

# 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  $\text{mg}\cdot\text{L}^{-1}$ 。77个嘧菌酯抗性菌株的 $EC_{50}$ 值全部大于100  $\text{mg}\cdot\text{L}^{-1}$ ,而吡唑醚菌酯抗性菌株的 $EC_{50}$ 平均值为67.680 7  $\text{mg}\cdot\text{L}^{-1}$ 。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  $\text{mg}\cdot\text{L}^{-1}$ )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 azoxystrobin and pyraclostrobin, fungicides resistance distribution and resistance phenotypes of *B. cinerea* isolates to azoxystrobin and pyraclostrobin. According to previous studies, the discriminatory concentration of azoxystrobin and pyraclostrobin was 10  $\text{mg}\cdot\text{L}^{-1}$ , and the plates were amended with 100  $\text{mg}\cdot\text{L}^{-1}$  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  $\text{mg}\cdot\text{L}^{-1}$  for sensitive isolates and 0, 3, 10, 30, 100 and 300  $\text{mg}\cdot\text{L}^{-1}$  for resistant isolates. The plates were amended by using 100  $\text{mg}\cdot\text{L}^{-1}$  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 GT-TATAGAATAG-3') + Qo14ext (5'-AACCATCTCCATCCACCATACCTAC 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 ( $EC_{50} < 1 \text{ mg}\cdot\text{L}^{-1}$ ) and decreased sensitivity isolates ( $1 \leq EC_{50} < 5 \text{ mg}\cdot\text{L}^{-1}$ ). The average  $EC_{50}$  values of the 15 sensitive isolates and 4 decreased sensitivity isolates to azoxystrobin were 0.269 8  $\text{mg}\cdot\text{L}^{-1}$  and 4.159 6  $\text{mg}\cdot\text{L}^{-1}$ , respectively. The  $EC_{50}$  values of the 18 sensitive isolates were 0.025 7–0.112 7  $\text{mg}\cdot\text{L}^{-1}$  to pyraclostrobin. The average values were 0.055 9  $\text{mg}\cdot\text{L}^{-1}$  and the 1 decreased sensitivity isolates were 1.598 8  $\text{mg}\cdot\text{L}^{-1}$ . The  $EC_{50}$  values of the whole 77 azoxystrobin-resistance isolates were more than 100  $\text{mg}\cdot\text{L}^{-1}$ . The 77 pyraclostrobin-resistance isolates were divided into moderate resistance isolates ( $10 \leq EC_{50} < 100 \text{ mg}\cdot\text{L}^{-1}$ , Pyr<sup>MR</sup>) and highly resistance isolates ( $EC_{50} \geq 100 \text{ mg}\cdot\text{L}^{-1}$ , Pyr<sup>HR</sup>). The average  $EC_{50}$  values of the 64 Pyr<sup>MR</sup> (83.12%) were 53.070 9  $\text{mg}\cdot\text{L}^{-1}$ . 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  $\text{mg}\cdot\text{L}^{-1}$ ) 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  $\text{mg}\cdot\text{L}^{-1}$ ) 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