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Mechanisms of resistance to QoI fungicides in phytopathogenic fungi

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Summary. The major threat to crops posed by fungal diseases results in the use by growers of enormous amounts of chemicals. Of these, quinol oxydation inhibitors (QoIs) are probably the most successful class of agricultural fungicides. QoIs inhibit mitochondrial respiration in fungi by binding to the Qo site of the cytochrome bc_1 complex, blocking electron transfer and halting ATP synthesis. Unfortunately, the rapid development of resistance to these fungicides and consequent control failure has become increasingly problematic. The main mechanism conferring resistance to QoIs is target site modification, involving mutations in the cytochrome *b* gene *CYTB*, such as the substitution of glycine by alanine at position 143 (G143A) that occurs in several phytopathogenic fungi. The impact of other mechanisms, including alternative respiration and efflux transporters, on resistance seems to be limited. Interestingly, in some species QoI resistance is not supported by mutations in *CYTB*, while in others the structure of the gene is such that it is unlikely to undergo G143A mutations. Better understanding of the biological basis of QoI resistance in a single pathogen species will facilitate the development of resistance diagnostic tools as well as proper anti-resistance strategies aimed at maintaining the high efficacy of these fungicides. [**Int Microbiol** 2008; 11(1):1-9]

Key words: alternative respiration · cytochrome *b* · efflux transporters · fungicide resistance · strobilurins

Introduction

Fungicides have long been used to reduce crop losses. During the last century until about 1960, most agricultural fungicides were protectants and multi-site inhibitors, which restricted their use to external plant applications. Two distinct classes of these fungicides, with multiple target sites, were most frequently employed. The first class included the inorganic Bordeaux mixture, sulfur and copper compounds. The second class comprised organic compounds, such as the phthalimides, dithiocarbamates, dinitrophenols, and aromatic hydrocarbons. All these compounds were used commonly on a small range of crops. Unfortunately, the costs were such that applications were only economical on high-value crops, and their use was restricted by the intrinsic problem of residues remaining on produce.

The introduction of systemic fungicides with specific modes of action offered the benefit of internal plant therapy. Benomyl, a benzimidazole, was the first successful systemic product, commercially launched in 1970, followed shortly afterwards by morpholine and aminopyrimidine com-

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pounds. Options for disease control were expanded in the 1980s through the introduction of several triazole-containing agents, the first members of the sterol demethylation inhibitor (DMI) class of fungicides, and additional members of the morpholine group.

Over the last decade, the standards of disease control have been further improved by the development of the strobilurins. These were the first members of the class of quinol oxydation inhibitors (QoIs) and were commercialized in 1996 [19]. The strobilurins (QoIs) quickly became one of the most important agricultural fungicides, accounting for over 20% of the global fungicide market within the first ten years of their commercial offering. However, one of the apparent strengths of these systemic fungicides, their highly specific mode of action, has proven to be a serious weakness, since a significant number of target pathogens have developed resistance to QoIs. Indeed, shortly after their commercial introduction, resistant isolates were detected in field populations of several plant pathogens. In most cases, resistance was due to modification of the target site. Nevertheless, an increasing amount of experimental evidence has attributed QoI resistance to other mechanisms. This article reviews our current knowledge of the mechanisms of QoI fungicide resistance in phytopathogenic fungi, and discusses the implications for the evolution of resistance and for risk assessment.

The QoI fungicides

The discovery of QoI fungicides was inspired by a group of natural fungicidal derivatives of β-methoxyacrylic acid, such as strobilurin A and oudemansin A, produced by the basidiomycetes *Strobilurus*, *Mycena*, and *Oudemansiella* [22]. Strobilurin A, the first QoI molecule, was obtained from liquid cultures of *Strobilurus tenacellus* in 1977 [2], and the mode of action of this group of compounds was elucidated in 1981. The fungicidal activity of QoIs relies on their ability to inhibit mitochondrial respiration by binding at the so-called Qo site (the outer quinol-oxidation site) of the cytochrome bc_1 enzyme complex (complex III) (Fig. 1). This inhibition blocks electron transfer between cytochrome *b* and cytochrome c_1, which, in turn, leads to an energy deficiency in fungal cells by halting the production of ATP. QoI fungicides usually have low toxicity towards birds, mammals (including humans), and bees at limited doses and are

