Ciência Rural, Santa Maria, v.54:06, e20220115, 2024
http://doi.org/10.1590/0103-8478cr20220115
ISSNe 1678-4596
CROP PROTECTION

Biological characteristics of a new pathogen in *Eucommia ulmoides* black spot

Shuying Li[1,2] Shujiang Li[1,2] Shan Han[1,2] Tiantian Lin[3] Hanmingyue Zhu[4] Tianhui Zhu[1,2*]

[1]College of Forestry, Sichuan Agricultural University, 611130, Chengdu, Sichuan Province, China. E-mail: zhuth1227@126.com. [*]Corresponding author.
[2]Key Laboratory of Forest Protection of Sichuan Education Department, Chengdu, Sichuan Province, China.
[3]School of Ecology and Environment, Yunnan University, Kunming, Yunnan Province, China.
[4]Graduate School, Sichuan Agricultural University, Chengdu, Sichuan Province, China.

ABSTRACT: In this study, the causative pathogen was isolated from *Eucommia ulmoides*, verified using Koch's postulates, identified by morphology and molecular biology, and assessed the biological characteristics. The effects of agar media, pH, temperature, light, carbon sources, and nitrogen sources on hyphal growth of *Pestalotiopsis trachicarpicola* were under investigation, and the influences of time, liquid matrix, light, temperature on conidial germination were studied. The results showed that *P. trachicarpicola* was a new pathogen causing black spot disease of *E. ulmoides*, and the mycelial growth of *P. trachicarpicola* was the best in the potato dextrose agar (PDA) medium, pH 7.0, the temperature was 20~25 °C, light, and glucose and beef extract were the best for carbon and nitrogen, respectively. The germination rate of the conidia increased from the time, darkness, and a proper supply of sugar. The lethal temperatures of the hyphae and conidia were 52 °C and 72 °C, respectively. We can conclude that appropriate environmental conditions are more conducive to the growth of the pathogen and the germination of spores, which leads to the occurrence of disease. This study constituted the first report on the new causative pathogen in *E. ulmoides* black spot and the biological characteristics. Theis study provided a theoretical basis to prevent the occurrence of this disease.
Key words: black spot, *Eucommia ulmoides*, biological characteristic, *Pestalotiopsis trachicarpicola*.

Características biológicas de um novo patógeno na mancha preta de *Eucommia ulmoides*

RESUMO: Neste estudo, o patógeno causador foi isolado de *Eucommia ulmoides*, verificado pelos postulados de Koch, identificado por morfologia e biologia molecular, e avaliadas as características biológicas. Os efeitos do meio de ágar, pH, temperatura, luz, fontes de carbono e fontes de nitrogênio no crescimento de hifas de *Pestalotiopsis trachicarpicola* foram investigados, e as influências do tempo, matriz líquida, luz e temperatura na germinação dos conídios foram estudadas. Os resultados mostraram que *P. trachicarpicola* é um novo patógeno causador da doença da mancha preta de *E. ulmoides*, e o crescimento micelial de *P. trachicarpicola* foi o melhor no meio de ágar batata dextrose (PDA), pH 7.0, a temperatura é de 20~25 °C, luz e extrato de glicose e carne são os melhores para carbono e nitrogênio, respectivamente. A taxa de germinação dos conídios aumentou com o tempo, escuridão e um suprimento adequado de açúcar. As temperaturas letais das hifas e dos conídios foram 52 °C e 72 °C, respectivamente. Podemos concluir que as condições ambientais adequadas são mais propícias ao crescimento do patógeno e à germinação de esporos, o que leva à ocorrência da doença. Este estudo constitui o primeiro relato sobre o novo patógeno causador da mancha negra de *E. ulmoides* e as características biológicas. O objetivo deste estudo é fornecer uma base teórica para prevenir a ocorrência desta doença.
Palavras-chave: mancha negra, *Eucommia ulmoides*, característica biológica, *Pestalotiopsis trachicarpicola*.

INTRODUCTION

Eucommia ulmoides Oliver, also known as bakelite, silk cotton, and cotton bark, belongs to the Hamamelidae, Eucommiales, Eucommiaceae. *Eucommia*, a deciduous tree, is a unique plant considered to be a "living fossil" in China. It has high economic and ecological value. *E. ulmoides* is a traditional and valuable Chinese herbal medicine (LIU et al., 2022). The tree is widely used in medicine, food, the rubber and timber industries, urban and rural greening, and soil and water conservation. *E. ulmoides* is endemic to China, widely distributed among the mountains south of the Qinling Mountains, Sichuan, Guizhou, Hubei, Hunan, Shanxi, Gansu, Guangxi, Guangdong, Yunnan, Henan, Zhejiang, Anhui, Jiangsu, Jiangxi, and other provinces and autonomous regions (WU et al., 2011; YANG, 2013). Black spot disease of *E. ulmoides* can damage the plant leaves, petioles, twigs, peduncles, and young fruit, which pose a serious threat to the growth and health of plants.

Many fungi can cause black spot disease. For example, the pathogens that can cause black spot disease are primarily *Alternaria brassicae* (AYER & PENARODRIGUEZ, 1987), *Diplocarpon rosae* Wolf

Received 03.03.22 Approved 09.19.23 Returned by the author 11.23.23
CR-2022-0115.R1
Editors: Leandro Souza da Silva Abdullah Al-Sadi

(MARCHANT et al., 1998), *Alternaria alternata* (ADACHI & TSUGE, 1994), and *Alternaria brassicicola* (MACKINNON et al., 1999).

To date, there have been few reports on the black spot of *E. ulmoides* in China and other parts of the world. However, the biological characteristics of the pathogens of *E. ulmoides* black spot disease in China have not yet been reported. In this study, the causative pathogen was isolated and identified from fresh tissues of *E. ulmoides* from a forest farm and then measured biological characteristics. Morphological and molecular characterizations were performed. It is not known how the disease was introduced into China or why the disease emerged. This study provided a theoretical basis for the prevention and treatment of black spot disease.

MATERIALS AND METHODS

Plant materials

Beginning in April 2017, field investigations were conducted on the *E. ulmoides* cultivation areas (30.62N, 103.33E), which were located in Dayi County, Chengdu, Sichuan Province, China. The characteristics of the black spot disease and the symptoms of the disease were recorded, and fresh *E. ulmoides* plants were collected.

Test medium

Starting with the formula for Fang medium (FANG, 1998), we made appropriate improvements on this basis. The formula primarily includes the following seven kinds of media: (1) Potato dextrose agar (PDA), (2) Potato sucrose agar (PSA), (3) Czapek (CZ), (4) Oat meal agar (OMA), (5) Corn meal agar (CMA), (6) Carrot agar (CA), and (7) 2% Water agar (WA).

Isolation and pathogenicity test

To isolate the causative pathogen, the diseased *E. ulmoides* leaf tissues were sectioned to 5×5 mm pieces with the help of a sterile blade and surface-sterilized for 30 s in 3% sodium hypochlorite and 30 s in 75% ethanol, rinsed three times with sterile distilled water, dried and transferred to Petri dishes containing PDA medium (one piece per plate). The Petri dishes were sealed with Parafilm and incubated for 3-8 days in a 25 °C incubator. Upon mycelial growth, a small colony was reisolated and transferred to a new Petri dish.This process was repeated several times to isolate single colonies that were distinctly different due to colony morphology. Fungal strains exhibiting good growth and spore production traits were selected and purified as test strains.

To confirm pathogenicity and fulfil Koch's postulates, the obtained pure cultures was inoculated on a PDA plate, cultured in a 25 °C incubator for 10-15 days, and the conidia were washed with sterile water to prepare a spore suspension with a concentration of approximately 1×10^6/mL. Fresh and healthy *E. ulmoides* leaves of the same size were washed with clean tap water to remove any soil debris, the surface sterilized with 75% alcohol, and kept in trays. The surface of the leaves was wounded using a sterile disposable syringe (1 mL) inoculation needle, and the spore suspension was sprayed evenly on the leaves by wound inoculation with 20 mL of pathogenic fungus suspension, with sterile water sprayed as a control. Trays were covered with plastic film, moisturized and cultured in a 25 °C incubator. Ten leaves were inoculated per treatment, and marked. This whole inoculation assay was repeated three times. The condition of the leaves was observed every day. Finally, after the pathogenicity test, reisolation from the symptomatic leaves was carried out, the fungal colonies and conidial features were compared to the original isolates. Actively growing pure cultures were transferred to PDA slants medium. After the establishment of the culture, these were refrigerated at temperatures of 4 °C as stock cultures and were checked periodically for contamination and desiccation. Before each experiment, fungi were grown on fresh PDA and incubated in a 25 °C incubator.

