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Evolutionary context of fungicide resistance: A comprehensive review

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Abstract
Plants are attacked by numerous phytopathogenic fungi. Synthetic fungicides have been used to manage plant diseases for a long time. Despite the fact that synthetic fungicides are quite effective, frequent use has resulted in issues such environmental contamination, the emergence of resistance, and residual toxicity. The sequential emergence of variant genotypes of these pathogens with decreased sensitivity to methyl benzimidazole carbamates, demethylation inhibitors, quinone outside inhibitors, and succinate dehydrogenase inhibitors-the most potent single-site fungicides-illustrates an ongoing evolutionary process in response to the introduction and use of various chemical classes. Although it is now possible to detect and track the prevalence of resistance in field populations more quickly and precisely thanks to analysis of the molecular mechanisms and genetic basis of resistance, it is still difficult to forecast when or where resistance will arise. It is examined to what extent pathogen comparison, laboratory mutagenesis investigations, fitness assessments and the reconstruction of evolutionary routes can increase the predictability of resistance evolution. Currently, risk models are being improved to incorporate new characteristics related to the pace of pathogen evolution, which are based on the life cycles of fungi, the characteristics of fungicides, and exposure to fungicides. To extend the useful life of fungicides and ensure their continued use in crop protection, comprehensive resistance management based on solid scientific data is essential.

Keywords: Fungicide resistance, resistance mechanism, resistance risk, single-site inhibitors

Introduction
A key component of contemporary agriculture's intensification has been the routine application of fungicides to manage crop diseases. This has increased crop yields, improved crop quality, and ensured production stability. A variety of powerful pesticides that are active at low dosages and offer great levels of disease control are available to farmers and producers (Russell, 2005) [32].

Since fungicides have been used in agriculture for well over a century, there haven't been any instances of their losing their effectiveness in the field. There is no mention of resistance in Horsfall (1945) [20], a thorough early treatise on fungicides and their activity. The initial cases were first noted in the 1960s and involved dodine in *Venturia inaequalis*, a fungus that causes apple scab and reduced sensitivity to aromatic hydrocarbons in *Penicillium* species that cause citrus storage rots (Brent, 2012) [5]. The adaptation to organomercurial fungicides by some strains of *Pyrenophora avenae*, the oat-borne pathogen, was unexpected (Noble *et al*. 1966) [28]. However, until the 1970s, when new classes of antifungal agents with particular modes of action were produced and widely employed, verified cases of fungicide resistance remained uncommon (Brent, 2012) [5]. Since then, an extensive spectrum of plant pathogenic fungus have reported an increasing number of instances. According to Urech *et al*. (1997) [34], product stewardship and use in practice are directly impacted by resistance, which has become a reality of life for the crop protection sector.

There is a sizable and growing body of research on fungicide resistance that covers various fungal diseases and crops, differing fungicide modes of action and methods for managing resistance. Several of these topics are covered in a recent book on fungicide resistance in crop protection with case studies from several nations (Thind, 2012) [33].

Through this review, we investigate how resistance evolves over time and whether this research might help us anticipate future issues and enhance risk assessment. Finally, we go over how this mechanical and evolutionary information might be used to effectively control resistance.

Fungicidal resistance

In contrast to earlier fungicide classes, which acted on a variety of cellular processes and were referred to as multisite inhibitors, modern selective fungicides disrupt particular cellular processes and bind to specific protein targets. As a result, they are referred to as single-site (site-specific). Insensitivity to single-site fungicides can develop from a change in a single target protein, but numerous modifications are thought to be necessary for multisite fungicides. Additionally, extremely active and frequently systemic (taken up and dispersed in plant tissues), single-site fungicides provide effective disease control at very low dose rates. Because most members of the pathogen population are either eliminated or prevented from finishing their life cycle after fungicide application, there is strong selection for any resistant individuals. Numerous plant pathogenic fungus produce enormous amounts of propagules (often spores) that can be transported over great areas because of their quick generation durations and rapid reproduction rates. Due to the high fungicide efficacy and vast size of the pathogen population, even uncommon mutations that affect the sensitivity to the fungicide will be selected, survive, and spread if there isn't a significant fitness penalty to the change. These several characteristics, including the fungicide mode of action and therapeutic usage, high efficacy, pathogen biology, and epidemiology, make single-site fungicides vulnerable to the emergence of resistance.

