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‘沃柑’果斑病病原菌鉴定及防治药剂筛选

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摘 要:【目的】明确重庆开州和长寿‘沃柑’果园发生果斑病的主要致病菌及其致病力。**【方法】**对‘沃柑’病果进行组织分离、致病性测试,利用传统形态学鉴定和分子生物学(ITS、TUB2、GPDH及LSU条码)鉴定方法鉴定病原真菌;以菌丝生长速率法进行室内药剂筛选。**【结果】**明确‘沃柑’果斑病病原菌为小新壳梭孢(*Neofusicoccum parvum*)。在致病力测试中,WC2菌株对‘大雅柑’‘尤力克柠檬’‘塔罗科血橙’和有核‘沃柑’的叶片、果实和枝条均致病,其中对枝条的致病力最强。室内毒力测定结果显示,18种杀菌剂中仅苯醚甲环唑、肟菌•戊唑醇、戊唑醚菌酯和啶酰菌胺4种杀菌剂对WC2菌株有效,抑制EC_{50}分别为0.24、0.72、1.04和5.28 μg·mL^{-1}。**【结论】**‘沃柑’果斑病的致病菌为小新壳梭孢,对‘沃柑’叶片、果实和枝条同样具有致病力,苯醚甲环唑和肟菌•戊唑醇对其具有明显的抑菌效果。

关键词:‘沃柑’;小新壳梭孢;致病力测试;药剂筛选

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Pathogen identification and fungicide screening of ‘Orah’ fruit spot disease

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Abstract:【Objective】‘Orah’ is a kind of wide-skinned citrus introduced from Israel. It has the advantages of beautiful skin, late ripening, high sugar content, early bearing and high yield. And then it has high economic value and broad planting prospect. In recent years, a large area of fruit spot disease has occurred in ‘Orah’ planting area of Kaizhou, Chongqing. When it happened seriously, it damaged the newly sprouted branches and young fruits, resulting in the death of new sprouted branches, yellowing of fruits and serious fruit drop. The disease weakened the seedling vigor and reduced fruit yield, which brought serious economic losses to local fruit growers. In order to identify the main pathogen of citrus fruit spot disease, the present experiment was carried out to provide theoretical basis and practical guidance for local fruit growers.【Methods】The diseased citrus fruits collected from the high incidence area of Kaizhou county, Chongqing were isolated by tissue separation method. The colony, mycelium, sporulation structure and spore phenotype of pathogen were observed under a microscope. The shape and size of mycelium on the slide were observed and measured under the microscope. Under the microscope, the hyphae were colorless and transparent. We observed the change of mycelium color during the growth of pathogenic strains cultured on PDA medium for 20 days. The genomic DNA of pathogenic fungi was extracted and identified based on molecular biology (ITS, TUB2, GPDH and LSU bar code). Furthermore, the pathogens were inoculated on citrus fruits, leaves and branches, and their pathogenicity was determined. After inoculating pathogen strain WC2, the citrus fruits and leaves were kept moist on the wet absorbent cotton, and cultured in the incubator at 28 ℃ for 12 hours, lasting 3-6 days, during

