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Evolutionary Patterns and Molecular Surveillance in Population Genomics of Fungicide Resistance in Agricultural Fungal Pathogens

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Abstract
Fungicide resistance is not just a series of "target site mutations", it is a population level issue in crop protection. Population genomics elucidates the origins and maintenance of resistant fungal lineages, mediated by standing variation, recurrent mutation, recombination, migration and heterogeneous selection. This is a critical narrative review of literature covering the period 2019 to 2026 focusing on Zymoseptoria tritici, Cercospora beticola, Botrytis cinerea, Alternaria solani and new pathogens in agriculture. We compare independent origins of resistance with geographic spread, look at the multilocus architectures and discuss the meaning of genome wide associations and sweeps. Comparative evidence shows that the same resistance-associated mutations can be found in different genomic backgrounds while different resistance-associated mutations can lead to identical phenotypes. We test phenotypic sensitivity assays, mutation-specific diagnostics, amplicon sequencing, quantitative allele-frequency methods, whole genome resequencing and long-read sequencing. They are only useful if they are representative and if the fungicide has been tested in the experiment and if exposure record is accurate. Additional topics of fitness trade-offs, mitochondrial heteroplasmy, host-associated structure, and temporal genomic collections are discussed. Integrated surveillance will provide an improved ability to detect the presence of emerging resistance compared to single mutation screening, but the latter does not necessarily establish causality or predict field performance. We endorse geographically coordinated, longitudinal monitoring associated with evolution and fungicide stewardship and uncertainty that is transparent. In order to achieve sustainable crop protection, this integration is of critical importance.

Keywords
Adaptive Evolution;
Fungicide Resistance;
Molecular Surveillance;
Population Genomics;
Selective Sweeps

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Introduction

Crop protection relies on the ability to reduce the incidence of fungal disease when populations of the fungus have evolutionary capacity left over for the fungus to react to fungicides. A spray doesn't have a homogenous enemy. It alters the likelihood of success for different lineages that vary in their susceptibility, reproductive success, dispersal ability and ecological performance. Selected alleles can become common after successive epidemics although the original variation may have been essentially absent in the normal sampling process.

The well-known description of fungicide resistance as a "single mutation which renders a chemical target inactive" would then describe a mechanism rather than the whole population process. Resistance is generated in landscapes that are composed of fields that are connected, cultivars are distinct, that have sexual and asexual generations, and that have varying treatments across space and time. Identifying the reason that a fungicide is no longer effective on a target is not enough to explain how the resistant genotype can become common.

Now it's supported by genome scale studies. McDonald et al. [18] demonstrated that the recent introduction of European resistant populations of Zymoseptoria species would not be sufficient to reduce the resistance mechanisms in Australian populations of these fungi. Pereira et al. [22] found multilocus associations with azole sensitivity in Parastagonospora nodorum and Spanner et al. [27] demonstrated that tetraconazole resistance in Cercospora beticola was due to genomic regions outside of the known target locus.

More recent studies highlight the extent of the problem. Puccetti et al. [23] studied a large European genomic diversity panel of Z. tritici and Lukasko et al. [16] examined the genome of Botrytis cinerea in the greenhouse for contributors to resistance to multifungicides. Such complementary studies illustrate the value of making genomic discovery and population history a part of phenotypes, and resistance.

The population-genetic starting point

To evolve resistance, there must be a difference or differences that give the fungicide resistant individuals an edge in survival, and that difference(s) must be heritable, but it does not have to have arisen after the compound was introduced into crop culture. In the case of standing variation, the alleles may have been once neutral, mildly deleterious or once favorable to some other environment. The same allele can obtain a huge selective advantage when exposure to the environment shifts. Hawkins et al. [8] added this evolutionary perspective to pesticides, noting that new pesticide regimes may also expose biological variation that goes back to before the time of modern pesticides.

This is important because it is not just an adaptation from the standing variation that can occur right away, but an adaptation from a new mutation, which takes many years for a mutation to appear and then to survive the early stochastic loss. Rare variants may be present in a field already without being detected using routine diagnostics, due to the fact that there are millions of fungal propagules present. However, mutation supply is not out of the picture. This will depend on the number of reproductive individuals, the mutation rate, how long the exposure happens and how many molecular changes will be tolerated for survival. Recurrence of target-site mutations has been particularly well demonstrated for some of the mitochondrial targets, and less clearly for the sterol-demethylation target CYP51, by Hawkins and co-workers [9].

