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江苏丘陵地区草莓灰霉病菌(*Botrytis cinerea*)对QoIs类杀菌剂的抗药性研究

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摘 要:【**目的**】明确江苏丘陵地区草莓灰霉病菌(*Botrytis cinerea*)对甲氧基丙烯酸酯类杀菌剂(QoIs)的抗药性区域分布和抗药性分子机制,为抗药性治理提供依据。【**方法**】区分计量法和抑制中浓度法测定草莓灰霉病菌对嘧菌酯和吡唑醚菌酯的抗药性区域分布和对药剂的敏感性表型,采用草莓果实离体测定法评估药剂对不同药剂敏感性菌株的防效,通过药剂靶标基因的序列分析确定抗药性分子机制。【**结果**】236株草莓灰霉病菌中对嘧菌酯和吡唑醚菌酯呈抗性的菌株有192株,2个药剂呈正交互抗性。嘧菌酯和吡唑醚菌酯敏感菌株的平均抑菌中质量浓度(EC_{50}值)分别为0.269 8和0.055 9 mg·L^{-1}。77个嘧菌酯抗性菌株的EC_{50}值全部大于100 mg·L^{-1},而吡唑醚菌酯抗性菌株的EC_{50}平均值为67.680 7 mg·L^{-1}。接种抗性菌株后再防治的试验证明,嘧菌酯和吡唑醚菌酯在推荐剂量(a.i 166.67 mg·L^{-1})下失去防效;所有抗性菌株的*cyt b*基因上都只含有G143A点突变,且第143位氨基酸后均不含有内元(Bcbi-143/144);敏感菌株*cyt b*基因分为2种,47.4%的敏感菌株不含有Bcbi-143/144,而52.6%的敏感菌株含有Bcbi-143/144。【**结论**】江苏丘陵地区田间草莓灰霉病菌群体中,对QoIs类药剂产生高水平抗药性的种群已成为主导种群,生产中不宜再用该类药剂防治草莓灰霉病。所有田间采集灰霉病菌抗性菌株的抗性分子机制均为*cyt b*基因G143A点突变,未发现其他位点突变类型。

关键词:草莓;灰霉病菌;嘧菌酯;吡唑醚菌酯;抗药性;分子机制

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Resistance to QoIs fungicides in *Botrytis cinerea* populations for strawberries in hilly area of Jiangsu

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Abstract:【Objective】Gray mold caused by *Botrytis cinerea* is a major fungal disease for strawberries in Jiangsu province, which is considered as one of the most important strawberry-producing areas in China. Recently, effective management of this disease has become an urgent need for the farmers as a result of the failure of many fungicides in controlling this pathogen. In order to investigate the resistance distribution and molecular mechanisms of methoxy-acrylates fungicides (QoIs) in *Botrytis cinerea* isolates, samples were collected from the strawberry fields in Jiangsu province and were used to provide measures of the resistance management.【Methods】Employing the methods of discriminative dose (a concentration that fully inhibits mycelial growth of the sensitive strains) and effective concentration (inhibits mycelia growth by 50% relative to the control, EC_{50}) values were identified to distinguish sensitivity to azosxystrobin and pyraclostrobin , fungicides resistance distribution and resistance phenotypes of *B. cinerea* isolates to azosxystrobin and pyraclostrobin. According to previous studies, the discriminatory concentration of azosxystrobin and pyraclostrobin was 10 mg·L^{-1}, and the plates were amended with 100 mg·L^{-1} salicylhydroxamic acid (SHAM) to inhibit the alternative respiratory pathways. Then the effective concentration that inhibits mycelia growth by 50% relative to the control (EC_{50}) values of azoxystrobin was determined

