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葡萄炭疽病菌对4种杀菌剂的敏感性分析

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摘 要:【目的】了解辽宁地区葡萄炭疽病菌(*Colletotrichum gloeosporioides*)对代森锰锌、戊唑醇、咪鲜胺和苯醚甲环唑4种常用杀菌剂的抗药性现状,为其有效的化学防治提供依据和指导。**【方法】**采用区分剂量法和菌丝生长速率法,对辽宁的6个葡萄产区的120个葡萄炭疽菌株进行敏感性测定。**【结果】**辽宁地区葡萄炭疽病菌对不同药剂的敏感性检测结果表明,戊唑醇对供试菌株的EC_{50}值为0.677 1~2.691 0 mg·L^{-1},平均EC_{50}值为1.474 9 mg·L^{-1},29.17%的菌株对戊唑醇表现出抗药性,沈阳地区葡萄炭疽抗性频率最低为16.67%,葫芦岛地区葡萄炭疽最高抗性频率为45%;代森锰锌对供试菌株的EC_{50}值为11.156 5~65.934 3 mg·L^{-1},平均EC_{50}值为19.834 6 mg·L^{-1},28.33%的菌株对代森锰锌表现出抗药性,其中,葫芦岛地区葡萄炭疽菌对代森锰锌抗性频率最高,达45%;葡萄炭疽病菌供试群体对咪鲜胺和苯醚甲环唑的敏感性较强,平均EC_{50}值分别为0.011 5 mg·L^{-1}和0.061 1 mg·L^{-1},不同地区的EC_{50}值差异并不明显,且未在试验中发现抗性菌株。**【结论】**葡萄炭疽病菌对戊唑醇和代森锰锌表现出中低的抗药性;对咪鲜胺和苯醚甲环唑敏感性较强。

关键词:葡萄炭疽病菌;代森锰锌;戊唑醇;咪鲜胺;苯醚甲环唑;敏感性

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Sensitivity analysis of *Colletotrichum gloeosporioides* to four fungicides

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Abstract:【Objective】*Colletotrichum gloeosporioides* is the causal agent of grapevine anthracnose, causing a sharp decline in grape growth and yield. Infection of grape industry by *Colletotrichum gloeosporioides* has led to big yield losses in recent years. The disease will break out in warm and rainy period just before and during maturing time and the conidia are transferred by wind or rain and glues itself to the grape fruit surfaces. After falling on grape fruit, it produces germ tubes and differentiates into specialized appressorium structure. After infection, *Colletotrichum gloeosporioides* propagates into neighboring cells, causing lesions on grape. In practice, the grapevine anthracnose is generally controlled through fungicides. In order to investigate the sensitivity of *C. gloeosporioides* to mancozeb, tebuconazole, prochloraz and difenoconazole, diseased berries were collected from different grape vineyards, and the obtained pathogen isolates were tested for their resistance to mancozeb, tebuconazole, prochloraz and difenoconazole. This study could offer a theoretical direction for scientific preventing of grape anthracnose effectively.【Methods】Collected diseased berries were first surface disinfected with 75% ethanol for 30 seconds and then washed with sterile water, two millimeter sized tissues from junction of healthy and diseased grape anthracnose were placed on PDA including chloramphenicol and cultured at 25 ℃ for four days. For getting purified strains, disk containing mycelium picked from medium