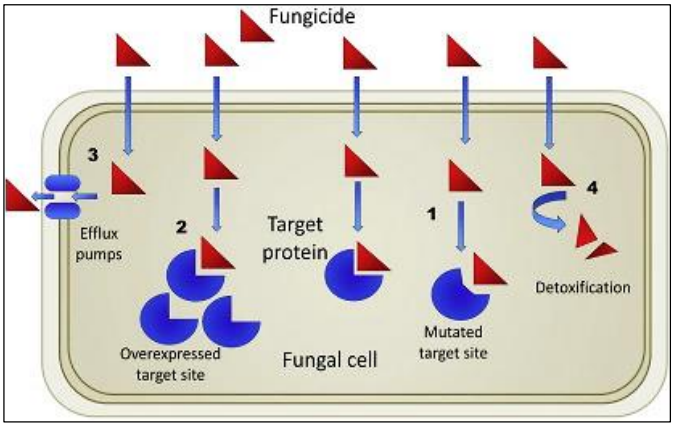
The presence of heritable heterogeneity in the pathogen population's susceptibility to the fungicide is the initial prerequisite for the development of resistance (Georgopoulos and Skylakakis, 1986) [11]. The type of fungicide and the genetic resistance determinant(s) affect the rate and pattern of resistance emergence. With single-site inhibitors, which can bestow a high level of resistance with a single target protein mutation, a qualitative shift occurs, giving rise to two separate populations with a bimodal sensitivity distribution. A unimodal distribution is seen with multisite inhibitors or some single-site drugs where more than one gene or allele contributes to resistance. There is directional selection for reduced sensitivity in both cases, but in the first one it is working on discrete variation rather than the continuous distribution seen in the second, which is characterized by incremental shifts towards resistance over time.

Another distinction that needs to be made is between acquired resistance, which forms in response to selection caused by exposure to the fungicide, and inherent resistance, which some fungal species have to specific classes of fungicides. The basis of intrinsic resistance, which occurs when some fungal taxa are inherently resistant to a particular chemical class and fungicides as a result have a particular range of activity, was very little understood until relatively recently. For the strobilurin (quinone outside inhibitors, QoI) fungicides, which are themselves naturally occurring by specific basidiomycete fungi, one possible explanation that has been proven is molecular polymorphism in the target site. Alternatively, extra copies of the encoding gene may result in redundancy in the protein target.

Resistance mechanisms to single-site inhibitors

Understanding the mechanisms resulting in decreased sensitivity to the chemical and the genetic underpinnings of the resistance trait are crucial to comprehend the evolution of fungicide resistance in field populations of pathogens. Numerous reasons influencing lower sensitivity have been suggested by studies using a variety of plant pathogenic fungi and numerous types of single-site inhibitors. Work on drug resistance in model species including yeast (Fisher and Meunier, 2005) [9] and clinically significant fungi like *Candida* spp. and *Aspergillus* (Camps *et al*., 2012; Cowen *et al*., 2000) [6, 7] has provided more evidence.

Acquired resistance to fungicides has been associated with four primary pathways. For numerous single-site fungicides, including the MBCs, azoles, QoIs, and SDHIs, it has been proven that target protein alteration brought on by mutations in the encoding gene has occurred. A typical mechanism in clinically significant fungi like Candida is the efflux of the fungicide due to the activity of ABC or other transporters, which has been described in various plant pathogens (Hiller *et al.* 2006) [15]. Although it has been confirmed in a few instances (Ma and Michailides, 2005) [24], overexpression of the target due to upregulation of the encoding gene does not seem to be a common mechanism in plant pathogens. In cases of weeds and insects developing resistance to herbicides and insecticides, respectively, degradation of the pesticide to detoxification by metabolic enzymes such as cytochrome P450s or glutathione transferase is a frequently reported scenario (Powles and Yu, 2010; Puinean *et al*., 2010) [30, 31]. However, it does not appear to be common in cases of resistance to fungicides, with only one report on the degradation of the QoI fungicide.



***Source*:** Lucas *et al*. 2015 [23].

Fig 1: Mechanisms of resistance to single-site fungicides

1. Alteration of the target protein prevents fungicide binding (target-site resistance).
2. Overexpression of target protein increases concentration of fungicide necessary for inhibition.
3. Efflux pumps expel fungicide from cell.
4. Degradation of fungicide by metabolic enzymes.

