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导致南方梨早期落叶的果生炭疽菌致病力分化分析

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摘 要:【目的】明确我国南方梨产区造成早期落叶的果生炭疽菌(*Colletotrichum fructicola*)的致病力分化状况并建立其室内快速测定方法。**【方法】**以梨的枝条、叶片和果实为材料,采用不同方法造成伤口后接种果生炭疽菌的强致病力菌株PAFQ32,通过比较各处理的测定效果筛选其室内快速测定方法,并对供试菌株的致病力进行观测和致病类型划分,分析不同菌株致病力分化与其地理来源之间的相关性。**【结果】**采用梨枝条、叶片和果实对果生炭疽菌致病力的观测结果显示,梨叶片经针刺后接种菌丝块的测定效果明显优于其他处理。供试菌株致病力的测定结果表明,来源于我国南方梨产区的111个果生炭疽菌菌株其致病力可划分为强、中、弱3个类型,其中强致病力菌株17个(15.3%);中等致病力菌株89个(80.2%);弱致病力菌株5个(4.5%)。不同地理来源的果生炭疽菌菌株,其致病类型的分布比例有异。**【结论】**果生炭疽菌的菌丝块针刺接种梨叶片的方法,可用于其致病力的室内快速测定。来源于我国南方梨产区导致早期落叶的果生炭疽菌存在明显的致病力分化,以中等致病力菌株为优势群体。

关键词:梨;果生炭疽菌;菌株;室内测定方法;致病力分化

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Pathogenicity differentiation of *Colletotrichum fructicola* causing precocious defoliation in pear in southern China

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Abstract:【Objective】In recent years, precocious defoliation of pear, mainly caused by *Colletotrichum fruticola* in southern China has become serious and resulted in large losses of fruit production in these areas. The aim of this study is to determine the pathogenicity differentiation of *C. fruticola* and establish the laboratory methods determining virulence of this pathogen.【Methods】The strain PAFQ32 of *C. fruticola*, which has strong virulence, was isolated from diseased leaves of *Pryus pyrifolia* cv. Cuiguan in Fujian. Mycelial discs (5 mm in diameter) were taken from the colony margins of 5-d-old cultures on PDA medium, and fresh PDA discs were used as controls. Isolates were cultured on SNA (synthetic nutrient-poor agar) medium for 3 days at 28 ℃. Conidia were harvested and put into a 2.0 mL sterilized Eppendorf tube, and the conidial suspension was diluted with sterile water to a final concentration of 1×10^6 conidia per mL. The strong pathogenic strain PAFQ32 of *C. fructicola* was inoculated from the twigs (*P. pyrifolia* 'Cuiguan'), leaves (*P. pyrifolia* 'Cuiguan'), and fruit (*P. bretschneideri* 'Huangguan') wounded with various methods. Inoculation of detached twigs was performed by using 1-year-old twigs (10.0 cm in length) with mycelial plugs on wounds and incubated at 25 ℃ in plastic contain-

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ers covered with plastic film. The lesion lengths were recorded at 9 dpi. Inoculation of detached mature pear fruit was performed by placing mycelium plugs or dropping an aliquot of 6.0 μL conidial suspension (10^6 conidia per mL) on wounds. Fresh PDA discs (5 mm in diameter) and sterile water were used as the controls, respectively. The lesion lengths were recorded at 7 dpi. Inoculation of detached leaves was performed by placing mycelium plugs or dropping a 6.0 μL aliquot of conidial suspension on the left side of a leaf after wounding. The lesion lengths were recorded at 4 dpi. The results of each treatment were compared, and then chose a laboratory method to determine the pathogenicity of 111 strains of *C. fruticola* that were isolated from the leaves and fruits of *P. pyrifolia* and *P. bretschneideri* in 7 orchards in Anhui, Fujian, Guangxi, Hubei, Jiangsu, Jiangxi, and Zhejiang. The pathogenicity of strains from different sources were observed and divided into different pathogenic types, and the relationship between the pathogenicity differentiation of the strains and their sources were analyzed.【Results】Laboratory determination of virulence showed that, on inoculated twigs, the incidence rate was 100% by scald holing and holing, 80% by girdling, 40% by puncturing with 10 needles, and 20% by puncturing with 3 needles after three days of inoculation. The average diameters of lesions treated with scald holing were significantly bigger than the latter four wounding treatments, but it varied greatly. On inoculated fruit, the average diameters of lesions produced by inoculating with mycelium plugs were larger than by inoculating with conidial suspensions under the same wound treatment, and there was no significant difference among the average diameters of lesions produced by inoculating mycelial plugs with different wounding treatments. On inoculated leaves, the average diameters of lesions produced by puncturing with 3 needles were significantly larger than with 1 needle on the right and the back side of the leaves. Under the same wound treatment, the expansion of the lesions produced by inoculating with mycelial plugs was faster than that by inoculating with conidial suspensions. The results showed that the method of inoculating with mycelial plugs on punctured pear leaves had good material uniformity and significant effect, and took less time, and thus was better than the other inoculation treatments. The pathogenicity of 111 strains were determined by this treatment. The results showed that there were significant differences in pathogenicity among *C. fructicola* strains from different provinces in southern China. According to cluster analysis, the strains could be divided into three types including 17 strains with strong pathogenicity (15.3%), 89 strains with middle pathogenicity (80.2%) and 5 strains with weak pathogenicity (4.5%). There was a significant difference in the proportions of the pathogenic types of the strains from different areas. This difference was related to climate differences.【Conclusion】It was suggested that rapid laboratory determining the virulence of *C. fruticola* could be carried out by inoculating with mycelial plugs on leaves wounded by puncturing. There was significant pathogenicity differentiation in *C. fructicola* causing leaf early defoliation in pear in southern China, and the strains with middle pathogenicity were the dominant group.

