Governing Principles Can Guide Fungicide-Resistance Management Tactics

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Abstract

Fungicide-resistance management would be more effective if principles governing the selection of resistant strains could be determined and validated. Such principles could then be used to predict whether a proposed change to a fungicide application program would decrease selection for resistant strains. In this review, we assess a governing principle that appears to have good predictive power. The principle states that reducing the product of the selection coefficient (defined as the difference between the per capita rate of increase of the sensitive and resistant strains) and the exposure time of the pathogen to the fungicide reduces the selection for resistance. We show that observations as well as modeling studies agree with the predicted effect (i.e., that a specific change to a fungicide program increased or decreased selection or was broadly neutral in its effect on selection) in 84% of the cases and that only 5% of the experimental results contradict predictions. We argue that the selection coefficient and exposure time principle can guide the development of resistance management tactics.

INTRODUCTION

Fungicides are a key part of the control of crop plant diseases. However, there have been repeated cycles of introductions of new fungicide modes of action and subsequent losses of efficacy due to the emergence and selection of resistant pathogen strains. For a range of pathogen-crop combinations, the rate of loss of effective fungicides threatens to exceed the rate of introduction (12, 65, 70, 75). It is thus critical to develop, validate, and implement effective fungicide-resistance management methods. Several approaches have been proposed to minimize resistance problems. These include (*a*) choice of application dose, (*b*) constraint on the number of applications, (*c*) mixtures of modes of action, (*d*) alternation of modes of action, and (*e*) adjustment of the timing of applications (5). Ideally, the choice of such resistance management methods should be based on sound experimental and theoretical evidence. In practice, when resistance management methods are advocated, the evidence that the method proposed is appropriate for the pathogen-crop-fungicide combination under consideration is often not made explicit. In this review, we present and evaluate the existing published evidence on fungicide-resistance management.

Most resistance management methods are developed on a case-by-case basis. In this review, we elucidate patterns that appear to hold true across fungal pathosystems and fungicide combinations. Specifically, we consider a governing principle introduced more than 25 years ago (14, 53, 76) and test how effectively this principle predicts the outcome of fungicide-resistance management methods. The governing principle we describe predicts qualitative trends, i.e., it asks whether an adjustment of the spray program reduces or increases the buildup of the fraction of the pathogen population resistant to the fungicide. Whether the size of the change in resistance buildup is large enough to be of practical relevance is not predicted by the principle and needs to be assessed in further model studies and field experiments. The qualitative trends identified do, however, greatly narrow down the number of methods that need to be field tested.

Our findings are generalizations for fungal plant pathogens and fungicides only. They do not necessarily apply to resistance against antibiotics, insecticides, or herbicides because of the differences in genetic systems and the reproduction biologies of the species groups involved (for further explanation on this point, see "Interpretations" below). We start with an introduction of the selection coefficient because this coefficient plays a central role in the paper.

Emergence: when a fungicide mode of action is introduced and the population is uniformly sensitive, resistance has to arise through mutations in sensitive strain(*s*). Emergence occurs when the resistant population increases so that it is large enough not to die out because of demographic stochasticity

Selection: when a fungicide is used and a resistant genotype is present in the population, its proportional contribution to the next pathogen generation is larger relative to that of the fungicide sensitive strain(*s*) in the population

Management methods: we use method rather than strategy so that we can use the words strategy and tactic in their exact and specific meaning in the paper

Principle: general rule that has a range of applications across a wide field. A principle of fungicide-resistance management is a rule that applies to a wide range of specific resistance management methods

THE SELECTION COEFFICIENT

The selection rate is measured by the selection coefficient (*s*). The selection coefficient is defined as the difference in fitness between the resistant and sensitive strains due to the application of the fungicide. Fitness is measured by the per capita rate of increase (*r*) of a population. Thus, the selection coefficient is

$$s = r_R - r_S, \qquad 1.$$

where r_R and r_S are the average per capita rate of increase, also often called the intrinsic rate of increase. Resistance management thus tries to minimize *s*. Below, we see how this simple equation helps to explain how changes in the spray application program affect selection for resistance (53). For more information on the selection coefficient see sidebar, Further Explanation of the Selection Coefficient.

FURTHER EXPLANATION OF THE SELECTION COEFFICIENT

Consider a pathogen population made up of a fungicide-sensitive and a fungicide-resistant strain, with densities $P_S(t)$ and $P_R(t)$, respectively. In the absence of severity dependence, the epidemic dynamics of the two strains can be modeled by two exponential growth equations,

$$\frac{dP_S(t)}{dt} = r_S P_S(t), \quad \text{S1.}$$

$$\frac{dP_R(t)}{dt} = r_R\, P_R(t) \quad \text{S2.}$$

where r_S and r_R are the intrinsic rate of increase of the sensitive strain and the resistant strain, respectively. The intrinsic rate of increase is the net increase in density per time unit per unit of pathogen density. We loosely describe this parameter as the epidemic growth rate. In population genetics, the fitness of a pathogen strain is defined as the intrinsic rate of increase, r (13), of that pathogen strain.

The frequency of resistance, which is the fraction of the pathogen population resistant to the fungicide, ρ, at time t is given by

$$\rho(t) = \frac{P_R(t)}{P_R(t) + P_S(t)}. \quad \text{S3.}$$

Differentiating $\rho(t)$ with respect to time, substituting Equation S1 and Equation S2 into Equation S3 and rearranging the expression yields

$$\frac{d\rho(t)}{dt} = s\rho(t)[1 - \rho(t)]. \quad \text{S4.}$$

Equation S4 shows that the frequency of resistance in a pathogen population grows logistically through time. The parameter $s = r_R - r_S$ is the selection coefficient. From this logistic growth equation, it is seen that decreasing s decreases the rate of increase of the fraction of the population resistant to the fungicide. Note that in population genetics selection is often referred to as $1 - (r_R/r_S)$.

