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Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain

D. Fernández-Ortuño[1], A. Pérez-García[2,]*, F. López-Ruiz[1], D. Romero[2], A. de Vicente[2], and J. A. Torés[1]
[1]*Estación Experimental "La Mayora" (CSIC), Algarrobo-Costa, E-29750, Málaga, Spain;* [2]*Grupo de Microbiología y Patología Vegetal-Unidad Asociada a CSIC, Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain *Author for Correspondence (Fax: +34-952131889; E-mail: aperez@uma.es)*

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Abstract

Cucurbit powdery mildew caused by *Podosphaera fusca* limits crop production in Spain. Since its management is strongly dependent on chemicals, the rational design of control programmes requires a good understanding of the fungicide resistance phenomenon in field populations. Fifty single-spore isolates of *P. fusca* were tested for sensitivity to three quinone-outside inhibiting (QoI) fungicides: azoxystrobin, kresoxim-methyl and trifloxystrobin. Minimum inhibitory concentration (MIC) values for QoI-sensitive isolates were found to range from 0.25 to 10 μg ml^{-1} for azoxystrobin to 5–25 μg ml^{-1} for kresoxim-methyl, using a leaf disc-based bioassay. High levels of cross-resistance to QoI fungicides were found. Eleven isolates showed resistance to the three QoI fungicides tested with MIC and EC_{50} values >500 μg ml^{-1} resulting in RF values as high as >715 and >1000 for trifloxystrobin and azoxystrobin, respectively. A survey of *P. fusca* QoI resistance was carried out in different provinces located in the south central area of Spain during the cucurbit growing seasons in 2002, 2003 and 2004. Examination of a collection of 250 isolates for QoI resistance revealed that 32% were resistant to the three fungicides tested; the provinces of Ciudad Real, Córdoba and Murcia being the locations with the highest frequencies of resistance (44–74%). By contrast, no resistance was found in Badajoz, and relatively low frequencies were observed in Almería and Valencia (10–13%). Nearly 50% of resistant isolates were collected from melon plants. Based on these data, recommendations about the use of QoI fungicides for cucurbit powdery mildew management in the sampled areas are made.

Introduction

Powdery mildew is a devastating disease of cucurbits worldwide (Sitterly, 1978) and one of the most important diseases affecting these crops in the Mediterranean basin. Like other powdery mildew diseases, its symptoms are characterised by the whitish, talcum-like, powdery fungal growth developing on both leaf surfaces, petioles and stems (Zitter et al., 1996). The disease can be caused by either *Golovinomyces cichoracearum* or *Podosphaera fusca*, obligate biotrophic ectoparasites that induce identical symptoms but can be easily distinguished by light microscopy (Braun et al., 2002). In southern Spain, *P. fusca* has been identified as the sole cause of the disease (Torés et al., 1990; Del Pino et al., 2002), and is responsible for important yield losses in cucurbit crops under field and greenhouse conditions, where the disease is particularly devastating for melon and zucchini crops, although accurate yield loss data are not available (Olalla, 2001).

Fungicide applications and the use of resistant cultivars are the main means of disease control, but in spite of these measures, powdery mildew continues to impose serious limitations on cucurbit production throughout the world (Zitter et al., 1996). In practice, application of fungicides is presently the principal tool in most cucurbit crops for managing powdery mildew (McGrath, 2001). The intensive use of chemicals often results in the development of resistance, and consequently, in the reduction of control efficacy. Unfortunately, this has been especially true for *P. fusca*, which has been described as a pathogen with a high potential for fungicide resistance development (McGrath, 2001).

Qo inhibitors (QoI) represent a relatively new and important class of agricultural fungicides, the discovery of which was inspired by a group of natural fungicidal derivatives of β-methoxy-acrylic acid (Anke et al., 1977). QoI have a single-site mode of action. They inhibit mitochondrial respiration by binding to the Qo site (the outer, quinone oxidizing pocket) of the cytochrome bc_1 enzyme complex (complex III), thus blocking electron transfer in the respiration pathway and leading to an energy deficiency due to a lack of ATP (Becker et al., 1981). QoI have a wide range of efficacy against many commercially important fungal diseases (Bartlett et al., 2002). Nevertheless, shortly after the commercial introduction of QoI in 1996, resistant isolates were detected in field populations of several plant pathogens, including *P. fusca*, in many parts of the world (Heaney et al., 2000). For most of the pathogens in which QoI resistance has been reported, resistance was conferred by a point mutation in the mitochondrial cytochrome b (*cyt b*) gene leading to an amino acid change from glycine to alanine at position 143 (G143A) (Gisi et al., 2002). In *P. fusca*, the same mutation has been found in two isolates resistant to QoI (Ishii et al., 2001).

