Within-Season Shift in Fungicide Resistance Profiles of *Botrytis cinerea* in California Strawberry Fields

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Abstract

Sensitivity of *Botrytis cinerea* to seven fungicide chemical classes was determined for 888 isolates collected in 2016 from 47 California strawberry fields. Isolates were collected early season (minimum fungicide exposure) and late season (maximum fungicide exposure) from the same planting block in each field. Resistance was determined using a mycelial growth assay, and variable frequencies of resistance were observed to each fungicide at both sampling times (early season %, late season %): boscalid (12, 35), cyprodinil (12, 46), fenhexamid (53, 91), fludioxonil (1, 4), fluopyram (2, 7), iprodione (25, 8), isofetamid (0, 1), penthiopyrad (8, 25), pyraclostrobin (77, 98), and thiophanate-methyl (81, 96). Analysis of number of chemical class resistances (CCRs) revealed an increasing shift in CCR from the early to late season. Phenotypes of 40 isolates that were resistant or sensitive to different chemical classes were associated with presence or absence of

mutations in target genes. Fungicide-resistance phenotypes determined in the mycelial growth assay closely matched (93.8%) the genotype observed. Previously described resistance-conferring mutations were found for each gene. A survey of fungicide use from 32 of the sampled fields revealed an average of 15 applications of gray mold–labeled fungicides per season at an average interval of 12 days. The most frequently applied fungicides (average number of applications during the 2016 season) were captan (7.3), pyraclostrobin (2.5), cyprodinil (2.3), fludioxonil (2.3), boscalid (1.8), and fenhexamid (1.4). Multifungicide resistance is widespread in California. Resistance management tactics that reduce selection pressure by limiting fungicide use, rotating among Fungicide Resistance Action Committee codes, and mixing/rotating site-specific fungicides with multisite fungicides need to be improved and implemented.

When environmental conditions are favorable for *Botrytis cinerea* Pers.:Fr., the causal agent of strawberry gray mold, fungicides are the most effective management method (Mertely et al. 2000). Over 20 fungicide applications can be made per season for prevention of this disease (Legard et al. 2001). Populations of *B. cinerea* in strawberry fields with multiple chemical class resistances (CCRs) are a rising concern (Fernández-Ortuño et al. 2014; Konstantinou et al. 2015; Saito et al. 2016). Multiple fungicide applications per season may be selecting for increasingly resistant populations of *B. cinerea* (Adaskaveg and Gubler 2006).

Currently, all site-specific fungicides labeled for gray mold of strawberry in the United States belong to eight chemical classes, none of which are allowed for use in organic strawberry production. Fungicide Resistance Action Committee (FRAC) codes 1, 2, 7, 9, 11, 12, 17, and 19 represent the methyl benzimidazole carbamate (MBC), dicarboxamide (DC), succinate dehydrogenase inhibitor (SDHI), anilinopyrimidine (AP), quinone outside inhibitor (QoI), phenylpyrrole, hydroxylanilide, and polyoxin chemical classes, respectively. Active ingredients are placed into FRAC codes according to their cross-resistance behavior (FRAC 2018). In the United States, *B. cinerea* isolates collected from strawberries have been reported to be resistant to fungicides within every chemical class except for the polyoxins (Dowling et al. 2016; Fernández-Ortuño et al. 2015). In strawberry fields within the southeastern United States, individual isolates of *B. cinerea* have been found to be resistant to up to seven chemical classes (7CCR) (Fernández-Ortuño et al. 2015). Although 5CCR isolates have been found to be competitively disadvantaged compared with sensitive isolates, multi-CCR subpopulations likely

persist because of high selection pressure from the frequent application of site-specific fungicides (Chen et al. 2016; Hu et al. 2016a).

Reports of extensive frequencies of resistance (>20%) to APs, DCs, MBCs, QoIs, and SDHIs in *B. cinerea* collected from strawberries in the Eastern United States demonstrate the necessity for the characterization of resistance levels within California, the leading strawberry producing state (Fernández-Ortuño et al. 2014). Understanding the distribution and frequency of resistant phenotypes of *B. cinerea* in California will aid resistance management efforts. Previous studies in California strawberries showed that resistance to popular fungicides exists (Mercier et al. 2010; Pokorny et al. 2016) and that the frequency of resistance in a field population can change within a season (Adaskaveg and Gubler 2006). The objectives of this study were to determine (i) the frequency of fungicide resistance in the Southern (Ventura county), Central (San Luis Obispo and Santa Barbara counties), and Northern (Monterey and Santa Cruz counties) strawberry growing districts of California to active ingredients of seven currently registered chemical classes; (ii) shifts in fungicide sensitivity profiles within a production season; (iii) the genetic basis of resistance, and (iv) fungicide use during the same season.