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edge and inoculated to new PDA and cultured at 25 ℃ for eight 8 days, collected conidia were diluted into 10^5 per mL conidial suspensions. Thirty microlitre conidial suspensions were evenly spread on agar medium plates and incubated for twenty hours, and single conidium was selected under a microscope. The strains collected by single spore isolation were observed after culturing on PDA medium for 8 days at 25 ℃ under a light microscope. Furthermore, a droplet of 5 μL fresh conidial suspensions was inoculated on slightly wound grape berry made by toothpick prick, and then placed in a box chamber under 25 ℃. The pathogenecity of grape anthracnose was examined through lesions condition after three or four days after inoculation. Each assay was repeated four times with four duplications for every time. Finally, 120 *Colletotrichum gloeosporioides* isolates from 6 regions of Liaoning were indentified through cultivation and pathogenicity characteristic observation. Distinguishing dosages and the mycelium growth rate method were used to detect the resistance of 120 grape anthracnose isolates. Hyphae were transferred into PDA plates covering different dosages of fungicides, and the diameter of the colony was measured after eight days. The effective inhibiting medium concentrations (EC_{50}) value was calculated according to colony diameter through a software.【Results】EC_{50} of tebuconazole ranged from 0.677 1 to 2.691 0 mg·L^{-1} with an average value of 1.474 9 mg·L^{-1}; 29.17% of the 120 isolates showed resistance to tebuconazole in sensitive assay; no strain showed high resistance and thirty strains showed low resistance to tebuconazole. The resistant frequency of grape anthracnose was from 16.67% to 45.00% in tebuconazole plate from six different regions. The lowest resistant frequency of grape anthracnose was 16.67% from Shenyang and the highest resistance of grape anthracnose was 45.00% from Huludao area. However, the EC_{50} values of different strains from different regions were varied. The EC_{50} of mancozeb ranged from 11.156 5 to 65.934 3 mg·L^{-1} with a mean value of 19.834 6 mg·L^{-1}; 28.33% of the 120 isolates showed certain resistance to mancozeb in sensitive test. The grape anthracnose from Huludao area was the most resistant to mancozeb-resistant strains, reaching 45%. From the analysis of EC_{50} distribution values, there were higher resistant strains from Dalian and Benxi areas, reaching 65.934 3 mg·L^{-1} and 50.009 1 mg·L^{-1}, respectively; There was no colony on PDA plates containing 1 mg·L^{-1} prochloraz and resistant prochloraz strain was not found. The EC_{50} value of the strain was less than 5 times of the sensitive baseline in difenoconazole resistant experiment and studies showed that grape anthracnose from different regions had lower resistance to difenoconazole. It was found that EC_{50} values of different strains from different regions were less different in prochloraz and difenoconazole plate. All 120 isolates were highly sensitive to prochloraz and difenoconazole with an average EC_{50} of 0.011 5 mg·L^{-1} and 0.061 1 mg·L^{-1}, respectively.【Conclusion】In this study it was showed that *C. gloeosporioides* had certain resistance to mancozeb and tebuconazole, and a high sensitivity to prochloraz and difenoconazole. Because mancozeb was a multi-acting site fungicide and had a long history of application, it was very effortless to produce some low resistant strains. It was single site of action that may be an important reason for the development of resistant strains like tebuconazole fungicide. Therefore, growers need to consider the condition of the resistance situation in different regions when tebuconazole and mancozeb will be used in manufacture. Although strains with a certain resistance to prochloraz and difenoconazole fungicide were not found in this test, it was recommended to use fungicides with different target sites in order to avoid the emergence of resistant strains.

Key words: Grape anthracnose; Mancozeb; Tebuconazole; Prochloraz; Difenoconazole; Sensitivity

葡萄是世界上种植面积最广的水果，其产量稳居水果排名的前三位，在水果生产中占据极其重要的地位[1]。葡萄炭疽病(*Anthracnose*)又名晚腐病，是世界葡萄生产中一种多发性真菌病害，特别是在欧

洲葡萄、美洲葡萄和圆叶葡萄种植区，严重影响了葡萄的产量和质量[2]。由于连年种植葡萄的原因，造成我国大多数葡萄栽培地区的葡萄炭疽病原菌数量积累。国内外相关研究表明，该病菌一直是葡萄生产上的主要病害[3]，多种病原菌可以引起葡萄炭疽病，其中报道最多的病菌是胶孢炭疽(*Colletotrichum gloeosporioides*)和尖孢炭疽菌(*C. acutatum*)[4]。我国的辽宁省、云南省、新疆等地关于葡萄炭疽病的病原菌研究结果表明，葡萄炭疽病菌均为胶孢炭疽菌[5-7]。有关尖孢炭疽菌侵染引起葡萄炭疽病的相关报道[2]在我国尚未发现。胶孢炭疽菌是一种普遍存在的植物病原菌，该菌侵染的寄主范围非常广泛，而且其不同的专化型可以侵染水果、蔬菜、花卉和豆类[8]，侵染种类达1 000多种植物。