WHAT DO WE WANT TO ACHIEVE WITH RESISTANCE MANAGEMENT?

Reducing Selection

The aim of resistance management is to slow the selection for fungicide-resistant strains in the pathogen population. Resistance management thus tries to minimize the selection coefficient (53). In the following paragraphs, we see that this simple equation (Equation 1) helps to explain how changes in the spray application program affect selection for resistance.

Milgroom & Fry (53), who were the first to introduce the idea of reducing the selection coefficient as the governing principle of fungicide-resistance management and derived strategies from this principle, state that "The efficacy of most of the tactics for preventing or delaying resistance problems has not been conclusively demonstrated," although there are "several anecdotal examples that seem to support the principle." Twenty-five years later, published evidence has accumulated to verify the practical relevance of the governing principle.

In experiments and models, the final frequency of the resistant strain is often used to compare the effect of different fungicide programs on selection for resistance. This gives a quantitative assessment of the differences between fungicide application programs. We have found more than

Strategies: the "what" aspect of resistance management. What do we want to achieve with resistance management?

Tactics: the "how" aspects of resistance management. How do we achieve our strategy of resistance management?

50 publications that use selection and the difference in selection between treatments to assess resistance management tactics.

Effective disease control: disease control of a sufficient level to keep yield losses below a required level

Sustainability of Disease Control

Fungicides are used to control plant disease, so any resistance management strategy of practical relevance needs to provide effective disease control. It is easy to find ineffective fungicide programs that minimize selection, but such programs are of no practical use. Fungicide treatment inevitably imposes a selection pressure on the pathogen population, and the frequency of resistance might eventually increase to a level at which effective control fails. The time from introduction of the fungicide to the point at which effective control can no longer be obtained is termed the effective life. By using effective life to compare resistance management strategies, the reduction in selection and the need for effective disease control are both taken into account.

Sustainability of Financial or Production Gain

For growers, it is not disease control but financial gain that is the ultimate objective. Instead of the effective life of effective disease control we can then consider the effective life of financial gain. There is no published work in this area and we therefore do not discuss it here further.

REDUCING SELECTION: PRINCIPLES, STRATEGIES, AND TACTICS

Reducing the Selection Coefficient

Milgroom & Fry (53) stated that the guiding principle of resistance management should be to reduce the selection coefficient, as defined in Equation 1. They used this principle to derive guiding strategies and then discussed how the effects of various tactics (mixing, adjusting dose, etc.) would explain results from their modeling study. The first two of the strategies discussed by Milgroom & Fry (53) are:

Strategy 1: Selection for fungicide resistance is reduced when the per capita rates of increase of both the sensitive and resistant strains (r_S and r_R, respectively) are reduced.

Strategy 2: Selection for fungicide resistance is reduced when the per capita rate of increase of the resistant strain (r_R) is reduced relative to that of the sensitive strain (r_S).

Staub & Sozzi (76) also considered exposure time. After a fungicide is sprayed on a crop, its efficacy decreases over time because of the decay of the fungicide (caused, variously, by plant metabolism, sunlight breaking down the fungicide, runoff by rain, etc.). The increase in frequency of the resistant strain thus depends not only on the numeric value of the selection coefficient just after the application but also on the time span over which the selection operates. From this, we can define a third strategy as:

Strategy 3: Selection for fungicide resistance is reduced when the time span over which selection takes place (exposure time) is reduced.

We can thus generalize Equation 1 to become

$$sT = (r_R - r_S)T, \qquad 2.$$

where T is the exposure time of the pathogen population to the fungicide. The aim of fungicide-resistance management then is to reduce sT, i.e., to reduce the product of the selection coefficient and exposure time.

Fungicide-Resistance Management Tactics

There are six tactics (**Figure 1**) that change just one aspect of a basic fungicide program. We first discuss what the effect of each tactic would be according to the three strategies (defined above) and

