Pesticide Biochemistry and Physiology 171 (2021) 104737

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Cross-resistance to the new fungicide mefentrifluconazole in DMI-resistant fungal pathogens

Hideo Ishii [a,b,*], Patricia Karen Bryson [b], Miyuki Kayamori [c], Takuya Miyamoto [d], Yuichi Yamaoka [a], Guido Schnabel [b,*]

[a] University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan
[b] Clemson University, 105 Collings St., Clemson, SC 29634, USA
[c] Tokachi Agricultural Experiment Station, Hokkaido Research Organization, Memuro, Kasai, Hokkaido 082-0081, Japan
[d] Horticultural Research Institute, Ibaraki Agricultural Centre, 3165-1 Ago, Kasama, Ibaraki 312-0292, Japan

ARTICLE INFO

Keywords:
Cucumber
DMIs
Endocrine disrupting
Mefentrifluconazole
Peach
Powdery mildew

ABSTRACT

In the European Union (EU), regulation of sterol demethylation inhibiting (DMI) fungicides is tightened due to their suspected endocrine disrupting properties. However, the new DMI fungicide mefentrifluconazole was reported to have high fungicidal activity with minimal adverse side effects. In addition, some evidence suggests inconsistent cross resistance between mefentrifluconazole and other azoles. In this study, mefentrifluconazole and other triazoles were examined for activity to select pathogens sensitive or resistant to DMIs using mycelial growth tests on fungicide-treated culture medium or spray trials using cucumber plants. Cross-resistance was confirmed for all of the fungal species tested but activity levels varied. The sensitivity of *Monilinia fructicola* from peach to mefentrifluconazole was higher compared to other DMIs. In contrast, the inhibitory activity of mefentrifluconazole was equal or slightly inferior compared to difenoconazole, tebuconazole, propiconazole in *Colletotrichum* spp., *Alternaria alternaria* sp. complex and *Cercospora beticola* isolated from peach and sugar beet, respectively. Similar tendencies (*i.e.* equal or slightly inferior activity and cross-resistance) were observed for cucumber powdery mildew (*Podosphaera xanthii*) resistant to triflumizole, myclobutanil, and difenoconazole. Despite cross-resistance to other DMIs, mefentrifluconazole is a promising fungicide for fungal disease control on peach and other crops, with a reportedly more favorable toxicity profile.

1. Introduction

Sterols such as ergosterol, ergosta-5,24(24[1])-dien-3β-ol, and ergosta-5,7-dien-3β-ol are essential constituents of fungal cell membranes regulating their stability and permeability. Therefore, fungicides inhibiting key enzymes involved in fungal sterol biosynthesis have become very effective plant disease management tools (Loeffler et al. 1992; Shirane et al. 1996; Chapuis et al. 1996; Ziogas and Malandrakis 2015). Sterol biosynthesis inhibitors (SBIs) are currently divided into four groups by the Fungicide Resistance Action Committee (FRAC) depending on the target: G1: C14-demethylase; G2: Δ14-reductase and Δ8 → Δ7-isomerase; G3: 3-keto reductase in C4-demethylation; and G4: squalene epoxidase. Among them, G1 azoles (triazole and imidazole) in particular (DMIs, FRAC Code 3) have become the most widely used disease control agents not only in agriculture but also in the medical field. In agriculture, thirty-seven DMI compounds are listed by FRAC as of July 2020 (https://www.frac.info/) with over 30% of the fungicide market share worldwide (Mehl et al. 2019). However, the following serious concerns exist for azoles: (1) development of azole resistance in fungal plant pathogens (Köller and Scheinpflug 1987; Brent 2012; Ziogas and Malandrakis 2015), (2) suspected endocrine disrupting properties (Vinggaard et al. 2005; Taxvig et al. 2008; Draskau et al. 2019), and (3) dispersal of *Aspergillus fumigatus* strains resistant to azoles from agricultural environment to humans (Verweij et al. 2013; Chowdhary et al. 2013; Berger et al. 2017; Santoro et al. 2017; Beer and Jackson 2019).

In contrast to methyl benzimidazole carbamates (MBCs) and quinone-outside inhibitors (QoIs), resistance development for DMIs proceeds slowly and levels of resistance increase gradually in general (Brent and Hollomon 2007). A decrease of control efficacy has occurred for this class of fungicides for a number of plant pathogens on various crops since early 1980s. For example, resistance to DMIs has been a

* Corresponding authors at: Clemson University, 105 Collings St., Clemson, SC 29634, USA.
E-mail addresses: hi481204@yahoo.co.jp (H. Ishii), schnabe@clemson.edu (G. Schnabel).

https://doi.org/10.1016/j.pestbp.2020.104737
Received 2 September 2020; Received in revised form 15 October 2020; Accepted 16 October 2020
Available online 19 October 2020

major issue in *Zymoseptoria tritici* (teleomorph: *Mycosphaerella graminicola*), the causal agent of Septoria tritici blotch which is one of the most devastating diseases of wheat (Cools and Fraaije 2012; Torriani et al. 2015; Heick et al. 2017; Sykes et al. 2018). Resistance has also impacted control of *Cercospora beticola*, the pathogen of Cercospora leaf spot of sugar beet (Karaoglanidis et al. 2003; Secor et al., 2010; Khan, 2015; Rangel et al., 2020). DMI resistance in *Podosphaera xanthii*, the pathogen of cucurbit powdery mildew, has spread worldwide (Schepers, 1985; López-Ruiz et al. 2010; McGrath 2015). For tree-fruit diseases, DMIs are essential IPM tools but resistance has been reported for peach brown rot caused by *Monilinia fructicola* (Schnabel et al. 2004; May-De-Mio et al. 2011; Chen et al. 2013b). Anthracnose of peach is mainly caused by the two species complexes *Colletotrichum gloeosporioides* and *C. acutatum*. *C. fructicola* and *C. siamense* belong to the former complex and were sensitive to DMIs, but *C. fioriniae* subgroup 2 isolates of the *C. acutatum* sp. complex were less sensitive to DMIs (Chen et al. 2016). Difenoconazole was effective for the control of Alternaria disease on apple (Reuveni and Sheglov 2002), but isolates of *A. alternata* sp. complex from DMI-treated pistachio orchards were less sensitive to difenoconazole (Avenot et al. 2016).

Mefentrifluconazole, (2*RS*)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1- (1*H*-1,2,4-triazol-1-yl) propan-2-ol, is an active ingredient from the new sub-class of isopropanol-triazole agricultural fungicides (Fig. 1). The mode of action of this fungicide is the inhibition of C14-demethylase in sterol biosynthesis in membranes and belongs to the FRAC Code 3 (action class of G1; https://www.frac.info/). Although triazoles and imidazoles in general adversely effect reproduction, neither embryofetal toxicity nor teratogenicity has been shown for mefentrifluconazole (EFSA, 2018; Tesh et al. 2019). Mefentrifluconazole revealed high efficacy to control key fungal diseases of pome and stone fruit, grapevine, potato, soybean (Heinecke et al. 2019) and other crops and was recently registered in the EU, USA, and other areas of the world (https://agriculture.basf.com/global/en/innovations-for-agriculture/innovation- for-fungicides/revysol.html). Interestingly, excellent efficacy of this fungicide was found against a wide spectrum of existing triazole-resistant (referred to 'triazole-adapted' in literature) isolates of *Z. tritici* of wheat (Jørgensen et al., 2020; Strobel et al. 2020). In their research, high intrinsic activity and a low correlation of resistance was observed with representative DMIs used for Septoria leaf blotch control such as prothioconazole and epoxyconazole. These findings raise an important question; to what extent is mefentrifluconazole effective against isolates resistant to existing DMIs, i. e., the presence or absence of cross-resistance, in other pathogens. Therefore, the objective of this study was to test inhibitory activity of mefentrifluconazole against isolates sensitive and resistant to older DMIs using *M. fructicola*, *Colletotrichum* spp., and *A. alternata* sp. complex, from peach, *C. beticola* from sugar beet, and *P. xanthii* from cucumber.

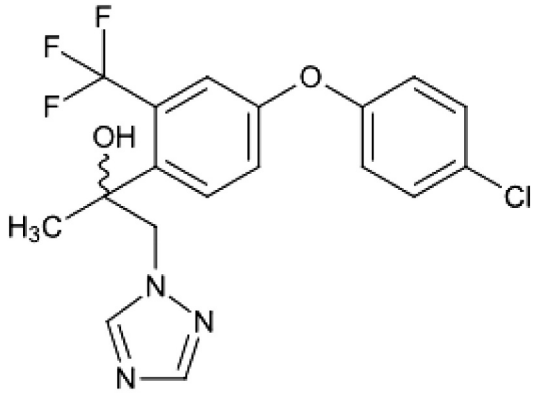

Fig. 1. Chemical structure of mefentrifluconazole. IUPAC: (2*RS*)-2-[4-(4-chlorophenoxy)-2- (trifluoromethyl)phenyl]-1-(1*H*-1,2,4- triazol-1-yl) propan-2-ol. CAS registration number: 1417782–03-6.