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which statistical analysis was carried out for several times. The virulence of 18 fungicides was tested by mycelium growth rate method. There were three repeats for each treatment, and the untreated PDA medium was used as the control.【Results】In the aspect of morphology, the colony of pathogenic strain WC2 was cultured on PDA plate, the colony of WC2 was round with neat edge and developed aerial hyphae on PDA plate; the colony was white at the initial stage and gray at the later stage. Through microscopic observation, it was found that the conidia were spindle-shaped, single-celled, colorless and transparent. In terms of molecular biology, WC2 and *Neofusicoccum parvum* are clustered into one branch, so it is clear that WC2 is *N. parvum*. In the pathogenicity test, obvious spots were found in all of four varieties. With the increase of the disease onset time, the disease spots increased gradually. The maximum diameter of the disease spots could reach 40 mm after 4 days of culture. The surface of leaves and branches was gray brown, and the surface of young fruits was black and slightly sunken. When the humidity was kept above 70%, there were white hyphae in the diseased area. The fruits and branches were hard and firm without rotten and soft phenomenon. The pulp was also black due to the invasion of pathogenic fungi, without obvious decay and special smell. WC2 had stronger virulence to Eureka lemon, tarocco and ‘Orah’ except *Citrus reticulatea* ‘Dayagan’, and WC2 had stronger pathogenicity to branches than to leaves and fruits. On the basis of the pathogenicity test *in vitro* plant tissue, the experiment in pot was carried out. Eight days after inoculation with pathogenic fungi, the plants began to suffer disease symptoms. Most of the buds on the damaged branches would be damaged. The infected buds can’t germinate normally, or they would wither just after germination, and the newly sprouted young leaves would become brown from the petiole. Then the disease spot spread along the vein, leading to the new sprouting leaves falling off, the branches were in the shape of bare stalks, and finally the whole branches died. The disease weakened the seedling vigor of ‘Orah’ and reduced the fruit yield caused by a large number of fruit drop, which brought serious economic losses to local fruit growers. In the toxicity test of fungicides, difenoconazole, trifloxystrobin-tebuconazole, tebuconazole-kresoxim-methyl and boscali had obvious antifungicidal effect on WC2 strain, with EC_{50} of 0.24, 0.72, 1.04 and 5.28 $\mu g \cdot mL^{-1}$, respectively. However, pyraclostrobin, mancozeb, prochloraz, thiophanate-methyl, benziothiazolinone, captan, thiamethoxam, diniconazole, copper (Ⅰ) oxide, bromothalonil, oxine-copper, buprofezin, copper chloride oxide, azoxystrobin, pyrazole-metiram had almost no inhibitory effect on WC2. These fungicides should be avoided in the field application to prevent and control the disease so as to reduce the economic expenditure.【Conclusion】The disease caused by *Neofusicoccum parvum* was pathogenic to many citrus varieties, mainly to the branches. Among them, difenoconazole, trifloxystrobin- tebuconazole, tebuconazole- kresoxim-methyl and boscali had obvious antifungicidal effect on WC2 strain, which was recommended for field control of the disease. This paper provides theoretical basis and practical guidance for early diagnosis and timely prevention and control of the disease.

Key words: ‘Orah’; *Neofusicoccum parvum*; Fungicide screening; Pathogenicity tests

‘沃柑’为‘坦普尔’橘橙与‘丹西’红橘的杂交种，种植起源于以色列，后引入中国广西、重庆、云南等地，具有外皮美观、晚熟、高糖、早结丰产等优势。加上树势生长强旺，耐寒抗旱，后期不易落果，易贮藏运输，大多在水果销售淡季收获，自身市场售价较高，当前具有较强的种植优势。该品种主要以早秋梢为结果母枝，结果的枝梢多为有叶花枝，一般树冠中下部坐果率较高，株产可达17.2 kg，666.7 m^2产量为1400 kg左右。‘沃柑’的病虫害种类多样，其中病害主要有溃疡病、炭疽病、褐斑病等；虫害主要有潜叶蛾、红蜘蛛、木虱等。近年来，在重庆开州‘沃柑’果园发生了大面积的柑橘果斑病，在果实膨大期，果实表面出现大片褐色革质病斑，发病后期病斑遍布全果，与砂皮病症状不同。果园在病害出现前期针

对褐斑病和砂皮病进行药剂防控,但带褐色革质病斑的病果未见减少。

小新壳梭孢(*Neofusicoccum parvum*)是重要的植物病原真菌,寄主范围广,可侵染柑橘、桉树、石榴、葡萄、珍珠李和核桃等多种植物,主要引起枝枯症状以及引起柑橘、杧果等产生溃疡流胶症状,并可引起葡萄果实干缩、掉粒、果梗干枯,树势减弱等症状[1],为害严重。此前,在柑橘上有关于小新壳梭孢侵染柑橘‘伦晚’果实最终致果实腐烂的报道,该果实腐烂病引起‘伦晚’脐橙果皮褐化、坚硬、缩水[2]。2020年在重庆开州和长寿‘沃柑’果园中发现大量果斑病,严重影响果实外观,产品收益锐减,给当地果农带来严重的经济损失。为了明确该果斑病的主要致病菌及其发病规律,并依此制定防控方案,笔者以开州和长寿‘沃柑’病害高发区域采集的病果为实验材料,对病菌进行分离鉴定及致病力测定,同时选择生产上常用的18种杀菌剂进行室内毒力测定。

1　材料和方法

1.1　致病菌分离纯化和鉴定

‘沃柑’病果采自重庆开州和长寿果园,采用组织分离法进行致病菌分离[3]。选取带有典型病斑的果皮组织,剪切、消毒、清洗后放置于PDA平板上,28 ℃培养2 d,挑取典型菌落边缘的菌丝置于新的PDA平板上,28 ℃培养5 d,然后将纯的单孢子培养物转移到PDA斜面上,保存在4 ℃条件下进行下一步研究。

