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海棠叶斑病病原菌的鉴定、致病性及杀菌剂敏感性测定

郭胜豪[1],杨 红[1*],葛 云[1],潘一可[2],田永生[3],章 瑶[1],吕国忠[1]

([1]大连民族大学环境与资源学院,辽宁大连 116600;[2]沈阳农业大学园艺学院,沈阳 110866;
[3]辽宁省本溪满族自治县农业综合发展服务中心,辽宁本溪 117100)

摘 要:【目的】明确引起唐纳德海棠(*Malus*‘Donald Wyman’)叶斑病的病原菌种类,确定其分类地位和致病性,并筛选高效杀菌剂以防治该病害。**【方法】**以唐纳德海棠叶斑病为研究对象,在大连市金州区部分地区采集病叶样本,分离纯化病原菌后通过形态特征和最大似然法(ML)进行多基因系统发育分析,确定病原菌分类地位;并对苹果属7种植物(印第安魔幻海棠、绚丽海棠、火焰海棠、西府海棠、垂丝海棠、山荆子、毛荆子)进行离体致病性测定;采用菌丝生长速率法对11种杀菌剂进行室内敏感性测定。**【结果】**通过形态学与多基因(ITS、ACT和SSU)序列分析确定唐纳德海棠叶斑病的病原菌为*Alternaria alternata*。离体致病性测定结果表明*A. alternata*可侵染5种寄主植物,其中对唐纳德海棠致病力最强,对垂丝海棠致病力最弱,而对山荆子和毛荆子则无明显侵染迹象。杀菌剂室内敏感性试验结果表明复配型药剂抑菌作用优于单剂,其中苯甲•丙环唑、异菌脲、咪鲜胺对病原菌的抑菌效果较好,EC_{50}分别为0.179 μg·mL^{-1}、1.735 μg·mL^{-1}、5.495 μg·mL^{-1}。**【结论】**唐纳德海棠叶斑病的病原菌为*A. alternata*,复剂抑菌作用优于单剂,50%苯甲•丙环唑对病原菌抑菌效果最好,可为唐纳德海棠叶斑病杀菌剂防治提供参考依据。

关键词:唐纳德海棠;叶斑病;病原鉴定;寄主范围;杀菌剂敏感性

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Identification, host range and fungicide screening of the pathogen causing leaf spot on *Malus*‘Donald Wyman’

GUO Shenghao[1], YANG Hong[1*], GE Yun[1], PAN Yike[2], TIAN Yongsheng[3], ZHANG Yao[1], LÜ Guozhong[1]

([1]College of Environment and Resources, Dalian Minzu University, Dalian 116600, Liaoning, China; [2]College of Horticulture, Shenyang Agricultural University, Shenyang 110866, Liaoning, China; [3]Benxi Manchu Autonomous County Agricultural Comprehensive Development Service Center of Liaoning Province, Benxi 117100, Liaoning, China)

Abstract:【Objective】Donald crabapple (*Malus*‘Donald Wyman’) has experienced widespread outbreaks of leaf spot disease within four consecutive years in Dalian region, resulting in substantial defoliation and plant mortality. This disease poses a serious threat to the cultivation, management, and ornamental value of Donald crabapple. The primary objective of this study was to identify the fungal pathogen responsible for the leaf spot disease. Furthermore, a series of chemical fungicide screenings were conducted to evaluate the efficacy of different fungicides against the pathogen, with the aim of providing a scientific basis for the effective prevention and control of *Malus*‘Donald Wyman’ leaf spot disease.【Methods】The field sampling and pathogen isolation field surveys were conducted in multiple locations across Dalian city, Liaoning Province, China, including roadsides and scenic areas with concentrated plantings of *Malus*‘Donald Wyman’. Symptomatic leaves were collected during the summer and autumn seasons of 2023 and 2024 from Tongniuling and Daheishan scenic areas in Jinpu New District, Dalian city. The collected samples were placed in sterile plastic bags and transported to the laboratory

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作者简介:郭胜豪,男,在读硕士研究生,主要从事植物病害诊断与防治方面的研究。E-mail:guoshenghao2022@163.com