Identification of pathogenic fungi

The major identification of fungi referred to the "Fungi identification manual" (WEI, 1979) describing the fungal colony morphology, and it conducted fungal microstructure observation and identified segregative fungi to the genus. The purified strain was inoculated onto PDA media and placed in a constant temperature culture at 25 °C for 7 days, and the mycelium was scraped with a sterilizing blade and placed on a sterile filter paper to absorb water. The pathogenic fungal DNA was extracted using a plant genomic DNA extraction kit (centrifugal column type) from the Tiangen Biochemical Technology (Beijing) Co., Ltd, according to the manufacturer's instructions. The extracted genomic DNA of the strain was used as a template, and the fungal ribosomal gene transcribed spacer (ITS) fungal universal primers ITS4/ITS5, β-tubulin gene (*tub*) amplification primers BT2A/BT2B were used. The *tef1* gene (*tef-1α*) amplification primers EF1-526F/EF1-1567R were used to amplify the DNA of the test strain, and the reaction system and conditions were as described by GLASS & DONALDSON (1995), O'DONNELL & CIGELNIK

(1997), BENSCH (2012), and WHITE et al. (1990). The PCR product obtained after amplification was detected using 1% agarose gel electrophoresis and sent to Shanghai Sangon Biotechnology Company for DNA sequencing, and homology comparison between the sequence measured and the sequence that had been reported in GenBank database through the Internet (http://www.ncbi.nlm.nihgov/blast.cgi) to determine the status of the test fungi in the microbial phylogeny.

Hyphal growth assay of Pestalotiopsis trachicarpicola

Colonies were assayed by plating. All the test strains were cultured on a PDA medium plate at 25 °C exception for the specific condition experiment. After 5 days, the colony diameter was measured using the cross method to evaluate hyphal growth. The mycelial plugs were punctured on the outer edge of the colony with a sterilized diameter of 5 mm puncher and inoculated to the center of each plate. All experiments were repeated five times.

The punctured mats were inoculated separately onto PDA, PSA, CZ, OMA, CMA, CA, and WA media to determine the effects of medium composition on mycelial growth.

To determine the effects of temperature on mycelial growth, the punctured mats were cultured at 5, 10, 15, 20, 25, 30, 35, and 40 °C in a constant-temperature incubator.

To determine the effects of pH on mycelial growth, the pH of the PDA medium was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 using 1 mol/L hydrochloric acid and 1 mol/L sodium hydroxide solutions. The illumination was set to one of three conditions: complete light, complete darkness, or light and darkness for 12 h to determine the effect of light conditions on mycelial growth.

Study on the effects of different carbon sources and nitrogen sources on mycelial growth of *P. trachicarpicola*, based on the fungal physiological medium (formulation: nitrogen source 1 g, potassium dihydrogen phosphate 0.5 g, magnesium sulfate heptahydrate 0.5 g, carbon source 5 g, agar 20 g, distilled water 1000 mL). Potassium nitrate was used as the nitrogen source, sucrose was the carbon source, and it was replaced by different carbon sources such as glucose, fructose, lactose, maltose, and soluble starch. A plate with no carbon source was used as a control. Using glucose as the carbon source and sodium nitrate as the typical nitrogen source, the sodium nitrate was replaced by five kinds of nitrogen sources, such as potassium nitrate, ammonium nitrate, beef extract, peptone, and yeast powder. A plate without a nitrogen source was used, but sodium chloride was added instead as a control. The punctured mats were inoculated into the medium.

Before each determination of the lethal temperature for the hyphae, five punctured mats were collected in a sterile test tube, and 2 mL of sterile water was added. The test tubes were placed in a constant-temperature water bath at 45, 50, 55, 60, 65, 70, and 75 °C, respectively, for 10 minutes and then moved to cold water until the test tubes cooled down to room temperature. The lethal temperature range was initially determined. A gradient of 1 °C was set to examine the hyphae and determine the final lethal temperature.

Spore germination assay of Pestalotiopsis trachicarpicola

The spore germination tests used the germination method of the slide (FANG, 1998). The spore-forming strains cultured on a PDA plate at 25 °C for 10-15 days were rinsed with sterile water to prepare a spore suspension at a concentration of 1×10^6/mL. After the prepared spore suspension was subjected to various treatments, it was placed in a constant temperature incubator to moisturize the culture, and the spores were germinated. The number of spores per microscopic examination was at least 300. The germination criterion was that when the length of the germ tube exceeds the length of the spore diameter (not a perfectly round spore, it refers to the side of the smaller diameter), it was considered as a spore that had already germinated. All experiments were repeated three times.

To determine the lethal temperature for the conidia, two hundred microliters of spore suspension (1×10^6/mL) in each sterilized 1.5 mL microcentrifuge tube were placed in a constant-temperature water bath at 45, 50, 55, 60, 65, 70, and 75 °C for 10 minutes, respectively. After that, the temperature was rapidly cooled to room temperature, and 20 μL of the treated spore suspension was pipetted onto the PDA medium. The suspension was uniformly coated to the surface of the plate using a sterilized triangular rod, and the plate was placed in a 25 °C incubator. The colony growth was observed after 3 days. After initially determining the lethal temperature range, the gradient was set to 1 °C to determine the final lethal temperature of the conidia.

The spore suspensions were cultured in a 25 °C, 95% bumidity artificial intelligence incubator of 12 h, 24 h, 36 h, and 48 h, and the spore germination was observed to determine the effects for time on spore germination.

To determine the effects of liquid matrix on spore germination, different sterile liquid substrate treatments were prepared at the same time as the spore suspension: 1% glucose solution, 1% sucrose solution, and sterile water. The spore germination rate

was examined after moisture treatment for 12 h, 24 h, 36 h, and 48 h in a 25 °C, 95% humidity artificial intelligence incubator system.

To determine the effects of light conditions on spore germination, the spore suspension slides prepared for sterile water were set to one of three conditions, complete light, complete darkness, and alternating light and darkness, and placed in a 25 °C, 95% humidity artificial intelligence incubator for 12 h. After 24 h, 36 h, 48 h, the spore germination rate was examined microscopically.

Data analysis

The data were analyzed and processed using DPS data processing software. All data represent repeated experiments and are expressed as mean ± standard deviation. Statistical significance of difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. The level of statistical significance was set at $P < 0.05$. The processed data were plotted using Microsoft Excel 2010.

RESULTS AND DISCUSSION

E. ulmoides black spot disease primarily harms the leaves (Figure 1). Initially, the disease appears as hazel and yellow dots on the edge or middle of the leaf and gradually expands into round or irregular shaped lesions with a greyish central colour and a dark brown edge. At the beginning of the disease, reddish brown to purple brown spots appear on the surface of the leaves, gradually expanding into round or amorphous dark black lesions. There are often yellow halos around the lesions; the edges are radial, and finally, the leaves fall off or become perforated. The edges of the holes are ragged. The black spots on the late lesions are scattered and represent the conidial discs of the fungi. In severe cases, the leaves of the lower part of the plant are yellow, and defoliated earlier, causing individual branches to die.

A total of 30 fungal strains were isolated from infested leaf fragments by tissue separation. Among them, the colony morphology of 22 strains was basically the same as that of *P. trachicarpicola*, and the separation frequency was 73.3%. This strain as a dominant was used for further measurement. According to Koch's postulates, this strain was proven to be the causative pathogen of black spot of *E. ulmoides*.