Key words：*Pyrus; Colletotrichum fructicola*; Strain; Laboratory determination; Pathogenicity differentiation

近些年来，在我国南方梨产区包括福建、浙江、湖北、安徽、江西、江苏、云南、四川、重庆、贵州、云南、广西等省份，6月至8月梨树生长期间发生大量异常落叶，导致梨果产量锐减，严重时当年秋末冬初出现返青与二次开花，因无效消耗树体营养而影响翌年的开花结果，造成巨大的经济损失[1-5]。张鹏飞等[6]、Fu等[7]研究证实，我国南方梨产区发生的早期落叶主要是由果生炭疽菌（*Colletotrichum fructicola*）侵染所致，且病原研究结果显示果生炭疽菌为我国南方梨产区炭疽病的优势种。目前，已有研究

证实，果生炭疽菌的地理分布广泛，可侵染17个属的植物[8-13]。

在泰国咖啡果实上首次发现果生炭疽菌[14]，之后陆续报道该病菌还可侵染苹果、梨、杧果、柑橘和葡萄，引起叶片黑点和果实腐烂[6,11-12,15-16]。我国首次发现该病菌为苹果炭疽叶枯病的病原菌[17]，李河等[18]报道该病菌为油茶炭疽病的致病菌，Diao等[19]研究证实该病菌是我国辣椒炭疽病病原菌的优势种。我国梨产区的地理跨度大，各地的气候条件差异明显，且各地区栽培的梨品种不同。因此，进一步系统研究梨果生炭疽菌的致病性及遗传多样性、致病机制及其与寄主的分子互作机制，对于制定梨炭疽病有效的防治对策具有重要意义。本研究以梨叶片、枝条和果实为材料，采用不同方法造成伤口后接种梨果生炭疽菌的强致病力菌株，通过比较不同处理的测定效果筛选其致病力室内快速测定方法，并对来源于不同梨产区的果生炭疽菌进行致病力分化分析，可为了解梨果生炭疽菌的遗传多样性和致病性提供新的信息，并为梨资源抗性评价及防治药剂筛选奠定技术基础。

1 材料和方法

1.1 供试菌株和寄主材料

室内测定方法研究所用菌株PAFQ32为本实验室从福建地区‘翠冠’梨叶片中分离获得的具有强致病力的菌株[7]。致病力分化分析所用菌株为本实验室保存的从我国湖北、江西、福建、江苏、浙江、安徽和广西7个省份梨产区砂梨（*Pyrus pyrifolia*）和白梨（*P. bretschneideri*）叶片和果实中分离获得的111个纯化果生炭疽菌株。

致病力分化分析的供试寄主材料为2019年4月到6月从湖北砂梨种质资源圃采集的‘翠冠’梨完全展开、叶龄一致的健康叶片。测定方法研究的供试寄主材料为‘翠冠’梨叶片和1年生枝条及‘黄冠’果实。

1.2 接种体的制备

1.2.1 菌丝块的制备 梨果生炭疽菌PAFQ32在PDA（potato dextrose agar）培养基上培养5 d后，用直径为5 mm的打孔器在菌落边缘打取菌丝块作为接种体。

1.2.2 分生孢子悬浮液的制备 使用SNA（synthetic nutrient-poor agar）培养基诱导梨果生炭疽菌产生分生孢子。挑取供试菌株的少量新鲜分生孢子置于灭菌的2.0 mL离心管中，加入适量无菌水，使用血球计数板将其配置成1×10^6个$\cdot mL^{-1}$的分生孢子悬浮液。

1.3 接种方法

1.3.1 叶片有伤接种 叶片经无菌水洗净后晾干，叶柄处用灭菌的湿润脱脂棉包裹保湿。伤口处理设有：正反面无伤，1、3、6和10针刺伤。菌丝块接种于叶片伤口处，以空白PDA培养基作为对照。吸取6 μL的分生孢子悬浮液接种至伤口处，对照接种等量无菌水。滴有无菌水的灭菌纱布铺于盘底，保鲜膜封口以保湿，将接种后的叶片置于白塑料盘（长39 cm、宽27 cm、高3 cm）中，在25 ℃条件下培养4 d后，用十字交叉法测量病斑直径。每一接种处理设10次重复，试验设2次重复。

