

## 不同杀菌剂对猕猴桃软腐病菌 (*Botryosphaeria dothidea*) 的防治效果评价

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**摘要:**【目的】筛选对猕猴桃软腐病的最佳药剂, 探明猕猴桃软腐病的化学防治方法, 为猕猴桃软腐病的科学防治提供依据。【方法】通过菌丝生长抑制法测定了 8 种杀菌剂对猕猴桃软腐病原菌葡萄座腔菌 (*Botryosphaeria dothidea*) 的室内毒力, 开展了 6 种杀菌剂的离体防效评价, 选用毒力较强的杀菌剂评价田间和贮藏期的防治效果, 并对贮藏期杀菌剂的残留量进行了安全性评价。【结果】室内毒力测定结果表明, 97%咪鲜胺对病菌菌丝生长的抑制活性最强, 97.5%吡唑醚菌酯最弱,  $EC_{50}$  分别为  $0.1349 \mu\text{g}\cdot\text{mL}^{-1}$ 、 $9.9987 \mu\text{g}\cdot\text{mL}^{-1}$ ; 在室内离体防效测定中, 75%肟菌酯·戊唑醇水分散粒剂 WG 表现出较好的预防效果, 防效达 78.15%, 而 25%咪鲜胺乳油 EC 却表现出较好的治疗效果, 防效达 89.38%。田间试验中, 75%肟菌酯·戊唑醇 WG 和 42.4%唑醚·氟酰胺悬浮剂 SC 在田间喷施对软腐病的防治效果较好, 防效分别为 92.30%和 80.90%; 贮藏期防效测定中, 25%咪鲜胺 EC 在贮藏前浸果对软腐病的防治效果较好, 贮藏 90 d 后, 防效达 95.98%, 并且农药残留低于最大残留限量 (MRL), 未出现超标。【结论】综合室内、生长期及贮藏期药效试验结果, 在猕猴桃生长期田间防治中可选用肟菌酯·戊唑醇、唑醚·氟酰胺交替使用, 此外在贮藏期选用咪鲜胺对果实进行浸果处理有较好的防治效果。

**关键词:** 猕猴桃软腐病; 葡萄座腔菌; 杀菌剂; 防效

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## Control efficacy of different fungicides against kiwifruit soft rot caused by *Botryosphaeria dothidea*

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**Abstract:** 【Objective】Kiwifruits (*Actinidia chinensis* Planch.) are becoming increasingly popular because of their nutritional and delicious properties. However, kiwifruit is highly perishable after harvest and is susceptible to infection by various pathogenic microorganisms, especially *Botryosphaeria dothidea*. Kiwifruit soft rot, caused by *B. dothidea*, spreads rapidly in confined spaces. A single diseased fruit easily contaminates others, causing a whole box of fruits to soften or rot quickly. In addition, fruits that appear healthy may also turn out to be decayed after their

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skins are peeled. Therefore, the significance of kiwifruit soft rot in the value chain is often underestimated. Currently, chemical control is the most effective approach for kiwifruit soft rot prevention and healing. Nevertheless, there is limited knowledge on fungicides that can be applied in the growth and storage periods to prevent kiwifruit soft rot. The lack of scientific evidence for the preventive use of fungicides has led to insufficient recommendations, widespread misuse and abuse of these chemicals. Therefore, we aimed at identifying high-efficiency, low-toxicity and low-residue fungicides for kiwifruit soft rot prevention and control during the growth and storage periods. **【Method】** Indoor toxicity tests. Indoor toxicity effects of tebuconazole, prochloraz, fluazinam, propiconazole, imazalil sulfate, pyraclostrobin, flusilazole and azoxystrobin on *B. dothidea* were assessed via the mycelial growth inhibition method. For evaluation of *in vitro* preventive and curative activities of prochloraz, azoxystrobin, pyraclostrobin·fluxapyroxad, trifloxystrobin·tebuconazole, imazalil sulfate and fludioxonil against kiwifruit soft rot, the fruits were treatment in preventive (applied 24 and 72 h pre-inoculation) and curative (applied 24 and 72 h post-inoculation), preventive as well as curative effects evaluated after kiwifruits had softened. In 2020, to the screening field trials of flusilazole, pyraclostrobin·fluxapyroxad, azoxystrobin, trifloxystrobin·tebuconazole was conducted in the modern agricultural research and development base of Sichuan Agricultural University. The fungicides were applied using a spray on 20<sup>th</sup> April 2020 (after shedding), 20<sup>th</sup> May 2020 (young fruit stage), 24<sup>th</sup> June 2020 (fruit expansion stage) and 24<sup>th</sup> July 2020 (30 days before harvest). Collected fruits from each treatment and stored at 25°C, the disease index incidence and control efficiencies were calculated after 15 days of storage. For storage trials, the fruits were soaked in trifloxystrobin·tebuconazole, prochloraz, fludioxonil, imazalil sulfate and myclobutanil for one min, air dried and stored. The disease index incidence and control efficiencies were calculated after 90 days of storage. After 90 days of storage, the kiwifruit samples treated with the most effective fungicide and water were taken for quantitative analysis and detection of fungicide residues. The national food safety standard GB 2763-2019 guidelines on maximum residue limits of fungicides in food were used to assess the residual safety of fungicides. **【Results】** In this study, based on the results of indoor toxicity and *in vitro* control efficacy assessments, we selected different fungicides for subsequent field and storage efficacy tests and identified three fungicides with great potential for control of kiwifruit soft rot. Prochloraz (a.i. 97%) had the strongest inhibitory activities against *B. dothidea* mycelial growth whereas pyraclostrobin (a.i. 97.5%) had the weakest inhibitory activities. The EC<sub>50</sub> values of prochloraz (a.i. 97%) and pyraclostrobin (a.i. 97.5%) were 0.1349 µg·ml<sup>-1</sup> and 9.9987 µg·ml<sup>-1</sup>, respectively. *In vitro*, trifloxystrobin·tebuconazole had preventive effects of 78.15% whereas prochloraz had curative effects of 89.38%. During growth in field trials, four fungicide applications were effective against kiwifruit soft rot. Among them, three fungicides had better control efficacies on the disease after applying them four times at different growth stages. Trifloxystrobin·tebuconazole successfully controlled kiwifruit soft rot with control efficacies of 92.30%, compared with other fungicides ( $p < 0.05$ ). It was followed by pyraclostrobin·fluxapyroxad and azoxystrobin with