In Spain, as in other parts of the world, soon after registration in 1997 cucurbit growers began extensively using QoI, and subsequently control failures of powdery mildew by these fungicides were frequently reported in some cucurbit-growing areas. Proper disease management requires a good understanding of the pathogen responsible. A biological characterization of a collection of isolates of *P. fusca* obtained in a three year survey (2002–2004) is being carried out in our laboratory. Part of this effort has been aimed at determining levels of QoI resistance in cucurbit powdery mildew populations present in different areas within south central Spain. In this way, epidemiological data on fungicide resistance has been obtained, which can be applied to the rational design of disease management programmes.

Materials and methods

Sampling

A total of 264 powdery mildew-diseased cucurbit plants were collected from 33 greenhouses and 70 fields at 31 localities, from the following six provinces in south central Spain: Almería (AL) and Córdoba (CO) from Andalucía; Badajoz (BA) from Extremadura; Ciudad Real (CR) from Castilla-La Mancha; Murcia (MU) from Región de Murcia; and Valencia (VA) from Comunidad Valenciana (Figure 1). Sampling was carried out during the cucurbit-growing seasons in 2002, 2003 and 2004. Each sample consisted of 1–3 fresh leaves colonized by sporulating powdery mildew colonies.

Figure 1. Sampling areas of powdery mildew infected cucurbit plants in Spain. AL, Almería; BA, Badajoz; CR, Cuidad Real; CO, Córdoba; MA, Málaga; MU, Murcia; VA, Valencia.

Pathogen isolates

From each diseased sample, 3–5 single-spore isolates were obtained. Single conidia were picked from independent powdery mildew colonies and transferred to separate cotyledons of zucchini cv. Negro Belleza (Semillas Fitó, Barcelona, Spain) maintained *in vitro* as previously described (Álvarez and Torés, 1997). Single-spore isolates were then identified based on characteristics of the conidial stage (Braun, 1987). After identification, isolates were kept at −80 °C (Pérez-García et al., 2006) until use in fungicide sensitivity tests. A total of 250 isolates of *P. fusca* from this survey were used. Additionally, 21 isolates obtained in previous studies from Almería (AL) and Málaga (MA), southern Spain, and reference strains from France (1 isolate) and Greece (3 isolates) were also included in the sensitivity tests.

Fungicides

Commercial formulations of azoxystrobin (Ortiva, Syngenta, Switzerland), kresoxim-methyl (Stroby, BASF, Germany) and trifloxystrobin (Flint, Bayer, Germany) were used. Fungicide stock solutions were prepared by dissolving the fungicides in sterile deionized water at a concentration of 10 mg ml^{-1} and stored at −20 °C until use. For sensitivity testing, stock solutions were diluted in sterile deionized water to give solutions with the desired final concentration.

Sensitivity tests

For fungicide sensitivity testing, a leaf disc assay was conducted (Délye and Corio-Costet, 1998). Zucchini cotyledons from 8 day-old plants were used. Leaf discs (11 mm diam) were cut with a corkborer and placed upside down in Petri dishes containing sterile filter paper imbibed with 3 ml of the corresponding fungicide solution. Ten discs were used per dish and fungicide concentration. After 24 h incubation at 22 °C and 16 h photoperiod, the discs were aseptically transferred onto sterile filter paper imbibed with 1.5 ml of sterile water deposited on agarized medium (40 g sucrose, 30 mg benzimidazole, 10 g agar, 1 l distilled water) in 5 cm diam Petri dishes (Álvarez and Torés, 1997), and the upper side of each disc was inoculated with conidia of the selected *P. fusca* isolate with the aid of an eyelash. After 8 days of incubation at 22 °C and 16 h photoperiod, powdery mildew symptoms on each leaf disc were recorded according to a 0–3 scale of values: 0, absence of visible symptoms; 1, < 25% of disc surface covered by powdery mildew; 2, 25–50%; and 3, > 50% of disc surface covered by powdery mildew. Disease severity (DS) was calculated as $[(0a + 1b + 2c + 3d)/3\ N] \times 100$, where a, b, c and d were the number of discs corresponding to the scale values 0, 1, 2 and 3, respectively, and N was the total number of leaf discs assessed (a + b + c + d) (Texeira de Sousa, 1985). Percentage of growth inhibition (I) was calculated as 100-DS. Fungicide testing was repeated three times with a range of concentrations adapted to the response of each isolate to the QoI tested in order to obtain a dose-response curve. Minimal inhibitory concentrations (MIC) were deduced directly from data and fungicide concentrations inhibiting 50% of growth (EC_{50}) were graphically determined from log-transformation of percentages of inhibition and regression against the natural logarithm of fungicide concentrations. Resistance factors (RF) were calculated for resistant isolates as the ratio between the EC_{50} value of the isolate and the mean of the EC_{50} values of sensitive isolates.