1.3.2 果实有伤接种 果实经无菌水洗净，75%（φ，后同）乙醇消毒后晾干，伤口处理设有无伤、1针、5针和10针刺伤。菌株、接种方法、对照设置、病斑测定方法同上，接种处理设3次重复，试验设2次重复，25 ℃条件下培养7 d后测定病斑直径。

1.3.3 枝条有伤接种 枝条经无菌水洗净晾干，75%乙醇消毒后将其剪成10 cm小段，两端用石蜡封口。伤口处理设有：无伤、3和10针刺伤（用灭菌的解剖针刺伤树皮，针点均匀分布在直径5 mm的圆形区域）、烫打（用烧热的直径5 mm打孔器取下树皮韧皮部）、打孔（用灭菌的直径5 mm打孔器取下树皮韧皮部）、环割（用灭菌的解剖刀环割枝条1周）。菌株、接种方法、对照设置、病斑测定方法同上，25 ℃条件下培养9 d后测定其病斑的长度。每个接种处理设5次重复。

1.4 致病力评价与分化分析

利用筛选出的室内快速测定方法，对不同来源菌株的致病力进行观测和比较。每一接种处理设16次重复，试验设2次重复。采用SAS 9.4软件的中间距离法（Median）进行聚类分析，并依据聚类结果划分致病类型，分析果生炭疽菌的致病力分化与其菌株来源之间的相关性。

1.5 数据统计分析

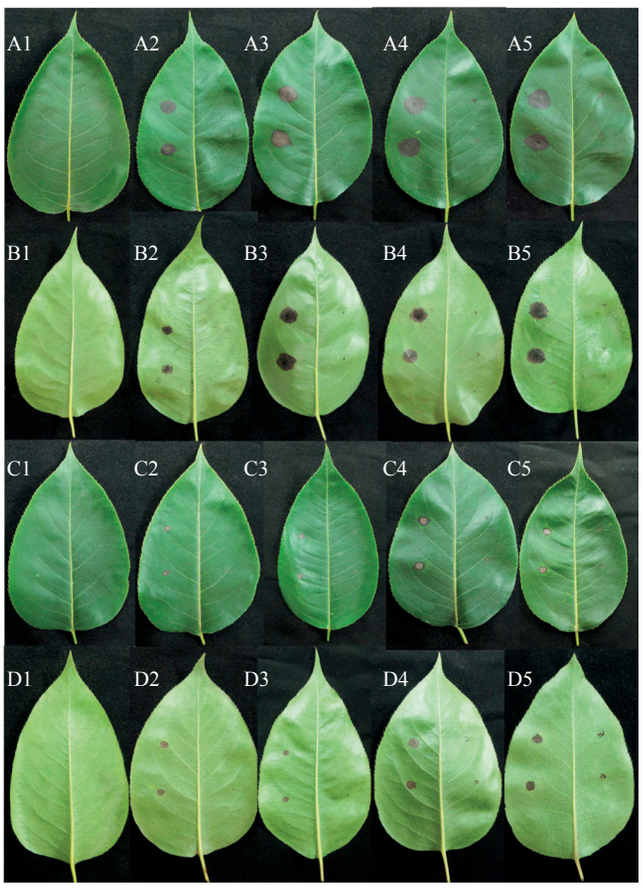
采用IBM SPSS statistics 21.0软件的新复极差法（Duncan's multiple range test）和均值比较的方法（$\alpha \leqslant 0.05$）对实验数据进行相应统计和差异性显著分析，试验结果为平均值（mean）±标准差（*SD*）表示。

2　结果与分析

2.1　室内快速测定致病力的方法

2.1.1　叶片有伤接种的测定结果　叶片上接种菌株PAFQ32菌丝块的结果表明：无伤口接种PAFQ32和空白培养基的叶片均未发病；有伤口叶片接种PAFQ32，发病率均为100%，接种1 d后针孔处均变褐色，随后病斑逐渐变黑，形成近圆形黑色坏死斑（图1），接种4 d后所有伤口处理产生的病斑均超出菌丝块，有伤口接种空白培养基4 d时，经正面和反面1针、3针针刺接种的叶片不发病，而经正面和反面6针、10针针刺接种的叶片部分轻微发病。叶片上接种菌株PAFQ32分生孢子悬浮液的结果表明：叶片上无伤口接种PAFQ32分生孢子悬浮液和无菌水接种均未发病，而经正面和反面10针针刺接种的叶片部分轻微发病；有伤口接种4 d时的发病率，经正面1针、3针、6针和10针针刺接种的叶片发病率分别为70%、100%、100%和100%；经反面1针、3针、6针和10针针刺接种的叶片发病率分别为75%、95%、90%和100%，接种4 d后形成近圆形坏死斑（图1）。

对叶片经不同伤口接种菌丝块和分生孢子悬浮液4 d后的病斑平均直径统计分析的结果显示（表1），不同伤口接种菌丝块时，经正反面1针、3针、6针及10针刺伤处理产生的病斑大小存在显著性差异，正反面3针刺伤处理产生的病斑平均直径显著大于正反面1针刺伤处理产生的病斑平均直径，其中仅3针针刺处理在叶片正面及背面接种产生的病斑平均直径无显著差异，其他伤口处理在叶片正面接种产生的病斑平均直径显著大于在叶片背面接种产生的病斑平均直径。不同伤口接种分生孢子悬浮液时，经6针和10针刺伤处理产生的