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for 19 sensitive isolates and 77 resistant isolates. Azoxystrobin or pyraclostrobin solution was added to PDA to produce final concentrations of 0, 0.01, 0.03, 0.1, 0.3 and 1 mg·L^{-1} for sensitive isolates and 0, 3, 10, 30, 100 and 300 mg·L^{-1} for resistant isolates. The plates were amended by using 100 mg·L^{-1} SHAM to further inhibit the alternative respiratory pathway. For each isolate, three replicates per concentration were used. Disease severity on detached strawberry fruit for different resistance phenotypes of *B. cinerea* isolates to azoxystrobin and pyraclostrobin were tested. Furthermore, the molecular mechanisms of QoIs fungicides were determined by the sequence analysis of target genes. First, DNA from fungal mycelia was extracted using a DNA kit. Two primer pairs, cytb129-F(5’-GCATAAAGC ATTGGGGCTAA-3’)+ cytb129- R (5’ - CCGTCTGGCGTCACTATAAAT- 3’), Qo13ext (5’ - GGTATAACCCGACGGG GTTATAGAATAG-3’)+ Qo14ext(5’-AACCATCTCCATCCACCATACCTAC A AA-3’)were used. PCR products were examined by electrophoresis in a 1.2 % agarose gel in 1×TAE buffer.【Results】236 isolates were collected from the Jiangning district of Nanjing city and the Jurong district of Zhenjing city in Jiangsu province. Among the 236 isolates, 192 (81.4%) showed resistance and 44 (18.6%) showed sensitivity to QoIs fungicides by using the methods of the discriminative dose. A positive cross resistance existed between azoxystrobin and pyraclostrobin. There were different frequencies of resistance in different sampling areas. The frequency of resistance from high to low were Huayang town (100%), Baitu town (88.9%), Shishi town (83.3%), Chunhua town (83.3), Maoshan town (80%), Tuqiao town (70.8%), Tianwang town (70.0%) and Houbai town (52.0%). The 19 sensitive isolates were divided into sensitive isolates（EC_{50}<1 mg·L^{-1}）and decreased sensitivity isolates（1≤EC_{50}< 5 mg·L^{-1}）. The average EC_{50} values of the 15 sensitive isolates and 4 decreased sensitivity isolates to azoxystrobin were 0.269 8 mg·L^{-1} and 4.159 6 mg·L^{-1}, respectively. The EC_{50} values of the 18 sensitive isolates were 0.025 7-0.112 7 mg·L^{-1} to pyraclostrobin. The average values were 0.055 9 mg·L^{-1}and the 1 decreased sensitivity isolates were 1.598 8 mg·L^{-1}. The EC_{50} values of the whole 77 azoxystrobin-resistance isolates were more than 100 mg·L^{-1}. The 77 pyraclostrobin-resistance isolates were divided into moderate resistance isolates（10≤EC_{50}<100 mg·L^{-1}, Pyr^{MR}）and highly resistance isolates（EC_{50}≥100 mg·L^{-1}, Pyr^{HR}）. The average EC_{50} values of the 64 Pyr^{MR} (83.12%) were 53.070 9 mg·L^{-1}. The detached strawberry fruit assay inoculated resistant isolates indicated that there was no or less control efficiency when using the recommended doses (a.i 166.67 mg·L^{-1}) of azoxystrobin and pyraclostrobin. All of the resistant isolates harbored the G143A point mutation. *B. cinerea* populations were divided into two types according to the structure of the *cyt b* gene, with or without the third intron (Bcbi-143/144). All the resistance isolates and some sensitive isolates (47.4%) were without Bcbi-143/144, however, the 52.6% sensitive isolates had Bcbi-143/144.【Conclusion】The examination of the population of high level resistance isolates from the strawberry fields of the Jiangsu province hilly area showed that the QoIs fungicides had become the dominant population (81.4%). Detached strawberry fruit assay inoculated resistant isolates indicated, that the control efficiencies were 3.02% and 18.91%, respectively, when using the recommended doses (a.i 166.67 mg·L^{-1}) of azoxystrobin and pyraclostrobin. We recommend that QoIs fungicides should not be used in the protection of strawberry gray mold. As tested, all the resistant isolates harbored the G143A point mutation. *B. cinerea* populations were divided into two types according to the structure of the *cyt b* gene, with or without the third intron Bcbi-143/144.

Key words: Strawberry; *Botrytis cinerea*; Azoxystrobin; Pyraclostrobin; Resistance to QoIs fungicides; Molecular mechanism

江苏省丘陵地区（镇江市和南京市）在国内最早种植草莓，其发展规模及产业化开发水平在国内乃至东南亚享有较高知名度，尤其是句容市早在20世纪80年代即被誉为“中国草莓第一乡”，2003年4月被授予“中国草莓之乡”称号。几十年来，草莓的栽培模式不断创新，由最初的露天栽培为主，到冬季设

施大棚栽培为主。然而，在封闭的设施大棚栽培模式下，由灰葡萄孢菌（*Botrytis cinerea*）引起的草莓灰霉病近几年来较难防控，因此其防治措施和常用药剂抗药性的研究都备受关注。

