Constructing optimal mixtures of high-risk fungicides depending on chemical choice, levels of resistance and pathogen sexual reproduction

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Abstract

Crop growers commonly use mixtures of high risk fungicides to control crop pathogens such as *Zymoseptoria tritici*. Existing work suggests that optimal mixtures of pairs of high risk fungicides select equally for single resistant strains in the first year. However, current modelling of optimal fungicide mixture construction does not address the important real-world case in which the initial levels of resistance to each fungicide differ. We show that the equal selection strategy often fails in this case and demonstrate a simple alternative approach which works in a wide variety of possible scenarios for different pathogen and fungicide parameters. Further, we show that inclusion of pathogen sexual reproduction in the model can qualitatively effect the optimal recommendation. The strategy we demonstrate gives useful intuition about general principles for constructing optimal fungicide mixtures depending on the characteristics of the pathosystem, the dose response curves and their decay rates.

Introduction

World food security faces multiple threats, including the growing global population (***Godfray et al., 2010***), climate change (***Tai et al., 2014***) and plant disease (***Strange and Scott, 2005***). Despite annual spending of roughly 16 billion US dollars on fungicides globally, estimated crop losses still stand at 20% (***Jorgensen et al., 2017***). Fungicide resistance challenges our ability to maintain control of plant diseases. Effective resistance management strategies prolong control of yield-limiting crop diseases and help growers maintain good yields for longer. An estimated 70% of the European cereal fungicide market is primarily targeted towards the management of *Zymoseptoria tritici* of winter wheat (***Torriani et al., 2015***), which is the pathosystem this study focuses on.

A common resistance management strategy is to use fungicide mixtures with more than one mode of action present in the mixture. Often in practice these mixtures contain two fungicides that are high-risk for development of resistance (). These mixtures are of increasingly relevance since there are few low-risk fungicides available and the high risk options are typically of higher efficacy (***van den Bosch et al., 2014b***). Previous modelling studies have found fungicide mixtures to be more effective as a resistance management tactic than alternating use of fungicides (***Elderfield et al., 2018***) or spatially concurrent applications (***Hobbelen et al., 2013***). That mixtures outperformed alternations or concurrent use was robust to fitness costs, partial resistance, changes in fungicide parameters and the initial frequency of the double resistant strain. For this reason we will pursue the optimal strategy for mixtures of high-risk fungicides and neglect the other two approaches.

It was reported by ***van den Bosch et al.*** (***2014b***) that, across 17 publications, mixtures of high-risk fungicides resulted in a reduction in selection for resistance in 20 out of 24 pathogen-crop-fungicide combinations. There is ongoing debate about how high-risk mixtures should be constructed. Although the so-called 'governing principles' (***van den Bosch et al., 2014a***) suggest that increasing fungicide dose increases selection for that mode of action, increasing dose of a mixing partner can reduce selection for the other mode of action in the mixture (***van den Bosch et al., 2014b***). Modelling work shows that the optimal way to mix a low-risk and a high-risk fungicide is to use the maximum dose of the low-risk chemical and the minimal viable dose of the high-risk chemical (***Hobbelen et al., 2011a***; ***Elderfield et al., 2018***). However, maximising the dose of one fungicide when the mixture contains two high-risk chemicals could lead to excessive selection pressure on that fungicide, so a new recommendation is required.

Modellers often separate fungicide resistance evolution into an emergence and a selection phase (***van den Bosch and Gilligan, 2008***; ***Milgroom, 1990***; ***van den Bosch et al., 2011***). The former concerns the initial stochastic phase where new resistant strains appear through random mutation and invasion. We focus on the selection phase, which is where the pathogen population changes once fungicide treatments are used and a corresponding selection pressure applied.

Hobbelen et al. (***2013***) use modelling to address the case where the fungicide mixture contains two high-risk chemicals. They consider four pathogen strains - one that is resistant to both chemicals, one that is sensitive to both, and two more that are sensitive to one fungicide but resistant to the other. Their results suggest that the choice of doses used is critically important to the resulting durability of the strategy. ***Hobbelen et al.*** (***2013***) suggest the optimal fungicide mixture has a dose pairing that is as weak as possible whilst achieving sufficient yield, and selects equally for both single resistant strains. These authors addressed the case where both single resistant strains are initially at the same frequency, and explored what happens for different amounts of the double resistant strain. However, that study did not address the common real-world scenario where the initial levels of resistance to the two chemicals differ. The optimal strategy in this case is not described in the literature and is the focus of this paper. This scenario would commonly occur due to differing natural incidences of resistant strains, or because one fungicide was introduced to market much earlier than its mixing partner.

Different fungicides can be described by 'dose-response curves' which are measures of their efficacy. These curves will differ depending on the mode of action and effectiveness of each chemical. The effect of the dose response curves and initial levels of resistance on the optimal dose combination has to the best of our knowledge not been described in the existing literature. A clear understanding of their effect on the optimal strategy would assist in decision making when constructing mixtures of pairs of existing fungicides as well as when new chemicals come on to the market.

Septoria, like many other crop pathogens, has a sexual stage to its reproductive cycle (). The proportion of sexual reproduction in the within-season and between-season is unknown, with different experiments finding different measured values of both (***Chen and McDonald, 1996***; ***Zhan et al., 1998***). It is known that sexual ascospores initiate Septoria epidemics (). It is unknown how much pathogen reproduction effects epidemic severity and pathogen population demographics. Most fungicide resistance modelling studies do not consider sexual reproduction (). We seek to understand the effect of inclusion of sexual reproduction of the pathogen on the resulting recommendation.

In this paper we show that the initial resistance frequencies are a critical factor in determining the optimal strategy. We demonstrate a simple recommendation that works even if these initial resistance frequencies differ, in contrast to any tactic recommendation currently found in the literature. We extend the models from ***Hobbelen et al.*** (***2011b***, 2013) to consider the effect of sexual reproduction between growing seasons, and demonstrate that our results are valid even if sexual reproduction is present.