Materials and Methods

Isolate collection and characterization. In 2016, 888 bulk conidial isolates of *B. cinerea* were collected from 37 conventional and 10 organic strawberry fields dispersed throughout California's Southern (296 isolates), Central (200 isolates), and Northern (392 isolates) strawberry growing districts. Each isolate was randomly collected from a 10-acre, fall-planted block in each field by brushing an individually wrapped, sterile applicator stick (Thermo Fisher Scientific, Waltham, MA) against a sporulating lesion of gray mold on a strawberry fruit. If sporulating fruit could not be found in the field, green strawberry fruit were collected, surface sterilized for 1 min in 10% bleach, and incubated until signs of gray mold appeared as described previously (Fernández-Ortuño et al. 2014). The same 10-acre block in each field was sampled twice: early season (zero to a few fungicide applications; minimum fungicide exposure) and late season (10 to 20 fungicide applications; maximum fungicide exposure). Approximately 10 isolates were collected per field at each sampling time. Recent reports have found gray mold on strawberry caused by pathogens besides *B. cinerea*. To characterize the species causing

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gray mold in the early- and late-season samplings, the *NEP2* gene from 95 isolates was amplified with primers capable of differentiating between three important causal agents: *B. cinerea*, *B. fragariae*, and *B. mali* (Table 1). The objective was to determine any differences in species distribution between or among growing districts. These isolates were randomly selected from all sampled fields, equally representing all three growing districts.

Mycelial growth assay. A discriminatory fungicide dose was used to differentiate sensitive from resistant isolates. Formulated products containing single active ingredients were used to create a unique growing medium for each active ingredient tested (Table 2). Fungicide dosages and growing media from previous studies were utilized (Fernández-Ortuño et al. 2014; Weber and Hahn 2011). Sterile toothpicks were used to transfer spores from all 888 swabs to the different fungicide-amended media in wells of 24-well plates (Thermo Fischer Scientific, Waltham, MA). Plates were incubated at 22°C for 4 days, and diametric colony growth was visually assessed in each well as follows: sensitive (S) for less than 20% diametric growth and resistant (R) for more than 20% diametric growth with respect to the 15-mm well diameter. Isolates classified as S and R to each fungicide dose were confirmed as sensitive or resistant with a detached strawberry fruit assay in a previous study (Fernández-Ortuño et al. 2014). A subset of the collected isolates from conventional (71 early season and 334 late season) and organic fields (21 early season and 82 late season) were also tested for resistance to pyraclostrobin and characterized according to the number (n) of chemical classes they were resistant to (nCCR). CCR in *Botrytis* populations is used to monitor the prevalence of multifungicide resistance (Chen et al. 2016; Fernández-Ortuño et al. 2014).

Molecular basis of resistance and isolate identification. The validity of the mycelial growth assay was vetted by comparing resistant phenotypes to the presence of variations in target genes or transcription factors of 40 representative isolates from all growing districts. A single spore was excised from each representative isolate and cultured as a single-spore isolate. Genomic DNA was extracted from the single-spore isolates using a previously described method (Chi et al. 2009). The following target genes were screened for resistance-associated mutations: β -tubulin gene, cytochrome b (*cytb*) gene, histidine kinase (*bos1*) gene, keto-reductase (*erg27*) gene,