control efficacies of 80.90% and 76.46%, respectively, whereas flusilazole had the least control efficacy of 32.84%. After fruit immersion in all five fungicides before storage, prochloraz exhibited the highest control efficacy of 95.98%, followed by myclobutanil and trifloxystrobin·tebuconazole with control efficacies of 91.14% and 86.30%, respectively. None of the fungicides were associated with phytotoxic effects on the kiwifruits after treatment, suggesting that they were safe. When kiwifruits were stored for 90 days, residual levels of tebuconazole, trifloxystrobin and prochlorazin water control were 0.015 mg/kg, 0.011 mg/kg and <0.01 mg/kg, respectively, whereas tebuconazole and trifloxystrobin residues in triclostrobin·tebuconazole treatment were 0.45 mg/kg and 0.22 mg/kg, respectively. Fungicide residues in the three treatments were significantly low in kiwifruits than the MRL for grapes and no pesticide residues exceeded the standard. 【Conclusion】 Taken together, this is the first study to comprehensively assess the control effects of different fungicides on kiwifruit soft rot in the field and during storage. We found that alternate applications of trifloxystrobin·tebuconazole and pyraclostrobin·fluxapyroxad in the field during the growth period and soaking postharvest fruits in prochloraz before storage are potential useful and effective measures for kiwifruit soft rot management. Moreover, fruit damage during harvesting and transportation should be avoided as much as possible to effectively prevent and control postharvest kiwifruit soft rot.

**Key words:** Kiwifruit soft rot, *Botryosphaeria dothidea*, Fungicides, Control efficacy

猕猴桃(*Actinidia chinensis* Planch.)因其丰富的营养和独特的风味而深受消费者的青睐,已成为世界范围内重要的经济作物<sup>[1-4]</sup>。随着猕猴桃产业的快速发展,猕猴桃软腐病已成为制约产业发展的重要因素,在贮藏、运输、销售和货架期造成大量果实腐烂,病果率为20%~50%<sup>[5-6]</sup>,降低果实的贮藏品质并缩短其贮藏时间,对果农和产业造成严重损失<sup>[7]</sup>。

软腐病是一种真菌性病害,又称熟腐病和褐腐病,其主要致病菌为葡萄座腔菌(*Botryosphaeria dothidea*)<sup>[8-12]</sup>。一般在果实后熟期显现症状,病斑圆形或椭圆形,褐色,病健交界处呈暗绿色水渍状环形晕圈,病部表面无明显凹陷。从出现水渍状褐色小圆斑至全果软腐一般约3 d,病害极易在密闭空间内迅速传播,甚至一个病果可引起整箱果实快速变软甚至腐烂,并挥发出难闻的酸臭味,影响果实的品质<sup>[8-13]</sup>。此外,大量看似健康的果实去皮后也有腐烂现象<sup>[14]</sup>,因此猕猴桃软腐病对果农和生产造成的损失往往被低估。

目前猕猴桃软腐病的防治措施中,化学防治仍然为首选,生产上广泛使用的药剂有甲基硫菌灵、苯醚甲环唑等<sup>[15-16]</sup>,主要以生长期田间喷施为主。针对猕猴桃软腐病防治药剂的报道不多,胡容平等<sup>[17]</sup>、吴文能等<sup>[18]</sup>通过室内毒力测定,发现咪鲜胺、苯醚甲环唑、丙环唑对猕猴桃软腐病菌的抑制效果较好,但未进一步开展田间试验验证;莫飞旭等<sup>[19]</sup>通过田间试验发现,四霉素与戊唑醇复配后在生长期施用对采后猕猴桃软腐病有较好的预防效果,但未与其他药剂进行对比;对于贮藏期病害的防治,一般选择在贮藏前对果实进行浸药处理,该措施已广泛应用且效果显著<sup>[20-23]</sup>,但针对猕猴桃软腐病在贮藏前浸药鲜有报道。

*B. dothidea* 寄主范围广，可危害苹果、梨、蓝莓、猕猴桃等多种重要的经济作物<sup>[9, 24-26]</sup>。前人针对 *B. dothidea* 已筛选出啞菌酯、戊唑醇、吡唑醚菌酯、咯菌腈、咪鲜胺等低毒、低残留杀菌剂。其中，啞菌酯对葡萄溃疡病菌 (*B. dothidea*) 有显著的抑制作用且田间防效较好<sup>[27]</sup>，戊唑醇、吡唑醚菌酯、氟硅唑已用于防治苹果轮纹病菌 (*B. dothidea*)<sup>[28-30]</sup>。咯菌腈、咪鲜胺对梨轮纹病菌 (*B. dothidea*) 的菌丝生长也有很强的抑制作用<sup>[31]</sup>。氟硅唑、吡唑醚菌酯对芒果蒂腐病病原菌 (*B. dothidea*) 的生长有较好的抑制作用<sup>[32]</sup>。但用于猕猴桃软腐病的生长期及贮藏期防治的药剂鲜有报道，由于缺乏科学的用药指导，导致防效不佳，乱用、滥用农药的现象极普遍。为此，本文针对猕猴桃软腐病，通过室内高效药剂筛选，结合生长期和贮藏期的药效试验，旨在筛选出高效、低毒、低残留杀菌剂用于该病害的防治。

## 1 材料和方法

### 1.1 试验材料

供试原药（毒力测定）：95%戊唑醇、97%咪鲜胺、98%氟啶胺、97.5%吡唑醚菌酯、95%氟硅唑、93%丙环唑、75%抑霉唑硫酸盐，均由四川农业大学无公害农药实验室提供。

供试杀菌剂（药效试验）：具体名称、剂型、施药浓度及生产厂家见表 1。施药浓度采用厂家推荐的最佳浓度。

供试菌株：猕猴桃软腐病原菌 *B. dothidea*（菌株编号为 ZC56），对猕猴桃果实具有强致病性，分离自四川省都江堰市胥家镇猕猴桃产区。

PDA 培养基：马铃薯 200 g，葡萄糖 20 g，琼脂粉 10 g，水 1 L。

试验地位于四川农业大学现代农业研发基地，4 年生红阳猕猴桃果园（地理坐标 30°56'26" N，103°65'16" E），平均海拔为 538 m，属四川盆地亚热带湿润季风气候，年平均气温为 15.9 °C，年降雨量达 973.5 mm，雨热同期。供试果园土壤类型为壤土，地势平坦、肥力均匀。