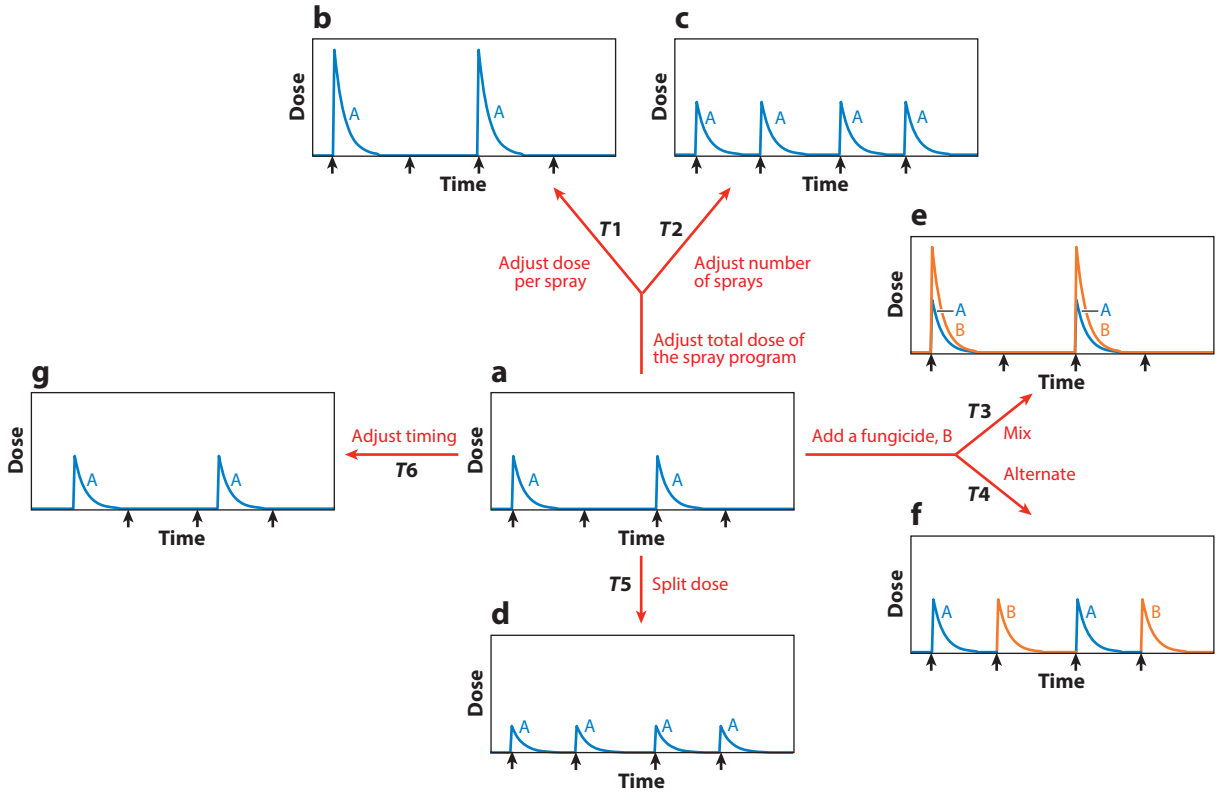

Figure 1

Resistance management tactics that change only one aspect of the default treatment program. The time points at which fungicide treatments may be applied are marked with arrows pointing to the x-axis in all the panels. For ease of representation, the fungicide dose decays rapidly so that the time windows in which the fungicide is active do not overlap. There are two fungicides of differing modes of action, fungicide A (*blue*) and fungicide B (*orange*), that can be used in the treatment program. The figure defines six tactics (numbered *T*1 to *T*6 and shown in panels *b* to *g*, respectively) that may increase or decrease selection or be neutral in their effect on selection for fungicide resistance. Each tactic is numbered and discussed in the text according to its number. Numbers *T*1, *T*2, etc. also correspond to those used in **Table 1**.

to the selection coefficient and exposure time principle. Next, we compare the prediction resulting from the reduced selection coefficient and exposure time with experimental and modeling evidence. Then, three further tactics (**Figure 2**) that change more than one aspect of the fungicide program but that describe practical program changes that maintain effective disease control are considered.

The available published evidence is given in tables that can be found in the online **Supplementary Material** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**); these tables are summarized in **Tables 1** and **2**. Most of the evidence relates to foliar-applied fungicide sprays.

Supplemental Material

Tactic (*T*1), adjusting fungicide dose. Changing dose is an example of reducing the growth rate of the sensitive strain (Strategy 2). The higher the dose applied, the greater the reduction in fitness of the sensitive strain, whereas the fitness of the resistant strain is unchanged or changes to a lesser extent compared to the resistant strain. This increases the fitness difference and thus increases selection. Strategy 2 thus predicts that an increasing dose should increase selection for fungicide resistance. All modeling studies and almost all experimental studies show increased

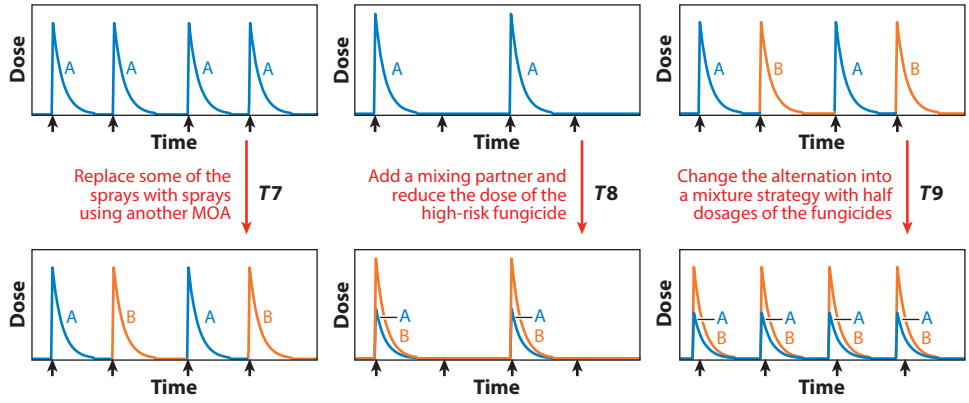

Figure 2

Resistance management tactics that change more than one aspect of the fungicide program. The possible time points at which a fungicide treatment can be done are marked with arrows pointing to the x-axis. Fungicide A (*blue*) and fungicide B (*orange*) are used in the treatment program. Each of the three tactics is numbered and discussed in the text according to its number. *T*7, *T*8, and *T*9 correspond to their counterparts in **Table 2**. Abbreviation: MOA, mode of action.

selection when the dose is increased (**Table 1**). We have summarized the explanation in the section "Interpretations," where we also discuss four hypothetical exceptions, although no experimental evidence currently exists to support these exceptions.

Conclusion (*T*1): The majority of the evidence suggests that an increased dose of fungicide increases selection for fungicide resistance.

Tactic (*T*2), adjusting the number of applications. Increasing the number of spray applications to a crop increases the time span over which the selection for fungicide resistance takes place (Strategy 3). We thus expect that increasing the number of sprays should increase selection for fungicide resistance. Although the number of published studies is small, both experimental and modeling studies show an increased selection with increasing number of sprays (**Table 1**).

Conclusion (*T*2): All current evidence suggests that an increased number of sprays increases selection.

Tactic (*T*3), mixing. The concept that a second fungicide might protect a high-resistance risk fungicide from the development of resistance arises from the expectation that mutants that are resistant to one fungicide mode of action would normally be sensitive to another with a different mode of action. Hence, the mixing partner has the same effect on the fitness of the resistant and sensitive strains. Therefore, both r_S and r_R are reduced by a similar percentage and, according to Strategy 1, the selection coefficient is decreased. We thus predict that mixing decreases selection for fungicide resistance.