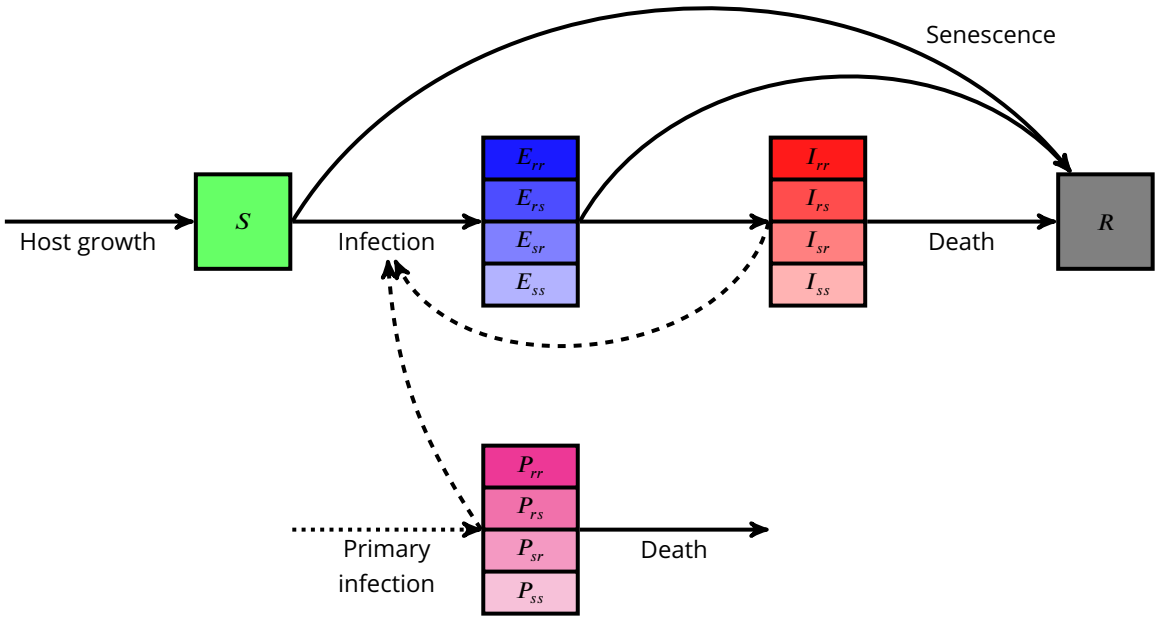

Figure 1. Model for pairs of high risk fungicides. The diagram depicts the within-season dynamics. The solid lines represent transitions, the dashed lines effects (for example the amount of infectious tissue affects the rate of infection) and the dotted line represents the instantaneous arrival of primary inoculum at the beginning of each season in the model. The four pathogen strains considered are denoted ss, rs, sr and rr. These strains are (respectively) double sensitive, two single-resistant, and the double resistant strain.

Methods

The model is an adapted version of one presented by (***Hobbelen et al., 2013***), which addresses a two high-risk fungicides used together to control Septoria. A full explanation of the model structure is given in Appendix 3.

The model is a compartment-based model – we deal with different categories of tissue. After infection, healthy (susceptible) tissue (S) transitions to exposed tissue (E) (infected but not infectious) and then to infectious tissue (I), before removal (R). The initial infection is given by a primary inoculum (P). The model also includes growth and senescence of living tissue. The subscripts s and r denote whether the pathogen strain is sensitive or resistant to fungicide application (Figure 1).

We adjust the model presented by (***Hobbelen et al., 2013***) in several ways. We consider one field instead of two, since we neglect the less effective concurrent field strategy. We extend the model to consider the possibility of between-season sexual reproduction of the pathogen, because Septoria's sexual ascospores are reported to contribute to a large proportion of the primary inoculum that initialises each epidemic (***Eriksen et al., 2001***). We denote this proportion p_X, and initially set $p_X = 0$ in line with ***Hobbelen et al.*** (***2013***) before exploring its effect when $p_X > 0$.

Definitions

Effective life

The term *effective life* is used by ***Hobbelen et al.*** (***2013***, 2011a) to denote the number of years for which a fungicide application tactic, or a fungicide itself, will be effective. By effective we mean we achieve a yield above a certain threshold, which we set at 95% of the disease-free yield, matching the assumption used by ***Elderfield et al.*** (***2018***). The assumption is that a grower requires a yield above this threshold for economic reasons. Below this yield the crop becomes uneconomic and the grower would look for alternative options.

Selection ratio

Hobbelen et al. (***2011b***) use the *selection ratio* as a measure of how strongly a particular tactic results in selection for the resistant strain.

They define the selection ratio to in terms of the frequency of resistant strains in consecutive years. We modify this definition to be the ratio at the start and end of a growing season. This is equivalent in the case where there is no sexual reproduction, and it is a better representation on the effect of the strategy chosen if there was sexual reproduction and the initial population used in the model was in frequencies that differed greatly from a fully sexual population.

The selection ratio can be written as:

$$SR_{i,N} = \phi_{i,N_{end}} / \phi_{i,N_{start}}. \tag{1}$$

Here ϕ could represent a single pathogen strain, or the sum of more than one pathogen strain. For example, we might consider the sum of one of the single resistant strains and the double resistant strain, since this combination represents the total fraction of the pathogen population which has resistance against one of the fungicides.

If the selection ratio for a fungicide is greater than 1 then the resistance frequency has increased in year N. Successful resistance management strategies are described by ***Hobbelen et al.*** (***2013***) in terms of minimising the selection ratio.

Results

Initially we will assume that there is no sexual reproduction in the model. Later on we will change the parameter p_X to explore what happens when there is a non-zero proportion of sexual reproduction between seasons in the model.

Through the course of the season, the four pathogen strains are affected by the fungicide mixture to different extents (Figure 2). This leads to selection for resistant strains and a gradual loss in yield due to reduced control of the pathogen (Figure 3).

What is the optimal way to mix an identical pair of high risk fungicides?

Initially, we explore the scenario where both chemicals used are of equal efficacy, and the initial frequency of resistant strains is the same for both chemicals. We seek to find the most durable fixed dose strategy (the strategy which gives the longest effective life). This case is introduced as a readily understandable base case, but we will relax this assumption later.

Equal efficacy means that we assume that the decay rate and dose response curves are identical for the chosen pairing. Both are set to match the parameterised curve and decay rate for pyraclostrobin as used in ***Hobbelen et al.*** (***2011a***); ***Elderfield et al.*** (***2018***).

We will develop a prescription for the optimal strategy, which depends on the initial frequencies of resistance. Later we will relax the assumption that the chemicals are of equal efficacy.

Generalising the equal initial resistance frequency scenario

We are interested in the optimal region within 'dose space', corresponding to the dose combinations which result in the longest effective life. This is a region rather than a single pair of doses, since there may be multiple dose combinations which break down in the same (optimal) year. Typically these optimal combinations are all clustered close to each other in dose space. For a given model parameterisation, the optimal region differs for different initial resistance frequencies.

To find this optimal region, we use a grid of dose choices for the first chemical and the second (labelled fungicide A and B respectively). This method was previously used by ***Hobbelen et al.*** (***2013***) to explore dose space. ***Hobbelen et al.*** (***2013***) assert that the most durable strategy usually exerts 'approximately equal selection pressure on both single-resistant strains', with the exception of 'the scenario where strains were completely resistant to fungicide A and partially resistant to fungicide B'. They considered the case where the frequencies of the single resistant strains were