Results

Sensitivity of P. fusca *to QoI fungicides*

To determine discriminatory concentrations between isolates of *P. fusca* sensitive and resistant to QoI, 50 randomly chosen single-spore isolates were initially tested for sensitivity to the QoI fungicides azoxystrobin, kresoxim-methyl and trifloxystrobin, and MIC and EC_{50} values were determined (Table 1). Two different groups of isolates could be clearly identified. The first group was composed of 39 isolates that showed MIC values ranging from 0.25 to 10 μg ml^{-1} for azoxystrobin, from 1 to 25 μg ml^{-1} for trifloxystrobin and from 5 to 25 μg ml^{-1} for kresoxim-methyl, the predominant MIC values being 5 μg ml^{-1} for azoxystrobin and trifloxystrobin (38% and 34% of the isolates, respectively) and 25 μg ml^{-1} for kresoxim-methyl (62%) (Figure 2). The mean EC_{50} values calculated for this group of isolates

Table 1. Sensitivity of 50 randomly chosen isolates of *P. fusca* to the QoI fungicides azoxystrobin, kresoxim-methyl and trifloxystrobin in terms of minimal inhibitory concentration (MIC) and concentration inhibiting 50% of growth (EC_{50})

Isolate	Year of isolation	Location	Crop	MIC (μg ml^{-1})			EC_{50} (μg ml^{-1})		
				AZ[1]	KM[2]	TF[3]	AZ	KM	TF
Sf 8	1988	Málaga	Melon	5	5	5	0.6	0.6	0.6
Sf 9	1988	Málaga	Zucchini	5	5	10	0.9	0.4	0.8
Sf 26	1989	Málaga	Melon	1	25	2.5	0.2	1.3	0.4
Sf 29	1993	Málaga	Melon	1	5	2.5	0.1	0.8	0.3
Sf 30	1993	Málaga	Zucchini	2.5	25	2.5	0.5	8	0.5
Sf 41	1995	Almería	Melon	1	5	5	0.01	0.5	0.5
Sf 45	1996	Almería	Melon	2.5	10	2.5	0.1	0.8	0.2
Sf 48	1996	Málaga	Melon	2.5	25	5	0.4	7	0.7
2086	1997	Greece	Melon	2.5	25	5	0.3	3.7	0.8
P27.2	1997	Almería	Cucumber	5	25	5	1.3	2.5	0.9
98Sm32	1998	France	Melon	5	25	2.5	1.4	8	0.4
S111	1998	Almería	Watermelon	0.25	25	2.5	0.2	2.3	0.3
S112	1998	Almería	Watermelon	0.25	25	5	0.2	1.8	0.7
C46.8	1999	Almería	Zucchini	> 500	> 500	> 500	> 500	> 500	> 500
Sf 53	1999	Málaga	Melon	5	25	5	1	11	0.6
Sf 56	1999	Almería	Melon	5	25	10	0.3	4.2	0.8
Sf 60	1999	Greece	Zucchini	2.5	25	5	0.3	2.5	0.7
Sf 61	1999	Greece	Cucumber	2.5	25	5	0.3	2.5	0.8
Sf 202	1999	Málaga	Melon	> 500	> 500	> 500	> 500	> 500	> 500
Sf 213	1999	Málaga	Pumpkin	5	25	25	1.6	9	2.3
Sf 220	1999	Málaga	Melon	> 500	> 500	> 500	> 500	> 500	> 500
P47.12	1999	Almería	Cucumber	> 500	> 500	> 500	> 500	> 500	> 500
P47.14	1999	Almería	Cucumber	> 500	> 500	> 500	> 500	> 500	> 500
Sf 222	2000	Almería	Melon	2.5	25	2.5	0.4	1	0.3
2147	2002	Almería	Melon	> 500	> 500	> 500	> 500	> 500	> 500
2201	2002	Almería	Zucchini	2.5	25	2.5	0.4	7	0.5
2208	2002	Almería	Zucchini	5	25	1	0.6	2	1
2237	2002	Almería	Zucchini	10	25	5	0.8	5.2	0.5
2243	2002	Almería	Zucchini	5	25	2.5	0.1	6	0.2
3164	2002	Murcia	Melon	> 500	> 500	> 500	> 500	> 500	> 500
3167	2002	Murcia	Melon	10	25	10	1	4.3	1
3177	2002	Murcia	Melon	> 500	> 500	> 500	> 500	> 500	> 500
3181	2002	Murcia	Melon	> 500	> 500	> 500	> 500	> 500	> 500
31430	2002	Murcia	Melon	5	25	25	0.5	3	1
11024	2003	Málaga	Melon	5	25	2.5	0.8	9	0.6
21369	2003	Almería	Melon	> 500	> 500	> 500	> 500	> 500	> 500
22014	2003	Almería	Zucchini	5	25	5	0.5	10	0.7
22249	2003	Almería	Zucchini	5	25	25	0.1	5.3	3.5
22251	2003	Almería	Zucchini	2.5	25	25	0.5	9	1
22252	2003	Almería	Zucchini	5	25	5	0.6	5	0.1
22261	2003	Almería	Zucchini	2.5	10	2.5	0.02	1	0.25
22263	2003	Almería	Zucchini	2.5	25	5	0.6	2.6	0.9
22267	2003	Almería	Zucchini	2.5	25	5	0.1	5	0.7
22304	2003	Almería	Zucchini	> 500	> 500	> 500	> 500	> 500	> 500
22305	2003	Almería	Zucchini	2.5	10	2.5	0.2	3	0.4
22306	2003	Almería	Zucchini	5	10	10	0.7	3.5	0.9
22308	2003	Almería	Zucchini	2.5	25	5	0.3	1.6	0.7
22317	2003	Almería	Zucchini	5	25	5	0.3	3	0.6
22320	2003	Almería	Zucchini	2.5	25	5	0.5	6	0.9
23030	2003	Almería	Cucumber	5	25	2.5	0.6	4.5	0.1