长期以来，化学防治一直是防控葡萄炭疽病的主要手段，而由于长期使用药剂，关于胶胞炭疽菌对多种农药产生抗药性的报道屡见不鲜。陈聃等[9]报道称对甲基硫菌灵高水平抗药性而对戊唑醇表现为低水平抗药性的葡萄炭疽菌(*C. gloeosporioides*)已被发现。孙行杰等[10]报道吡唑醚菌酯对葡萄炭疽病菌的孢子萌发抑制效果最显著，而戊唑醇、吡唑醚菌酯、多菌灵、苯醚甲环唑对抑制葡萄炭疽病菌菌丝生长具有良好效果。由于防治方法单一、防治时期和方法不当及病原菌抗药性等问题[11]，致使农户遭受巨大的经济损失，大大打击了农户的积极性，严重影响了现代高效农业的推进。因此，需要筛选出防治葡萄炭疽病的高效药剂，指导生产中的农药应用和炭疽病的高效防控。本研究采用菌丝生长速率法测定4种杀菌剂对该病菌的室内毒力，明确生产中炭疽病菌的抗药性水平，以期为葡萄炭疽病菌高效防控的药剂选择与合理使用提供依据。

1 材料和方法

1.1 供试菌株

2019年7—9月，分别从辽宁沈阳、大连、葫芦岛、朝阳、营口和本溪6个地区采集葡萄炭疽病果120份。病原菌的分离及单孢纯化参照王美玉等[12]的方法，略作修改。将葡萄炭疽样品表面用75%乙醇消毒30 s后，无菌水冲洗2~3次，剪取葡萄炭疽病果病健交界处2~3 mm组织块置于含100 mg·L^{-1}氯霉素的PDA平板上，25 ℃黑暗培养4 d，从中挑取菌丝进行再次培养8 d，刮取分生孢子配置稀释的分生孢子液(1×10^{4}个•mL^{-1})，取30 μL分生孢子液涂布于水琼脂平板上，于25 ℃培养箱培养12 h，利用解剖显微镜挑取已萌发的单个分生孢子，进行培养。根据病原菌的形态特点、培养性状，结合相关资料进行病原菌的鉴定[13]。

致病性测定参照汪家胜等[14]的方法，略作改动。将单孢分离后得到的纯菌株在含有100 mg·L^{-1}氯霉素PDA上培养6 d，用直径为4 mm的打孔器打成菌饼。把菌饼的菌丝面粘于健康并轻微刺伤的寄主葡萄果实上(套袋果)，以不含菌丝的4 mm PDA菌饼作对照，然后将其放入25 ℃左右的培养箱中进行黑暗培养，每处理4个重复，每隔1 d观察1次，记录观察结果，对侵染成功的病果进行组织分离，确认使果实致病的菌株。通过对其进行菌落形态、分生孢子形态的观察和致病性检测，共获得供试葡萄炭疽病菌菌株120个(表1)。

表1 采集和分离获得的葡萄炭疽菌株

Table 1 *Colletotrichum gloeosporioides* isolates collected and isolated from different areas

采集地点 Collection area	菌株编号 Isolate code
辽宁沈阳 Shenyang, Liaoning	LS1, LS2-1, LS3-2, LS4-5, LS6-1, LS7, LS8-2, LS9-2, LS10-1, LS11-3, LS12-2, LS13-2, LS14-5, LS15-6, LS16-1, LS17-2, LS18-1
辽宁大连 Dalian, Liaoning	LD1-2, LD2, LD3-2, LD4-1, LD5-2, LD6-2, LD7, LD8-2, LD9-2, LD10-2, LD11, LD12-2, LD13, LD14-1, LD15-3, LD16, LD17, LD18, LD19-2, LD20-1, LD21-2, LD22-2
辽宁葫芦岛 Huludao, Liaoning	LH1-2, LH2-1, LH3, LH4-2, LH5-1, LH6-2, LH7-3, LH8-2, LH9-2, LH10-2, LH11-2, LH12-3, LH13-2, LH14-2, LH15-6, LH16-3, LH17-2, LH18-1, LH19-2, LH20-2
辽宁朝阳 Chaoyang, Liaoning	LC1-9, LC2-1, LC3-2, LC4-1, LC5-6, LC6-6, LC7-2-1, LC8-2, LC9-2, LC10-2, LC11-6, LC12-3, LC13-4, LC14-5, LC15-2-1,LC16-1, LC17-2, LC18-3, LC19-5, LC20-6, LC21-6
辽宁营口 Yingkou, Liaoning	LY1-2, LY2-3, LY3-2, LY4-5, LY5-6, LY6-4, LY7-5, LY8-4, LY9-5, LY10-2, LY11-2, LY12-1, LY13-2, LY14-4, LY15-2, LY16-4, LY17-3, LY18-3, LY19-8
辽宁本溪 Benxi, Liaoning	LB1-2, LB2-3, LB3-2, LB4-5, LB5-2, LB6-3, LB7-3, LB8-6, LB9-3, LB10-6, LB11-2, LB12-2, LB13-4, LB14-2, LB15-3, LB16-1, LB17-1, LB18-3, LB19-2, LB20-2