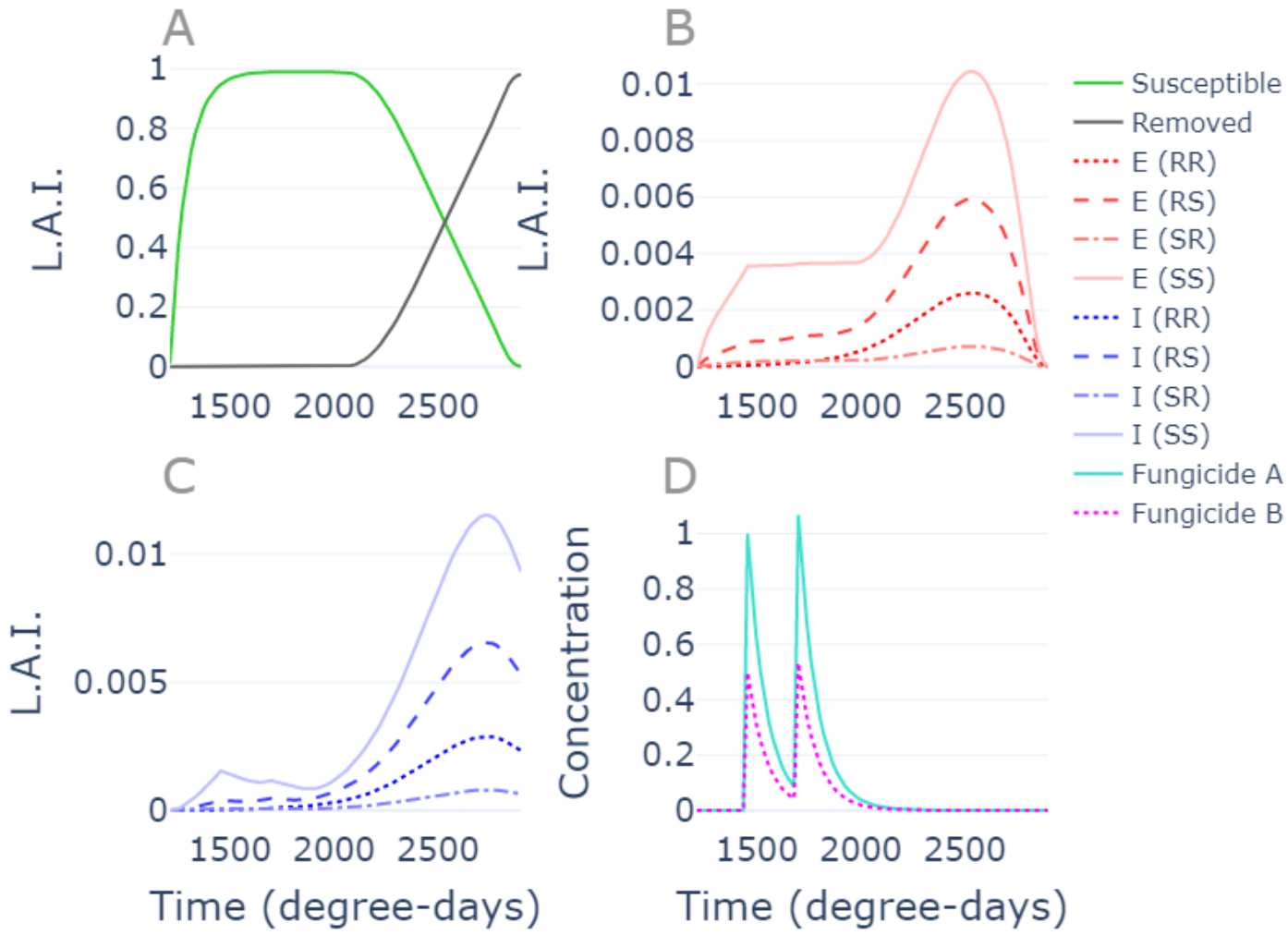

Figure 2. Disease progress curves from the model, which includes host growth, senescence, fungicide decay over time and density dependent pathogen growth.
Parameter values: default values as used by ***Hobbelen et al.*** (***2013***), doses: $(1, 0.5)$. Resistance frequencies: $(2 \times 10^{-1}, 5 \times 10^{-2})$. Fungicides A and B parameterised to match efficacy of pyraclostrobin.

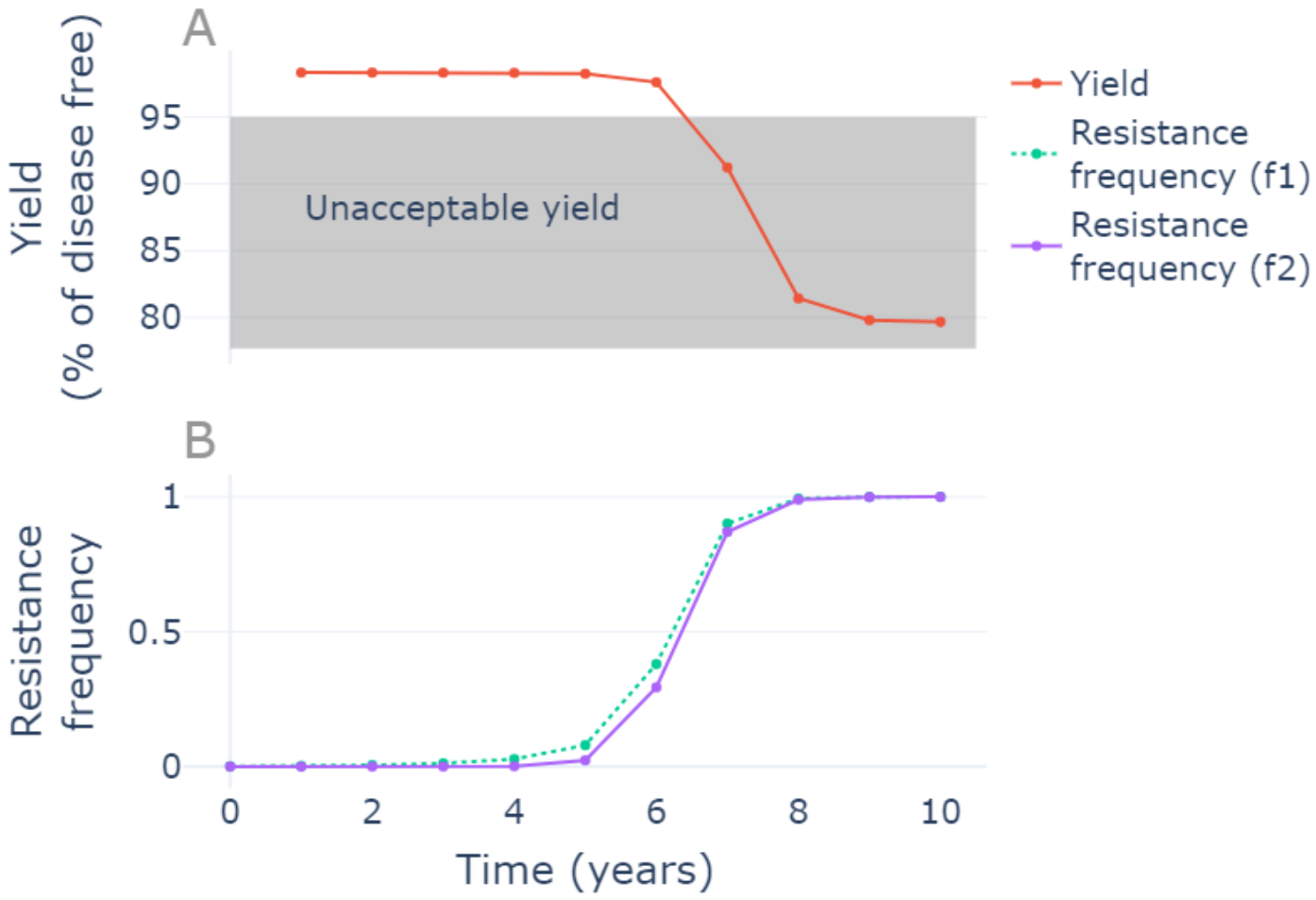

Figure 3. Plot A shows how the yield declines very slowly at first before a steep drop from years 6-8 takes it below the 95% 'unacceptable yield' threshold. Plot B shows how the levels of resistance correspondingly increase. The levels are initially very low but their rapid increase causes the loss in disease control and yield breakdown.
Parameter values: default values as used by ***Hobbelen et al.*** (***2013***), doses: $(1, 0.5)$. Resistance frequencies: $(2 \times 10^{-3}, 5 \times 10^{-5})$. Fungicides A and B parameterised to match efficacy of pyraclostrobin.

equal initially. In the supporting information, they show how equal selection in the first growing season gives the best outcome.