***通信作者** Author for correspondence. E-mail:yangqianran136@163.com

for pathogen isolation. Leaf tissues with clear lesion boundaries were selected, and 5 mm × 5 mm sections were excised from the junction between diseased and healthy tissues. The samples were surface-sterilized by immersion in 75% ethanol for 30 seconds, followed by 3% sodium hypochlorite solution for 30 seconds, and subsequently rinsed 2-3 times with sterile distilled water. The sterilized tissues were placed into potato dextrose agar (PDA) medium and incubated at 25 ℃ in complete darkness for 5-7 days. The emerging fungal colonies were subcultured and purified through hyphal tip transfer. The purified isolates were stored at 4 ℃ for further use and were also maintained on PDA medium supplemented with streptomycin to suppress bacterial contamination. All fungal cultures were incubated at 25 ℃ in the dark until colonies were fully developed. Pathogenicity was confirmed through wound inoculation assays conducted under aseptic laboratory conditions. The purified fungal isolates were cultured on PDA plates at 26 ℃ in darkness for 7 days prior to inoculation. The healthy leaves were surface-cleaned with ultrapure water, wiped with 75% ethanol, and rinsed three times with sterile water before being dried using sterile tissue paper. Wounding was performed with sterile insect pins under a laminar flow hood. The mycelial plugs (8 mm in diameter) were excised from the colony margin using a sterile cork borer and placed on the wounded sites. The sterile PDA plugs served as negative controls. The inoculated leaves were incubated at 25 ℃ under dark conditions for 5 days. Each treatment was conducted with nine replicates, and symptom development was monitored and recorded throughout the incubation period. Preliminary identification of the isolated pathogen was conducted by observing the morphological characteristics of reproductive structures under a light microscope. For molecular identification, the genomic DNA was extracted from the purified isolates, and the internal transcribed spacer (ITS), actin (ACT), and large subunit (LSU) gene regions were amplified by polymerase chain reaction (PCR). PCR products were purified and sequenced by Shanghai Sangon Biotech Co., Ltd. The obtained sequences were analyzed and aligned using BioEdit software. A multi-locus phylogenetic tree was constructed using the maximum likelihood (ML) method implemented in RAxML software to confirm species identity. An *in vitro* mycelial growth inhibition assay was employed to evaluate the sensitivity of the isolated pathogen to various fungicides. Eleven commercial fungicides were tested, formulated as suspension concentrates, aqueous solutions, emulsifiable concentrates, wettable powders, microemulsions, and water-dispersible granules. The mycelial discs were inoculated onto PDA plates amended with the respective fungicides at designated concentrations and incubated at 25 ℃ in darkness. The colony diameters were measured after incubation to assess fungal growth inhibition.【Results】The ITS, ACT, and LSU gene fragments of strain SH0019 were amplified and sequenced, yielding fragments of 542 bp, 218 bp, and 1291 bp in length, respectively. BLAST analysis against the GenBank database revealed that the nucleotide sequence identities of the rDNA ITS, ACT, and SSU regions of strain SH0019 with *Alternaria alternata* were 99.81%, 99.04%, and 100%, respectively. A concatenated multi-locus phylogenetic tree was constructed using the maximum likelihood (ML) method implemented in RAxML, based on the GTR+ nucleotide substitution model. The phylogenetic analysis showed that strain SH0019 clustered together with *A. alternata*. Combined with the morphological characteristics and molecular data, the strain SH0019 was identified as *A. alternata*. The artificial inoculation experiments were conducted on seven test plant species. The pathogenicity determination results of this pathogen on the following seven *Malus* plants (*Malus*‘Indian Magic’, *Malus*‘Radiant’, *Malus*‘Flame’, *Malus micromalus*, *Malus halliana, Malus baccata*, *Malus mandshurica*) showed that *A. alternata* could infect five host plants. It had the strongest pathogenicity to *Malus*‘Donald Wyman’ and the weakest effect to *Malus* halliana, while it did not obviously infected *Malus baccata* and *Malus mandshurica*. Although strain the SH0019

exhibited pathogenicity across different *Malus* species, the lesion size varied among the species, with the smallest average lesion diameter (4.3 mm) observed on *Malus halliana*, indicating differences in host resistance levels. The fungicide sensitivity tests were conducted using 11 fungicides. The mycelial growth inhibition assay showed that among the single agents tested, iprodione and prochloraz exhibited the strongest inhibitory effects against the strain SH0019, with EC_{50} values of 1.735 mg·L^{-1} and 5.495 mg·L^{-1}, respectively, which were significantly lower than those of the other fungicides. In contrast, mancozeb demonstrated the weakest activity, with an EC_{50} of 222.923 mg·L^{-1}. Further screening of fungicide combinations revealed that the 50% benzyl·difenoconazole formulation exhibited the highest inhibitory activity, with an inhibition rate of 71.65% and an EC_{50} value of 0.179 mg·L^{-1}. The inhibitory effect of 50% benzyl·difenoconazole was superior to that of 43% tebuconazole and 25% difenoconazole single formulations.【Conclusion】The causal agent for the leaf spot of *Malus*‘Donald Wyman’was identified as *Alternaria alternata* based on Koch’s postulates, morphological characteristics, and multi-locus sequence analysis of ITS, ACT, and SSU genes. Among the tested fungicides, benzyl·difenoconazole demonstrated the highest antifungal activity and may serve as an effective candidate for controlling the disease although the field trials should be conducted to further testify the efficiency of the chemicals before practical application.

Key words: *Malus*‘Donald Wyman’; Leaf spot disease; Pathogen identification; Host range; Fungicide sensitivity

海棠(*Malus* spp.)为蔷薇科苹果属落叶乔木,以花朵绚丽、叶色丰富多变及果实美观的特点而闻名,是我国城市园林绿化和家庭园艺的优选植物之一[1-3]。海棠原种起源于中国,后被引入欧美地区,与当地苹果属植物杂交选育出众多观赏价值较高的海棠杂交品种[4]。北美海棠因环境适应性强,在中国各地适宜栽培,具有广阔的推广前景[5]。唐纳德海棠由于花朵和果实色彩艳丽,近年来在大连市道路两旁、公园及景区等地的种植规模不断扩大,已成为兼具观花、观叶和观果价值的优良园林树种。

观赏海棠多源于自然杂交后代,通常具有较强的抗病性[6],因此病害发生的种类相对较少且危害程度较轻。目前,关于海棠植物病害的研究报道较为有限。例如,在美国的北美海棠上已报道了锈病(*Gymnosporangium yamadae*)[7]、果实斑点病和叶枯病(*Phacidiopycnis washingtonensis*)[8];在土耳其的多花海棠上报道了枯萎病(*Erwinia amylovora*)[9]。在中国的亚斯特海棠(*Malus*‘Ester’)上报道了腐烂病(*Valsa mali*)[10];在河北省雄安新区的海棠上发现锈病(*Gymnosporangium yamadae*)[11],在怀来县的八棱海棠报道了立枯病(*Rhizoctonia solani*)[12];在泰山的湖北海棠上发现枝干溃疡病[13]。这些病害对植株造成了严重危害。