A 与 B. 接种菌丝块；C 与 D. 接种分生孢子悬浮液；A 与 C. 叶片正面；B 与 D. 叶片反面；1-5 为无伤、1 针、3 针、6 针、10 针刺伤处理。
A and B. Inoculation with mycelial plugs; C and D. Inoculation with conidial suspensions; A and C. On the right side; B and D. On the back side; 1-5 showed no wound, puncturing one needle, three needles, six needles, and ten needles.

图1　在‘翠冠’梨叶片上不同伤口接种菌株PAFQ32菌丝块和分生孢子悬浮液4 d的病斑形态

Fig. 1　The lesions feature on leaves (*P. pyrifolia* ‘Cuiguan’) wounded by different methods 4 days after inoculation with mycelial plugs or conidial suspensions of PAFQ32 isolate

表1 菌株PAFQ32接种‘翠冠’梨不同伤口叶片4 d后产生病斑的平均直径

Table 1 The average diameters of lesions produced on leaves (*P. pyrifolia* ‘Cuiguan’) wounded by different methods 4 days after inoculation with PAFQ32 isolate

接种体 Inoculum	接种方向 Inoculum direction	病斑平均直径 Average diameters of lesions/mm				
		无伤 No wound	1针 One needle	3针 Three needles	6针 Six needles	10针 Ten needles
菌丝块 Mycelial plugs	正面 Right	0	7.4±1.5 d	8.6±1.2 bc	9.3±1.4 bc	10.5±1.4 a
	反面 Back	0	5.6±1.5 e	8.8±1.9 bc	8.4±1.7 c	9.4±1.4 b
分生孢子悬浮液 Conidial suspensions	正面 Right	0	2.4±0.9 C	3.2±0.7 B	4.2±1.0 A	3.8±0.7 A
	反面 Back	0	2.7±1.2 BC	3.0±0.8 B	3.8±0.9 A	4.4±1.2 A

注：不同小写字母、大写字母分别表示在 $p < 0.05$ 和 $p < 0.01$ 上差异显著。下同。

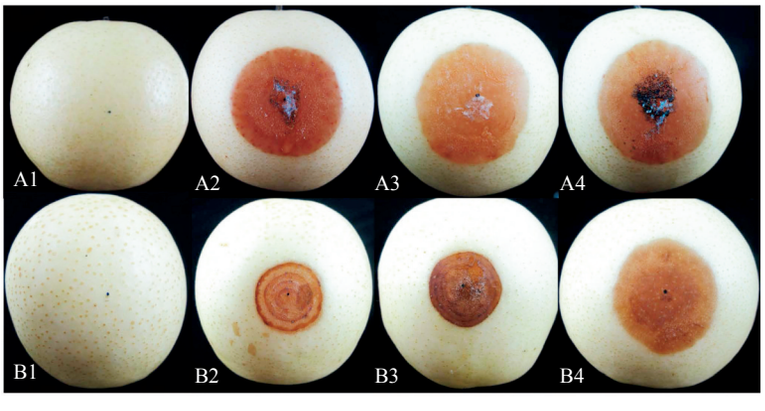
Note: Different small and capital letters indicate the significant difference at $p < 0.05$ and $p < 0.01$, respectively. The same below.

病斑直径较大，与1针和3针刺伤处理产生的病斑平均直径在大小上存在显著性差异，但在叶片正面接种产生的病斑与在叶片背面接种产生的病斑大小无显著差异。

比较菌丝块和分生孢子悬浮液接种梨叶片的结果显示（表1），在相同伤口处理下，接种菌丝块的病斑扩展速度较接种分生孢子悬浮液快，表明经针刺后接种菌丝块比接种分生孢子悬浮液可以更快速地测定果生炭疽菌的致病力。综上分析可知，叶片接种可采用简单的正面针刺3针接种菌丝块的方法。

2.1.2 果实有伤接种的测定结果 果实无伤口接种PAFQ32菌丝块、分生孢子悬浮液、空白培养基和无菌水均未发病。有伤接种菌株PAFQ32菌丝块和分生孢子悬浮液后，发病率均为100%。有伤接种菌株PAFQ32菌丝块时，1 d后各针刺处理均出现黄褐色坏死，2 d后病斑均超出菌丝块，有伤接种菌株PAFQ32分生孢子悬浮液时，3 d后1针、5针和10针刺伤的果实在伤口处出现黄褐色坏死，两者均随接种时间的增加病斑继续扩展，形成黄褐色的近圆形的坏死斑，7 d时病斑上有黄色分生孢子（图2）。

对果实经不同伤口接种菌丝块和分生孢子悬浮液7 d后的病斑平均直径统计分析的结果显示（表2），各针刺处理果实接种菌丝块产生的病斑平均直径均大于分生孢子悬浮液，果实有伤接种分生孢子悬浮液的病斑是随着伤口变大而增大的，但接种菌丝块产生的病斑大小无明显差异，因此果实接种可采用操作简单的1针针刺接种菌丝块法。



A. 接种菌丝块；B. 接种分生孢子悬浮液；1-4为无伤、1针、5针、10针刺伤。无伤接种点位于蓝色记号笔点。

A. Inoculation with mycelial plugs; B. Inoculation with conidial suspensions; 1 to 4 showed no wound, puncturing one needle, five needles, ten needles. Under unwounded conditions, inoculated positions are indicated with blue spots.