Fungicide targets, pathways and the limits of a single-gene explanation

Biochemical action of fungicides involves disruption of processes that fungi need to survive, such as ergosterol biosynthesis, mitochondrial respiration and microtubule assembly. Demethylation inhibitors target primarily sterol 14-alpha-demethylase (CYP51);

succinate dehydrogenase inhibitors target the respiratory succinate dehydrogenase complex; Quinones outside inhibitors target the cytochrome bc1 system; and Methyl benzimidazole carbamates target beta-tubulin. A change in the target protein can lead to reduced binding, but if it also leads to resistance in the field, this will depend on the compound and its dose as well as the genetic background and the pathogen's ability to maintain its physiological functions. Dorigan et al. [4] surveyed target-site and non-target-site action of the major groups of fungicides. CYP51 represents a difference between molecular mechanism and genomic architecture. Some amino acid changes can lead to resistance to other azoles and multiple changes may be correlated on a single haplotype.

One substitution and one haplotype cannot replace the phenotyping of a population, as shown by McDonald et al. [18] and Oreiro et al. [21]. The situation is complicated by non-target-site mechanisms. Hu et al. [11] highlighted the presence of environmentally conditional and/or transient physiological responses that can complicate the categorization of resistance as stable heritable. Additionally, sensitivity can be modified by transport, detoxification and regulatory processes which are discussed by Dorigan et al. [4].

Multilocus resistance and genomic background

Fungicide susceptibility is frequently distributed in a quantitative manner throughout the genome and it is genetically heterogeneous. Pereira et al. [22] found 34 significant SNPs that were associated with resistance to azoles in a worldwide collection of 159 P. nodorum isolates, including candidates near a major facilitator superfamily transporter. There were distinct combinations of resistance associated alleles carried by the strains that indicated that no single marker was able to be used to describe the range of sensitivity. Importantly, the authors' assays of growth and pigmentation did not lend themselves to a simple, universal fitness penalty. Spanner et al. [27] re-sequenced 190 isolates of C. beticola and used phenotyping, genome-wide association and selective-sweep analyses. The other genomic regions were included with the associations: CYP51. The inference is stronger than marker scanning alone, as independent approaches that are informative came to the same conclusion about elements of the resistance architecture. In fact, the analysis of Puccetti et al. [23] also identified a diverse genetic contribution to azole resistance in a panel of 248 diverse strains, suggesting the importance of considering population structure when examining candidate loci.

Parallel evolution, convergence and recurrent origins

The similarity of resistance mutations in remote areas can be explained by at least two distinct mechanisms. A resistant lineage might have been introduced, or a similar selective pressure might have been responsible for the independent mutational events. Resistant isolates with highly similar genome-wide ancestry may be due to recent migration or expansion. If they tend to vary more across the genome but are more similar at the limited number of selected targets then repeated local adaptation is more convincing. However, the pattern of standing variation in antiquity may introduce a pattern similar to recurrent mutation, and ancestry may be obscured by recombination. Inferences about origin should, therefore, be made in a probabilistic way and be based on several genomic summaries.

Australian Z. tritici populations were reported to have developed azole resistance in a parallel evolution process in a few years [18]. They were based not just on familiar CYP51 haplotypes but on more general genetic differences among the Australian and European populations. After that, Hartmann et al. [7] found the continuous adaptation of pesticides over continents recurrent and also population-specific traits. Resistance-associated substitutions were identified in different genomic backgrounds by Einspanier et al. (5), which indicates several

evolutionary sources or recombination with the spread of resistant clone(s).

Selection scans and their interpretive hazards

No single pattern is diagnostic of fungicide exposure and selection leaves patterns in the genome. Nearby variants may increase in frequency if there is linkage, which could result in the loss of local diversity as well as an increase in haplotype homozygosity when a favorable allele takes over quickly. A hard selective sweep is frequently referred to as the expansion of a single adaptive origin; soft sweeps may be a result of many origins or background. The emergence of fungicide resistance may be especially prone to creating complex signatures due to the frequent occurrence of recurrent mutation, standing variation and recombination. The resulting patterns may not be as strong as textbook selective sweeps even in the face of biological strong selection. Genome scans usually look at the differences in nucleotide diversity, frequency spectra or linkage disequilibrium or differences between population groups. Selective-sweep analysis was combined with association mapping to improve the interpretation of C. beticola resistance by Spanner et al. [27]. Hartmann et al. [7] studied how the candidate resistance loci in Z. tritici overlapped with signals of recent positive selection.