观察在PDA培养基生长3、6、10和15 d的菌落形态,挑取生长15 d的菌丝和典型病斑上的孢子器制作临时玻片,在Olympus显微镜下观测载孢体、产孢结构的形态特征并进行显微拍照。用加热裂解法(参照吴望等[4]报道的加热裂解法,略有改进)获得模板DNA后,采用常规鉴定引物ITS1/ITS4、T2a/T2b、Gpd1/Gpd2和LSU-F/LSU-R(表1)直接扩增真菌基因组片段[5-6]。25 μL PCR反应体系:T5 Plant 12 μL,模板1 μL,上下游引物各1 μL,ddH_2O 10 μL。ITS、TUB2、GPDH和LSU扩增反应程序为:98 ℃预变性2 min,94 ℃变性30 s,退火40 s(ITS、TUB2为55 ℃、GPDH为60 ℃、LSU为50 ℃),72 ℃延伸45 s,35个循环;72 ℃补充延伸10 min[6]。PCR扩增产物电泳检测后,送擎科公司测序。测序结果在NCBI数据库中进行比对分析,用MEGA 7.0邻接法(Neighbor-Joining)进行聚类分析,构建系统进化树(1000次重复)(NCBI:http://blast.ncbi.nlm.nih.gov/Blast.cgi)。

表1 PCR引物序列

Table 1　Primer sequences of PCR

基因位点 Locus	扩增片段长度 Product size/bp	引物 Primer	序列(5’-3’) Sequence (5’-3’)
ITS	500~750	ITS1	TCCGTAGGTGAACTGCGC
		ITS4	CCTCCGCTTATTGATATGC
TUB2	450	T2a	GGTAACCAAATCGGTGCTGCTTTC
		T2b	ACCCTCAGTGTAGTGACCCTTGGC
GPDH	500~600	Gpd-1	CAACGGCTTCGGTCGCATTG
		Gpd-2	GCCAAGCAGTTGGTTGTGC
LSU	500~600	LSU-F	GTACCCGCTGAACTTAAGC
		LSU-R	ATCCTGAGGGAAACTTC

1.2　致病性测试

致病性测试参照王雪莲[3]报道的方法进行。采集‘大雅柑’‘尤力克柠檬’‘塔罗科血橙’和‘沃柑’的健康膨大期果实、展开未转绿叶片和夏梢,75%(φ)乙醇中浸泡30 s后,用无菌水冲洗漂洗3次,自然风干,针刺10针接种分离株菌饼(28 ℃光照培养7 d的分离株菌饼,直径6 mm),以接种PDA培养基的叶片、果实和枝条为对照,每个处理60个重复,28 ℃光照保湿培养(12 h光照/12 h黑暗),接种4 d后移去菌饼(若病斑明显大于菌饼,可保留菌饼拍照),记录病状并拍照,测量病斑直径。拍照并测量后,将原始菌饼再次放上叶片,并继续保湿,观察4 d后病斑扩展情况。用SPSS软件对病斑直径进行方差分析。从发病叶片和病果中重新分离真菌,进行形态学观察及分子鉴定。

1.3　室内药剂筛选

选用表2中的18种杀菌剂进行室内毒力测定,采用菌丝生长速率法测定杀菌剂的毒力[7]。所有杀菌剂均配置为1 mg·mL^{-1}的母液备用。再稀释成一系列质量浓度梯度,制成含有不同质量浓度药剂的含药培养基,备用。然后用直径6 mm打孔器打取菌饼接种于含药平板上。以加入等量无菌水的PDA平板为空白对照,每处理3次重复。28 ℃恒温培养5 d后用十字交叉法测量菌落直径,计算各杀菌剂对致病菌生长的抑制作用。抑制率/%=(对照菌落平均直径−处理菌落平均直径)/对照菌落平均直径×100[6]。采用农药室内生物测定数据处理系统(PBT)对各药剂试验结果进行统计分析,获得各药剂对菌株的毒力回归方程、抑制中浓度(EC_{50})和相