The published experimental evidence (**Table 1**) shows that in the vast majority of cases, adding a mixture partner decreases selection for resistance. The published modeling evidence also shows that in most cases, mixing reduces selection (**Table 1**). Note, however, that nearly all the evidence is for cases in which resistance is only evolving to one of the mixture partners during the period studied.

Conclusion (*T*3): The vast majority of the evidence shows that adding a mixing partner to a high-resistance-risk fungicide reduces selection for fungicide resistance.

Table 1 Summary of the published evidence of the effectiveness of fungicide resistance management tactics[a,b,c]

Adjust the total dose of the spray program				
(*T*1) Adjust the dose of the sprays				
Increasing dose has the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	16	1	2	19
Models	8	0	0	8
(*T*2) Adjust the number of sprays				
Increasing the number of treatments has the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	6	0	0	6
Models	2	0	0	2
Adding a fungicide to the spray program				
(*T*3) Mixing				
Adding a mixing partner has the following effect on selection, experiments				
	Increase	No effect	Decrease	Total
Experiments	1	5	43	49
Adding a mixing partner has the following effect on selection, models				
	Reduce selection if spray coverage <95%; no difference if coverage >95%	Always reduce	Depends on parameter values	Total
Models	1	7	1	9
(*T*4) Alternation				
Alternation have the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	1	4	0	5
Models	0	2	0	2
Other tactics				
(*T*5) Split dose				
Splitting dose has the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	10	0	1	11
Models	2	0	0	2
(*T*6) Adjust spray timing				
Early sprays have the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	2	1	3	6
Models	1	0	1	2

[a]The tactics are explained in **Figure 1**; the numbers *T*1, *T*2, etc. in the table correspond to the numbers in **Figure 1**.
[b]For details, see the **Supplementary Materials**.
[c]References: 1, 3, 4, 6, 9–11, 15–21, 23–28, 30, 33–35, 37–44, 46–51, 53, 57–67, 68, 73, 74, 77, 80–83.

Supplemental Material

Table 2 Summary of the published evidence of the effectiveness of fungicide resistance management tactics[a,b,c]

(*T*7) Replace part of the sprays by sprays with another mode of action, alternation				
Alternation has the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	0	3	12	15
Models	0	0	3	3
(*T*8) Add a mixing partner and reduce the dose of the high-risk fungicide				
Mixing and reducing dose has the following effect on selection, experiments				
	Increase	No effect	Decrease	Total
Experiments	1	5	17	23
Adding a mixing partner has the following effect on selection, models				
	Reduce selection if spray coverage <95%; no difference if coverage >95%	Always reduce	Depends on parameter values	Total
Models	1	0	0	1
(*T*9) Change the alternation into a mixture tactic with half dosages of the fungicide				
Mixtures as compared to alternation has the following effect on selection				
	Increase	No effect	Decrease	Total
Experiments	2	4	6	12
Mixtures as compared to alternation has the following effect on selection				
	Reduce selection if spray coverage <95%; No difference or increase if >95	Always reduce	Depends on parameter values	Total
Models	2	2	1	5

[a]The tactics are explained in **Figure 2**; the numbers *T*7, *T*8, and *T*9 in the table correspond to the numbers in **Figure 2**.
[b]For details see the **Supplementary Materials**.
[c]References: 3, 6, 7, 11, 15, 16, 20, 21, 25, 32, 35, 36, 38, 41, 42, 45, 49, 57, 66, 68, 71, 74, 78, 83, 87.

Supplemental Material

Tactic (*T*4), alternating. Referring to **Figure 1**, here we discuss alternation, which is the process of including additional sprays with a fungicide that has a different mode of action between sprays of the basic spray program (**Figure 1*a–f***) (The replacement of sprays by another mode of action is considered later.). In a situation in which the decay rate is fast, meaning that the time windows during which the fungicides are active do not overlap, fungicide B is applied when there is no selection for resistance to fungicide A. This implies that fungicide B does not alter the selection coefficient, as there is no selection. The selection for resistance to fungicide A in the spray program found in **Figure 1*a*** is therefore the same as that in the spray program found in **Figure 1*f***. We thus predict that this type of alternation does not have an effect on fungicide-resistance buildup. The published experimental and modeling evidence agrees with this prediction, although the number of studies is small.[1]

[1]One study shows greater selection resulting from the alternation of iprodione and captan to control *Botrytis cinerea* in vineyards (57). The resistance of *B. cinerea* to iprodione increased at a higher rate when captan sprays were added to the treatment program. This can be explained if there is cross-resistance between the two fungicides. Fourie & Holz (22) report cross-resistance in *B. cinerea* for iprodione, and folpet is a member of the same fungicide group (phthalimides) as captan; thus it is possible that there is cross-resistance between iprodione and captan. Moreover, enhanced glutathione biosynthesis in *B. cinerea* is being discussed as a possible resistance mechanism in iprodione, folpet, and captan.

Conclusion (*T*4): The available evidence, although limited, suggests that alternating with a fungicide that has a different mode of action does not alter selection for the high-risk fungicide if the number of applications of the high-risk fungicide remains constant with and without alternation.

Tactic (*T*5), splitting fungicide applications. What happens if the total dose applied to a crop is constant but is distributed over two sprays? The dose per application is halved, and the time span over which selection takes place is doubled. These two changes act in opposing directions on selection, so it is possible that they could negate each other, leaving the effect on the frequency of the resistant strain unchanged. However, the selection coefficient is not linearly dependent on the fungicide dose. For small fungicide doses, an increase in dose strongly increases selection, whereas at high dose an increase in dose has a smaller increasing effect on selection. Hence, we expect the total change in the frequency of resistance to be larger for the split-dose treatment program (**Figure 1*d***) than for the standard program (**Figure 1*a***). In the section "Interpretations," we make this reasoning explicit and show that for known plausible shapes of the fungicide dose response curve, a split dose selects more strongly for fungicide resistance. Both the modeling and the experimental evidence are in line with this expectation, although the number of studies is small.

