



## **FRAC recommendations for fungicide mixtures designed to delay resistance evolution.**

### **INTRODUCTION**

Designing a disease control programme to include effective measures to combat the development of resistance to the fungicide(s) used is a complex subject. However, within all resistance management programmes there are certain common practices relating to how the fungicide(s) is used. These practices frequently involve advice on appropriate fungicide dose rates, limitations on how often the fungicide should be used in a spray programme and programmes designed around the use of two or more fungicides which have different modes of action in controlling the same pathogen.

When considering how best to use fungicides with different modes of action in a resistance management programme there are two basic alternatives: the fungicides can be applied in alternation or they can be applied together in a mixture. Alternation programmes can also include mixtures. Such programmes can include simple alternation where fungicide A is applied followed by fungicide B, then A, then B etc or products can be arranged in different sequences to include, for instance, a block of A sprays followed by a single spray or a block of B sprays. It may even be appropriate to include a third fungicide in the sequence. Where blocks are used, it is common practice to limit the number of applications of a fungicide in a block. For mixtures, the two or more active ingredients are applied together. The mixture may have been designed and produced by the manufacturer as a 'co-formulation' in which the active ingredients are combined in the same formulation or the mixture may be prepared by the user by physically mixing the mixture components in the spray tank; the latter are commonly referred to as tank mixes. In certain cases the manufacturer may provide the components of a tank mixture as individual containers in a common product package; these are usually referred to as 'twin packs' or 'combi-packs'. For both alternation and mixture programmes, considerations based on dose rates and limitations on the number of applications used for a specific fungicide still apply.

There is no clear evidence to suggest that either strategy, alternation or mixtures, is the better for resistance management and the choice of which to adopt must be made according to the pathogen to be controlled, the crop variety to be protected, and the availability of suitable fungicides. In crops with a high number of applications per cropping cycle and in which only a limited number of different modes of action are

available, alternation rather than combination of fungicides may be a more effective way to reduce selection pressure in commercial spray programs.

The advantage of a co-formulation is that the manufacturer has already selected the ingredients, the precise ratio and the dose rates best suited for the job. Tank mixes may provide some extra flexibility but need more expert knowledge to design the ideal combination of ingredients and the dose rates within the regulatory frame work.

Whatever strategy is adopted, alternations or mixtures, the objective should be to minimise the risk of resistance developing to any of the fungicides used in the programme.

The purpose of this document is to give general advice on the composition of fungicide mixtures designed to delay the onset of or manage resistance in plant pathogen populations, with special reference to the risks of resistance development.

### **WHY USE MIXTURES?**

Fungicides are often combined as co-formulations or tank mixes for several reasons. These can be conveniently divided into three categories:

1. Improved disease control. Mixtures can be used to broaden the spectrum of disease control of a product, to combine the specific characteristics of the components of the mixture to increase the effectiveness of the product (for example curative plus protectant activity, or systemic plus non-systemic), or to take advantage of additive or synergistic interactions leading to more potent disease control and greater flexibility. Even if the mixture does not in itself provide resistance management such mixtures can be used successfully within disease control programmes that require such management providing suitable strategies are included.
2. Disease control security when resistance is present. Resistance to fungicides can develop rapidly in plant pathogen populations and it is possible that the fungicide user may not be aware of the resistance status of the population to be controlled. It could be argued that the use of a mixture in these cases is better than an alternation strategy as the application programme would be more robust in terms of disease control.
3. Resistance management. When used for resistance management it is necessary for at least two components of the mixture to have activity against the field populations of the target pathogen when used alone. In addition the activity profiles of these components should be combined in such a way that effective disease management is achieved.

A key requirement for any mixture product applied to manage resistance is that the components of the mixture must not be cross-resistant and the dose rates of each component used in the mixture should provide sufficient control of sensitive isolates when used alone. The most common mixtures consist of single-site fungicides (with moderate or high resistance risk) mixed with multisite fungicides (with low resistance risk) either as tank mixes or as a co-formulation. However, since more regulatory restrictions are being imposed on multi-site fungicides and highly effective single site fungicides with different modes of action are available in most crops, mixtures between single-site fungicides are appearing in the market and it is clear that more

care regarding the resistance status in pathogen populations needs to be taken when recommending them.

## **DEFINITION OF RISK**

Care must be taken in how to interpret the term 'risk'. "Resistance risk" is defined as a combination of the inherent risk determined by the chemical class or compound concerned, its interaction with the target sites of the pathogen, the pathogen itself and several risk modifying factors (see FRAC Monograph 2).

The major modifying factor is called the 'Agronomic Risk' and is determined by the geographical area in which the crop is grown, the crop variety, the expected severity of disease in that area and the disease control practices used, for example, application number and timing. The disease control practices are particularly important because these factors can be modified by growers and advisors and are also influenced by precautionary statements on fungicide labels.