2. Materials and methods

2.1. Fungal isolates and fungicides

Ten isolates of *M. fructicola*, 8 isolates of *A. alternata* sp. complex, 12 isolates of *Colletotrichum* spp. including *C. fructicola*, *C. siamense*, *C. nymphaeae*, *C. fioriniae*-subgroup 1 and -subgroup 2, all laboratory stocks in Clemson Univ., SC, originated from the US, additional 4 isolates of *A. alternata* sp. complex supplied by Genebank Project, NARO, 10 isolates of *C. beticola*, and 3 isolates of *P. xanthii*, all from Japan, used in this study are listed in Table 1. DMIs employed were: propiconazole (CGA64250 45% WP, Syngenta, Greensboro NC), difenoconazole (Inspire 23.2% EC, gift from Syngenta and 10% DF, purchased), tebuconazole (Elite 45% DF, supplied by Bayer CropScience, Research Triangle Park NC), triflumizole (Trifumin 30% WP, purchased), myclobutanil (Rally 10% WP, purchased), and mefentrifluconazole (Cevya; 40% SC, BASF, Raleigh NC).

2.2. Activity against mycelial growth on culture medium

Isolates of *M. fructicola*, *Colletotrichum* spp., *A. alternata* sp. complex, and *C. beticola* were cultured on potato dextrose agar (PDA) plates in darkness. The isolates of the former three species and *C. beticola* were cultured at 22 to 24 °C for 5 days and 15 days, respectively. Mycelial discs, 4 mm in diameter, were cut from actively growing colony margins and transferred onto PDA plates containing each DMI fungicide at 0, 0.1, 0.3, 1, 3, and 10 mg/L of active ingredient (AI). After incubation at 22 to 24 °C in the dark for 3 days (*M. fructicola*, *Colletotrichum* spp., and *A. alternata*) and for 7 days (*C. beticola*), the colony diameter (minus 4 mm for the plug) of two replicates per treatment was measured using a handheld digital caliper. For *M. fructicola*, growth at 0.3 mg/L, the discriminatory concentration to distinguish propiconazole-resistant from -sensitive isolates, relative to that on untreated PDA plates was calculated (Luo et al. 2008). The values of 50% effective concentration (EC_{50}) were calculated for other species by regressing percentage mycelial growth inhibition against the log of fungicide concentration using software kindly gifted by K. So, ZEN-NOH (Tokyo). Experiments were conducted two times and the 95% confidence interval (95% CI = $\pm 1.96 \times$ SE) of mean values, calculated by Excel, was compared. When the 95% CI of the mean do not overlap, there is no statistical difference between treatment at the alpha = 0.05 level.

2.3. Activity against disease development on plants

One DMI-resistant isolate N-E4 of *P. xanthii*, isolated from Ibaraki, Japan (Miyamoto et al. 2020b), was maintained on healthy cucumber cotyledons according to the methods of Miyamoto et al. (2020a). Two other isolates Chikusei-1 and Tsukuba-1, collected by mass isolation from sporulating cucumber leaves in commercial greenhouses in June 2020, were used directly. Seedlings of cucumber cv. Shin Suyo Tsukemidori (Tohoku Co., Ltd., Tochigi, Japan) in plastic pots were prepared at 24 °C under fluorescent light, and plants at 1st leaf stage were used. Distilled water (DW)-diluted suspensions, 50 mg AI/L for each fungicide, were sprayed onto whole plants until run-off using a hand sprayer, and plants were kept at 24 °C until inoculation on the following day. DW was used as a control, and three replicate plants were served for each treatment. Concentration (AI) of each fungicide labeled for cucumber powdery mildew in Japan is as follows: triflumizole, 60–100 mg/L; myclobutanil, 12.5–25 mg/L; and difenoconazole, 50 mg/L. Mefentrifluconazole has not been registered yet.

Fresh conidia were collected into DW from powdery mildew-sporulating cotyledons or leaves using a sterile paint brush, their concentrations were adjusted to ca 1–2.5 $\times$ 10^5/mL, and suspensions sprayed to fungicide or DW treated plants. Inoculated plants were kept at 24 °C under fluorescent light and then powdery mildew development was recorded on each cotyledon and leaf at 10 days after inoculation

Table 1
List of isolates used in this study.

Species	Isolate	Origin	Host	Year of isolation	Reference
Monilinia fructicola	GADL133	GA, USA	Peach	2003	Luo et al. 2008
	GAAP10	GA, USA	Peach	2003	Luo et al. 2008
	Bmpc7	GA, USA	Peach	2006	Luo et al. 2008
	GADL7	GA, USA	Peach	2003	Luo et al. 2008
	SC.dap3	SC, USA	Peach	2008	Chen et al. 2013a
	NY12B	NY, USA	Cherry	2007	Luo et al. 2008
	GADL133	GA, USA	Peach	2003	Luo et al. 2008
	NY9C	NY, USA	Cherry	2007	Luo et al. 2008
	GAAP12	GA, USA	Peach	2003	Luo et al. 2008
	SCDL28	SC, USA	Peach	2001	Luo et al. 2008
Colletotrichum fructicola	Cg_RR12_3	SC, USA	Peach	2012	Hu et al. 2015
	Cg_RR12_1	SC, USA	Peach	2013	Hu et al. 2015
Colletotrichum siamense	Cg_Ey12_1	SC, USA	Peach	2012	Hu et al. 2015
	Cg_Ey12_10	SC, USA	Peach	2012	Hu et al. 2015
Colletotrichum nymphaeae	CaPH40	SC, USA	Peach	2004	Chen et al. 2016
	CaPH44	SC, USA	Peach	2004	Chen et al. 2016
Colletotrichum fioriniae-subgroup 1	CaEy12_2	SC, USA	Peach	2004	Chen et al. 2016
	CaPH4	SC, USA	Peach	2004	Chen et al. 2016
Colletotrichum fioriniae-subgroup 2	C.2.4.2	GA, USA	Peach	2015	Chen et al. 2016
	C.2.2.2	GA, USA	Peach	2015	Chen et al. 2016
	C.2.3.2	GA, USA	Peach	2015	Chen et al. 2016
	C.2.1.1	GA, USA	Peach	2015	Chen et al. 2016
Alternaria alternata sp. complex	EY12-4	SC, USA	Peach	2012	Yang et al. 2015
	SE12-12	SC, USA	Peach	2012	Yang et al. 2015
	SE12-3	SC, USA	Peach	2012	Yang et al. 2015
	RR13-5	SC, USA	Peach	2013	Yang et al. 2015
	SE12-5	SC, USA	Peach	2012	Yang et al. 2015
	EY12-6	SC, USA	Peach	2012	Yang et al. 2015
	RR13-28	SC, USA	Peach	2013	Yang et al. 2015
	RR13-39	SC, USA	Peach	2013	Yang et al. 2015
	MAFF425020	Tokyo, Japan	Yama-zakura (*Cerasus jamasakura*)	Before 1987	Watanabe et al. 1987
	MAFF425333	Tokyo, Japan	Yama-zakura (*Cerasus jamasakura*)	1984	Watanabe et al. 1987
	MAFF425334	Tokyo, Japan	Yama-zakura (*Cerasus jamasakura*)	1984	Watanabe et al. 1987
	MAFF645011	Akita, Japan	Apple	1959	Sawamura 1962
Cercospora beticola	KM52	Hokkaido, Japan	Sugar beet	2017	This study
	J24	Hokkaido, Japan	Sugar beet	2015	This study
	HT-K10	Hokkaido, Japan	Sugar beet	2019	This study
	Sika 19–11	Hokkaido, Japan	Sugar beet	2019	This study
	Oto 19–28	Hokkaido, Japan	Sugar beet	2019	This study
	Oto 19–05	Hokkaido, Japan	Sugar beet	2019	This study
	J27	Hokkaido, Japan	Sugar beet	2015	This study
	HT-K2	Hokkaido, Japan	Sugar beet	2019	This study
	Memu 19–06	Hokkaido, Japan	Sugar beet	2019	This study
	Memu 19–12	Hokkaido, Japan	Sugar beet	2019	This study
Podosphaera xanthii	N-E4	Ibaraki, Japan	Cucumber	2017	Miyamoto et al. 2020b
	Chikusei-1	Ibaraki, Japan	Cucumber	2020	This study
	Tsukuba-1	Ibaraki, Japan	Cucumber	2020	This study

using the following scale: 0 = no visible mildew development; 1 = 1–5%; 2 = 6–25%; 3 = 26–50%; 4 = 51–75%; 5 ≥76% of cotyledon and leaf area covered with the powdery mildew fungus. Disease severity (DS) was calculated as $[(5A + 4B + 3C + 2D + E)/5F] \times 100$, where A, B, C, D, and E are the number of cotyledons and leaves corresponding to the scales, 5, 4, 3, 2, and 1, respectively, and F is the total number of cotyledons and leaves assessed. Control (%) of individual fungicides was calculated as [(DS on untreated cotyledons and leaves – DS on treated cotyledons and leaves)/DS on untreated cotyledons and leaves] × 100 (Ishii et al. 2001). Experiments were conducted twice and data are represented as the mean and 95% CI to indicatesignificant differences between treatments.