After culturing on PDA medium for 7 days at 25 °C, round colonies with a diameter of approximately 60 mm were formed with fringed edges, dense aerial hyphae on the surface, and white to pale yellow, reverse yellow, with black spherical mucilaginous tissue containing copious conidia produced on white colony tissue in the later stage of culture (Figure 2). The white colony tissue produces

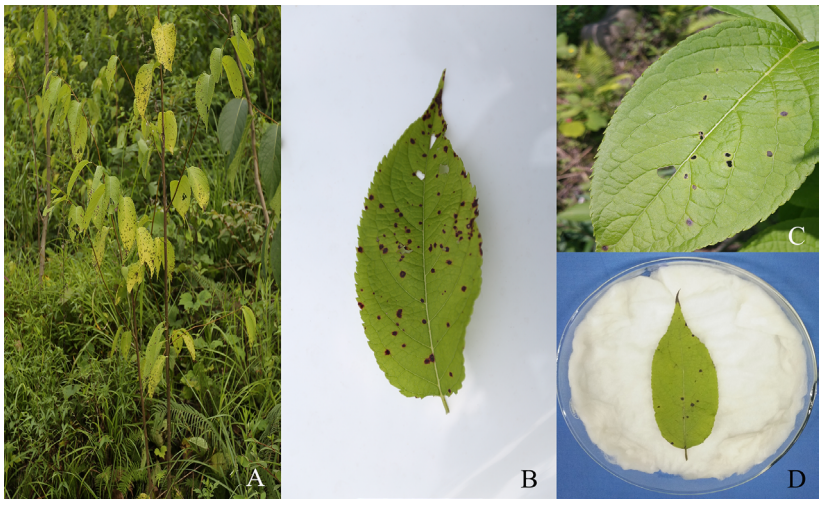

Figure 1 - Black spot symptoms on the leaves of *Eucommia ulmoides*. (A, B, C) Leaf symptoms of *E. ulmoides* black spot in the field. (A) In severe cases, leaves show symptoms of *E. ulmoides* black spot in the field. (B) Leaf symptoms of *E. ulmoides* black spot at the beginning of the disease. (C) At a later stage, the leaf symptoms of *E. ulmoides* black spot become perforated. (D) Leaf symptoms of *E. ulmoides* black spot after artificial inoculation. The photograph was taken on 15 days post inoculation.

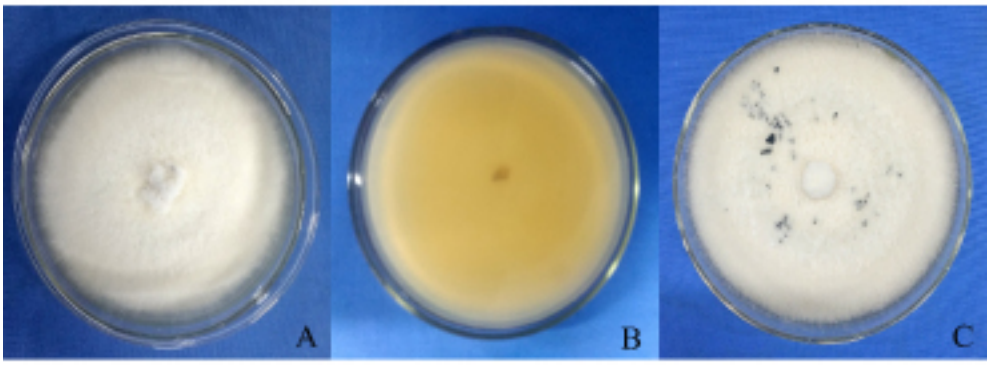

Figure 2 - Colony morphology of *Pestalotiopsis trachicarpicola* on PDA. A: The front side of the plate; B: The back side of the plate; C: Late colony morphology. A, B: Photographs were taken after 7 days of incubation at 25 °C; C: The photograph was taken after 10 days of incubation at 25 °C.

a black mucus tissue containing a large number of spherical conidia. The conidiogenous cells are fusiform, hyaline, short, and thin-walled. The conidia (each size is 21.5-25.8 μm × 5.5-7.5 μm) contain five cells, and the size of the basal cell is 2.8-6.8 μm, sharp, colorless, and transparent, thin-walled, and with a ridge-like bulge. The apical cell size is 2.7-6.4 μm, conical to cylindrical, colorless, and transparent. The middle three cells are all olive in color. From the basal cell, the second cell size is 2.9-7.4 μm, the third cell with a dimension of 3.4-6.8 μm, and the fourth cell with a magnitude of 3-5.8 μm. The apical appendages are tubular, 2-4 (mostly 3) arising from the apex of the apical cell, 5-17 μm, and the basal appendages are filiform, single, 2.2-8.3 μm. The primers ITS4/ITS5, BT2A/BT2B, and EF1-526F/EF1-1567R were used to PCR and sequence the rDNA-ITS, β-tubulin, and TEF-1α gene sequences of the pathogen, yielding 592 bp, 435 bp, and 951 bp gene fragment, respectively. The GenBank accession numbers are MG827240, MG833724, and MG833725. The results retrieved showed that the test strain was highly nucleotide sequence similar with *P. trachicarpicola*, more than 99% (registration numbers: KX609685.1, MG740742.1, JQ845946.1). The rDNA-ITS, β-tubulin gene and TEF-1α gene sequences of the pathogen were homologously aligned in GenBank. Constructed phylogenetic tree analysis showed that the test strain was closely related to *P. trachicarpicola* and was located in the same branch. According to the pathogenicity test, the morphological characteristics of the pathogens (Figure 3), combined with the description of JAYAWARDENA et al. (2015) and others, and molecular identification, the causative pathogen of black spot disease was determined to be *P. trachicarpicola*.

For the effects of medium composition on mycelial growth, it was found that the pathogens exhibited macroscopic differences on their growth (Figure 4). When grown on a variety of media, pathogens exhibit differences in the macroscopic experience of their growth. When cultured for 5 d, the mycelial growth rate was PDA > PSA > CA > CZ > CMA > OMA > WA. The pathogens grew best on the PDA and PSA media; the colonies were dense, and; the growth was smooth, and the edges were tassel-like. The fungus grows better on PDA and PSA media than it does on CA medium. The colonies have concentric circles; the aerial hyphae were dense, and the edges were regular. The colonies growth of the CZ medium was uneven, and the aerial hyphae were thin. The colony morphology was similar on the CMA and OMA media. The aerial hyphae on both media were thin and extremely sparse. The margins were regular. The colony growth of the OMA was lower than that with the CMA, but the aerial hyphae were slightly thicker on the CMA. The colonies grew the slowest on the WA medium; the aerial hyphae were transparent, extremely thin and sparse, and the shape was regular. Based on these factors, the pathogen grows best on PDA media, followed by PSA and CA medium.

Figure 5 shows that the temperature has a substantial influence on the mycelial growth of *P. trachicarpicola*. There was a significant difference in mycelial growth at the different temperatures ($P < 0.05$). Hyphae can grow at 10~30 °C, and the optimal growth temperature was 20~25 °C. After incubation for 5 days on PDA media, the colonies had an average diameter of 7.21 cm and 7.30 cm at 20 °C and 25 °C, respectively. When the temperature was between 10

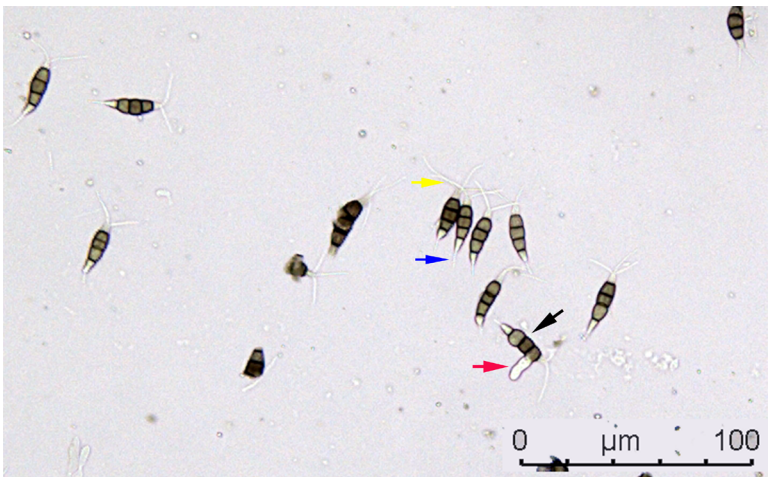

Figure 3 - The conidia of *Pestalotiopsis trachicarpicola*. The black arrows indicate germinating conidia, and the red arrow indicates the germ tube. The yellow arrows indicate apical appendages arising from the apex of the apical cell, and the blue arrow indicates basal appendages.

