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Fitness Penalties in the Evolution of Fungicide Resistance

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Abstract

The evolution of resistance poses an ongoing threat to crop protection. Fungicide resistance provides a selective advantage under fungicide selection, but resistance-conferring mutations may also result in fitness penalties, resulting in an evolutionary trade-off. These penalties may result from the functional constraints of an evolving target site or from the resource allocation costs of overexpression or active transport. The extent to which such fitness penalties are present has important implications for resistance management strategies, determining whether resistance persists or declines between treatments, and for resistance risk assessments for new modes of action. Experimental results have proven variable, depending on factors such as temperature, nutrient status, osmotic or oxidative stress, and pathogen life-cycle stage. Functional genetics tools allow pathogen genetic background to be controlled, but this in turn raises the question of epistatic interactions. Combining fitness penalties under various conditions into a field-realistic scenario poses an important future challenge.

INTRODUCTION

Control of fungal plant diseases has a long history, but chemical control on an industrial scale took off only during the second half of the nineteenth century (115). Initially, preparations based on inorganic sulfur, lime, and copper compounds were produced. Organic broad-spectrum multisite-acting fungicides with protectant properties such as dithiocarbamates and phthalimides were developed from 1940–1960. Further advances in crop protection were made beginning in the 1970s with the introduction of systemic single-site fungicides with protectant and eradicant properties, such as methyl benzimidazole carbamates (MBCs), sterol biosynthesis inhibitors [different classes, including demethylation inhibitors (DMIs; azoles) and amines], quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs). Systemic fungicides have become key components of disease management programs ensuring profitable and high-quality production of a range of commodities, including staple crops such as wheat, rice, and soybeans, and, therefore, providing an important contribution to food security and safety (e.g., reduction of mycotoxins). **Figure 1** shows the importance of different fungicide groups in controlling foliar cereal diseases in the United Kingdom over time.

Fungicides targeting a single biochemical step generally have a low toxicological profile for humans and other non-target organisms but are also prone to resistance development. Resistance development and tighter regulations on the registration and usage of fungicide active substances, resulting in the removal of products from the market as well as a slowing pipeline of new products due to increased costs, are reducing the range of available chemical classes (80). A greater

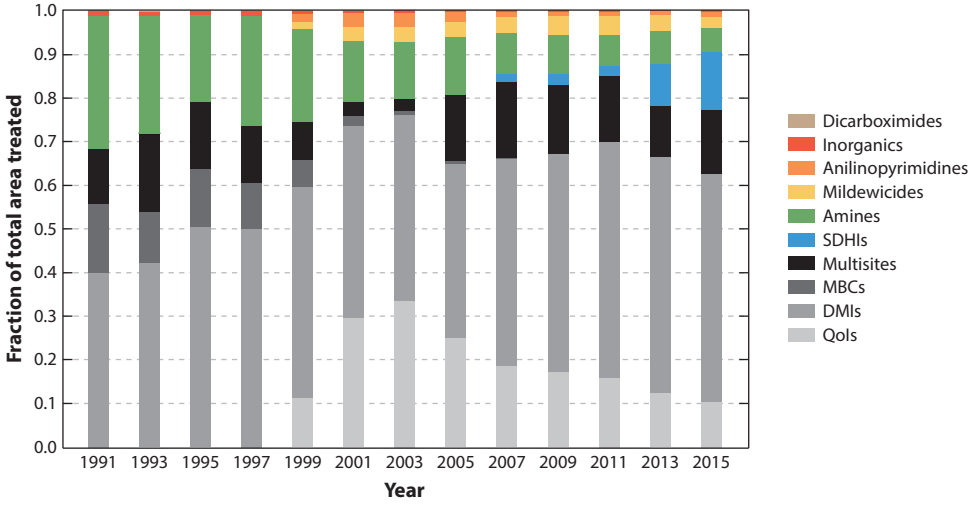

Figure 1

Relative importance of different fungicide groups used as foliar sprays to control fungal cereal diseases in the United Kingdom over time. In total 56 active substances were identified, belonging to dicarboximides (1), inorganics [sulfur and copper compounds (5)], anilinopyrimidines (1), mildewicides [azanaphtalenes (2), arylphenylketones (2), and phenylacetamides (1)], amines (4), succinate dehydrogenase inhibitors (SDHIs) (6), multisites [dithiocarbamates (3), phthalimides (1), and chloronitriles (1)], methyl benzimidazole carbamates (MBCs; 3), demethylation inhibitors (DMIs; 18), and quinone outside inhibitors (QoIs; 8). Area treated refers to the active substance treated area. This is the basic area treated by each active substance, multiplied by the number of times the area was treated. Figure is based on data collected by the Pesticide Usage Survey Teams at Fera Science Ltd, the Scottish Agricultural Science Agency, and the Agri-Food and Biosciences Institute of Northern Ireland (**https://secure.fera.defra.gov.uk/pusstats/**).

dependence on fewer modes of action increases the selective pressure for further cases of resistance. In order to increase the shelf life of new and currently available actives, evolution-smart integrated pest management strategies are needed. Strategies based on different dose rates, alternations, and mixtures of fungicides have been advocated to reduce the selection of resistance. Owing to resistance development to QoIs and azoles in field populations of *Zymoseptoria tritici*, the most important foliar wheat pathogen in the United Kingdom, multisite fungicides, chlorothalonil in particular, are increasingly being used as mixing partner for azoles and/or SDHI fungicides (**Figure 1**). The emergence and further selection of individuals with genetic changes conferring reduced fungicide sensitivity are dependent on their ability to compete with other individuals in the population in the presence or absence of fungicides. The eventual outcome is dependent on the fitness costs associated with the mutations that contribute to resistance. Developing rational resistance management strategies depends on whether the evolutionary outcomes of such strategies are predictable, and there is a need for more empirical data on the fundamental evolutionary processes underlying the selection of resistance. This article provides a review of pathogen fitness penalties associated with the evolution of fungicide resistance to the most important groups of systemic fungicides with broad-spectrum activity.

EVOLUTIONARY BIOLOGY OF RESISTANCE

Fungicide use exerts a selective pressure, under which resistant genotypes are fitter than sensitive genotypes (89). The resistant genotypes may emerge de novo under fungicide selection (124) or be selected from standing genetic variation (58) and may result in effectively full resistance with a single mutation (6) or a more continuous shift as multiple mutations accumulate (37).

Although biocides can exert a very strong selective pressure in favor of resistance, this selective advantage may be offset by fitness costs. This has been reported for some classes of antibiotic resistance (150). Fitness penalties may reduce the overall selective advantage of resistance under fungicide treatment (129), but more significantly they may even lead to reversals of resistance evolution given sufficient gaps in treatment (1). When resistance mutations are considered in terms of a fitness landscape, resistance is the fitness component used as the vertical dimension (110); however, where those mutations have other impacts on fitness, the adaptive landscape could be very different in the absence of fungicide selection or at lower doses (101).

However, subsequent compensatory mutations can sometimes reduce the fitness penalties associated with resistance-conferring mutations (150). More generally, epistatic interactions mean the overall fitness impact of a resistance mutation may be affected by other resistance mutations, compensatory mutations, or the genetic background in which they occur (110).

The main molecular mechanisms of fungicide resistance are listed in **Table 1**. Direct fitness penalties may result from allocation costs or functional trade-offs depending on the resistance mechanism, whereas indirect costs depend on the evolutionary origin and strength of selection of resistance.

Target-site resistance involves point mutations in the coding region of the gene, which are therefore subject to functional constraints. The mutations must lead to decreased fungicide-enzyme binding, resulting in potential trade-offs with the need to retain the original, often essential enzyme function (36). Penalties for enzyme function may be detected through their impact on the affected metabolic pathway: for example, altered sterol composition for azole-resistant *CYP51* mutants (11); increased osmotic sensitivity for dicarboximide resistance (85); or respiratory impairment or oxidative stress sensitivity for QoI or SDHI resistance (6, 16). However, functional constraints have been detected even in the case of metabolic resistance to antibiotics, where

Table 1 Overview of resistance mechanisms in key groups of systemic fungicides and selection of references discussed in this paper

Group name	Target site and code[a]	Target-site alterations	Target-site overexpression	Altered efflux pump activity	Metabolic circumvention	Metabolic detoxification
Methyl benzimidazole carbamates (MBCs)	β-tubulin assembly in mitosis (B1)	✓ (9, 19, 32)	✓ (105)	–	–	–
Succinate dehydrogenase inhibitors (SDHIs)	Complex II: succinate dehydrogenase (C2)	✓ (3, 54, 79)	–	✓ (106)	–	–
Quinone outside inhibitors (QoIs)	Complex III: Qo site of cytochrome bc1 (C3)	✓ (76, 97, 120)	–	✓ (106)	✓ (139)	✓ (65)
Anilinopyrimidines	Methionine biosynthesis (D1)	? (102)	–	✓ (102)	–	–
Phenylpyrroles	MAP/histidine kinase in osmotic signal transduction (E2)	✓ (48, 113)	–	✓ (77)	–	–
Dicarboximides	MAP/histidine kinase in osmotic signal transduction (E3)	✓ (48, 113)	–	✓ (77)	–	–
Demethylation inhibitors (DMIs) (SBI: class I)	C14 demethylase in sterol biosynthesis (G1)	✓ (23, 36, 75)	✓ (33, 35, 58)	✓ (77, 106, 142)	✓ (27)	✓ (74)
Amines or morpholines (SBI: class II)	Δ14-reductase and Δ8→Δ7-isomerase in sterol biosynthesis (G2)	–	–	–	–	–

[a]Group name, target site, and code based on FRAC Code List 2017 (**http://www.frac.info**).
Abbreviation: MAP, mitogen-activated protein.

resistance is directly linked to increased enzyme activity, due to trade-offs between enzyme activity and protein stability (137); stability trade-offs also apply to target-site mutations.

Some mutations can result in negative cross-resistance. Fitness penalties are generally defined as making the resistant strain less fit than the sensitive strains in the absence of a fungicide, whereas negative cross-resistance can be seen as a fitness penalty in the presence of a different fungicide. Negative cross-resistance is generally associated with target-site resistance, as the effects on fungicide binding may be compound-specific binding, whereas overexpression tends to produce positive cross-resistance across a mode of action. For example, the two *Z. tritici CYP51* mutations V136A and I381V each have opposite effects on sensitivity to the two azole fungicides prochloraz and tebuconazole (38), whereas a *CYP51* promoter insertion causes reduced sensitivity to all azoles (35).