3. Results

3.1. Activity against mycelial growth on culture medium

M. fructicola isolates were considered resistant to propiconazole when mycelium growth exceeded 20% (Luo et al. 2008) rather than 50% (Cox et al. 2007) at 0.3 mg/L, the discriminatory concentration of this fungicide, relative to that on untreated PDA plates. Out of 5 stock isolates recorded as resistant previously (Luo et al. 2008), GAAP10, GADL133, Bmpc7, and GADL7 were confirmed to be resistant in the present study because the relative growth was 47.3, 39.3, 28.6, and 25.2%, respectively. However, isolate SC.dap3 (Chen et al. 2013a) seemed to have converted to be sensitive because the relative growth was 15.8% at 0.3 mg/L (Fig. 2). Five sensitive stock isolates, NY12B, GADL3, NY9C, GAAP12, and SCDL28 maintained sensitivity to propiconazole as their relative growth was less than 20% at the discriminatory concentration. Cross-resistance was observed in four propiconazole-resistant isolates to difenoconazole, tebuconazole, and mefentrifluconazole but mefentrifluconazole tended to show higher activity than the three other DMIs (Figs. 2 and 3).

The *A. alternata* sp. complex isolates MAFF425020, MAFF425333, MAFF425334, and MAFF645011 derived from trees that had never been treated with DMIs. The baseline sensitivity EC_{50} values ranged from 0.320 to 1.057 mg/L for propiconazole, 0.164 to 1.445 mg/L for difenoconazole, 0.105 to 0.801 mg/L for tebuconazole, and 0.065 to 0.682 mg/L for mefentrifluconazole (Fig. 4). When comparing these four baseline isolates with eight isolates collected peach orchards where DMIs had been applied for years, propiconazole exhibited similar levels of activity on the latter isolates (average EC_{50} values ranged from 0.390 to 1.374 mg/L). Difenoconazole showed higher activity (average EC_{50} values ranged from 0.020 to 0.218 mg/L), whereas activity of

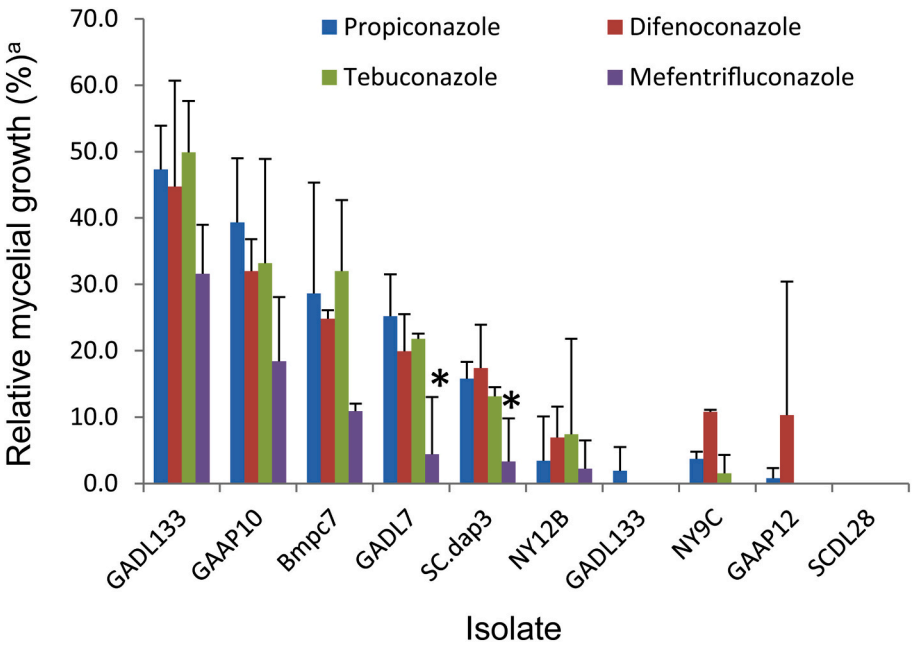

Fig. 2. Relative mycelial growth (%) of *Monilinia fructicola* isolates on potato dextrose agar plates amended with the four DMIs, propiconazole, difenoconazole, tebuconazole, and mefentrifluconazole at 0.3 mg/L. [a]Mean values and their corresponding 95% CIs. *Significantly different between mefentrifluconazole and three other DMIs.

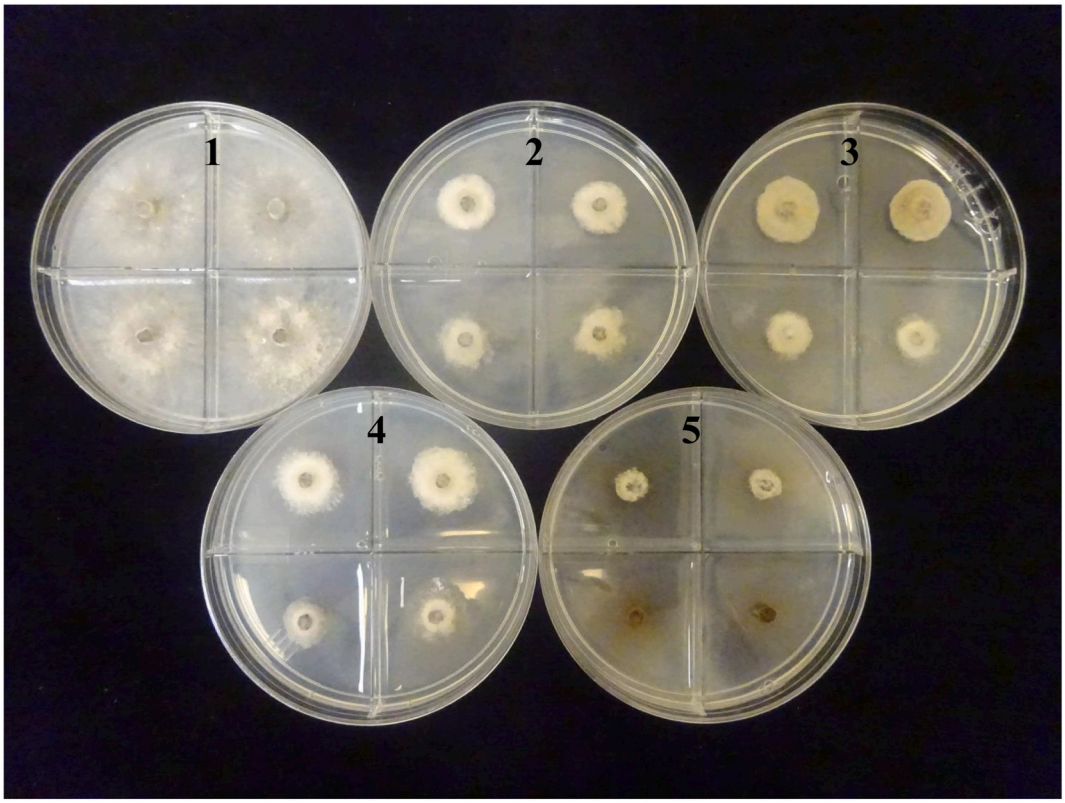

Fig. 3. Comparison of mycelial growth of the resistant isolate Bmpc7 (upper two colonies in each plate) and the sensitive isolate GADL7 (lower two colonies in each plate) of *Monilinia fructicola* on potato dextrose agar amended with no fungicides (**1**), 0.3 mg AI/L each of the four DMIs, propiconazole (**2**), difenoconazole (**3**), tebuconazole (**4**), and mefentrifluconazole (**5**). The photo was taken 3 days after incubation.

tebuconazole was lower (average EC_{50} values ranged from 1.243 to 2.675 mg/L) against the six peach isolates SE12-5, EY12-4, SE12-12, SE12-3, RR13-5, and EY12-6 compared to the baseline isolates (Fig. 5). Mefentrifluconazole was more active on peach isolates (average EC_{50}: 0.001 to 0.242 mg/L) than on baseline isolates. Mefentrifluconazole tended to be more effective against isolates SE12-3, RR13-5, SE12-5, EY12-6, RR13-28, and RR13-39 compared with the other DMIs, but the difference of EC_{50} values was not statistically significant (Fig. 5).