1.2　供试药剂

10%苯醚甲环唑（Difenoconazole)微乳剂（山东省联合农药工业有限公司）；430 g·L^{-1}戊唑醇（Tebuconazole）悬浮剂（山东烟台科达化工有限公司）；80%代森锰锌（Mancozeb）可湿性粉剂（陶氏益农农业科技有限公司）；25%咪鲜胺(Prochloraz)水乳剂（山东省联合农药工业有限公司）。

1.3　方法

采用区分剂量法[15]和菌丝生长速率法[16]，挑取快速生长期菌落边缘生长状态相同的葡萄炭疽菌丝（直径约 0.2 cm 的菌丝团），置于含有不同剂量杀菌剂的 PDA 平板上（以不加药而加入等量无菌水为对照，每个处理 4 次重复），25 ℃恒温培养箱中黑暗培养 8 d 后用十字交叉法测量不同培养基上菌丝的生长速度。通过测量每个处理的菌落直径（cm），求取平均值。统计各地区的敏感、抗性菌株的频率，抗性频率/%=（抗性菌株数/测定总菌株数）×100。

计算不同农药对葡萄炭疽菌的抑制百分率（%）、有效抑制中浓度（EC_{50}值）、毒力回归方程及相关系数（r）。求有效抑制中浓度 EC_{50}值。

代森锰锌的区分剂量设置为 5、10、20、50、100 mg·L^{-1}，以不含药剂而加入等量无菌水为对照（CK），每个处理 4 次重复。按 FAO 推荐的菌落直径法，从纯化的葡萄炭疽菌丝中取 0.2 cm 的菌丝团，将其转接到含有不同浓度代森锰锌的 PDA 平板中央。25 ℃培养箱下培养 8 d 后观察:该菌不能在 5 mg·L^{-1}代森锰锌 PDA 培养基上生长的为敏感菌株（Ben S）；能在 5 mg·L^{-1}而不能在等于或高于 20 mg·L^{-1}PDA 培养基上生长的为低水平抗药性菌株（Ben LR）；能在 20 mg·L^{-1}而不能在等于或高于 100 mg·L^{-1}PDA 培养基上生长的为中等抗药性菌株（Ben MR）；100 mg·L^{-1}PDA 培养基上能生长的为高水平抗药性菌株（Ben HR）[17]。

戊唑醇的剂量分别设置为 0、1、5、10、20 mg·L^{-1}，抗性水平依据陈聃等[9]：在含 5 mg·L^{-1}戊唑醇的 PDA 平板上不能生长的菌株为敏感性菌株（S）；能在 5 mg·L^{-1}上生长而不能在等于或高于 20 mg·L^{-1}的 PDA 平板上生长的为低水平抗性菌株（LR）；在 20 mg·L^{-1}的 PDA 平板上能够生长的为高水平抗性菌株（HR）。

咪鲜胺的剂量分别为 0、0.1、1、5 mg·L^{-1}，抗性水平划分：在含 1 mg·L^{-1}咪鲜胺的 PDA 平板上不能生长的为敏感性菌株（S）；能在 1 mg·L^{-1}上生长而不能在等于或高于 5 mg·L^{-1}的 PDA 平板上生长的为低水平抗性菌株（LR）；在 5 mg·L^{-1}的 PDA 平板上能生长的为高水平抗性菌株（HR）[12]。

苯醚甲环唑的剂量分别为 0、0.1、1、5 mg·L^{-1}，抗性水平依据陈蕾丽的敏感基线 0.45±0.11 mg·L^{-1}进行判别，当菌株的 EC_{50}值小于敏感基线的 5 倍时，即为敏感菌株；当菌株的 EC_{50}值处于敏感基线的 5~10 倍时，即为低抗菌株，当菌株的 EC_{50}值处于敏感基线 10~40 倍，即为中抗菌株；当某菌株的 EC_{50}值大于敏感基线的 40 倍，即为高抗菌株[18-19]。