'Resistance risk' is thus determined by how a particular fungicide is being used to control a particular pathogen under certain conditions. For convenience, 'Resistance risk' is divided into three categories: Low, High and Medium. The 'Low' and 'High' risk categories tend to be easily determined. The 'Medium' category is more difficult. In some cases, e.g. the multi-site compounds such as the dithiocarbamates, the term 'low risk' is attached to the chemical irrespective of which pathogen it is used to control. This is because the nature of the chemistry and its mode of action precludes resistance development and the biology of the target pathogen is not important. However, in the majority of cases, particularly with modern single site inhibitors, the classification of risk is based on a consideration of all the above factors. It is thus quite possible that one fungicide – pathogen combination will be classified as 'high risk', while another combination of the same fungicide with a different pathogen or in locations with generally low disease pressure could be classified as 'low risk'. By utilising all available mitigation measures (agronomic risk factors), the resistance risk of a particular combination may be reduced.

It is important to realise that for new chemistry (new mode of action), the risk associated with the chemistry will not be known and decisions may have to be made based on experiences with the target pathogens. In such circumstances a precautionary approach may be wise.

### **Definition of low risk**

To qualify as a 'low risk' use, the fungicide or the fungicide-pathogen use combination must have a confirmed history of a lack of or very rare instance of resistance development. As indicated above, several low risk fungicides have a multi-site mode of action (e.g. dithiocarbamates), but this is not a general requirement.

### **Definition of high risk**

A fungicide – pathogen combination can be classed as 'high risk' based on the expectation of resistance developing quickly if no resistance management is practiced or the actual development of resistance during product use.

Criteria for a high resistance risk include:

- Resistance based mainly on single target site mutations, highly conserved within all affected pathogens, monogenic resistance (known or suspected): e.g. QoIs: G143A; MBCs: E198A/G/K, F200Y; Dicarboximides: I365S. Such mutations are usually associated with high levels of resistance.
- Resistant isolates are still virulent after several generations without selection pressure and without significant fitness costs.
- Appearance of resistance in field populations a few years (2-5) after product launch.
- Rapid increase of resistance frequency over time and area.
- Significant decrease of disease control under commercial field conditions when the fungicide is used as a solo product and/or at low rates according to the product label. This may include complaints of insufficient disease control.
- Product failure associated with confirmed presence of resistant isolates in field populations of the pathogen.

Phenylamide, QoI, MBC, and Dicarboximides are considered as high risk fungicides and a 'high risk' category is justified for most pathogens. All are single-site inhibitors.

### **Definition of medium risk**

The normally accepted definition of 'medium risk' is applied to situations where the fungicide or its intended use cannot be categorised as presenting a low risk, yet the risk posed is not sufficient that resistance would be expected to develop to the solo product as rapidly as to an accepted high risk situation. Criteria can be similar to those described in the definition for "high risk" but are usually less severe, e.g. mutants can be created but confer reduced fitness, resistance is polygenic, i.e. significant sensitivity shifts in field populations are only observed with stepwise selection of multiple gene mutations; or inheritance of resistance is recessive. Modifying factors like limited spread of resistance can apply. At appropriate dosages, the fungicides will continue to provide good control of the pathogens.

Many single-site fungicides can be considered to bear a medium resistance risk, e.g. DMIs (polygenic resistance, good field performance at appropriate rate), APs (limited spread of resistance), CAA fungicides (recessive inheritance of resistance, limited spread of resistance).

### **MIXTURE OPTIONS AND THEIR RISK POTENTIAL**

There are various combinations of individual fungicides that can be placed together in a 'mixture'. When discussing fungicide mixtures designed to manage resistance, it is convenient to consider the mixture to be made up of (usually two) components; each being a particular fungicide targeted at the same pathogen. Each component will present its own 'Resistance risk'. It is thus necessary to consider how different components with the same or different risk levels can be used together in a mixture and whether a particular mixture is a valid resistance management option in the presence or absence of resistance. In all cases, the relative component dose rates used in the mixture must be carefully balanced based on the individual properties of each mixing partner (e.g. lasting effect, dose response curve, etc.) to ensure that, for instance, the concentration of one component in or on the plant does not decrease

below an acceptable level much faster than the other component and so leave an 'at risk' component without any protection.

It must also be remembered that no mixture is likely to completely prevent the eventual development of resistance to a mixture component. Used wisely, however, mixtures can significantly delay the process and lead to a longer fungicide life.

The various options are considered below.

**1. Mixing two low risk fungicides.**

This poses no change of risk to the use of either component used solo.

**2. Mixing a high or medium risk single site fungicide with a low risk multisite.**

No resistance to high or medium risk component present: This has been, and still is, a firm favourite for managing resistance development to the high or medium risk fungicide. In many cases, reduced rates (compared to recommended solo use rates) of both the high or medium risk and the low risk components are used. The critical requirement for such a mixture is that the dose rates used for the individual components must be capable of providing good disease control if used solo. This is governed by the dose response curve for the individual component but usually needs dose rates of no less than 50% of the recommended rate of the solo product. For some components and particularly for the multisite component, dose rates of 75% of the solo rate may be more appropriate in order to achieve long lasting protection for the at risk component.