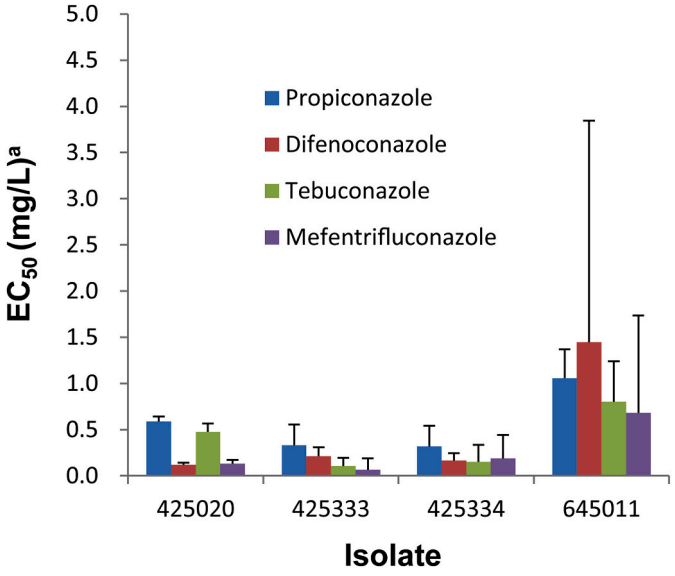

Fig. 4. 50% effective concentration (EC_{50}) for DMIs against baseline isolates of *Alternaria alternata* sp. *complex* on potato dextrose agar plates amended with the four DMIs, propiconazole, difenoconazole, tebuconazole, and mefentrifluconazole. [a]Mean values and their corresponding 95% CIs.

The average EC_{50} values of *Colletotrichum* spp. isolates for propiconazole, difenoconazole, tebuconazole, and mefentrifluconazole ranged from 0.033 to 0.481 mg/L, 0.009 to 0.354 mg/L, 0.005 to 0.368 mg/L, and 0.000 to 1.359 mg/L, respectively (Fig. 6). As reported previously (Chen et al. 2016), the sensitivity of *C. fioriniae*-subgroup 2 isolates to propiconazole was lower (EC_{50} values ranging from 0.343 to 0.481 mg/L) compared to the isolates of *C. fioriniae*-subgroup 1 (EC_{50} values ranging from 0.033 and 0.075 mg/L). The activity of mefentrifluconazole was lowest among all DMIs tested against 11 out of 12 isolates regardless of species difference and DMI cross-resistance pattern. The only exception was isolate CaPH4 of *C. fioriniae*-subgroup 1.

The EC_{50} values of *C. beticola* isolates for difenoconazole ranged from 1.880 to greater than 10 mg/L for seven DMI resistant isolates, J24, Oto 19–28, Oto 19–05, HT-K7, HT-K10, Sika 19–11, and KM52 (Table 2). By contrast, the EC_{50} values of the sensitive isolates J27, HT-K2, Memu 19–06, and Memu 19–12 ranged from 0.007 to 1.089 mg/L. For tebuconazole, the EC_{50} values ranged from 1.267 to greater than 10 mg/L for resistant isolates and from 0.001 to 0.590 mg/L for sensitive isolates. Mefentrifluconazole was effective against sensitive isolates (EC_{50} values between 0.030 and 1.079 mg/L), but cross-resistance to mefentrifluconazole was present in isolates resistant to difenoconazole and tebuconazole (Table 2).

3.2. Activity of DMIs against cucumber powdery mildew

Powdery mildew disease severity was 65.0 on plants inoculated with sensitive isolate Chikusei-1 and 68.9 on plants inoculated with resistant isolate Tsukuba-1. For the sensitive isolate, preventive treatment with 50 mg/L of triflumizole, myclobutanil, difenoconazole and mefentrifluconazole provided 97.4%, 89.7%, 74.3%, and 69.2% control, respectively (Fig. 7a). Cross-sensitivity to the four DMIs was thus observed, although intrinsic activity of mefentrifluconazole seemed to have been slightly lower than that of the three other fungicides. In contrast, isolate Tsukuba-1 showed moderate level of cross-resistance to all fungicides sprayed at 50 mg/L with disease control being 64.6%, 51.7%, 67.8%, and 51.7% for triflumizole, myclobutanil, difenoconazole, and mefentrifluconazole, respectively. Cross-resistance was also confirmed among the four DMIs for stock isolate N-E4 exhibiting high levels of resistance to DMIs based on A372G, I374V, V449L, and G461S mutations in CYP51 (Miyamoto et al., unpublished; Fig. 7b). The isolate exhibited the highest disease severity (75.6 ± 17.35 95% CI) among the three isolates tested in the two independent experiments. Preventive application of DMIs at 50 mg/L reduced the severity but the control efficacy was only 40.4%, 54.0%, 26.9%, and 20.9% for triflumizole, myclobutanil, difenoconazole, and mefentrifluconazole, respectively (Fig. 7b). There was no statistical difference in the control efficacy among fungicides employed.

4. Discussion

Azole compounds such as triazoles and imidazoles have been contributing to maintaining food security worldwide since the 1980s.

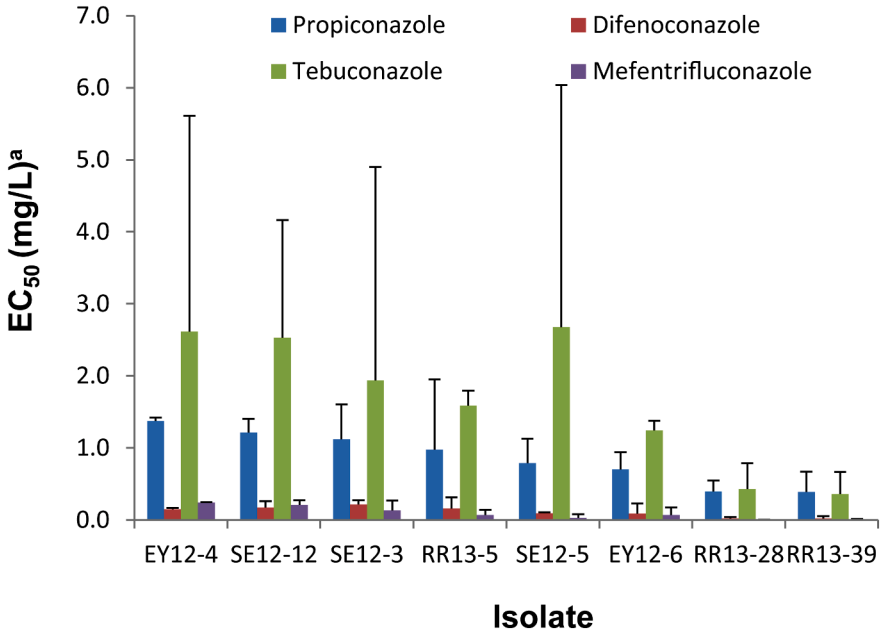

Fig. 5. 50% effective concentration (EC_{50}) for DMIs against peach isolates of *Alternaria alternata sp. complex* on potato dextrose agar plates amended with the four DMIs, propiconazole, difenoconazole, tebuconazole, and mefentrifluconazole. [a]Mean values and their corresponding 95% CIs.