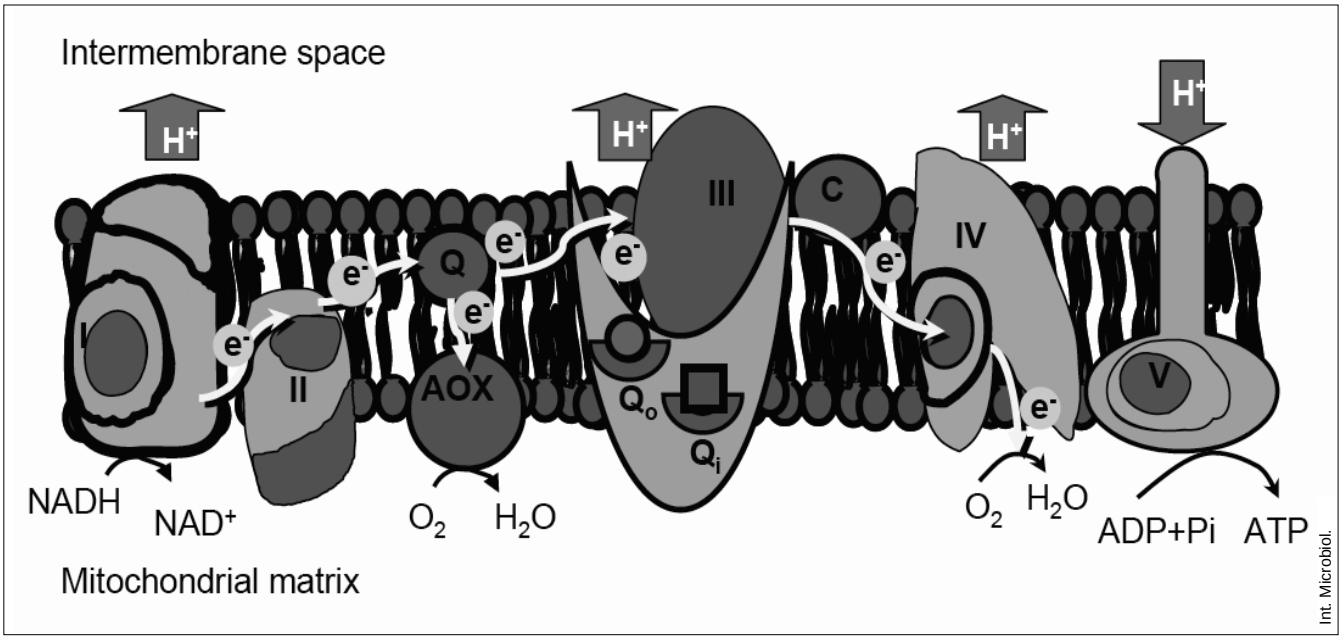

Fig. 1. Schematic representation of the mitochondrial electron transport system. I, II, III, IV are the different complexes of the transfer chain. V is the ATP synthase complex; Q, the ubiquinone pool; and C, the peripheral protein cytochrome *c*. The arrows inside the membrane indicate the direction of electron flow. Qo and Qi binding sites of the cytochrome bc_1 enzyme complex (complex III) are delineated by a circle and a square representing Qo- and Qi-inhibitor molecules, respectively. In some fungi, inhibitors of the respiratory pathway induce the synthesis of alternative oxidase (AOX), which diverts electrons at the ubiquinone pool (Q), although generating much less energy.

Table 1. The QoI fungicides

Classes	Fungicides	Company	Announced	First sales
I. Methoxyacrylates	Azoxystrobin	Syngenta	1992	1996
	Picoxystrobin	Syngenta	2000	2002
	Enestrobin	SRICI[a]	In progress	In progress
I. Methoxycarbamates	Pyraclostrobin	BASF	2000	2002
III. Oximinoacetates	Kresoxim-methyl	BASF	1992	1996
	Trifloxystrobin	Bayer	1998	1999
IV. Oximinoacetamides	Metominostrobin	Shionogi	1993	1999
	Dimoxystrobin	BASF	2005	2006
	Orysastrobin	BASF	In progress	In progress
V. Oxazalidinediones	Famoxadone	Dupont	1996	1997
VI. Dihydro-dioxazines	Fluoxastrobin	Bayer	2004	2005
VII. Imidazolinones	Fenamidone	Bayer	1998	2001
VIII. Benzyl-carbamates	Pyribencarb	K-I Chemical	In progress	In progress

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thus considered low risk, but they do show dose-dependent toxicity towards aquatic organisms. Nevertheless, since all QoI dissipate in the environment relatively quickly, there is little potential for long-term exposure and hence there is low chronic risk [5].