transcription factor *mrr1* gene, and *sdhB* gene. Mutations within these genes are known to confer resistance to FRAC 1, 11, 2, 17, 12, and 7, respectively, and previously published thermocycling protocols and primers were used for each gene (Table 1). The target site of FRAC 9 fungicides was unknown and therefore was not included. Polymerase chain reactions (PCRs) were conducted using a BioRAD T100 Thermal Cycler in a total volume of 20 μ l and contained AccuPower Hotstart PCR PreMix (Bioneer Corp., Alameda, CA) and 0.2 μ l of DNA. PCR products were electrophoresed for 1 h at 100-V constant voltage on 1.0% agarose gel with Tris-acetate-EDTA buffer. For sequencing, the PCR products were purified using the ExoSAP-IT PCR purification kit (USB Corporation, Cleveland, OH) following the manufacturer's instructions, and DNA was sequenced by CORE Laboratories at Arizona State University. Sequences were assembled and compared using DNASTAR software (DNASTAR, Madison, WI). We chose to sequence the β -tubulin gene from 9 resistant and 2 sensitive isolates, the *cytb* gene from 10 resistant and 1 sensitive isolates, the *bos1* gene from 11 resistant and 1 sensitive isolates, the *erg27* gene from 8 resistant and 6 sensitive isolates, the *mrr1* gene from 3 resistant and 1 sensitive isolates, and the *sdhB* gene from 16 resistant and 1 sensitive isolates. There was at least one isolate from each district represented for each gene.

Fungicide use survey. In California, all pesticide use is reported to the California Department of Pesticide Regulation by the Agricultural Commissioner offices in each county. In 2017, a public records request for 2016 pesticide use information was submitted to the Agricultural Commissioner offices in the counties of the Southern, Central, and Northern strawberry growing districts. Fungicide use data of products labeled for gray mold of strawberry in 32 of the sampled conventional fields was summarized by application interval and applications per season for each field. No fungicides used in organic production were known to select for resistance to the site-specific fungicides tested in this study.

Statistical analysis. JMP software (version 12.1.0; SAS Institute, Cary, NC) was used to perform statistical analyses. An analysis of variance was used to compare the average CCR from early- and late-season sampling times from conventional and organic ranches. Outliers were excluded to meet the assumption of homogeneity of variance, which was tested with a Levene's test ($P = 0.186$). The

Table 1. Oligonucleotide primers used in this study

Location	Name	Primer sequence (5'—3')	Reference
<i>β-Tubulin</i>	Tub-F1	GCTTTTGATCTCCAAGATCCG	Banno et al. (2008)
	Tub-R1	CTGGTCAAAGGAGCAAATCC	
<i>bos1</i>	BF2	CAACGTTATGGCACAAAATCTCA	Ma et al. (2007)
	BR2	AAGTTTCTGGCCATGGTGTTC	
<i>erg27</i>	F412_F	GACATTACGTTCTCGCACACG	Grabke et al. (2013)
	F412_R	CAACCAGGAACCTCGGTTCG	
	F412S_int	CTTCCCATCCATCTTACAAGGTAGG	
	F412I_int	CTTCCCATCCATCTTACAAGGTAGAT	
	F412C_int	CTTCCCATCCATCTTACAAGGTAGCA	
<i>mrr1</i>	Mrr1_TF1-1	CCAATCATTCCCAATCATTTCA	Kretschmer et al. (2009)
	Mrr1_TF1-4	GGATAGGGTATTGCGTAGATCG	
	MfsM2-pfor	TAGCCAATGGATCCTACG	
	MfsM2-prev	CGAGATGGATGCCATTTTCAGAG	
<i>nep2</i>	CFM_NEP2	GTAGGAACAGTTTATGAG	Dowling et al. (2017)
	C_NEP2	GACCCATTGAGTGATCGACG	
	F_NEP2	TAGTTTGGATCTGTAAGGAGGTGA	
	M_NEP2	ACCCTAAGAAACGTTAGAGACATG	
<i>QoI</i>	Qo13ext	GGTATAACCCGACGGGGTTATAGAATAG	Fernández-Ortuño et al. (2012)
	Qo14ext	AACCATCTCCATCCACCATACTACAAA	
<i>sdh</i>	N230I-fw	TCTACCCACTACCCACACC	Amiri et al. (2014)
	N230I-rev	TCCGCAATTGCCAAACCAGGATTC	
	H272Y-fw	ATCTTGCCCCCTCTACTGGT	
	H272Y-rev	CTCCAGCAGTTGAGAATAGTGA	
	272Y/R-F	AAGGAGGATCGTAAGAAGCTTG	Yin et al. (2011)
	272R-R	TCGAGCAGTTGAGAATAGTGC	
	SdhB-F	AAGGTATCTGCGGCAGTTGTG	
	SdhB-R	TAGCAATAACCGCCCAAAAC	

exclusion did not affect the outcome of the analysis. Multiple comparisons of average CCR were conducted using a post hoc Tukey's honest significant difference test. The relationship between the resistance observed and the sampling time for each fungicide treatment was compared using a Fisher's exact test and adjusted with a Bonferroni correction.