2023年,笔者在对大连地区海棠病害进行调查时发现,唐纳德海棠(*Malus*‘Donald Wyman’)叶片上普遍发生一种叶斑病,严重影响了该树的栽培管理及观赏价值。叶斑病不仅会干扰植物的叶片光合作用,严重时还可导致叶片枯死和提早脱落,给城市绿化造成严重经济损失。为了明确该病害的病原菌种类及其寄主范围,开展了病原菌鉴定、致病性测定及杀菌剂筛选工作,以期为海棠叶斑病的科学防治提供理论依据。

1 材料和方法

1.1 样品来源

分别于2023年和2024年的夏、秋两季,在大连市金普新区铜牛岭和大黑山风景区采集唐纳德海棠叶斑病病叶。样品装入无菌袋中,带回实验室用于病原菌分离。

1.2 病原菌分离纯化

采用组织分离法[14]进行叶斑病病菌分离。选取具有典型叶斑病症状的病叶,在病健交界处切取5 mm×5 mm的组织块,经75%酒精消毒30 s,3%次氯酸钠溶液消毒30 s,再用无菌水冲洗2~3次后,接种在PDA平板上,置于25 ℃培养箱中暗培养5~7 d。待长出新鲜菌落后进行转接和纯化,获得纯化菌种

后置于4 ℃冰箱保存备用。

1.3　病原菌致病性测定

1.3.1　病原菌活化培养　将保存的菌株在PDA平板上活化培养6 d，用无菌打孔器（直径8 mm）在菌落边缘打取菌苔备用。

1.3.2　离体叶片接种　将采集的唐纳德海棠植株健康新鲜叶片，在实验室用自来水冲洗干净，经75%酒精擦洗后再用无菌水冲洗3次。在超净工作台中用无菌接种针刺伤叶片，平铺在底部铺有滤纸和玻璃棒的大培养皿（直径10 cm）中，皿底加入适量无菌水以保湿。取一块制备好的菌苔，菌丝面朝下放置在经刺伤的叶片部位，以接种无菌PDA培养基作为对照，盖好皿盖后置于25 ℃恒温培养箱中培养，设置3次重复，接种后定期观察发病情况。

1.3.3　人工接种发病叶片症状观察和病菌再分离　待上述人工接种叶片出现叶斑病的典型症状后，对发病叶片进行症状观察和病原菌再分离，并根据形态特征进行病菌鉴定。观察病原菌的菌落形态、菌丝颜色、菌丝致密性等培养特性和分生孢子、孢子囊、产孢结构等孢子结构特征，以确定是否与上面人工接种病菌为同一种真菌。

1.4　病原菌鉴定

1.4.1　形态特征观察　将供试纯化菌株接种于PDA培养基上，于25 ℃恒温培养箱中暗培养7 d，观察菌落特征。在光学显微镜下观察病原菌的子实体形态特征，参考《中国真菌志·第十六卷 链格孢属》[15]的分类进行病原菌形态鉴定。

1.4.2　分子生物学鉴定　将供试菌株接种在铺有灭菌玻璃纸的PDA平板上，于25 ℃恒温培养箱中暗培养7 d，收集菌丝体。采用Ezup柱式真菌基因组DNA抽提试剂盒（上海生工）提取菌株的基因组DNA，通过1.5%琼脂糖凝胶电泳检测基因组DNA提取质量。分别用引物ITS1（5'-TCCGTAGGTGAACCTGCGG- 3'）/ITS4（5'- TCCGTAGGTGAACCTGCGG- 3'）、ACT- 512F（5'- ATGTGCAAGGCCGGT TTCGC- 3'）/ACT- 783R（5'- TACGAGTCCTTCTGGCCCAT- 3'）、NS1（5'- GTAGTCATATGCTTGTCTC- 3'）/NS6（5'- GCATCACAGACCTGTTATTGCCTC-3'）对病原菌的ITS[16]、ACT[17]及LSU[18]基因进行PCR扩增，扩增产物经1.0%琼脂糖凝胶电泳检测正确后，送至上海生工生物工程有限公司进行纯化和测序。将测序结果提交至NCBI网站（https://www.ncbi.nlm.nih.gov）GenBank数据库，获取登录号，然后进行BLAST同源性比对，删去低质量的比对区域。使用RAXML软件，采用ML法构建多基因串联系统发育树。使用Phylosuite V1.2.2软件对组合的ITS、ACT和SSU基因序列首尾串联，使用RAXML软件构建多基因串联系统发育树。

1.5　不同海棠品种致病性测定

1.5.1　供试寄主　在田间选取与唐纳德海棠同属北美海棠系列的5个常见品种：火焰海棠（*Malus* ‘Flame’）、印第安魔幻海棠（*Malus* ‘Indian Magic’）、垂丝海棠（*Malus halliana*）、西府海棠（*Malus micromalus*）及绚丽海棠（*Malus* ‘Radiant’）。另外，选取2种常用作海棠嫁接砧木的山荆子（*Malus baccata*）和毛荆子（*Malus baccata* var. *mandshurica*），并对7种亲缘关系相近的植物进行致病性测定。

1.5.2　致病性测定　采用菌饼刺伤接种方法对供试寄主进行致病性测定（方法同1.3）。选取健康的供试海棠叶片，将叶片表面清洗干净后，以接种无菌PDA培养基的植株作为对照。每个处理设置3次重复，接种后定期观察并记录各个品种海棠的症状发展情况。同时，再次分离病原物，该分离操作重复3次。对再次分离获得的菌株，通过形态学特征和分子生物学方法进行对比，验证其与原始菌株的一致性，从而确定原分离菌的致病性。