表 2 供试菌剂

Table 2 Tested fungicides

供试药剂 Fungicides	英文通用名 English generic name	生产厂家 Manufacturer
250 g·L^{-1}吡唑醚菌酯EC	Pyraclostrobin	巴斯夫欧洲公司 BASF SE
80%代森锰锌 WP	Mancozeb	拜耳作物科学(中国)有限公司 Bayer Crop Science (China) Co., Ltd
450 g·L^{-1}咪鲜胺EC	Prochloraz	安道麦马克西姆有限公司 Adama Maxim Co., Ltd
50%甲基硫菌灵 SC	Thiophanate-methyl	陕西汤普森生物科技有限公司 Shaanxi Thompson Biotechnology Co., Ltd
70%苯醚甲环唑 WG	Difenoconazole	瑞士先正达作物保护有限公司 Rui Shi Xian Zheng Da crop protection Co., Ltd
3%噻霉酮 ME	Benziothiazolinone	江苏辉丰农化股份有限公司 Jiangsu Huifeng Agrochemical Co., Ltd
75%肟菌•戊唑醇 WG	Trifloxystrobin.tebuconazole	德国拜耳作物科学公司 Bayer Crop Sciences, Germany
80%克菌丹 WP	Captan	安道麦(北京)农业技术有限公司 Adama (Beijing) Agricultural Technology Co., Ltd
70%戊唑•醚菌酯 WG	Tebuconazole.Kresoxim-methyl	陕西康禾立斗生物科技药业有限公司 Shaanxi Kanghe Lidou Biotechnology Pharmaceutical Co., Ltd
12.5%烯唑醇 WP	Diniconazole	江苏托球农化股份有限公司 Jiangsu Tuoqiu Agrochemical Co., Ltd
40%氧化亚铜 WG	Copper (I) oxide	挪威.劳道克斯公司 NORDOX
25%溴菌腈 EC	Bromothalonil	江苏托球农化股份有限公司 Jiangsu Tuoqiu Agrochemical Co., Ltd
33.5%喹啉铜 SC	Oxine-copper	浙江海正化工股份有限公司 Zhejiang Haizheng Chemical Co., Ltd
25%噻嗪酮 SC	Buprofezin	山东澳得利化工有限公司 Shandong Aodeli Chemical Co., Ltd
30%王铜 SC	Copper chloride oxide	江西禾益化工股份有限公司 Jiangxi Heyi Chemical Co., Ltd
50%嘧菌酯 WG	Azoxystrobin	巴斯夫欧洲公司 BASF SE
50%啶酰菌胺 WG	Boscalid	巴斯夫欧洲公司 BASF SE
60%吡唑•代森联 WG	Pyrazole.metiram	巴斯夫欧洲公司 BASF SE

关系数(r)。

2 结果与分析

2.1 受害症状和致病菌分离纯化

受害‘沃柑’果实症状如图1所示。在果实膨大期,果实上出现大片褐色革质病斑,稍凹陷,呈单点或片状分布,不光滑,发病后期病斑遍布全果,严重影响果实外观。

采用组织分离法获得了一系列菌株(WC1、WC2、WC3和WC4),分别接种于健康‘沃柑’果实和叶片上,以无菌水作对照,针刺接种5 d后,其中分离株WC2接种叶片和果实上出现感病症状。如图2所示,从接种后发病的果实上分离致病菌,获得了与原始菌株形态学一致的菌株。基本可以确定菌株