Metabolic circumvention also results in functional trade-offs. Alternative metabolic pathways that bypass the inhibited catalytic step may enable survival but are generally less efficient than

the primary pathway that has evolved in the absence of fungicide selection. Co-option of alternative oxidase (AOX) as a terminal electron acceptor allows aerobic respiration to continue when cytochrome *b* is inhibited but with 2.5-fold lower ATP production per NADH (139). *ERG3* loss-of-function mutations avoid production of 14-methyl-3,6-diol under *CYP51* inhibition, resulting in the production of 14-methyl fecosterol, which is nontoxic but less optimal than ergosterol for fungal cell membrane function (66).

Resistance mechanisms involving overexpression, whether of the target site, efflux pumps, or detoxification enzymes, incur a resource allocation cost. Such allocation costs have previously been described in relation to pathogen resistance in host plants and associated yield penalties (134). Overexpression of a protein, at levels above those required in the absence of fungicide, requires the diversion of energy and nutrients from growth and reproduction. These costs are more applicable where simple repeats or transposon insertions in the promoter lead to constitutive upregulation. Costs are less for inducible upregulation, where the extra enzyme is produced only when needed (i.e., in the presence of the fungicide), but such changes to transcriptional regulation require more complex adaptations, which are less likely to evolve de novo. In the case of efflux, active transport also incurs a direct energetic cost, with ATP-binding cassette transporters hydrolyzing ATP directly and transporters belonging to the major facilitator superfamily (MFS) utilizing a transmembrane proton-motive force (42).

Constitutive overexpression may also result in functional trade-offs, with problems caused by excess protein and loss of regulation (138). Conversely, if a fungus is able to compensate for reduced enzyme activity of target-site mutants through upregulation (11), this shifts the trade-off to an allocation cost.

Where a de novo mutation spreads rapidly, resulting in a hard selective sweep, the associated alleles of linked genes increase in frequency and diversity in linked genes decreases (21). Fungicide selection has been shown to lead to reduced diversity in neutral genetic markers (133). Whether this can also result in fitness penalties and/or affect potential mechanisms for reversion of resistance in the population depends on whether any linked alleles are deleterious; whether linked diversity includes conditionally useful alleles, such as virulence on different host varieties or adaptation to different climates; and whether subsequently selected mutations (such as resistance to further fungicides) occur in a sensitive or resistant genetic background. Furthermore, the occurrence of fungicide resistance in a locally adapted genetic background may reduce its ability to spread to regions where that genetic background is maladapted, resulting instead in multiple independent origins in different regions (20).

DETECTING FITNESS COSTS

Evidence of fitness costs can range from field observations that resistance to a given class of fungicides has not emerged as quickly as might otherwise be expected to the elucidation of the precise biochemical basis of a deleterious effect of a resistance mutation. The different methods for experimentally testing a hypothesis of fitness penalties generally involve a compromise between adding inclusive fitness measurements and reducing confounding variables. On a scale from inclusive but variable to controlled but limited, experiments may use field populations, field isolates, laboratory mutants, or isogenic transformants; may be conducted in the field, in planta in a glasshouse or controlled environment, in vitro with fungal cultures, or in vitro with isolated organelles or enzymes; and may measure competition outcomes, inclusive fitness of single isolates, or individual fitness components.

In field populations, fitness penalties are sometimes invoked as the reason why resistance has not occurred in some species (60) or why some mutations generated in the laboratory do not

emerge in the field (59). However, emergence of new mutations depends on mutation rate, and the strength of positive selection is influenced by the resistance factors of different mutations. Furthermore, once the first highly resistant mutation emerges, there may be no further positive selection for alternative resistance mechanisms; thus, a single resistance allele is selected and alternative mutations generated in the laboratory do not evolve in the field population. The presence or absence of fitness penalties can also be inferred from whether resistance mutations decrease or persist between growing seasons (55) or once a fungicide class is withdrawn (132). However, reversions should be compared to a null model that includes genetic drift and may be due to indirect costs from linked genes or clonal replacement under selection by different fungicides and host plant varieties. Where resistance has emerged but not reached fixation, field trials can be conducted with natural inoculum, comparing treated and untreated plots or seasons (84). Field trials can control the host variety and use of other fungicides, but minor penalties may not have a significant effect within the experimental timescale.

The use of single isolates, alone or in defined mixtures, means inoculum can be controlled in common garden experiments, whether as inoculated field trials (56), in controlled in planta environments (5), or in vitro (26), but there are still (usually unknown) differences in genetic backgrounds between sensitive and resistant isolates. Where possible, multiple sensitive and resistant isolates from different genetic backgrounds should be used. If the relevant mutations can be generated spontaneously or with UV or chemical mutagenesis in laboratory mutants, they can be compared against the parental strain (51). This ensures similar genetic background, but other de novo mutations occur elsewhere in the genome, and extra in vitro subculturing steps may result in loss of pathogenicity. Two main approaches can be used for in vitro selection. Selecting single colonies, usually with a single round of mutagenesis and initial selection, will produce a set of strains that includes any viable mutations regardless of any nonlethal fitness penalties, which can then be further characterized or used in competition assays. Allowing mutants to grow in a mixed culture, potentially with multiple rounds of selection, results in intermutant competition, so the fittest mutants dominate (54).

Transformation of sensitive or resistant alleles into the same parental isolates provides the greatest consistency in genetic background. Heterologous expression allows functional testing of alleles from nontransformable and nonculturable fungi, and conditional expression vectors for yeast enable confirmation of lethal penalties. However, interspecific differences may affect the results: *Z. tritici* CYP51 with Y137F did not complement yeast CYP51 but has been found in *Z. tritici* in the field, so evidence of more subtle fitness penalties is less reliable (38). Where possible, homologous gene replacement is preferable to establish fitness effects of specific resistance mutations (78), but field isolates with higher relative fitness than strain-specific transformants may indicate the occurrence of compensatory mutations.

Field trials provide a measure of inclusive fitness over the timescale of the experiment, usually throughout a growing season and often over multiple years (84). However, conditions such as temperature cannot be controlled (143). Furthermore, field trials either rely on natural infection or are inoculated with field isolates with various genetic backgrounds, as resistant mutants or transformants cannot be released into the field. In planta testing in a glasshouse or controlled environment chamber allows more conditions to be controlled (5), and isogenic transformants or mutants may be used (117) while still taking account of pathogenicity, but these experiments do not always encompass the whole life cycle, e.g., overwintering states. In vitro testing allows growth conditions to be closely controlled and often allows a larger number of strains, replicates, or different conditions to be tested, but measurements are limited to certain fitness components such as hyphal growth or conidial production; pathogenicity or tolerance of host defenses cannot be measured; and rich growth media may compensate for some fitness penalties. Relative fitness

may depend on temperature (149), growth medium (14), and time of measurement (112) as well as on mechanism-specific factors such as osmotic or oxidative stress. Proof-of-concept studies may use extreme conditions to maximize penalties (78) rather than attempting to replicate field-realistic conditions. Isolated organelles (117) or enzymes (100) can indicate the mechanism of a fitness penalty but not the size of the penalty that would apply at the organismal level, and enzyme properties may differ in purified versus native conditions (100).

Single-isolate growth assays can generally detect only major reductions in vegetative growth or asexual spore production, whereas competition assays offer greater sensitivity for smaller fitness differences. A review of bacterial studies indicates that individual growth rate differences must be >5% per generation to be detectable, whereas competition assays can detect differences of 1% or even 0.1%, depending on the detection methods used to quantify genotype frequencies (4). In addition, for plant pathogens, different levels of aggressiveness may be optimal in single or mixed infections (143); thus, competition assays are more informative regarding the impact of pathogenicity changes on fitness where mixed infections are common in the field. Competition assays may use isolate pairs or more complex mixtures, with an equal starting ratio or a range of starting points (111).

Mutagenesis studies often refer to the stability of resistance as well as the fitness of mutants (97). This may reflect the basis of the trait, with reduced sensitivity through plasticity or epigenetic changes being unstable, whereas reduced sensitivity through genetic mutations is stable. A single mutant in pure culture is unlikely to spontaneously back-mutate to enable the resulting sensitive isolates to be selected even where resistance does carry penalties. A notable exception is mitochondrial mutations: if mutants are heteroplasmic, a mixed mitochondrial population is present within single-spore isolates, and fitness costs may result in a reduction in intracellular resistance frequency and declining resistance in the isolate overall (145), effectively producing a competition assay within a single heteroplasmic isolate.

FITNESS COSTS IN AZOLE RESISTANCE

Phenotypic Correlations

Experimental evolution and competition assays with the clinical pathogen *Candida albicans* demonstrated that fluconazole resistance was associated with fitness costs in some evolving lineages, but this was followed by compensatory evolution to reduce the fitness penalties while maintaining resistance (39). In vitro selection studies on plant pathogens have given mixed results. In *Cladosporium cucumerinum*, resistant mutants had reduced sporulation or spore viability, and in some rust species no resistant mutants were obtained (51). These results were taken as evidence of low resistance risk, but this has not been fully borne out in the field as azole resistance has emerged in many plant pathogens, albeit more slowly and with lower resistance factors than for MBC resistance (52). In contrast, tebuconazole-selected laboratory mutants of *Fusarium graminearum* with azole-specific resistance had normal in vitro growth over four temperatures, with no decrease in sporulation, germination, or in planta virulence; mycotoxin production varied between mutants but was not correlated with resistance phenotype (12). Azole-resistant mutants of *Aspergillus parasiticus* displayed lower in planta mycotoxin production, but no consistent reduction in pathogenicity or in vitro fitness components (44).

For field isolates, azole-resistant isolates of *Venturia inaequalis* showed no reduction in in vitro growth or sporulation (26), and resistant isolates of *Phomopsis obscurans* (119) and *Alternaria alternata* (96) showed no significant differences in growth, sporulation, or pathogenicity. In contrast, resistant postharvest isolates of *Penicillium expansum* had reduced mycelial growth, mycotoxin

production, and pathogenicity (70). In *Sclerotinia homoeocarpa*, resistant isolates showed no significant difference in in vitro growth rate but some reduction in pathogenicity (63).