[1]Azoxystrobin (Ortiva, Syngenta, Switzerland)
[2]Kresoxim-methyl (Stroby, BASF, Germany)
[3]Trifloxystrobin (Flint, Bayer, Germany)

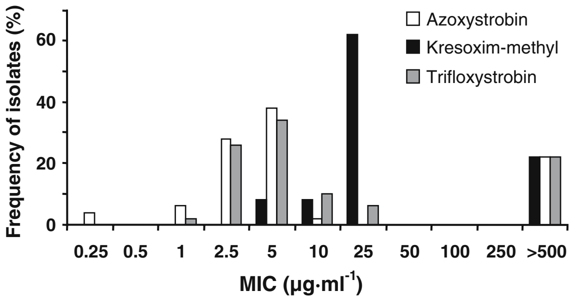

Figure 2. Frequencies of sensitivity (MIC values) to the QoI fungicides azoxystrobin, kresoxim-methyl and trifloxystrobin in 50 randomly chosen isolates of *P. fusca*.

were 0.5 μg ml^{-1} for azoxystrobin, 0.7 μg ml^{-1} for trifloxystrobin and 4.2 μg ml^{-1} for kresoxim-methyl, respectively. These results indicated that against *P. fusca*, azoxystrobin and trifloxystrobin displayed similar intrinsic activities and were 8 and 6 times more active than kresoxim-methyl. The second group consisted of 11 isolates that showed, in all cases, MIC and EC_{50} values estimated as >500 μg ml^{-1} for azoxystrobin, kresoxim-methyl and trifloxystrobin (Table 1). Phytotoxicity problems prevented the use of fungicides at higher concentrations. These 11 isolates were considered to be resistant to QoI fungicides and their resistance factors (RF) were estimated as >1000 for azoxystrobin, >715 for trifloxystrobin and >120 for kresoxim-methyl.