图2 在‘黄冠’梨果实上不同伤口接种菌株PAFQ32菌丝块和分生孢子悬浮液7 d的病斑形态

Fig. 2 The lesions feature on fruit (*P. bretschneideri* ‘Huangguan’) wounded by different methods 7 days after inoculation with mycelial plugs and conidial suspensions of PAFQ32 isolate

2.1.3 枝条有伤接种的测定结果 枝条上无伤口接种PAFQ32和空白培养基均未发病。有伤枝条接种PAFQ32 3 d后在伤口处开始出现黑色病斑，经打孔和烫打处理的发病率均为100%，环割处理为80%，

表2　菌株PAFQ32接种‘黄冠’梨不同伤口果实7 d后产生病斑的平均直径

Table 2　The average diameters of lesions produced on fruits (*P. bretschneideri*‘Huangguan’) wounded by different methods after 7 days of inoculation with PAFQ32 isolate

接种体 Inoculum	病斑直径 Average diameters of lesions/ mm			
	无伤 No wound	1针 One needle	5针 Five needles	10针 Ten needles
菌丝块 Mycelial plugs	0	34.1±2.9 a	37.1±1.9 a	33.3±4.3 a
分生孢子悬浮液 Conidial suspensions	0	19.9±1.8 b	21.6±5.8 b	31.8±2.3 a

10针刺伤处理为40%，3针刺伤处理为20%，随接种时间的增加病斑继续扩展，形成黑色的凹陷病斑（图3），接种30 d后经打孔和烫打处理的枝条整枝坏死，而经环割、3针和10针刺伤的枝条仅在伤口周围有病斑。

A1~A6：依次为无伤、3针刺伤、10针刺伤、环割、打孔、烫打伤口处理。

A1 to A6 showed no wound, puncturing three needles, ten needles, girdling, holing and scald holing.

图3　在‘翠冠’梨枝条上不同伤口接种菌株PAFQ32菌丝块9 d的病斑形态

Fig. 3　The lesions feature on twigs (*P. pyrifolia*‘Cuiguan’) wounded by different methods 9 days after inoculation with mycelial plugs of PAFQ32 isolate

对枝条经不同伤口处理接种9 d后的病斑平均直径统计的分析结果显示（表3），经3针、10针刺伤、打孔和环割4种处理的病斑平均直径明显小于经烫打处理的病斑平均直径，存在显著差异，前4种处理的病斑平均直径之间无显著差异，但是经烫打处理后发病不稳定，病斑平均直径标准误差较大，故不利于准确测定果生炭疽菌的致病力。

果生炭疽菌致病力室内测定方法研究的结果表明，叶片正面针刺3针接种菌丝块的方法材料均一性好、效果显著、试验周期短，发病稳定和操作简单，故选其为梨果生炭疽菌致病力的室内快速测定方法。

2.2　不同来源菌株致病力的测定结果与分化分析

2.2.1　*供试菌株致病力的测定结果*　对111份不同来源的梨果生炭疽菌在翠冠梨叶片上进行致病力测定，结果表明这些菌株致病力存在显著差异（表4）。菌株PAFQ32致病力最强，病斑平均直径为10.3 mm；菌株JSNJ-2-1致病力最弱，病斑平均直径为2.8 mm。

2.2.2　*致病力分化类型的划分*　采用SAS 9.4软件对111份梨果生炭疽菌在翠冠梨叶片上4 d的病斑长度进行聚类分析的结果表明，以聚类距离（cluster distance）1.0划分，供试菌株的致病力划分为3组（图4），其中聚类组1包括89个菌株，聚类组2共17个菌株，聚类组3共5个菌株。按照聚类分析结果将我国梨果生炭疽菌致病力划分为3

表3　菌株PAFQ32接种‘翠冠’梨不同伤口枝条9 d后产生病斑的平均直径

Table 3　The average diameters of lesions produced on twigs (*P. pyrifolia*‘Cuiguan’) wounded by different methods 9 days after inoculation with PAFQ32 isolate