草莓灰霉病的化学常规用药以多菌灵、腐霉利、异菌脲、嘧霉胺等杀菌剂为主[1-2]，与其相关的抗药性研究也已经有很多报道。近年来，在国内外田间已检测到该病原菌对苯并咪唑类、二甲酰亚胺类、苯胺基嘧啶类、甲氧基丙烯酸酯（QoIs）类及琥珀酸脱氢酶抑制剂（SDHIs）类杀菌剂产生抗性的菌株，这些抗性菌株可能与靶标位点的改变或多药抗药性机制有关[3-6]。甲氧基丙烯酸酯类杀菌剂（QoIs）具有抗菌谱广、作用活性高、作用机制独特和与其他现有的杀菌剂不存在正交互抗性等优点，是近年来防治草莓病害的主打药剂，生产应用主要包括嘧菌酯、吡唑醚菌酯、肟菌酯等。该类药剂作用于真菌线粒体呼吸链上的复合体Ⅲ，通过与线粒体*cyt b*和c1复合体Qo部位的结合而抑制呼吸链上的电子传递，进而阻碍病原真菌的ATP合成，干扰细胞正常分裂和生长，造成菌体死亡，故这类化合物被称为QoIs类杀菌剂[7-10]。研究表明，灰霉病菌*cyt b*基因第143、129和137位氨基酸密码子的突变是引起灰霉病菌对QoIs药剂呈现出不同抗性表型的分子机制[11]，其中含G143A突变的菌株表现为高抗，含F129L和G137R突变的菌株则表现为中抗，而灰霉病菌田间抗性种群的产生主要是G143A，且该突变引起的抗性水平稳定[12]。

嘧菌酯（25%阿米西达SC）和吡唑醚菌酯（25%凯润EC）是江苏丘陵地区（镇江、南京）草莓和蔬菜病害防治的主打药剂。在一季草莓生产全程中应用4次以上，长期多频率高剂量应用，使得草莓灰霉病菌和炭疽病菌等病原菌对该类药剂（QoIs）产生了严重的抗药性。最近2 a，生产上出现防治效果下降甚至失去防效的反馈。鉴于此，本研究着眼于本地区生产实际，结合农户化学防治主打药剂，检测本地区草莓灰霉病菌对QoIs类药剂的抗性，明晰抗性区域分布，并通过分子手段揭示抗性机制，以期为生产中指导科学用药提供依据。

1　材料和方法

1.1　菌株来源

2015年4月分别在南京市江宁区和镇江市句容市等8个镇30个草莓园随机采取草莓灰霉病菌，实验室内分离获得236株单孢菌株。采用滤纸片法保存于−20 ℃冰箱，备用。

1.2　供试药剂

95.4%嘧菌酯（azoxystrobin）原药，由先正达中国有限公司提供；99%吡唑醚菌酯（pyraclostrobin）原药，由拜耳中国有限公司提供。药剂均用丙酮配制成10 000 mg·L^{-1}的母液。99%水杨肟酸（salicylhydroxamic acid，SHAM），从Sigma-Aldrich公司购得，用甲醇配制成20 000 mg·L^{-1}的母液。所有母液均置于4 ℃保存备用。

1.3　灰霉病菌对嘧菌酯和吡唑醚菌酯抗药性检测

嘧菌酯和吡唑醚菌酯的区分剂量均设置为10 mg·L^{-1}，为防止旁路氧化[13]，培养基（MEA）中均含有SHAM 100 mg·L^{-1}，设不加药剂为空白对照，23 ℃培养3 d后观察。平板上正常生长的确定为抗性菌株，不能正常生长的确定为敏感菌株。所有抗性菌株均进行2次重复测定确认。

1.4　灰霉病菌对嘧菌酯和吡唑醚菌酯的敏感性

采用菌丝生长速率法，挑取19株敏感菌株（事先在MEA平板上活化培养3 d）直径4 mm的菌丝块，移到含系列质量浓度0.01、0.03、0.1、0.3和1 mg·L^{-1}的嘧菌酯和吡唑醚菌酯MEA平板（同时含100 mg·L^{-1} SHAM）正中央；同样方法分别测定77株抗性菌株的敏感性，所不同的是嘧菌酯和吡唑醚菌酯的质量浓度设为3、10、30、100和300 mg·L^{-1}，以不含药剂但含100 mg·L^{-1} SHAM的平板为对照，每处理4次重复。23 ℃培养3 d后用十字交叉法测量各处理的菌落直径（cm），取平均值计算抑制率（%），利用DPS软件，通过浓度对数值（x）与抑制率几率值（y）之间的线性回归关系，求出毒力回归方程和有效抑制中浓度（EC_{50}）。菌株敏感性表型划分按如下方法：EC_{50}值小于1 mg·L^{-1}的菌株视为敏感菌株（S），EC_{50}值1~5 mg·L^{-1}的菌株视为敏感性下降菌株（RS），EC_{50}值5~10 mg·L^{-1}的菌株定为低抗菌株（LR），EC_{50}值10~100 mg·L^{-1}的菌株定为中抗菌株（MR），EC_{50}值大于100 mg·L^{-1}的菌株定为高抗菌株（HR）。