°C and 30 °C, mycelial growth was obviously inhibited, and the growth was slow. When the temperature was 5 °C, 35 °C, or 40 °C, the mycelia hardly grew, and the colony diameter was always maintained at approximately 0.80 cm. To sum up, the pathogens can only grow better when the temperature is appropriate, and excessively high or low temperatures will cause serious interference in their growth.

Figure 6 shows that the pathogen has wide adaptability to pH and can grow at a pH of 4.0 to 10.0. With the increase in the pH value, the colony growth diameter generally increases first and then decreases. After culturing for 5 days on PDA media, the pH values ranged from 6.0 to 10.0, and the average diameter of the colonies was greater than 7.0 cm. The pH value ranging from 6.5 to 8.5 was the optimal pH for the growth of *P. trachicarpicola*. The average diameter of the colonies reached 7.45 cm, 7.63 cm, 7.52 cm, 7.50 cm, and 7.48 cm, and the colonies grew best at pH 7.0. In general, the colony growth was inhibited from both acid and alkaline conditions, but the colony growth was more inhibited from acidic conditions, and the colony growth was more favorable to alkaline conditions. After 10 days of culture, the pathogens sporulated, and the sporulation amount was high, indicating that the pathogen's spore growth could not be inhibited from the partial acidic or alkaline conditions.

The experiment indicated that the pathogens could grow well under the three conditions of illumination, and the colonies grew the most quickly under full light conditions (Figure 7). The average diameter of the colonies was determined by incubation on PDA media and a 25 °C incubator for 5 days. The average diameter of the colonies was 7.28 cm under the full light conditions, and the second-largest average diameter was under the alternating 24 h light and dark conditions. The colonies of the pathogens cultured in the dark grew the slowest, and the average diameter of the colonies was 6.97 cm. There was a significant difference ($P<0.05$) between the pathogens cultured in the constant light and constant dark, and the difference was not significant ($P<0.05$) compared with the pathogens alternately cultured with 24 h light and dark. The pathogens cultured under the three test conditions were all flats, and the mycelium was dense, but the morphology of the mycelium was also slightly different. The pathogens cultured under the alternating conditions of 24 h lights and dark had concentric circles, but there was only one under full illumination. The circle has obvious ring marks, while the pathogens of the cultured pathogens in the dark condition have no ring marks, but the hyphae appear denser.

The pathogens were able to grow on the seven carbon source mediums examined (Figure 8). After 5 days of culture, the growth rate of the colonies was glucose > maltose > soluble starch > sucrose > fructose > lactose > CK, and the average diameter of the colonies were 5.31 cm, 5.14 cm, 5.10 cm, 5.08

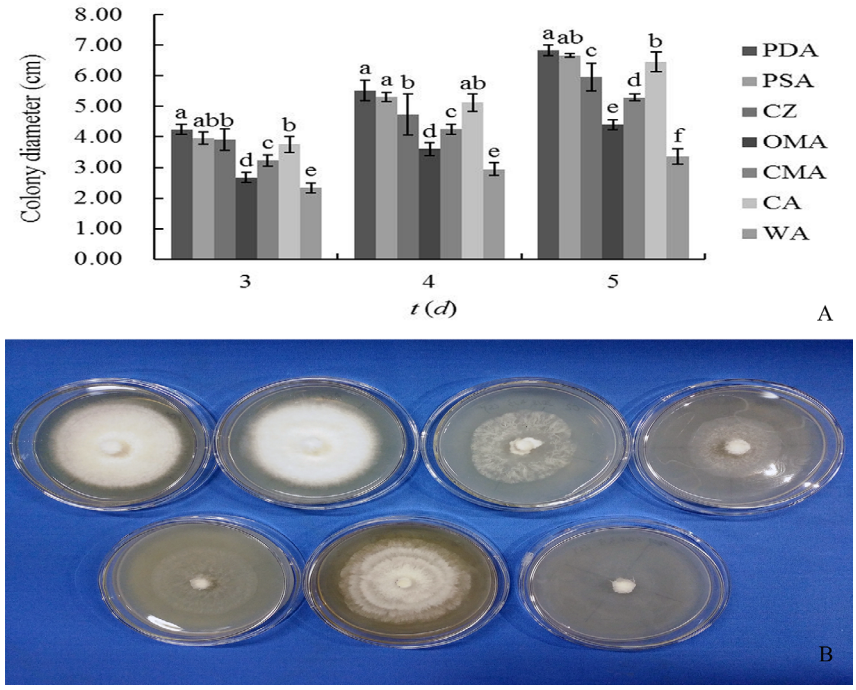

Figure 4 - Effects of medium type on the mycelium growth of *P. trachicarpicola*. (A) Statistical analysis of the indicated *P. trachicarpicola* growth under different medium types. The X-axis represents culture time (unit: day), and the Y-axis represents colony diameter (unit: cm). The colony diameters of the indicated strains were measured on days 3, 4, and 5, respectively. Results are mean ± SD (n = 5). All experiments were repeated at least five times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level. (B) Mycelial radial growth of the indicated *P. trachicarpicola* on different medium types. The media names are ordered from left to right and top to bottom as follows: PDA, PSA, CZ, OMA, CMA, CA, and WA. The photographwas taken after 5 days of incubation at 25 °C.

cm, 4.89 cm, 4.75 cm, and 4.44 cm, respectively. From the morphology of colonies, the mycelial density of the pathogens cultured on the five media containing glucose, maltose, soluble starch, sucrose, and fructose as carbon sources was glucose > sucrose > maltose > fructose > soluble starch, and the surface of the colony was uneven, except for that with the soluble starch. The mycelial growth of the central part of the other four carbon sources of the starch was thicker. After 10 days of culture, the pathogens on the five media showed signs of sporulation, and the medium with fructose as the carbon source was the most noticeable. The mycelium of the pathogen was cultured on the medium with lactose and no-carbon sources as the control was thin, transparent, and sparse, and no sporulation was observed after 10 days of culture.

The pathogens were able to grow on the seven types of nitrogen source culture media (Figure 9). After 5 days of culture, the colony growth rate based on the nitrogen source was in order beef extract > sodium nitrate > yeast powder > protein > ammonium nitrate > CK > potassium nitrate; the average colony diameters were not significantly different and were 4.85 cm, 4.83 cm, 4.79 cm, 4.73 cm, 4.64 cm, 4.58 cm, and 4.21 cm, respectively. After 10 days of culture, the morphology of the colonies was examined. The pathogens of the other six types of test media cultured, except for the control medium without a nitrogen source, the CK, grew better and were villous, sporulated, and had dense mycelia. Regarding the mycelial growth density, the optimal order for the nitrogen sources was

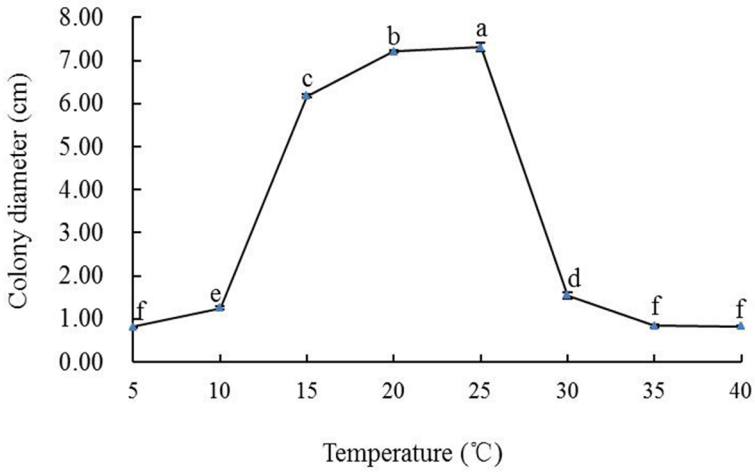

Figure 5 - Effects of temperature on the mycelium growth of *P. trachicarpicola*. Statistical analysis of the indicated *P. trachicarpicola* growth under different temperatures. The X-axis represents culture temperature (unit: °C), and the Y-axis represents colony diameter (unit: cm). The colony diameters of the indicated strains were measured on day 5. Results are mean ± SD (n = 5). All experiments were repeated at least five times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level.

beef extract > ammonium nitrate > yeast powder > peptone > potassium nitrate > sodium nitrate, in which the mycelia on the media with ammonium nitrate and peptone as nitrogen sources gradually turn greyish white, with potassium nitrate, peptone and yeast powder as a nitrogen source, the surface of the colony of the culture medium has a concentric pattern, and that of the pathogen cultured on the medium with yeast powder as a nitrogen source was the most obvious, with regular colony edges. Based on these factors, the beef extract is the most suitable nitrogen source for pathogen growth and facilitates spore germination.