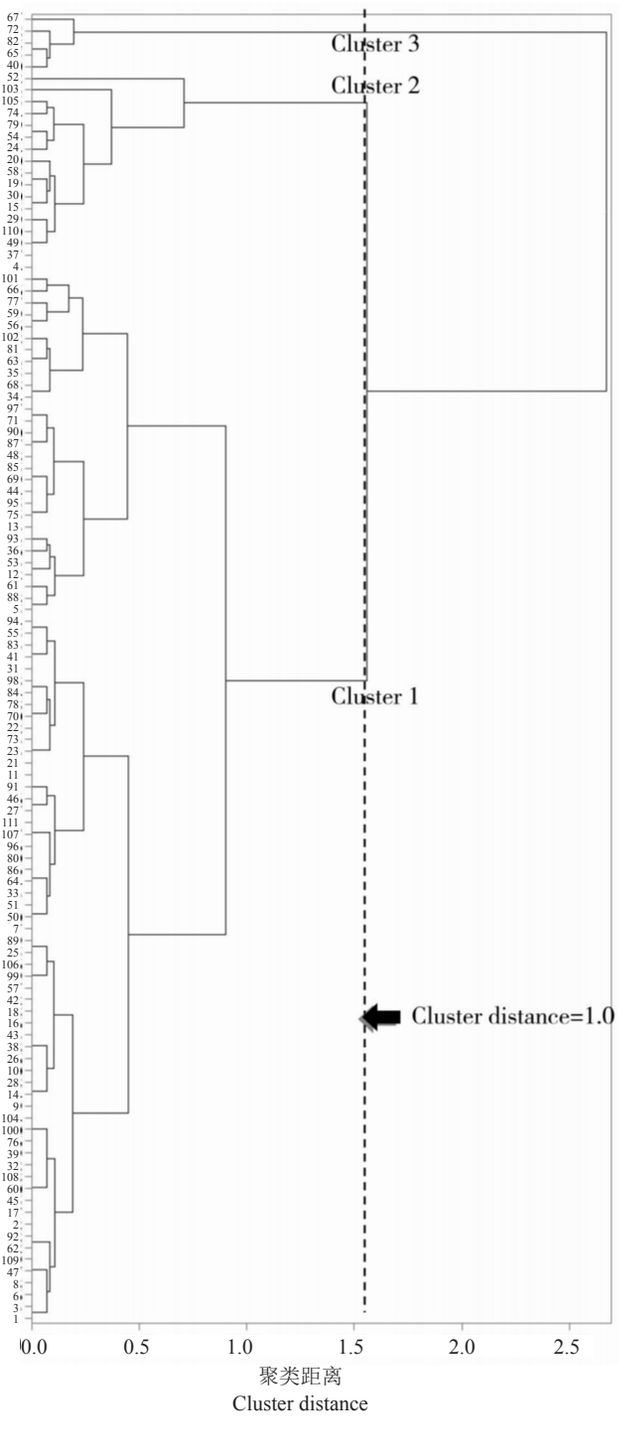
接种体 Inoculum	病斑平均直径 Average diameters of lesions/mm					
	无伤 No wound	3针 Three needles	10针 Ten needles	环割 Girdling	打孔 Holing	烫打 Scald holing
菌丝块 Mycelial plugs	0	3.1±1.6 b	3.6±0.7 b	4.5±1.8 b	3.6±0.9 b	9.9±3.4 a

表4 供试菌株菌丝块有伤接种‘翠冠’梨叶4 d后产生病斑的平均直径

Table 4 The average diameters of lesions on leaves (*P. pyrifolia* ‘Cuiguan’) 4 days after inoculation with mycelial plugs of tested strains