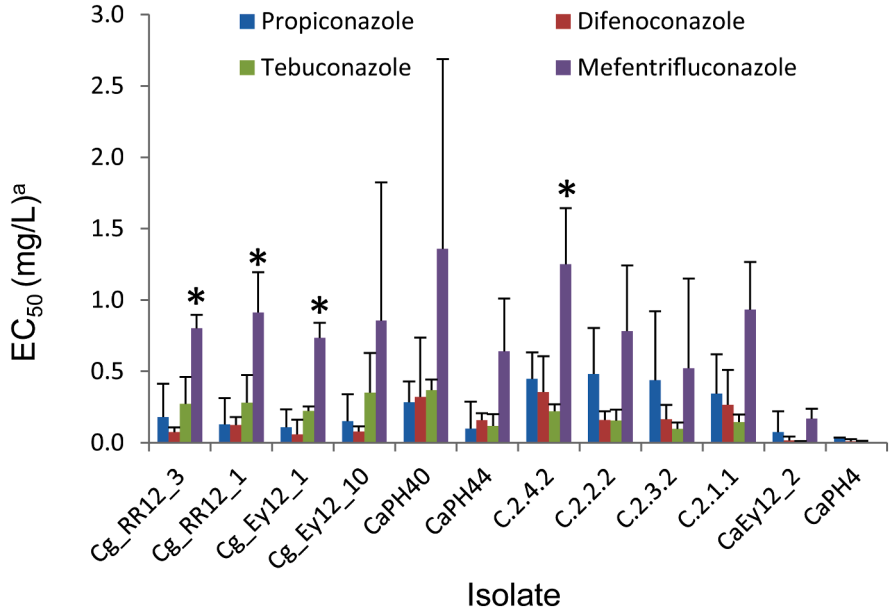

Fig. 6. 50% effective concentration (EC_{50}) for DMIs against isolates of *Colletotrichum* spp. on potato dextrose agar plates amended with the four DMIs, propiconazole, difenoconazole, tebuconazole, and mefentrifluconazole. [a]Mean values and their corresponding 95% CIs. *Significantly different between mefentrifluconazole and three other DMIs.

Table 2
Values of 50% effective concentration (EC_{50}) for DMI fungicides against isolates of *Cercospora beticola* on potato dextrose agar plates.

Isolate	Difenoconazole	Tebuconazole	Mefentrifluconazole
J24	>10[a]	>10	>10
Oto19-28	>10	>10	>10
Oto19-05	>10	9.445 (± 4.353)	>10
HT-K7	>10	4.703 (± 3.918)	>10
HT-K10	3.830 (± 1.093)	>10	2.078 (± 0.530)
Sika19-11	2.243 (± 0.596)	1.267 (± 0.033)	3.185 (± 1.429)
KM52	1.880 (± 0.076)	1.300 (± 0.305)	9.135 (± 0.241)*
HT-R3	1.135 (± 0.162)	0.531 (± 0.335)	5.106 (± 7.569)
Memu19-06	1.078 (± 0.311)	0.590 (± 0.035)	1.179 (± 0.301)
HT-K2	0.103 (± 0.139)	0.014 (± 0.026)	0.181 (± 0.177)
J27	0.014 (± 0.022)	0.001 (± 0.001)	0.030 (± 0.052)
Memu19-12	0.007 (± 0.013)	0.001 (± 0.001)	0.033 (± 0.018)

[a] Mean values and their corresponding 95% CIs in parenthesis.
* Significant differences between mefentrifluconazole and the two other DMIs.

Very recently, however, due to the suspected risk as endocrine disruptors (https://www.europarl.europa.eu/ RegData/etudes/STUD/2019/608866), some azole fungicides were banned in the EU countries where precautionary measures are prioritized and where standards are among the most stringent for human and environmental safety in the world (https://endocrinesciencematters.org/wp-content/uploads/2016/ 02/24442_ECPA-Toolkit-Regulating-Endocrine-Disruptors). The number of effective fungicides is declining in Europe, due to development of pathogen resistance and consequences of the regulation EC 1107/2009 (Bryson et al. 2016; Strobel et al. 2020). Consequently, fungicides with safer toxicity profiles to human and environment and with lower risk of resistance development must be developed for sustainable crop production.

Emergence and development of pathogen resistance to azole fungicides have become a critical issue not only in agriculture but also in the medical field (Becher and Wirsel 2012; Fisher et al. 2018). Multiple biochemical mechanisms are involved in resistance to DMIs. They include (1) modifications in target-site proteins caused by the mutations of *CYP51* gene resulting in decreased binding affinity of fungicides, (2) overexpression of the *CYP51* gene during sterol biosynthesis, (3) decreased intracellular DMI accumulation mediated by an increased energy-dependent efflux, and others (Ziogas and Malandrakis 2015). For the new triazole fungicide mefentrifluconazole, the high structural flexibility has been proposed to cause limited cross-resistance (Strobel et al. 2020). Flexible isopropanol linker allows to fit into the binding pocket of the target enzyme CYP51, leading to strong inhibition of enzyme activity even where amino-acid substitutions may have already caused alterations in the target site.

In this study, inhibitory activity of mefentrifluconazole was tested using pathogen isolates resistant and sensitive to existing DMIs. Mefentrifluconazole exhibited high activity against mycelial growth of sensitive *M. fructicola* isolates in the tests conducted on agar medium. The level of activity was equal or superior to that of the existing DMIs, propiconazole, difenoconazole, and tebuconazole. High control efficacy of mefentrifluconazole on brown rot of peach caused by *M. fructicola* was also confirmed in field experiments carried out in SC, USA (Schnabel et al., unpublished). Mefentrifluconazole and its mixture with the SDHI fungicide fluxapyroxad were tested on peach brown rot previously and high field efficacy similar to a commercial reference fungicide observed in both products (Ritchie 2018). These results supported registration of mefentrifluconazole in stone fruits and other crops (https://www.basf.com/us/en/media/news-releases/2019/011/). The level of mefentrifluconazole activity was also equal or higher against resistant isolates than that of three other DMIs tested. In contrast to previous studies (Jørgensen et al., 2020; Strobel et al. 2020), cross-resistance was evident between mefentrifluconazole and propiconazole, difenoconazole, and tebuconazole. In a previous study, mefentrifluconazole provided better control against *Z. tritici* compared to older DMIs, although *CYP51* mutations had accumulated in resistant populations and sensitivity of this pathogen to the older fungicides had decreased in the European populations (Jørgensen et al., 2020). Different patterns of sensitivity to mefentrifluconazole were detected compared to prothioconazole and epoxyconazole, and field performance of mefentrifluconazole did not seem to be influenced by the currently dominating *CYP51* mutations (Jørgensen et al., 2020).

Molecular mechanisms of DMI resistance have been *intensively* studied in *M. fructicola*, arguably the most important causal agent of brown rot of stone fruits in major production areas worldwide. Luo et al. (2008) found overexpression of the *MfCYP51* gene and a corresponding 65-bp genetic element 'Mona', inserted in the upstream region of