Resistance risk estimation

Three different reasons, including the fungicide mode of action and use, high efficacy, and pathogen biology and epidemiology, contribute to the vulnerability of single-site fungicides to the development of resistance. In order to quantify the total risk of resistance developing in various

pathogens and industrial systems to various chemical classes, these factors have been utilized in combination (Brent and Hollomon, 2007) [4]. Such risk models can offer a basic direction and are originally based on real-world experience with various fungicides and pathogens, but they are unable to foresee where or when resistance will arise or how quickly it might spread to impair disease control (Lucas, 2011) [22]. The selection coefficient (the difference in fitness between the resistant and the sensitive strain due to application of the fungicide (Bosch *et al.* 2014) [3], as well as other factors influencing the survival and invasion of resistant strains (Gubbins and Gilligan, 1999) [14], would need to be measured with extreme precision in order to achieve this. While the system had useful predictive power for the broad risk categories (low, moderate and high) for type of fungicide, pathogen, and agronomic system, it had limited predictive value within the currently dominant single-site fungicide group. This conclusion was reached after a recent analysis of such risk matrix fungicide x pathogen assessment schemes, in which the time from the introduction of a fungicide to the first emergence of resistance was compared. Now, 61 recorded occurrences of fungicide resistance and candidate features that may be related to the rate of evolution to resistance have been compared by the same authors, leading to the development of a new scheme (Grimmer *et al.* 2014) [12]. This risk assessment identified some key characteristics that are significant determinants of resistance risk, such as the number of crop species infected by the pathogen (narrow versus wide host range, with less intensive fungicide selection acting on the latter), pathogen latent periods per year (a measure of duration of the disease epidemic divided by the time from infection to pathogen reproduction), protected versus open field production system with the confined environment all but guarant. In a model combining these essential characteristics, 61 per cent of the variation in the number of years before the onset of single-site fungicide resistance was explained. Additionally, it is suggested that these trait-based resistance risk assessments could be used to forecast the prospective resistance risk status of fungicides with novel modes of action even in the absence of prior understanding of their behavior in actual use.

Depending on how a pathogen reacts to new fungicide classes or changes in fungicide use, pathogen risk levels may alter over time. Overall, it has been believed that the rust fungi are not particularly likely to become resistant to other fungicide classes, however this belief has recently come under scrutiny (Oliver, 2014) [29]. The danger of selection for resistance evolution is lower for agricultural and horticultural usage and higher for veterinary and medical ones. The use of some fruit and seed treatments, as well as applications for wood preservation, were acknowledged to carry some danger, although the far lower exposure levels in fungicide-sprayed crops would only have a little effect on the selection of fungi in the soil or crop residues (Gisi 2014) [12].

Evaluation of management strategies

Reduced directional selection for resistance in the pathogen population is the goal of all resistance management measures. Practically speaking, the difficulty is to do this without sacrificing disease prevention efforts or the agricultural production system's economic viability. Options for managing resistance include lowering the dose or number of applications of the fungicide used, as well as combining or alternating treatments with fungicides that have a different mode of action. Since its inception 25 years ago (Milgroom and Fry, 1988) [27], the selection coefficient, which measures the fitness difference between resistant and sensitive strains in the presence of fungicide, has served as the general principle guiding resistance management, for the most part supported only by empirical observations rather than experimental data. This has recently been revisited with a modeling approach and detailed analysis of the literature to validate or challenge assumptions about the most effective resistant management tactics (Bosch *et al*., 2014) [3].