序号菌株 Strain number	菌株 Strain	病斑平均直径 Average diameter of lesions/mm	序号菌株 Strain number	菌株 Strain	病斑平均直径 Average diameters of lesions/mm	序号菌株 Strain number	菌株 Strain	病斑平均直径 Average diameters of lesions/mm
1	HBQJ-7	7.5	38	FJ-35	7.9	75	ZJTL-3-3	5.5
2	HBQJ-43-1	7.8	39	JN-139	7.7	76	ZJTL-4-1	7.7
3	HBQJ-48	7.5	40	FJ-103	3.1	77	ZJTL-11-1	4.8
4	HBQJ-24	8.3	41	FJ-85	6.8	78	ZJTL-3-1	6.5
5	HBQJ-8	6.2	42	FJ-45	8.2	79	ZJTL-15-1	8.9
6	HBQJ-49	7.4	43	FJ-1	7.9	80	ZJTL-9-1	7.1
7	HBQJ-4	7.0	44	FJ-42	5.6	81	ZJHZ-1-1	5.2
8	HBQJ-13	7.4	45	FJ-14	7.8	82	ZJTL-14-2	3.0
9	HBQJ-14	8.0	46	FJ-110	7.3	83	ZJTL-8-4	6.8
10	HBQJ-15	7.9	47	FJ-39-1	7.4	84	ZJTL-12-1	6.4
11	HBQJ-11-1	6.6	48	FJ-119	5.7	85	ZJTL-10-4	5.6
12	HBGJ-20	6.1	49	FJ-116	8.3	86	ZJTL-7-1	6.9
13	HBQJ-2	5.5	50	FJ-60	7.0	87	ZJTL-1-3	5.7
14	HBQJ-28	8.0	51	FJ-2	7.0	88	ZJTL-5-2	6.2
15	HBQJ-38	8.7	52	PAFQ32	10.3	89	ZJTL-13-1	8.1
16	HBQJ-47	8.2	53	JSNJ-15-2	6.1	90	ZJHZ-12-1	5.7
17	HBQJ-32	7.8	54	JSNJ-G-9-1	8.9	91	ZJHZ-4-1	7.2
18	HBQJ-21	8.2	55	JSNJ-9-1	6.7	92	ZJHZ-9-1	7.6
19	HBQJ-44	8.6	56	JSNJ-4-1	4.9	93	ZJHZ-2-1	6.0
20	HBQJ-1	8.5	57	JSNJ-G-5-3	8.2	94	ZJHZ-6-1	6.7
21	HBQJ-43-2	6.6	58	JSNJ-G-4-1	8.6	95	ZJHZ-10-1	5.5
22	JX-40	6.5	59	JSNJ-12-2	4.9	96	ZJHZ-11-1	7.1
23	JX-25	6.6	60	JSNJ-G-8-1	7.8	97	ZJHZ-5-1	5.8
24	JX-12	8.8	61	JSNJ-14-4	6.3	98	ZJHZ-3-1	6.4
25	JX-27	8.1	62	JSNJ-G-6-1	7.6	99	AHHG-2-4	8.2
26	JX-68	7.9	63	JSNJ-7-1	5.2	100	AHHG-17-6	7.7
27	JX-1	7.3	64	JSNJ-13-3	6.9	101	AHHG-16-4	4.5
28	JX-35	8.0	65	JSNJ-G-1-1	3.0	102	AHHG-15-62	5.3
29	JX-21	8.4	66	JSNJ-G-2-1	4.6	103	AHHG-18-3	9.4
30	JX-66	8.7	67	JSNJ-2-1	2.8	104	AHHG-4-8	7.7
31	JX-33	6.8	68	JSNJ-5-3	5.1	105	AHHG-3-1	9.1
32	JX-72	7.7	69	JSNJ-G-3-1	5.6	106	AHHG-1-1	8.2
33	JX-41-1	6.9	70	JSNJ-8-1	6.5	107	AHHG-4-11	7.1
34	JX-44	5.1	71	JSNJ-G-10-1	5.8	108	AHHG-20-12	8.0
35	JX-16	5.2	72	JSNJ-10-1	3.2	109	GX-40	7.4
36	JX-30	5.9	73	JSNJ-G-7-2	6.6	110	GX-18	8.3
37	FJ-73	8.3	74	ZJHZ-7-1	9.0	111	GX-19	7.1

个等级，病斑平均直径（ADL）≥8.3 mm 为强致病力（strong pathogenicity，缩写为 SP）；8.3 mm>ADL≥4.0 mm 为中致病力（middle pathogenicity，缩写为 MP）；ADL<4.0 mm 为弱致病力（weak pathogenicity，缩写为 WP）。依据划分标准，111 份供试菌株中表现为弱致病力的菌株 5 份，占所有菌株的 4.5%；中等致病力 89 份，占 80.2%；强致病力的菌株 17 份，占 15.3%，结果表明，梨果生炭疽菌不同来源的菌株存在明显的致病力分化，以中等致病力菌株为优势群体。



图中的编号与表4的菌株序号一致。

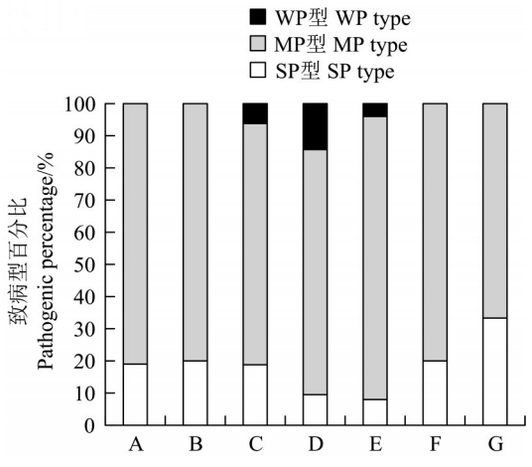
The number of Fig.4 is the same as the strain number of Table 4.

图4　111份果生炭疽菌在翠冠梨叶上有伤接种4 d后产生病斑平均直径的聚类分析

Fig. 4　Cluster analysis of the average diameter lesion of 111 *C. fructicola* strains on leaves (*P. pyrifolia*‘Cuiguan’) 4 days after inoculation

2.2.3　*不同菌株的致病力分化与其地理来源之间的关系*　来源于不同地区的菌株致病类型的分布比例：从图5可以看出，来源于福建、江苏和浙江3个地区的菌株存在SP、MP和WP三种致病类型，而来源于湖北、江西、安徽和广西仅有SP和MP两种致病类型。结果表明，梨果生炭疽菌致病力的分化因其地理来源不同存在明显的差异，这种差异与我国南方梨产区地理气候的差异之间具有一定的相关性。

来源于南方梨产区砂梨和白梨菌株致病类型



A-G 依次为湖北，江西，福建，江苏，浙江，安徽，广西。

A to G showed Hubei，Jiangxi，Fujian，Jiangsu，Zhejiang，Anhui and Guangxi.