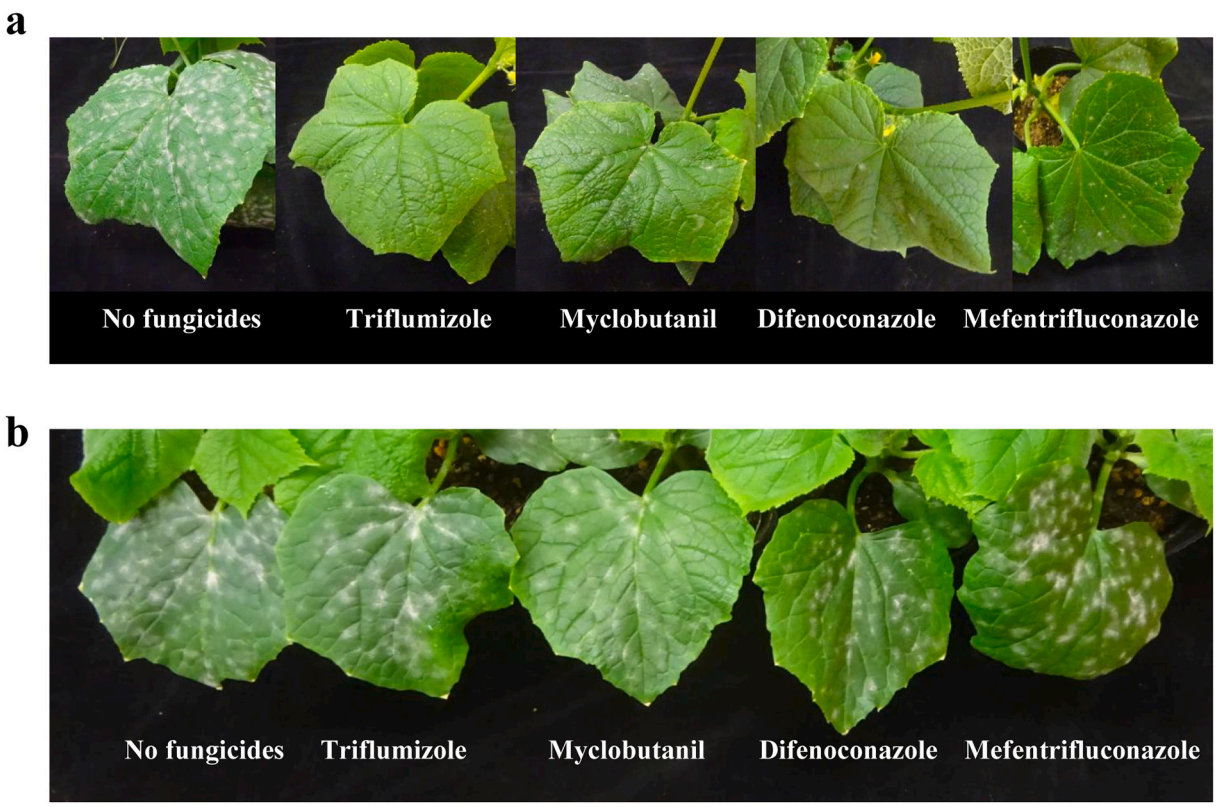

Fig. 7. Control efficacy of the four DMIs, triflumizole, myclobutanil, difenoconazole, and mefentrifluconazole at 50 mg AI/L against cucumber powdery mildew disease caused by (a) DMI-sensitive isolate (Chikusei-1) and (b) -resistant isolate (N-E4) of *Podosphaera xanthii*. The photo was taken 10 days after inoculation.

MfCYP51, linked to propiconazole resistance. The 'Mona' was predicted to be a transposable element (Luo and Schnabel 2008) and the promoter activity of this element was demonstrated recently (Chen et al. 2017). Separately, the target-site G461S mutation was associated with tebuconazole resistance in Brazilian isolates where the *MfCYP51* gene was overexpressed but 'Mona' not found (Lichtemberg et al. 2017). Yet other studies identified field isolates resistant to DMIs with unknown genetic determinants (Villani and Cox 2011). In the current study, mefentrifluconazole showed high inhibitory activity against isolates sensitive to other DMIs. As brown rot is the main disease of stone fruits worldwide (Pereira et al. 2020), it will be interesting to examine the potential effect of mefentrifluconazole exposure on overexpression of the *MfCYP51* gene and whether this fungicide exhibits cross resistance patterns in pathogens with altered target sites.

In China, difenoconazole has been sprayed widely for more than 20 years to control *A. alternata*, which was replaced by *A. solani* as the main pathogen causing potato early blight, but no resistance has been found suggesting that the risk of developing resistance is low (He et al. 2019). However, Zhang et al. (2019) published a first report of cross-resistance between propiconazole and difenoconazole in *Alternaria* sect. Isolates on potato in China and suggested the importance of induced expression of *CYP51* gene in resistance. Data on DMI sensitivity was not available for peach isolates of *A. alternata* sp. complex. It stands to reason that the sensitivity to DMIs in *Alternaria* spp. isolates in peach orchards decreases over time due to frequent use of DMIs for the control of blossom blight, brown rot, and peach scab. Therefore, in this study, baseline DMI sensitivity of *A. alternata* sp. complex was determined using isolates originating from two Rosaceous woody plants Yama-zakura (*Cerasus jamasakura*) and apple which had never been treated with DMIs. When California populations of *A. alternata* on pistachio were tested using isolates collected prior to DMI use, the average EC_{50} values of propiconazole, difenoconazole, and tebuconazole was 1.29, 0.17, and 0.96 μg/mL, respectively (Avenot et al. 2016). The sensitivity of our peach isolates to propiconazole, difenoconazole, and mefentrifluconazole was within the range of baseline but sensitivity to tebuconazole was lower compared with our baseline isolates. Mefentrifluconazole tended to show higher *in vitro* activity to those isolates than propiconazole and tebuconazole.

Sensitivity of *C. acutatum* sp. complex (*C. fioriniae* and *C. nymphaeae*), *C. gloeosporioides* sp. complex (*C. fructicola* and *C. siamense*), and *C. truncatum* isolates from peach to some DMIs were determined *in vitro* previously (Chen et al. 2016). In the latter study, propiconazole and difenoconazole showed high inhibitory activity to all five species with EC_{50} values of 0.2 to 2.7 mg/L and isolates of *C. fructicola* and *C. siamense* were sensitive to all fungicides (EC_{50} ranging from 0.2 to 13.1 mg/L). In contrast, isolates of *C. fioriniae* subgroup 2 were less sensitive (EC_{50} ranging from 0.5 to 16.2 mg/L) than those of *C. fioriniae* subgroup 1 (EC_{50} ranging from 0.03 to 2.1 mg/L). In the present study, activity of mefentrifluconazole was lowest against most isolates tested and no variation in sensitivity among species or lack of cross-resistance was recognized. Recently, the role of two paralogous *CYP51A* and *YP51B* genes in DMI sensitivity has been characterized in *C. fioriniae* and *C. nymphaeae* (Chen et al. 2020). The results indicated species-specific differential binding of DMIs onto the two CYP51 enzymes which explains the difference in fungicide sensitivity. Wei et al. (2020) observed positive cross-resistance between tebuconazole and difenoconazole or propiconazole in the isolates of *C. gloeosporioides* from chili pepper in China. They divided resistant isolates into three groups based on deduced amino acid substitutions in CgCYP51. Genotype I carried four substitutions (V18F, L58V, S175P, and P341A) at the *CgCYP51A* gene but no substitutions at *CgCYP51B*, while genotype II possessed five substitutions (L58V, S175P, A340S, T379A, and N476T) at *CgCYP51A*, concomitant with three substitutions (D121N, T132A, and F391Y) at *CgCYP51B*. Genotype III had two substitutions (L58V and S175P) at *CgCYP51A*, concomitant with a substitution (T262A) at *CgCYP51B*. Furthermore, a molecular docking study indicated that the binding

affinity of tebuconazole to the CgCYP51 protein was decreased in resistant isolates when compared with that in sensitive isolates (Wei et al. 2020).

Mefentrifluconazole activity against *C. beticola* was equal to slightly inferior to difenoconazole and tebuconazole except for one isolate employed and cross-resistance to mefentrifluconazole was observed in this study. Molecular mechanisms of DMI resistance weres studied in *C. beticola* previously (Bolton et al. 2012). No mutations or haplotypes of *CbCyp51* were associated with resistance or sensitivity although five nucleotide haplotypes leading to three amino acid substitutions were identified. Instead, *CbCyp51* was overexpressed constitutively as well as *inducibly* in isolates with reduced DMI sensitivity indicating that resistance was a result of overexpression of the fungicide-targeted gene (Bolton et al. 2012).

In the cucumber powdery mildew pathogen *P. xanthii*, cross-resistance is well known although resistance is quantitative and inherent activity differs among DMIs (McGrath 2015). In inoculation tests on cucumber plants after preventive application performed in the current study, the intrinsic activity of mefentrifluconazole seemed to be lower than that of triflumizole, myclobutanil, and difenoconazole. Additionally, cross-resistance to mefentrifluconazole was confirmed in isolates exhibiting resistance to triflumizole, myclobutanil, and difenoconazole. Molecular mechanisms were studied previously and the four amino-acid substitutions A372G, I374V, V449L, and G461S in CYP51 were associated with high levels of DMI resistance in this pathogen (Kubo 2007; Kubo et al. 2008). In contrast, only G461S was detected in isolates with lower level of resistance indicating that differential binding affinity between the fungicide and CYP51 enzyme might account for distinct levels of resistance (Kubo 2007).

In conclusion, cross-resistance between mefentrifluconazole and other DMIs was confirmed both *in vitro* and *in planta* experiments in this study. To our knowledge, this is the first paper to clearly demonstrate positive cross-resistance. Reasons for the inconsistent cross resistance observed in *Z. septoria* (Jørgensen et al., 2020; Strobel et al. 2020) remain to be elucidated. Just like other DMIs, mefentrifluconazole is at risk for resistance development and strict resistance management practices must be applied to extend efficacy. The lower-risk in terms of its toxicological aspects, however, make it an attractive IPM tool.

Acknowledgements

We are grateful to K. Uchida and Cham Le for technical assistance. BASF, Syngenta, and Bayer CropScience are also appreciated for suppling fungicides. Technical Contribution No. 6898 of the Clemson University Experiment Station. This material is based upon work supported by the NIFA/USDA, under project number SC-1700530.

References

European Food Safety Authority (EFSA), 2018. Peer review of the pesticide risk assessment of the active substance BAS 750F (mefentrifluconazole). EFSA J. 16 (7), 5379. https://doi.org/10.2903/j.efsa.2018.5379.