1.6　杀菌剂药效测定

1.6.1　供试杀菌剂　供试杀菌剂共11种，选取悬浮剂、水剂、乳油、可湿性粉剂、水乳剂、水分散粒剂共6种剂型（表1）。

1.6.2　杀菌剂药效测定　采用菌丝生长速率法[19]进行杀菌剂抑菌试验。供试药剂为复配型药剂和广谱性的杀菌剂单剂，参考每种杀菌剂使用说明书中的使用浓度，配制相应浓度的杀菌剂PDA培养基，具体杀菌剂浓度如表1。在PDA平板中央接种1片菌饼，以不含杀菌剂的PDA平板作为对照，设置3次重复，然后置于25 ℃恒温培养箱中暗培养7 d，采用十字交叉法测量菌落直径，根据下列公式计算和统计相对抑菌率。使用SPSS 22.0软件拟合毒力回归方程，并计算EC_{50}值。

$$I/\% = \frac{Dc - Dt}{Dc - 8\ \text{mm}} \times 100。$$

式中：Dc为不含杀菌剂平板菌落直径；Dt为含杀菌剂平板菌落直径；菌饼直径8 mm。

表1 11种杀菌剂对应信息

Table 1 Information on 11 fungicides

序号 Index	药剂 Fungicide	浓度 Concentration/%	剂型 Formulation	来源 Source	质量浓度梯度 Concentration gradient/（μg·mL^{-1}）
1	腈菌唑 Myclobutanil	40	悬浮剂 Suspension Concentrate (SC)	江西禾益化工股份有限公司 Jiangxi Heyi Chemical Co., Ltd.	4、2、1、0.5、0.25
2	异菌脲 Iprodione	50	悬浮剂 Suspension Concentrate (SC)	苏州富美实植物保护剂有限公司 Suzhou FMC Plant Protection Co., Ltd.	1、0.5、0.1、0.05、0.025
3	噁霉灵 Mefenoxam	30	水剂 Aqueous Solution (AS)	山东邹平农药有限公司 Shandong Zouping Pesticide Co., Ltd.	1、0.5、0.1、0.05、0.025
4	丙环唑 Propiconazole	25	乳油 Emulsifiable Concentrate (EC)	先正达作物保护有限公司 Syngenta Crop Protection Co., Ltd.	1、0.5、0.1、0.05、0.025
5	代森锰锌 Mancozeb	80	可湿性粉剂 Wettable Powder (WP)	利民化学有限公司 Limin Chemical Co., Ltd.	2、1、0.5、0.25、0.125
6	多菌灵 Carbendazim	50	可湿性粉剂 Wettable Powder (WP)	四川润尔有限公司 Sichuan Runer Co., Ltd.	0.5、0.25、0.125、0.062 5、0.031 25
7	戊唑醇 Tebuconazole	43	悬浮剂 Suspension Concentrate (SC)	拜尔股份公司 Bayer AG	1、0.5、0.1、0.05、0.025
8	咪鲜胺 Prochloraz	45	水乳剂 Emulsion in Water (EW)	苏州富美实植物保护剂有限公司 Suzhou FMC Plant Protection Co., Ltd.	1、0.5、0.1、0.05、0.025
9	肟菌•戊唑醇 Trifloxystrobin• Tebuconazole	75	水分散粒剂 Water Dispersible Granule (WDG)	拜尔股份公司 Bayer AG	4、2、1、0.5、0.25
10	苯甲•丙环唑 Propiconazole• Difenoconazole	50	水乳剂 Emulsion in Water (EW)	江西巴菲特化工有限公司 Jiangxi Buffett Chemical Co., Ltd.	2、1、0.5、0.25、0.125
11	氟菌•肟菌酯 Fluopyram• Trifloxystrobin	43	悬浮剂 Suspension Concentrate (SC)	拜尔股份公司 Bayer AG	2、1、0.5、0.25、0.125

Y=a+bX。

式中：Y为抑制机率值；X为药剂浓度对数。

1.7 数据分析

利用SPSS 22. 0软件对数据进行统计分析，采用Origin pro 2024软件制图，通过Duncan多重比较法进行差异显著性检验（$P<0.05$）[20]。

2 结果与分析

2.1 叶斑病症状

田间症状：唐纳德海棠叶斑病多于7—10月发生，主要侵染叶片。发病初期病斑呈点状退绿斑，后逐渐扩大，呈近圆形或椭圆形，边缘呈淡黄绿色，中央呈棕褐色，后期呈灰白色，直径为0.6~1.2 cm（图1）。在湿度较高的条件下，病斑背面可以观察到黑色霉层。发病严重时，病斑可相互融合，导致叶片枯萎并提早脱落。