图5 来源于南方不同梨产区菌株3种致病类型的分布比例

Fig. 5 The proportion of three pathogenic types of strains from different areas in southern China

的分布比例：111 个供试菌株中共有 101 个菌株来源于砂梨，10 个菌株来源于白梨，来源于砂梨的菌株存在 SP、MP 和 WP 三种致病类型，而来源于白梨的菌株仅有 SP 和 MP 两种致病类型。结果显示，梨果生炭疽菌致病力的分化因其来源的梨系统不同存在明显的差异。我国南方梨产区栽培的绝大多数为砂梨，白梨很少，因此明确来源于不同梨系统菌株的致病类型，还需进一步从我国北方梨产区采样分离果生炭疽菌。来源于叶片和果实菌株致病类型的分布比例：111 个供试菌株中共有 100 个菌株来源于叶片，11 个菌株来源于果实，来源于果实和叶片的菌株均存在 SP、MP 和 WP 三种致病类型。结果显示，梨果生炭疽菌致病力的分化与分离菌株的梨组织之间无明显的相关性。南方梨果生炭疽菌主要为害叶片，为害果实的很少，因此明确来源于不同分离组织菌株的致病类型还需进一步从果实、枝条等梨组织中分离果生炭疽菌。

3 讨 论

梨果生炭疽菌在有伤条件下对果实（或叶片）发病迅速，但相同时间内无伤条件下则很难发病或发病较晚，这种现象与刺盘孢属真菌侵染特性有关，多数刺盘孢属真菌为半活体营养型病原菌，而这种无症状时期很可能是其正处于潜伏期[7,20-22]。此外，刺伤可以打破 *C. fructicola* 的潜育期，增强其侵染能力，使果实更快的腐烂[23]。因此在室内主要利用伤口接种病原菌测定致病力。但伤口类型、接种部位或接种材料的不同，均可影响病原菌致病力的测定结果。韦洁玲等[24]建立了以离体叶片针刺接种作为室内准确、快速评价苹果腐烂病的方法，孙洁莹等[25]通过叶片针刺接种鉴定了梨种质资源对炭疽病的抗性，张蕊[26]等建立了以离体叶片针刺接种作为室内快速测定芒果炭疽菌致病力的方法，刘威等[27]、李扬等[28]和李江华等[29]通过针刺造成伤口分别鉴定了茶树炭疽病菌、油茶炭疽病菌和杧果炭疽病菌的致病力，但尚未见有梨果生炭疽菌的室内快速测定方法的报道。

本研究采用梨叶片、果实和枝条对梨果生炭疽菌进行致病力测定效果比较，结果表明采用 1 年生枝条作为接种材料，经烫伤处理的病斑直径最大，但发病不稳定，这与 Bessho 等[30]认为烫伤时间的一致性很难把握，而烫伤时间上的差异对其测定结果影响很大的研究结果一致，且枝条的试验周期较长（需要 5~15 d）[30-32]，不适用于致病力的快速测定；而采用果实作为接种材料，虽然发病迅速，试验周期短，但果实即使表面无伤痕也极有可能存在储藏病害，且不同批次果实成熟度的差异易对试验结果造成影响；而健康的叶片正反两面接种菌丝块，发病迅速，试验周期短，且以完全展开叶作为接种材料的致病力鉴定体系，材料本身均一性较好，误差小，鉴定结果稳定，综合比较，采用正面针刺 3 针接种菌丝块的方法，25 ℃保湿培养，4 d 测量病斑直径，发病稳定、快速，可准确可靠鉴定菌株间的致病力差异。

目前已有研究证实，真菌和寄主在长期共进化的过程中，其致病力也发生分化，如烟草赤星病菌[33]、杧果炭疽病菌[29]、黄瓜褐斑病菌[34]、水稻纹枯病菌[35]和薯蓣炭疽病菌[36]等。近些年在我国南方梨产区早期落叶发生危害较重，主要是由炭疽病所致[6]，并已研究证实不同地区引起炭疽病的病原种类组成有异，果生炭疽菌为我国南方梨产区的优势种群[7]。本研究结果表明，我国南方梨果生炭疽菌存在明显的致病力分化，以中等致病类型菌株居多，这与梨腐烂病菌[37]、杧果炭疽病菌[29]和薯蓣炭疽病菌[36]的致病力分化相似。梨果生炭疽菌的不同地区来源的菌株之间，其致病类型的分布比例存在明显差异，结果与李江华等[29]、吴桥等[34]和丛子文等[36]

的研究相似，表明植物病原菌致病力的分化与寄主植物种类及环境因素有一定的关联。本研究研究结果对制定梨炭疽病的综合防治规程具有重要的指导意义。

4　结　论

果生炭疽菌的菌丝块针刺接种梨叶片的方法，可用于其致病力的室内快速测定。来源于我国南方梨产区导致早期落叶的果生炭疽菌存在明显的致病力分化，以中等致病力菌株为优势群体。

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