供试品种：选用四川省猕猴桃主产区大面积种植的红阳品种，易感猕猴桃软腐病。

表 1 供试杀菌剂概况

Table 1 Information on the fungicides that were used in this study

| 杀菌剂          | 活性成分 <sup>x</sup>  | 施药浓度                           | 生产厂家  | 预防和治疗效果 <sup>y</sup>                 | 田间防效 <sup>y</sup>                   | 贮藏期防效 <sup>y</sup>                  |
|--------------|--|--------------------------------|---|--------------------------------------|-------------------------------------|-------------------------------------|
| Fungicide    | Active ingredient <sup>x</sup>   | Amount/ (mg kg <sup>-1</sup> ) | Manufacturer  | Preventive and curative <sup>y</sup> | Field control efficacy <sup>y</sup> | Store control efficacy <sup>y</sup> |
| Agcelence®   | 吡唑醚菌酯 21.2% + 氟酰胺 21.2% (SC)<br>pyraclostrobin 21.2% + fluxapyroxad 21.2% (SC) | 169.6                          | 巴斯夫农药有限公司<br>BASF SE [China] Co., LTD                                   | T                                    | T                                   | NT                                  |
| Celest®      | 咯菌腈 25 g·L <sup>-1</sup> (SC)<br>fludioxonil 25 g·L <sup>-1</sup> (SC)         | 12.5                           | 先正达农药有限公司<br>Syngenta Crop Protection [Sichuan] Co., LTD                | T                                    | NT                                  | T                                   |
| Amistar®     | 啶菌酯 250 g·L <sup>-1</sup><br>azoxystrobin 250 g·L <sup>-1</sup> (SC)           | 166.67                         | 先正达农药有限公司<br>Syngenta Crop Protection [Sichuan] Co., Ltd.               | T                                    | T                                   | NT                                  |
| Prochloraz®  | 咪鲜胺 25%<br>prochloraz 25% (EC)   | 250                            | 四川国光农化有限公司<br>Sichuan Guoguang Agrochemical Co.<br>LTD                  | T                                    | NT                                  | T                                   |
| Flusilazole® | 氟硅唑 400 g·L <sup>-1</sup> (EC)<br>flusilazole 400 g·L <sup>-1</sup> (EC)       | 50                             | 广东茂名绿银农化有限公司<br>Guangdong Maoming Green Silver<br>Agrochemical Co., LTD | NT                                   | T                                   | NT                                  |

|                   |   |       |  |    |    |   |
|-------------------|---|-------|--|----|----|---|
| Imazalil sulfate® | 抑霉唑硫酸盐 75% (SG)<br>imazalil sulfate 75% (SG)                          | 250   | 安道麦马克西姆有限公司<br>Addo Maxim [Beijing] Co., LTD | T  | NT | T |
| Nativo®           | 肟菌酯 25% + 戊唑醇 50% (WG)<br>trifloxystrobin 25% + tebuconazole 50% (WG) | 187.5 | 拜耳农药有限公司<br>Bayer [China] Co., LTD           | T  | T  | T |
| Systhane®         | 腈菌唑 40% (SG)<br>myclobutanil 40% (SG)                                 | 57.14 | 美国陶氏益农公司<br>Dow Agrochemical Co., LTD        | NT | NT | T |

注：<sup>x</sup> 括号内的值表示配制的类型：SC=悬浮剂；EC=乳油；WG =水分散粒剂；SG=可湿性粉剂。<sup>y</sup>T 表示进行了测试，NT 表示未测试。

Note: <sup>x</sup> Values in parentheses indicate the formulated type: SC=suspension concentrate; EC=emulsifiable concentrate; WG=water-dispersible granule; SG=soluble granule agent. <sup>y</sup> T indicates testing was conducted, NT indicates untested.

## 1.2 方法

### 1.2.1 室内毒力测定

采用菌丝生长速率法进行供试药剂的室内毒力测定。将 7 种原药分别溶于丙酮，配置成  $10 \text{ g}\cdot\text{L}^{-1}$  母药备用（现配现用）。用 0.05%吐温 80 的无菌水将 7 种原药母液按下列梯度进行稀释，其中戊唑醇、咪鲜胺、氟啶胺、丙环唑和抑霉唑硫酸盐的浓度梯度均为 50、25、12.5、6.25、 $3.175 \mu\text{g}\cdot\text{mL}^{-1}$ ，吡唑醚菌酯的浓度梯度为 200、100、50、25、 $12.5 \mu\text{g}\cdot\text{mL}^{-1}$ ，氟硅唑的浓度梯度为 100、50、25、12.5、 $6.25 \mu\text{g}\cdot\text{mL}^{-1}$ 。以无菌吐温水中加入相同体积丙酮处理作为空白对照。将不同浓度的 7 种原药母液加入  $45^\circ\text{C}$  的 PDA 培养基中（PDA 培养基：原药=9：1，体积比），制备成含药 PDA 培养基。其中戊唑醇、咪鲜胺、氟啶胺、丙环唑和抑霉唑硫酸盐的浓度梯度均为 5、2.5、1.25、0.625、 $0.3175 \mu\text{g}\cdot\text{mL}^{-1}$ ，吡唑醚菌酯的浓度梯度为 20、10、5、2.5、 $1.25 \mu\text{g}\cdot\text{mL}^{-1}$ ，氟硅唑的浓度梯度为 10、5、2.5、1.25、 $0.625 \mu\text{g}\cdot\text{mL}^{-1}$ 。

将猕猴桃软腐病菌菌株 ZC56 用 PDA 培养基暗培养（ $25^\circ\text{C}$ ）约 7 d，用打孔器在菌落边缘等距离切取直径 5 mm 的菌饼。将菌饼接种于不同浓度的含药 PDA 培养基和对照培养基，每个处理 3 次重复，置于  $25^\circ\text{C}$  恒温培养箱中暗培养，7 d 后采用“十字交叉法”测量菌落直径，并按照下式计算菌丝生长抑制率。

$$\text{菌丝生长抑制率}/\% = \frac{(\text{对照菌落直径} - \text{处理菌落直径})}{\text{对照菌落直径} - \text{接种菌饼直径}} \times 100$$

运用 SPSS 2.0 软件对数据进行分析，以药剂质量浓度的对数值为横坐标、抑制率对应的几率值为纵坐标进行作图，得到毒力回归方程及有效抑制中浓度（ $\text{EC}_{50}$ ）值。

### 1.2.2 离体防效评价

取健康的红阳猕猴桃用 75%乙醇浸泡 10 min 后置于超净工作台中紫外杀菌晾干，并标记接种点，备用。选用 6 种杀菌剂，以无菌水为空白对照，分预防与治疗效果测定两组，共 14 个处理，每个处理 3 次重复，每个重复 15 个果实。预防效果测定：先将药剂喷施于果实表面，晾干后分别于 24 h 和 72 h 接种 5 mm 的菌饼；治疗效果测定：先将 5 mm 菌饼接种在果实表面，保湿培养 24 h 和 72 h 后再喷施药剂。所有果子在  $25^\circ\text{C}$  和相对湿度 85% 条件下贮藏，待果实软化后测定病菌侵染直径，按下式计算防效<sup>[33]</sup>。

$$\text{防效}/\% = \frac{(\text{对照病斑直径} - \text{处理病斑直径})}{\text{对照病斑直径}} \times 100$$

### 1.2.3 生长期田间药效试验

（1）试验设计 采取随机区组进行田间试验，试验田四周设保护行。根据前期药剂筛选结果选用氟硅唑、唑醚·氟酰胺、啉菌酯、肟菌酯·戊唑醇 4 种杀菌剂，以清水为空白对照，共 5 个处理，每个处理 3 个重复小区，每个小区 5 棵树。分别于 2020 年 4 月 20 日（谢花后）、5 月 20 日（幼果期）、6 月 24 日（果实膨大期）、7 月 24 日（采前 30 d）喷施药剂。

（2）试验调查 至果实成熟时，每棵树随机采收 30 个果子，每个处理共计 450 个果子，

置于 25℃恒温贮存。待果实软熟后调查果实发病率及病害严重程度，统计果实发病情况，计算病情指数、发病率及防治效果<sup>[34]</sup>，病害严重程度分级标准见表 2。

表 2 猕猴桃软腐病病害严重程度分级标准

Table 2 Grading standard for severity of kiwifruit soft rot

| 代表值<br>representative value | 分级标准<br>Grading standard   |
|-----------------------------|--|
| 0                           | 无症状<br>No disease  |
| 1                           | 病斑占果实面积 5%以下<br>Diseased spots accounted for less than 5% of fruit area  |
| 3                           | 病斑占果实面积 >5%~15%<br>Diseased spots account for >5%-15% of fruit area      |
| 5                           | 病斑占果实面积 >15%~25%<br>Diseased spots account for >15%-25% of fruit area    |
| 7                           | 病斑占果实面积 >25%~50%<br>Diseased spots account for >25%~50% of fruit area    |
| 9                           | 病斑占果实面积 50%以上<br>Disease spots accounted for more than 50% of fruit area |

$$\text{发病率}/\% = \frac{\text{发病果数}}{\text{调查总果数}} \times 100 ;$$

$$\text{病情指数} = \frac{\sum(\text{各级病果数} \times \text{各级代表值})}{\text{调查总果数} \times \text{最高级代表值}} \times 100$$

$$\text{防治效果}/\% = \frac{\text{空白对照区病情指数} - \text{药剂处理区病情指数}}{\text{空白对照区病情指数}} \times 100$$

#### 1.2.4 贮藏期药效试验

(1) 试验设计 果实采收后，除去病、虫、机械伤果，根据前期药剂筛选结果并参考生产常用药剂选用脲菌酯·戊唑醇、咪鲜胺、咯菌腈、抑霉唑硫酸盐、腈菌唑 5 种杀菌剂，施药浓度采用厂家推荐的最佳浓度，以清水为对照，共 6 个处理，每个处理 3 次重复，每个重复 100 个果。将果实放入各药液或清水中均匀浸果 1 min，随后捞起晾干，入库贮藏。冻库温度为 (1.5±0.5) °C，湿度为 93%±0.5%。贮藏 90d 后调查果实发病情况，计算果实发病率及病情指数。(分级标准见表 2)

(2) 数据分析 运用 SPSS 19.0 软件对数据进行分析。在田间生长期和贮藏期药效试验中，均采用单因素方差分析 (ANOVA) 和双尾检验比较不同组间的显著性水平。所有的



统计细节可以在表及图例中找到。

(3) 安全性评价 入库贮藏 90 d 后, 取清水处理和防治效果最佳处理的猕猴桃样品, 送于诺安实力可商品检验(青岛)有限公司进行单个农药定量分析检测, 基于 EQuEChERS 方法建立戊唑醇、肟菌酯、咪鲜胺、吡唑醚菌酯残留的提取和纯化步骤, 然后使用高效气相色谱-质谱法(GC-MS)对戊唑醇进行定量检测, 使用高效液相色谱-质谱法(LC-MS)对肟菌酯和咪鲜胺进行定量检测。依据《食品安全国家标准食品中农药最大残留限量》GB 2763—2021<sup>[35]</sup>规定的最大残留限量, 对本次试验中的杀菌剂进行残留安全性评价。

## 2 结果与分析

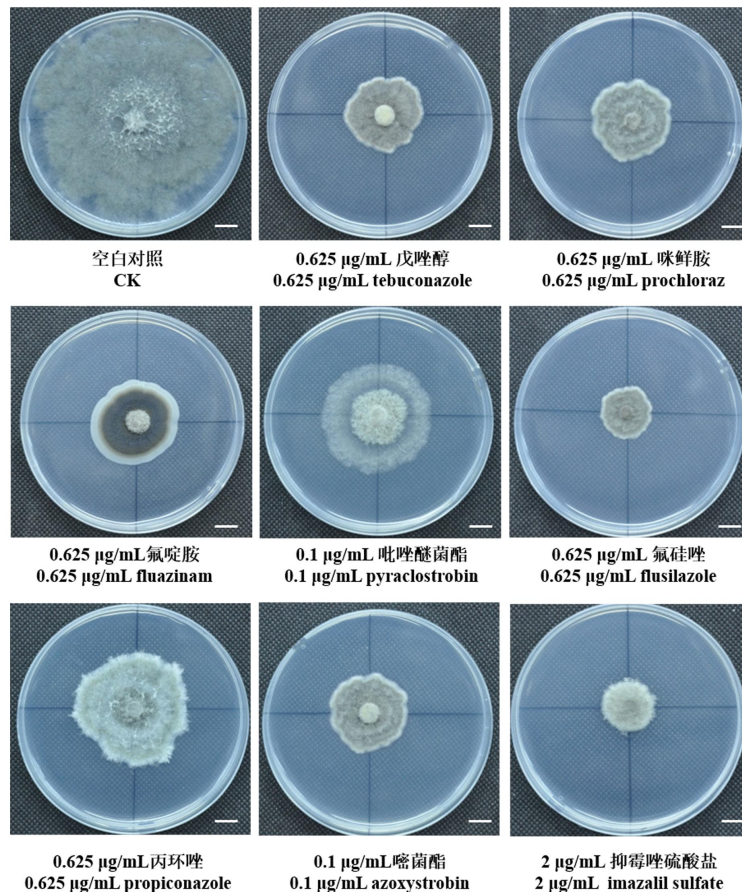
### 2.1 不同药剂对猕猴桃软腐病菌的室内毒力测定

由表 3 和图 1 可知, 在浓度测试范围内, 8 种药剂均在一定程度上抑制猕猴桃软腐病菌菌丝的生长, 但毒力存在明显差异。97%咪鲜胺毒力最强, EC<sub>50</sub> 值仅为 0.134 9 μg·mL<sup>-1</sup>, 其次是 98%氟啶胺、95%戊唑醇、75%抑霉唑硫酸盐、95%氟硅唑、96%嘧菌酯、93%丙环唑效果较好, EC<sub>50</sub> 分别为 0.180 1、0.240 9、0.261 3、0.326 1、0.638 1、0.781 9 μg·mL<sup>-1</sup>, EC<sub>50</sub> 值均小于 0.80 μg·mL<sup>-1</sup>; 97.5%吡唑醚菌酯虽然对病原菌生长有一定的抑制作用, 但抑制效果相对较差, EC<sub>50</sub> 为 9.998 7 μg·mL<sup>-1</sup>, 毒力最低。

表 3 8 种药剂对猕猴桃软腐病菌菌丝生长的抑制作用

Table 3 Inhibitory activities of eight fungicides against mycelial growth of *B. dothidea*

| 供试药剂<br>Tested fungicides         | 回归方程<br>Regression equation | EC <sub>50</sub> /<br>(μg·mL <sup>-1</sup> ) | 相关系数<br>Correlation coefficient, <i>r</i> |
|-----------------------------------|-----------------------------|--|---|
| 95%戊唑醇 95% tebuconazole           | $y = 1.3474x + 5.8329$      | 0.2409                                       | 0.9985                                    |
| 97%咪鲜胺 97% prochloraz             | $y = 1.3288x + 6.1559$      | 0.1349                                       | 0.9853                                    |
| 98%氟啶胺 98% fluazinam              | $y = 1.0982x + 5.8175$      | 0.1801                                       | 0.9632                                    |
| 97.5%吡唑醚菌酯 97.5% pyraclostrobin   | $y = 1.7617x + 3.2384$      | 9.9987                                       | 0.9715                                    |
| 95%氟硅唑 95% flusilazole            | $y = 1.8003x + 5.8760$      | 0.3261                                       | 0.9619                                    |
| 93%丙环唑 93% propiconazole          | $y = 1.6491x + 5.1762$      | 0.7819                                       | 0.9845                                    |
| 96%嘧菌酯 96% azoxystrobin           | $y = 0.7460x + 5.1455$      | 0.6381                                       | 0.9858                                    |
| 75%抑霉唑硫酸盐<br>75% imazalil sulfate | $y = 1.9380x + 6.1295$      | 0.2613                                       | 0.9780                                    |



标尺=1 cm。Bar=1 cm.

图 1 不同浓度的 8 种杀菌剂对葡萄座腔菌 (*B. dothidea*) 菌丝生长的抑制作用

**Fig. 1 Inhibitory activities of different concentrations of fungicides against mycelial growth of *B. dothidea***

## 2.2 离体防效评价

### 2.2.1 不同药剂对猕猴桃软腐病的预防效果评价

由表 4 和图 2 可知, 6 种杀菌剂对猕猴桃软腐病的预防效果不一, 防效由强到弱依次为 75% 肟菌酯·戊唑醇 WG > 25 g·L<sup>-1</sup> 咯菌腈 SC > 42.4% 唑醚·氟酰胺 SC > 25% 咪鲜胺 EC > 75% 抑霉唑硫酸盐 SG > 250 g·L<sup>-1</sup> 啞菌酯 SC; 其中 75% 肟菌酯·戊唑醇 WG 对葡萄座腔菌 (*B. dothidea*) 的预防效果最好, 在喷药后 24 h、72 h 接种病原菌, 防效分别为 78.15% 和 70.65%, 25 g·L<sup>-1</sup> 咯菌腈 SC 对葡萄座腔菌 (*B. dothidea*) 的预防效果较好, 在喷药后 24 h、72 h 接种病原菌, 防效分别为 71.98% 和 68.36%; 250 g·L<sup>-1</sup> 啞菌酯 SC 预防效果最差, 防效分别为 46.17%、35.61%。

表 4 6 种杀菌剂在离体果实上对葡萄座腔菌 (*B. dothidea*) 的预防效果

Table 4 The preventive effects of six fungicides in fruits with *B. dothidea*

| 处理<br>Treatment   | 喷药后 24 h 接种<br>Inoculation 24h after spray fungicide  |                          | 喷药后 72 h 接种<br>Inoculation with 72h after spray fungicide |                          |
|---|---|--------------------------|---|--------------------------|
|   | 病斑直径<br>Spot diameter/mm                              | 平均防效<br>Control effect/% | 病斑直径<br>Spot diameter/mm                                  | 平均防效<br>Control effect/% |
|   | 75% 肟菌酯·戊唑醇 WG<br>75% trifloxystrobin·tebuconazole WG | 6.35±0.60 c              | 78.15±2.07 a  | 7.12±0.80 e              |
| 25 g·L <sup>-1</sup> 咯菌腈 SC<br>25 g·L <sup>-1</sup> fludioxonil SC    | 8.14±0.61 d   | 71.98±2.08 b             | 7.67±0.92 e   | 68.36±3.78 a             |
| 42.4% 唑醚·氟酰胺 SC<br>42.4% pyraclostrobin·fluxapyroxad SC               | 10.14±0.69 c  | 65.11±2.36 c             | 10.53±1.10 d  | 56.59±4.54 b             |
| 25% 咪鲜胺 EC<br>25% prochloraz EC                                       | 10.80±2.66 c  | 62.84±9.16 c             | 11.56±1.68 cd   | 52.33±6.93 bc            |
| 75% 抑霉唑硫酸盐 SG<br>75% imazalil sulfate SG                              | 15.13±0.83 b  | 47.93±2.85 d             | 13.13±2.20 c  | 45.85±9.08 c             |
| 250 g·L <sup>-1</sup> 啞菌酯 SC<br>250 g·L <sup>-1</sup> azoxystrobin SC | 15.65±0.27 b  | 46.17±0.92 d             | 15.62±0.99 b  | 35.61±4.10 d             |
| 清水对照<br>CK  | 29.07±1.52 a  | —                        | 24.25±0.83 a  | —                        |

注：每个值为平均值±标准差，平均值包括 3 次生物独立的重复。同一列不同字母表示以  $p < 0.05$  为差异有统计学意义（采用单因素方差分析和最小显著差异法 LSD 检验进行多重比较）。

Note: Each value is mean ± SD and the average include three biologically independent replicates. Different letters indicate significant differences at  $p < 0.05$  in the same column (one-way ANOVA followed by two-sided least significant difference (LSD) test for multiple-comparisons).

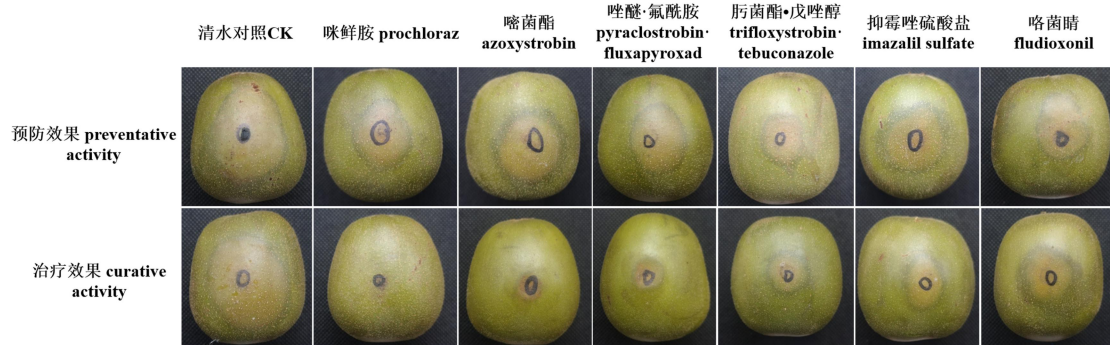
### 2.2.2 不同药剂对猕猴桃软腐病的治疗效果评价

由表 5 和图 2 可知，6 种杀菌剂对猕猴桃软腐病均有一定的治疗效果，接种 72 h 后，25% 咪鲜胺 EC 和 250 g·L<sup>-1</sup> 啞菌酯 SC 效果显著 ( $p < 0.05$ ) 高于其他药剂，防效由强到弱依次为 25% 咪鲜胺 EC > 250 g·L<sup>-1</sup> 啞菌酯 SC > 42.4% 唑醚·氟酰胺 SC；其中 25% 咪鲜胺 EC 对葡萄座腔菌 (*B. dothidea*) 的治疗效果最好，在接种病原菌 24 h、72 h 后喷药，防效分别为 89.38%、84.24%；25 g·L<sup>-1</sup> 咯菌腈 SC 治疗效果最差，防效分别为 52.30%、45.33%。

表 5 6 种杀菌剂在离体果实上对葡萄座腔菌 (*B. dothidea*) 的治疗效果

Table 3 The curative effects of six fungicides in fruits with *B. dothidea*

| 处理<br>Treatment  | 接种后 24 h 喷药                            |                          | 接种后 72 h 喷药                            |                          |
|--|--|--------------------------|--|--------------------------|
|  | Spray fungicide 24 h after inoculation |                          | Spray fungicide 72 h after inoculation |                          |
|  | 病斑直径<br>Spot diameter/mm               | 平均防效<br>Control effect/% | 病斑直径<br>Spot diameter/mm               | 平均防效<br>Control effect/% |
| 25%咪鲜胺 EC 25% prochloraz EC  | 2.64±0.50 d                            | 89.38±2.03 a             | 2.94±0.25 e                            | 84.24±1.32 a             |
| 250 g·L <sup>-1</sup> 啶菌酯 SC 250 g·L <sup>-1</sup> azoxystrobin SC | 2.82±0.71 d                            | 88.66±2.87 ab            | 3.73±1.16 e                            | 79.97±6.25 a             |
| 42.4%唑醚·氟酰胺 SC 42.4%<br>pyraclostrobin·fluxapyroxad SC             | 4.14±0.83 d                            | 83.31±3.33 b             | 6.24±0.89 d                            | 66.49±4.79 b             |
| 75%肟菌酯·戊唑醇 WG 75%<br>trifloxystrobin·tebuconazole WG               | 7.94±1.52 c                            | 68.03±6.13 c             | 7.95±0.67 c                            | 57.30±3.58 c             |
| 75%抑霉唑硫酸盐 SG 75% imazalil sulfate SG                               | 9.17±1.45 c                            | 63.07±5.83 c             | 8.83±2.41 bc                           | 52.59±12.95 cd           |
| 25 g·L <sup>-1</sup> 咯菌晴 SC 25 g·L <sup>-1</sup> fludioxonil SC    | 11.84±1.05 b                           | 52.30±4.21 d             | 10.18±0.98 b                           | 45.33±5.29 d             |
| 清水对照 CK  | 24.83±2.32 a                           | —                        | 18.63±0.57 a                           | —                        |



注：每个值为平均值±标准差，平均值包括 3 次生物独立的重复。同一列不同字母表示以  $p < 0.05$  为差异有统计学意义(采用单因素方差分析和最小显著差异法 LSD 检验进行多重比较)。

Note: Each value is mean ± SD and the average include three biologically independent replicates. Different letters indicate significant differences at  $p < 0.05$  in the same column (one-way ANOVA followed by two-sided least significant difference (LSD) test for multiple-comparisons).

图 2 猕猴桃软腐病预防效果（喷药 72 h 后接种）和治疗效果（接种 72 h 后喷药）的病斑扩展情况

Fig. 2 Representative soft rot lesion development in preventive (applied 72 h pre-inoculation) and curative (applied 72 h post-inoculation) treatments

### 2.3 不同药剂对猕猴桃软腐病的田间防治效果

由表 6 可知，空白对照区猕猴桃软腐病发病严重，平均病情指数达到 31.18，生长期不

同时期喷雾施药 4 次后, 4 种杀菌剂中有 3 种对猕猴桃软腐病的防治效果较好, 75% 肟菌酯·戊唑醇 WG 防效显著高于其他药剂 ( $p < 0.05$ ), 为 92.30%; 其次是 42.4% 唑醚·氟酰胺 SC 和 250 g·L<sup>-1</sup> 嘧菌酯 SC, 防效分别为 80.90% 和 76.46%; 以 400 g·L<sup>-1</sup> 氟硅唑 EC 防治效果最差, 仅 32.84%。从施药到调查结束, 各杀菌剂处理后对猕猴桃均未产生药害。