For the lethal temperature of hyphae and conidia determinations, table 1 indicates that after the pathogenic fungal mat and conidia were treated with a water bath at 52 °C and 72 °C for 10 min, the hyphae no longer grew on PDA media, and the conidia no longer germinated and grew. These results indicated that the mycelial lethal temperature of the pathogen was 52 °C and that the conidia lethal temperature was 72 °C.

The results of figure 10 show that the germination rate of the conidia increased gradually over time, but there was no significant difference between the conidial germination rates at 36 h and 48 h, which were 1.00% and 3.38%, 8.78%, and 9.50%, respectively.

Figure 11 indicates that the germination rate of the conidia in the three liquid substrates tested was best with 1% glucose solution; the 1% sucrose solution was the second, and the sterile water was the lowest; and after 48 h, the conidial germination rate was 29.24%, 25.24%, and 9.50%, respectively. There was a significant difference between the spore suspensions treated with the substrate and those treated for 12 h and with the 1% sucrose solution or the spore suspension treated with sterile water for 24 h. The results showed that the glucose and sucrose solution promoted the conidial germination of the pathogen and increased the germination rate of the conidia.

Figure 12 indicates that the conidia can germinate into the three conditions of the test, and the conidia germination rate for different light treatment conditions was significantly different from the same germination time. Under the conditions of total darkness, the conidial germination of the pathogen was the best, followed by alternating light and darkness, and the lowest germination rate was under

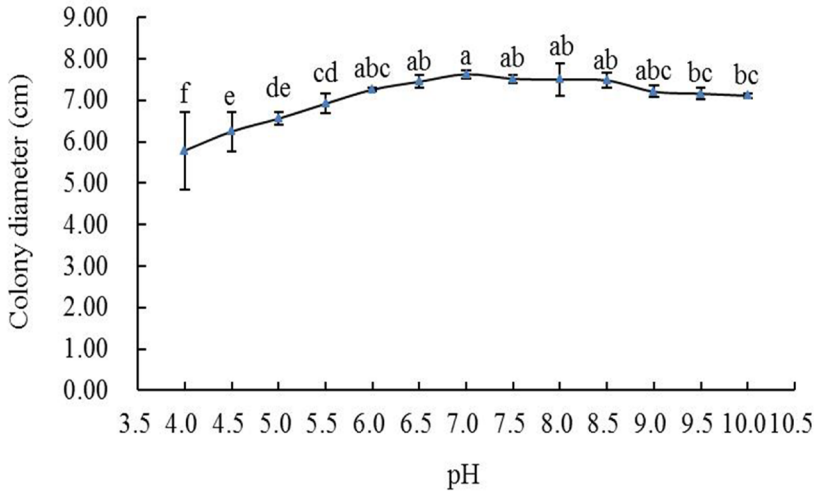

Figure 6 - Effects of pH on the mycelium growth of *P. trachicarpicola*. Statistical analysis of the indicated *P. trachicarpicola* growth under different pH. The X-axis represents pH, and the Y-axis represents colony diameter (unit: cm). The colony diameters of the indicated strains were measured on day 5. Results are mean ± SD (n = 5). All experiments were repeated at least five times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level.

full light; the germination rate of the conidia after 48 h was 18.49%, 10.47%, and 9.50%, respectively. This result indicated that darkness is more conducive to the germination of the conidia of the pathogen.

The traditional classification of *Pestalotiopsis* is primarily based on the morphology of the host and conidia, such as conidial length, width, intermediate cell length, intermediate cell color, and apical appendage length (HU et al., 2007, JEEWON et al., 2003, MAHARACHCHIKUMBURA et al., 2012, MAHARACHCHIKUMBURA et al., 2014, SEONJU et al., 2006). However, this classification results in identification problems and difficulties differentiating species, and research has shown that molecular phylogeny helps to establish species concepts; using multilocus DNA sequence analysis offered a better phylogeny to resolve taxonomic questions, as it can easily distinguish *Pestalotiopsis* with unique characters (HU et al., 2007, LIU et al., 2010).

Pestalotiopsis is a common plant pathogen that can cause a variety of diseases, such as ulcer disease, blight disease, leaf spot, needle blight, top blight, grey blight, yellow wilt, and fruit rot (CROUS et al., 2011; MAHARACHCHIKUMBURA et al., 2012; MAHARACHCHIKUMBURA et al., 2013a; MAHARACHCHIKUMBURA et al., 2013b; ZHANG et al., 2012; ZHANG et al., 2013). The host plants infected by this type of fungus have a wide range, and different species can cause different diseases. For example, SUWANNARACH et al. (2013) confirmed that leaf spot disease on oil palm caused by *P. theae* in Thailand. RIZWANA et al. (2012) proved that a postharvest disease of *Mangifera indica* fruit of Saudi Arabia caused by *P. mangiferae*, and CHEN et al. (2017) described that *P. camelliae* caused grey blight disease on *Camellia sinensis* in China.

There are few reports on the pathogens causing black spot disease of *E. ulmoides* in China. In this study, the biological characteristics of *P. trachicarpicola*, a new pathogen of *E. ulmoides* black spot disease determined. The pathogenic isolation test also proved that *P. trachicarpicola* was the pathogen causing the black spot of *E. ulmoides*. Some researchers have shown that the pathogen also causes palm leaf spot disease (MAHARACHCHIKUMBURA et al., 2012), grape stem disease, and fruit soft rot (JAYAWARDENA et al., 2015). The test results showed that the optimum medium for the pathogen was PDA and that the optimum temperature was 20~25 °C. Both

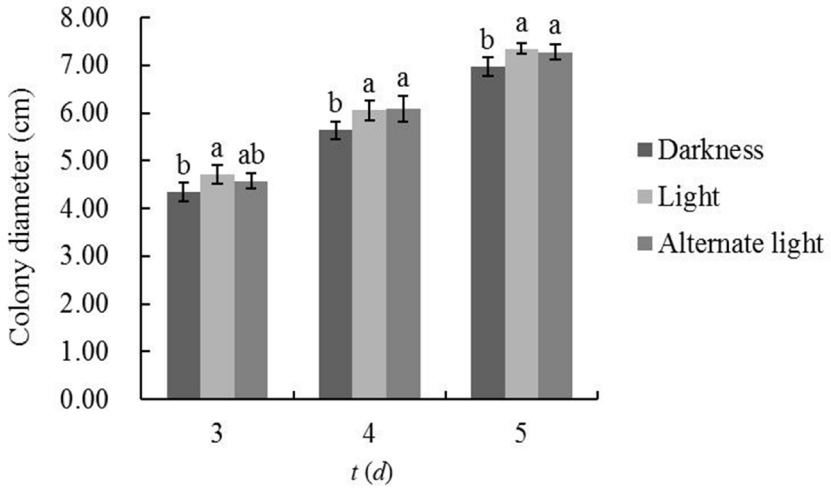

Figure 7 - Effects of light on the mycelium growth of *P. trachicarpicola*. Statistical analysis of the indicated *P. trachicarpicola* growth under different lights. The X-axis represents culture time (unit: day), and the Y-axis represents colony diameter (unit: cm). The colony diameters of the indicated strains were measured on days 3, 4, and 5. Results are mean ± SD (n = 5). All experiments were repeated at least five times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level.

high temperature and low temperature can inhibit the growth of the pathogen. The study reported that the leaves began to develop in April and May. Entering the peak period of the disease in June, and *E. ulmoides* was more seriously affected in July and August by the environmental conditions with suitable temperature and rain. The optimum pH was 7.0, and the mycelial growth of alkaline conditions was better than that under the acidic conditions, which is required for the separation of the genus *Pestalotiopsis* from HOPKINS & MCQUILKEN (2000), JANG et al. (1997). The optimum pH was slightly acidic. The colony growth was best under light, but total darkness was more conducive to the conidial germination of the pathogen. In addition, the pathogen has a wide range of adaptations to carbon and nitrogen sources, in which glucose was the most suitable carbon source, and the beef extract was the most suitable nitrogen source. The conidia were compared to 1% glucose solution and 1% sucrose solution. The germination rate was higher under the condition of fungal water, indicating that proper nutrition improves the growth of the hyphae and the germination of spores. In addition, the lethal temperatures of the hyphae and conidia were significantly different, 52 °C and 72 °C, respectively, which proved that the conidia were more resistant to high temperatures than were the hyphae. The germination rate of the conidia increased gradually over time, but it only reached 9.80% after 48 h. It was proven that the conidia of the pathogen are more likely to germinate into appropriate environmental conditions, which may also be the primary reason for the low rate of conidial germination of the pathogen.