Based on structural similarities, eight chemical classes of Qo inhibitors can be distinguished [for an extensive review on strobilurin fungicides, see 4] (Table 1). They share a common mode of action that involves binding to the Qo site of the cytochrome bc_1 complex and are generally cross-resistant when tested in different assays such as in-planta tests, spore germination, mycelial growth, cell-free enzyme tests, and on artificial mutants, although their spectra and intrinsic levels of biological activity are quite different [Heaney et al. (2000) Proceedings of the Brighton Conference–Pests and Diseases, BCPC, Farnham, pp 755-762]. The toxophore is similar in all compounds and always carries a carbonyl oxygen moiety, thought to be responsible for binding [5]. Presently, there are ten QoI-containing active ingredients commercially available for agricultural use, including azoxystrobin, the world's most commonly used fungicide [4] (Fig. 2).

The QoI target, cytochrome bc_1, is an integral membrane protein complex essential for fungal respiration. In eukaryotes, it comprises 10 or 11 different polypeptides with a combined molecular mass of about 240 kDa, and operates as a structural and functional dimer. Cytochrome *b*, cytochrome c_1 and the Rieske iron-sulfur protein (ISP) form the catalytic core of the enzyme. The catalytic mechanism, called the Q-cycle, requires two distinct quinone-binding sites: Qo, the quinol oxidation site, and Qi, the quinone reduction site [14]. The location of the quinol/quinone binding sites of bc_1, both within the cytochrome *b* subunit, has been resolved by X-ray crystallography using bound inhibitors. Detailed informa-

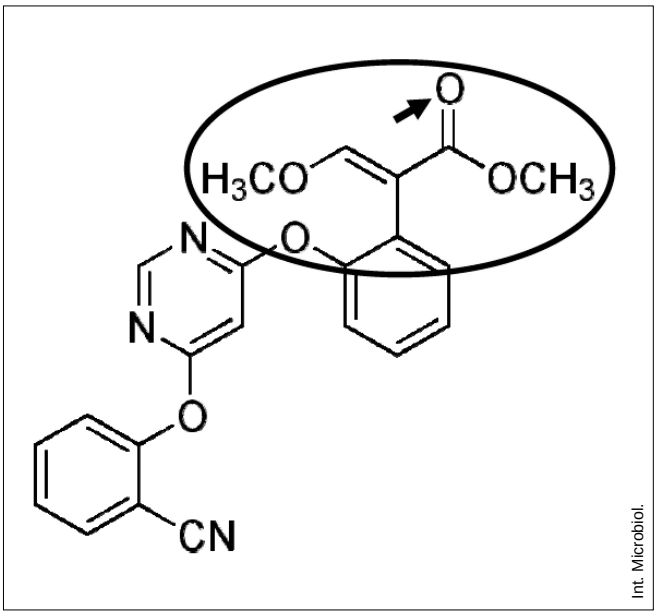

Fig. 2. Chemical structure of azoxystrobin. The toxophore is highlighted with a circle and the carbonyl oxygen moiety responsible for binding is indicated by the arrow.

tion about interactions between bc_1 and several inhibitors has since become available for several QoI fungicides. It is now known that despite differences in binding between the different Qo inhibitors, their fit to the enzyme pocket is very similar [11].

Since their introduction, QoIs have become essential components of plant disease control programs because of their wide-ranging efficacy against many agriculturally important fungal diseases. QoIs have also been registered in numerous countries for use on several different crops, including cereals, turf grass, grapevine, and a number of vegetables and ornamentals. Unfortunately, the intrinsic risk of the development of resistance to this group of fungicides is high, and strict anti-resistance strategies, including treatment limitations and the use of mixtures or alternations, are therefore needed [27]. In Spain, for example, there are cucurbit-growing areas where cucurbit powdery mildew, *Podosphaera fusca*, has developed high levels of resistance (up to 51% in Córdoba, and 74% in Murcia) to QoI fungicides, such that disease control based on these compounds is virtually ineffective, presumably due to the frequent use of these popular fungicides among growers [12].