1.5　抗药性分子机制

为明确抗性菌株的抗性机制，选取19株敏感菌株和77株抗性菌株，按DNA试剂盒（OMEGA）提取基因组DNA。引物序列[14-15]：cytb129-F（5’-GCATAAAGCATTGGGGCTAA-3’）+ cytb129-R（5’-CC-

GTCTGGCGTCACTATAAA T－3'）和Qo13ext（5'－GGTATAACCCGACGGGGTTATAGAATAG－ 3'）＋ Qo14ext（5'－AACCATCTCCATCCA CCATACCTA-CAAA-3'）。采用50 μL反应体系，引物cytb129-F/cytb129-R的反应参数为：95 ℃预变性3 min，94 ℃变性40 s，55 ℃退火40 s，72 ℃延伸1.5 min，40个循环，最后72 ℃延伸5 min。引物Qo13ext/Qo14ext反应参数为：95 ℃预变性3 min，95 ℃变性30 s，60 ℃退火30 s，68 ℃延伸1 min，40个循环，最后68 ℃延伸4 min。

PCR产物经琼脂糖凝胶电泳检测后由南京金斯瑞生物科技有限公司进行产物纯化和序列测定。抗性靶标基因序列用DNASRAR进行序列编辑，比对分析利用NCBI中的BLAST程序。

1.6 草莓果实室内接种灰霉病菌防效测定

选取对嘧菌酯和吡唑醚菌酯呈双抗和双敏感的菌株B15-9（Azo^{S}Pyr^{S}）和B15-187（Azo^{HR}Pyr^{MR}），23 ℃室内培养3 d，用打孔器打孔（直径2 mm）备用。将田间采回的'红颊'草莓（7~8成熟）洗净，用75%酒精进行表面消毒，超纯水冲洗3次后风干。将嘧菌酯（25%阿米西达SC）和吡唑醚菌酯（25%凯润EC）分别按推荐剂量稀释1 500倍（a.i 166.67 mg·L^{-1}）后，用喉头喷雾器（扬州平安医疗器械有限公司）均匀喷施于草莓果实，置于吸水纸上风干，对照用清水喷雾后风干，每处理3次重复（18个/处理）。将处理和对照草莓果实置于铺有吸水纸的塑料盒中保湿，用昆虫针（00号）在草莓果实表面刺3个微伤口后接种菌丝块，置于23 ℃光照培养箱（光：暗＝16 h：8 h）中，72 h后检查结果，测量病斑直径，计算防治效果。

2 结果与分析

2.1 草莓灰霉病菌对嘧菌酯和吡唑醚菌酯的抗药性检测

为准确快速区分出草莓灰霉病菌对QoIs类药剂的抗药性，以能在10 mg·L^{-1}吡唑醚菌酯或嘧菌酯的MEA平板上正常生长的菌株判定为抗性菌株，不能正常生长的菌株判定为敏感菌株。研究显示，灰霉病菌对吡唑醚菌酯和嘧菌酯的抗药性呈现出正交互抗性，因此检测结果中灰霉病菌对嘧菌酯和吡唑醚菌酯的抗性频率完全相同。按该方法获得对嘧菌酯和吡唑醚菌酯都表现出抗性和都表现敏感的菌株分别为192株（81.4%）和44株（18.6%）（表1）。不同取样地区抗性频率由高到低分别为华阳镇（100%）、白兔镇（88.9%）、石狮镇（83.3%）、淳化镇（83.3%）、茅山镇（80.0%）、土桥镇（70.8%）、天王镇（70.0%）和后白镇（52.0%）（图1）。以上结果表明，检测地区田间草莓灰霉病菌对嘧菌酯和吡唑醚菌酯呈抗性的种群成为主导种群。

表1　江苏草莓灰霉病菌菌株信息

Table 1 Information of *Botrytis cinerea* isolates collected from field strawberry in Jiangsu