As one of the traditional "the three wood medicine materials," *E. ulmoides* has high economic value. The black spot disease caused by *P. trachicarpicola* severely restricts the growth and planting of *E. ulmoides*. This experiment determined the causative pathogen and the biological characteristics of *P. trachicarpicola* that caused the disease of *E. ulmoides* black spot and provided a theoretical basis to prevent and treat the disease. However, the pathogenic mechanism of the pathogen and whether there are other pathogens mixed with the pathogen to infect the leaves of *E. ulmoides* should be determined by additional research. Future research should also attempt to understand the disease cycle and epidemiology of the disease, screen pesticides,

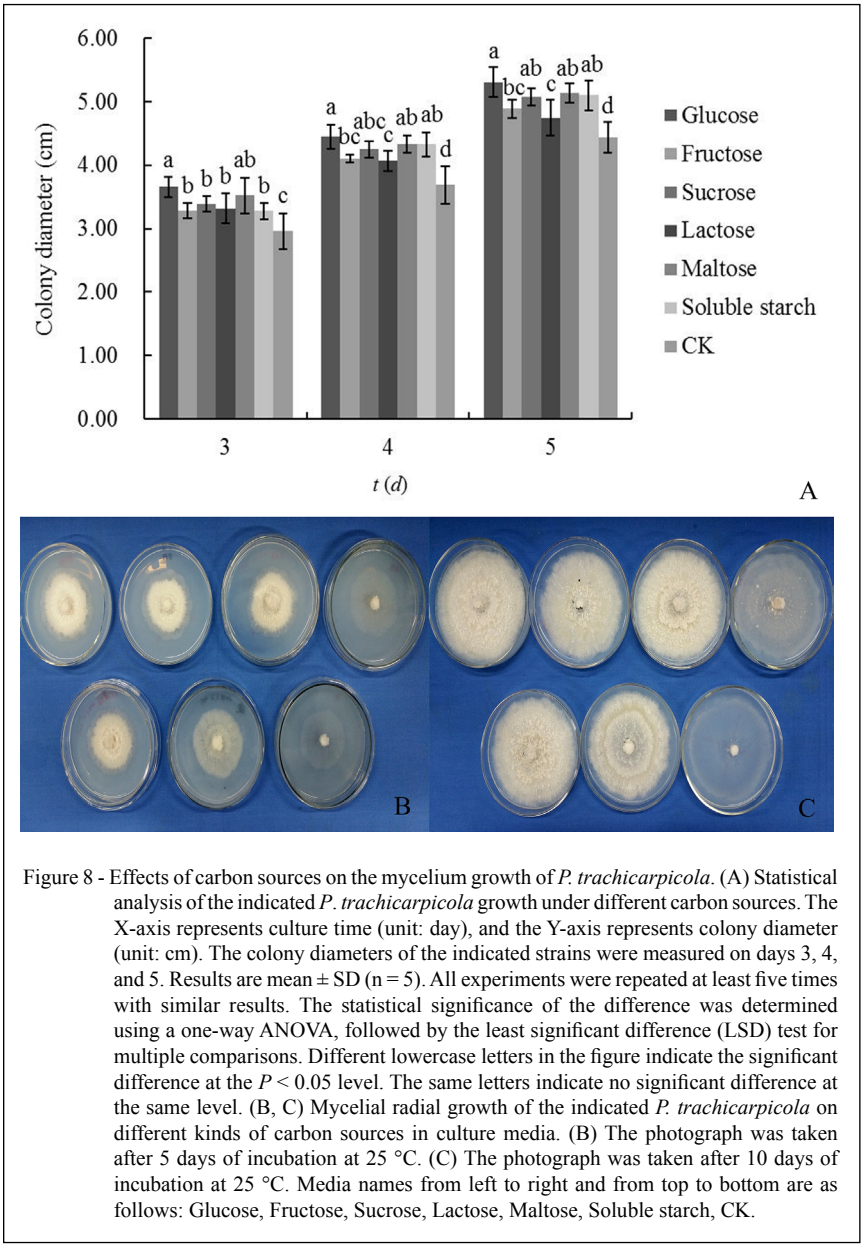

Figure 8 - Effects of carbon sources on the mycelium growth of *P. trachicarpicola*. (A) Statistical analysis of the indicated *P. trachicarpicola* growth under different carbon sources. The X-axis represents culture time (unit: day), and the Y-axis represents colony diameter (unit: cm). The colony diameters of the indicated strains were measured on days 3, 4, and 5. Results are mean ± SD (n = 5). All experiments were repeated at least five times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at the $P < 0.05$ level. The same letters indicate no significant difference at the same level. (B, C) Mycelial radial growth of the indicated *P. trachicarpicola* on different kinds of carbon sources in culture media. (B) The photograph was taken after 5 days of incubation at 25 °C. (C) The photograph was taken after 10 days of incubation at 25 °C. Media names from left to right and from top to bottom are as follows: Glucose, Fructose, Sucrose, Lactose, Maltose, Soluble starch, CK.

facilitate the breeding of good seedlings that have disease resistance. These are all necessary measures to prevent and treat the disease.

CONCLUSION

Factors such as temperature, humidity, pH, and the host will affect the growth of pathogens, thereby affecting the occurrence and development of plant diseases. Suitable environmental conditions are more conducive to the growth of pathogens, and as a result, the occurrence and prevalence of plant diseases. Therefore, mastering the species and their biological characteristics of pathogens is of great significance in preventing and controlling plant diseases.