Population connectivity and regional fungicide use

Fungi hardly discriminate between farm boundaries. The evolutionary unit of resistance can, therefore, be the regional, especially if inoculum dispersal is large. A new resistance allele may emerge on a local scale and be picked up by migration, or the same chemical may select similar alleles in many of the fields. These scenarios result in distinct histories of the genome but can result in similar frequency maps of resistance without considering neutral ancestry and temporal information. Long-running surveys of French Z. tritici resistance have been analysed by Garnault et al. [6] and the region of occurrence of each race has been correlated with the history of fungicide use. Their findings based on observations from 2004 to 2017 were incorporated into the work, which put resistance evolution within landscape scale agricultural management. Importance of results: sustained selection in surrounding populations can interfere with the ability to steward the field. Hellin et al. [10] reported on geographical and temporal changes in European Z. tritici populations concerning key resistance-associated target changes. This heterogeneity suggests that the selection, dispersal and sampling history of agriculture should be taken into account in comparing regions.

Host-associated structure and environmental reservoirs

In pathogen-host interactions, selection by fungicides occurs in a structured environment. Infestation success, pathogen density, funghiological opportunity and fungicide management strategies are influenced by the crop species and cultivar. Therefore, host adaptation may also relate to fungicide resistance if the genes involved in the resistance are not related. Host-associated variants might appear as resistance determinants when this structure cannot be modeled in genomic association analyses. On the other hand, host specialization can also impact the alleles that are selected for resistance, due to the different ecological conditions of the pathogen.

Naegele et al. [20] looked into the population structure of Botrytis from California specialty crops and concluded that host associations and fungicide resistance were factors in population structure. This discovery warns against the mixing of dissimilar samples, without regard for their source. Taliadoros et al. [29] examined 326 C. beticola isolates from cultivated and wild Beta hosts, and pieced together several aspects of host-associated differentiation.

Zymoseptoria tritici as a comparative model

Zymoseptoria tritici is a very informative species as its resistance evolution has been studied using the target sequencing, population genomics, field surveillance, and phenotypic testing approaches. It has large effective populations, recombination, genetic diversity, and dispersal which can provide multiple pathways to adaptation. But the properties that make inference difficult are additional properties. A resistant genotype recovered following treatment can be due to either local selection, to a new recombinant background, to immigration or to the maintenance of standing variation.

When multiple lines of evidence are combined, as in the literature presented here for this species, the supporting evidence is strongest; it cannot be dismissed as simply one molecular marker telling the evolutionary story [7, 18]. McDonald et al. [18] studied the continental origin of resistance, Hartmann et al. [7] studied parallel adaptation, Garnault et al. [6] modeled landscape-scale selection, Hellin et al. [10] characterized distributions of target-site alterations, and Oreiro et al. [21] studied resistance-associated haplotypes and neutral backgrounds in a large European diversity panel. One particularly instructive recent observation is that there may be a lot of phenotypic variation even in a single field. A great variation in fungicide-sensitivity and cultivar-associated differences was observed in experimental populations of Z. tritici by Talas et al. [28].

Some plant resistance to Cercospora beticola.

In sugar-beet systems, repeated exposure to demethylation inhibitors has led to the selection of resistance, and pathogen population structure has been found to be affected by host history, geography and pathogen reproduction. Whole genome resequencing and phenotyping are useful for uncovering more than one genomic determinant of tetraconazole sensitivity, as shown by Spanner et al. [27]. They found that they had found familiar target-site variation, but other regions as well, that a target mutation was a possible explanation but not the whole story for a quantitative phenotype. The following study by Taliadoros et al. [29] extended the scope to wild as well as treated plants by investigating population-genomic relationships of C. beticola on both wild and cultivated hosts. An ideal next step would be to marry the benefits of these studies: repeated phenotyping of a geographically and ecologically representative set of populations, high-density genomic variation data and historical information regarding both the host and the use of the chemical. The integrated designs would better detect the genetic pathways that lead to the establishment of resistance in farming systems.