However, if the initial frequencies of the resistant strains differ, then this recommendation can fail to give the optimal outcome even in cases where both single resistant strains are completely resistant to their respective fungicides (Figure 4). Further, we would typically expect initial levels of resistance to differ, and if the fungicides were introduced to market at different times we would expect large differences in levels of resistance when the candidate high risk mixture is first used.

Contours in dose space

Equal selection contour

We will define a metric Δ_S as follows:

$$\Delta_S = \frac{SR_{A,1}}{SR_{A,1} + SR_{B,1}}, \tag{2}$$

where $SR_{i,1}$ is the selection ratio for fungicide i in year 1. Then $\Delta_S = 0.5$ means that there is equal selection for both fungicides. This relates to the first year of chemical application.

Then there is a contour in dose space defined as the line along which $\Delta_S = 0.5$. This corresponds to all the doses which select equally in year one for both fungicides. This contour is crucial to the prescription from ***Hobbelen et al.*** (***2013***) that equal selection in the first year, and minimal doses are best. In general we will explore any dose along this contour rather than only considering minimal doses. This gives the Equal Selection strategy a better chance at finding a good dose combination than if we just considered the minimal dose case.

Equal resistance frequency at breakdown contour

To explore the difference in effects of the two chemicals on the system, we will define another quantity: Δ_{RFB}. This is defined in terms of the (logits of the) resistance frequencies at breakdown:

$$\Delta_{RFB} = \text{logit}(R_A) - \text{logit}(R_B), \tag{3}$$

where

$$\text{logit}(x) = \log_{10}\Big(\frac{x}{1-x}\Big), \tag{4}$$

and, taking I^*_{mn} to be the amount of strain mn at the end of the breakdown season:

$$R_A = (I^*_{rr} + I^*_{rs})/\Big[\sum_{m,n\in\{s,r\}} I^*_{mn}\Big], \tag{5}$$

$$R_B = (I^*_{rr} + I^*_{sr})/\Big[\sum_{m,n\in\{s,r\}} I^*_{mn}\Big]. \tag{6}$$

This quantity informs us about the state of the system in the breakdown year, and whether our strategy led to a greater degree of resistance to one fungicide more than its mixing partner.

If the frequencies are equal then $\Delta_{RFB} = 0$. If there is more resistance to fungicide A than B, Δ_{RFB} is positive, but if there is more resistance to fungicide B than A then Δ_{RFB} is negative. In general there is a contour described by $\Delta_{RFB} = 0$. We will refer to this as the Δ_{RFB} contour.

For this model parameterisation, some points along the Δ_{RFB} contour lie in the optimal region. For these points, resistance frequencies in the breakdown year are equal. We seek to test whether there are optimal doses along the Δ_{RFB} contour for other fungicide and pathogen parameterisations (Figure 4).

Note that there are also points along the Δ_{RFB} contour that are not optimal - this is because the strength of the mixture must be carefully chosen according to the fungicide/pathogen parameters. This process is examined in the wider parameter scan later in the results section.

In the case where the initial resistance frequencies are the same, this recommendation is equivalent to equal selection in the first year as found in (***Hobbelen et al., 2013***). However, the equal selection recommendation may not work if the initial resistance frequencies (and fungicide parameterisations) are not identical.

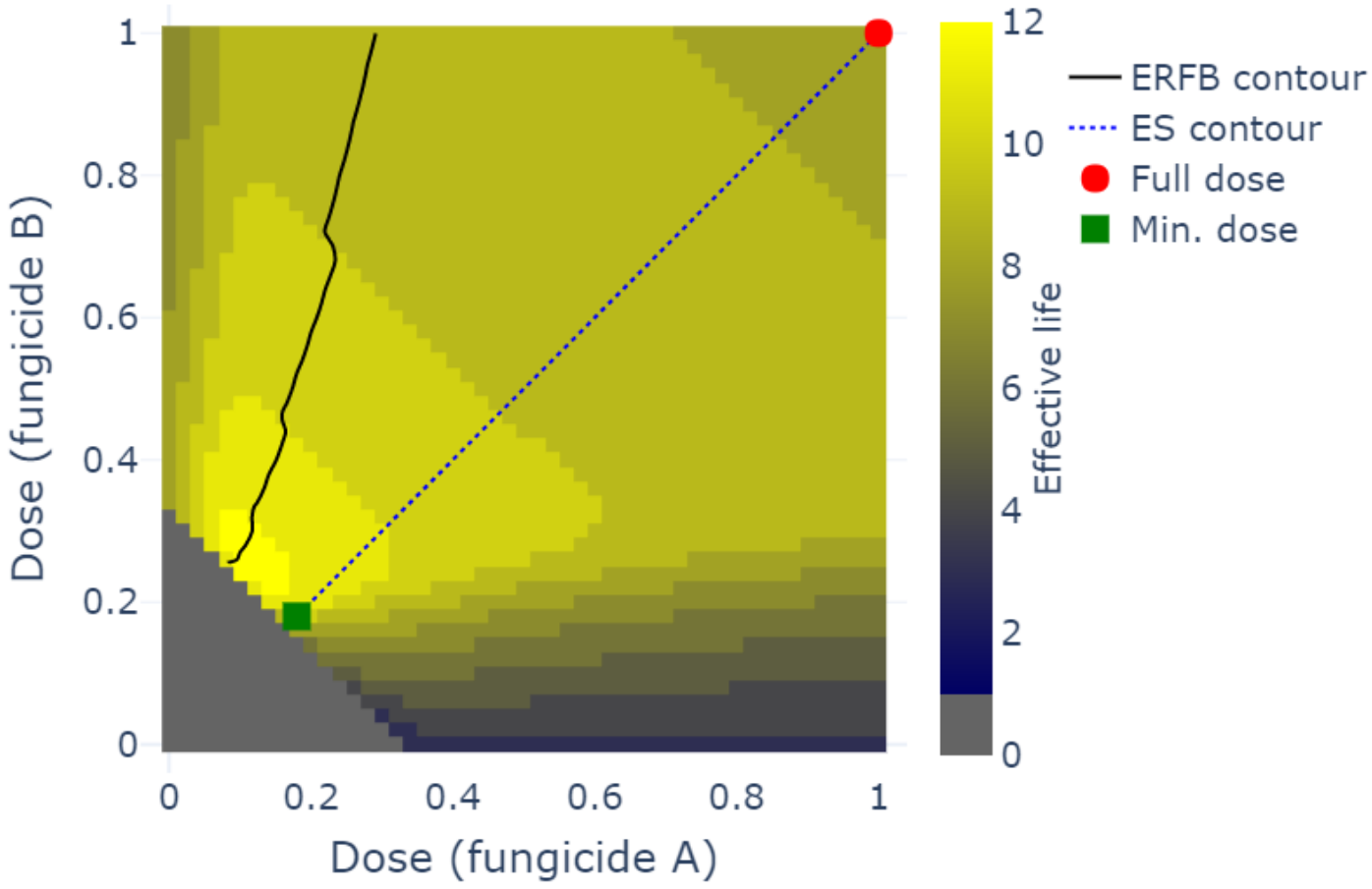

Figure 4. This figure shows 'dose space' for a scenario in which initial levels of resistance to the two fungicides are not equal, but the two fungicides are assumed to act with equal efficacy. The points in this square represent all of the possible choices of chemical concentrations that a grower could make. In this work we assume they apply the same dose every year. Different choices lead to different effective lives, which is the number of years for which this strategy gives acceptable yields.