Avenot, H.F., Solorio, C., Morgan, D.P., Michailides, T.J., 2016. Sensitivity and cross-resistance patterns to demethylation-inhibiting fungicides in California populations of *Alternaria alternata* pathogenic on pistachio. Crop Prot. 88, 72–78.

Becher, R., Wirsel, S.G.R., 2012. Fungal cytochrome P450 sterol 14α-demethylase (CYP51) and azole resistance in plant and human pathogens. Appl. Microbiol. Biotechnol. 95, 825–840.

Beer, K., Jackson, B., 2019. Impacts of the global emergence of azole resistance among *Aspergillus* spp. on humans in a medical setting. Phytopathology 109. S2.200 (Abstr.).

Berger, S., Chazli, Y.E.L., Babu, A.F., Coste, A.T., 2017. Azole resistance in *Aspergillus fumigatus*: a consequence of antifungal use in agriculture? Front. Microbiol. 8, 1024. https://doi.org/10.3389/fmicb.2017.01024.

Bolton, M.D., Birla, K., Rivera-Varas, V., Rudolph, K.D., Secor, G.A., 2012. Characterization of *CbCyp51* from field isolates of *Cercospora beticola*. Phytopathology 102, 298–305.

Brent, K.J., 2012. Historical perspectives of fungicide resistance. In: Thind, T.S. (Ed.), Fungicide Resistance in Crop Protection – Risk and Management. CABI, Wallingford, pp. 3–18.

Brent, K.J., Hollomon, D.W., 2007. Fungicide Resistance in Crop Pathogens: How Can it be Managed? FRAC Monograph No. 1, second revised ed. Fungicide Resistance Action Committee, Brussels www.frac.info.

Bryson, R.J., Brix, H.D., Bruns, J., 2016. How will EU pesticide legistration impact the crop protection innovation pipeline? Proc. Crop Protect. North. Brit. Conf 9–14. Dundee. https://www.cabdirect.org/cabdirect/abstract/20163306819.

Chapuis, L., Corio-Costet, M.-R., Malosse, C., 1996. Sterol composition of the woody plant pathogenic fungus *Eutypa lata*. Phytochemistry 42, 1599–1601.

Chen, F., Liu, X., Chen, S., Schnabel, E., Schnabel, G., 2013a. Characterization of *Monilinia fructicola* strains resistant to both propiconazole and boscalid. Plant Dis. 97, 645–651.

Chen, F., Liu, X., Schnabel, G., 2013b. Field strains of *Monilinia fructicola* resistant to both MBC and DMI fungicides isolated from stone fruit orchards in the eastern United States. Plant Dis. 97, 1063–1068.

Chen, S.N., Luo, C.X., Hu, M.J., Schnabel, G., 2016. Sensitivity of *Colletotrichum* species, including *C. fioriniae* and *C. nymphaeae*, from peach to demethylation inhibitor fungicides. Plant Dis. 100, 2434–2441.

Chen, S., Yuan, N., Schnabel, G., Luo, C.X., 2017. Function of the genetic element 'Mona' associated with fungicide resistance in *Monilinia fructicola*. Mol. Plant Pathol. 18, 90–97.

Chen, S., Hu, M., Schnabel, G., Yang, D., Yan, X., Yuan, H., 2020. Paralogous *CYP51* genes of *Colletotrichum* spp. mediate differential sensitivity to sterol demethylation inhibitors. Phytopathology 110, 615–625.

Chowdhary, A., Kathuria, S., Xu, J., Meis, J.F., 2013. Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. PLoS Pathog. 9 https://doi.org/10.1371/journal.ppat.1003633.

Cools, H.J., Fraaije, B.A., 2012. Resistance to azole fungicides in *Mycosphaerella graminicola*: mechanisms and management. In: Thind, T.S. (Ed.), Fungicide Resistance in Crop Protection – Risk and Management. CABI, Wallingford, pp. 64–77.

Cox, K.D., Bryson, P.K., Schnabel, G., 2007. Instability of propiconazole resistance and fitness in *Monilinia fructicola*. Phytopathology 97, 448–453.

Draskau, M.K., Boberg, J., Taxvig, C., Pederson, M., Frandsen, H.L., Christiansen, S., Svingen, T., 2019. *In vitro* and *in vivo* endocrine disrupting effects of the azole fungicides triticonazole and flusilazole. Environ. Pollut. 255, 113309.

Fisher, M.C., Hawkins, N.J., Sanglard, D., Gurr, S.J., 2018. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science 360, 739–742.

He, M.-H., Wang, Y.-P., Wu, E.-J., Shen, L.-L., Yang, L.-N., Wang, T., Shang, L.-P., Zhu, W., Zhan, J., 2019. Constraining evolution of *Alternaria alternata* resistance to a demethylation inhibitor (DMI) fungicide difenoconazole. Front. Microbiol. 10, 1609. https://doi.org/10.3389/fmicb.2019.01609.

Heick, T.M., Justesen, A.F., Jørgensen, L.N., 2017. Anti-resistance strategies for fungicides against wheat pathogen *Zymoseptoria tritici* with focus on DMI fungicides. Crop Prot. 99, 108–117.

Heinecke, M., Rocha, L.F., Fakhoury, A.M., Bond, J.P., 2019. Efficacy of fungicides containing mefentrifluconazole to manage Frogeye leaf spot of soybean. Phytopathology 109, S2.63 (Abstr.).

Hu, M.-J., Grabke, A., Schnabel, G., 2015. Investigation of the *Colletotrichum gloeosporioides* species complex causing peach anthracnose in South Carolina. Plant Dis. 99, 797–805.

Ishii, H., Fraaije, B.A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T., Hollomon, D.W., 2001. Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. Phytopathology 91, 1166–1171.

Jørgensen, L.N., Matzen, N., Havis, N., Holdgate, S., Clark, B., Blake, J., Glazek, M., Korbas, M., Danielewicz, J., Maumene, C., Rodemann, B., Weigand, S., Kildea, S., Bataille, C., Brauna-Morževska, E., Gulbis, K., Ban, R., Berg, G., 2020. Efficacy of common azoles and mefentrifluconazole against septoria, brown rust and yellow rust in wheat across Europe. In: Deising, H.B., Fraaije, B., Mehl, A., Oerke, E.C., Sierotzki, H., Stammler, G. (Eds.), Modern Fungicides and Antifungal Compounds, IX, pp. 27–34.

Kahn, M.F.R., 2015. Sugar beet diseases: cercospora leaf spot. In: Ishii, H., Hollomon, D. W. (Eds.), Fungicide Resistance in Plant Pathogens – Principles and a Guide to Practical Management. Springer Japan, Tokyo, pp. 379–387.

Karaoglanidis, G.S., Karadimos, D.A., Ioannidis, P.M., 2003. Detection of resistance to sterol demethylation-inhibiting (DMI) fungicides in *Cercospora beticola* and efficacy of control of resistant and sensitive strains with flutriafol. Phytoparasitica 31, 373–380.

Köller, W., Scheinpflug, H., 1987. Fungal resistance to sterol biosynthesis inhibitors: a new challenge. Plant Dis. 71, 1066–1074.

Kubo, M., 2007. Molecular mechanism of demethylation inhibitor (DMI) fungicide-resistance in cucumber powdery mildew fungi (*Sphaerotheca fliginea*). In: Abstr. 17th Symp. Res. Com. Fungic. Resist., Phytopathol. Soc. Jpn, pp. 61–68 (in Japanese with English abstr.).

Kubo, M., Nomura, K., Uekusa, H., Takeda, T., Kita, N., 2008. Occurrence of DMI-resistant isolates of cucumber powdery mildew fungus in Kanagawa. Jpn. J. Phytopathol. 74, 269–270 (Japanese abstr.).

Lichtemberg, P.S.F., Luo, Y., Morales, R.G., Muehlmann-Fischer, J.M., Michailides, T.M., May De Mio, L.L., 2017. The point mutation G461S in the *MfCYP51* gene is Aasociated with tebuconazole resistance in *Monilinia fructicola* populations in Brazil. Phytopathology 107, 1507–1514.

Loeffler, R.S.T., Butters, J.A., Hollomon, D.W., 1992. The sterol composition of powdery mildews. Phytochemistry 31, 1561–1563.