Results

Fungicide resistance profiles. Overall resistance frequencies of isolates collected from conventional and organic fields were 94 and 73% resistant to pyraclostrobin, 89 and 72% to thiophanate-methyl, 73 and 42% to fenhexamid, 29 and 13% to cyprodinil, 24 and 18% to boscalid, 17 and 6% to penthiopyrad, 16 and 6% to iprodione, 5 and 3% to fluopyram, 2 and 2% to fludioxonil, and 1 and 1% to isofetamid, respectively. Resistance frequencies of isolates from conventional fields were significantly different ($P < 0.05$) between the early and late season for all active ingredients except fluopyram and isofetamid (Fig. 1). Resistance frequencies of isolates from organic fields were not significantly different ($P < 0.05$) between early and late season for any active ingredients. Frequencies of resistance varied from district to district and from field to field within the same district. Resistance to isofetamid was only observed in three isolates from the late-season sampling of the Northern district.

Resistance phenotypes. The average CCR of isolates from late-season conventional fields was statistically higher than early-season conventional fields and organic fields at both sampling times (Fig. 2). From conventional fields, there were 45 distinct resistance patterns among the 405 isolates tested for resistance to all seven FRAC codes (including pyraclostrobin). The most common resistance pattern was resistance to fenhexamid, pyraclostrobin, and thiophanate-methyl. Consequently, the most common CCR phenotype was 3CCR both in the early-season and late-season collections. We only detected four 7CCR isolates during the late season and only from the Northern district (Fig. 3).

Molecular basis of resistance and isolate identification. A 95-isolate subset of the early- and late-season isolate collections representing all districts was identified to be *B. cinerea* (94 isolates) and *B. mali* (1 isolate). To determine the range of target gene variations in California isolates, a total of 40 isolates from the Southern, Central, and Northern districts were subjected to genetic analysis. They were determined as resistant or sensitive to multiple fungicides in the mycelial growth assay. Several known resistance-conferring mutations were found within each target gene: F196C, F412I, and F412S within *erg27*; I356N, I365N, and I365S within *bos1*; E198A within the β -tubulin gene; G143A within the *cytb* gene; R351C and the Δ L497 deletion within *mrr1*; and H272R, H272Y, N230I, and P225F within the *sdhB* gene. The Δ L497 deletion within the *mrr1* gene is only found in *Botrytis* group S genotypes and is referred to as MDR1h (Leroch et al. 2013). P225F was the only mutation associated with resistance to isofetamid. Isolates with this mutation were also cross-resistant to penthiopyrad, fluopyram, and boscalid. One isolate sensitive to pyraclostrobin contained a

previously reported type I intron after codon 143 of the *cytb* gene (Leroux et al. 2010).

The fungicide resistance phenotype determined in the mycelial growth assay matched largely (61/65 matches; 93.8% accuracy) with the absence or presence of target gene mutations. Exceptions include two isolates (660 and 118A) that were sensitive to pyraclostrobin but revealed the G143A mutation; mycelial growth assay retesting of the single-spore isolates confirmed both were resistant to pyraclostrobin. Another two isolates (44 and 746) were sensitive to boscalid but had the H272R mutation; retesting of the single-spore isolates confirmed both were resistant to boscalid.

Fungicide use survey. From our survey, we observed that fungicide applications were made throughout the harvest season. Harvest began and ended at different times for the three regions, but the average time between the first and last fungicide application was approximately 6 months. There was an average of 15 fungicide applications at an average interval of 12 days. The resistance management recommendation of rotating modes of action was universally employed. Captan, a multisite fungicide, was the most frequently applied fungicide, whereas thiram (also multisite) was rarely used (Fig. 4). The cyprodinil + fludioxonil premix, boscalid + pyraclostrobin premix, and fenhexamid were the most frequently applied site-specific products in the 32 surveyed fields.