Conclusion (*T*5): Most evidence suggests that splitting a given fungicide dosage between two or more applications increases selection.

Tactic (*T*6), adjusting spray timing. At first sight, when the time of the application is shifted, neither the selection coefficient nor the time span over which the selection takes place changes. This may suggest that the change in the fraction of the pathogen population resistant to the fungicide should remain the same under two different spray timings. However, the mechanism by which a reduction in the growth rate of both pathogen strains reduces selection (as in Strategy 1) also applies to changes in growth rate caused by environmental and host variables. The specific timing of growth and unfolding of leaf layers, adult plant or seedling expression of cultivar resistance, changes in weather patterns, etc., may all cause the pathogen population to grow faster or slower during particular time periods or developmental phases of crop growth. Because crop-pathogen combinations differ in their responses to weather, cultivar resistance, crop growth dynamics, etc., it is not predictable whether the sprays will hit the pathogen at a higher or lower growth rate when the spray time is shifted, and thus whether this results in an increase or decrease, respectively, in the selection coefficient. This leads us to predict that specific attributes of the pathogen species, the crop cultivar, and the weather patterns determine whether a shift in spray timing affects selection. Although there are few studies, the experimental as well as the modeling evidence show that selection can increase, decrease, or remain unchanged (**Table 1**). We discuss these results in relation to protectant and curative use of fungicides in the section "Interpretations."

Conclusion (*T*6): The existing evidence suggests that the specific case and the specific spray program determine whether a shift in spray timing increases or decreases selection for fungicide resistance.

Reducing Selection While Achieving Effective Control

The tactics discussed so far only change one aspect of the spray program. This reductionist approach tests the strategies suggested by Milgroom & Fry (53). However, some of the tactics may compromise effective disease control and would thus not be acceptable. In practice, the choice is between different spray programs that exert a comparable level of effective disease control. There are three widely studied tactics that manage resistance while maintaining similar pathogen control levels.

Tactic (*T*7), replacing one or more sprays with a fungicide with another mode of action. Reducing the number of sprays with the high-risk fungicide reduces the time span over which selection takes place in the spray program, as covered by Strategy 3, which predicts that selection decreases. The sprays with the high-risk fungicide that are taken out of the program are replaced by applications with another mode of action. As discussed above, this does not affect selection. In general, we expect that selection decreases when this tactic is used and the experimental and modeling evidence supports this prediction (**Table 2**).

Conclusion (*T*7): The evidence suggests that replacing some of the applications in the fungicide program with a fungicide with a different mode of action reduces selection for fungicide resistance.

Tactic (*T*8), mixing the high-risk fungicide with a fungicide with another mode of action and reducing the dose of the high-risk fungicide. This tactic reduces the growth rate of both pathogen strains (Strategy 1) and also decreases the difference between the growth rates of the strains (Strategy 2). We thus predict that selection for fungicide resistance will decrease. The experimental evidence clearly supports this prediction (**Table 2**). There is only one modeling study that looks into this tactic and its conclusions support the prediction.

Conclusion (*T*8): Adding a mixing component to a high-risk fungicide and reducing the dose of the high-risk fungicide reduces selection for fungicide resistance.

Tactic (*T*9), comparing alternation and mixing. These two spray programs keep the total dose applied throughout the spray program constant for both the high-risk fungicide and the alternation/mixing partner fungicide. To keep pathogen control at a similar level throughout the spray program, the mixture uses half the dose of the alternative fungicide in each spray. Going from the alternation to the mixture tactic, we thus split the dose of the high-risk fungicide, A, and mix the high-risk fungicide with another fungicide, B. As discussed above, splitting the dose increases selection, and mixing reduces selection. It is then a matter of the balance between increasing and decreasing selection whether one or the other tactic is the better resistance management method. This suggests that this choice between alternation and mixing is a case-by-case decision.

The experimental and modeling evidence shows a variable pattern. Half of the experimental cases measure a smaller selection for the mixture spray program. Only two of the 12 cases show a smaller selection for the alternation treatment, and the other cases do not measure a difference between the selection imposed by mixing or alternation. The modeling evidence generally agrees with the trend in the experimental data, with many cases showing less selection in mixtures but some showing less selection when using alternation.

Conclusion (*T*9): Whether mixing reduces selection compared to alternation depends on the balance between increased selection due to dose splitting and decreased selection due to mixing. The experimental and modeling evidence shows that, in many cases, mixing is the better strategy, but for any single case this needs to be established before implementing.

A Fourth Strategy

Milgroom & Fry (53) introduced another strategy:

Strategy 4: Reducing the initial frequency of resistance has a major effect on the success of resistance management strategies.

There are four modeling studies that consider the effect of the initial frequency of resistance in the pathogen population (29, 37, 43, 53). Each study found that initial frequency has a surprisingly strong effect on the time it takes for the frequency of resistance to reach such levels that effective control starts to be compromised. For example, Kable & Jeffery (37) found that

it takes many applications for the frequency of resistance to grow from 10^{-9} to 10^{-2}, with no consequences for effective control, but only a few sprays to bring it from 1% up to 100%, completely compromising effective control. Simulations by Hobbelen et al. (29) suggest that it takes approximately 5 years for the resistant strain to increase in frequency from 10^{-12} to 1% but only 2 years to subsequently increase to virtually 100%. There is no experimental evidence because the analytical techniques available have, up until now, not been sensitive enough to quantify such small resistance frequencies.

This strategy is different from the other strategies discussed because there are no treatment programs that reduce the initial frequency of resistance, but it implies that the appropriate tactic is to introduce a resistance management program at the moment a new mode of action is introduced. Waiting until resistance has emerged and is detected in field populations and then putting a resistance management program in place is unlikely to be effective, unless the resistance is of a slow-shifting type. We are not the first to make this important point, yet discussions about resistance management often start at the moment resistance is found in field populations.