Mechanisms of resistance to QoI fungicides

Mutations in the cytochrome *b* gene. QoI resistance primarily arises from a target-site-based mechanism involving mutations in the mitochondrial cytochrome *b* gene (*CYTB*). These result in peptide sequence changes that prevent fungicide binding. Mutations affecting sensitivity have been found in two regions of *CYTB*, corresponding to amino acid positions 120–155 and 255–280 of the encoded protein. These domains lie close to each other in the folded protein and play a role in ligand binding. Two important amino acid substitutions, from glycine to alanine at position 143 (G143A) and from phenylalanine to leucine at position 129 (F129L), have been detected in cytochrome *b* of several phytopathogenic fungi and oomycetes that are resistant to QoIs [17]. Recently, a new amino acid change, from glycine to arginine at position 137 (G137R), also has been linked to QoI resistance [34]. Isolates carrying F129L or G137R express moderate (partial) resistance, which is usually controlled by the recommended field levels of QoIs. By contrast, isolates with G143A express high (complete) resistance, which is always associated with the failure of QoIs to control disease. The G143A substitution has been detected in resistant isolates of more than twenty species, including phytopathogenic ascomycetes and oomycetes, such as several powdery mildews and *Alternaria* species, and the major *Mycosphaerella* pathogens, *M. fijensis* and *M. graminicola* (Fungicide Resistance Action Committee, FRAC, QoI Working Group) [www.frac.info].

To understand the molecular basis of natural resistance to strobilurins in the strobilurin-producing basidiomycetes, Kraiczy et al. [22] compared the sequences of the two regions of cytochrome *b* spanning the Qo site from *S. tenacellus* and *Mycena galopoda*. Five amino acid changes in cytochrome *b* were found, including G143A, which was postulated to account for resistance to β-methoxyacrylates. Furthermore, one of those substitutions, from asparagine to aspartate at position 261 (N261D), was also found in *Schizosaccharomyces pombe*, which is naturally resistant to QoIs [22]. Thus, it is not surprising that the main mutation conferring field resistance to QoIs in plant pathogens is one of the five substitutions detected in strobilurin-producing basidiomycetes (G143A).

Although many reports have attributed QoI resistance in plant pathogens to the G143A substitution, the role of this amino acid change in resistance has been very difficult to establish conclusively because the cytochrome *b* target is encoded by a mitochondrial gene. Consequently, functional genetic studies have been undertaken only in *M. fijiensis* [17]. In that study, the transformation of sensitive isolates with fragments of *CYTB* amplified from resistant isolates containing the mutation confirmed that G143A conferred full QoI resistance. In light of the obstacles in demonstrating the role of *CYTB* mutations in QoI resistance by molecular approaches, most studies have been based on demonstrating a correlation between QoI resistance and the G143A substitution. This statistical approach, however, has not been always as thorough as required due to the low number of resistant isolates analyzed, at least as determined from the published literature and sequences available in gene databases. Nonetheless, a correlation between QoI resistance and the G143A mutation has been clearly shown for *Blumeria graminis* f. sp. *tritici*, *M. graminicola*, and *Pyricularia grisea* [15,16,21].

The fact that QoIs inhibit a target site encoded by a mitochondrial gene implies several important differences in terms of the evolution of fungicide resistance. A fungal cell may contain >100 mitochondria and an equal copy number of mitochondrial DNA (mtDNA). Thus, a point mutation in a single copy of mtDNA would have little effect on fungal survival. Accordingly, the evolution of QoI resistance in a

fungal population must be the combined result of recurrent mutations and the selection process imposed by the fungicide [17]. Once homoplasmic resistant individuals have emerged in a population, the selection process for QoI resistance is expected to follow mechanisms similar to those of nuclear-encoded resistance, with the key to effective resistance being the transition process from hetero- to homoplasmic cells. How this mutation is selected from within a population of wild-type, sensitive mitochondria is not clear. Some reports have described that mitochondrial heteroplasmy with respect to the G143A mutation results in different levels of QoI resistance [23]; however, the question of how many mutated mitochondria per cell are required to produce a fully QoI-resistant phenotype has not yet been answered.

Because of the relevance of the G143A substitution in agriculture, the single nucleotide polymorphism (SNP) giving rise to it has been used as the basis to detect and quantify resistance by molecular methods, such as allele-specific PCR [24] and quantitative real-time PCR (Q-PCR) [15]. Both techniques allow for detection of the mutation even when it is present at very low frequencies. PCR-based diagnostics have been designed and are currently being used to follow the response of plant pathogen populations to selection by QoI fungicides and to test the risk of resistance development prior to the possible appearance of disease control failures, such as those already shown for the wheat pathogens *B. graminis* f. sp. *tritici* and *M. graminicola* [15,16].