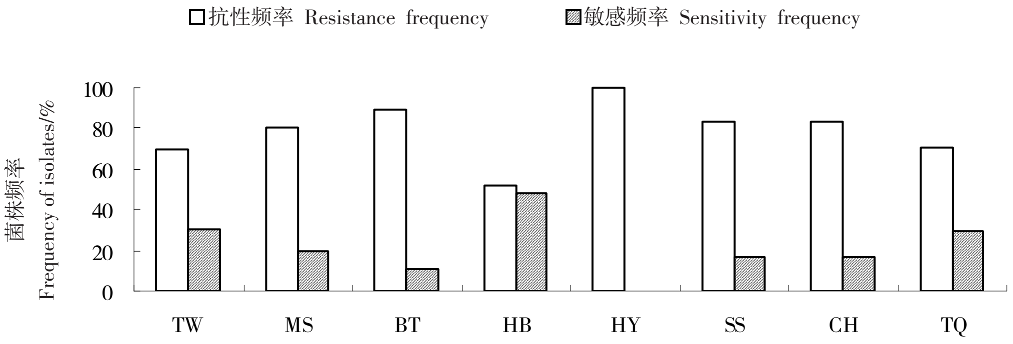
采样地点 Sampling location	菌株数 No. of isolates	敏感菌株数 No. of Azo^{S}Pyr^{S}	抗性菌株数 No. of Azo^{R}Pyr^{R}
句容市天王镇 Tianwang town of Jurong city	10	3	7
句容市茅山镇 Maoshan town of Jurong city	40	8	32
句容市白兔镇 Baitu town of Jurong city	90	10	80
句容市后白镇 Houbai town of Jurong city	25	12	13
句容市华阳镇 Huayang town of Jurong city	23	0	23
句容市石狮镇 Shishi town of Jurong city	6	1	5
南京市淳化镇 Chunhua town of Nanjing city	18	3	15
南京市土桥镇 Tuqiao town of Nanjing city	24	7	17
总数 Total	236	44	192

注：Azo^{S}Pyr^{S}表示对嘧菌酯和吡唑醚菌酯都呈现出敏感的菌株，Azo^{R}Pyr^{R}表示对嘧菌酯和吡唑醚菌酯都呈现出抗性的菌株。

Note: Azo^{S}Pyr^{S} indicates sensitive to azoxystrobin and pyraclostrobin; Azo^{R}Pyr^{R} indicates resistance to azoxystrobin and pyraclostrobin.

2.2 灰霉病菌对嘧菌酯和吡唑醚菌酯的敏感性

对嘧菌酯敏感的19株草莓灰霉病菌中，根据EC_{50}值分布范围可细分为敏感菌株（EC_{50}<1 mg·L^{-1}，Azo^{S}）和敏感性下降菌株（1≤EC_{50}<5 mg·L^{-1}，Azo^{RS}）；15株Azo^{S}的EC_{50}值为0.062 8~0.875 5 mg·L^{-1}，平均值为0.269 8 mg·L^{-1}，4株Azo^{RS}的平均EC_{50}值为4.159 6 mg·L^{-1}（图2-A）；77株嘧菌酯抗性菌株的EC_{50}值全部大于100 mg·L^{-1}，表现出高抗（EC_{50}>100 mg·L^{-1}，Azo^{HR}）（图2-B）。同样方法区分出19株对吡唑醚菌酯敏感的草莓灰霉病菌中，有18株敏感菌株（Pyr^{S}）和1株敏感性下降菌株（Pyr^{RS}），18株Pyr^{S}的EC_{50}值为0.025 7~0.112 7 mg·L^{-1}，平均值为0.055 9 mg·L^{-1}，1株Pyr^{RS}的EC_{50}值为1.598 8 mg·L^{-1}（图2-C）；对吡唑醚菌酯抗性的77株菌株中，根据EC_{50}的



TW. 句容市天王镇;MS. 句容市茅山镇;BT. 句容市白兔镇;HB. 句容市后白镇;HY. 句容市华阳镇;SS. 句容市石狮镇;CH. 南京市淳化镇;TQ. 南京市土桥镇。

TW. Tianwang town of Jurong; MS. Maoshan town of Jurong; BT. Baitu town of Jurong; HB. Houbai town of Jurong; HY. Huayang town of Jurong; SS. Shishi town of Jurong; CH. Chunhua town of Nanjing; TQ. Tuqiao town of Nanjing.