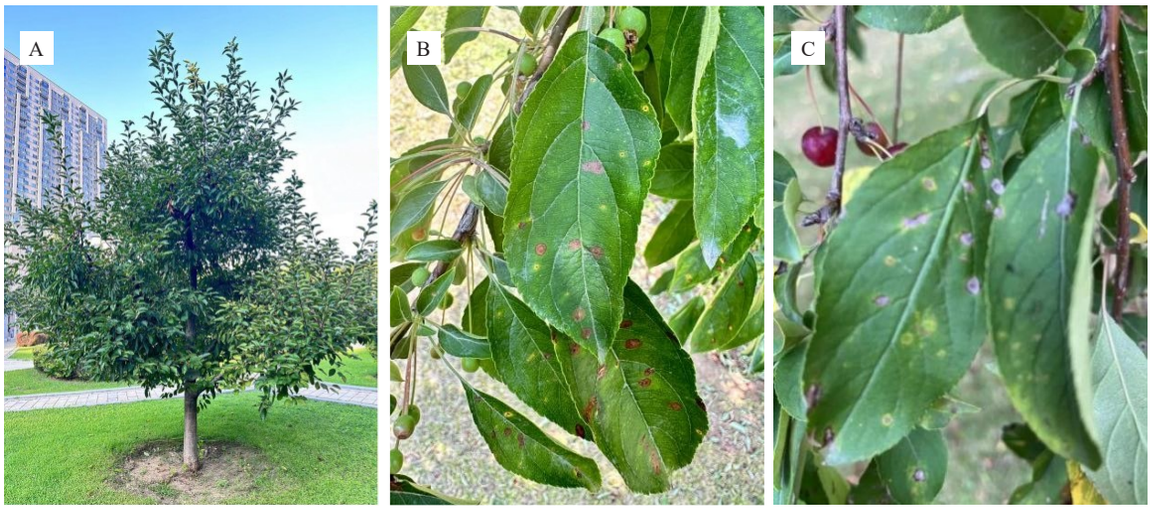
2.2 病原菌的致病性

通过人工接种试验发现（图2），菌株SH-0019可侵染唐纳德海棠叶片，人工诱发症状（图2-B~C）与自然发病症状（图1-B）一致，病斑逐渐变为深褐色（图2-D~E）。从接种发病的叶片中重新分离病原物，再次获得与原接种菌一致的菌株。上述结果符合柯赫氏法则，证实*A. alternata*是引起唐纳德海棠叶斑病的病原菌，后续试验均以菌株SH-0019为代表性菌株。

2.3 病原菌鉴定

2.3.1 *形态特征* 该病菌在PDA培养基上于25 ℃条件下生长较快，培养7 d后菌落直径可达8 cm。初期菌落呈白色，逐渐转为灰白色或灰褐色，絮状，背面深褐色或暗褐色（图3-A）；菌丝无色，具隔膜；分生孢子梗发达，多弯曲，暗褐色，顶端具1~3个产孢痕；产孢方式为孔出式；分生孢子倒梨形、椭圆形或卵圆形，褐色，具3~7个横膈膜，0~4个纵斜隔膜，孢子大小（26.5~38.0）μm×（6.5~10.3）μm；顶端细胞较细并具喙，喙部大小（7.2~19.5）μm×（2.5~4.2）μm（图3-B）。根据菌落形态及分生孢子的特征，将菌株鉴定为链格孢（*Alternaria alternata*）。

2.3.2 *分子生物学鉴定* 通过对致病菌株SH0019的ITS、ACT及LSU基因片段序列进行测序，经鉴定长度分别为542 bp、218 bp及1291 bp，并在GenBank

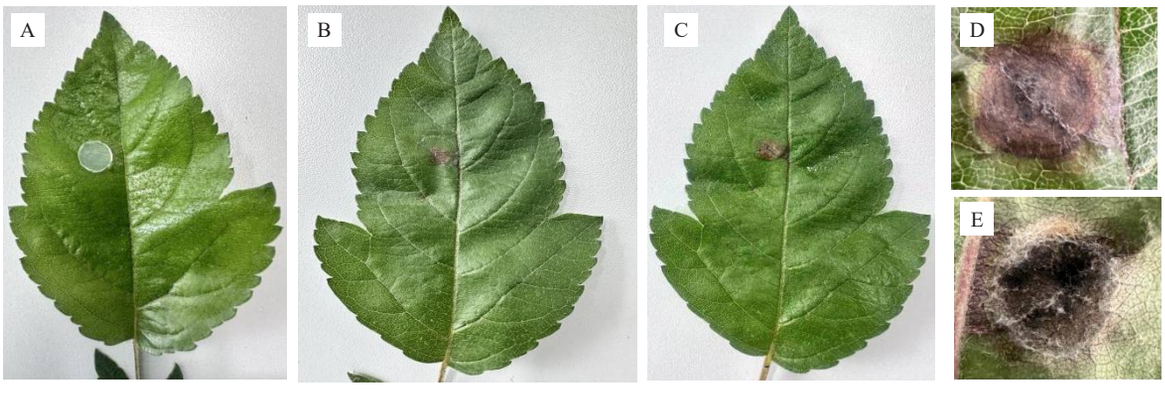


A. 发病植株；B. 发病初期点状退绿斑；C. 中后期灰白干枯叶片。

A. Infected plants in the field; B. Initial chlorotic spots at the early stage of infection; C. Grayish-white, withered leaves at later stages.

图 1　唐纳德海棠叶斑病症状

Fig. 1　Symptoms of leaf spot of *Malus* ‘Donald Wyman’



A. 接种无菌 PDA 培养基的对照组；B. 接种第 4 天的照片；C. 接种第 7 天的照片；D~E. 最终发病状况。

A. Control leaves inoculated with sterile PDA; B. Photos taken on the fourth day of vaccination; C. Photo taken on the 7th day after vaccination; D-E. The final disease status.