In *Cercospora beticola*, single-isolate in planta inoculation and re-isolation were carried out in the absence of fungicide selection, resulting in some reductions in isolate resistance levels after cycling at the lowest temperature of −20°C but resistance was maintained under all other conditions (5). In mixed inoculation in planta assays of *Blumeria graminis* (2), the average EC50 of re-isolated populations decreased, leading to the conclusion that in the absence of fungicide treatment, the resistant isolates were less fit and outcompeted by the sensitive isolates. For the eyespot pathogens *Oculimacula acuformis* and *Oculimacula yallundae*, prochloraz resistance persisted in untreated plots of inoculated field trials over multiple years (84). In the wider field population, prochloraz resistance emerged but later declined again as other fungicides took over (107). Field surveys of *Z. tritici* in Oregon reported reductions in propiconazole resistance frequency over the winter period when no fungicides were applied (55).

Target-Site Functional Constraints

The target site of azole fungicides is CYP51. Basidiomycetes and yeasts possess a single *CYP51* ortholog, apart from a few cases of recent genome duplications in basidiomycetes. In filamentous ascomycetes, a gene duplication produced paralogs *CYP51A* and *CYP51B*, but some lineages have subsequently lost *CYP51A*. In species retaining both paralogs, mutations more commonly emerge in *CYP51A*, suggesting that the constitutively expressed workhorse *CYP51B* is subject to tighter functional constraints than the additional, inducible *CYP51A* (36).

The most widely reported CYP51 alteration is Y137F, with the equivalent substitution reported in rusts, yeasts, and CYP51B of ascomycetes (36). Other mutations are more variable between species (36), but some of the same mutations have been reported in related species, such as *Z. tritici* and *Pseudocercospora fijiensis* (23).

In *Z. tritici*, heterologous expression studies have elucidated some of the functional constraints at play, including lethal mutations, compensatory mutations, and epistatic interactions. I381V as a sole alteration results in loss of enzyme function when heterologously expressed in yeast, but function is restored if I381V occurs in combination with Y461H (38). S524T is also found only in the field in combination with other alterations, but S524T as a sole alteration does not disrupt enzyme function; thus, in this case the mutation combinations found in the field reflect historical factors, namely the introduction over time of different azoles with incomplete cross-resistance (37). The equivalent substitution S508T has since been reported as a sole mutation in *Pyrenopeziza brassicae* (24).

The yeast conditional expression system was able to demonstrate mutations causing complete loss of enzyme function, but other mutations may result in more subtle reductions in fitness. In *Monilinia fructicola*, Y136F laboratory mutants had slower in vitro growth and produced smaller fruit lesions than sensitive strains (30); azole resistance in *M. fructicola* in the field is mainly associated with *CYP51* overexpression (90), but a substitution equivalent to *Z. tritici* G460S has recently been reported in Brazil (86). Field isolates of *Phakopsora pachyrhizi* with CYP51 substitutions F120L + Y131H, Y131F + K142R, or Y131F + I475T all decreased in frequency in competition with wild-type isolates on untreated detached leaves (75). In contrast, Y136H laboratory mutants of the rice false smut pathogen *Villosiclava virens* showed no significant reduction in in vitro growth or sporulation (135).

Moderately resistant field isolates of *Cercospora beticola* with CYP51 substitution I330T showed growth rates significantly lower than the sequenced wild-type isolate but similar to the slowest-growing sensitive isolate (104). Another study of resistant field isolates reported significantly

lower virulence and sporulation but similar germination and hyphal growth compared to sensitive isolates, whereas competition experiment outcomes varied by isolate pair (71). This demonstrates the importance of reference isolate choice and variability within sensitivity groups. Similarly, in *Pyrenophora teres*, significant differences in fitness were detected within populations, but these were not correlated with fungicide resistance (108). Resistance mechanisms were not elucidated for these US and German *P. teres* isolates, but a later study of Australian isolates (94) found CYP51A substitution F489L as well as inducible *CYP51A* overexpression.

Clinical *A. fumigatus* isolates with CYP51A substitutions G54W or M220K were found to be competitive with wild-type strains in vitro and in vivo (128), whereas *A. parasiticus* UV mutants with CYP51A-G54W had reduced spore production, spore germination, and in planta aflatoxin production (44).

A further fitness trade-off in *CYP51* mutations can be found in cases of negative cross-resistance between different azoles. In *Z. tritici*, I381V confers reduced sensitivity to tebuconazole but increased sensitivity to prochloraz, with V136A conferring the inverse effect (36). However, field isolates with both alterations are now common, and other resistance mechanisms such as *CYP51* and/or *MgMFS1* efflux pump overexpression confer positive cross-resistance across all azoles (35, 106). In *O. acuformis*, prochloraz-resistant isolates showed positive cross-resistance, but one group showed negative cross-resistance to triflumizole, whereas another group showed no significant difference in triflumizole sensitivity (82). However, more commonly, cross-resistance between azoles is positive in sign but varying in magnitude (36).

Altered Sterol Composition

Reduced CYP51 efficiency may be evident from altered sterol composition. In *Ustilago maydis*, alterations in the sterol composition of azole-resistant laboratory mutants implied reduced CYP51 enzyme activity (67), but this did not result in significantly impaired growth, indicating that plasticity in sterol metabolism is a potential compensatory mechanism for impaired CYP51 function. In *Erysiphe necator*, sterol composition was similar in triadimenol-resistant and triadimenol-sensitive field isolates (40), with triadimenol-resistant isolates subsequently found to have the target-site substitution Y136F (43). In *Z. tritici*, sterol composition and *CYP51* expression were measured and CYP51 efficiency calculated for isolates with various *CYP51* haplotypes (11). In that study, Y137F, as well as V136A and I381V, was associated with reduced CYP51 enzyme efficiency, whereas no significant effects were detected for L50S, S188N, A379G, Δ459/460, G460D, Y461H, or N513K, or for interactions between mutations within the combinations studied. Variation in *CYP51* expression appeared to provide another possible compensatory mechanism for reduced enzyme efficiency even in *Z. tritici*, which has only the *CYP51B* paralog.

Altered sterol metabolism as a resistance mechanism has been reported in clinical *C. albicans* strains, with sterol-C5-desaturase (ERG3) loss-of-function mutants producing less toxic alternative sterols when CYP51 is inhibited (27). The altered sterol content may lead to increased sensitivity to osmotic stress, or to substrates usually removed by efflux, because of altered membrane permeability (87). However, this resistance mechanism has not yet been identified in any plant pathogens.

CYP51 Overexpression

Where overexpression evolves de novo through a simple promoter insertion or tandem repeat, this commonly results in constitutive overexpression. In contrast, in species with lower intrinsic sensitivity due to the presence of the *CYP51A* paralog, *CYP51A* upregulation is inducible. This

reduces the allocation costs of overexpression because the extra enzyme is produced only when needed, but inducible upregulation is a more complex trait to evolve unless it is already present as a standing variation as in *Rhynchosporium commune* (58).

In *C. beticola*, azole-resistant field isolates with constitutive *CYP51* overexpression showed no significant difference in growth rate compared to the sensitive reference isolate (104). Similarly, in *M. fructicola*, *CYP51* overexpression due to the Mona promoter insert was not associated with any significant differences in mycelial growth, sporulation, or pathogenicity, as confirmed through insertion/deletion transformants (33). In *A. parasiticus* laboratory mutants overexpressing *CYP51A*, spore production and germination were not significantly different, whereas aflatoxin production was higher in vitro but lower in planta (44). In *ERG11*(*CYP51*)-overexpressing clinical *C. albicans* isolates, no fitness cost was apparent in vitro but pathogenicity was reduced, possibly reflecting the different requirements for regulation of sterol biosynthesis in hyphal versus yeast-like growth (88); therefore, fitness testing in plant pathogens should also consider different stages in their life cycles.

Non-Target-Site Resistance

The impacts of efflux-mediated multidrug resistance have been most extensively studied in *Botrytis cinerea*. Field monitoring of MDR3 strains, with a combination of *atrB* and *mfsM2* efflux pump overexpression, showed that following positive selection by fungicide use in summer, there was no consistent decrease over winter when no fungicide sprays were applied (77). However, subsequent analysis of longer-term field trial data found significant fitness penalties associated with MDR1 (*atrB* overexpression) and some evidence of penalties for MDR2 (*mfsM2* overexpression) based on decreasing resistance frequencies over the winter months (133). MDR1h isolates, with higher *atrB* overexpression than MDR1, in a genetic background that also has multiple target-site resistance mutations were characterized in vitro, revealing increased osmotic sensitivity compared to sensitive isolates but no other detectable reduction in mycelial growth or spore production (47). A subsequent study compared MDR1h isolates and sensitive isolates with isolates with target-site mutations but without MDR1h (31). MDR1h was associated with reduced growth at the lowest temperature tested but similar growth at higher temperatures; similar oxidative stress tolerance; and similar aggressiveness and sporulation in planta. The reduced osmotic stress tolerance relative to sensitive isolates was associated with (one or more of) the target-site mutations rather than MDR1h, demonstrating the importance of comparing strains with as similar genetic backgrounds as possible.

Tebuconazole-selected mutants of *F. graminearum* with cross-resistance to azoles and morpholines had reduced growth at higher temperatures and lower virulence on untreated plants than both the parental strain and mutants with azole-specific resistance (12). Flusilazole-selected mutants of *A. parasiticus* with *mdr* overexpression had reduced growth, sporulation, and spore germination, as well as a loss of aflatoxin production (44), with cyprodinil-selected MDR mutants showing similar phenotypes (99). In contrast, propiconazole-resistant *M. fructicola* field isolates with non-target-site resistance (no *CYP51* mutations or overexpression) displayed no fitness penalties (growth/sporulation/virulence) (29). Furthermore, *Z. tritici* isolates with reduced cyproconazole sensitivity but no *CYP51* mutations had higher virulence than sensitive isolates, possibly due to efflux pumps being effective against defensive toxins produced by the host plant as well as against fungicides (142). Next-generation sequencing approaches are revealing more non-target-site resistance alleles, including efflux transporters and other resistance mechanisms. In *Z. tritici*, the *PKS1* quantitative trait locus is associated with increased melanization, correlated with reduced azole sensitivity but slower growth (81). Metabolization of prochloraz was shown in a resistant

Fusarium fujikuroi isolate (73). The degradation product showed that hydrolysis of the amine moiety of prochloraz was likely, but no fitness cost associated with this mechanism was reported.