SUSTAINABILITY OF DISEASE CONTROL: THE FUNGICIDE EFFECTIVE LIFE

Fungicides are used to control disease, so fungicide programs designed for resistance management must also deliver effective control. The key question is, therefore, which strategies yield effective control and at the same time maximize the effective life of a fungicide?

Direct experiments on fungicide effective life would be extremely time consuming and difficult or impossible to conduct. Hence, we must use theoretical and modeling data. Here, we summarize whether conclusions on the effectiveness of fungicide-resistance management tactics are different when effective life rather than selection is considered. The few papers written about effective life (28, 29, 85) suggest the following:

1. Effective life increases if selection decreases when studying the effect of fungicide dose, fungicide mixtures, alternation, and split dose of sprays (28, 29, 31, 84). For dose mixing, alternating, and splitting, we can thus use selection to show qualitative trends in the effectiveness of fungicide-resistance tactics and infer qualitative trends in effective life.
2. Selection may not work in the same direction as effective life when studying fungicide application timing (84). For spray timing we have, for example, found that there are time periods during which applications result in high selection rates as well as high levels of disease control and other time periods during which low selection and low disease control coincide. In the particular case of *Mycosphaerella graminicola* and a QoI-type fungicide, fungicide effective life was often maximized by a spray timing that resulted in relatively high selection rates (84). The reason for this phenomenon is that high levels of effective control coincide with the high levels of selection, making it possible to reduce the fungicide dose to such an extent that effective control was maintained over a longer series of cropping seasons.

Further work on effective life and its relationship to selection rates is needed. Selection may be a good indicator to test fungicide-resistance management tactics when considering dose, mixtures, alternation, and split dose, but not when studying spray timing.

INTERPRETATIONS

In this section, we further discuss the results of certain tactics analyzed in the previous sections. We discuss fungicide dose and compare it with insecticide resistance. We also discuss in more detail

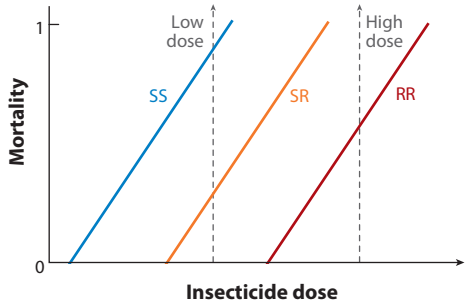

Figure 3

The mortality rate of an insect species as a hypothetical function of the insecticide dose applied. The line marked SS is the dose response of homozygote-sensitive individuals, the line marked SR is the response for heterozygotes, and the line marked RR is the response for homozygote-resistant individuals.

the reasons why split-dose treatments increase selection. Lastly, we connect the findings from the tactic of adjusting spray timing (*T6*) to the discussion on protectant versus curative fungicide treatment.

Further Notes on Tactic *T1*: The Fallacy That High Dosages Reduce the Risk of Resistance

The use of a high dose to reduce the risk of resistance is often suggested in discussions on fungicide-resistance management. Yet almost all the objective evidence suggests that for a given number of applications, increasing dose increases selection for fungicide resistance. We have discussed the effects of dose amounts with plant pathologists, agronomists, and the crop protection industry. What we found is that some of the ideas about using high doses to reduce the risk of resistance may have arisen from the insecticide resistance literature. Many insect species are diploid and reproduce sexually. This has major consequences for the effect of dose on selection for resistance. Consider **Figure 3**, where the kill rate of the insecticide is plotted as a hypothetical function of dose. There are three genotypes, the homozygote sensitive (SS), the homozygote resistant (RR), and the heterozygote (SR). The sensitivity of the heterozygote to the insecticide is intermediate to that of the two homozygotes. When the frequency of resistance is small in sexually reproducing populations, virtually all resistance genes are found in heterozygote individuals.

This is because any homozygote-resistant individual is most likely to mate with a homozygote-sensitive individual because the homozygote-resistant individuals make up the vast majority of individuals in the population. The outcome of this cross is heterozygote individuals only. Applying a high dose of the insecticides removes many resistance genes from the population before the frequency of resistance becomes high enough for a significant density of homozygote-resistant individuals to build up. In this case, applying a high dose reduces the probability of a homozygote-resistant individual crossing with a homozygote-resistant individual, and may therefore be a good method to delay the buildup of resistance.

In contrast, most plant pathogens are either haploid during the time window when fungicide is applied or are clonal. In both cases, the reasoning of insecticide resistance does not apply. A higher dose reduces the density of sensitive individuals more than the density of resistant individuals and thus a high dose increases selection for resistance. We refer the reader to a paper by van den Bosch et al. (86) for more detail.

We have found four hypothetical mechanisms through which a high fungicide dose may decrease selection (86).

1. Fungicides increase mutation rates. It is known for bacteria that stress (including antibiotic treatments) can increase the mutation rate by suppressing proofreading during DNA replication (2, 8, 55, 64, 79). There is currently only one conference abstract (69) in which an experiment suggests an effect of the fungicide on mutation rates. If fungicides do increase mutation rates, the balance between stress-induced mutation rates and the effect of the fungicide on pathogen density determines whether an increased dose reduces or increases the extent of emerging fungicide resistance.
2. Mutation limitation. An increased fungicide dose decreases the population density of the sensitive population, which reduces the number of resistance mutations per time unit, potentially slowing down the emergence of resistance. The decreased density of the sensitive strain decreases competition, thus making it easier for the resistant mutant to establish and build up a population. It is the balance between these two processes, mutation and establishment, that determines whether an increased dose increases, reduces, or does not affect the length of time it takes for a resistant strain to emerge. There is some evidence from mathematical models (31, 52) that this mechanism does not lead to dosage increases reducing the risks of fungicide resistance.
3. Pathogen refugia. Shaw (72) suggested that the high-dose refugia strategy advocated in insecticide resistance management may operate in plant pathogens even though they are mostly haploid and/or clonal.
4. Converging dose response curves where the dose response curve of a population consisting of sensitive strains only and the dose response curve of a population consisting of resistant strains only converge at higher fungicide dosage. The larger the distance between the dose response curves, the larger the relative fitness of the resistant strain. This implies that when, at larger dosages, the dose response curves converge (within the range of doses that are permitted), selection for fungicide resistance decreases. This mechanism has been shown for herbicide resistance (56) and causes high dosages to select less for fungicide resistance.