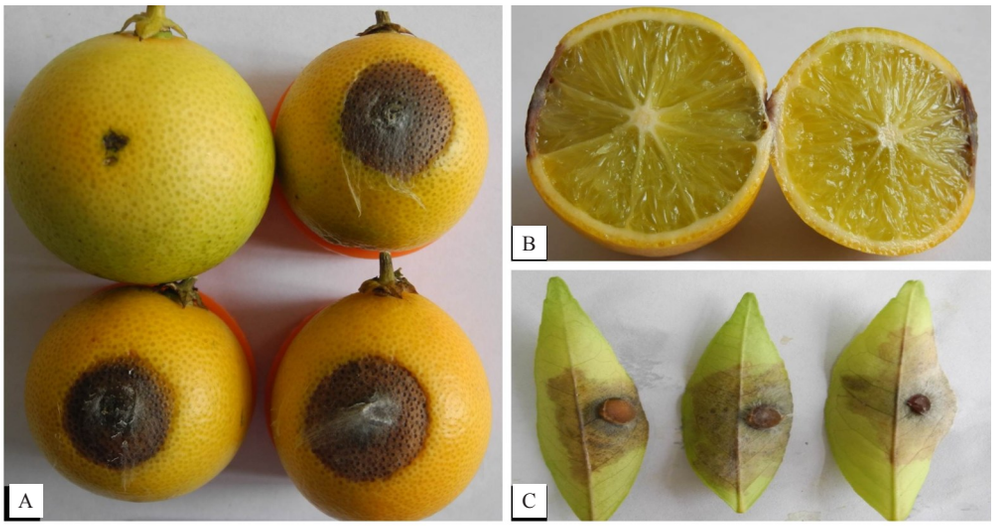
图 1 ‘沃柑’果实受害症状

Fig. 1 Symptoms on ‘Orah’ fruits

WC2为致病菌。由此对WC2菌株进行形态学鉴定以及分子生物学鉴定。

2.2 菌株形态学特征和分子鉴定

如图3所示,WC2菌株气生菌丝发达,继代培养的真菌迅速长成棉状气生菌丝体,生长高度可以达到并紧贴培养皿上盖;生长初期基质、菌丝和气生菌丝均为白色(图3-A~D),6~8 d气生菌丝变为灰色,气生菌丝呈网状结构(图3-E~F),20 d后产生黑色子实体。分生孢子透明,长椭圆形,无横隔,薄壁,两端钝圆(图3-G),大小为(4.7~7.9) μm ×(12.86~

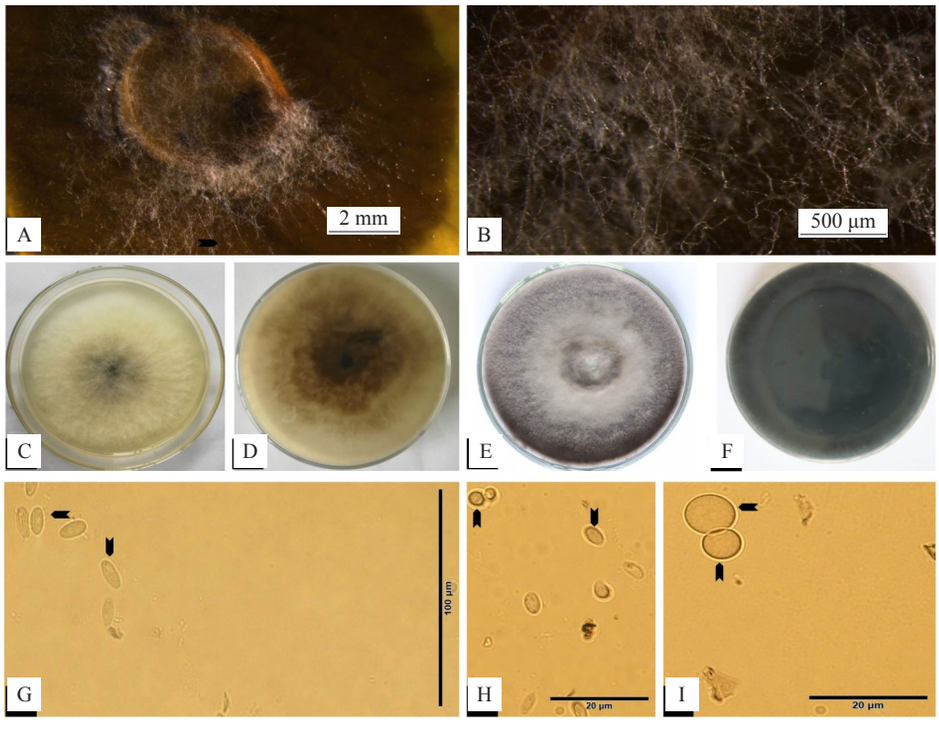


A~B. 接种 WC2 菌株后沃柑果实症状(5 d);C. 接种 WC2 菌株后沃柑叶片症状(5 d)。

A-B. Symptoms of Orah fruit after inoculation with WC2 strain (5 days); C. Symptoms of Orah leaves after inoculation with WC2 strain (5 days).

图 2　WC2 菌株接种‘沃柑’症状

Fig. 2　Pathogenicity test on ‘Orah’



A~B. 菌丝;C~F. PDA 上培养 6 d 和 15 d 的菌落正反面;G~H. 分生孢子;I. 厚垣孢子。

A-B. Hyphae; C-F. Positive and negative sides of colonies cultured on PDA for 6 days and 15 days; G-H. Conidia; I. Chlamydospores.