Alternative respiration. The second mechanism of resistance to QoI fungicides is mediated by the induction of alternative, cyanide-resistant respiration, which is sustained by alternative oxidase (AOX) [37]. With this rescue mechanism, mitochondrial electron transfer is diverted by circumventing the inhibitory site of QoI, the cytochrome bc_1 complex (Fig. 1). Alternative respiration, however, only seems to provide energy to counteract QoI effects in vitro but not in planta. Under field conditions, the impact of alternative respiration on the protective activities of QoI fungicides is limited for two main reasons. Firstly, this pathway provides only 40% of the normal efficiency for energy conservation. This is due to the fact that complexes III and IV of the mitochondrial electron transport system are bypassed and AOX lacks proton pump activity. Consequently, processes that demand large amounts of energy, such as spore germination and host-penetration, which are critical steps for successful colonization of the plant, are not supported. Secondly, plant antioxidants, such as flavones, are released during infection and interfere with the induction of alternative respiration by quenching the reactive oxygen species which are necessary to induce the *AOX* gene and which are generated by QoIs [37].

Nevertheless, in contrast to the widely accepted minor role of AOX in QoI resistance, recent reports using alternative-oxidase-deficient mutants and specific inhibitors of this enzyme have revealed that alternative respiration also limits QoI effectiveness in planta, especially once the infection has been established [3,28,31]. A general explanation is that a decreasing demand for energy efficiency during the later stages of the infection process, such as mycelial growth and sporulation, enables AOX to become more effective as infection progresses [37]. This is consistent with the lack of eradicant activity of QoI fungicides on many fungi; after visible symptoms have appeared, control is usually ineffective.

Moreover, alternative respiration may provide an opportunity for the selection of a point mutation in *CYTB* by decreasing levels of damaging reactive oxygen species and ensuring some ATP synthesis. Both these functions contribute to cell survival while selection increases the frequency of resistant mitochondria [28,37]. The QoI-targeted cytochrome *b* protein is encoded by mtDNA, which is known to mutate at a higher frequency than nuclear DNA—a frequency that is increased by the accumulation of reactive oxygen species as a result of the inhibition of the electron transport system by QoIs [6]. Under such circumstances, alternative respiration could represent an essential pathway in the transition from sensitivity to full resistance in phytopathogenic fungi in the presence of sublethal concentrations of QoI fungicides. In any case, as previously pointed out by Wood and Hollomon [37], despite the lack of a clear-cut example in which AOX plays a role in field resistance to QoIs, involvement of the enzyme should not be discarded. Transcriptome analysis of infected plants treated with strobilurin could provide useful information to clarify the function of oxidative stress response genes in QoI resistance [7,30].

Efflux transporters. Efflux transporters enable fungi to survive exposure to toxic compounds by preventing their accumulation to toxic concentrations inside fungal cells. These membrane-bound proteins are known to provide protection against a wide range of natural toxic compounds and xenobiotics [10]. The family of ATP-binding cassette (ABC) transporters and the major facilitator superfamily (MFS) are the most important efflux pumps involved in the protection of fungi against fungicides [9,36]. The first efflux transporter involved in resistance to QoI was detected in *Aspergillus nidulans*. This ABC transporter, encoded by the

AtrB gene, is involved in protection against compounds from all major classes of fungicides, including strobilurins [1]. In agriculture, however, obvious cases of multi-drug resistance to fungicides in plant pathogens are few. The efflux transporter gene involved in QoI insensitivity first reported for a plant pathogen is *MgMfs1*, a MFS transporter gene of *M. graminicola* [33]. Most natural isolates of *M. graminicola* resistant to strobilurins and overexpressing *MgMfs1* also contain the G143A mutation, which suggests that the contribution of *MgMfs1* to QoI resistance in field strains should be small.