FITNESS COSTS IN RESISTANCE TO OTHER FUNGICIDES

Methyl Benzimidazole Carbamates

MBC-resistant isolates show normal growth and pathogenicity in many pathogen species, including *Oculimacula* sp. (19), *B. cinerea* (62), *Colletotrichum musae* with β-tubulin substitution F200Y (131), and *M. fructicola* with E198A (32). Virulence was higher in *P. expansum* isolates with β-tubulin alterations F167Y or E198A/V/K (9) and in MBC-resistant isolates of *Pseudocercosporella fijiensis* (114) and *Didymella bryoniae* (72).

However, some fitness penalties due to target-site functional trade-offs are temperature dependent. Microtubule function requires both assembly and disassembly, so hyperstability leads to loss of function at some temperatures (105). Temperature sensitivity has been identified in some laboratory mutations that have not been found in the field as well as in some field isolates (59). In *Saccharomyces cerevisiae*, six laboratory mutants were heat sensitive and four were cold sensitive, including F167Y (123); in *Aspergillus nidulans*, four mutants were heat sensitive (68). *M. fructicola* H6Y field isolates were cold sensitive and E198A isolates were heat sensitive (93), whereas resistant isolates of *P. obscurans* (119) and *F. graminearum* (28) showed no penalties over a range of temperatures.

Some species display no temperature sensitivity in the absence of fungicide, but resistance itself is temperature dependent. Resistant *Fusarium moniliforme* (141), *C. beticola* (125), and E198V *B. cinerea* (144) isolates are sensitive at low temperatures, and *Monilinia laxa* L240F isolates are sensitive at high temperatures (92). This reduces the selective advantage of the mutation in the presence of fungicide but does not lead to reversion in its absence. In contrast, the heat-sensitivity of *A. nidulans* lab mutants is reversed by MBCs, as they inhibit hyperstable microtubule binding (105). Compensatory mutations have also been generated in *A. nidulans* lab mutants, including mutations in α-tubulin to restore the correct binding affinity with β-tubulin (105).

Mutations at codon 198 confer negative cross-resistance to diethofencarb, but F200Y confers resistance to both MBCs and diethofencarb. In *B. cinerea*, E198A persisted after withdrawal of MBCs but F200Y declined, possibly due to fitness penalties; the negative cross-resistance of one mutation plus the fitness penalty of the other could in theory be combined in a resistance management strategy, but diethofencarb is no longer authorized (132). *B. cinerea* E198K isolates are cross-resistant to zoxamide, whereas M233I mutants are resistant only to zoxamide and have fitness penalties (22). The authors conclude that zoxamide use without MBCs is unlikely to lead to resistance, as zoxamide-specific resistance carries penalties, but selection for zoxamide resistance and against fitness penalties could also select for E198K even in the absence of MBCs.

Overexpression of β-tubulin in a yeast model was toxic, producing the wrong ratios of tubulins for correct microtubule formation (138). β-tubulin overexpression is not known in field isolates, except in intrinsically resistant *Colletotrichum acutatum*, in which upregulation is inducible (103). One study also generated moderately resistant mutants with an unknown non-target-site mechanism, with fitness penalties (148).

Quinone Outside Inhibitors

The highest fitness cost is associated with G143A in species with an intron following codon 143, as the mutation prevents proper splicing and is therefore lethal. Yeast mutagenesis experiments have produced compensatory mutations (127), but in the field, pathogen species with the intron predominantly evolve F129L or G137R instead (120). In *B. cinerea*, some isolates have the intron

but others do not, resulting in the selection of G143A in backgrounds without the intron (83); in this case, lack of intron is a cryptic standing variant and not a de novo compensatory mutation.

Other penalties result from functional trade-offs at the protein level. Of 22 cytochrome *b* alterations reported in eukaryotes as of 1996, nine were associated with respiration deficiency or impaired protein stability, but F129L, G137R, and G143A were not (16). A site-directed mutagenesis study in yeast, using site-directed mutagenesis to mimic the surrounding residues of different fungal species, found G143A to be slightly deleterious in *V. inaequalis*, *Podosphaera fuliginea*, and *Phytophthora megasperma*, but not in *B. graminis*, backgrounds (49). *V. inaequalis* G143A mutants were also unstable when maintained on fungicide-free media (145), but G143A has subsequently emerged in *V. inaequalis* field isolates (76).

Testing of fitness components has demonstrated penalties in a few cases, with reduced virulence in *Magnaporthe oryzae* (91) and *Z. tritici* (57) field isolates with G143A and reduced growth and pathogenicity in some *C. beticola* lab mutants with G143S or F129V (97) and in some resistant mutants of *U. maydis* (147). However, in many cases, resistant isolates showed normal growth, sporulation, and pathogenicity and/or competitiveness, for example in *Plasmopara viticola* field isolates (41), *B. graminis* field isolates (34), and *Magnaporthe grisea* mutants (8) with G143A; in *P. pachyrhizi* isolates with F129L (75); and in unsequenced QoI-resistant isolates of *E. necator* (111) and *A. alternata* (69). In *B. cinerea*, laboratory mutants were less competitive in vitro, but resistant field isolates showed no reduction in pathogenicity, sporulation, or growth under a range of conditions (130).

Reports of non-target-site resistance to QoIs are limited. *M. fructicola* isolates with QoI resistance but no cytochrome *b* mutations had reduced growth and sporulation (32). Alternative respiration, utilizing AOX as the terminal electron acceptor, gives lower ATP yields; this reduced efficiency, as well as possible sensitivity of AOX to plant flavones, means this mechanism probably has limited impact in planta (139). The only report of metabolization of QoIs has been for kresoxim-methyl in *V. inaequalis* (65). An external fungal esterase produced in planta was shown to hydrolyze the ester moiety in the toxophore of kresoxim-methyl.

Succinate Dehydrogenase Inhibitors

Several mutagenesis studies that included fitness measurements were carried out before SDHI resistance emerged in the field. *A. alternata* lab mutants with target-site mutations resulting in sdhB-S221P, B-H267N, B-H267Y, or D-D129E had no apparent reduction in hyphal growth, except under oxidative stress (6). Field isolates with sdhD-D123E showed reduced sporulation and increased oxidative sensitivity, but there were no differences for other fitness components or other mutations (B-H277Y/R, C-H134R, and D-H133R) (45), whereas no reports of significant difference in hyphal growth or spore viability for *A. alternata* or *Alternaria solani* field isolates with sdhD-D123E, sdhB-H277/8 Y/R, sdhC-H134R, or sdhD-H133R were reported, including for genetic backgrounds with QoI resistance mutations G143A or F129L (79).

There was also no apparent loss of pathogenicity in *Corynespora cassiicola* or *D. bryoniae* field isolates (7) or in *Ramularia collo-cygni* (109) or *P. expansum* (98) mutants. However, *Rhizoctonia solani* mutants had reduced pathogenicity and sclerotia production (7). *Rhizoctonia cerealis* mutants showed fitness variations depending on the parental isolates' genetic background, but it is not clear whether this is related to resistance or other mutations (121).

In *B. cinerea*, results varied between studies for lab mutants (7) and field isolates. One study found no evidence of major fitness penalties in growth and sporulation in vitro or in planta, sclerotia production, or osmotic sensitivity for sdhB-H272R, sdhB-H272Y, sdhB-H272L, sdhB-P225F, and sdhB-N230I (3), but another study found that H272R/Y/L, N230I, and P225F isolates were outcompeted by sensitive strains in in planta competition assays, and most resistant

isolates showed reductions in fitness components in planta and in vitro; H272R was less impaired than other resistant strains but was more sensitive to oxidative stress (130). Interestingly, isolates in the second study had G143A, but because all were field isolates, there would have been other genetic differences too. In a study of isogenic transformants (78), H267L/R caused slower growth at high temperatures on some media but grew faster at low temperatures on a minimal medium. P225L/H272R had significantly reduced sclerotial production, and other mutants had reduced sclerotial survival at higher temperatures. Organismal fitness components were not correlated with differences in sdhB enzyme activity in isolated mitochondria (78). This further demonstrates that observed differences in fitness depend on which fitness components are measured and which growth conditions are used, even when correcting for genetic background, and makes the application of measured fitness differences to field conditions more complex.

Similarly, a range of SDHI-resistant laboratory mutants of *Z. tritici* showed no obvious loss of organism-level fitness or pathogenicity (50), even where mitochondrial respiration rates were markedly reduced (117). However, experimental evolution revealed dose-dependent selection, implying fitness penalties in sdhC-H152R mutants (54); this is supported by the as yet limited spread of H152R but has not been confirmed by competition assays with isogenic transformants.

Some *sdhB* mutations confer no or negative cross-resistance to fluopyram, but other mutations, especially in *sdhC*, are highly resistant to all current SDHIs (50). Some MDR efflux pumps (see section titled Non-Target-Site Resistance) also affect SDHI sensitivity. More recently, an unknown SDHI-specific non-target-site resistance mechanism, most likely not associated with altered efflux pump activity, was reported to be present as a standing genetic variant in *Z. tritici* field populations (140).

Other Fungicide Classes

For phenylpyrrole-dicarboximide double-resistant mutants carrying modifications in the osmosensing class III histidine kinase, multiple fitness parameters, including increased osmotic sensitivity, decreased sporulation, and loss of pathogenicity, are often affected (48). Therefore, the resistance mechanisms in field strains may involve loss of function, or loss-of-binding mutations with deleterious effects on function, of a component in this signaling pathway, resulting in fitness penalties due to functional trade-offs. Field strains with low levels of resistance to phenylpyrrole and dicarboximides are often associated with MDR (85) and do not always carry fitness penalties (31). Experimental evolution from fludioxonil-resistant mutants of *A. nidulans* demonstrated the possibility of compensatory evolution, with evolved lines restoring or exceeding the original in vitro fitness of the sensitive parent without loss of resistance (118), and this might also explain the recent report on the emergence of *B. cinerea* field strains carrying mutations in the class III HK gene in China (113).

For anilinopyrimidines, fitness penalties have been proposed as a reason for the slower spread of resistance compared to other fungicides in *B. cinerea* (132), but results from testing fitness components of resistant isolates have proven inconsistent in *B. cinerea* (46) and other species (70). More recently, a mitochondrial target site has been proposed for cyprodinil, with a mitochondrial inner membrane transporter and a NADH kinase as potential candidates (102).