图 3　WC2 菌株形态学特征

Fig. 3　Morphological characteristics of WC2 strain

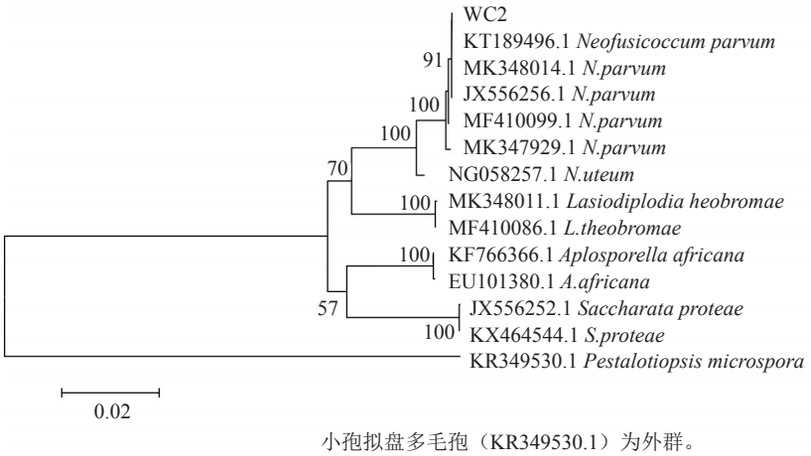
22.04) μm(n =50)。将菌丝处于营养和水分缺乏的条件下培养20 d,无性世代的分生孢子和厚垣孢子同时存在(图3-H),厚垣孢子为扁圆形(图3-I),由断裂方式产生,壁厚,寿命长,能抵御不良外界环境。这些形态特征均与前人描述的*N. parvum*相符[8-11]。

对分离菌株WC2的ITS、TUB2、GPDH和LSU基因进行PCR扩增并测序。经测序,病原菌ITS、TUB2、GPDH和LSU基因序列分别为574、425、577和878 bp,与NCBI登录号为MN904795.1、MN318109.1、KY009944.1和KT189496.1等*N. par-*

vum 序列的相似度分别为100%、100%、95%和100%。从GenBank中选取含模式菌株在内的相关菌株 *N. parvum*、*L. heobromae* 和 *S. proteae* 的ITS、TUB2、GPDH和LSU序列联合构建系统发育树，以 *Pestalotiopsis microspora* 为外群（NCBI登录号为KR349530.1）进行多重序列比较及系统发育学分析，结果显示，WC2菌株与 *N. parvum* 遗传距离最小，聚为一支。结合形态学特征（图3）和分子鉴定结果（图4），确定WC2菌株为 *N. parvum*。

2.3 致病力分析

将菌株WC2接种于‘大雅柑’‘尤力克柠檬’‘塔罗科血橙’和‘沃柑’的叶片、果实和枝条上，28 ℃光



小孢拟盘多毛孢（KR349530.1）为外群。

Pestalotiopsis microspora (KR349530.1) represents the out-group.

图4 基于ITS、TUB2、GPDH和LSU序列以最大似然法构建的系统发育树

Fig. 4 Phylogenetic tree based on maximum-likelihood analysis of combined ITS，TUB2，GPDH and LSU sequence data

暗交替培养2 d后，整个实验过程对照叶片、果实和枝条均不发病，与对照相比，接种2 d后每个品种都可见明显的病斑，随发病时间增长，病斑逐渐增大，培养至4 d时病斑直径最大可达40 mm。WC2菌株接种样品4 d后，叶片和枝条表面产生灰褐色病斑，幼果表面为黑色略凹陷的病斑，培养时保持湿度大于70%，发病区域为褐色病斑，菌饼上迅速生长出更多的白色菌丝。果实和枝条坚硬结实，无腐烂变软现象，内部瓤瓣被病原菌入侵变为黑色，无明显腐烂和特殊气味（图5）。第6天实验样品枝条和果实几乎被病斑全部覆盖，叶片也开始腐烂。WC2菌株除了对‘大雅柑’致病力相对较轻以外，对‘尤力克柠檬’‘塔罗科血橙’和有核‘沃柑’都有较强的致病力，而WC2对各个品种枝条的致病力明显较叶片和果实强。如表3所示，WC2菌株在枝条（平均病斑直径为18.8 mm）上形成的病斑较大，远远大于在叶片（平均病斑直径为8.7 mm）和果实（平均病斑直径为9.9 mm）上形成的病斑。4个品种中，WC2菌株在‘塔罗科血橙’叶片、‘沃柑’果实、‘尤力克柠檬’枝条上形成的病斑较大，在‘大雅柑’叶片、枝条、果实上形成的病斑最小。

