsequent studies.

(Continued on page 53)

Chemical Research, 2020, 31(3): 263 - 269. (in Chinese).

[3] DENG LY. Research progress on pre-treatment methods for multi-residue veterinary drugs in animal-derived foods[J]. Animal Husbandry and Veterinary Science & Technology Information, 2020(3): 15 - 16. (in Chinese).

[4] QIAN C, LI J, CHEN G L. Research progress of sample preparation methods in veterinary drug residue analysis for animal-derived foods[J]. Journal of Food Safety and Quality, 2015, 6(5): 1666 - 1674. (in Chinese).

[5] GAO PF, GONG W, TANG YY, *et al.* Research progress on detection technology and analysis of existing circumstance of veterinary drug residues in beef, mutton, cattle milk and goat milk[J]. Chinese Journal of Veterinary Drug, 2022, 56(1): 77 - 85. (in Chinese).

[6] HU YN. Research on a liquid chromatography-mass spectrometry screening method for multiple residues involving 63 veterinary drugs in animal-derived foods[D]. Hubei: Huazhong Agricultural University, 2021. (in Chinese).

[7] WIERUCKA M, BIZIUK M. Application of magnetic nanoparticles for magnetic solid-phase extraction in preparing biological, environmental and food samples[J]. TrAC, Trends in Analytical Chemistry (Regular ed.), 2014, 59: 50 - 58.

[8] WANG C, LIANG RQ, CAO J, *et al.* Application progress of high resolution mass spectrometry in food analysis[J]. Journal of Food Safety and Quality, 2018, 9(5): 1038 - 1044. (in Chinese).

[9] LEON N, ROCA M, IGUALADA C, *et al.* Wide-range screening of banned veterinary drugs in urine by ultra high liquid chromatography coupled to high-resolution mass spectrometry[J]. J Chromatogr A, 2012, 1258: 55 - 65.

[10] HU MZ, MENG XS, WANG C, *et al.* Advances in application of mass spectrometry in high-throughput and rapid detection[J]. Journal of Instrumental Analysis, 2018, 37(2): 127 - 138. (in Chinese).

[11] CHEN G, SCHNEIDER MJ. A rapid spectrofluorometric screening method for enrofloxacin in chicken muscle[J]. J Agric Food Chem, 2003, 51(11): 3249 - 3253.

[12] SU Y. Research on high-throughput screening methods for hundreds of veterinary drug residues in animal-derived foods[D]. Sichuan: Sichuan Agricultural University, 2019. (in Chinese).

[13] GUO TR, YE M, KE H, *et al.* Application of high resolution mass spectrometry in the detection of veterinary drug residues in animal foods [J]. Food Science and Technology, 2020, 45(7): 371 - 377. (in Chinese).

[14] WU YN, LI JG, ZHENG MH, *et al.* Research on trace and ultra-trace detection techniques for dioxins, polychlorinated biphenyls, and chloropropanols[Z]. 2004. (in Chinese).

[15] WANG W, CAI WS, SHAO XG. Progress in Fourier transform ion cyclotron resonance mass spectroscopy[J]. Progress in Chemistry, 2005 (02): 336 - 342. (in Chinese).

[16] SENYUVA HZ, GOKMEN V, SARIKAYA EA. Future perspectives in Orbitrap-high-resolution mass spectrometry in food analysis: A review [J]. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 2015, 32(10): 1568 - 1606.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 49)

References

[1] WANG TY LI MH, WU ZC, *et al.* Evaluation model of yellow peach climatic quality rating in hilly mountainous areas[J]. Journal of Applied Meteorological Science, 2024, 35(4): 456 - 466.

[2] LIANG R. Loudi: "Ziquejie Tribute Rice" and "Shuiyunfeng Yellow Peach" approved as national geographical indication products[J]. The Food Industry, 2025, 46(8): 9.

[3] WANG F, HUANG JB, LI GH. Occurrence of and progress in fruit tree diseases caused by *Botryosphaeria* spp. [J]. Plant Protection, 2013, 39 (6): 7 - 13.

[4] WANG JL. Systematic study on *Botryosphaeria* and related genera from some regions of China[D]. Beijing: Beijing Forestry University, 2003.

[5] ZHAO JP. Systematic taxonomy of tree canker pathogens—*Botryosphaeria* and related fungi [D]. Beijing: Chinese Academy of Forestry, 2007.

[6] WU XQ, HE YQ, LIU ZH. Occurrence and progress on tree cankers caused by *Botryosphaeria* spp. [J]. Journal of Nanjing Forestry University, 2001(1): 61 - 66.

[7] FANG ZD. Research methods in plant pathology[M]. Beijing: China Agriculture Press, 1998.

[8] SU ZG, YANG ZQ. Spatial distribution of high-temperature damage during the maturation period of yellow peach fruits[J]. Journal of Anhui Agricultural Sciences, 2015, 43(19): 168 - 169, 189.

[9] SLIPPERS B, WINGFIELD MJ. *Botryosphaeria*ceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact [J]. Fungal Biology Reviews, 2007, 21: 90 - 106.

[10] WANG F, ZHAO L, LI G, *et al.* ldentification and characterization of *Botryosphaeria* spp, causing gummosis of peach trees in Hubei Province, central China[J]. Plant Disease, 2011, 95: 1378 - 1384.

[11] WANG F, HUANG JB, LI GH. Diseases caused by *Botryosphaeria* species in fruit trees and research advances[J]. Plant Protection, 2013, 39 (6): 7 - 13.

[12] LIU Y, WANG FR, WANG HL, *et al.* Transcriptomic analysis of peach response to *Botryosphaeria* infection [J]. Molecular Plant Breeding, 2024, 22(6): 1842 - 1849.

[13] ZHOU YY, ZHANG W, LI XH, *et al.* Identification of *Botryosphaeria dothidea* associated with cherry leaf spot disease[J]. Acta Phytopathologica Sinica, 2021, 51(4): 636 - 640.

[14] WANG XJ, LI SY, LI YW, *et al.* Pathogen identification of kiwifruit soft rot and fungicide screening for control of the disease[J]. Journal of Plant Protection, 2017, 44(5): 826 - 832.

[15] WEAVER DJ. A gummosis disease of peach trees caused by *Botryosphaeria dothidea*[J]. Phytopathology, 1974, 64: 1429 - 1432.

[16] YU FS, QIANG Y, QIN SL, *et al.* Identification of a strain in *Botryosphaeria* infecting fruit of yellow peach variety "Jinxiu"[J]. Acta Agriculturae Universitatis Jiangxiensis, 2021, 43(3): 566 - 573.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU