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Full Length Article

Sensitivity and Cross-Resistance of *Botrytis cinerea* (Cause of Strawberry Grey Mould) to Three New Fungicides in Hunan Province, China

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Abstract

This study aimed to clarify the sensitivity and cross-resistance of *Botrytis cinerea* on strawberries to fluazinam (FAN), boscalid (BCI) and phenazine-1-carboxylic acid (PCA) to guide the rational use of these drugs. By measuring the mycelial growth rate, the sensitivity of 126 strains of *B. cinerea* on strawberries to FAN, BCI and PCA in 9 regions of Hunan Province was determined. The results showed that highest half maximal effective concentration (EC_{50}) values for FAN, BCI and PCA against *B. cinerea* on strawberries were 0.9840 μg/mL, 9.0564 μg/mL and 11.2239 μg/mL, respectively; the minimum values were 0.0135 μg/mL, 0.1347 μg/mL and 1.3540 μg/mL, respectively, while the mean values were 0.1349 ± 0.1710 μg/mL, 2.1238 ± 1.9095 μg/mL and 5.2601 ± 2.2908 μg/mL, respectively. The highest values were 70.22-, 73.15- and 8.29-times higher, respectively, than the minimum values. The sensitivity frequencies of the three fungicides against *B. cinerea* on strawberries were not normally distributed, suggesting that sub-populations with reduced sensitivity were present in the *B. cinerea* population. Cross resistance to FAN, BCI and PCA existed. Field experiment results showed that the best control of *B. cinerea* occurred with FAN at 84.87%, which was 1.28-times that of the control, followed by BCI. PCA had the lowest control effect, which was lower than that of the control drug. This study provides a basis for decision-making in the future development of new policies for the prevention and control of *B. cinerea.* © 2019 Friends Science Publishers

Keywords: *Botrytis cinerea*; Cross-resistance; Sensitivity; New fungicides; Mancozeb

Introduction

Botrytis cinerea growth on strawberries is an important disease during the strawberry flowering stage. *B. cinerea* mainly harms the fruit of the strawberry and presents as a grey mildew layer. In severe cases, it can cause yield losses of up to 25% and can even result in no yield (Williamson *et al*., 2007; Ceredi *et al*., 2009; Essghaier *et al*., 2009). During production, chemical agents are often used for disease control, but long-term use of chemical agents can cause pathogenic bacteria and fungi to develop drug resistance (Rosslenbroich and Stuebler, 2000; Ugolini *et al*., 2014). Many scholars abroad have shown that *B. cinerea* on strawberries is resistant to chemical agents. Grabke *et al*. (2014) demonstrated that, in the United States, *B. cinerea* resistance to iprodione commonly exists, but that there are few highly resistant strains. Washington and Shanmuganathan (1992) found that in Victoria, Australia, strawberry-associated *B. cinerea* was resistant to iprodione, chlorothalonil, and dichlofluanid and that there was cross-resistance between iprodione and dichlofluanid. A study by FernándezOrtuño *et al*. (2013a) found that in north and south carolina, while there were few highly resistant strains, *B. cinerea* isolated from strawberries was resistant to cyprodinil and exhibited cross-resistance to cyprodinil and fludioxonil, however, no resistance to fludioxonil was found. However, further research showed that *B. cinerea* was resistant to fludioxonil in Virginia (FernándezOrtuño *et al*., 2013b). Yan *et al*. (2014) found that *B. cinerea* isolated from apples was resistant to pyrimethanil and that therefore, it is necessary to explore new methods for controlling apple grey mould.

In recent years, many scholars in China have also demonstrated that *B. cinerea* on strawberries is resistant to chemical pesticides. *B. cinerea* in East China was resistant to carbendazim, diethofencarb, procymidone and pyrimethanil and that the frequency of resistance was high (Sun *et al*., 2010). The results of the study by Zhou *et al*. (2017) showed that *B. cinerea* on strawberries was resistant to fenhexamid, but no highly resistant strains were identified. The results of the study by Xu *et al*. (2018) showed that *B. cinerea* on strawberries was resistant to pyrimethanil and the pathogenicity was obviously different. Zhao *et al*. (2016) demonstrated that the frequency of strawberry-associated *B. cinerea* multidrug resistance to carbendazim, diethofencarb, procymidone, pyrimethanil,

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and kresoxim-methyl in Nanjing and Zhenjiang, China, was as high as 97.24%. *B. cinerea* on strawberries in Hunan Province, China, is resistant to carbendazim, procymidone and pyrimethanil but has low drug resistance to iprodione (Zhang *et al*., 2016). In some parts of Zhejiang Province the fungus has resistance to iprodione, but in most areas, no resistant strains were found (Li *et al*., 2007). Chen *et al*. (2015) showed that the resistance frequency of *B. cinerea* on strawberries to iprodione is high in the Beijing area of China and that the frequencies of different strains are largely different, which may be related to the level of drug use.

Why is *B. cinerea* susceptible to drug resistance? There are many reasons for this, of which the irrational use of drugs is an important factor. In particular, the multiple repeated uses of agents only acting on single site increases the risk of pathogen drug resistance. To address this issue, it is of great urgency to explore new methods for controlling *B. cinerea*. The development of novel fungicides with unique mechanisms of action is an important method to alleviate the drug resistance of pathogens. Fluazinam (FAN) is a dinitroaniline fungicide that interferes with mitochondrial oxidative phosphorylation coupling agents and mainly affects bacterial respiration. FAN has been registered for use in the prevention and control of diseases, including clubroot, anthracnose, and sheath blight, among others, but it has not been used for the control of *B. cinerea* and no drug resistance has been reported (Xiao, 1996; Qi, 2013). Boscalid (BCI) is a fungicide and is a type of nicotinamide. It can inhibit the succinate dehydrogenase activity of pathogenic bacteria, block the tricarboxylic acid cycle, and interfere with cell division and growth. BCI is active against a variety of fungal diseases and is less prone to resistance. It is mainly used for the prevention and treatment of a variety of crop diseases (Zhang, 2007; Yan *et al*., 2008). Currently, BCI has been registered for the control of grey mould and its sensitivity baselines have been reported in the Jiangsu and Shanghai regions (Liu *et al*., 2018; Xiao *et al*., 2018). However, there are no reports on the sensitivity of *B. cinerea* on strawberries to BCI in Hunan Province. In recent years, phenazine-1-carboxylic acid (PCA) has been developed as a novel fungicide. It can be reduced by nicotinamide adenine dinucleotide (NADH) to become an electron transport intermediate, which can disturb the normal redox of cells and thus can inhibit the growth of microorganisms (Price-whelan *et al*., 2007). Currently, PCA is mainly used for the control of watermelon wilt disease, phytophthora blight in peppers, and stem blight in watermelon and melon, and there are no reports on the sensitivity of *B. cinerea* from strawberries (Shen, 2011; Fang *et al*., 2014). Therefore, this paper studied the sensitivity of *B. cinerea* on strawberries to FAN, BCI and PCA in nine regions of Hunan Province, investigated the cross-resistance among these three fungicides and evaluated the field control effects of these fungicides, in order to provide a theoretical basis for controlling the resistance of *B. cinerea* in different areas of Hunan Province and developing prevention and control strategies.

Materials and Methods

Preparation of Potato Dextrose Agar Medium

Potato dextrose agar culture medium (PDA) was prepared using the Fang's method (Fang, 1998).

Sensitivity Analysis of *B. cinerea* on Strawberries to FAN, BCI and PCA

Stock solutions of 1000 μg/mL were prepared by diluting FAN, BCI and PCA in acetone, after which the stocks were diluted with sterile water to the following concentrations: FAN, 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, 10 μg/mL and 100 μg/mL; BCI, 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, 10 μg/mL and 100 μg/mL and PCA, 6.25 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL and 100 μg/mL. All drug solutions were stored at 4°C for later use. After PDA culture medium was thawed, drug-containing mediums were prepared at the following final concentrations: FAN, 0.001 μg/mL, 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL and 10 μg/mL; BCI, 0.001 μg/mL, 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL and 10 μg/mL; and PCA, 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL, and 10 μg/mL. The ratio between the drug solutions and the PDA culture medium was 1:9.

The mycelial growth rate was measured. The *B. cinerea* strain was inoculated into the centre of the PDA plates and cultured at 22 ± 0.5°C in the dark for 3 days. A 5 mm diameter fungal cake was then taken along the edge of the colony and inoculated onto the centre of a drug-containing PDA plate. A drug-free plate was used as blank control. Each treatment was repeated 3 times and the inoculated plate was cultured in the dark at 22 ± 0.5°C for 3 d. When the colonies on the control plate were 2/3 grown, the colony diameter was measured using the cross method and the inhibition rate (IR) was calculated according to Equation 1. The measured data were preliminarily processed with Excel to calculate the IR of the different treatments against *B. cinerea*. The IR was plotted on the y-axis and the logarithm of the drug concentration was plotted on the x-axis. The regression curve (y = a + bx), the half maximal effective concentration (EC_{50}) and the correlation coefficient (r) were calculated. The EC_{50} values of the strains from different regions treated with different reagents were analysed. Differences in the sensitivity of *B. cinerea* from different regions were analysed by one-way analysis of variance (ANOVA) using DPSv6.55 software. In addition, according to the EC_{50} ranges corresponding to FAN, BCI, and PCA, each EC_{50} value was equally divided into 24, 11 and 11 stages, respectively, starting from 0 μg/mL and increasing by 0.04 μg/mL, 1 μg/mL and 1 μg/mL, respectively. The median EC_{50} value at each stage was plotted on the x-axis, the number of strains and frequency of

occurrence at each stage were counted, and the sensitivity frequency distribution of *B. cinerea* was plotted to establish sensitivity baselines of *B. cinerea* to different drugs.

$$IR(\%) = \frac{DCC\,(mm) - DTC\,(mm)}{DCC\,(mm) - DFC\,(mm)} \times 100 \quad (1)$$

IR = inhibition rate; DCC = diameter of the control colony; DTC = diameter of the treated colony; DFC = diameter of the fungal cake.

Cluster Analysis of the Susceptibility of *B. cinerea* to FAN, BCI and PCA

Cluster analysis of *B. cinerea* strains from different regions was performed using the DPSv6.55 software. The correlation between the sensitivity of *B. cinerea* to different agents and the geological origin of the strains was analysed using cluster analysis.

Control Effect of FAN, BCI and PCA on *B. cinerea* Strains with Weak Sensitivity

Experimental sites were selected in a strawberry plot at Hunan Agricultural University with serious and uniform occurrence of *B. cinerea* every year. The land ploughing, fertilization, watering, and strawberry cultivation (Benihope strawberry) of each experimental site were conducted according to conventional methods. A total of 4 treatments were set up as follows: 50 g/L FAN suspensions (concentrations were prepared according to the recommended dosage), 50% BCI water-dispersible granules, 1% PCA suspensions and 50% procymidone wettable powder (control drug) and sterile water was used as control. Each treatment was repeated 3 times, with a treatment area of 10 m^2. Different test sites were randomly arranged and used for each treatment. The prepared solution was sprayed evenly at 10:00 am in the initial stage of *B. cinerea* growth (approximately March 15 each year) using a 3WBD-16L knapsack sprayer (Lantian Plant Protection Instrument Factory, Pei County, Jiangsu Province). Seven days after the spray, the 5-point sampling method was used and 40 fruits were taken at each point. The total fruit count and diseased fruit count were recorded and the control effect was calculated using Equation (2) (Xiao *et al*., 2018).

$$CF(\%) = \frac{DFR - TFR}{DFR} \times 100 \quad (2)$$

CF: control effect; DFR: blank diseased fruit rate; TFR: treatment diseased fruit rate.

Cross-resistance of *B. cinerea* to FAN, BCI and PCA

The logarithms of the FAN, BCI and PCA EC_{50} values were calculated for all the tested strains using Excel. In addition, the correlations of the EC_{50} values among the different agents were analysed using DPSv6.55 software to determine the cross-resistance among FAN, BCI and PCA.

Data Processing

Regression analysis, correlation analysis and ANOVAs were performed using the DPSv6.55 statistical software. Duncan's new multiple range test was used to test the significance of differences among the different treatments. The normality of the sensitivity frequency distribution of the tested strains to FAN, BCI and PCA was tested by the Shapiro-Wilk test (Luo and Zhang, 2018).

Results

The Sensitivity of *B. cinerea* to FAN, BCI and PCA

Table 1 shows the EC_{50} values for FAN, BCI and PCA against *B. cinerea* on strawberries. The highest EC_{50} values were 0.9840 μg/mL, 9.0564 μg/mL and 11.2239 μg/mL for FAN, BCI and PCA, respectively. The minimum values were 0.0135 μg/mL, 0.1347 μg/mL and 1.3540 μg/mL, respectively and the mean values were 0.1349 ± 0.1710 μg/mL, 2.1238 ± 1.9095 μg/mL and 5.2601 ± 2.2908 μg/mL, respectively. The highest values were 70.22-, 73.15- and 8.29-times that of the minimum values, respectively. The mean EC_{50} values for *B. cinerea* strains from different regions were significantly different. One-way ANOVA was performed for the mean EC_{50} values for *B. cinerea* strains from different regions using DPSv6.55 software. The ANOVA results were compared by Duncan's new multiple range test, and the EC_{50} values of FAN, BCI and PCA against *B. cinerea* strains from different regions were found to be significantly different ($P \leq 0.05$).

Establishment of *B. cinereal* Sensitivity Baselines for FAN, BCI and PCA

Fig. 1 shows that the sensitivity of *B. cinerea* to FAN, BCI, and PCA displayed a continuous single peak distribution. DWSv6.55 software was used to conduct non-parametric normality tests of FAN, BCI and PCA using the K-W method. The following results were obtained: W = 0.6301, P = 0.000 (<0.05), W = 0.8363 and P = 0.000 (<0.05). The sensitivity frequencies of *B. cinerea* to FAN, BCI and PCA were not normally distributed but were positively skewed. These results indicated that the sensitivity of *B. cinerea* from different regions to FAN, BCI and PCA varied and that there were sub-populations with reduced sensitivity. In addition, the EC_{50} values of the 126 *B. cinerea* strains to FAN were between 0.0135 and 0.9480 μg/mL and the 0.04–0.08 μg/mL interval had the highest frequency of occurrence at 58. The frequency of the other intervals decreased successively (Fig. 2). In addition, the EC_{50} value of *B. cinerea* strain CS42 from Changsha, Hunan, to FAN was the highest at 0.9480 μg/mL.

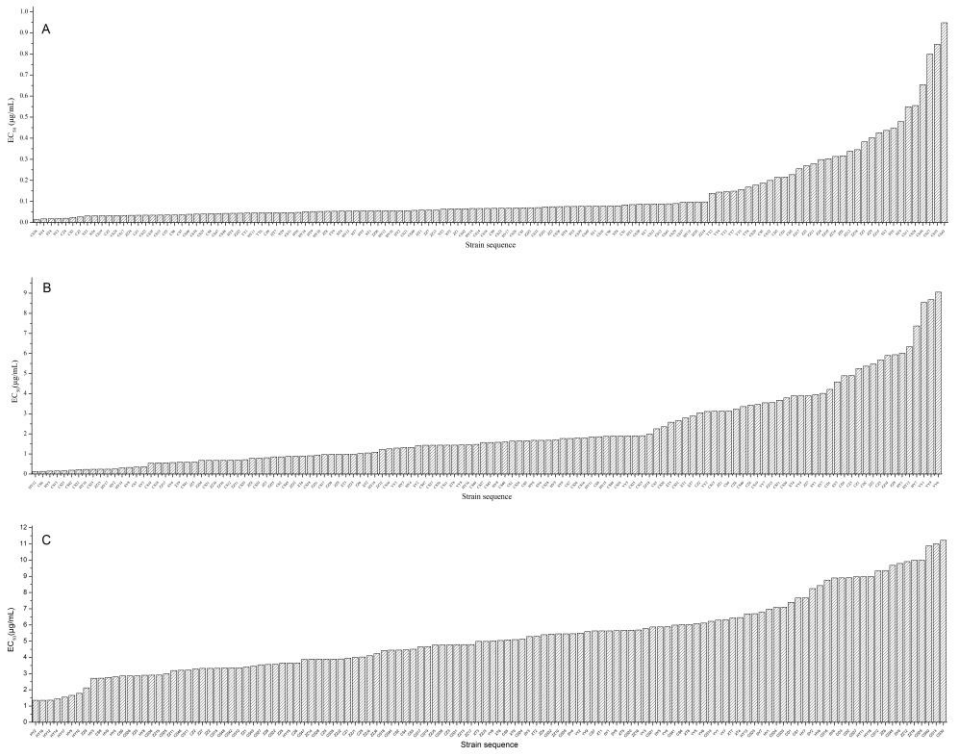

Fig. 1: Sensitivity distribution of *B. cinerea* to three fungicides
A: Fluazinam; CS from Changsha, CD from Changde, YY from Yueyang, ZZ from Zhuzhou, XT from Xiangtan, HY from Hengyang, CZ from Chenzhou, SY from Shaoyang, ZJJ from Zhangjiajie. B: Boscalid; CS from Changsha, CD from Changde, YY from Yueyang, ZZ from Zhuzhou, XT from Xiangtan, HY from Hengyang, CZ from Chenzhou, SY from Shaoyang, ZJJ from Zhangjiajie. C: Phenazine-1-carboxylic acid; CS from Changsha, CD from Changde, YY from Yueyang, ZZ from Zhuzhou, XT from Xiangtan, HY from Hengyang, CZ from Chenzhou, SY from Shaoyang, ZJJ from Zhangjiajie

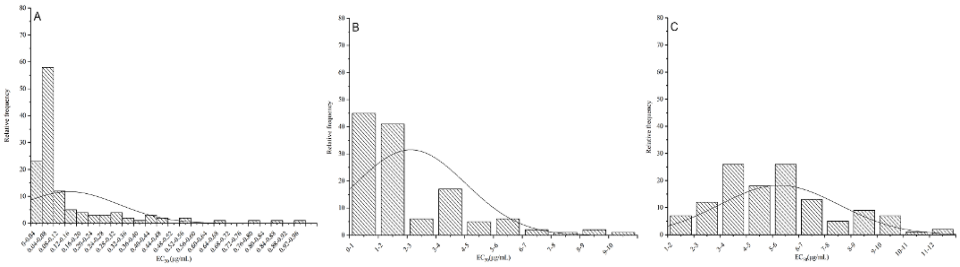

Fig. 2: Frequency distribution of EC_{50} values for three fungicides against *B. cinerea*
A: Fluazinam; Horizontal ordinate: 0-0.96; EC_{50} value ranges: 0-0.04, 0.04-0.08, 0.08-0.12, 0.12-0.16, 0.16-0.20, 0.20-0.24, 0.24-0.28, 0.28-0.32, 0.32-0.36, 0.36-0.40, 0.40-0.44, 0.44-0.48, 0.48-0.52, 0.52-0.56, 0.56-0.60, 0.60-0.64, 0.64-0.68, 0.68-0.72, 0.72-0.76, 0.76-0.80, 0.80-0.84. B: Boscalid; Horizontal ordinate: 0-10; EC_{50} value ranges: 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10. C: Phenazine-1-carboxylic acid; Horizontal ordinate: 1-12; EC_{50} value ranges: 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12

The EC_{50} values of the 126 *B. cinerea* strains to BCI were between 0.1238 and 9.0564 μg/mL and the 0–1.0 μg/mL interval had the highest frequency at 45. The frequency of the other intervals decreased successively. The EC_{50} value of *B. cinerea* strain YY6 from Changsha, Hunan, to BCI was the highest at 9.0564 μg/mL. The EC_{50} values of the 126 *B. cinerea* strains to PCA were between 1.3540–11.2293 μg/mL and the 3.0–4.0 μg/mL and 8.0–6.0 μg/mL intervals had the highest frequency at 26. The frequency of the other intervals decreased successively. The EC_{50} value of *B. cinerea* strain CS30 from Changsha, Hunan, to PCA was the highest at 11.2239 μg/mL.

Cluster Analysis of the Sensitivity of *B. cinerea* to FAN, BCI and PCA

Fig. 3 shows the cluster analysis of the FAN, BCI and PCA EC_{50} values for *B. cinerea* strains using 0.09, 0.70 and 0.55 as the cut-off points, respectively, with 4, 3 and 4 categories, respectively. For the FAN EC_{50} values for the *B. cinerea* strains, the first category had 1 *B. cinerea* strain (CS43), the second category included 2 strains (CS10, CS27), the third category included 1 strain (CS44) and the fourth category included 122 strains (all remaining strains). For the BCI EC_{50} values for the *B. cinerea* strains,

Table 1: Sensitivity of *B. cinerea* from different areas of the Hunan region of China to three fungicides

Fungicide	District	Number of isolates	EC_{50} (μg/mL)	
			Range	Mean±SD
FAN	Changsha	48	0.0135-0.9480	0.1349±0.1710
	Changde	3	0.2143-0.2268	0.1356±0.1675
	Zhuzhou	18	0.0529-0.4258	0.13580±1722
	Xiangtan	9	0.0312-0.0789	0.1023±0.1272
	Shaoyang	9	0.0168-0.4786	0.1325±0.1763
	Zhangjiajie	6	0.0339-0.0975	0.1332±0.1665
	Chenzhou	6	0.0198-0.0463	0.1328±0.1667
	Hengyang	18	0.0434-0.0971	0.1173±0.1468
	Yueyang	9	0.0447-0.1679	0.1261±0.1628
Total		126	0.0135-0.9480	0.1349±0.1710
BCI	Changsha	48	0.1347-3.7896	2.1228±1.9095
	Changde	3	4.2154-5.3690	2.1612±1.9455
	Zhuzhou	18	0.2389-5.9369	2.1378±1.9212
	Xiangtan	9	0.5876-3.8906	2.1915±1.8696
	Shaoyang	9	0.3267-4.5862	2.0498±1.8210
	Zhangjiajie	6	0.6754-0.9867	2.1058±1.9296
	Chenzhou	6	3.0499-5.6789	2.1931±1.9636
	Hengyang	18	0.1238-7.3689	2.1176±1.8770
	Yueyang	9	1.2937-9..0564	2.1505±1.9830
Total		126	0.1238-9.0564	2.1238±1.9095
PCA	Changsha	48	2.7207-11.2239	5.2601±2.2908
	Changde	3	7.3994-8.9076	5.2393±2.3193
	Zhuzhou	18	2.1209-9.9873	5.2050±2.2636
	Xiangtan	9	4.9875-6.4567	5.2822±2.2097
	Shaoyang	9	5.2846-9.7902	5.1968±2.3065
	Zhangjiajie	6	3.8996-4.9876	5.2086±2.3014
	Chenzhou	6	3.2861-4.7786	5.2059±2.3031
	Hengyang	18	1.3540-8.9888	5.2139±2.2838
	Yueyang	9	5.0123-6.3211	5.3578±2.3297
Total		126	1.3540-11.2239	5.2601±2.2908

the first category included 3 strains (YY1, YY6 and YY9), the second category included 1 strain (HY7) and the third category included 122 strains (all remaining strains). For the PCA EC_{50} values for the *B. cinerea* strains, the first category included 3 strains (CS40, CS13, CS30), the second category included 8 strains (HY2, HY12, HY16, HY14, HY15, HY17, HY10 and ZZ9), the third category included 12 strains (all remaining strains), and the fourth category included 12 strains (SY2, HY6, CS16, SY6, CD3, CD2, CS37, HY11, HY18, CS12, ZZ6, CS45, SY9, ZZ12, ZZ18, and CS29). In addition, a series of strains with different geographic origins appeared in the same cluster group, indicating that the sensitivity of pathogenic bacteria to drugs did not have a significant correlation with geographical origin. A series of strains from the same geographic origin also appeared in different cluster groups, indicating that the sensitivity of these pathogenic bacteria to the drugs had a correlation with a specific geographical origin.

The Control Effect of FAN, BCI and PCA on the Most Sensitive and Least Sensitive *B. cinerea* Strains

As shown in Table 2, FAN achieved the best control against *B. cinerea* of 84.87% in 2018 which was 1.28-times that of the control drug and was followed by BCI. PCA showed the lowest control effect, which was significantly lower than that of the control drug.

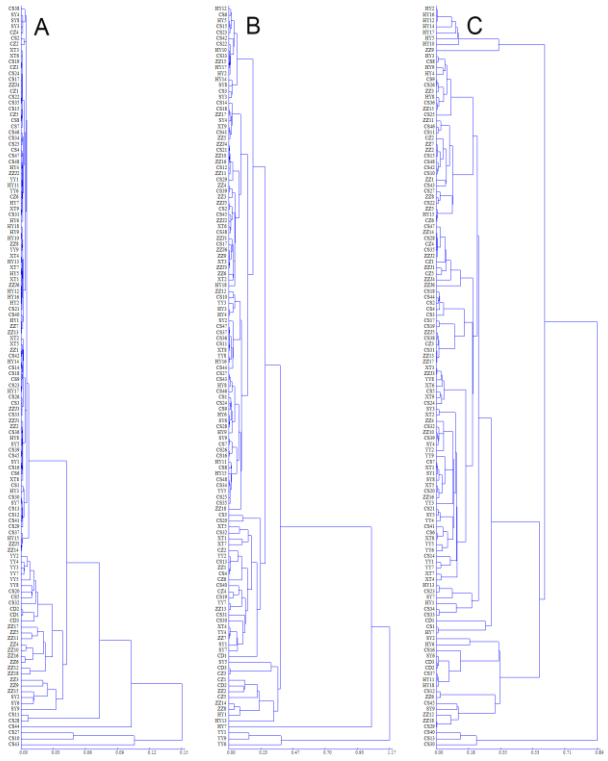

Fig. 3: System clustering analysis of the EC_{50} value for the three fungicides against *B.cinerea*
A: fluazinam; B: boscalid; C: phenazine-1-carboxylic acid. The x-axis is the similarity distance and the y-axis is the EC_{50}

ANOVA results also demonstrated that the control of *B. cinerea* by FAN was significantly different compared to the other treatments (P <0.05). The control of *B. cinerea* in 2017 was basically the same as that in 2018, but the overall control effect in 2017 was better than that in 2018, which may be related to the external environmental conditions of the strawberry plot. These results indicated that FAN and BCI have application prospects in the control of *B. cinerea*, while PCA has poor prospects. In addition, in future applications, it will be necessary to consider the use of alternative agents with different mechanisms of action.

Cross-resistance of *B. cinerea* to FAN, BCI and PCA

As shown in Fig. 4, the correlation coefficient (r) of the FAN and BCI EC_{50} values against *B. cinerea* strains was 0.9313 ($P < 0.05$), reaching a significant level and indicating that there is cross-resistance between FAN and BCI. The correlation coefficient (r) of the BCI and PCA EC_{50} values against *B. cinerea* strains was 0.9857 ($P < 0.05$), which also reached a significant level and indicated that there is cross-resistance between BCI and PCA. The correlation coefficient (r) of the FAN and PCA EC_{50} values against *B. cinerea* strains was 0.9360 ($P <0.05$), reaching a significant level and indicating that there is cross-resistance between FAN and PCA.

Table 2: Control effect of three fungicides against *B. cinerea* in 2017 and 2018

Fungicide	2017		2018	
	Disease fruit rate (%)	Control effect (%)	Disease fruit rate (%)	Control effect (%)
Boscalid	6.07±0.63[c]	69.89±5.38[b]	7.98±0.66[c]	65.80±2.46[b]
Fluazinam	3.75±0.45[c]	81.40±4.18[a]	3.83±0.25[d]	84.87±1.68[a]
Phenazine-1-carboxylic acid	7.50±0.64[b]	62.78±1.98[b]	9.99±0.55[b]	60.58±3.98[c]
Procymidone	6.25±0.58[b]	69.00±4.51[b]	8.53±0.49[c]	66.32±2.60[b]
CK	20.16±2.21[a]		25.34±1.20[a]	

CK is the control. Values ± SD followed by different letters indicate significantly different scores in the same phase according to Duncan's multiple range tests at the $P < 0.05$ significance level

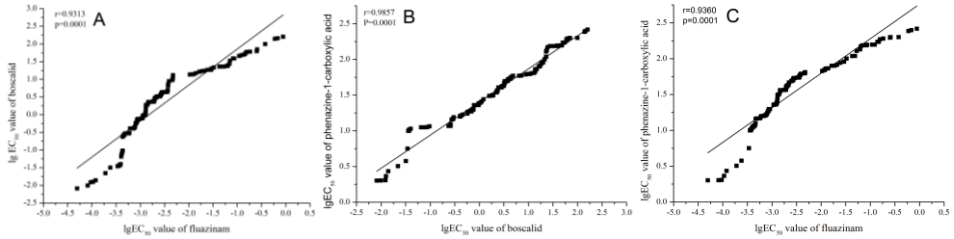

Fig. 4: Cross-Resistance between different fungicides
A: fluazinam and boscalid; B: boscalid and phenazine-1-carboxylic acid; C: fluazinam and phenazine-1-carboxylic acid