There is a grey region for which the mixture isn't sufficiently strong to give acceptable yields even in the first year. There is also an important region for which the effective life is at its maximum of 12. The position and size of this region depend on the initial resistance frequencies and the fungicide parameters.

The solid black line represents those doses which lead to equal resistance frequencies to the two fungicides in the final year. This is described by $\Delta_{RFB} = 0$ where $\Delta_{RFB} = \text{logit}(R_A) - \text{logit}(R_B)$ as in 3. Although the initial resistance frequencies in this scenario differ, applying different doses of the two chemicals allows the grower to shift the resistance frequencies so that they are equal by the final year of acceptable yields. Note that there are doses along this contour which fall in the optimal region.

The dotted blue line represents those doses for which selection is equal after the first year of treatment. Note that this falls almost along the line $y = x$, since the chemicals have equal efficacy and in the first year the initial resistance frequencies are so low that density dependent effects do not have a drastic effect on selection. Note that there are no doses along this contour which lie in the optimal region of dose space. This is an example of a case in which the recommendation given by ***Hobbelen et al.*** (***2013***) fails.

Two other strategies which we will analyse are marked on this plot - firstly applying full dose and secondly applying minimal equal doses. Minimal equal doses means the minimal dose along the contour $x = y$ which still gives acceptable yields.

Parameter values: both fungicides with dose response curves and decay rate parameter as per pyraclostrobin (***Hobbelen et al., 2013***), initial resistance frequencies: $(10^{-7}, 10^{-3})$.

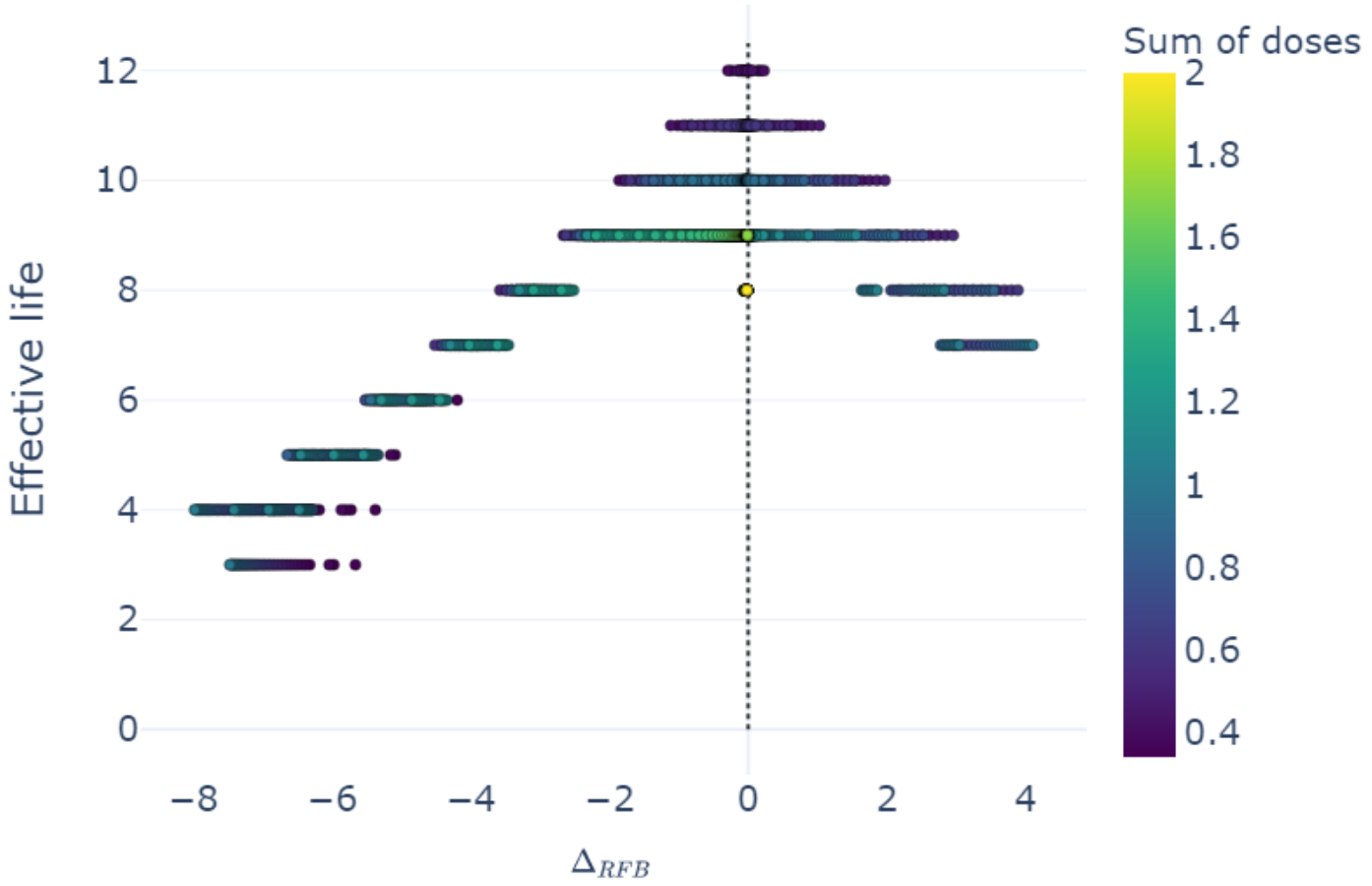

Figure 5. Each point on this scatter plot corresponds to a different pair of doses. On the x-axis we have Δ_{RFB} which relates to the difference in the (logit of the) resistance frequencies. On the y-axis we plot the effective life - more durable strategies have a longer effective life. The colour of the markers corresponds to the *dose-sum*, which is the sum of the two chemical concentrations. A higher dose-sum means a stronger/more concentrated fungicide mixture.
The dotted line is the line $\Delta_{RFB} = 0$. This corresponds to outcomes where resistance frequencies to both fungicides are equal at breakdown. Note that the optimal effective life of 12 years is acheived with doses where $\Delta_{RFB} = 0$. However, there are doses where $\Delta_{RFB} = 0$ and the effective life is sub-optimal. For this parameter combination, these are found for dose combinations where the dose-sum is higher.
In general, doses with larger positive or negative Δ_{RFB} lead to lower effective lives, suggesting that strategies which lead to higher resistance in one of the fungicides are less effective.
Parameter values: as in Figure 4. Both fungicides with dose response curves and decay rate parameter as per pyraclostrobin (***Hobbelen et al., 2013***), initial resistance frequencies: $(10^{-7}, 10^{-3})$.

Parameter scan

We seek to test the robustness of the prescription that a dose combination along the Δ_{RFB} contour will be optimal. We will test this against different pathogen and fungicide parameterisations.

Pathogen parameters

The model uses a proportion of between-season sexual reproduction p_X. In the previous section this was set to 0 as in ***Hobbelen et al.*** (***2013***). However, we know that ascospores are sexual offspring, so in this section we naively allow p_X to vary between 0 and 1, since we do not have a precise figure that we can set it to be.

To characterise the pathogen population with respect to the two fungicides in use, we allow the initial level of resistance to vary too. Since we have a mixture of sexual and asexual reproduction, these figures need not be coupled, so we independently choose values for the proportion of the population taken up by the two single resistant strains and for the double resistant strain, so that the remaining proportion of the population is the double sensitive strain.