盆钵‘沃柑’苗喷雾接种8 d后开始发病，受害枝条上的大多数芽受到侵染危害，不能正常萌发，或刚萌发即枯死（图6-A），整个枝条呈光秆状。而已经抽发的嫩叶也会从叶柄部分开始褐化（图6-B），病斑沿叶脉扩展，结果导致新抽发嫩叶脱落（图6-C），

表3 接种WC2 6 d后各品种的病斑平均直径

Table 3 The average diameter of lesion in different varieties 6 days after inoculation with WC2

品种 Varieties	叶斑平均直径 Average diameter in leaves/mm	果斑平均直径 Average diameter in fruits/mm	枝斑平均直径 Average diameter in branches/mm
大雅柑 Dayagan	1.8±3.1 c	3.2±2.0 c	11.5±4.0 b
尤力克柠檬 Eureka Lemon	10.3±6.6 b	10.0±4.0 b	26.1±7.3 a
塔罗科血橙 Tarocco	15.6±8.5 a	6.9±3.1 b	21.8±3.3 a
沃柑 Orah	10.0±8.8 b	12.8±5.8 a	14.5±5.0 b

注：同列数据不同小写字母表示在 $p<0.05$ 差异显著。

Note: Different small letters in the same column showed significant difference at $p<0.05$.

A. 大雅柑;B. 尤力克柠檬;C. 塔罗科血橙;D. 沃柑。

A. Dayagan; B. Eureka Lemon; C. Tarocco; D. Orah.

图 5　WC2 菌株对不同品种柑橘叶片、果实和枝条的致病性

Fig. 5　Pathogenicity of WC2 to different citrus leaves, fruits and branches

最终整个枝条枯死。叶片染病初期呈现棕色圆形水浸状小病斑,后期扩展为圆形或半圆形水浸状病斑,其上散生小黑点,病健交界处有一明显的深褐色坏死带。受害初期果实果皮呈水浸状凸起,随着病情发展,油胞肿胀溃破,病斑由中心至边缘开始变黑(图6-D),病健交界明显,发病柑橘果实黑褐色、软腐。

2.4　室内药剂筛选

对18种田间常用的杀菌剂进行室内药剂筛选中,质量浓度为4 μg·mL^{-1}的吡唑醚菌酯、溴菌腈、甲基硫菌灵、代森锰锌、咪鲜胺、噻嗪酮、噻霉酮、王铜、

A. 芽枯死;B. 叶柄褐化;C. 嫩叶脱落;D. 果实病斑变黑。

A. The buds withered; B. Petiole browning; C. Tender leaves fall off; D. The disease spot of fruit turns black.

图 6　盆栽‘沃柑’苗喷雾接种 WC2 菌株后的发病情况

Fig. 6　Incidence of ‘Orah’ inoculated with WC2 strain

嘧菌酯、喹啉铜、氧化亚铜、克菌丹、吡唑代森联和烯唑醇对WC2几乎无抑制作用，这些药剂应避免在田间使用。苯醚甲环唑、肟菌•戊唑醇、戊唑•醚菌酯和啶酰菌胺4种杀菌剂对WC2菌株有效，如表4所示，EC_{50}分别为0.235、0.723、1.036和5.28 μg·mL^{-1}，可进一步进行田间药效评测。

表4 杀菌剂对菌株WC2的毒力

Table 4 Fungicides virulence to strain WC2

药剂 Fungicides	毒力回归方程 Toxic regression equation	抑制中浓度 EC_{50}/(μg·mL^{-1})	相关系数 Correlation coefficient, *r*	95%置信限 95% confidence intervals
70%戊唑•醚菌酯 WG Tebuconazole.Kresoxim-methyl	*Y*=4.981 3+1.220 2*X*	1.04	0.98	0.12~8.79
75%肟菌•戊唑醇 WG Trifloxystrobin.tebuconazole	*Y*=5.168 6+1.196 9*X*	0.72	0.99	0.08~5.94
50%啶酰菌胺 WG Boscalid	*Y*=4.607 1+0.543 7*X*	5.28	0.97	0.06~1 712.46
37%苯醚甲环唑 WG Difenoconazole	*Y*=5.544 5+0.866 9*X*	0.24	0.97	0.01~7.33

3 讨 论

本研究通过致病菌分离、致病性测定、形态特征观察及ITS、TUB2、GPDH和LSU序列分析，鉴定了引起‘沃柑’果斑病的病原菌为小新壳梭孢 *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A. J. L Phillips[10]，是子囊菌葡萄座腔菌科Botryosphaeriaceae的一种无性型菌物。其为林木和果树常见的病原真菌，寄主非常广。Sakalidis等[12]2013年统计在全球6个洲29个国家有90种植物受*N. Parvum*侵染，寄主有桉树、葡萄、蓝莓、石榴、杨树、丝瓜、甜橙等，我国也有关于*N. Parvum*侵染柑橘、蓝莓的报道[13-14]。