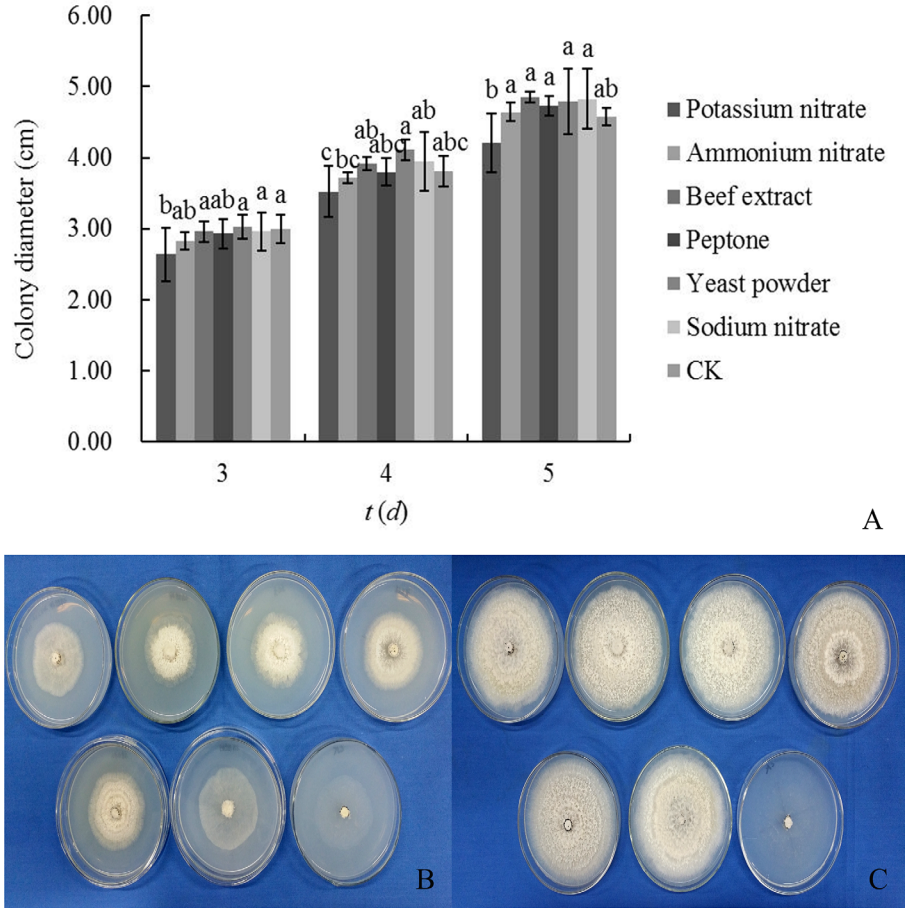

Figure 9 - Effects of nitrogen sources on the mycelium growth of *P. trachicarpicola*. (A) Statistical analysis of the indicated *P. trachicarpicola* growth under different nitrogen sources. The X-axis represents culture time (unit: day), and the Y-axis represents colony diameter (unit: cm). The colony diameters of the indicated strains were measured on days 3, 4, and 5. Results are mean ± SD (n = 5). All experiments were repeated at least five times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level. (B, C) Mycelial radial growth of the indicated *P. trachicarpicola* on different kinds of nitrogen sources in culture media. (B) The photograph wwas taken after 5 days of incubation at 25 °C. (C) The photograph was taken after 10 days of incubation at 25 °C. Media names from left to right and from top to bottom are as follows: Potassium nitrate, Ammonium nitrate, Beef extract, Peptone, Yeast powder, Sodium nitrate, CK.

Table 1 - Mycelium and conidia lethal temperature.

Temperature/°C	Mycelial growth	Spore germination
45	+	+
50	+	+
51	+	+
52	-	+
53	-	+
54	-	+
55	-	+
60	-	+
65	-	+
70	-	+
71	-	+
72	-	-
73	-	-
74	-	-
75	-	-

Note: "+" indicates mycelial growth, spore germination, and growth; "-" indicates that the hyphae do not grow, and the spores do not germinate or grow. The lethal temperature for the hyphae experiment was repeated at least five times with similar results (n = 5). The lethal temperature for the spore germination experiment was repeated at least three times with similar results (n = 3).

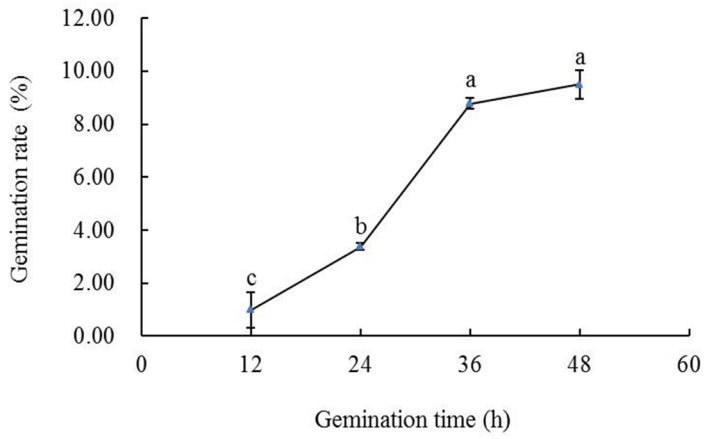

Figure 10 - Effects of germination time on spore germination of *P. trachicarpicola*. Statistical analysis of the indicated *P. trachicarpicola* spore germination at various germination times. The X-axis represents germination time (unit: hour), and the Y-axis represents colony germination rate (unit: %). The colony germination rates of the indicated strains were measured at the 12th, 24th, 36th, and 48th hours. Results are mean ± SD (n = 3). All experiments were repeated at least three times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level.

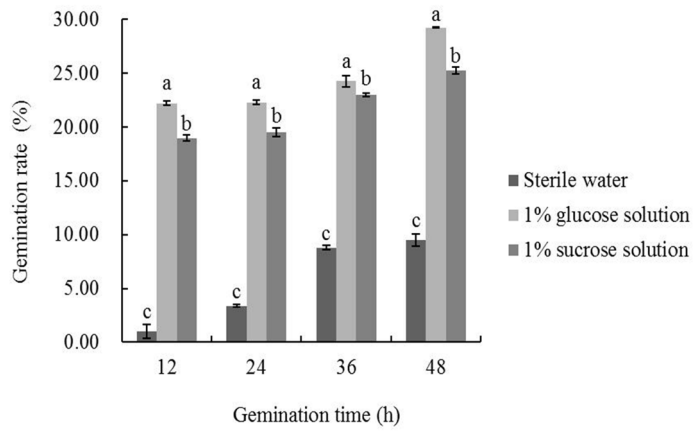

Figure 11 - Effect of liquid matrix on spore germination of *P. trachicarpicola.* Statistical analysis of the indicated *P. trachicarpicola* spore germination in various liquid matrixes. The X-axis represents germination time (unit: hour), and the Y-axis represents colony germination rate (unit: %). The colony germination rates of the indicated strains were measured at the 12th, 24th, 36th, and 48th hours. Results are mean ± SD (n = 3). All experiments were repeated at least three times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level.

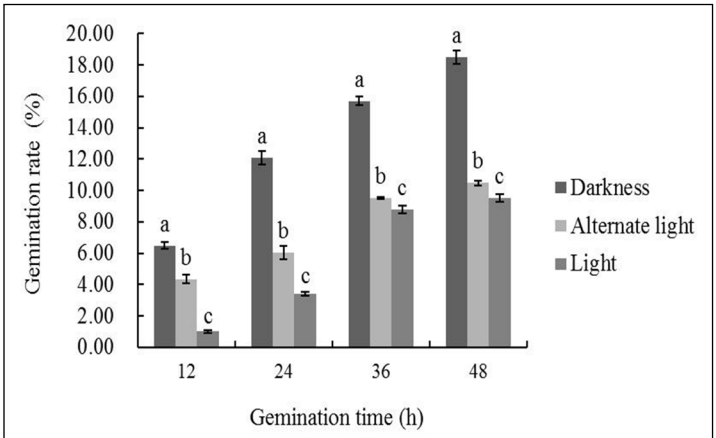

Figure 12 - Effects of light on spore germination of *P. trachicarpicola.* Statistical analysis of the indicated *P. trachicarpicola* spore germination under various lighting conditions. The X-axis represents germination time (unit: hour), and the Y-axis represents colony germination rate (unit: %). The colony germination rates of the indicated strains were measured at the 12th, 24th, 36th, and 48th hours. Results are mean ± SD (n = 3). All experiments were repeated at least three times with similar results. The statistical significance of the difference was determined using a one-way ANOVA, followed by the least significant difference (LSD) test for multiple comparisons. Different lowercase letters in the figure indicate the significant difference at $P < 0.05$ level. The same letters indicate no significant difference at the same level.

ACKNOWLEDGEMENTS

This research was funded by the Foundation of National Science and Technology Platform Infrastructure (Grant No. 2005DK21207-13).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

ADACHI, Y.; TSUGE, T. Coinfection by different isolates of *Alternaria alternata* insingle black spot lesions of Japanese pear leaves. **Phytopathology**, v.84, n.5, p.447-451, 1994. Available from: <http://europepmc.org/article/AGR/IND20443868>. Accessed: Aug. 02, 2018. doi: 10.1094/Phyto-84-447.

AYER, W. A.; PENARODRIGUEZ, L. M. Metabolites produced by *Alternaria brassicae*, the black spot pathogen of canola. Part 1, the phytotoxic components. **Journal of Natural Products**, v.50, n.3, p.400-407, 1987. Available from: <https://pubs.acs.org/doi/abs/10.1021/np50051a010>. Accessed: Aug. 17, 2018. doi: 10.1021/np50051a010.

BENSCH, K. et al. The genus Cladosporium. **Studies in Mycology**, v.72, p.1-401, 2012. Available from: <https://doi.org/10.3114/sim0003>. Accessed: Jun. 13, 2017. doi: 10.3114/sim0003.

CHEN, Y. et al. First Report of *Pestalotiopsis camelliae* causing grey blight disease on *Camellia sinensis* in China. **Plant Disease**, v.101, n.6, 2017. Available from: <http://agri.ckcest.cn/ass/66252127-1b59-4c34-b688-ad58a549372c.pdf>. Accessed: Jun. 15, 2018. doi: 10.1094/PDIS-01-17-0033-PDN.