Prior to the isolation of *MgMfs1*, Reimann and Deising [32] described an efflux-transporter-mediated mechanism of resistance to QoI fungicides in field isolates of the wheat pathogen *Pyrenophora tritici-repentis*. The involvement of this type of mechanism in fungicide resistance was determined by using inhibitors of efflux transporters, such as the hydroxyflavone derivative 2-(4-ethoxy-phenyl)-chromen-4-one, in combination with fungicides, which shifted resistant isolates back to normal sensitivity levels, preventing infection of wheat leaves. In addition, those authors reported the induction of efflux transporter activity under field conditions and after in vitro applications of sublethal amounts of fungicides [32]. A similar mechanism of adaptation to fungicidal stress through efflux transporter activity was recently described in several *Colletotrichum* species [Deising et al. (2007) 15th Int. Reinhardsbrunn Symp. on Modern fungicides and antifungal compounds, Friedrichroda].

In *Phytophthora infestans*, laboratory mutants with high multi-drug resistance to chemically unrelated oomycetes fungicides, including QoI, have been described [39]. Experimental evidence for the molecular basis of the multi-drug resistance phenomenon in *P. infestans* has not yet been provided, but the increased expression of energy-dependent efflux transporters has been suggested as the most probable mechanism. Although data regarding resistance to QoI fungicides based on efflux transporters are scarce, the contribution of these energy-dependent mechanisms to the in planta adaptation to fungicides by phytopathogenic fungi should be seriously considered. Regardless, the novel disease management strategy based on the inhibition of fungicide efflux, suggested by Reimann and Deising [32], should be explored.

Other mechanisms. In recent studies, resistance to QoI fungicides based on the G143A substitution has been deemed unlikely in phytopathogenic basidiomycetes, (such as *Puccinia* spp.) and in several ascomycetes (such as *Alternaria solani* and *Pyrenophora teres*) because both carry a type I intron immediately after this codon. Consequently, a nucleotide substitution in codon 143 of *CYTB* would prevent splicing of the intron, and thus a deficiency in cytochrome *b*, a presumably lethal mutation [18]. Indeed, so far, serious resistance problems have not developed among *Puccinia* species, despite the extensive use of QoIs on crop hosts of this group of pathogens. Nevertheless, one isolate of *Puccinia horiana* resistant to QoI has been reported to have *CYTB* sequences identical to sensitive genotypes, including the type I intron and, therefore, without the G143A substitution [18].

The cucurbit powdery mildew *Podosphaera fusca* and the apple scab pathogen *Venturia inaequalis* are two pathogens in which G143A has been described [20,35]. However, the mutation does not always explain the QoI-resistant phenotype. This is the case for a set of highly resistant field isolates of *P. fusca* examined in our laboratory, which did not show G143A or any other consistent mutation in cytochrome *b*. Moreover, in both species, the role of alternative respiration in resistance has been ruled out and the effective mechanisms responsible for QoI resistance remain to be characterized [13,35]. Considering the pattern of cross-resistance to different QoIs, the high levels of resistance of the resistant *P. fusca* isolates, and the absence of consistent mutations in *CYTB*, a structural change in the Rieske protein may well be responsible for QoI resistance [13]. Experimental evidence regarding the role of the Rieske protein in resistance to QoI fungicides has not yet been obtained, but its nature as a nuclear-encoded target makes it an ideal candidate to explain QoI resistance in pathogens in which *CYTB* mutations have not been found.

Fitness costs associated with QoI resistance

The fitness of pathogen isolates resistant to fungicides is significant in disease management. If fitness costs are associated with fungicide resistance, the frequency of resistant isolates will decline in the absence of fungicide. Despite its practical relevance, little is known about the fitness cost associated with QoI resistance. Fitness penalties have been observed in QoI-resistant populations of the oomycete *Plasmopara viticola* and the rice pathogen *P. grisea*, carrying the G143A substitution [3]. By contrast, fitness penalties are not apparent in the case of *B. graminis* [Heaney et al. (2000) Proceedings of the Brighton Conference–Pests and Diseases, BCPC, Farnham, pp 755-762]. The lack of fitness

cost associated with *CYTB*-based resistance was confirmed by competition assays between resistant and sensitive field isolates of *B. graminis* f. sp. *tritici*. The results provided evidence that QoI resistance is stable in the absence of selection over three generations, and that resistant isolates appear to be equal in fitness to sensitive isolates found on untreated plants [8].