Distribution of QoI resistance in Spain

To simplify the analysis of field resistance to QoI fungicides in *P. fusca*, only three concentrations were used: 0, 50 and 250 μg ml^{-1}. Isolates were considered resistant when they were able to grow at 250 μg ml^{-1} of azoxystrobin, kresoxim-methyl or trifloxystrobin, and sensitive when unable to grow at 50 and 250 μg ml^{-1} of fungicide. The concentration of 50 μg ml^{-1} was included to detect intermediate resistance (growth at 50 but not at 250 μg ml^{-1}). We analysed the sensitivities to azoxystrobin, kresoxim-methyl and trifloxystrobin of 250 single-spore isolates of *P. fusca* derived from 264 samples obtained from different locations in the most important cucurbit production areas of south central Spain (Almería, Badajoz, Córdoba, Ciudad Real, Murcia and Valencia) during 2002–2004 growing seasons. Frequencies of resistance to QoI fungicides in *P. fusca* by location and year of sampling are shown in Table 2. During the sampling period, 81 isolates (32.4%) were identified as resistant, all to azoxystrobin, kresoxim-methyl and trifloxystrobin. No isolates with intermediate resistance were found. This overall QoI resistance was differentially distributed by location. The highest frequencies of resistance were observed in Murcia (73.9%), Córdoba (51%) and Ciudad Real (43.6%). By contrast, the lowest frequencies of resistance were detected in Valencia (12.5%), Almería (10.4%) and especially in Badajoz, where no resistance was found. QoI resistance generally increased slightly during the sampling period but not in all provinces. Resistance frequency increased in Ciudad Real (20–53.3%), Córdoba (41.6–66.7%) and Valencia (0–20%), while it remained at similar levels in Badajoz (0%), Almería (8.7–12.5%) and Murcia (71.4–76.5%).

Frequency of QoI resistance relative to the cucurbit crops from where the isolates were collected was also investigated. Most of the resistant isolates were obtained from melon (46.5%) mainly from Murcia (34 out of 46 melon isolates were resistant), Córdoba and Ciudad Real (Table 3). In pumpkin, 33.3% of isolates were resistant,

Table 2. Frequency of QoI resistance in *P. fusca* populations in six different cucurbit production areas of south central Spain in a three-year survey. Frequencies of resistance are shown and number of isolates analysed are given in parentheses

Locations	Sampling year			Overall (by location)
	2002	2003	2004	
Almería	12.5 (24)	8.7 (23)	10 (20)	10.4 (67)
Badajoz	0 (15)	0 (10)	0 (10)	0 (35)
Ciudad Real	66.7 (9)	20 (15)	53.3 (15)	43.6 (39)
Córdoba	46.7 (15)	41.6 (12)	66.7 (12)	51 (39)
Murcia	73.3 (15)	71.4 (14)	76.5 (17)	73.9 (46)
Valencia	0 (9)	20 (15)	–	12.5 (24)
Overall (by year)	31 (87)	25.8 (89)	41.9 (74)	32.4 (250)

Table 3. Frequency of QoI resistance in *P. fusca* population in different cucurbit crops in six cucurbit production areas of south central Spain in a three-year survey. Frequencies of resistance are expressed as number of resistant isolates/total number of isolates analysed. Overall resistance obtained by crop is also given

Location or Year	Crops				
	Cucumber	Melon	Pumpkin	Watermelon	Zucchini
Almería	2/7	3/11	–	1/9	1/40
Badajoz	0/1	0/24	0/7	0/1	0/2
Ciudad Real	0/3	14/31	1/2	–	2/3
Córdoba	–	16/28	1/2	1/5	2/4
Murcia	–	34/46	–	–	–
Valencia	0/3	0/4	3/4	0/3	0/10
2002	1/9	19/40	2/5	1/4	4/29
2003	0/4	19/52	3/10	0/4	1/19
2004	1/1	29/52	–	1/10	0/11
Overall	2/14	67/144	5/15	2/18	5/59
Frequency (%)	14.3	46.5	33.3	11.1	8.5

although the low number of isolates analysed (n = 15) makes this frequency less reliable than the others. In other crops, resistance frequencies were lower. In zucchini, 8.5% of isolates were resistant with only 1 out of 40 isolates from zucchini in Almería being resistant. No fluctuations in resistance over years were observed with the exception of zucchini, where a decrease in frequency of QoI resistance was detected.

Discussion

As a preliminary step before QoI resistance frequencies could be investigated, it was necessary to identify test concentrations to use to differentiate QoI sensitive and resistant isolates of *P. fusca* to the three QoI fungicides tested, azoxystrobin, kresoxim-methyl and trifloxystrobin. It was found with a leaf disc assay that field isolates of *P. fusca* could be easily distinguished as sensitive (EC_{50} 0.01–9 μg ml^{-1}) and highly resistant ($EC_{50} > 500$ μg ml^{-1}) to azoxystrobin, kresoxim-methyl and trifloxystrobin (Table 1) with RF values of up to >1000. There are few reports describing levels of sensitivity and resistance to QoI fungicides in *P. fusca*; nevertheless, data resembling those described here have been previously detailed (Ishii et al., 2001; McGrath, 2001).