As Neve & Powles (56) discuss, the dose response curves of a population consisting of the sensitive strain and of a population consisting of the resistant strain do not converge when there is a single target site mutation conferring a high level of resistance (at least not within the permitted dose range). The resistance developing against single-site fungicides is usually of this type. It is thus unlikely that the mechanism is of relevance to most cases of resistance against single-site fungicides. If resistance develops by successive mutations, each adding a small contribution to the level of resistance of the pathogen to the fungicide (such as in the case of azole insensitivity in *M. graminicola*), the mechanism might operate. Although we consider this a possible mechanism that may result in increased fungicide dosage reducing selection for fungicide resistance, there is no evidence in the current literature that shows convergence of dose response curves of sensitive and resistant strains.

We refer the reader to van den Bosch et al. (85) for more details on each of these mechanisms. We have to stress here, however, that these are four hypothetical mechanisms that, if they operate, could in certain circumstances cause a higher fungicide dose to select less strongly for resistance. There is currently no evidence that suggests that these mechanisms operate for fungicides.

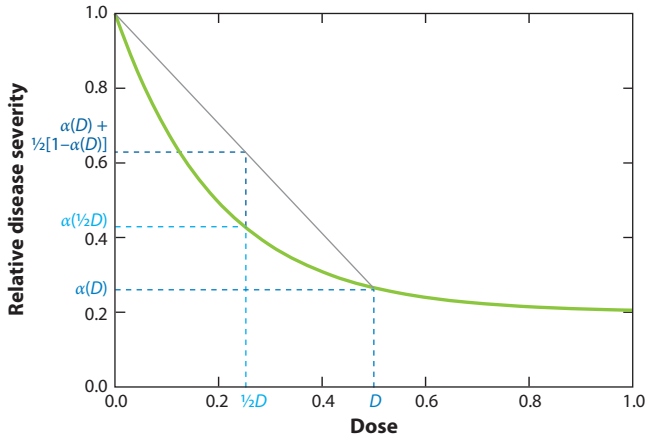

Figure 4

Dose response curve of a fungicide. Dose amount is found on the x-axis, and the fraction, α, of the rate of increase of the sensitive strain, r_S, as a function of application dose is found on the y-axis. For the split dose, we need to consider the original dose, D, as well as half that dose, $1/2D$. The figure shows that $\alpha(1/2D)$ is smaller than the value of α half-way between the straight line between the points (0, 1) and $[D, \alpha(D)]$.

Further Notes on Tactic *T5*: Split-Dose Treatments

Splitting dose increases selection for fungicide resistance. In this section, we make the reasoning in the text explicit and show that, independent of the exact shape of the dose response curve, the selection due to a split dose is higher than the selection due to a single higher dose application.

Here, we restrict the analysis to fungicides for which absolute resistance evolves. The same findings also hold for cases where partial resistance develops but the notational complexity increases. The fungicide affects the rate of increase of the sensitive strain, and we denote by $\alpha(D)$ the fractional reduction in the rate of increase of the sensitive strain when experiencing dose D of the fungicide. Obviously, $\alpha(D)$ decreases with dose. We thus have

$$r_S = r\alpha_S(D),$$

$$r_R = r.$$

The selection coefficient for the single dose application is given by

$$s\,T_{single\ application} = r_R - r_S = r[1 - \alpha(D)].$$

In the split application, the dose is halved but the fungicide is applied twice. The selection coefficient for the split dose application is thus given by

$$s\,T_{split\ dose} = 2r[1 - \alpha(0.5\,D)].$$

Published fungicide dose response curves from field experiments are seldom expressed as the theoretical sigmoid-shape on a log-log scale but rather are decreasing functions of dose on a linear scale throughout the dose range. Any published fungicide dose response curve has the following properties (see **Figure 4**):

$$\alpha(0) = 1, \frac{d\alpha}{dD} < 0 \text{ and } \frac{d^2\alpha}{dD^2} > 0.$$

The figure shows that $\alpha(0.5\ D) < \alpha(D) + 0.5\ [1 - \alpha(D)]$. This then implies that $\alpha(0.5\ D) < 0.5 + 0.5\ \alpha(D)$ or $-\alpha(0.5\ D) > -0.5 - 0.5\ \alpha(D)$. Adding 1 to both sides and multiplying both sides by $2r$ we find that $2r\ [1 - \alpha(0.5\ D)] > r\ [1 - \alpha(D)]$, showing that the selection due to a split dose is larger than the selection of the single dose, independent of the exact shape of the dose response curve.

Further Notes on Tactic *T6*: Selection for Fungicide Resistance and Protectant versus Curative Fungicide Treatment

The terms protectant and curative fungicide applications are sometimes used loosely to describe fungicide applications early(ier) and late(r) in the epidemic season. Strictly, protectant refers to preventing infections from occurring, i.e., reducing the pathogen's infection efficiency. Curative refers to fungicides that are systemic and change life-cycle parameters other than infection efficiency, i.e., increased latent period, reduced spore production rate, and/or shortened infectious period.