Hydroxyanilide-resistant field isolates of *M. laxa* showed normal mycelial growth but reduced sporulation and virulence (95). In *B. cinerea*, field frequency of fenhexamid resistance fluctuated over ten years of selection (14). Resistant field isolates had lower growth and survival rates in culture (116), and laboratory mutants had reduced sporulation and virulence (146). Isogenic transformants with *ERG27* target-site mutations had reductions in various fitness components, depending on temperature and nutrient levels (14).

Few studies have been carried out for other fungicide groups. Phospholipid-biosynthesis inhibitor (dithiolane/phosphorothiolate)-resistant isolates of *M. oryzae* had no reduction in sporulation (64) and increased virulence even on untreated plants (74).

Melanin biosynthesis inhibitor-dehydrase (MBI-D) resistance frequencies declined in *M. oryzae* populations after withdrawal of those fungicides (122). *Alternaria alternata* isolates with lower sensitivity to mancozeb showed increased aggressiveness, but all fitness components were variable within sensitivity groups (96). Dodine-resistant field isolates of *V. inaequalis* showed no significant difference in in vitro growth or sporulation (26). Resistance to phenylamide oomyceticides showed no decline in frequency in populations of *Phytophthora erythroseptica* (25) in untreated field trials. In contrast, resistance to carboxylic-acid amide (CAA) oomyceticides was accompanied by reduced growth and pathogenicity in laboratory mutants of *Phytophthora litchi* (136).

PRACTICAL IMPLICATIONS

Knowledge of fitness penalties can improve the accuracy of resistance risk assessments (61). Not considering fitness penalties leads to overestimates of risk: For example, mechanisms found in laboratory mutants but with prohibitive fitness costs may be of little practical importance (53). However, overestimates of the practical significance of fitness penalties leads to underestimates of risk if, for example, costs are expressed only at temperatures rarely occurring in the field or if compensatory mutations subsequently emerge. Where costs depend on genetic background, resistance risk may be higher for outcrossing pathogens as resistance alleles can be recombined into the most favorable background (18).

It is also important not to assume a reduction in risk where some lab mutants have fitness penalties, if other, equally or more resistant mutants do not, but this knowledge will improve predictions of which resistance mechanisms are likely to emerge, helping to know in advance what to look for, informing monitoring programs and the design of molecular diagnostics.

Resistance management is also affected by fitness penalties. The effectiveness of many resistance management guidelines depends on the fitness of resistant isolates during breaks (temporal or spatial) in spraying (17). For strategies based on temporal gaps between sprays, on alternating different modes of action, or on spatial mosaics or refugia, fitness costs lead to reversions of resistance (1), whereas mutations that are neutral in the absence of fungicide selection persist, and selection of resistance is simply stretched out in a zero-sum manner. Fitness penalties also change the relationship between fungicide dose and resistance selection. In the absence of fitness penalties, the mutation is neutral below the minimum inhibitory concentration of sensitive strains, and positive selection increases with dose throughout the dose range in which sensitive pathogens are controlled and resistant strains are not. In contrast, fitness penalties lead to a specific tipping point at which a resistance mutation changes from deleterious to advantageous, as positive selection for resistance outweighs selection against the fitness penalties.

However, major complications arise when fitness penalties depend on life stage or environmental conditions (14), as models must combine different selection coefficients, weighted for prevalence and adjusted for conditions in different regions. For example, in Northern Europe, *B. cinerea* is actively growing during the summer months and sclerotia are produced for overwinter survival in low temperatures, whereas in Southern Europe, especially in glasshouses, growth occurs during the winter, and sclerotia or saprophytic mycelium must survive in high summer temperatures (112). However, comparisons of resistance emergence between climate zones are confounded by effects of climate on disease levels and corresponding differences in fungicide use, and therefore regional differences in resistance levels cannot be related directly to different fitness costs of resistance under different climatic conditions.

FUTURE PROSPECTS

For recent fungicide groups, mutagenesis studies have been carried out prior to release, with the awareness that fitness penalties and resistance levels of mutants influence resistance risk. For SDHIs, mutations generated by in vitro selection (117) and evidence of differential fitness of mutants (54) can be compared to the emerging situation in the field. For the novel oomyceticide oxathiapiprolin, fitness components (growth, sporulation, germination, and pathogenicity) of mutants were measured as part of the resistance risk assessment, although results varied in different genetic backgrounds within as well as between *Phytophthora* species (15). However, there is not yet a consensus on a standard method of fitness testing to use as part of resistance risk assessments.

Many studies provide initial hints at fitness penalties, but these are often inconsistent between studies. Such inconsistencies may reflect genuine differences between pathogen species, or they may be an artifact of the measurement of different fitness components, the use of different growth conditions, or intraspecific variation in genetic background. Increasing use of functional genetics tools, such as homologous recombination with site-directed mutagenesis, allows mutations to be assessed within common genetic backgrounds within species, and genome editing may allow multiple mutations to be combined. Whole-genome sequencing of UV mutants or field isolates could provide insights into other contributing or compensatory mutations. Molecular diagnostics enable higher-resolution tracking of allele frequencies in the field, for example throughout the growing season and winter season (10), and more precise detection of competition experiment outcomes (4).

In terms of the development of future crop protection, penalties for resistance are clearly desirable but are not something that can readily be incorporated into the discovery process. Gene drives could be used to improve the relative inclusive fitness of sensitive alleles, but there is a risk of resistance to gene drives (126) or of resistance mutations occurring de novo within the gene drive cassette. As with antibiotics, the notion of evolution-proof products may not be achievable in practice but can at least lead to insights regarding the most durable deployment strategies for existing options (13).

Resistance management strategies also stand to benefit from conceptual and modeling advances regarding fitness coefficients and fitness landscapes. However, empirical results so far indicate that many penalties are condition dependent, depending on temperature, host genetics, or pathogen life stage. The impact of fitness penalties on selection for resistance will also change with spatial and temporal variations in fungicide use and dose. An outstanding question is whether these shifting alternative landscapes could be combined into a single, field-realistic weighted average or whether it would be too dependent upon local or seasonal conditions.

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LITERATURE CITED

1. Allen RC, Engelstädter J, Bonhoeffer S, McDonald BA, Hall AR. 2017. Reversing resistance: different routes and common themes across pathogens. *Proc. R. Soc. B* 284:20171619

2. Almughrabi KI, Gray AB. 1995. Competition between triadimefon-sensitive and triadimefon-resistant isolates of *Erysiphe graminis* f.sp. *tritici*. *Plant Dis.* 79:709–12
3. Amiri A, Heath SM, Peres NA. 2014. Resistance to fluopyram, fluxapyroxad, and penthiopyrad in *Botrytis cinerea* from strawberry. *Plant Dis*. 98:532–39
4. Andersson DI, Hughes D. 2010. Antibiotic resistance and its cost: Is it possible to reverse resistance? *Nat. Rev. Microbiol.* 8:260–71
5. Arabiat S, Khan MFR, Bolton M, Secor G. 2017. Stability of tetraconazole-resistant isolates of *Cercospora beticola* after exposure to different temperature and time treatments. *J. Plant Pathol.* 99:177–84
6. Avenot H, Sellam A, Michailides T. 2009. Characterization of mutations in the membrane-anchored subunits AaSDHC and AaSDHD of succinate dehydrogenase from *Alternaria alternata* isolates conferring field resistance to the fungicide boscalid. *Plant Pathol*. 58:1134–43
7. Avenot HF, Michailides TJ. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot*. 29:643–51
8. Avila-Adame C, Köller W. 2003. Characterization of spontaneous mutants of *Magnaporthe grisea* expressing stable resistance to the Qo-inhibiting fungicide azoxystrobin. *Curr. Genet.* 42:332–38
9. Baraldi E, Mari M, Chierici E, Pondrelli M, Bertolini P, Pratella GC. 2003. Studies on thiabendazole resistance of *Penicillium expansum* of pears: pathogenic fitness and genetic characterization. *Plant Pathol*. 52:362–70
10. Barres B, Micoud A, Corio-Costet MF, Debieu D, Fillinger S, et al. 2016. Trends and challenges in pesticide resistance detection. *Trends Plant Sci*. 21:834–53
11. Bean TP, Cools HJ, Lucas JA, Hawkins ND, Ward JL, et al. 2009. Sterol content analysis suggests altered eburicol 14α-demethylase (*CYP51*) activity in isolates of *Mycosphaerella graminicola* adapted to azole fungicides. *FEMS Microbiol. Lett.* 296:266–73
12. Becher R, Hettwer U, Karlovsky P, Deising HB, Wirsel SGR. 2010. Adaptation of *Fusarium graminearum* to tebuconazole yielded descendants diverging for levels of fitness, fungicide resistance, virulence and mycotoxin production. *Phytopathology* 100:444–53
13. Bell G, MacLean C. 2018. The search for 'evolution-proof' antibiotics. *Trends Microbiol*. 26:471–83
14. Billard A, Fillinger S, Leroux P, Lachaise H, Beffa R, Debieu D. 2012. Strong resistance to the fungicide fenhexamid entails a fitness cost in *Botrytis cinerea*, as shown by comparisons of isogenic strains. *Pest Manag. Sci.* 68:684–91
15. Bittner RJ, Sweigard JA, Mila AL. 2017. Assessing the resistance potential of *Phytophthora nicotianae*, the causal agent of black shank of tobacco, to oxathiopropalin with laboratory mutants. *Crop Prot*. 102:63–71
16. Brasseur G, Saribas AS, Daldal F. 1996. A compilation of mutations located in the cytochrome *b* subunit of the bacterial and mitochondrial bc_1 complex. *Biochim. Biophys. Acta* 1275:61–69
17. Brent KJ, Hollomon DW. 2007. *Fungicide Resistance in Crop Pathogens: How Can it be Managed?* Brussels, Belg.: FRAC
18. Brent KJ, Hollomon DW. 2007. *Fungicide Resistance: The Assessment of Risk*. Brussels, Belg.: FRAC
19. Brown MC, Taylor GS, Epton HAS. 1984. Carbendazim resistance in the eyespot pathogen *Pseudocercosporella herpotrichoides*. *Plant Pathol*. 33:101–11
20. Brunner PC, Stefanato FL, McDonald BA. 2008. Evolution of the *CYP51* gene in *Mycosphaerella graminicola*: evidence for intragenic recombination and selective replacement. *Mol. Plant Pathol.* 9:305–16
21. Brunner PC, Torriani SFF, Croll D, Stukenbrock EH, McDonald BA. 2014. Hitchhiking selection is driving intron gain in a pathogenic fungus. *Mol. Biol. Evol.* 31:1741–49
22. Cai M, Lin D, Chen L, Bi Y, Xiao L, Liu XL. 2015. M233I mutation in the β-tubulin of *Botrytis cinerea* confers resistance to zoxamide. *Sci. Rep.* 5:13
23. Canas-Gutierrez GP, Angarita-Velasquez MJ, Restrepo-Florez JM, Rodriguez P, Moreno CX, Arango R. 2009. Analysis of the *CYP51* gene and encoded protein in propiconazole-resistant isolates of *Mycosphaerella fijiensis*. *Pest Manag. Sci*. 65:892–99
24. Carter HE, Fraaije BA, West JS, Kelly SL, Mehl A, et al. 2014. Alterations in the predicted regulatory and coding regions of the sterol 14α-demethylase gene (*CYP51*) confer decreased azole sensitivity in the oilseed rape pathogen *Pyrenopeziza brassicae*. *Mol. Plant Pathol*. 15:513–22