Once a criterion for differentiating QoI sensitive and resistant isolates of *P. fusca* was established, a study for monitoring QoI resistance in field populations of *P. fusca* from the main cucurbit production areas in south central Spain was undertaken. This is a crucial aspect in fungicide resistance research, because virtually all our knowledge about distribution and impact of resistance in the field is dependent on monitoring. Data from the three year survey presented here have revealed interesting information. Ciudad Real, Córdoba and especially Murcia were, by far, the locations that showed the highest frequencies of QoI resistance. By contrast, in Almería and Valencia resistance frequencies were relatively low, and in Badajoz no resistance was detected (Table 2). Unfortunately, we do not have accurate data on fungicide use and applications that can support the high differences observed in QoI resistance frequencies in terms of a differential use of mildewicides in the different locations. Nevertheless, in spite of such limitation, two obvious conclusions can be drawn from these data. Firstly, in Ciudad Real, Córdoba and Murcia, QoI fungicides must be removed, at least temporarily from the management strategies against cucurbit powdery mildew; and secondly, in the rest of the analysed locations the use of QoI should result in good disease control. Furthermore, most of the *P. fusca* isolates resistant to QoI were collected from melon (Table 3). This is probably because melon is the main crop in most cucurbit production areas of Spain with the exception of Almería, which may result in the highest fungicide use pressure occurring in this crop.

In Spain there is limited use of cucurbit cultivars resistant to powdery mildew. Instead, disease management is practically based on fungicide applications. In addition, there are no annual guidelines for managing cucurbit powdery mildew with fungicides, and management programmes are arranged locally at cooperative or farmer level. Therefore, growers using exclusively high-risk fungicides such as QoI with frequencies of application abnormally high, may select resistant strains and thereby thwart efforts of growers who are using a proper resistance management programme (Brent and Hollomon, 1998). QoI resistance in cucurbit powdery mildew is known to occur globally in major cucurbit growing regions and field performance in those countries has been reduced (Anon. 2005). The QoI working group of the Fungicide Resistance Action Committee (FRAC) has defined recommendations for management of QoI fungicide resistance in cucurbit vegetables, which should serve as a fundamental guide for development of local resistance management programmes for 2006 (Anon. 2005).

In Almería, Valencia and Badajoz, the cucurbit production areas of south central Spain where QoI resistance frequencies in *P. fusca* are still relatively low, these recommendations should be seriously considered in order to achieve successful disease control and to keep QoI resistance at low levels. By contrast, the high levels of QoI resistance found in Ciudad Real, Córdoba and Murcia, suggest that the local QoI resistance management programmes in cucurbits, if they exist, have been overcome, and therefore, there is an urgent need to reconsider the use of QoI fungicides against cucurbit powdery mildew. In these areas, other mildewicides from different cross-resistance groups such us demethylation inhibitors (DMI), and multi-site inhibitors such as sulphur, should be used. To illustrate this, a similar strategy was recently put into practice in a study to evaluate the use of fungicides for prevention and management of powdery mildew on watermelon in southern USA (Keinath and DuBose, 2004). In that study, one of the most effective treatments included the use of three fungicides: mancozeb (a multi-site inhibitor) alternated with azoxystrobin (QoI) during the entire season, and myclobutanil (DMI), added when powdery mildew was detected.

A rapid appearance of fungicide resistance suggests the occurrence of a single resistance conferred by a point mutation in the gene encoding the fungicide-targeted protein. The QoI-targeted cytochrome b protein is encoded by mitochondrial DNA (mtDNA), which is thought to mutate at a higher frequency than nuclear DNA (Bohr and Anson, 1999). Several amino acid substitutions in two regions of the mitochondrial *cyt b* gene have been identified in yeast and fungi naturally resistant to QoI (Kraiczy et al., 1996). For most of the plant pathogens in which QoI resistance has been reported, resistance was conferred by a point mutation in *cyt b* leading to an amino acid change from glycine to alanine at position 143 (G143A) (Gisi et al., 2002). In *P. fusca*, the same mutation was found in two isolates resistant to QoI (Ishii et al., 2001). Whether this or other mutations in the *cyt b* gene are responsible for QoI resistance in the isolates of *P. fusca* resistant to QoI identified in this study is unknown, but the qualitative nature of resistance exhibited by the isolates examined and occurrence of cross-resistance support this hypothesis.

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