图1　草莓灰霉病菌对嘧菌酯和吡唑醚菌酯的抗药性检测

Fig. 1 Frequency distribution of different resistant populations of *Botrytis cinerea* collected from different strawberry locations

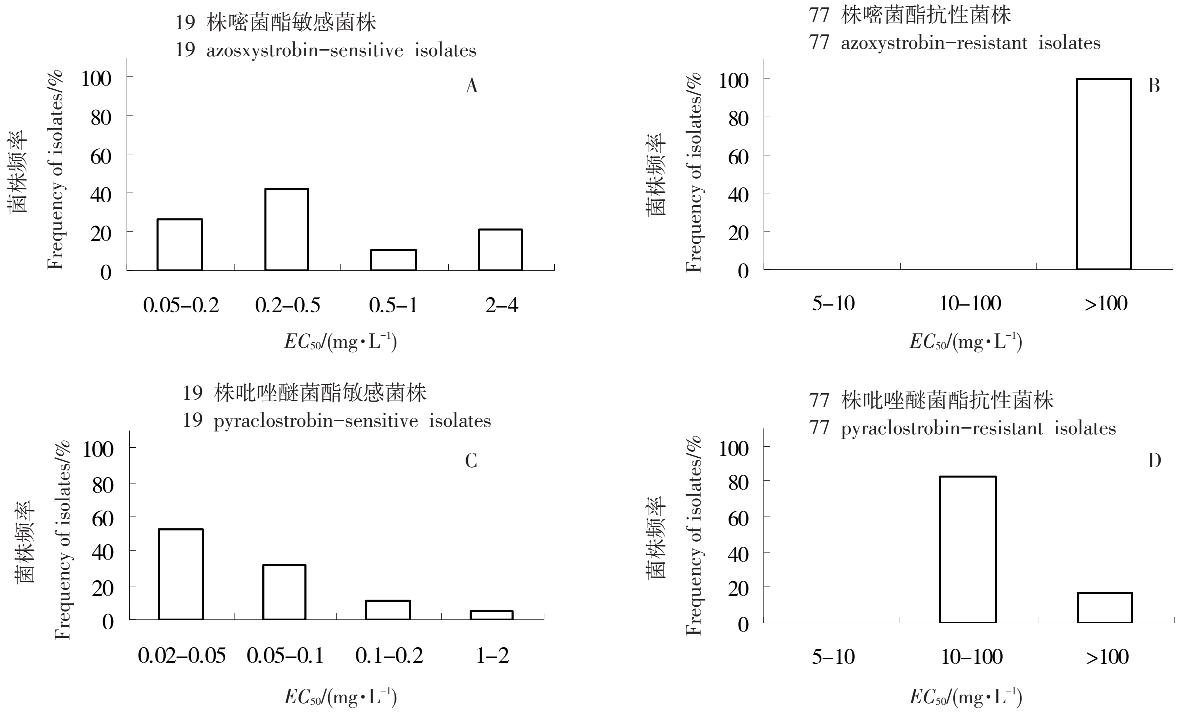


图2　草莓灰霉病菌对嘧菌酯和吡唑醚菌酯 EC_{50} 分布频率

Fig. 2 Frequency distribution of the effective concentrations resulting in 50% mycelial growth inhibition（EC_{50}）

范围分为中抗菌株（$10\leqslant EC_{50}<100$ mg·L^{-1}, Pyr^{MR}）和高抗菌株（$EC_{50}\geqslant100$ mg·L^{-1}, Pyr^{HR}）, Pyr^{MR}有64株占总抗性菌株的83.12%,其EC_{50}均值为53.070 9 mg·L^{-1}, Pyr^{HR}有13株（图2-D）,本研究中未发现对嘧菌酯和吡唑醚菌酯的低抗菌株（$5\leqslant EC_{50}<10$ mg·L^{-1}, Pyr^{LR}）。

2.3　草莓灰霉病菌对嘧菌酯和吡唑醚菌酯的抗药性分子机制

对19株敏感菌株和77株抗性菌株的抗性靶标基因(*cyt b*)测序结果分析表明,所有77株抗性菌株*cyt b*基因第143位密码子由GGC（编码甘氨酸,G）突变成为GCC(编码丙氨酸,A)即G143A突变;而19株敏菌株*cyt b*基因上均不含有该突变(表2)。研究发现根据灰霉病菌*cyt b*基因G143位点后是否含有

表2 草莓灰霉病菌 *cyt b* 基因突变及敏感性表型检测

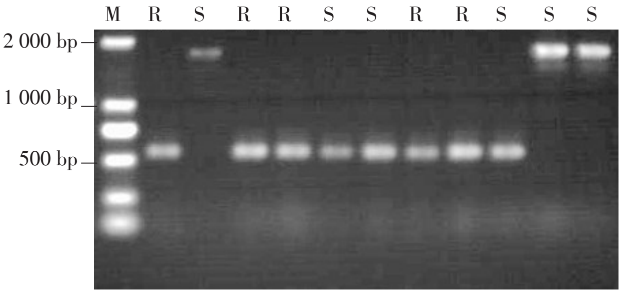
Table 2 Mutation in *cyt b* gene and corresponding resistant phenotypes in *Botrytis cinerea* isolates collected from field strawberry in Jiangsu