This allows us to explore the effect of sexual reproduction and of fungicides having differing levels of resistance when the strategy is to be decided. This could occur naturally or due to the effect of previous use, particularly if one fungicide has only been recently developed.

Fungicide parameters

The fungicides in the model are characterised by three parameters: the decay rate of the chemical; the curvature parameter; and asymptote of the dose-response curve. See Appendix 4 for a more in depth description of this parameterisation.

Fungicides with a higher curvature value are more efficacious. However, we do not vary the curvature parameter in the parameter scan. This is because a change in curvature for a fixed dose is equivalent to a change in dose for a fixed curvature. That is, two fungicide/dose combinations are equivalent if the curvature multiplied by chemical concentration is constant (and the curvature and decay rate parameters are equal). This is explained more fully by the mathematical argument in Appendix 4.

This means we can explore all possible fungicide/dose combinations without varying the curvature parameter. We can nevertheless infer that if we have two choices of mixing partner for a fungicide, the fungicide with the higher curvature requires a lower dose (if the other fungicide parameters are held constant).

Iterative Process

For each iteration in the parameter scan, we sample randomly from distributions for each parameter. We repeated 1024 times, giving 1024 scenarios with different fungicide and pathogen characterisations. The distributions are described in Table 1.

For each parameter combination, we ran the model over a 41×41 grid of dose pairings until the yield dropped below 95%.

For some dose pairings 95% was never achievable. In dose space, we found the Equal Resistance Frequency at Breakdown contour and the Equal Selection contour (Δ_{RFB} and Δ_S contours respectively).

We tested doses along both contours and compared the best doses along these contours with the optimal dose(s) on the grid. These doses do not lie exactly on the 41×41 grid, but are found through interpolation.

Restriction on Parameter Space

To test the Δ_{RFB} contour strategy, we restricted the parameter scan to only those fungicides for which equal resistance at breakdown was possible. For certain particularly weak fungicides, the initial resistance to one fungicide could be so much stronger that the resistance frequencies at breakdown were always in favour of that chemical.

Table 1. This table shows the ranges/values taken by parameters in the scan. Table 1 in Appendix 2 describes the other default parameter values, their sources and their units.

Parameter	Symbol	Range/Value	Distribution
Pathogen between-season sex prop.	p_X	$[0, 1]$	Uniform
Single resistant path. strain init. freqs.	$I_{mn}(0)$	$[10^{-8}, 10^{-2}]$	Log-uniform
Double resistant path. strain init. freq.	$I_{rr}(0)$	$[10^{-15}, 10^{-3}]$	Log-uniform
Baseline fung. decay rate	Δ_0	1.11×10^{-2}	-
Fungicide decay rates	Δ_i	$\left[\frac{1}{2}\Delta_0, 2\Delta_0\right]$	Uniform
Fungicide asymptotes	ω_i	$[0.4, 1]$	Uniform
Fungicide curvature	θ_i	9.6	-

Table 2. This table shows the results of the parameter scan.
'Cases worked' is the percentage of runs in which the strategy was as durable as the optimal strategy from the 41×41 grid.
'Minimal equal dose' is the strategy of applying the same smallest dose of fungicides A and B that gives an acceptable yield in the first year.
Equal resistance frequency at breakdown is a more effective strategy than any of the alternative options. Of those cases where this strategy did not give the optimal result, it was one year worse than the optimal, and the optimal was only achieved by at most two points on the grid.
For some model runs, equal selection was not possible for any dose in the first year, due to ???. For this reason only 646 cases were considered for this strategy (we ignored cases where the strategy was not possible).

Strategy	Cases worked
Equal resistance frequency at breakdown	$792/800 = 99.0\%$
Equal selection in the first year	$326/646 = 50.4\%$
Minimal equal dose	$129/800 = 16.1\%$
Full dose	$21/800 = 2.6\%$

We rejected any of these problematic parameter combinations by restricting our scan to those for which a full dose of either chemical on its own could give sufficient control ($> 95\%$) at least in the first year of application.

This restriction meant every run in the parameter scan had some dose combinations which favoured either resistance frequency at breakdown. This is because we could choose to only apply one chemical and therefore only really increase one resistance frequency, thus negating whatever difference there was in initial resistance frequencies. See Appendix 5 for further details about how the parameter ranges were constructed.

Scan results

Equal resistance at breakdown is optimal

Table 2 describes the results of the parameter scan. It shows that in 92.3% of cases, there is at least one point along the Δ_{RFB} contour which is optimal. This means there is no dose combination in the grid that gives a better outcome than this point. Therefore we can use the Δ_{RFB} contour to reduce the problem of dose selection from a two-dimensional problem (choice of two doses) to a one-dimensional problem: 'how far along the Δ_{RFB} contour is best?'

Table 2 also shows that the Equal Selection in First Year strategy frequently does not work in cases where the Equal Resistance at Breakdown strategy does work.

Selecting mixture strength

Yet to analyse, but I'm hoping to write something like Table 3.

