

Exploiting Genotypic Monitoring, Mixtures and Alternations for Sustainable Fungicide Management; Sym-Mixes, Allo-Mixtures and the Hogwarts Staircase

Oliver R, Lopez-Ruiz F

Centre for Crop and Disease Management, Curtin University, Australia

Richard.Oliver@curtin.edu.au

Fungicide resistance is a major problem limiting agricultural and productivity. The tools we have at our disposal to combat fungicide resistance are rapidly improving. These tools include more efficient ways to screen pathogen populations for resistance using both phenotypic and genotypic methods, deeper if still incomplete knowledge of the resistance factors and fitness penalties imposed by different fungicide resistance mechanisms in major resistance-impacted pathogens, and, most importantly, a more sophisticated model of resistance development based firmly on evolutionary principles. The question is therefore “can we improve the effective life of fungicides and reduce the burden of resistance by devising better resistance management strategies?”

The evolutionary perspective allows the rationalisation of much of the history of fungicide resistance (van den Bosch *et al.* 2014a). The rate with which a pathogen population develops resistance is a function of the selection intensity (i.e. the efficacy at the applied dose, the exposure time and the geographic spread of the fungicide) and the difference between the growth rate of the resistant pathogen and the sensitive in the presence of the fungicide.

These simple principles support all the current resistant management strategies. These include measures that are in the domain of the grower (the use of resistant cultivars, crop and soil hygiene measures, timely spraying, not spraying when disease pressure gives no economic benefit) and measures that appear on the “label”, (limitations on the dose and the number of sprays in a season and the use of mixtures and alternations (M and A)).

Most labels on modern fungicides specify a maximum number of times a fungicide can be sprayed in a season. The theoretical basis of this is that each spray causes a step increase in the frequency of the resistant strains in the population. If the frequency of sprays is high, the resistant population may reach 100% and be fixed. But if the resistant population has not reached fixation, the prevalence of the resistant strains in the population will decay in the gaps between sprays, due to dilution from non-sprayed areas (if coverage of the respective fungicide is below a certain threshold, (Parnell *et al.* 2006)) and by re-colonisation by the sensitive strains especially if a fitness penalty applies in times when the respective fungicide is

not used. Hence, if the fungicide is sprayed infrequently, the prevalence of the resistant population will remain low for a long time. An alternative legislative framework for determining the number of permitted sprays could be to monitor the pathogen population for the frequency of resistant isolates and to permit more sprays if the frequency remained below a (to be determined) threshold. Such a system might enable a powerful fungicide to be used more often than currently, giving better overall disease control.

The threshold chosen must take into account the selection intensity applied by the fungicide as well as the sampling strategy used to monitor the population. In practice all such thresholds would need to be 1% or less and hence many hundreds of strains would need to be screened from a substantial numbers of sites. New developments in genotypic monitoring now make this a possibility (provided the molecular basis of resistance is fully understood), but the implementation of a contingent monitoring program would require substantial discipline from the farming community as well as revised label provisions.

The most actively debated aspect of fungicide resistance management is the use of mixtures and alternations (M and A). We can rationalise how M and A reduces the risk of resistance to one or all of the fungicides in the program by reference to the concept of cross resistance (Van Den Bosch *et al.* 2014b). The key issue is whether the levels of resistance (normally measured by EC50s) to both (all) fungicides are positively, negatively or uncorrelated. In most cases, resistance to fungicides from different mode of action groups is uncorrelated; the main exception would be cases of multi-drug resistance (MDR) (Kretschmer *et al.* 2009). Many fungicide mixtures are composed of actives from the same mode of action class such as “Tilt xtra” comprising propiconazole and cyproconazole. We can call these “sym-mixes” to distinguish them from “allo-mixtures” made up from actives of different modes of action. Most cases of sym-mixing show positive cross resistance whereby a fungal strain resistant to one fungicide is also resistant to the other. Clear cases of this are provided by the QoI fungicides and the G143A mutation. All QoI fungicides are reported to be essentially inactive against the G143A mutants (Sierotzki 2015). Even the QoI fungicides reveal a subtlety here in that the F129L mutation has a much less comprehensive impact. In the cases of DMI and SDHI fungicides, cross resistance within the MOA class is generally modest with often low degrees of positivity and a few isolated , cases of negative cross resistance (NCR). NCR was noted some years ago for B1 and B2 fungicides (FRAC coding) and has been mooted as a possible active means of resistance management with DMIs as well (Leroux & Gredt, 1989, Elad *et al.* 1988, Fraaije *et al.* 2007). In cases of NCR, the strains resistant to one fungicide are hypersensitive to a second fungicide. This would make an alternation strategy particularly powerful forcing the pathogen population back and forth along a linear evolutionary path.