The impact of fungicide dosage on selection for resistance has long been a topic of discussion. Higher doses should theoretically apply more selection pressure, according to logic. However, a closer look indicates that it's possible that this supposition isn't always true. It is anticipated that high dosages will significantly select for the resistant group where there is a definite separation into a sensitive and a resistant subpopulation. One could argue that a high dose might control all of the different resistance classes, along with the sensitive wild types, thereby reducing the selection coefficient, whereas a lower dose might allow the most resistant types to survive where resistance manifests as a continuous series of slight shifts in sensitivity. The fact that a fungicide is unlikely to be dispersed equally inside a crop and that the amount may decrease due to weathering or degradation adds another layer of complexity. As a result, not every member of the pathogen population will be exposed to the same amount of fungicide. Experimentation is the only method to address these theoretical issues. In order to determine if azole resistance in *Z. tritici* would be selected for, Mavroeidi and Shaw (2006) [25] examined the effects of various dosages of a triazole fungicide, with or without a QoI partner. The inclusion of the QoI at higher azole dosages was shown to diminish selection, whereas resistance selection was shown to rise proportionally to dose. However, depending on the dose rate and the various degrees of disease control in the various treatments, the effects of the mixture differed. These findings confirmed that resistance selection is strongly correlated with fungicide dose, however the effects of combinations on selection may vary. The focus on phenotypes rather than specific genotypes where selection of certain genes or alleles giving resistance could be detected was one drawback of this and other earlier investigations on selection. To test their predictions, Hobbelen *et al.* (2011) [18] used data from field trials with powdery mildew (B. *Graminis f. sp*. hordei) on barley in which the ratio of QoI-sensitive and QoI-resistant (G143A) alleles was quantified after treatments differing in the overall dose and number of sprays of the QoI fungicide azoxystrobin. The model correctly predicted between 75 and 90 per cent of the variation in the mean selection ratio for the majority of sites and seasons. Selection was demonstrated to increase with increasing dose. This strategy could be expanded to assess the management of resistance in any pathosystem where resistance is provided by well-known genes or alleles with significant effects. In their evaluation of further data on the relationship between fungicide resistance risk and dose rate, Bosch *et al*. 2011 [2] came to the conclusion that the majority of experimental investigations and models that have been published to date support the idea that greater doses favour resistance.

Presently, combining a high-risk, single-site inhibitor with a low-risk, multisite fungicide is a widely used tactic meant to

lessen selection for resistance. As a result, it is anticipated that the low-risk fungicide will continue to be effective and aid in lowering the selection pressure for resistance to the mixture's high-risk component. The high-risk fungicide's dose may also be lowered without impairing control. Hobbelen *et al*. 2011 [18] put this hypothetical situation to the test using a modelling method in which resistance to the high-risk fungicide carries no fitness cost. They found that keeping the low-risk partner at its full dose while varying the dose of the high-risk fungicide delayed selection and lengthened the latter's useful life. In more recent years, Mikaberidze *et al.* 2014 [26] examined this issue while incorporating a fitness cost of resistance into their model to account for potential effects. The results showed that mixing can postpone resistance in the absence of fitness cost and that it may be able to identify the ideal ratio of the two fungicides to avoid de novo emergence of resistance in the presence of fitness cost. In accordance with mutation probabilities, fitness costs of resistance, and sensitivity of the resistant strain, models are now being expanded to estimate time to emergence (Hobbelen *et al.* 2014) [17]. This theoretical framework might be put to the test experimentally in a pathosystem where mutations that could lead to resistance are known through research conducted in the lab or can be deduced from the establishment of resistance in fungi that are related to the pathogen. Molecular techniques can provide a more accurate assessment of their possible consequences, albeit in the lab or glasshouse rather than in a crop setting, therefore it is vital to collect more in-depth experimental estimates of fitness costs of specific genetic modifications linked with resistance. Using recombinant strains with various sdhB mutations in a fixed genetic background, a recent study of SDHI fungicide resistance in the grey mould pathogen *B. cinerea* (Laleve *et al.* 2014) assessed the impact of these changes on a range of fitness parameters, including growth, reproduction, survival, sensitivity to stress, pathogenicity, and competitiveness and showed that different mutations varied in their effects. Evidence for potential compensating mechanisms that would reduce the impact of some mutations and allow them to persist in field populations was gathered.

Conclusion

For the foreseeable future, fungicides will probably continue to play a significant role in disease control and crop protection (Lucas, 2011) [22]. But there are a number of factors putting pressure on their continued use and effectiveness. The threat of resistance is spreading over the globe, and there are few effective treatment solutions. The pipeline of new drugs is becoming depleted due to the regulatory environment for new chemistry and rising research costs. The withdrawal of some current goods due to stricter new hazard standards may reduce flexibility and have an influence on disease control. For example, discontinuing the use of azole fungicides would make it more difficult to control diseases like *Fusarium* ear blight, raise the risk of mycotoxin contamination, and have an adverse effect on both human and animal health. All of this is happening at a time when fungal pathogen dangers are rising rather than falling (Fisher *et al*., 2012) [10]. A more coordinated approach is needed, integrating plant breeding and biotechnology, chemical discovery, coherent policies on sustainable use of pesticides, as well as ongoing innovation in alternative crop protection technologies, if we are to counter the global risks to plant health (Fears *et al*. 2014) [8] and meet the challenge of food security in a changing environment. To protect both current and future chemistry, fungicide resistance must be managed more successfully through greater information, risk assessment and monitoring.

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