Diseases caused by Botrytis cinerea and heterogeneous multidrug resistance.

In the case of Botrytis cinerea, multiple fungicide resistance should not be considered one molecular trait. Gray mold management involves multiple exposure to pathogen populations of various mode-of-action groups in the same crop, same season and same production system. Resistant isolates can have multiple target site substitutions, regulatory changes and transporter-associated mechanisms. Phenotypic resistance to multiple compounds may thus be due to multiple independent genetic changes in a single background, rather than to a common multidrug mechanism. Shao et al. [25] reviewed the available resistance mechanisms in Chinese B. cinerea populations, and identified differences in resistance to different chemical classes and production systems.

In California specialty-crop isolates, Naegele et al. [20] showed that population structuring resulted from host and fungicide resistance. Amini et al. [3] reported the presence of benzimidazole resistance in Iranian B. cinerea strains, thus emphasizing the necessity of developing genetic markers in a local context. Liu et al. [14] investigated the effect of structural changes and genetic transformation to the succinate dehydrogenase complex on fungicide

response. A novel mutation linked with resistance to the succinate dehydrogenase inhibitors was described by Malandrakis et al. [17] and further supports the necessity of interpreting the compounds individually. Lukasko et al. [16] applied a genome-wide strategy to screen for regions associated with resistance to several fungicides in B. cinerea in the greenhouse. These associations call for analysis in function rather than assuming a common cause of the multiple fungicide phenotypes.

Alternaria spp. and emerging pathosystems

Alternaria solani is an example of a fungus that develops resistance within a population that has also been geographically differentiated, admixed and recombined. A recent study by Einspanier et al. [5] applied whole genome sequencing to look at European populations and found that the resistance patterns in the species are not as clonal as they are often described. Changes associated with resistance were observed over a variety of genomic backgrounds, which further indicate independent emergence or recombinational redistribution.

This result is important because it allowed to distinguish the history of a particular locus from the history of the organism that bears it. A common mutation does not necessarily mean a common recent epidemic. Less intensively studied pathosystems also raise similar evolutionary questions, but with smaller genomic datasets, there are additional constraints. Long-read and hybrid assembly methods were applied to study the genomic features of Clarireedia [26] and diversity and resistance associated candidates of Neopestalotiopsis from strawberry [1]. An error of particular danger is the adoption from a well-established pathogen of validated mutations that can be assumed to have the same phenotype in a different genetic background for emerging pathogens.Fitness costs, compensation and persistence

One of the common assumptions is that the resistance costs will lead to the extinction of resistant alleles when fungicides are not used. This is a basic assumption that is being made too easily. Mutations that lead to a lowered binding to the fungicide can impact on the function of the target, but the type of penalty will vary with the mutation, the genetic background and environmental conditions. A mutation that is 'costly' at one temp may not have a measurable effect at another. A slower growing resistant isolate can be very successful via sporulation, infection efficiency or survival. Fitness should thus be defined as an experimentally determined aspect of pathogen performance, not be taken for granted. A panel of P. nodorum was tested for growth and melanin related proxies by Pereira et al. [22] and no universal trade-off was detected. Spanner et al. [27] also showed that demethylation inhibitor resistance in C. beticola does not come with an obvious cost in the terms of simple growth measure.

Mitochondrial genomes and overlooked allele dynamics

Mitochondrial cytochrome b may also be associated with quinone outside inhibitor resistance and mitochondrial DNA can have unique copy number, inheritance and within-cell heterogeneity. In conclusion, molecular diagnostics for measuring a resistance-associated mitochondrial allele will thus need to be interpreted differently from diagnostics of single-copy nuclear genes. In particular, the allele frequency in extracted DNA is not necessarily the same as the percentage of independently resistant individuals [12, 24]. Schoettner et al. [24] experimentally examined mitochondrial heteroplasmy in Z. tritici and showed that mitochondrial resistance-associated alleles are able to persist in a transient manner within single cells under varying fungicide selection. They suggest a mechanistic explanation for which to differentiate within-cell allele mixtures from mixtures of resistant and sensitive individuals. Biological sampling unit should be considered when using molecular monitoring of mitochondrial resistance. In pooled assays, calibration is important, as noted by Isa et al. [12]

who tested the quantitative sequencing method for estimation of the frequency of alleles associated with resistance.