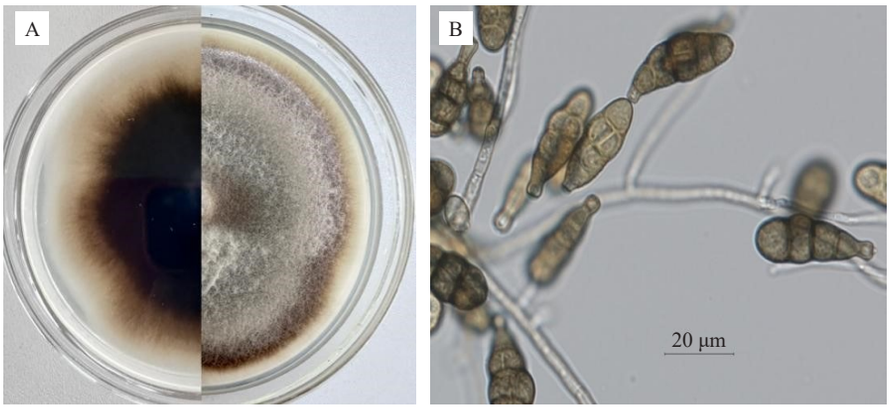
图 2　唐纳德海棠人工接种病菌产生的症状观察

Fig. 2　Pathogenicity test results of *Malus* ‘Donald Wyman’

中的登录号分别为 PV523935（ITS）、PV768877（ACT）及 PQ809778（SSU）。通过 BLAST 同源比对，发现菌株 SH0019 的 rDNA ITS、ACT 及 SSU 序列与 *A. alternata* TJU_JUL50（OM236783；535/536 bp）、*A. alternata*（OK664978；207/209 bp 及 *A. alternata* CRB-3（OM630609.；1291/1291 bp）序列的一致性分别为 99.81%、99.04% 及 100%。选取与目标菌株序列相似度较高的模式菌株及已报道的菌株作为参考序列（表 2），使用 RAXML 并采用 GTR+ 的核苷酸替代模型构建多基因串联系统发育树，自举检验（bootstrapping）重复 1000 次获得各分支支持率。菌株 SH0019 与 GenBank 中 *A. alternata* 聚在一支，结合上述形态学特征与分子生物学特征综合评估，将病原菌 SH0019 鉴定为链格孢菌 *A. alternata*（图 4）。

2.4　不同海棠品种致病性测定

7 种供试海棠的人工接种试验结果表明，菌株 SH-0019 能够侵染火焰海棠、印第安魔幻海棠、绚丽海棠、垂丝海棠及西府海棠，且引发的叶斑症状与在唐纳德海棠上观察的症状一致，而对山荆子和毛荆子无侵染能力（图 5）。尽管菌株 SH-0019 对以上 5 个海棠品种均具有致病性，但其致病性程度存在差



A. 在 PDA 上菌落形态的正反面（7 d）；B. 分生孢子（比例尺=20 μm）。

A. The front and back of the colony morphology（7 d）; B. Conidia bar (scale=20 μm).

图 3 *Alternaria alternata* 的形态特征

Fig. 3 Morphological characteristics of *Alternaria alternata*

表 2 构建系统发育树所用菌株的 GenBank 登录号

Table 2 The GenBank accession numbers of the strains used for the construction of phylogenetic tree

菌株 Species	登录号 Accession numbers	ITS	ACT	SSU
莲子草链格孢 *Alternaria alternantherae*	CBS 124392	KC584179.1	OR797155.1	KC584506.1
链格孢 *A. alternata*	TJU_JUL50	OM236783.1	OK664978.1	OM630609.1
巴恩斯链格孢 *A. burnsii*	CBS 107.38	KP124420.1	JQ671685.1	KP125043.1
A. jacinthicola	CPC 25267	KP124439.1	HQ413699.1	KP125063.1
番茄链格孢 *A. tomato*	CBS 114.35	KP124446.1	JQ671686.1	KP125070.1
茄链格孢 *A. solani*	CBS 116651	KC584217.1	MN813551.1	KC584562.1
A. carotiincultae	CBS 109381	KC584188.1	EU141969.1	KC584518.1
葡萄孢链格孢 *A. botrytis*	CBS 197.67	KC584243.1	LC481887.1	KC584609.1
纸链格孢 *A. chartarum*	CBS 200.67	AY625071.1	LC481879.1	KC584614.1
A. consortialis	CBS 104.31	KC584247.1	PQ468098.1	KC584615.1
A. atra	AC88	LC440623.1	LC481885.1	KC584608.1
A. radicina	HB1	FJ958190.1	EU141971.1	KC584555.1
细极链格孢 *A. tenuissima*	—	HQ402558.1	ON996402.1	KC584567.1
A. petroselini	Ap95	EU807868.1	AB744037.1	KM102529.1
A. cumini	CBS 121329	KC584191.1	LC481873.1	KC584523.1
人参链格孢 *A. panax*	CBS 482.81	KC584209.1	MK451990.1	KC584549.1
葱链格孢 A. porri	CBS 116698	JF422724.1	JQ671726.1	KC584553.1
A. selini	EGS 25-198	AF229455.1	JQ671676.1	AF229515.1

异，其中垂丝海棠叶片产生的病斑扩展直径最小，平均直径为4.3 mm，表明不同海棠品种对该病原菌的抗性存在明显差异。

2.5 杀菌剂筛选

11种供试杀菌剂的抑菌试验结果（表3）表明，各药剂对病原菌的抑菌效果存在明显差异。毒力回归方程分析显示，50%苯甲•丙环唑抑菌效果最好，EC_{50}值为0.179 μg·mL^{-1}；其次为50%异菌脲和45%咪鲜胺，EC_{50}值分别为1.735 μg·mL^{-1}、5.495 μg·mL^{-1}；而80%代森锰锌对病菌的抑菌效果最弱，EC_{50}值高达222.923 μg·mL^{-1}。

3 讨 论

本研究通过形态学、多基因系统发育分析及致病性测定，明确引起大连地区唐纳德海棠叶斑病的病原菌为链格孢（*A. alternata*）。离体接种试验表明，该病原菌可侵染多个北美海棠品种，但对山荆子和毛山荆子无侵染能力。链格孢（*A. alternata*）寄主