Discussion

The results of this study demonstrated that 126 *B. cinerea* strains from 9 regions of Hunan Province were sensitive to FAN, BCI and PCA. The mean EC_{50} values for FAN, BCI, and PCA were 0.1349 ± 0.1710 μg/mL, 2.1238 ± 1.9095 μg/mL and 5.2601 ± 2.2908 μg/mL, respectively. The mean FAN EC_{50} value against *B. cinerea* was higher than that in the study by Shi *et al*. (0.0221 μg/mL), but lower than that in the study by Zheng *et al*. (4.663 μg/mL) (Zheng, 2010; Shi *et al*., 2016). The mean BCI EC_{50} value against *B. cinerea* was higher than that in the study by Liu *et al*. (1.973 μg/mL) (Liu *et al*., 2015). These differences are related to the physiology, inheritance, years of pesticide application, frequency of pesticide application and complexity of microbial communities among different hosts and strains with different geographic origins.

The mean EC_{50} frequency distributions of FAN, BCI, and PCA against *B. cinerea* were not normally distributed. Therefore, the mean EC_{50} values of these three fungicides could not be used as the sensitivity baselines. These results indicated that sub-populations with reduced sensitivity were present among the overall *B. cinerea* population, with the cause determined by many factors. For example, the selection pressure of fungicides, the suitability of resistant mutants, the incidences, and application levels. In addition, the differentiation of the mechanisms and factors involved in this sensitivity await further in-depth studies in the future.

The results of this study confirmed the existence of cross-resistance between FAN and BCI, BCI and PCA, and FAN and PCA. On the one hand, this indicated that *B. cinerea* possesses resistance to multiple fungicides, as well as different resistance genes or alleles. On the other hand, this also indicated that there is a high risk of *B. cinerea* resistance to the three fungicides. However, the study by Cheng *et al*. (2011) found that FAN and BCI did not show cross-resistance, which may be because of the small sample size, resistance to multiple fungicides, the age of fungus tested, or the amine groups in both FAN and BCI; however, the specific reasons remain to be investigated in the future. Therefore, the use of these three fungicides is reasonable, and particular attention should be paid to the dosage and frequency of drug use to prevent the development of drug resistant pathogens.

Results from field experiments showed that FAN, BCI and PCA still had high control effects against *B. cinerea*. The control effect of FAN on *B. cinerea* reached 84.87%. This result is basically consistent with the results reported by Xu (2015) indicating that this drug can be used to control *B. cinerea*, but it should be used cautiously and alternating its use is recommended. Second, the research results of Yang *et al*. (2010) showed that the control effect of BCI against *B. cinerea* may reach 91.84%, which is different from the results of this study, however, this may be related to differences in manufacturers, batches, concentrations, application times, and effective duration of the drug. In addition, *B. cinerea*, strains with different levels of pathogenicity are often mixed together in the field, with each strain making up a different proportion of the total population. Whether this has an impact on the control effect has yet to be determined and will require further study in the future.

Finally, because the strawberry mainly depends on stolon to carry out asexual reproduction, strawberry seedlings are frequently transported between different regions during the production process, which allows for pathogens to be transported over long distances during the seedling transport process. Therefore, it is impossible to completely separate pathogens from their geographical locations. This is one of the reasons why the *B. cinerea* strains in the same area with sensitivity to the 3 agents clustered together.

Conclusion

The results of this study showed that the mean FAN, BCI, and PCA EC_{50} values against *B. cinerea* were 0.1349 ± 0.1710 μg/mL, 2.1238 ± 1.9095 μg/mL and 5.2601 ± 2.2908 μg/mL, respectively. The sensitivity frequencies against *B. cinerea* were not normally distributed, indicating that sub-populations with reduced sensitivity were present among the *B. cinerea* population. Cross-resistance existed between FAN and BCI, BCI and PCA and FAN and PCA. Field experiment results showed that the control effect of FAN on *B. cinerea* reached 84.87% (the greatest effect observed, followed by BCI), which was 1.28-times that of the control drug. PCA had the lowest control effect, which was significantly lower than that of the control drug.

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