At a leaf layer level, these definitions are easily applied. If a new leaf layer of, for example, a wheat crop has just fully unfolded, a fungicide can be used to protect the leaf layer from infection. When the fungicide is applied at a later stage, when infections have already taken place and the fungicide mainly targets the other life-cycle parameters, this is curative use. At a crop level, it is less clear how protectant and curative use should be defined, unless infection events are restricted to small windows in time and occur infrequently. For many pathosystems in which infection conditions are frequent, any fungicide application timing provides both protection (on newly emerged leaves) and eradication (on earlier-emerged leaves that carry latent infections).

Antiresistance guidance (such as from the Fungicide Resistance Action Committee) advises against the curative use of fungicides. Two hypotheses that could provide a rationale for such a recommendation have been put forward (5).

1. Many fungicides come to the market as formulated or tank mixtures, where the mixing component is a multisite-acting, nonsystemic fungicide that acts only as a protectant. Hence, curative use of the mixture implies that the nonsystemic fungicide has a smaller contribution to disease control and thereby less effect on selection. In protectant applications, this nonsystemic protectant fungicide reduces the growth rate of both sensitive and resistant pathogen strains and thus reduces selection.
2. The opportunity for selection is lower when the population is kept permanently low by applying early/protective sprays to retard the buildup of the pathogen population. Delaying the fungicide application until a threshold pathogen population appears means that many sporulating lesions are exposed to the fungicide. We note here that this hypothesis has no mechanistic foundation with respect to selection because selection is concerned with the frequency of strains in the pathogen population, not with absolute densities. This hypothesis may have some logical basis for the emergence phase but that remains to be studied in more detail.

Milgroom & Fry (53) commented on the absence of evidence to support the view that curative use of fungicides should be avoided. Almost 20 years later, Brent & Hollomon (5) made a similar statement: "To the authors' knowledge there is no experimental evidence comparing the resistance risks of prophylactic versus threshold-based schedules, and research on this would be useful." The experimental and modeling evidence we have found (five and two papers, respectively) and discussed under Tactic *T6* is inconclusive. Both increased and decreased selection have been observed for earlier compared with later sprays. This inconclusiveness may be because the number

of studies is very small or because the outcome of changing spray timing and protective versus curative use differs from case to case.

DISCUSSION

We have summarized the existing experimental and theoretical evidence on fungicide-resistance management tactics. These tactics were compared with predictions made using the principle of minimizing the selection coefficient and exposure time as defined in Equation 2. This principle leads to three fungicide-resistance management strategies:

1. Reduce the rate of increase of both the sensitive and the resistant strain.
2. Reduce the difference in the growth rate of the strains.
3. Reduce the time span over which selection occurs.

We used these strategies to predict the effect on resistance development for a range of tactics and found that the vast majority of the evidence agrees with the predictions. In 84% of the cases, the outcome of an experiment agreed with the predictions made on the basis of the three strategies. In only 5% of the cases did the outcome of experiments contradict the predictions. Even with the caveat discussed below we conclude that the product of the selection coefficient and the exposure time, sT, provides a framework that can be used to develop fungicide-resistance management tactics. Where predictions were difficult or impossible to make with the sT strategies, such as in the case of spray timing, the experimental and modeling evidence was inconclusive. These tactics thus need to be tested for specific fungicide-pathogen-crop combinations.

Some care is needed when interpreting published evidence, as results of experiments in which no difference between treatments is found have a smaller chance of being published than results of experiments in which a difference is found. This may cause the effectiveness of a resistance management strategy to be overestimated. However, the balance between the number of studies showing increasing or decreasing selection shows a very clear pattern that closely follows the predictions based on principles governing fungicide-resistance management. Further, the available evidence mainly relates to cases in which a single mutation causes a high level of resistance against the fungicide. From an experimental viewpoint, it is easiest to study such big-step cases because the assessments are relatively easy. The published evidence is therefore short of cases in which genetic changes lead to small changes in sensitivity to the fungicide and of cases in which a range of mutations/genetic changes cause a slow shift of sensitivity over time.

Almost all experimental evidence is from foliar applied sprays. Very little evidence exists about selection for resistance due to seed treatment. This is a knowledge gap that needs to be considered urgently.

Our exhaustive survey of published material has shown that many of the fungicide-resistance management tactics have been studied, but for some the evidence is weak. Moreover, as discussed in the section "Interpretations," there are several aspects of resistance management that have not been considered. These areas justify more research. Industry and regulatory decisions about fungicide-resistance management often cannot wait for the accumulation of new evidence, so decisions should be made by weighing the existing evidence and making judgments about the consequences should those decisions prove to be wrong. With this review, we hope to make a contribution toward such evidence-based resistance management.

LIST OF KEY FINDINGS

1. **Governing principle:** A governing principle exists that predicts whether adjustments in spray programs will reduce or increase the selection for fungicide resistance. This principle

states that reducing the product of the selection coefficient and the exposure time, sT, reduces the buildup of fungicide resistance. The selection coefficient and exposure time are defined as

$$sT = (r_R - r_S)T,$$

where r_S is the growth rate of the sensitive strain, r_R is the growth rate of the resistant strain, and T is the exposure time of the pathogen population to the fungicide.

2. **Fungicide-resistance management strategies:** This governing principle leads to three fungicide-resistance management strategies.
   Strategy 1: Selection for fungicide resistance is reduced when the per capita rate of increase of both the sensitive and the resistant strain (r_R and r_S, respectively) is reduced.
   Strategy 2: Selection for fungicide resistance is reduced when the per capita rate of increase of the resistant strain (r_R) is reduced relative to that of the sensitive strain (r_S).
   Strategy 3: Selection for fungicide resistance is reduced when the time span over which selection takes place, also known as exposure time, is reduced.
3. **Fungicide-resistance management tactics:** In 84% of the cases, the outcome of an experiment agreed with the predictions made on the basis of the three strategies. In only 5% of the cases did the outcome of experiments contradict the predictions. The selection coefficient and the exposure time, sT, provide a framework that can be used to develop fungicide-resistance management tactics.

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