**表 6 4 种杀菌剂对猕猴桃软腐病的田间防治效果**

Table 6 Control efficacy of four fungicides on kiwifruit soft rot in fields

| 处理<br>Treatment   | 发病率<br>The percentage of<br>diseased/% | 病情指数<br>Disease index | 平均防效<br>Control effect/% |
|---|--|-----------------------|--------------------------|
| 75% 肟菌酯·戊唑醇 WG<br>75% trifloxystrobin-tebuconazole WG                 | 15.93±4.38 d                           | 2.51±0.96 c           | 92.30±2.96 a             |
| 42.4% 唑醚·氟酰胺 SC<br>42.4% pyraclostrobin-fluxapyroxad SC               | 35.21±5.87 c                           | 6.22±1.90 c           | 80.90±5.84 b             |
| 250 g·L <sup>-1</sup> 嘧菌酯 SC<br>250 g·L <sup>-1</sup> azoxystrobin SC | 26.71±4.34 c                           | 6.91±1.92 c           | 78.78±5.89 b             |
| 400 g·L <sup>-1</sup> 氟硅唑 EC<br>400 g·L <sup>-1</sup> flusilazole EC  | 76.46±6.32 b                           | 25.10±2.13 b          | 32.84±6.53 c             |
| CK  | 87.57±7.80 a                           | 31.18±9.81 a          | —                        |

注: 每个值为平均值±标准差, 平均值包括 3 次生物独立的重复。同一列不同字母表示以  $p < 0.05$  为差异有统计学意义 (采用单因素方差分析和最小显著差异法 LSD 检验进行多重比较)。

Note: Each value is mean ± SD and the average include three biologically independent replicates. Different letters indicate significant differences at  $p < 0.05$  in the same column (one-way ANOVA followed by two-sided least significant difference (LSD) test for multiple-comparisons).

## 2.4 贮藏期防效验证及安全评价

### 2.4.1 果实贮藏前浸药的防效验证

由表 7 可知, 5 种杀菌剂对猕猴桃软腐病均有一定的防治效果, 冻库贮藏 90 d 后调查, 25% 咪鲜胺 EC 防治效果最佳, 达 95.98%, 其次为 40% 腈菌唑 SG 和 75% 肟菌酯·戊唑醇, 防效分别为 91.14% 和 86.30%。肟菌酯·戊唑醇、咪鲜胺及腈菌唑在果实采后浸果处理对猕猴桃软腐病的防治效果较好, 从药剂浸果到调查结束, 猕猴桃未发生任何异常情况, 各杀菌剂处理后对猕猴桃均未产生药害。

**表 7 5 种杀菌剂对贮藏期猕猴桃软腐病的防治效果**

Table 7 Control efficacy of five fungicides on kiwifruit soft rot during storage period

| 处理 | 发病率 | 病情指数 | 平均防效 |
|----|-----|------|------|
|----|-----|------|------|

| Treatment   | The percentage of diseased/% | Disease index | Control effect/% |
|---|------------------------------|---------------|------------------|
| 25%咪鲜胺 EC 25%prochloraz EC                                      | 0.51 de                      | 0.25 f        | 95.98 a          |
| 40%腈菌唑 SG 40%myclobutanil SG                                    | 0.81 d                       | 0.54 de       | 91.14 a          |
| 75%腈菌酯·戊唑醇 WG 75%trifloxystrobin·tebuconazole WG                | 1.51 d                       | 0.84 cd       | 86.30 ab         |
| 75%抑霉唑硫酸盐 SG 75%imazalil sulfate SG                             | 2.93 c                       | 1.24 c        | 79.81 b          |
| 25 g·L <sup>-1</sup> 咯菌晴 SC 25 g·L <sup>-1</sup> fludioxonil SC | 3.91 b                       | 2.15 b        | 64.97 c          |
| 清水对照 CK   | 10.51 a                      | 6.13 a        | —                |

注：每个值为平均值±标准差，平均值包括3次生物独立的重复。同一列不同字母表示以  $p < 0.05$  为差异有统计学意义(采用单因素方差分析和最小显著差异法 LSD 检验进行多重比较)。

Note: Each value is mean ± SD and the average include three biologically independent replicates. Different letters indicate significant differences at  $p < 0.05$  in the same column (one-way ANOVA followed by two-sided least significant difference (LSD) test for multiple-comparisons).

#### 2.4.2 果实贮藏前浸药的安全评价

猕猴桃采后软腐病发病率极高，猕猴桃果实在贮藏期的杀菌剂最大残留量评估对规范猕猴桃采后病害防治具有重要意义。由表 8 可见，清水对照处理的猕猴桃中戊唑醇、腈菌酯、咪鲜胺的残留量：0.015、0.011 和  $< 0.01 \text{ mg} \cdot \text{kg}^{-1}$ ；用 75%腈菌酯·戊唑醇 WG 浸果处理 90 d 的猕猴桃中戊唑醇、腈菌酯的残留量分别为  $0.45 \text{ mg} \cdot \text{kg}^{-1}$  和  $0.22 \text{ mg} \cdot \text{kg}^{-1}$ ；用 25%咪鲜胺 EC 浸果处理 90 d 的猕猴桃中咪鲜胺的残留量为  $0.59 \text{ mg} \cdot \text{kg}^{-1}$ 。由于腈菌酯和咪鲜胺在猕猴桃上并未进行登记，为此参考浆果和其他小粒水果分类中葡萄上的最大残留限量（MRL），经对比，3 种处理中的农药残留均远小于在猕猴桃或葡萄上的 MRL 值，未出现农药残留超标。