2　结果与分析

2.1　葡萄炭疽病菌对代森锰锌的敏感性分析

利用菌丝生长速率法分析代森锰锌对来自不同葡萄栽培区的 120 株葡萄炭疽病菌菌株的 EC_{50}值及抗感菌株数量，结果见表 2。总体看，辽宁产区分离的 120 株葡萄炭疽病菌对代森锰锌的抗药性菌株为 34 株，抗性频率为 28.33%，其中低抗菌株 21 株，抗性频率为 17.5%，中抗菌株均为 13 株，抗性频率为 10.83%，抗性水平较高。其中，葫芦岛地区葡萄炭疽菌对代森锰锌抗性频率最高，达 45%。从 EC_{50}分布值分析，其中大连和本溪地区存在较高抗性菌株，EC_{50}最高分别达到 65.934 3 mg·L^{-1}和 50.009 1 mg·L^{-1}。

2.2　葡萄炭疽病菌对戊唑醇的敏感性分析

利用菌丝生长速率法分析戊唑醇对来自不同葡萄栽培区的 120 株葡萄炭疽病菌菌株的 EC_{50}值及抗感菌株数量，结果见表 3。在测定的 120 个菌株中，葡萄炭疽病菌对戊唑醇的抗药性菌株 35 株，其中高抗菌株为 0 株，低抗菌株 35 株，抗性频率为 29.17%。不同地区的葡萄炭疽病菌对戊唑醇的抗药性频率 16.67%~45.00%，沈阳地区葡萄炭疽抗性频率最低为 16.67%，葫芦岛地区葡萄炭疽病菌最高达 45.00%，可能与不同地区栽培葡萄的历史长短有关系。但不同地区不同菌株的 EC_{50}值相差较少。

2.3　葡萄炭疽病菌对咪鲜胺的敏感性分析

利用菌丝生长速率法分析咪鲜胺对来自不同葡萄栽培地区的 120 株葡萄炭疽病菌菌株的 EC_{50}值及抗感菌株数量，结果见表 4。葡萄炭疽病菌在含有 0.1 mg·L^{-1}咪鲜胺的 PDA 平板上生长受到抑

表2 不同栽培区的葡萄炭疽病菌对代森锰锌的抗性及抗性频率

Table 2 Resistance frequency of *C. gloeosporioides* to mancozeb in different viticulture zones

葡萄栽培区 Viticulture zones	菌株数 No. of isolates	EC_{50}范围 Scope of EC_{50}/(mg·L^{-1})	抗性菌株表现 Phenotype of resistant isolates				抗性菌株数(抗性频率) Resistant isolates No.(Resistance frequency/%)
			敏感 Sensitive	低抗 Low resistant	中抗 Middle resistant	高抗 High resistant	
沈阳 Shenyang	18	11.156 5~21.859 2	13	3	2	0	5(27.78)
大连 Dalian	22	14.616 2~65.934 3	18	2	2	0	4(18.18)
葫芦岛 Huludao	20	15.589 7~21.843 7	11	5	4	0	9(45.00)
朝阳 Chaoyang	21	16.300 2~19.345 1	15	3	3	0	6(28.57)
营口 Yingkou	19	13.085 1~17.004 7	14	4	1	0	5(26.32)
本溪 Benxi	20	15.488 0~50.009 1	15	4	1	0	5(25.00)
合计 Total	120	11.156 5~65.934 3	86	21	13	0	34(28.33)

表3 不同栽培区的葡萄炭疽病菌对戊唑醇的抗性及抗性频率

Table 3 Resistance frequency of *C. gloeosporioides* to tebuconazole in different viticulture zones