菌株数 No. of isolates	*cyt b* 基因突变位点 Mutation site of *cyt b* gene	抗性表型 Resistance phenotype		Bcbi-143/144 intron	菌株 Isolate
		嘧菌酯 Azoxystrobin	吡唑醚菌酯 Pyraclostrobin		
9		S	S	-	B15-27、B15-47、B15-70、B15-72、B15-117、B15-123、B15-222、B15-228、B15-245
6		S	S	+	B15-8、B15-9、B15-10、B15-71、B15-124、B15-255
3		RS	S	+	B15-126、B15-197、B15-211
1		RS	RS	+	B15-116
64	G143A	HR	MR	-	B15-1、B15-2、B15-7、B15-11、B15-13、B15-14、B15-28、B15-35、B15-39、B15-40、B15-43、B15-44、B15-53、B15-54、B15-56、B15-59、B15-64、B15-65、B15-68、B15-78、B15-79、B15-83、B15-85、B15-87、B15-93、B15-95、B15-96、B15-106、B15-108、B15-113、B15-121、B15-134、B15-136、B15-137、B15-139、B15-143、B15-147、B15-151、B15-154、B15-155、B15-156、B15-157、B15-160、B15-165、B15-166、B15-168、B15-177、B15-178、B15-180、B15-184、B15-187、B15-188、B15-189、B15-192、B15-206、B15-208、B15-213、B15-225、B15-230、B15-239、B15-248、B15-250、B15-251、B15-254
13	G143A	HR	HR	-	B15-4、B15-32、B15-48、B15-60、B15-62、B15-80、B15-82、B15-109、B15-146、B15-152、B15-158、B15-194、B15-223

注：S. 敏感；RS. 敏感性下降；LR. 低抗；MR. 中抗；HR. 高抗；+. 含 Bcbi-143/144。

Note: S. Sensitivity; RS. Reduced sensitivity; LR. Low resistance; MR. Moderately resistance; HR. Highly resistance；+ represents containing Bcbi-143/144 intron.

1 205 bp的内元（Bcbi-143/144）可分为2种类型。本研究中所有抗性菌株的*cyt b*中不含Bcbi-143/144；敏感菌株*cyt b*中有的含Bcbi-143/144（52.7%），有的不含Bcbi-143/144（47.3%）（图3）。



M. DNA ladder；R. 抗性菌株；S. 敏感菌株。

M. DNA ladder; R. Resistant isolates; S. Sensitive isolates.

图3 引物 Qo13ext/Qo14ext 扩增灰霉病菌 *cyt b* 基因

Fig. 3 PCR amplification of partial *cyt b* gene in *Botrytis cinerea* isolates with primers Qo13ext/Qo14ext

2.4 草莓果实接种灰霉病菌防效测定

选取对嘧菌酯和吡唑醚菌酯呈双抗和双敏感表型菌株B15-9（$\mathrm{Azo^SPyr^S}$）和B15-187（$\mathrm{Azo^{HR}Pyr^{MR}}$）进行果实离体接种防效试验。结果表明，在草莓果实有微伤口且有病原菌菌丝接种的条件下，嘧菌酯和吡唑醚菌酯在推荐质量浓度（166.67 mg·L^{-1}）下对接种敏感菌株的防治效果分别为64.61%和79.63%，而对接种抗性菌株的防治效果分别只有3.02%和18.91%（表3）。室内接种防治试验充分说明QoIs类药剂对灰霉病菌抗性种群失去防效或防效很低。

表3 嘧菌酯和吡唑醚菌酯对不同敏感性菌株的离体防治

Table 3 Disease severity on detached fruit for different resistance phenotype isolates of azoxystrobin and pyraclostrobin

处理 Treatments	ρ/(a.i mg·L^{-1})	病斑直径 Lesion size/mm		防效 Control efficiency/%	
		$\mathrm{Azo^{HR}Pyr^{MR}}$	$\mathrm{Azo^SPyr^S}$	$\mathrm{Azo^{HR}Pyr^{MR}}$	$\mathrm{Azo^SPyr^S}$
嘧菌酯 Azoxystrobin	166.67	26.78	9.77	3.02	64.61
吡唑醚菌酯 Pyraclostrobin	166.67	22.39	5.63	18.91	79.63
对照 Control		27.61	26.00		

注：$\mathrm{Azo^SPyr^S}$. 对嘧菌酯和吡唑醚菌酯均敏感；$\mathrm{Azo^{HR}Pyr^{MR}}$. 对嘧菌酯高抗和对吡唑醚菌酯中抗。

Note: $\mathrm{Azo^SPyr^S}$. Sensitive to azoxystrobin and pyraclostrobin; $\mathrm{Azo^{HR}Pyr^{MR}}$. High resistant to azoxystrobin and moderate resistance to pyraclostrobin.