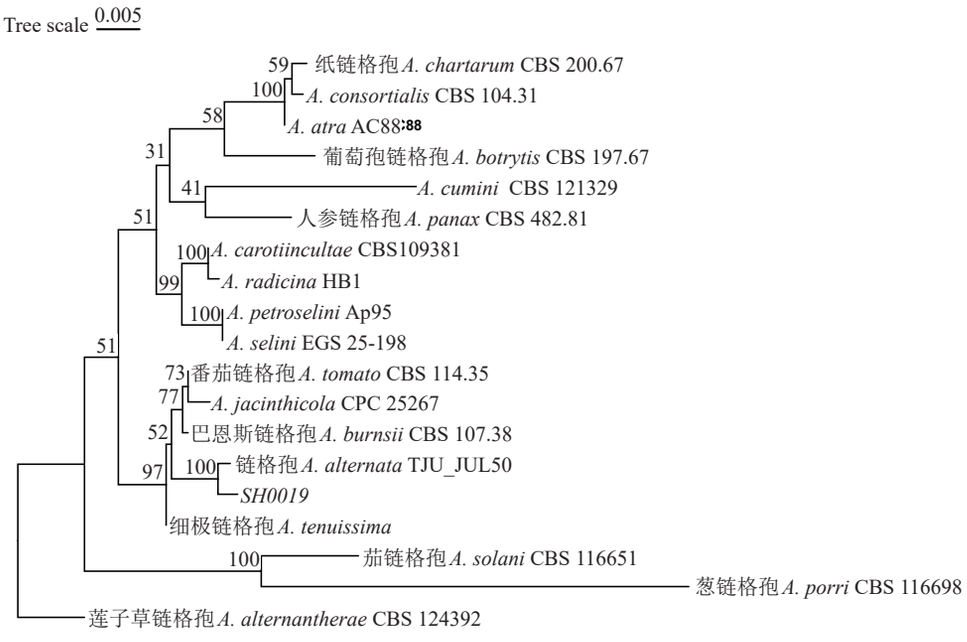
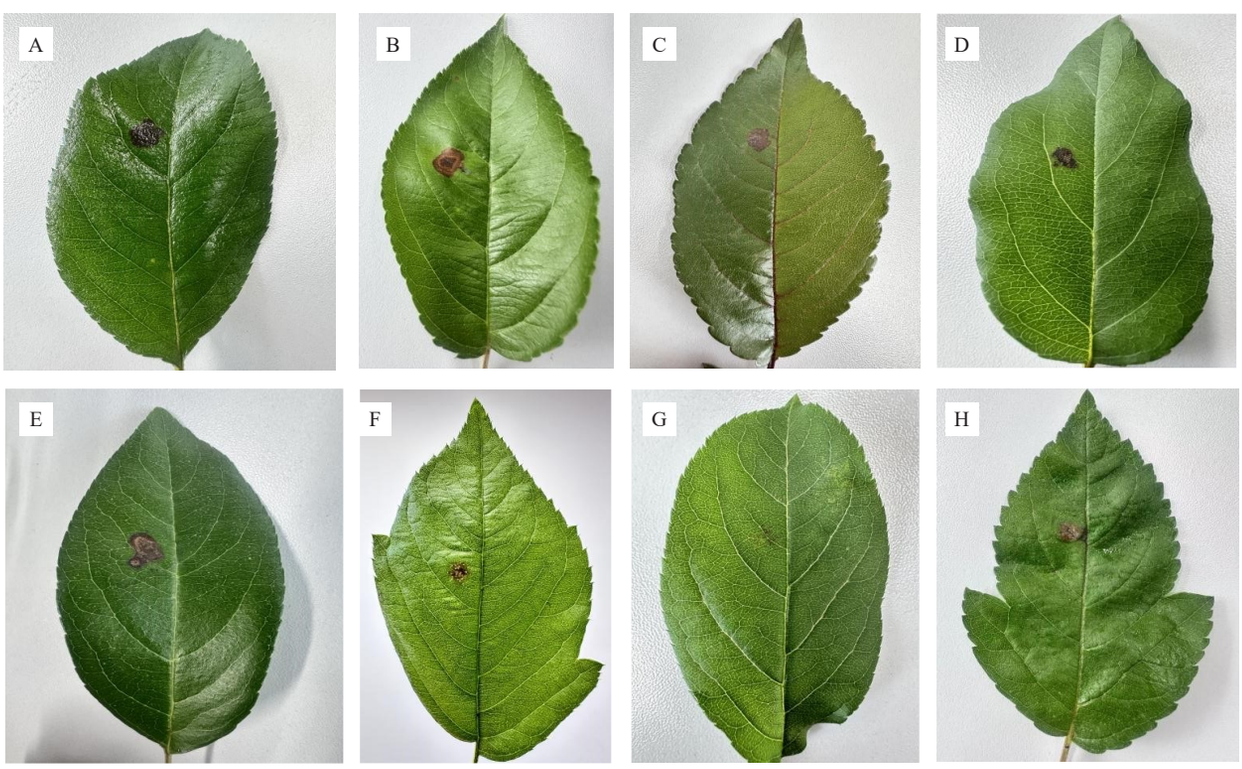


图 4　基于分离菌 SH0019 的 ITS、ACT 和 LSU 序列使用 ML 法并采用 GTR+的核苷酸替代模型构建多基因串联系统发育树

Fig. 4　Phylogenetic tree constructed based on multi-gene *ITS-ACT-LSU* sequences using Maximum Likelihood method and GTR+ nucleotide substitution model of isolate SH0019



A. 火焰海棠；B. 印第安魔幻海棠；C. 绚丽海棠；D. 垂丝海棠；E. 西府海棠；F. 山荆子；G. 毛荆子；H. 唐纳德海棠（对照）。

A. Flame; B. IndianMagic; C. Radiant; D. *Malus halliana*; E. *Malus*×*micromalus*; F. *Malus baccata*; G. *Malus baccata* var. *mandshurica*; H. Donald Wyman (Control).