The impact of evolutionary forces of the DMI fungicides on the Cyp51 gene is particularly complex (Hawkins & Fraaije 2017, Lucas *et al.* 2015). In addition to MDR and Cyp51 gene(s) overexpression profiles, non-synonymous coding region changes have been found at up to 30 variant sites and found in 70 combinations in *Z. tritici* (Cools *et al.* 2013). Different fungicides

in the DMI group have different EC50s for each of the genotypes; in other words cross resistance patterns vary from strongly positive to weakly negative.

How can M and A resistance management strategies be adapted to exploit the complex patterns of cross resistance as exemplified by DMIs and pathogens such as *Z. tritici*? M and A strategies vary in two dimensions. One dimension is the area being sprayed under each regime; the second is the period between one fungicide and the second fungicide spray (Fig. 1). If both fungicides are applied to the same field at the same time (i.e. a tank mixture) both parameters are minimised and this is a mixture. Alternation can apply from areas as small as a single field up to a continental land-mass. Times can extend from a few days to several years.

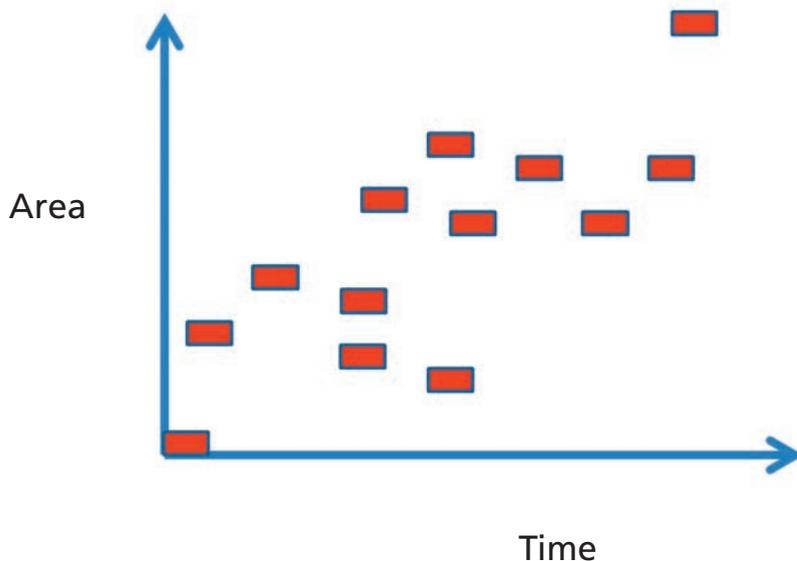


Figure 1: Schematic diagram illustrating the matrix of options for mixtures and for alternations. The area to which a specific fungicide application is sprayed can vary from a single field to a continental region; the time between different fungicide sprays can vary from zero (equivalent to a tank mix) to several years.

The Australian cropping scene is characterised by very large fields and small numbers of farming enterprises and is similar in this context to many secondary fungicide markets in N. and S. America. Fungicides were rarely used until ten or so years ago. Even then, a very small number of actives of a single MOA class was used (Tucker *et al.* 2015). As a result the fungal pathogen population currently comprises small numbers of genotypes selected by the few fungicides in heavy use. In this case, one could imagine a “large area, long time” (LALT) alternation strategy. The current actives would need to be embedded into a multi-season spray program and temporarily replaced with one or a few actives that were most active on the

current genotypes. The new fungicide(s) would then “drive” the pathogen towards the genotype that were best adapted to it and would counter-select the existing genotypes. It may take several seasons before the new population structure emerges, whereupon the original fungicides could replace the new ones. Such a strategy would require significant discipline from the fungicide suppliers, regulatory authorities and growers. Furthermore it would be ineffective if genotypes resistant to both groups of fungicides could evolve.

In mature markets, like Northern Europe, a hyper-complex mix of genotypes exists presumably because many different DMI fungicides have been adopted successively and used simultaneously within the landscape. Historical analysis of fungicide choice suggests that farmers changed their fungicide choices when the pathogen population developed noticeable insensitivity. The pathogen population is challenged by what amounts to a “cafeteria” situation with each pathogen genotype proliferating on whichever of the multitude of local fungicide regimes/environments/host tolerances to which it was best adapted. A diverse array of fungicide regimes does at least have the merit of mitigating the risk that a single highly resistant genotype would be selected.