本研究分离到的*N. parvum*菌株在病斑组织或人工培养条件下不易产生有性繁殖结构，这与Shen等[14]的研究结果一致；*N. parvum*一般只在含有松针的水琼脂培养基中产生无性子实体，本实验室是将其菌丝接种在柑橘枝条和叶片上，在不保湿的恶劣条件下培养20 d才能获得分生孢子和厚垣孢子。笔者除在‘沃柑’果实上分离纯化到*N. parvum*以外，也发现*N. parvum*可造成‘沃柑’叶片枯死，并在致病力测试中发现*N. parvum*对‘大雅柑’‘尤力克柠檬’‘塔罗科血橙’和‘沃柑’的果实、叶片和枝条都致病。并且，笔者发现*N. parvum*对各个品种中的枝条的致病力明显较叶片和果实强，说明*N. parvum*对产量和树势都有较严重的危害。在盆钵实验中，受害枝条上的芽、未转绿叶片和果实同时受到侵染危害，说明该病害在新枝抽发、第1次生理落果时就已经危害‘沃柑’树苗。监测病害时，枝梢和叶片易与炭疽病等混淆，也可能在修剪枝条时被剪掉，叶片在叶柄基部染病时就掉落，造成落果时又与生理落果混淆，使得该病害不易发现，而被忽视。针对膨大期染病果实进行药剂防控，已经为时已晚。因此有必要开展小新壳梭孢在柑橘上发病规律及防治措施的深入研究。

关于药剂防控，邵雪花等[15]报道针对*N. parvum*引起的番石榴枝枯病，使用30%咪鲜胺乳油和60%苯醚甲环唑水分散粒剂防效最好，达到56.87%和52.40%，有较大的应用潜力。唐利华等[16]报道针对*N. parvum*引起的杧果流胶病，多菌灵、戊唑醇、苯醚甲环唑、咪鲜胺、烯唑醇和丙环唑均可有效抑制其病菌的生长，可进一步用于田间药效试验。徐成楠等[17]报道针对*N. parvum*引起的蓝莓枝枯病菌，室内盆栽及田间药效试验结果表明，百菌清及戊唑醇对枝条的保护效果较好，但对已经发病的枝条无治疗效果。尹万瑞等[18]报道针对由*N. parvum*引起的核桃枝枯病，筛选出70%甲基托布津可湿性粉剂、50%多菌灵可湿性粉剂和75%百菌清可湿性粉剂3种高效药剂，其抑制率依次为66.67%、64.44%、44.44%。在本实验中，针对由*N. parvum*引起的‘沃柑’果斑病，共进行了18种杀菌剂的室内筛选，只有4种有效，其中苯醚甲环唑和肟菌•戊唑醇抑制效果最好，EC_{50}值分别是0.235和0.723 μg·mL^{-1}。这些实验表明，*N. parvum*抗药性很强，很多杀菌剂对它都不起作用，并且针对不同感病植物，适用的有效杀菌剂不同，这可能与不同物种上的*N. parvum*属于不同小种，不同地区施药情况不同，*N. parvum*抗药性也逐渐不同。这也解释了田间起初针对该病害所用的炭疽病或褐斑病的防控方案无效的原因。总体来说，应通过在春梢抽发期、谢花2/3期和幼果期加强该病原的防控，以及在果实膨大期加强该病原的监测来确保果树不受该病害的影响。防控时喷洒以上4种非常有效的杀菌剂（苯醚甲环唑、肟菌•戊唑醇、戊

唑•醚菌酯和啶酰菌胺）是必要的选择。

因此，针对不同感病植物不同地区的*N. parvum*，需要不同的防控对策，读者可以尝试以上报道的各类有效的杀菌剂，对自己种植区域的由*N. parvum*引起的枝枯等病进行防控。

4　结　论

本研究确定了重庆开州和长寿‘沃柑’果园发生的果斑病的主要致病菌为小新壳梭孢（*N. parvum*），对‘沃柑’叶片、果实和枝条都具有致病力，70%苯醚甲环唑和75%肟菌•戊唑醇对其具有明显的抑菌效果。本研究为探明该病的发生规律及制定有效的防治方案奠定了基础。

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