3　讨　论

笔者从镇江市和南京市辖区8个乡镇的30个草莓园中采集并分离获得了236株草莓灰霉病菌单孢菌株，并对QoIs类药剂（嘧菌酯和吡唑醚菌酯）进行了抗药性检测。抗药性检测结果与生产反馈结果相印证，即在采样检测的所有乡镇的草莓灰霉病菌对嘧菌酯和吡唑醚菌酯都已产生高水平抗药性，抗药性菌株频率为52%~100%，总体平均抗药性菌株频率为81.4%，且2种药剂间呈现出完全正交互抗性。通过用草莓果实接种抗性菌株后的离体防治试验证明，当接种抗性菌株时，在推荐浓度稀释1 500倍（a.i 166.67 mg·L^{-1}）下嘧菌酯失去防效（3.02%），而吡唑醚菌酯的防治效果低于20%。

在室内培养条件下，灰霉病菌敏感菌株和抗性菌株对温度的敏感性、产孢能力和孢子萌发能力等无显著劣势[16-17]。在田间人工接种灰霉病菌敏感菌株和抗性菌株混合群体后，再经过杀菌剂处理的试验，已清楚地证明了杀菌剂对病原菌的选择作用[18]。在田间条件下，用杀菌剂防治灰霉病有利于抗性菌株获得有利的选择优势，且药剂处理后其获得有利的选择优势大于其因药剂处理而可能造成的适合度缺陷[18]，因此在持续的药剂选择下，抗性菌群逐渐成为主导种群。以上结果说明，江苏丘陵地区草莓灰霉病对QoIs类药剂抗药性的群体已成为主导种群，和新型QoIs类药剂吡唑醚菌酯与嘧菌酯呈完全正交互抗性，是生产上应用该类药剂防治失效的根本原因。

灰霉病菌对QoIs类药剂敏感性表型研究表明，嘧菌酯抗性菌株均只呈现出高抗表型（EC_{50}>100 mg·L^{-1}，Azo^{HR}），未发现有对嘧菌酯低抗（1≤EC_{50}< 10 mg·L^{-1}，Azo^{LR}）和中抗（10≤EC_{50}<100 mg·L^{-1}，Azo^{MS}）表型的灰霉病菌株。与嘧菌酯抗性表型不同，吡唑醚菌酯抗性菌株中则大部分菌株（83.1%）表现出中抗。本研究中发现21.05%的菌株对嘧菌酯呈敏感性下降表型（1≤EC_{50}<5 mg·L^{-1}，Azo^{RS}），其平均EC_{50}值为4.159 6 mg·L^{-1}，与敏感菌株平均EC_{50}值（0.269 8 mg·L^{-1}）相比RF值大于15，但该类型菌株的*cyt b*基因未发生突变（与敏感菌株完全相同），说明田间存在敏感性下降的群体（或称之为抗药性亚群体），这一结果与皇甫运红等[16]的报道相同。

抗药性分子机制研究表明，所有抗性菌株的*cyt b*基因上都只含有G143A突变，没有发现其他突变类型[2,19-20]。研究发现根据灰霉病菌*cyt b*基因的143位点后是否含有长度为1 205 bp的内元（Bcbi-143/144）可分为2种类型。本研究中所有抗性菌株的*cyt b*基因均不含Bcbi-143/144，敏感菌株*cyt b*基因有的含Bcbi-143/144（52.6%），有的不含Bcbi-143/144（47.4%），该研究结果与浙江地区草莓灰霉病菌的类型相似[15-16]，但还没有确切的证据证明Bcbi-143/144的存在有利于菌株保持对QoIs类药剂的敏感性[21]。

新型QoIs类药剂吡唑醚菌酯与嘧菌酯的抗性菌株频率相同、抗性表型相似[22]，因此断定2种药剂呈完全正交互抗性，这一结果提示生产上不能运用吡唑醚菌酯来取代嘧菌酯，鉴于嘧菌酯在许多作物上已广泛长期应用，因此吡唑醚菌酯取代防治会产生极大抗药性风险，推测这一抗药性风险很有可能存在于其他农业作物病原真菌中。

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