The evolutionary forces set up in mature markets by using a diverse array of fungicides where the least effective actives are gradually dropped and replaced by the newest within a large local diversity of fungicide regimes led to the successive evolution of genotypes adapted to each new fungicide in turn. The key driver in the selection of actives are currently efficacy and immediate cost-effectiveness. We can expect that the genotypes selected by the first fungicides are subject to mutation and selection by the subsequent fungicides (Tucker *et al.* 2015). This evolutionary scenario can be likened to the Hogwarts staircase where each new fungicide provides an upward route for the selection of ever fitter genotypes. The ultimate result could be domination by genotypes resistant to all current and past fungicides from the DMI fungicide class.

Can we break this inevitable march towards total resistance? One obvious strategy is to use allo-mixtures, but even then MDR resistance will prove a challenge, albeit most cases of MDR are relatively weak. . The key to solving this conundrum for sym-mixtures is comprehensive knowledge of the selection pressures and fitness penalties placed on each genotype by each fungicide active and M and A strategy. It is then possible to envisage a scenario in which the selection of actives is made so as to drive evolution backwards towards the wild-type and/or into evolutionary cul-de-sacs where further mutations carry lethal fitness penalties. Such a fungicide strategy would need to be organised on at least a local area scale, governed in size by the epidemiological and dispersal characteristics of the pathogen. The strategy would also need to be highly dynamic with in-season choices of active and individual field treatments – a small area, short time (SAST) strategy. We note that both the LALT strategy for new markets and the SAST strategy for mature markets are highly ambitious requiring unprecedented levels of knowledge and cooperation to enact.

ACKNOWLEDGEMENTS

Research in our lab is supported by the CCDM and by AGWA. We thank numerous colleagues for stimulating conversations and ideas that have led to the development of the ideas expressed in this article.

REFERENCES

- Cools HJ; Hawkins NJ; Fraaije BA (2013). Constraints on the evolution of azole resistance in plant pathogenic fungi. *Plant Pathology* 62, 36-42.
- Elad Y; Shabi E; Katan T (1988). Negative cross resistance between benzimidazole and N-phenylcarbamate fungicides and control of *Botrytis cinerea* on grapes. *Plant Pathology* 37, 141-7.
- Fraaije BA; Cools HJ; Kim SH; Motteram J; Clark WS; Lucas JA (2007). A novel substitution I381V in the sterol 14 α -demethylase (CYP51) of *Mycosphaerella graminicola* is differentially selected by azole fungicides. *Molecular Plant Pathology* 8, 245-54.
- Hawkins NJ; Fraaije B (2017). Adaptive landscapes in fungicide resistance; fitness, epistasis, constraints and predictability. In: this volume, pp. 217-221.
- Kretschmer M; Leroux P; Mosbach A; Walker AS; Fillinger S; Mernke D; Schoonbeek HJ; Pradier JM; De Waard MA; Hahn M (2009). Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathogens* 5(12): e1000696. doi:10.1371/journal.ppat.1000696
- Leroux P; Gredt M (1989). Negative cross-resistance of benzimidazole-resistant strains of *Botrytis cinerea*, *Fusarium nivale* and *Pseudocercospora herpotrichoides* to various pesticides. *Netherlands Journal of Plant Pathology* 95, 121-7.
- Lucas JA; Hawkins NJ; Fraaije BA (2015). The Evolution of Fungicide Resistance. In: *Advances in Applied Microbiology*, Vol 90, 29-92.
- Parnell S; Van Den Bosch F; Gilligan CA (2006). Large-scale fungicide spray heterogeneity and the regional spread of resistant pathogen strains. *Phytopathology* 96, 549-55.
- Sierotzki H (2015). Respiration Inhibitors: Complex III. In: *Fungicide Resistance in Plant Pathogens. Principles and a Guide to Practical Management*, eds. H Ishii & DWHollomon, pp. 119-143. Springer Japan
- Tucker MA; Lopez-Ruiz F; Jayasena K; Oliver RP (2015). Origin of Fungicide-Resistant Barley Powdery Mildew in Western Australia: Lessons to Be Learned. In: *Fungicide Resistance in Plant Pathogens. Principles and a Guide to Practical Management*, eds. H Ishii & DWHollomon, pp. 329-40. Springer Japan.
- Van Den Bosch F; Oliver RP; Van Den Berg F; Paveley N (2014a). Governing principles can guide fungicide-resistance management tactics. *Annual Review Of Phytopathology* 52, 175-95.
- Van Den Bosch F; Paveley N; Van Den Berg F; Hobbelen P; Oliver R (2014b). Mixtures as a fungicide resistance management tactic. *Phytopathology* 104, 1264-73.