25. Chapara V, Taylor RJ, Pasche JS, Gudmestad NC. 2011. Competitive parasitic fitness of mefenoxam-sensitive and -resistant isolates of *Phytophthora erythroseptica* under fungicide selection pressure. *Plant Dis*. 95:691–96
26. Chapman KS, Sundin GW, Beckerman JL. 2011. Identification of resistance to multiple fungicides in field populations of *Venturia inaequalis. Plant Dis*. 95:921–26
27. Chau AS, Gurnani M, Hawkinson R, Laverdiere M, Cacciapuoti A, McNicholas PM. 2005. Inactivation of sterol Δ5,6-desaturase attenuates virulence in *Candida albicans*. *Antimicrob. Agents Chemother*. 49:3646–51
28. Chen C, Wang J, Luo Q, Yuan S, Zhou M. 2007. Characterization and fitness of carbendazim-resistant strains of *Fusarium graminearum* (wheat scab). *Pest Manag. Sci.* 63:1201–7
29. Chen F, Liu X, Schnabel G. 2013. Field strains of *Monilinia fructicola* resistant to both MBC and DMI fungicides isolated from stone fruit orchards in the eastern United States. *Plant Dis*. 97:1063–68
30. Chen FP, Fan JR, Zhou T, Liu XL, Liu JL, Schnabel G. 2012. Baseline sensitivity of *Monilinia fructicola* from China to the DMI fungicide SYP-Z048 and analysis of DMI-resistant mutants. *Plant Dis*. 96:416–22
31. Chen SN, Luo CX, Hu MJ, Schnabel G. 2016. Fitness and competitive ability of *Botrytis cinerea* isolates with resistance to multiple chemical classes of fungicides. *Phytopathology* 106:997–1005
32. Chen SN, Shang Y, Wang Y, Schnabel G, Lin Y, et al. 2014. Sensitivity of *Monilinia fructicola* from peach farms in China to four fungicides and characterization of isolates resistant to carbendazim and azoxystrobin. *Plant Dis*. 98:1555–60
33. Chen SN, Yuan NN, Schnabel G, Luo CX. 2017. Function of the genetic element 'Mona' associated with fungicide resistance in *Monilinia fructicola*. *Mol. Plant Pathol*. 18:90–97
34. Chin KM, Chavaillaz D, Kaesbohrer M, Staub T, Felsenstein FG. 2001. Characterizing resistance risk of *Erysiphe graminis* f.sp. *tritici* to strobilurins. *Crop Prot*. 20:87–96
35. Cools HJ, Bayon C, Atkins S, Lucas JA, Fraaije BA. 2012. Overexpression of the sterol 14α-demethylase gene (*MgCYP51*) in *Mycosphaerella graminicola* isolates confers a novel azole fungicide sensitivity phenotype. *Pest Manag. Sci.* 68:1034–40
36. Cools HJ, Hawkins NJ, Fraaije BA. 2013. Constraints on the evolution of azole resistance in plant pathogenic fungi. *Plant Pathol*. 62:36–42
37. Cools HJ, Mullins JGL, Fraaije BA, Parker JE, Kelly DE, et al. 2011. Impact of recently emerged sterol 14α-demethylase (CYP51) variants of *Mycosphaerella graminicola* on azole fungicide sensitivity. *Appl. Environ. Microbiol*. 77:3830–37
38. Cools HJ, Parker JE, Kelly DE, Lucas JA, Fraaije BA, Kelly SL. 2010. Heterologous expression of mutated eburicol 14α-demethylase (CYP51) proteins of *Mycosphaerella graminicola* to assess effects on azole fungicide sensitivity and intrinsic protein function. *Appl. Environ. Microbiol.* 76:2866–72
39. Cowen LE, Kohn LM, Anderson JB. 2001. Divergence in fitness and evolution of drug resistance in experimental populations of *Candida albicans*. *J. Bacteriol*. 183:2971–78
40. Debieu D, Coriocostet MF, Steva H, Malosse C, Leroux P. 1995. Sterol composition of the vine powdery mildew fungus, *Uncinula necator*: comparison of triadimenol-sensitive and resistant strains. *Phytochemistry* 39:293–300
41. Delmas CEL, Dussert Y, Delière L, Couture C, Mazet ID, et al. 2017. Soft selective sweeps in fungicide resistance evolution: recurrent mutations without fitness costs in grapevine downy mildew. *Mol. Ecol.* 26:1936–51
42. Del Sorbo G, Schoonbeek HJ, De Waard MA. 2000. Fungal transporters involved in efflux of natural toxic compounds and fungicides. *Fungal Genet. Biol.* 30:1–15
43. Délye C, Laigret F, Corio-Costet MF. 1997. A mutation in the 14α-demethylase gene of *Uncinula necator* that correlates with resistance to a sterol biosynthesis inhibitor. *Appl. Environ. Microbiol.* 63:2966–70
44. Doukas EG, Markoglou AN, Vontas JG, Ziogas BN. 2012. Effect of DMI-resistance mechanisms on cross-resistance patterns, fitness parameters and aflatoxin production in *Aspergillus parasiticus* Speare. *Fungal Genet. Biol.* 49:792–801
45. Fan Z, Yang JH, Fan F, Luo CX, Schnabel G. 2015. Fitness and competitive ability of *Alternaria alternata* field isolates with resistance to SDHI, QoI and MBC fungicides. *Plant Dis*. 99:1744–50
46. Fernández-Ortuño D, Chen FP, Schnabel G. 2013. Resistance to cyprodinil and lack of fludioxonil resistance in *Botrytis cinerea* isolates from strawberry in North and South Carolina. *Plant Dis*. 97:81–85

47. Fernández-Ortuño D, Grabke A, Li X, Schnabel G. 2014. Independent emergence of resistance to seven chemical classes of fungicides in *Botrytis cinerea*. *Phytopathology* 105:424–32
48. Fillinger S, Ajouz S, Nicot P, Leroux P, Bardin M. 2012. Functional and structural comparison of pyrrolnitrin- and iprodione-induced modifications in the class III histidine-kinase Bos1 of *Botrytis cinerea*. *PLOS ONE* 7(8):e52520
49. Fisher N, Brown AC, Sexton G, Cook A, Windass J, Meunier B. 2004. Modeling the Q(o) site of crop pathogens in *Saccharomyces cerevisiae* cytochrome *b*. *Eur. J. Biochem*. 271:2264–71
50. Fraaije BA, Bayon C, Atkins S, Cools HJ, Lucas JA, Fraaije MW. 2012. Risk assessment studies on succinate dehydrogenase inhibitors, the new weapons in the battle to control Septoria leaf blotch in wheat. *Mol. Plant Pathol.* 13:263–75
51. Fuchs A, Drandarevski CA. 1976. The likelihood of development of resistance to systemic fungicides which inhibit ergosterol biosynthesis. *Neth. J. Plant Pathol.* 82:85–87
52. Fungic. Resist. Action Comm. 2017. List of plant pathogenic organisms resistant to disease control agents. *Fungicide Resistance Action Committee*. **http://www.frac.info/publications/downloads**
53. Grimmer MK, van den Bosch F, Powers SJ, Paveley ND. 2014. Evaluation of a matrix to calculate fungicide resistance risk. *Pest Manag. Sci.* 70:1008–16
54. Gutiérrez-Alonso O, Hawkins NJ, Cools HJ, Shaw MW, Fraaije BA. 2017. Dose-dependent selection drives lineage replacement during the experimental evolution of SDHI fungicide resistance in *Zymoseptoria tritici*. *Evol. Appl.* 10:1055–66
55. Hagerty CH, Anderson NP, Mundt CC. 2016. Temporal dynamics and spatial variation of azoxystrobin and propiconazole resistance in *Zymoseptoria tritici*: a hierarchical survey of commercial winter wheat fields in the Willamette Valley, Oregon. *Phytopathology* 107:345–52
56. Hagerty CH, Graebner RC, Sackett KE, Mundt CC. 2017. Variable competitive effects of fungicide resistance in field experiments with a plant pathogenic fungus. *Ecol. Appl.* 27:1305–16
57. Hagerty CH, Mundt CC. 2016. Reduced virulence of azoxystrobin-resistant *Zymoseptoria tritici* populations in greenhouse assays. *Phytopathology* 106:884–89
58. Hawkins NJ, Cools HJ, Sierotzki H, Shaw MW, Knogge W, et al. 2014. Paralog re-emergence: a novel, historically contingent mechanism in the evolution of antimicrobial resistance. *Mol. Biol. Evol.* 31:1793–802
59. Hawkins NJ, Fraaije BA. 2016. Predicting resistance by mutagenesis: lessons from 45 years of MBC resistance. *Front. Microbiol.* 7:1814
60. Hellin P, Scauflaire J, Van Hese V, Munaut F, Legreve A. 2017. Sensitivity of *Fusarium culmorum* to triazoles: impact of trichothecene chemotypes, oxidative stress response and genetic diversity. *Pest Manag. Sci.* 73:1244–52
61. Hollomon DW. 2015. Fungicide resistance: facing the challenge. *Plant Prot. Sci.* 51:170–76
62. Hsiang T, Chastagner GA. 1990. Parasitic fitness of benzimidazole and dicarboximide resistant isolates of *Botrytis cinerea*, *B. elliptica* and *B. tulipae*. *Phytopathology* 80:978
63. Hsiang T, Yang L, Barton W. 1998. Relative virulence of isolates of *Sclerotinia homoeocarpa* with varying sensitivity to propiconazole. *Eur. J. Plant Pathol.* 104:163–69
64. Hu MJ, Ma QY, Li KB, Lin Y, Luo CX. 2014. Exploring mechanism of resistance to isoprothiolane in *Magnaporthe oryzae*, the causal agent of rice blast. *J. Plant Pathol.* 96:249–59
65. Jabs T, Cronshaw K, Freund A. 2001. New strobilurin resistance mechanism in apple scab (*Venturia inaequalis*). *Phytomedizin* 31:15–16
66. Jackson CJ, Lamb DC, Manning NJ, Kelly DE, Kelly SL. 2003. Mutations in *Saccharomyces cerevisiae* sterol C5-desaturase conferring resistance to the CYP51 inhibitor fluconazole. *Biochem. Biophys. Res. Commun.* 309:999–1004
67. Joseph-Horne T, Hollomon D, Loeffler RST, Kelly SL. 1995. Altered P450 activity associated with direct selection for fungal azole resistance. *FEBS Lett*. 374:174–78
68. Jung MK, May GS, Oakley BR. 1998. Mitosis in wild-type and β-tubulin mutant strains of *Aspergillus nidulans*. *Fungal Genet. Biol*. 24:146–60
69. Karaoglanidis GS, Luo Y, Michailides TJ. 2011. Competitive ability and fitness of *Alternaria alternata* isolates resistant to QoI fungicides. *Plant Dis*. 95:178–82