图 5　不同海棠品种人工接种发病情况

Fig. 5　Determination of host range of strains SH0019

表3 11种杀菌剂对菌株SH0019的毒力测定

Table 3 Toxicity of 11 fungicides fungicides to strain SH0019 (EC_{50})

供试药剂 Microbicide	毒力回归方程 Toxicityreg ression equation	致死中浓度 Median lethal concentration, EC_{50}/(μg·mL^{-1})	相关系数 Correlation coefficient, *r*	95%置信区间 95% confidence interval
40%腈菌唑 40% Myclobutanil	$Y = 1.448X + 5.282$	181.432	0.929	128.634~221.214
50%异菌脲 50% Iprodione	$Y = 1.309X + 8.674$	1.735	0.928	0.926~2.357
30%噁霉灵 30% Mefenoxam	$Y = 8.403X + 0.957$	129.453	0.965	99.276~135.287
25%丙环唑 25%Propiconazole	$Y = 0.131X + 0.475$	17.704	0.931	16.914~19.652
80%代森锰锌 80% Mancozeb	$Y = 1.658X + 3.894$	222.923	0.983	216.382 ~234.935
50%多菌灵 50% Carbendazim	$Y = 0.763X + 9.174$	25.963	0.923	21.636~30.168
43%戊唑醇 43% Tebuconazole	$Y = 1.231X + 4.450$	88.356	0.964	84.327~102.910
45%咪鲜胺 45% Prochloraz	$Y = 6.879X + 2.629$	5.495	0.912	3.153~8.504
75%肟菌•戊唑醇 75%Trifloxystrobin·Tebuconazole	$Y = 0.784X + 4.378$	46.667	0.967	43.969~69.589
50%苯甲•丙环唑 50%Propiconazole·Difenoconazole	$Y = 1.068X + 0.299$	0.179	0.978	0.108~2.932
43%氟菌•肟菌酯 43%Fluopyram·Trifloxystrobin	$Y = 2.891X + 8.192$	10.852	0.918	8.434~14.695

范围广泛，可侵染多种作物、药用及观赏植物，造成严重经济损失[21-22]。在之前的报道中，该病菌可侵染长柄水青冈[23]、草坪[24]、龙牙百合[25]、库尔勒香梨[26]、柑橘[27]、鸢尾[28]、猕猴桃[29]、芍药[30]等多种类型寄主植物，引起黑腐病、褐斑病、软腐病、轮纹病、果枯病等病害。本研究也证实其可危害多个北美海棠品种，进一步表明其寄主适应范围较为广泛，跨寄主传播风险值得警惕。在本研究中，*A. alternata* 对山荆子和毛荆子无明显侵染迹象，推测可能与砧木野生种的抗病遗传背景有关。罗红丽[31]曾指出，不同 *A. alternata* 菌株因产毒能力与致病因子存在差异，可表现出较明显的寄主专化性。这一发现为利用抗性砧木选育抗病海棠品种提供了依据，这与其他植物-病原互作过程中基于遗传抗性的防控策略相吻合[32]。此外，本研究还发现不同海棠品种对叶斑病的感病性存在明显差异，其中垂丝海棠病斑扩展范围最小，表明栽培品种间存在抗性遗传多样性[33]。本研究结果表明山荆子和毛荆子可作为抗病砧木材料，用于选育兼具优良观赏性和抗病性的海棠新品种，从而降低对化学杀菌剂的使用依赖程度，促进海棠病害的绿色防控。

化学药剂筛选结果表明，50%苯甲•丙环唑、50%异菌脲和45%咪鲜胺对海棠叶斑病菌株SH0019表现出较强的抑制活性，具有良好的应用潜力。李有德等[34]在苹果链格孢病害防治以及苏秀敏等[35]在番茄早疫病防治中均发现苯醚甲环唑类药剂对链格孢菌具有明显的抑菌效果，进一步支持了本研究中对苯甲•丙环唑抑菌效果的认定。此外，王媛媛等[36]和马伟丽等[37]均报道了异菌脲和咪鲜胺对玉米链格孢叶斑病具有较高毒力，表明这两种药剂对不同作物上的链格孢病害防治具有一定广谱性。综上所述，本研究筛选出的3种化学药剂对唐纳德海棠叶斑病的防治效果与已有研究相互印证，为北美海棠叶斑病的科学防控提供了可靠的药剂选择与理论依据。

尽管本研究明确了唐纳德海棠叶斑病病原种类并筛选出了有效药剂，但仍存在一定局限性。所有药剂试验均在室内条件下完成，尚未开展田间试验验证，其实际防效及潜在环境影响仍需进一步评估。未来将结合转录组学等分子手段进一步明确该病原菌的侵染机制，以期揭示致病与抗病机制，推动本研究成果向实际应用的转化。

4　结　论

本研究明确了大连地区唐纳德海棠叶斑病的病原菌为*Alternaria alternata*。室内杀菌剂筛选结果表明，50%苯甲•丙环唑对病原菌的抑菌效果最佳，具有良好的应用潜力。本研究为该病害的准确诊断和化学防治提供了科学理论依据，所筛选的高效杀菌剂为田间防治实践提供了候选药剂，对保障唐纳德海棠等部分北美海棠品种的观赏价值与生态效益具有重要意义。

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