Historical genomics and repeated sampling value

While they will be invaluable for understanding the diversity of the present, they will not necessarily reveal the sequence of evolutionary events in the past. Changes not discernable from the present-day population structure can be revealed by archived isolates and longitudinal field samples. Alkemade et al. [2] analyzed genomic diversity of historical samples of a number of plant-pathogenic fungi and presented an example of how historic samples can be used to illustrate adaptation to agricultural change. Minadakis et al. [19] analysed a time-series collection of wheat powdery mildew isolates, across the globe, to look into the historical distribution and emergence of resistance-associated mutations. This kind of evidence can be used to identify a recently expanded allele from one that has been in low frequency for decades.

Genome-wide connotation studies: detection and replication

The genome-wide association studies are attractive because they can scan the whole genome without the need of the causal genes to be nominated beforehand. The strategy is particularly useful when phenotypes are continua and/or mutations at the target site account for only a fraction of the observed response. Both new resistance and new genetic modifiers of sensitivity are available for studies in P. nodorum, C. beticola, Z. tritici and B. cinerea [22, 27]. However, association mapping doesn't identify molecular causality. The problem is the structure of the population. Thousands of different variants can be correlated with resistance if the area has been heavily treated with a fungicide and the area is genetically different. Balanced sampling is not fully replaced by the use of mixed models, kinship adjustment and "appropriate" population covariates that can reduce the inflation. Rare alleles when few individuals are assigned to them are often underpowered, especially if there is an imbalanced distribution of phenotypes.

Structural difference and long read sequencing

Single-nucleotide polymorphisms are easy to assay, but are not the full genetic signature of adaptation. Changes in expression or dosage may occur without an obvious coding substitution, such as copy-number differences, gene presence–absence variation, promoter rearrangements, and transposable-element insertions. Traditional short read alignment can be difficult to align these events, especially in repetitive areas or if the reference genome does not adequately cover the sampled population [22]. These blind spots may give the impression of isolates with resistance that cannot be accounted for by known mutations in apparently unexplained resistant strains, in resistance research. The advantages of long-read sequencing and hybrid assembly are that it offers greater continuity over many structural features, but that is dependent on sequence depth, base accuracy and assembly evaluation. Using comparative genomic analysis, Shi et al. [26] provided a picture of the use of nanopore and hybrid approaches in the assessment of Clarireedia. It is hoped that graph-based or multiple-reference approaches will eventually decrease the preferential bias towards a single reference genome, particularly in the case of diversity in fungal populations.

Transcriptomes, regulation and efficient tests

Comparisons of the transcriptomes can reveal pathways that are affected by fungicide exposure and candidate regulators that differentiate resistant from sensitive isolates. Liu et al. [15] identified genes that differed between fludioxonil-resistant and wild-type B. cinerea isolates and suggested processes linked to resistance. Joe et al. [13] discussed the use of genome and transcriptome sequencing to investigate the mechanisms of resistance. Interpretation means that there should be a clear

distinction between the resistance cause and the effects of resistance. Exposed isolate may induce detoxification genes not because those genes gave them the advantage for survival but the reason they survive longer. Liu et al. [14] used genetic transformation and structural modelling to analyse succinate dehydrogenase inhibitor responses in B. cinerea.

Molecular investigation

The most useful application of molecular surveillance is when there is a clearly stated question. For hundreds of samples, a targeted polymerase chain reaction assay may be the best approach to estimate prevalence of a validated substitution. Third-generation sequencing will be able to resolve structural changes and complex regions. Zulak et al. [30] made the point that no one molecular technology can meet all monitoring needs. Hellin et al. [10] correlated the occurrence of major resistance associated markers in Z. tritici samples from Europe; Oreiro et al. [21] interpreted the CYP51 diversity under genetic backgrounds by multilocus sequencing. Calibrated pooled molecular surveillance is demonstrated by Isa et al. [12] who studied the quantitative sequencing of resistance-associated variants of Colletotrichum scovillei. The false-positive and false-negative performance, the limit of detection, the technical reproducibility of the method, and the relationship between the allele signal and the respective phenotype should be quantified during method validation.