葡萄栽培区 Viticulture zones	菌株数 No. of isolates	EC_{50}范围 Scope of EC_{50}/(mg·L^{-1})	抗性菌株表现 Phenotype of resistant isolates			抗性菌株数(抗性频率) Resistant isolates No.(Resistance frequency/%)
			敏感 Sensitive	低抗 Low resistant	高抗 High resistant	
沈阳 Shenyang	18	0.920 1~1.202 0	15	3	0	3(16.67)
大连 Dalian	22	0.677 1~1.991 8	15	7	0	7(31.82)
葫芦岛 Huludao	20	1.551 5~2.691 0	11	9	0	9(45.00)
朝阳 Chaoyang	21	1.730 8~2.021 0	15	6	0	6(28.57)
营口 Yingkou	19	0.742 4~1.788 0	14	5	0	5(26.32)
本溪 Benxi	20	0.929 6~1.808 6	15	5	0	5(25.00)
合计 Total	120	0.677 1~2.691 0	85	35	0	35(29.17)

制，平均抑制率为96.37%，在含1 mg·L^{-1}咪鲜胺的PDA平板上不生长。咪鲜胺对葡萄炭疽病菌株的EC_{50}均值为0.011 46 mg·L^{-1}，没有发现抗性菌株。

2.4 葡萄炭疽病菌对苯醚甲环唑的敏感性分析

利用菌丝生长速率法分析葡萄炭疽病菌对苯醚甲环唑的敏感度，结果如表5所示。葡萄炭疽病菌在含有1 mg·L^{-1}苯醚甲环唑的PDA平板上生长受到抑制，平均抑制率为72.44%，在含5 mg·L^{-1}苯醚甲环唑的PDA平板上不生长。菌株的EC_{50}值小于敏感基线的5倍，未发现抗性菌株。研究表明各不同地区的葡萄炭疽病菌对苯醚甲环唑的抗性水平较低，且EC_{50}值相差较少。

3 讨 论

如前所论，葡萄栽培面积和产量在世界果树生产中占有重要地位，但长期遭受炭疽病等多种病害危害，需要倍加关注[20]。解决好葡萄炭疽病的防治及其病原菌的抗药性等问题，对保障葡萄安全生产具有重要实践意义。

通过区分剂量法发现，辽宁产区葡萄炭疽病菌已经对代森锰锌和戊唑醇产生抗性，代森锰锌属于多作用位点杀菌剂,主要抑制菌体内丙酮酸的氧化,使丙酮酸氧化过程的二硫辛酸脱氢酶中的硫氢基结合,进而杀死病菌[21-22]，因其内吸性差，一般使用较

表4　不同栽培区的葡萄炭疽病菌对咪鲜胺的抗性及抗性频率

Table 4　Resistance frequency of *C. gloeosporioides* to prochloraz in different viticulture zones

葡萄栽培区 Viticulture zones	菌株数 No. of isolates	EC_{50}范围 Scope of EC_{50}/(mg·L^{-1})	抗性菌株表现 Phenotype of resistant isolates			抗性菌株数(抗性频率) Resistant isolates No.(Resistance frequency/%)
			敏感 Sensitive	低抗 Low resistant	高抗 High resistant	
沈阳 Shenyang	18	0.010 1~0.018 0	18	0	0	0
大连 Dalian	22	0.009 4~0.014 0	22	0	0	0
葫芦岛 Huludao	20	0.009 0~0.012 0	20	0	0	0
朝阳 Chaoyang	21	0.009 6~0.014 0	21	0	0	0
营口 Yingkou	19	0.008 8~0.013 4	19	0	0	0
本溪 Benxi	20	0.009 8~0.014 0	20	0	0	0
合计 Total	120	0.008 8~0.018 0	120	0	0	0

表5　不同栽培区的葡萄炭疽病菌对苯醚甲环唑的抗性及抗性频率

Table 5　Resistance frequency of *C. gloeosporioides* to difenoconazole in different viticulture zones

葡萄栽培区 Viticulture zones	菌株数 No. of isolates	EC_{50}范围 Scope of EC_{50}/(mg·L^{-1})	抗性菌株表现 Phenotype of resistant isolates			抗性菌株数(抗性频率) Resistant isolates No.(Resistance frequency/%)
			敏感 Sensitive	低抗 Low resistant	高抗 High resistant	
沈阳 Shenyang	18	0.033 0~0.103 2	18	0	0	0
大连 Dalian	22	0.041 7~0.075 7	22	0	0	0
葫芦岛 Huludao	20	0.043 5~0.090 7	20	0	0	0
朝阳 Chaoyang	21	0.041 2~0.101 9	21	0	0	0
营口 Yingkou	19	0.035 7~0.065 7	19	0	0	0
本溪 Benxi	20	0.023 0~0.077 0	20	0	0	0
合计 Total	120	0.023 0~0.103 2	120	0	0	0