CROUS, P. W. et al. Fungal pathogens of *Proteaceae*. **Persoonia Molecular Phylogeny and Evolution of Fungi**, v.27, n.1, p.20-45, 2011. Available from: <https://www.researchgate.net/publication/221689353>. Accessed: Sep. 05, 2018. doi: 10.3767/003158511X606239.

FANG, Z. D. **Method of plant disease research**. 3. ed. Beijing: China, 1998. p. 37-145.

GLASS, N. L.; DONALDSON, G. C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. **Applied and Environmental Microbiology**, v.61, n.4, p.1323-1330, 1995. Available from: <http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC167388&blobtype=pdf>. Accessed: Jan. 20, 2021. doi: 10.1002/bit.260460112.

HU, H. L. et al. Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: Evidence from rDNA and β-tubulin gene phylogenies. **Fungal Diversity**, v.24, p.1-22, 2007. Available from: <https://www.researchgate.net/publication/298532536>. Accessed: Jan. 20, 2020.

HOPKINS, K. E.; MCQUILKEN, M. P. Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK. **European Journal of Plant Pathology**, v.106, n.1, p.77-85, 2000. Available from: <https://link.springer.com/content/pdf/10.1023%2FA%3A1008776611306.pdf>. Accessed: Aug. 03, 2018. doi: 10.1023/A:1008776611306.

JAYAWARDENA, R. S. et al. Identification and characterization of *Pestalotiopsis* like fungi related to grapevine diseases in China. **Fungal Biology**, v.119, n.5, p.348-361, 2015. Available from: <https://doi.org/10.1016/j.funbio.2014.11.001>. Accessed: Nov. 11, 2017. doi: 10.1016/j.funbio.2014.11.001.

JEEWON, R. et al. Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. **Molecular Phylogenetics and Evolution**, v.27, n.3, p.372-383, 2003. Available from: <https://doi.org/10.1016/S1055-7903(03)00010-1>. Accessed: Jan. 14, 2017. doi: 10.1016/S1055-7903(03)00010-1.

LIU, A. R. et al. Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in *Pestalotiopsis* taxonomy. **Molecular Phylogenetics and Evolution**, v.57, n.2, p.528-535, 2010. Available from: <https://www.researchgate.net/publication/258859244>. Accessed: Oct. 12, 2018. doi: 10.1016/j.ympev.2010.07.017.

LIU, T, K. et al. Rapid identification of the geographical origin of *Eucommia ulmoides* by using excitation-emission matrix fluorescence combined with chemometric methods. **Spectrochimica acta, Part A. Molecular and biomolecular spectroscopy**, v,277, p.1386-1425, 2022. Available from: <https://doi.org/10.1016/j.saa.2022.121243>. Accessed: Oct. 20, 2023. doi: 10.1016/j.saa.2022.121243.

MACKINNON, S. L. et al. Components from the phytotoxic extract of *Alternaria brassicicola*, a black spot pathogen of canola. **Phytochemistry**, v.51, n.2, p.215-221, 1999. Available from: <https://doi.org/10.1016/S0031-9422(98)00732-8>. Accessed: Jun. 18, 2019. doi: 10.1016/S0031-9422(98)00732-8.

MAHARACHCHIKUMBURA, S. S. N. et al. *Pestalotiopsis* species associated with *Camellia sinensis* (tea). **Mycotaxon**, v.123, p.47-61, 2013a. Available from: <http://www.ithaca-ny.com/journal-PDF/vol123/s8.pdf>. Accessed: Sep. 1, 2018. doi: 10.5248/123.47.

MAHARACHCHIKUMBURA, S. S. N. et al. A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. **Fungal Diversity**, v.56, n.1, p.95-129, 2012. Available from: <https://www.zhangqiaokeyan.com/academic-journal-foreign_other_thesis/02041367358.html>. Accessed: Sep. 01, 2018. doi: 10.1007/s13225-012-0198-1.

MAHARACHCHIKUMBURA, S. S. N. et al. A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangensis*. **Tropical Plant Pathology**, v.38, n.3, p.227-235, 2013b. Available from: <https://www.researchgate.net/publication/258884752>. Accessed: Sep. 01, 2018. doi: 10.1590/S1982-56762013005000002.

MAHARACHCHIKUMBURA, S. S. N. et al. Improving the backbone tree for the genus *Pestalotiopsis*; addition of *P. steyaertii*, and *P. magna*, sp. nov. **Mycological Progress**, v.13, n.3, p.617-

624, 2014. Available from: <https://link.springer.com/content/pdf/10.1007%2Fs11557-013-0944-0.pdf>. Accessed: Sep. 20, 2018. doi: 10.1007/s11557-013-0944-0.

MARCHANT, R. et al. Expression of a chitinase transgene in rose (*Rosa hybrida* L.) reduces development of black spot disease (*Diplocarpon rosae* Wolf). **Molecular Breeding**, v.4, n.3, p.187-194, 1998. Available from: <https://link.springer.com/article/10.1023/A:1009642707505>. Accessed: Aug. 02, 2018. doi: 10.1023/A:1009642707505.

O'DONNELL, K.; CIGELNIK, E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. **Molecular Phylogenetics and Evolution**, v.7, n.1, p.103-116, 1997. Available from: <http://europepmc.org/article/MED/9007025>. Accessed: Feb. 14, 2019. doi: 10.1006/mpev.1996.0376.

RIZWANA, H. et al. A post harvest disease of *Mangifera indica* fruit caused by *Pestalotiopsis mangiferae*, in Saudi Arabia. **African Journal of Microbiology Research**, v.6, n.27, p.5723-5724, 2012. Available from: <http://www.academicjournals.org/AJMR>. Accessed: Aug. 02, 2018. doi: 10.5897/AJMR12.713.

SEONJU, L. et al. Pestalotioid fungi from *Restionaceae* in the Cape Floral Kingdom. **Studies in Mycology**, v. 55, n.1, p.175-187, 2006. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2104724/pdf/0175.pdf>. Accessed: May, 02, 2017. doi: 10.3114/sim.55.1.175.

SUWANNARACH, N. et al. First report of leaf spot disease on *oil palm* caused by *Pestalotiopsis theae* in Thailand. **Journal of General Plant Pathology**, v.79, n.4, p.277-279, 2013. Available from: <https://link.springer.com/article/10.1007/s12600-014-0429-y>. Accessed: Aug. 02, 2018. doi: 10.1007/s12600-014-0429-y.

WEI, J. C. **Fungi identification manual**. Shang Hai: China, 1979. p. 1-802.

WHITE, T. J. et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A.; GELFAND, D.H. et al **Aguide to methods and applications**. New York: USA, 1990. p.315-322.

WU, M. Q. et al. An analysis of the genetic diversity and genetic structure of *Eucommia ulmoides* using inter-simple sequence repeat (ISSR) markers. **African Journal of Biotechnology**, v.10, n.84, p.19505-19513, 2011. Available from: <http://www.academicjournals.org/AJB>. Accessed: Aug. 10, 2018. doi: 10.5897/AJB11.1715.

YANG, L. Distribution prediction of suitable growth area for *Eucommia ulmoides* in China under climatic change background. **Meteorological and Environmental Research**. v.4, n.8, p.21-24, 2013. Available from: <http://qikan.cqvip.com/Qikan/Article/Detail?id=47470599>. Accessed: Jun. 02, 2018. doi: CNKI:SUN:MEVR.0.2013-08-007.

ZHANG, Y. M. et al. A novel species of *Pestalotiopsis* causing leaf spots of *Trachycarpus fortunei*. **Cryptogamie Mycologie**, v.33, n.3, p.311-318, 2012. Available from: <http://10.7872/crym.v33.iss3.2012.311>. Accessed: Nov. 05, 2019. doi: 10.7872/crym.v33.iss3.2012.311.

ZHANG, Y. M. et al. *Pestalotiopsis* species on ornamental plants in Yunnan province,China. **Sydowia**, v.65, n.1, p.113-128, 2013. Available from: <http://10.1016/j.riam.2013.01.005>. Accessed: May, 18, 2020. doi: 10.1016/j.riam.2013.01.005.