López-Ruiz, F.J., Pérez-García, A., Fernández-Ortuño, D., Romero, D., García, E., de Vicente, A., Brown, J., Torés, K.M., A., J., 2010. Sensitivities to DMI fungicides in

populations of *Podosphaera fusca* in south central Spain. Pest Manag. Sci. 66, 801–808.
Luo, C.-X., Schnabel, G., 2008. The cytochrome P450 lanosterol 14-demethylase gene is a demethylation inhibitor fungicide resistance determinant in *Monilinia fructicola* field isolates from Georgia. Appl. Environ. Microbiol. 74, 359–366.
Luo, C.-X., Cox, K.D., Amiri, A., Schnabel, G., 2008. Occurrence and detection of the DMI resistance-associated genetic element 'Mona' in *Monilinia fructicola*. Plant Dis. 92, 1099–1103.
May-De-Mio, L.L., Luo, Y., Michailides, T.J., 2011. Sensitivity of *Monilinia fructicola* from Brazil to tebuconazole, azoxystrobin, and thiophanate-methyl and implications for disease management. Plant Dis. 95, 821–827.
McGrath, M.T., 2015. Cucurbit powdery mildew in the USA. In: Ishii, H., Hollomon, D.W. (Eds.), Fungicide Resistance in Plant Pathogens – Principles and a Guide to Practical Management. Springer Japan, Tokyo, pp. 401–417.
Mehl, A., Schmitz, H., Stenzel, K., Bloomberg, J., 2019. DMI fungicides (FRAC Code 3): sensitivity status of key target pathogens, field versus laboratory resistance, and resistance mechanisms. In: Stevenson, K.L., McGrath, M.T., Wyenandt, C.A. (Eds.), Fungicide Resistance in North America, Second ed. APS Press, St. Paul, Minnesota, pp. 51–68.
Miyamoto, T., Hayashi, K., Ogawara, T., 2020a. First report of the occurrence of multiple resistance to flutianil and pyriofenone in field isolates of *Podosphaera xanthii*, the causal fungus of cucumber powdery mildew. Eur. J. Plant Pathol. 156, 953–963.
Miyamoto, T., Hayashi, K., Okada, R., Wari, D., Ogawara, T., 2020b. Resistance to succinate dehydrogenase inhibitors in field isolates of *Podosphaera xanthii* on cucumber: monitoring, cross-resistance patterns and molecular characterization. Pestic. Biochem. Physiol. (in press).
Pereira, W.V., Morales, R.G.F., Bauer, A.I.G., Kudlawiec, K., May De Mio, L.L., 2020. Discontinuance of tebuconazole in the field restores sensitivity of *Monillinia fructicola* in stone fruit orchards. Plant Pathol. 69, 68–76.
Rangel, L.I., Spanner, R.E., Ebert, M.K., Pethybridge, S.J., Stukenbrock, E.H., de Jonge, R., Secor, G.A., 2020. Cercospora beticola: The intoxicating lifestyle of the leaf spot pathogen of sugar beet. Mol. Plant Pathol. 21, 1020–1041.
Reuveni, M., Sheglov, D., 2002. Effects of azoxystrobin, difenoconazole, polyoxin B (polar) and trifloxystrobin on germination and growth of *Alternaria alternata* and decay in red delicious apple fruit. Crop Prot. 21, 951–955.
Ritchie, D.F., 2018. Three years evaluation of fungicide BAS 750F and BAS 752F (contains Revysol™) for controlling brown rot on peach, 2018. Plant Dis. Manag. Rept. 13, PF055.
Santoro, K., Matić, S., Gisi, U., Spadaro, D., Pugliese, M., Gullino, M.L., 2017. Abundance, genetic diversity and sensitivity to demethylation inhibitor fungicides of *Aspergillus fumigatus* isolates from organic substances with special emphasis on compost. Pest Manag. Sci. 73, 2481–2494.
Sawamura, K., 1962. Studies on spotted diseases of apple 1. Causal agent of Alternaria blotch. Bull. Tohoku Natl. Agric. Exp. Stn. 23, 163–175 (in Japanese with English summary).
Schepers, H.T.A.M., 1985. Development and persistence of resistance to fungicides in *Sphaerotheca fuliginea* in cucumbers in the Netherlands. PhD Thesis. Wageningen Agric. Univ.
Schnabel, G., Bryson, P.K., Bridges, W.C., Brannen, P.M., 2004. Reduced sensitivity in *Monilinia fructicola* to propiconazole in Georgia and implications for disease management. Plant Dis. 88, 1000–1004.
Secor, G.A., Rivera, V.V., Khan, M.F.R., Gudmestad, N.C., 2010. Monitoring fungicide sensitivity of *Cercospora beticola* of sugar beet for disease management decisions. Plant Dis. 94, 1272–1282.
Shirane, N., Takenaka, H., Ueda, K., Hashimoto, Y., Katoh, K., Ishii, H., 1996. Sterol analysis of DMI-resistant and -sensitive strains of *Venturia inaequalis*. Phytochemistry 41, 1301–1308.
Strobel, D., Bryson, R., Semar, M., Stammler, G., Kienle, M., Smith, J., 2020. Mefentrifluconazole (Revysol® – The first isopropanol-azole). In: Deising, H.B., Fraaije, B., Mehl, A., Oerke, E.C., Sierotzki, H., Stammler, G. (Eds.), Modern Fungicides and Antifungal Compounds, IX, pp. 259–264.
Sykes, E.M., Sackett, K.E., Severns, P.M., Mundt, C.C., 2018. Sensitivity variation and cross-resistance of *Zymoseptoria tritici* to azole fungicides in North America. Eur. J. Plant Pathol. 151, 269–274.
Taxvig, C., Vinggaard, A.M., Hass, U., Axelstad, M., Metzdorff, S., Nellemann, C., 2008. Endocrine-disrupting properties in vivo of widely used azole fungicides. Intr. J. Androl. 31, 170–177.
Tesh, S.A., Tesh, J.M., Fegert, I., Buesen, R., Schneider, S., Mentzel, T., van Ravenzwaay, B., Stinchcombe, S., 2019. Innovative selection approach for a new antifungal agent mefentrifluconazole (Revysol®) and the impact upon its toxicity profile. Regul. Toxicol. Phamacol. 106, 152–168.
Torriani, S.F.F., Melichar, J.P.E., Mills, C., Pain, N., Sierotzki, H., Courbot, M., 2015. *Zymoseptoria tritici*: a major threat to wheat production, integrated approaches to control. Fungal Genet. Biol. 79, 8–12.
Verweij, P.E., Kema, G.H.J., Zwaan, B., Melchers, W.J.G., 2013. Triazole fungicides and the selection of resistance to medical triazoles in the opportunistic mould *Aspergillus fumigatus*. Pest Manag. Sci. 69, 165–170.
Villani, S.M., Cox, K.D., 2011. Characterizing fenbuconazole and propiconazole sensitivity and prevalence of 'Mona' in isolates of *Monilinia fructicola* from New York. Plant Dis. 95, 828–834.
Vinggaard, A.M., Christiansen, S., Laier, P., Poulsen, M.E., Breinholt, V., Jarfelt, K., Jacobsen, H., Dalgaard, M., Nellemann, C., Hass, U., 2005. Perinatal exposure to the fungicide prochloraz ferminizes the male rat offspring. Toxicol. Sci. 85, 886–897.
Watanabe, T., Uematsu, S., Hayashi, K., 1987. Fungal isolates from seeds of two cherry species collected at the cherry tree preservation forest at Asakawa. Jpn. J. Mycol. 28, 475–481.
Wei, L.-L., Chen, W.-C., Zhao, W.-C., Wang, J., Wang, B.-R., Li, F.-J., Wei, M.-D., Guo, J., Chen, C.-J., Zheng, J.-Q., Wang, K., 2020. Mutations and overexpression of *CYP51* associated with DMI-resistance in *Colletotrichum gloeosporioides* from chili. Plant Dis. 104, 668–676.
Yang, J.H., Brannen, P.M., Schnabel, G., 2015. Resistance in *Alternaria alternata* to SDHI fungicides causes rare disease outbreak in peach orchards. Plant Dis. 99, 65–70.
Zhang, Y., Zhou, Q., Tian, P., Li, Y., Duan, G., Li, D., Zhan, J., Chen, F., 2019. Induced expression of *CYP51* associated with difenoconazole resistance in the pathogenic *Alternaria* sect. on potato in China. Pest Manag. Sci. 76, 1751–1760.
Ziogas, B.N., Malandrakis, A.A., 2015. Sterol biosynthesis inhibitors: C14 demethylation (DMIs). In: Ishii, H., Hollomon, D.W. (Eds.), Fungicide Resistance in Plant Pathogens – Principles and a Guide to Practical Management. Springer Japan, Tokyo, pp. 199–216.