Saccharomyces cerevisiae has been used as a model system to test the fitness associated with QoI *CYTB*-based resistance. Specific residues in the Qo site of yeast cytochrome *b* have been modified to obtain new forms that mimic the Qo binding site of several fungal plant pathogens and to study the impact of the G143A substitution on bc_1 complex activity. As expected, the G143A substitution caused high levels of resistance to QoI fungicides. However, G143A led to a slight reduction in the activity of the bc_1 complex in most of the Qo site mimics (e.g., *P. fusca*) but not in *B. graminis* f. sp. *tritici*. Based on these observations in the yeast model, it has been suggested that the G143A substitution affects the fitness of plant pathogens differentially [14]. This differential effect on fitness could explain the absence of the G143A substitution in the field populations of some pathogens resistant to QoI fungicides, such as the Spanish populations of *P. fusca* [13], but it could also explain the widespread detection of G143A in resistant strains of *B. graminis* f. sp. *tritici* from northern Europe [15].

Fitness studies also have been carried out with laboratory mutants of *Botrytis cinerea*, *Cercospora beticola*, and *Ustilago maydis* resistant to QoIs [25,26,38]. In all cases, fitness penalties in most QoI-resistant mutants were found. These mutations appear to be pleiotropic and to have significant adverse effects on fitness-determining characteristics, such as those related to development of fungal infection structures. Note that mutants are differentially affected in pathogenicity, which decreases in the *C. beticola* and *U. maydis* mutants and is unaffected in the *B. cinerea* ones. Furthermore, for the three species, resistance was reduced in the QoI-resistant mutants when they were grown on fungicide-free medium but recovered when the mutants were returned to selective medium. Moreover, competition assays between *B. cinerea* mutants and the wild-type strain have shown that, in vitro, all mutants are less competitive than the parental strain. The molecular bases of QoI resistance have been determined only for the *C. beticola* mutants, in which typical mutations in the cytochrome *b* gene can be linked to the different levels of resistance. For *B. cinerea* and *U. maydis* mutants, the mechanisms of resistance to QoI fungicides remain uncharacterized.

It has been also widely assumed that fitness costs are fixed. However, the cost of resistance to QoI fungicides seems to vary with environmental conditions such as temperature, being more costly under conditions that are suboptimal for the fungus, as shown for *B. graminis* and *M. graminicola* [Brown et al. (2006) 13th Conference of the Spanish Society for Phytopathology, Murcia, pp 15]. Although the G143A substitution can confer resistance to QoI fungicides, in the absence of such compounds it may also impose a fitness penalty by affecting mitochondrial respiration. There are virtually no data regarding the fitness cost associated with other mechanisms involved in QoI resistance. For *B. cinerea*, the usefulness of QoI fungicides is very limited and restricted to certain crops. Note that, in liquid cultures, *B. cinerea* produces high levels of AOX in the absence of any external stimulus, so it may be better adapted for dispensing with complexes III and IV of mitochondrial electron transport chain than species in which AOX is only induced as a rescue mechanism [37]. Moreover, for the MFS transporter gene of *M. graminicola MgMfs1*, the increased strobilurin efflux activity provided by this transporter may be a condition for normal fitness, since efflux may prevent strobilurin accumulation in fungal membranes and, hence, safeguard normal membrane functioning. Therefore, it would be of great interest to analyze the fitness associated with the different mechanisms of QoI resistance in fungal plant pathogens against which QoI fungicides are still in use. These studies, along with data about the genetic diversity of pathogen populations [29] should provide relevant information that will be used for the rational design of anti-resistance strategies.

Conclusions

While there is an extensive literature that emphasizes the contribution of the G143A substitution of cytochrome *b* in QoI resistance, this has not always been clearly substantiated. Although important insights into the mechanisms influencing the evolution of *CYTB*-based QoI resistance have been gained in recent years, many basic questions remain to be answered, most of which are related to the mitochondrial nature of the cytochrome *b* gene. An increasing amount of experimental evidence attributes the phenomenon of QoI resistance to mechanisms distinct from cytochrome *b* mutations, such as alternative respiration, efflux transporters, and other as-yet-unknown mechanisms. The cause of QoI resistance in phytopathogenic fungi could be more complex than

a simple point mutation, and the widely accepted role of cytochrome *b* in QoI resistance should be reconsidered. In the microbial world, there are examples of microbes that can develop resistance to a given antibiotic through different mechanisms. This could also be the case for QoI. Accordingly, broad assumptions should be avoided and molecular bases of QoI resistance for single pathogen species carefully clarified before rapid diagnostic methods are developed. This approach will help to maintain the high efficacy of this important class of agricultural fungicides for as long as possible.

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