表 8 腈菌酯、戊唑醇和咪鲜胺在猕猴桃中的最终残留量

Table 8 Terminal residues of trifloxystrobin, tebuconazole and prochloraz in kiwifruit

| 处理                              | 检测成分                   | 残留量<br>Amount of<br>residue/<br>( $\text{mg} \cdot \text{kg}^{-1}$ ) | 最大残留限量<br>Maximum residue<br>limit/ ( $\text{mg} \cdot \text{kg}^{-1}$ ) | 参考产品<br>Reference<br>product | 判定标准号<br>Decision standard<br>number |
|---------------------------------|------------------------|--|--|------------------------------|--------------------------------------|
| 75%腈菌酯·戊唑醇 WG                   | 戊唑醇 Tebuconazole       | 0.45   | 5.00   | 猕猴桃<br>Kiwifruit             | GB 2763—2021                         |
| 75%                             |                        |  |  |                              |                                      |
| trifloxystrobin·tebuconazole WG | 腈菌酯<br>Trifloxystrobin | 0.22   | 3.00   | 葡萄 Grape                     | GB 2763—2019                         |
| 25%咪鲜胺 EC 25%prochloraz EC      | 咪鲜胺 Prochloraz         | 0.59   | 2.00   | 葡萄 Grape                     | GB 2763—2019                         |

|         |                        |       |      |                  |              |
|---------|------------------------|-------|------|------------------|--------------|
|         | 戊唑醇 Tebuconazole       | 0.015 | 5.00 | 猕猴桃<br>Kiwifruit | GB 2763—2021 |
| 清水对照 CK | 肟菌酯<br>Trifloxystrobin | 0.011 | 3.00 | 葡萄 Grape         | GB 2763—2019 |
|         | 咪鲜胺 Prochloraz         | <0.01 | 2.00 | 葡萄 Grape         | GB 2763—2019 |

### 3 讨论

猕猴桃软腐病发病迅速且难以防治,已成为制约猕猴桃产业发展的主要限制因子,目前化学防治仍是猕猴桃软腐病的主要防治途径<sup>[8, 15-16]</sup>。甾醇脱甲基酶抑制剂(DMI)和甲氧基丙烯酸酯类(QoIs)的多个产品已注册用于由*B. dothidea*引起苹果轮纹病的化学防治,其中包括戊唑醇、肟菌酯及吡唑醚菌酯(<http://www.chinapesticide.org.cn>),然而在中国未有杀菌剂注册用于控制由*B. dothidea*引起的猕猴桃软腐病。本研究中,笔者依据室内药剂毒力测定和果实离体防效评价结果,选用了不同的药剂应用于后续的田间及贮藏期药效试验,筛选出可用于猕猴桃软腐病防治的三种杀菌剂,以咪鲜胺对软腐病菌菌丝生长的抑制活性最强,肟菌酯·戊唑醇的预防效果较好,而咪鲜胺的治疗效果较好;在最佳推荐浓度下,肟菌酯·戊唑醇和唑醚·氟酰胺在生长期田间喷施对软腐病防效较好,咪鲜胺在贮藏前浸果对软腐病的防效较好。本试验中筛选出的生长期和贮藏期的最佳防治药剂略有差别,究其原因可能是杀菌剂作用机制不同造成的。因此,笔者推测生长期喷施预防效果更好的杀菌剂,更能显著地降低软腐病的发生;而采摘和运输过程中,会对果实有一定程度的损伤,采后的果实更加有利于病原菌的侵染<sup>[36]</sup>,此时治疗效果更好的杀菌剂防控效果更加显著。

戊唑醇和咪鲜胺同属于甾醇脱甲基酶抑制剂(DMI)类杀菌剂<sup>[37-40]</sup>,抑制病原菌麦角甾醇的生物合成从而影响病原菌细胞壁的形成,属羟乙基三唑衍生物,戊唑醇和咪鲜胺对白粉菌属、柄锈菌属、喙孢属、核腔菌属和壳针孢属菌引起的病害防治效果较好<sup>[37-40]</sup>;而吡唑醚菌酯为广谱甲氧基丙烯酸酯类(QoIs)杀菌剂<sup>[41]</sup>,是线粒体呼吸作用抑制剂,通过影响细胞色素**b**和**c1**间电子传递而抑制线粒体呼吸作用,使线粒体不能产生和提供细胞正常代谢所需要的能量,最终导致细胞死亡,具有广谱、低毒和内吸性等特点,广泛应用于由子囊菌、担子菌和卵菌等真菌引起的病害,如葡萄霜霉病、葡萄白粉病、柑橘黑星病、苹果轮纹病等<sup>[29]</sup>。长期使用单一作用方式的药剂容易导致病原菌产生抗药性,若采用不同机制的杀菌剂交替或复配使用将能延缓和阻止病原菌抗药性加剧<sup>[42]</sup>。为了降低抗药性产生的风险,建议在田间及采后防治时,将上述药剂交替使用,也可同其他药剂混用或复配,同时限制喷施次数与浓度,并及时进行药剂的敏感性监测,延长药剂使用寿命,避免盲目用药。

笔者研究结果表明在生长期用药和采后用药对猕猴桃软腐病有显著的防效,但果品中农药残留是否达标是消费者最关心的问题。红阳猕猴桃一般在8月底至9月初采收,其货架期短,大多数果实采收后需及时入冷库贮藏。由于贮藏温度对果品中农药残留影响很大,一般农药在冷藏条件下消解速度比室温更慢,比如梨中的咪鲜胺和戊唑醇在25℃的半衰期为



8.8~10.3 d, 而在 2 °C 的半衰期为 173.3~346.6 d<sup>[43]</sup>。在本研究中, 对猕猴桃软腐病防效显著的两种药剂使用后其残留量均远小于在猕猴桃或葡萄上的 MRL 值, 未出现农药残留超标现象, 可推荐生产上使用。

除此之外, 增强猕猴桃果实的抗逆性也会极大地降低病害的发生率, 笔者研究发现吡唑醚菌酯在室内毒力测定中活性最弱, 但在田间试验中防效却达 80%, 能有效防控猕猴桃软腐病。据报道, 吡唑醚菌酯可以刺激大豆生长, 增加叶绿素含量, 并降低丙二醛的水平<sup>[44]</sup>, 吡唑醚菌酯拌种还可以提高小麦种子的发芽率, 促进小麦生物量的积累, 提高株高和鲜质量<sup>[45]</sup>。这可能是由于吡唑醚菌酯增强了猕猴桃的抗逆性, 从而控制了猕猴桃软腐病的发生。综上所述, 生产上推荐肟菌酯·戊唑醇、唑醚·氟酰胺在生长期田间交替轮换使用, 推荐咪鲜胺在贮藏前对果实进行浸果处理, 同时还应尽量避免采收、运输中果实损伤, 科学高效地防控猕猴桃软腐病。

#### 4 结论

本研究开展了不同杀菌剂的室内毒力测定及离体防效评价, 并应用于田间及贮藏期药效试验, 筛选出防治猕猴桃软腐病效果较好的三种杀菌剂。在田间防治中肟菌酯·戊唑醇、唑醚·氟酰胺有很好防治效果, 在贮藏期咪鲜胺对果实进行浸果处理有较好的防治效果, 可在生产中大面积推广, 交替使用。

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