Discussion

Resistance of *B. cinerea* to fungicides has been previously described in California (Adaskaveg and Gubler 2006; Mercier et al. 2010; Pokorny et al. 2016). This study builds on previous work by looking at a much larger number of isolates and fungicides across the major California strawberry fruit production districts and

Fig. 1. Early-season (white) and late-season (black) frequencies of resistance to 10 active ingredients with isolates of *Botrytis cinerea* collected in conventional fields of the Southern ($n = 229$), Central ($n = 165$), and Northern ($n = 308$) strawberry growing districts of California. Fewer isolates were tested for resistance to pyraclostrobin in the Southern ($n = 120$), Central ($n = 97$), and Northern ($n = 188$) districts. Statistical significance of frequency of resistance between sampling times is represented by an asterisk (*) ($P < 0.05$).

Table 2. Discriminatory concentrations and media used in the mycelial growth assay to monitor resistance in isolates of *Botrytis cinerea*^a

Trade name	Active ingredient (FRAC code)	Concentration ($\mu\text{g ai/ml}$)	Medium
Nonamended	...	...	CZA
Vangard	Cyprodinil (9)	4.0	CZA
Scholar SC	Fludioxonil (12)	0.5	MEA
Elevate	Fenhexamid (17)	50.0	MEA
Rovral	Iprodione (2)	10.0	MEA
Topsin M	Thiophanate-methyl (1)	100.0	MEA
Cabrio	Pyraclostrobin (11)	10.0	MEA + SHAM at 100 $\mu\text{g/ml}$
Endura	Boscalid (7)	75.0	YBA*
Luna Privilege	Fluopyram (7)	10.0	YBA
Fontelis	Penthiopyrad (7)	5.0	YBA
Kenja	Isofetamid (7)	5.0	YBA

^a Fungicide concentrations and media were previously described by Weber and Hahn (2011). FRAC = Fungicide Resistance Action Committee; CZA = Czapek-Dox agar, and this medium avoids the interference of amino acids with the assay (Leroux 2007); MEA = malt extract agar; SHAM = the alternative oxidase inhibitor salicylhydroxamic acid; YBA = yeast bacto acetate agar, and this medium avoids the interference of sugars with the assay (Weber and Hahn 2011).

addresses the question of within-season shifts in fungicide sensitivity. Our results document resistance to all major site-specific chemical classes in isolates from all major fruit growing regions of the state and establish that resistant populations are widespread in California strawberry production. High frequencies of resistance to fenhexamid, pyraclostrobin, and thiophanate-methyl were found in most fields. Our survey showed that although the use of MBCs in fruit production fields was rare in 2016, resistance was high. This is likely a result of the use of benomyl and later thiophanate-methyl during the 1970s, 1980s, and 1990s (Johnson et al. 1994; Young 2015). The common, MBC resistance-associated E198A mutation may not entail a significant fitness cost, and other studies have also observed high frequencies of resistance (Fernández-Ortuño et al. 2014; Johnson et al. 1994; Raposo et al. 1996). Because of very high levels of resistance to thiophanate-methyl (85%), this product should not be used for gray mold management but may still be effective against other diseases. Alternatively, pyraclostrobin and fenhexamid were used frequently within the 2016 season; their current popularity may be responsible for the high levels of resistance observed. The common G143A mutation that confers resistance to QoI fungicides was not found to accompany a loss in fitness in *B. cinerea* (Veloukas et al. 2014), and thus it is expected that the California population will maintain high frequency of resistance even if FRAC 11 usage is curtailed. In contrast, a fitness cost related to fenhexamid resistance has been observed; therefore, reducing or eliminating its use for a period may yield a restoration of its efficacy (Adaskaveg and Gubler 2006; Billard et al. 2012).

We observed a within-season shift in fungicide-resistance profiles, which was likely induced by the regular application of fungicides and the associated selection pressure. Although most applications were captan, a product that is not believed to select for resistance, site-specific fungicides were applied about half of the time. The frequency of resistance either increased or stayed the same for most fungicides.

Fig. 2. Average number of chemical class resistances (CCRs) of *Botrytis cinerea* isolates collected early and late season from conventional ($n = 405$) and organic ($n = 103$) California strawberry fields. Error bars are standard error of the mean. Bars with different letters are significantly different according to a post hoc Tukey's honest significant difference test ($\alpha = 0.05$).