70. Karaoglanidis GS, Markoglou AN, Bardas GA, Doukas EG, Konstantinou S, Kalampokis JF. 2011. Sensitivity of *Penicillium expansum* field isolates to tebuconazole, iprodione, fludioxonil and cyprodinil and characterization of fitness parameters and patulin production. *Int. J. Food Microbiol.* 145:195–204
71. Karaoglanidis GS, Thanassoulopoulos CC, Ioannidis PM. 2001. Fitness of *Cercospora beticola* field isolates – resistant and – sensitive to demethylation inhibitor fungicides. *Eur. J. Plant Pathol.* 107:337–47
72. Keinath AP, Zitter TA. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. *Plant Dis.* 82:479–84
73. Kim SH, Park MR, Kim YC, Lee SW, Choi BR, et al. 2010. Degradation of prochloraz by rice Bakanae disease pathogen *Fusarium fujikuroi* with differing sensitivity: a possible explanation for resistance mechanism. *J. Korean Soc. Appl. Biol. Chem.* 53:433–39
74. Kim YS, Kim KD. 2009. Evidence of a potential adaptation of *Magnaporthe oryzae* for increased phosphorothiolate-fungicide resistance on rice. *Crop Prot.* 28:940–46
75. Klosowski AC, Brahm L, Stammler G, De Mio LLM. 2016. Competitive fitness of *Phakopsora pachyrhizi* isolates with mutations in the *CYP51* and *CYTB* genes. *Phytopathology* 106:1278–84
76. Köller W, Parker DM, Turechek WW, Avila-Adame C, Cronshaw K. 2004. A two-phase resistance response of *Venturia inaequalis* populations to the QoI fungicides kresoxim-methyl and trifloxystrobin. *Plant Dis.* 88:537–44
77. Kretschmer M, Leroch M, Mosbach A, Walker A-S, Fillinger S, et al. 2009. Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLOS Pathog.* 5(12):e1000696
78. Lalève A, Fillinger S, Walker AS. 2014. Fitness measurement reveals contrasting costs in homologous recombinant mutants of *Botrytis cinerea* resistant to succinate dehydrogenase inhibitors. *Fungal Genet. Biol.* 67:24–36
79. Landschoot S, Carrette J, Vandecasteele M, De Baets B, Höfte M, et al. 2017. Boscalid-resistance in *Alternaria alternata* and *Alternaria solani* populations: an emerging problem in Europe. *Crop Prot.* 92:49–59
80. Leadbeater A. 2011. The impact of the European regulations on the management of crop diseases. In *Modern Fungicides and Antifungal Compounds VI*, ed. HW Dehne, HB Deising, U Gisi, KH Kuck, PE Russell, H Lyr, pp. 1–11. Braunschweig, Ger.: DPG Spectrum Phytomedizin
81. Lendenmann MH, Croll D, McDonald BA. 2015. QTL mapping of fungicide sensitivity reveals novel genes and pleiotropy with melanization in the pathogen *Zymoseptoria tritici*. *Fungal Genet. Biol.* 80:53–67
82. Leroux P, Chapeland F, Arnold A, Gredt M. 2000. New cases of negative cross-resistance between fungicides, including sterol biosynthesis inhibitors. *J. Gen. Plant Pathol.* 66:75–81
83. Leroux P, Gredt M, Leroch M, Walker A-S. 2010. Exploring mechanisms of resistance to respiratory inhibitors in field strains of *Botrytis cinerea*, the causal agent of gray mold. *Appl. Environ. Microbiol.* 76:6615–30
84. Leroux P, Gredt M, Remuson F, Micoud A, Walker A-S. 2013. Fungicide resistance status in French populations of the wheat eyespot fungi *Oculimacula acuformis* and *Oculimacula yallundae*. *Pest Manag. Sci.* 69:15–26
85. Leroux P, Walker A-S. 2013. Activity of fungicides and modulators of membrane drug transporters in field strains of *Botrytis cinerea* displaying multidrug resistance. *Eur. J. Plant Pathol.* 135:683–93
86. Lichtemberg PSF, Luo Y, Morales RG, Muehlmann-Fischer JM, Michailides TJ, De Mio LLM. 2017. The point mutation G461S in the *MfCYP51* gene is associated with tebuconazole resistance in *Monilinia fructicola* populations in Brazil. *Phytopathology* 107:1507–14
87. Löffler J, Einsele H, Hebart H, Schumacher U, Hrastnik C, Daum G. 2000. Phospholipid and sterol analysis of plasma membranes of azole-resistant *Candida albicans* strains. *FEMS Microbiol. Lett.* 185:59–63
88. Lohberger A, Coste AT, Sanglard D. 2014. Distinct roles of *Candida albicans* drug resistance transcription factors TAC1, MRR1, and UPC2 in virulence. *Eukaryot. Cell* 13:127–42
89. Lucas JA, Hawkins NJ, Fraaije BA. 2015. The evolution of fungicide resistance. *Adv. Appl. Microbiol.* 90:29–92
90. Luo CX, Schnabel G. 2008. The cytochrome p450 lanosterol 14α-demethylase gene is a demethylation inhibitor fungicide resistance determinant in *Monilinia fructicola* field isolates from Georgia. *Appl. Environ. Microbiol.* 74:359–66

91. Ma B, Uddin W. 2009. Fitness and competitive ability of an azoxystrobin-resistant G143A mutant of *Magnaporthe oryzae* from perennial ryegrass. *Plant Dis.* 93:1044–49
92. Ma Z, Yoshimura MA, Holtz BA, Michailides TJ. 2005. Characterization and PCR-based detection of benzimidazole-resistant isolates of *Monilinia laxa* in California. *Pest Manag. Sci.* 61:449–57
93. Ma Z, Yoshimura MA, Michailides TJ. 2003. Identification and characterization of benzimidazole resistance in *Monilinia fructicola* from stone fruit orchards in California. *Appl. Environ. Microbiol.* 69:7145–52
94. Mair WJ, Deng W, Mullins JGL, West S, Wang P, et al. 2016. Demethylase inhibitor fungicide resistance in *Pyrenophora teres* f. sp. *teres* associated with target site modification and inducible overexpression of Cyp51. *Front. Microbiol.* 7:1279
95. Malandrakis A, Koukiasas N, Veloukas T, Karaoglanidis G, Markoglou A. 2013. Baseline sensitivity of *Monilinia laxa* from Greece to fenhexamid and analysis of fenhexamid-resistant mutants. *Crop Prot.* 46:13–17
96. Malandrakis AA, Apostolidou ZA, Markoglou A, Flouri F. 2015. Fitness and cross-resistance of *Alternaria alternata* field isolates with specific or multiple resistance to single site inhibitors and mancozeb. *Eur. J. Plant Pathol.* 142:489–99
97. Malandrakis AA, Markoglou AN, Nikou DC, Vontas JG, Ziogas BN. 2006. Biological and molecular characterization of laboratory mutants of *Cercospora beticola* resistant to Qo inhibitors. *Eur. J. Plant Pathol.* 116:155–66
98. Malandrakis AA, Vattis KN, Markoglou AN, Karaoglanidis GS. 2017. Characterization of boscalid-resistance conferring mutations in the SdhB subunit of respiratory complex II and impact on fitness and mycotoxin production in *Penicillium expansum* laboratory strains. *Pestic. Biochem. Physiol.* 138:97–103
99. Markoglou AN, Doukas EG, Malandrakis AA. 2011. Effect of anilinopyrimidine resistance on aflatoxin production and fitness parameters in *Aspergillus parasiticus* Speare. *Int. J. Food Microbiol.* 146:130–36
100. Meini M-R, Tomatis PE, Weinreich DM, Vila AJ. 2015. Quantitative description of a protein fitness landscape based on molecular features. *Mol. Biol. Evol.* 32:1774–87
101. Mira PM, Meza JC, Nandipati A, Barlow M. 2015. Adaptive landscapes of resistance genes change as antibiotic concentrations change. *Mol. Biol. Evol.* 32:2707–15
102. Mosbach A, Edel D, Farmer AD, Widdison S, Barchietto T, et al. 2017. Anilinopyrimidine resistance in *Botrytis cinerea* is linked to mitochondrial function. *Front. Microbiol.* 8:2361
103. Nakaune R, Nakano M. 2007. Benomyl resistance of *Colletotrichum acutatum* is caused by enhanced expression of β-tubulin 1 gene regulated by putative leucine zipper protein CaBEN1. *Fungal Genet. Biol.* 44:1324–35
104. Nikou D, Malandrakis A, Konstantakaki M, Vontas J, Markoglou A, Ziogas B. 2009. Molecular characterization and detection of overexpressed C-14α-demethylase-based DMI resistance in *Cercospora beticola* field isolates. *Pestic. Biochem. Physiol.* 95:18–27
105. Oakley BR, Morris NR. 1981. A β-tubulin mutation in *Aspergillus nidulans* that blocks microtubule function without blocking assembly. *Cell* 24:837–45
106. Omrane S, Sghyer H, Audeon C, Lanen C, Duplaix C, Walker AS. 2015. Fungicide efflux and the MgMFS1 transporter contribute to the MDR phenotype in *Zymoseptoria tritici* field isolates. *Environ. Microbiol.* 17:2805–23
107. Parnell S, Gilligan CA, Lucas JA, Bock CH, van den Bosch F. 2008. Changes in fungicide sensitivity and relative species abundance in *Oculimacula yallundae* and *O. acuformis* populations (eyespot disease of cereals) in Western Europe. *Plant Pathol.* 57:509–17
108. Peever TL, Milgroom MG. 1994. Lack of correlation between fitness and resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. *Phytopathology* 84:515–19
109. Piotrowska MJ, Fountaine JM, Ennos RA, Kaczmarek M, Burnett FJ. 2017. Characterisation of *Ramularia collo-cygni* laboratory mutants resistant to succinate dehydrogenase inhibitors. *Pest Manag. Sci.* 73:1187–96
110. Poelwijk FJ, Kiviet DJ, Weinreich DM, Tans SJ. 2007. Empirical fitness landscapes reveal accessible evolutionary paths. *Nature* 445:383–86
111. Rallos LEE, Johnson NG, Schmale DG, Prussin AJ, Baudoin AB. 2014. Fitness of *Erysiphe necator* with G143A-based resistance to quinone outside inhibitors. *Plant Dis.* 98:1494–502