Sampling strategy, metadata and investigative comparability

Representative sampling is not always as easy as generating sequence data. A convenience sample from fields in which treatment failed can confirm resistance, but it cannot provide an idea of the level of resistance in an agricultural area. Likewise, if there appears to be a temporal rise, this may be due to more intensive farms being sampled in the later year [21]. Sampling frames need to be designed to include target crop area, field selection, sampling within the field, number of independent lesions or isolates and time from fungicide application, and how to avoid redundant clones. The structure of the fungal populations is hierarchical, with isolates nested within lesions, plants, fields, regions, and seasons; hierarchical designs are appropriate. If resources for sampling are restricted, it may be more helpful to have samples from strategically selected locations than to have extensive numbers from a single outbreak [10]. Continuity: changes in allelic frequencies can be differentiated from sampling artifacts with greater credibility when continuity in location and protocol is achieved each year. Interpretation can be enhanced with the use of assay controls, reference strains and interlaboratory comparisons [30].

Addition with crop controlling and resistance stewardship

The aim of fungicide resistance management is to minimise selection of resistant genotypes whilst maintaining adequate levels of disease control. Commonly, mixtures, alternation of modes of action, and treatment thresholds and integration with host resistance are considered. By using population genomics, the interventions can be evaluated not only for their ability to reduce a specific marker in one season, but also as to whether they can actually transform the entire population. In cases where multiple resistance mechanisms are present in the population, a selection pressure from a new mixture will not be evenly distributed across the different types of resistance. If migration is linked to fields, then the local value of using less may be affected by the practices in the region.

On the other hand, under application intensities which are lower, even if resistant alleles are still present, they may have a negative effect on selection [4, 6, 28]. Garnault et al. [6] offered evidence of the role of fungicide use at the regional level in explaining the dynamics of resistance in Z. tritici. Talas et al. [28] also showed that under field conditions there is

variability in fungicide sensitivity based on the host cultivar. The effects on reproductive system, fitness and cross-resistance vary greatly between pathosystems, as do the accessibility of mutations. A defensible translation workflow starts with surveillance – quantifying and characterizing the frequency and phenotype of existing resistance mechanisms, understanding the population connectivity and detecting unexplainable resistance.

Emerging subjects and research urgencies

There are many studies that are molecularly complex, but are epidemiologically cross-sectional. Others observe resistance over an extended period of time without genomic data to differentiate between lineage movement and repeated adaptation. To bridge these traditions, consistent sampling with documented fungicide application and repeated susceptibility assays and genetic characterisation studies that are relevant to the reproductive biology are needed [19]. The focus should be on estimating evolutionary pathways, and not just on describing novel variants. Secondly, it is the validation from the outside.

Samples taken from discovery panels may be over-sampled from a country, host or production system, and markers may not perform as expected in other countries. Inter-year and geographic replication should become commonplace. Qualifications for functional testing should be based on candidates that have plausible molecular functions and good cross-population association. If the collections are well documented, it is possible to determine from them whether putatively novel variants existed prior to recent exposure to high levels of fungicides [30].

Dangerous synthesis

At the population level, seemingly contradictory observations can be explained. The frequency and genomic context of a common target-site substitution might be hard to predict, while it may seem very predictable among species. Likewise, a dominant target-site mechanism does not preclude the association of several genome-wide polymorphisms with resistance. It may contain modifiers which affect susceptibility, associated ancestry, or pathways that are of primary importance in specific genetic backgrounds [28]. Another apparent paradox is that of maintaining diversity despite selection. The level of chemical exposure could generate significant directional selection, but not immediately eliminate variation from phenotype to genotype in the field. This was demonstrated in the case of Z. tritici by Talas et al. [28]. Repeated treatment can be used to maintain variation through diverse initial populations, host heterogeneity, alternating chemical regimes and recombination.

Conclusions

The most common way to conceptualize fungicide resistance is as adaptation occurring at genome, population and agriculture system levels. There can be multiple variants of the same phenotype, and the same target-site allele can have multiple histories. For the four pathogens, studies have shown that standing diversity, recombination, regional selection and migration are all contributing to resistance distributions. GWAS and selection scans are powerful discovery strategies that need to be carefully phenotyped and functionally annotated. Future molecular surveillance should include uniform sensitivity testing, temporal and spatial sampling and periodic genome-wide testing with validated targeted markers. These systems do not have the power to foresee all mutations, but they can recognize emerging threats more quickly than traditional methods, differentiate between possible evolutionary scenarios and supply more evidence for coordinated fungicide stewardship.

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