Resistance to high or medium risk component present: In situations where resistance to the high or medium risk fungicide in the mixture is already present, the use of a mixture with a low risk component will ensure disease management and can slow down the build up in frequency of resistant isolates. It is often recommended to impose limitations on spray numbers in a season and placement of such a mixture in the spray programme; these are determined according to the crop – pathogen system being considered.

There are notable cases where such mixtures can be expected to be particularly valuable:

1. In cases where the frequency of isolates resistant to the high or medium risk fungicide in field populations is low, mixtures with a low risk fungicide have been shown to delay the build up of resistance.
2. In situations where the fungal population resistant to the high or medium risk fungicide declines between seasons such that it is at a minimum at the start of the spray cycle. In these cases, use of the mixture may provide better control of the pathogen in early season than either mixture product alone. However, experience usually shows that resistance rapidly builds up to the at risk component with each subsequent spray application. The number of spray applications must thus be limited depending on the host-pathogen system.
3. In situations where it is proven that the current impact of resistance to the high or medium risk component is low in terms of disease control i.e. resistance can be detected but it is not causing great harm and the biological profile of the

target pathogen indicates that resistance development would be a slow process. Such situations could occur with control of, for example:

- a. monocyclic diseases.
- b. diseases of infrequent occurrence.
- c. pathogens where the rate of development of resistance has been shown to be restricted, for instance where genetic studies show that the inheritance of resistance is by recessive genes, as for the CAA fungicides and *Plasmopara viticola*.

Mixing a low risk fungicide with a high or medium risk component could thus delay further the development of resistance. It would, however, be wise to limit the number of applications in such circumstances and the situation would require careful monitoring.

### **3. Mixing single-site (high risk or medium risk) fungicides with different modes of action:**

#### **No resistance present to either component**

If no resistance has yet been found to either mixture component the use of a mixture can delay the development of resistance to the components. The extent of the delay cannot be predicted but should allow both components to remain effective for longer than if either had been used as a solo product. Reductions in dose rate of the mixture components to below an effective rate should be avoided. The number of applications needs to be restricted (i.e. a disease control programme should not be based on continuous and sole use of the mixture) but depending upon the pathogen it may be possible to recommend more applications of the mixture product than either component used solo. With such combinations disease management can be improved and thereby, resistance management in general is strengthened. Such cases must be considered on their individual merits.

#### **Resistance present to one or both components**

If resistance in field populations against one high or medium risk component has already evolved to an extent that this component used as a solo product does not provide sufficient disease control, the addition of a second fungicide bearing a moderate or a high resistance risk may place undue selection pressure upon the second mixture component which, if a recognised high risk one, could favour rapid development of resistance just as if it was being used as a solo product.

For these reasons two high risk components, a high plus a medium risk component or two medium risk components should not be recommended as a strategy to delay resistance evolution where resistance already occurs in current pathogen populations to either one or both component such that inadequate disease control would result if that component was used solo.

Examples would be a mixture between QoI and Phenylamide fungicides in *Plasmopara viticola* or between QoI and MBC fungicides in *Venturia inaequalis*. Note that such mixtures may still have a valid use for spectrum extension purposes. In this case other resistance management techniques should be included in the disease control programme e.g. alternating with a third component, a 3-way mixture combination etc.

#### **4. Mixtures between low risk single site fungicides and a moderate or high risk component.**

In these circumstances, the same considerations apply as if the low risk component was a multisite fungicide, although during the time of early product introduction it would be wise to monitor the performance of both components and not assume that resistance to the single site, low risk component could not happen. An example of a single site low risk category could be the use of DMI fungicides to control *Puccinia* spp. on cereals. Despite over 30 years of exposure, no resistance has occurred.

#### **SPECIAL NOTE: Mixture products used to control two or more pathogens on the same crop.**

Where the same mixture product is used to control two or more pathogens on the same crop and there are different resistance risks associated with each pathogen, the decision making process of how best to use the product is clearly more complex. Alongside a consideration of the various risk factors associated with the exposure of the individual pathogens to the mixture product, a consideration of the economic impact of the selected pattern of use of the mixture product becomes important. In some cases it has to be accepted that, for economic reasons, the priority will be to provide effective control of the most damaging pathogen, even if this means exposing a lower threat pathogen to a higher risk of resistance development. Such situations can only be analysed on a case by case basis.

#### **CONCLUSION**

Resistance Management is an important and crucial objective of any disease control programme and the incorporation of mixture products into the programme is an excellent means of achieving this objective. Mixtures can be designed and used to delay the onset of resistance to any fungicide or, if resistance has appeared, to manage the effects of such resistance. The result is to prolong the active life of a particular fungicide to the benefit of the grower and producer. This document has given practical general advice on how this can be achieved.

#### **FURTHER INFORMATION**

Further information on resistance risk and resistance management can be found on the FRAC webpage at [www.frac.info](http://www.frac.info)