112. Raposo R, Gomez V, Urrutia T, Melgarejo P. 2000. Fitness of *Botrytis cinerea* associated with dicarboximide resistance. *Phytopathology* 90:1246–49
113. Ren W, Shao W, Han X, Zhou M, Chen C. 2016. Molecular and biochemical characterization of laboratory and field mutants of *Botrytis cinerea* resistant to fludioxonil. *Plant Dis*. 100:1414–23
114. Romero RA, Sutton TB. 1998. Characterization of benomyl resistance in *Mycosphaerella fijiensis*, cause of black sigatoka of banana, in Costa Rica. *Plant Dis*. 82:931–34
115. Russell PE. 2005. Century review: a century of fungicide evolution. *J. Agric. Sci.* 143:11–25
116. Saito S, Cadle-Davidson L, Wilcox WF. 2013. Selection, fitness, and control of grape isolates of *Botrytis cinerea* variably sensitive to fenhexamid. *Plant Dis*. 98:233–40
117. Scalliet G, Bowler J, Luksch T, Kirchhofer-Allan L, Steinhauer D, et al. 2012. Mutagenesis and functional studies with succinate dehydrogenase inhibitors in the wheat pathogen *Mycosphaerella graminicola*. *PLOS ONE* 7:e35429
118. Schoustra SE, Debets AJM, Slakhorst M, Hoekstra RF. 2006. Reducing the cost of resistance; experimental evolution in the filamentous fungus *Aspergillus nidulans*. *J. Evol. Biol*. 19:1115–27
119. Shi HJ, Wu HM, Zhang CQ, Shen X. 2013. Monitoring and characterization of resistance development of strawberry Phomopsis leaf blight to fungicides. *Eur. J. Plant Pathol.* 135:655–60
120. Sierotzki H, Frey R, Wullschleger J, Palermo S, Karlin S, et al. 2007. Cytochrome *b* gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. *Pest Manag. Sci.* 63:225–33
121. Sun H-Y, Lu C-Q, Li W, Deng Y-Y, Chen H-G. 2017. Homozygous and heterozygous point mutations in succinate dehydrogenase subunits b, c and d of *Rhizoctonia cerealis* conferring resistance to thifluzamide. *Pest Manag. Sci*. 73:896–903
122. Suzuki F, Yamaguchi J, Koba A, Nakajima T, Arai M. 2010. Changes in fungicide resistance frequency and population structure of *Pyricularia oryzae* after discontinuance of MBI-D fungicides. *Plant Dis*. 94:329–34
123. Thomas JH, Neff NF, Botstein D. 1985. Isolation and characterization of mutations in the β-tubulin gene of *Saccharomyces cerevisiae*. *Genetics* 111:715–34
124. Torriani SFF, Brunner PC, McDonald BA, Sierotzki H. 2009. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. *Pest Manag. Sci*. 65:155–62
125. Trkulja N, Ivanović Ž, Pfaf-Dolovac E, Dolovac N, Mitrović M, et al. 2013. Characterisation of benzimidazole resistance of *Cercospora beticola* in Serbia using PCR-based detection of resistance-associated mutations of the β-tubulin gene. *Eur. J. Plant Pathol.* 135:889–902
126. Unckless RL, Clark AG, Messer PW. 2017. Evolution of resistance against CRISPR/Cas9 gene drive. *Genetics* 205:827–41
127. Vallieres C, Trouillard M, Dujardin G, Meunier B. 2011. Deleterious effect of the Q(o) inhibitor compound resistance-conferring mutation G143A in the intron-containing cytochrome *b* gene and mechanisms for bypassing it. *Appl. Environ. Microbiol.* 77:2088–93
128. Valsecchi I, Mellado E, Beau R, Raj S, Latge JP. 2015. Fitness studies of azole-resistant strains of *Aspergillus fumigatus*. *Antimicrob. Agents Chemother*. 59:7866–69
129. van den Bosch F, Oliver R, van den Berg F, Paveley N. 2014. Governing principles can guide fungicide-resistance management tactics. *Annu. Rev. Phytopathol.* 52:175–95
130. Veloukas T, Kalogeropoulou P, Markoglou AN, Karaoglanidis GS. 2014. Fitness and competitive ability of *Botrytis cinerea* field isolates with dual resistance to SDHI and QoI fungicides, associated with several sdhB and the cytb G143A mutations. *Phytopathology* 104:347–56
131. Vieira WAD, Lima WG, Nascimento ES, Michereff SJ, Reis A. 2017. Thiophanate-methyl resistance and fitness components of *Colletotrichum musae* isolates from banana in Brazil. *Plant Dis*. 101:1659–65
132. Walker A-S, Micoud A, Rémuson F, Grosman J, Gredt M, Leroux P. 2013. French vineyards provide information that opens ways for effective resistance management of *Botrytis cinerea* (grey mould). *Pest Manag. Sci.* 69:667–78
133. Walker A-S, Ravigne V, Rieux A, Ali S, Carpentier F, Fournier E. 2017. Fungal adaptation to contemporary fungicide applications: the case of *Botrytis cinerea* populations from Champagne vineyards (France). *Mol. Ecol.* 26:1919–35
134. Walters DR, Boyle C. 2005. Induced resistance and allocation costs: What is the impact of pathogen challenge? *Physiol. Mol. Plant Pathol.* 66:40–44

135. Wang F, Lin Y, Yin WX, Peng YL, Schnabel G, et al. 2015. The Y137H mutation of *VvCYP51* gene confers the reduced sensitivity to tebuconazole in *Villosiclava virens*. *Sci. Rep*. 5:13
136. Wang HC, Sun HY, Stammler G, Ma JX, Zhou MG. 2010. Generation and characterization of isolates of *Peronophythora litchii* resistant to carboxylic acid amide fungicides. *Phytopathology* 100:522–27
137. Wang X, Minasov G, Shoichet BK. 2002. Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs. *J. Mol. Biol.* 320:85–95
138. Weinstein B, Solomon F. 1990. Phenotypic consequences of tubulin overproduction in *Saccharomyces cerevisiae*: differences between α-tubulin and β-tubulin. *Mol. Cell. Biol.* 10:5295–304
139. Wood PM, Hollomon DW. 2003. A critical evaluation of the role of alternative oxidase in the performance of strobilurin and related fungicides acting at the Q(o) site of Complex III. *Pest Manag. Sci.* 59:499–511
140. Yamashita M, Fraaije BA. 2018. Non-target site SDHI resistance is present as standing genetic variation in field populations of *Zymoseptoria tritici*. *Pest Manag. Sci*. 74:672–81
141. Yan K, Dickman MB. 1996. Isolation of a β-tubulin gene from *Fusarium moniliforme* that confers cold-sensitive benomyl resistance. *Appl. Environ. Microbiol.* 62:3053–60
142. Yang L, Gao F, Shang L, Zhan J, McDonald BA. 2013. Association between virulence and triazole tolerance in the phytopathogenic fungus *Mycosphaerella graminicola*. *PLOS ONE* 8:e59568
143. Zhan J, McDonald BA. 2013. Experimental measures of pathogen competition and relative fitness. *Annu. Rev. Phytopathol.* 51:131–53
144. Zhang CQ, Liu YH, Zhu GN. 2010. Detection and characterization of benzimidazole resistance of *Botrytis cinerea* in greenhouse vegetables. *Eur. J. Plant Pathol.* 126:509–15
145. Zheng DS, Olaya G, Koller W. 2000. Characterization of laboratory mutants of *Venturia inaequalis* resistant to the strobilurin-related fungicide kresoxim-methyl. *Curr. Genet.* 38:148–55
146. Ziogas BN, Markoglou AN, Malandrakis AA. 2003. Studies on the inherent resistance risk to fenhexamid in *Botrytis cinerea*. *Eur. J. Plant Pathol*. 109:311–17
147. Ziogas BN, Markoglou AN, Tzima A. 2002. A non-Mendelian inheritance of resistance to strobilurin fungicides in *Ustilago maydis*. *Pest Manag. Sci*. 58:908–16
148. Ziogas BN, Nikou D, Markoglou AN, Malandrakis AA, Vontas J. 2009. Identification of a novel point mutation in the β-tubulin gene of *Botrytis cinerea* and detection of benzimidazole resistance by a diagnostic PCR-RFLP assay. *Eur. J. Plant Pathol.* 125:97–107
149. Zou G, Ying SH, Shen ZC, Feng MG. 2006. Multi-sited mutations of β-tubulin are involved in benzimidazole resistance and thermotolerance of fungal biocontrol agent *Beauveria bassiana*. *Environ. Microbiol*. 8:2096–105
150. zur Wiesch PA, Kouyos R, Engelstädter J, Regoes RR, Bonhoeffer S. 2011. Population biological principles of drug-resistance evolution in infectious diseases. *Lancet Infect. Dis.* 11:236–47