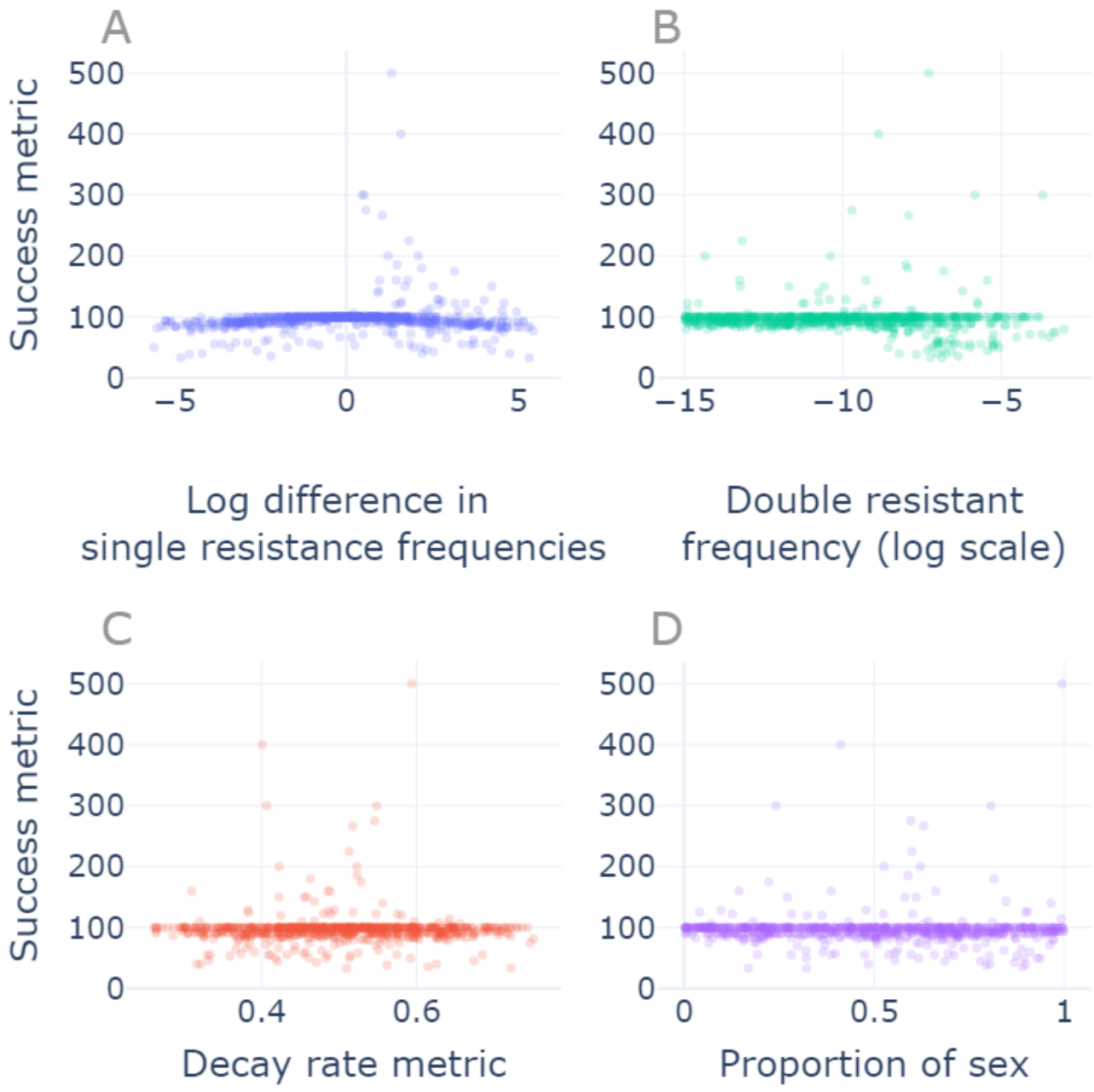

Figure 6. On the y-axis we plot the 'success metric' which compares the first year equal selection strategy to the equal resistance frequencies at breakdown strategy. Mathematically it is given by $100\%\max(EL_{ES})/\max(EL_{RFB})$. It takes values less than 100% if the latter strategy outperforms the former, and it equals 100% if the two strategies perform equally well.
NB: this is not the final version! In particular the success metric shouldn't ever cross 100% (ideally). Plot A might do absolute value of the log difference. I expect that my strat does better than the other one when the single res freqs differ greatly.
want to say as the difference in single resistance frequencies increases, the RFB strategy is more likely to outperform the ES strategy (A).
The decay rate metric (C) is given by $d_A/(d_A + d_B)$ so that 0.5 signifies the decay rates are equal and points further that this suggest that either fungicide remains active for longer than its mixing partner.
Not sure if there's much to get from the other two plots! Need to keep exploring once the parameter scan runs are more reliable.

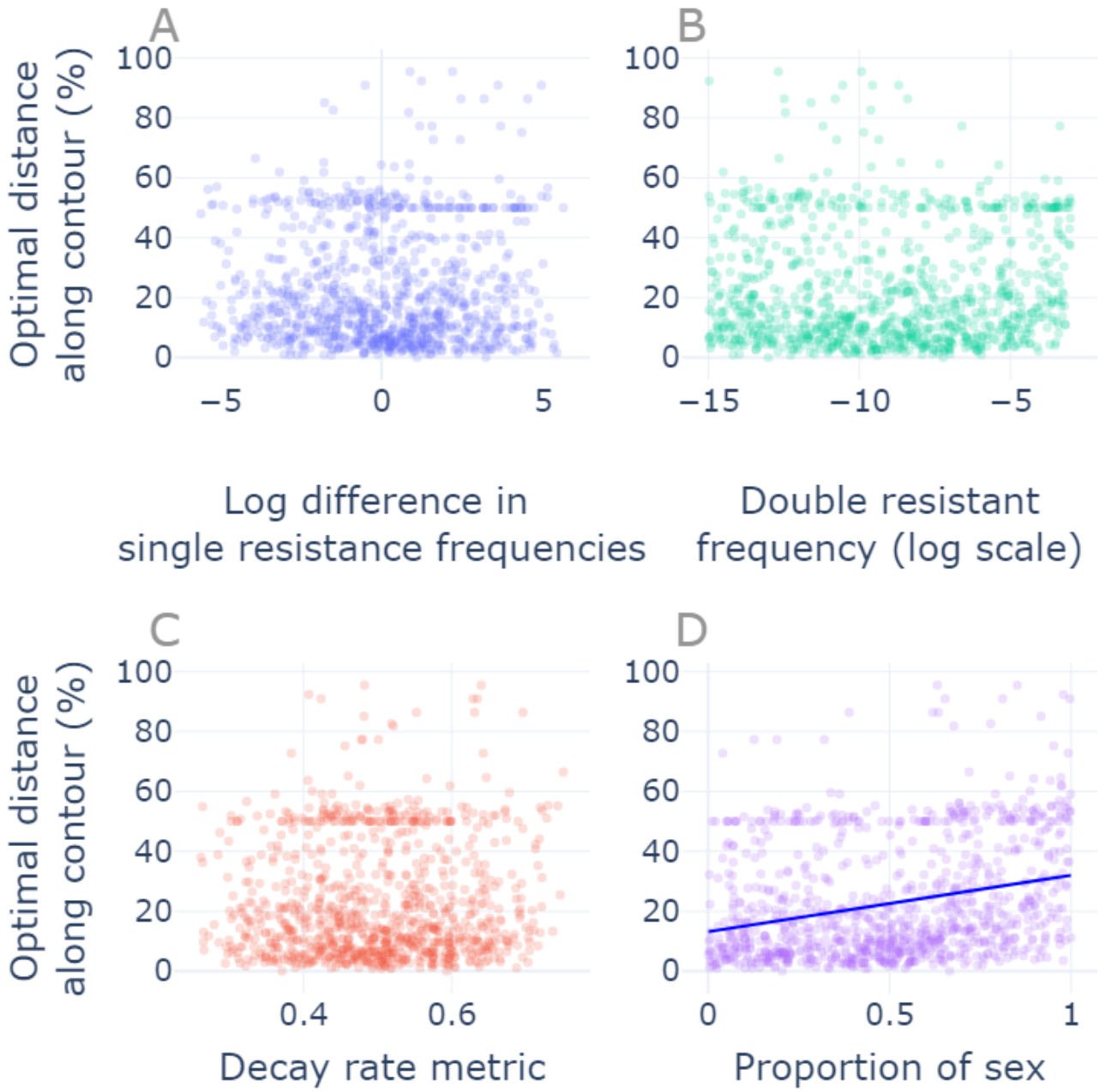

Figure 7. NB: this is not the final version!
On the y-axis of all plots we show the distance along the Δ_{RFB} contour which is in the middle of the optimal region, as a percentage. This tells us whether higher or lower mixture strengths are optimal. Mathematically this distance is given by $100 \times \frac{0.5(u+l)-L}{U-L}$, where u, l are the highest and lowest dose sums in the optimal region of the contour, and U, L are the highest and lowest dose sums along the whole contour. 100% would mean the only optimal point on the contour was that with the highest dose sum, and 0% would mean the only optimal point on the contour was that with the lowest viable dose sum.
The proportion of between-season sexual reproduction (D) has an interesting effect. The optimal dose tends to be higher when there is a larger amount of sexual reproduction between seasons. The other parameters shown (A,B,C) show no clear pattern.

Table 3. HYPOTHETICAL MIXTURE STRENGTH RESULTS
'Minimal strength' means within 10% of the minimum possible along the Δ_{RFB} contour for equal resistance frequencies at breakdown. 'Maximal strength' means within 10% of the maximum possible along the Δ_{RFB} contour, and medium means any mixture strength in between.