高剂量，加之代森锰锌使用历史较长，可能是产生高频率抗药性菌株的原因。有研究者从代森锰锌的合成、理化性质、毒理方面进行研究，为合理使用该类杀菌剂提供理论指导[23]。有研究发现，在防治番茄枯萎病中可以利用聚乙二醇(PEG)的特性进一步开发控释代森锰锌的纳米制剂，使其在施用后的一段合理的时间内，仍然可以有足够量的活性成分，从而减少了农药的施用次数[24]。DMIs 类杀菌剂戊唑醇，通过抑制真菌中麦角甾醇生物合成途径中关键酶 CYP51 的合成，进而阻碍麦角甾醇的合成，但该类药剂作用位点相对单一，可能是产生抗药性菌株的重要原因。DMIs 类杀菌剂戊唑醇虽然是防治炭疽病的常用药剂，但是长期不合理的使用该药剂促使抗药性菌株的产生[25]。有报道称，吡唑醚菌酯和戊唑醇以质量比 1∶1 混合后会对葡萄炭疽病产生明显的抑制作用，在果穗套袋前使用能使病害防治效果达到 90%以上[26]。该杀菌剂不仅能够对植物病害产生防御作用，也会影响动物代谢过程，研究者可以通过 mRNA 的表达量来评估戊唑醇的毒性[27]。本次研究发现，辽宁不同地区供试菌株对戊唑醇和代森锰锌的敏感性有明显不同，可能与所在地区农药的使用量及使用年限有关，为了使葡萄炭

疽菌的防效效果较好，尽量减少对戊唑醇和代森锰锌的使用量。

通过对病菌进行区分剂量法和菌丝生长速率法研究发现，葡萄炭疽病菌对咪鲜胺和苯醚甲环唑较为敏感，咪鲜胺和苯醚甲环唑对葡萄炭疽病菌的EC_{50}均值为0.011 46 mg·L^{-1}和0.061 07 mg·L^{-1}，未发现有抗性菌株。但是在杧果炭疽病 *Colletotrichum gloeosporioides* 和黄瓜枯萎病 *Fusarium oxysporum* f. sp. *cucumerinum* 防治时却发现咪鲜胺存在抗药性风险[28]。且有研究发现水稻恶苗病菌对咪鲜胺的敏感性有明显下降[29]。栗疫病菌 *Endothia parasitica* 及番茄灰霉病菌 *Botrytis cinera* 对苯醚甲环唑产生了低水平抗性[30-31]。在咪鲜胺安全试验中，通过农药动态降解试验，发现咪鲜胺在预防生姜炭疽病的过程中需要在发病早期喷药两次，两次间隔7 d效果最好[32]。咪唑类杀菌剂咪鲜胺通过影响甾醇分子的生物合成，从而破坏细胞膜的功能[33]，该药剂属于甾醇合成抑制剂。有研究表明,咪鲜胺和吡唑醚菌酯的复配对胶孢炭疽菌的抑菌效果较好[34]，多菌灵和咪鲜胺1∶7的比列混合液对控制枯萎病具有一定作用[35]。

苯醚甲环唑的致病原理是通过抑制细胞壁甾醇的生物合成，阻止真菌的生长，该药剂不仅属于甾醇合成抑制剂，也是甾醇脱甲基化抑制剂。植物病原菌对杀菌剂的抗性风险是由药剂和病害共同决定的[36]，需考虑到药剂的选择和病菌的自身抗性。因此，为延缓葡萄炭疽病菌对咪鲜胺及苯醚甲环唑抗药性的发展，需把握好农药的使用用量，根据不同农药的作用位点进行农药的交替使用。

4 结 论

辽宁产区葡萄炭疽病菌对戊唑醇和代森锰锌产生抗性，其中尤以葫芦岛地区抗性菌株频率较高，抗性菌株达45%。因此，使用戊唑醇和代森锰锌时要根据不同地区的抗性情况进行使用，葫芦岛地区建议暂时停止使用戊唑醇和代森锰锌。未发现对咪鲜胺和苯醚甲环唑产生抗药性的菌株。但建议要交替使用相关药剂，避免抗药性菌株的出现。

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