We observed a considerable increase in resistance frequency to cyprodinil, boscalid, and fenhexamid, likely owing to selection pressure (Adaskaveg and Gubler 2006), but not to fludioxonil. The scarcity of fludioxonil resistance supports the evidence that fludioxonil resistance is unstable within a population (Chen et al. 2016; Fernández-Ortuño et al. 2014). Resistance frequencies to thiophanate-methyl increased despite it being rarely applied in the 2016 season. Along with the lack of fitness penalty of the E198A mutation in the β -tubulin gene, the increasing shift in frequency of resistance may also be attributed to selection by association, a phenomenon suggesting that selection for resistance to a particular fungicide in populations with multifungicide-resistant isolates can occur without direct selection pressure (Hu et al. 2016a). Resistance to iprodione may have decreased from the early season to the late season because this fungicide is primarily used in nurseries, and resistance to this active ingredient may entail a fitness cost (Johnson et al. 1994; Raposo et al. 1996).

There was a general shift toward isolates with increased CCR. Studies have shown CCR to fungicides changing from year to year (Fernández-Ortuño et al. 2014). The results of this study show a rapid, within-season shift in resistance to fungicides, indicating that fungicides may lose efficacy during the course of the production season. The Southern district had the most drastic changes from early to late season, with resistance to boscalid, cyprodinil, and penthiopyrad increasing by over 40%. The Southern district's production occurs during the rainy season, which may bring higher disease pressure, leading to a more rapid resistance development. More research needs to be conducted to determine how different fungicides and fungicide programs affect frequencies of resistance over time within a field.

Isolates of *B. cinerea* varied in frequency of resistance to the four SDHI fungicides tested in this study. Specific mutations within the *sdhB* region are associated with different resistance patterns among the SDHI fungicides (Hu et al. 2016b; Leroux et al. 2010). Mutations within *sdhB* conferred the same resistance patterns as shown

Fig. 3. Early-season ($n = 71$) and late-season ($n = 334$) frequencies of chemical class resistance (CCR) phenotypes from *Botrytis cinerea* isolates collected in conventional fields of the Southern, Central, and Northern strawberry growing districts of California.

Fig. 4. Average number of applications per season of active ingredients labeled for gray mold of strawberry in 32 California conventional strawberry fields in 2016. Error bars are standard error of the mean. BLAD = Banda de *Lupinus albus* doce.

previously, except that this study includes isofetamid (Fernández-Ortuño et al. 2017). The P225F mutation that conferred resistance to boscalid, fluopyram, isofetamid, and penthiopyrad has also been associated with resistance to fluxapyroxad (Hu et al. 2016b). Boscalid and penthiopyrad are among the longest-used SDHIs and tended to have higher frequencies of resistance. SDHI active ingredients are sold under six different trade names currently labeled for gray mold of strawberry. For this reason, care must be taken to not make successive applications of the same chemical class.

None of the fungicides tested in this study are labeled for use in organic strawberry production, yet a similar resistance profile was observed in early-season organic fields to that of early-season conventional fields. Resistant subpopulations of *B. cinerea* can be introduced into strawberry fields from wind-dispersed spores from nearby host plants (Fitt et al. 1985) or by colonized strawberry transplants (Oliveira et al. 2017). Strawberry nurseries typically do not apply fungicides for gray mold management; however, similar chemical classes are applied for other diseases, and nontarget selection for resistant subpopulations of *B. cinerea* is possible. Resistance management cooperation between nurserymen and fruit growers is important so that selection pressure exerted in nurseries does not translate to loss of fungicide efficacy in fruit production fields.

Resistance management strategies are key for sustained site-specific fungicide efficacy. Isolates of *B. cinerea* with reduced sensitivity to the newest mode of action for gray mold of strawberry, FRAC 19, have already been found (Dowling et al. 2016). Therefore, multisite fungicides should be the primary defense against gray mold, leaving highly effective, site-specific fungicides for times of high disease pressure (Hu et al. 2016a). Applying fungicides according to environmental conditions can be equally effective to a calendar-based spray program and can greatly reduce the number of applications per season (Bulger et al. 1987; Pavan et al. 2011). Cultural methods can also be effective ways to decrease gray mold pressure, thereby reducing the need to apply fungicides (Janisiewicz et al. 2016; Legard et al. 2000; Xiao et al. 2001). With industry-wide cooperation and careful use of site-specific fungicides, selection pressure can be reduced and the effective life of fungicides for gray mold control can be extended.

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