Strategy	Percentage of model runs optimal	Average outcome
Minimal strength	70%	$x\%$
Medium mixture strength	20%	$y\%$
Maximal strength	10%	$z\%$

Discussion

Poor decision making by growers and policy makers can accelerate the loss in efficacy of chemical controls of crop disease. This leads to increased disease induced yield-losses (), which is an increasingly important issue with a growing global population (). We have shown that existing recommendations for mixing high risk fungicides are often sub-optimal in the common real-world case in which the initial levels of resistance to the chemicals are not equal. We have presented an alternative strategy which gave optimal results 99% of the time across a broad parameter scan. This was a dramatic improvement when compared to existing strategy recommendations which were optimal 50% of the time.

The strategy which consistently gives optimal results gives equal resistance frequencies at breakdown. That means that the levels of resistance to each fungicide in the mixture are equal in the final year before the yield becomes unacceptable due to pathogen evolution. More efficacious fungicides develop resistance faster, and if the dose of one fungicide is held fixed then increasing the dose of the mixing partner leads to its resistance developing more rapidly. This leads to a broader intuition about the approach when mixing fungicides - lower doses of more efficacious fungicides will give better resistance management results. More efficacious could be in terms of any of: the decay rate; the curvature; or the asymptote parameters. If there is a greater level of resistance to one chemical at the start of the disease management programme then its doses should also be reduced relative to the mixing partner. These principles allow both chemicals to optimally 'protect' each other, slowing the development of resistance and consequently maximising the effective life of the mixture.

The parameter scan in the results section demonstrates that these principles apply across almost the entire range of fungicide and pathogen parameters scanned. These included variable amounts of between-season reproduction, initial proportions of the resistant strains and all possible fungicide parameterisations. These results could potentially transfer to other pathosystems that are managed by mixtures of high risk fungicides, especially to diseases of winter wheat (unless within-season sexual reproduction is strongly present). Future work may consider extending these ideas to mixtures of more chemicals or to parameterisations of different pathosystems. The same ideas should generalise to three or more chemicals - all other components in the mixture would need balancing in a similar manner. However, further work would be required to confirm this and to explore other strategies possible with more fungicides.

There are various possible criticisms of the model used to generate these results. In particular, there is no detailed consideration of spatial effects, which would explore how resistance might develop on a regional scale. There is no stochasticity, so variation within a growing season and between different growing seasons is ignored. In reality these will have significant effects on the strength of mixture required, although different dosages of the two fungicides could still be used to give greater protection to the one at most urgent threat.

We did not explore the effect of partial resistance on the results and future work could also consider the exploring economic output as the main metric rather than effective life. This would involve incorporating the cost of each fungicide application into the calculations. We ignore within-

season sexual reproduction because the experimental work suggests the effect of ascospores on epidemic severity during one growing season is small (***Eriksen et al., 2001***). Future modelling work could consider its effect.

The breakdown in the final year recommendation may seem less practical than a first year recommendation, since it is too late once the final year is reached and the future evolution of the pathogen population can be very difficult to predict in practice. However, points in dose space that are close to the optimal ones usually also have a long effective life. This means that even if the estimates of the initial resistance frequencies or other parameters are imperfect, a good decision that is close to the optimal can be made. The model could be used to determine the best estimate for optimal dose combination given imperfect information about the levels of resistance or fungicide parameters. It can be difficult to estimate the proportion of resistance strains particularly when their incidence is very low. However, as the resistance frequencies increase and become easier to reliably estimate, the model output could be updated with improved estimate for the corresponding optimal dose combination. Further, recent work combining field and laboratory experiments should help improve estimates of the 'effective period' of new fungicides (which relates to the 'decay rate' in our model) (**?**).

There are other more practical criticisms which can be made. Growers typically apply doses in multiples of a quarter of a full dose (). This means that a very precise theoretical prescription for an optimal dose may not be used in practice. However, much better results could be achieved if thought was given to ensuring that the two fungicides are used in such a way as to maximise mutual protection and avoid either developing resistance too rapidly. Further, if modelling shows that more precise doses could lead to a dramatic increase in effective lifetimes of fungicide mixtures, then perhaps they should be used in practice.

Future work might consider addressing the parameter combinations for which equalising resistance frequencies at breakdown is not possible. This case may be more complex, since there are potential conflicting ideas at play: it is likely that minimising the difference in resistance frequencies is desirable, but the optimising the strength of mixture may play an important part in these cases. Doses which achieve the former may not achieve the latter, so the strategy which minimises the difference in resistance frequencies may not be the overall optimal strategy. Further investigation of this scenario is required. However, the case explored here in which equalising resistance frequencies at breakdown is possible is an interesting and practically relevant one, since it is possible as long as both mixing partners are sufficiently strong to control the disease on their own (at least initially).

A particularly interesting area for future work would be to consider time varying disease management strategies. Given that the resistant frequencies vary each year, it may be possible to prolong the effective lifetime by increasing dose as the level of resistance increases. Further, it would allow other strategies like alternating the use of mixtures that favour fungicide A or B, which could be better than strategies that are static in time. The optimal strategy over a few years need not match the beginning of a time-varying strategy intended to be used over a longer time period.

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Appendix 1

NB - haven't edited appendices recently so for now this is placeholder content rather than worthy of comment!

Appendix 2 Table 1. caption

Parameter	Symbol	Value	Units	Source
Pathogen between-season sex prop.	p_X	$[0, 1]$	-	A

Appendix 2

Parameter Values

We non-dimensionalise so that all of our tissue quantities are proportions that sum to a total of 1.

Appendix 3

Model structure

The model is split up into two distinct time periods - within and between seasons.

For parameter meanings see Table 1. For parameter values, see Appendix 2.

Pathogen strains

We denote the two fungicides A and B.

It is assumed that there are four key pathogen strains in the population; the double sensitive strain, two single resistant strains and the dangerous double resistant strain. These are denoted by ss, sr, rs and rr respectively. In general we use the indices m, n to refer to the response of the pathogen to fungicides A and B respectively, and adopt the notation strain mn to denote one of the pathogen strains more generally.

Within-season

Primary infection

The modelled season starts at T_{emerge}, which corresponds to the emergence of 'leaf five' (***Elderfield et al., 2018***; ***van den Berg et al., 2013***), rather than the start of the growing season. The dynamics between the start of the growing season and T_{emerge} are approximated by the initial conditions and the primary infection (***Elderfield et al., 2018***).

The initial infection comes from a primary inoculum P_{mn} for strain mn. This inoculum is assumed to decay exponentially, with the same rate for all strains. In fact aside from the effect of the fungicide application, the strains are treated as identical. This assumes no fitness cost to presence of fungicide resistance.

$$P_{mn} = \exp(-\nu t). \tag{7}$$

Host growth

We define the total amount of tissue

$$A = S + E_{ss} + E_{rs} + E_{sr} + E_{rr} + I_{ss} + I_{rs} + I_{sr} + I_{rr} + R. \tag{8}$$

Then the growth of the wheat crop is given by the following function g:

$$g(A) = r(1 - A). \tag{9}$$

The growth rate is density dependent – that is, the rate of host growth decreases as the total amount of tissue (A) increases. We non-dimensionalised the tissue quantities, so that after growth finishes, each quantity represents the proportion (out of 1) of tissue of that particular type. The growth function is scaled by growth rate r.

Senescence

We use the following senescence function Γ:

$$\Gamma(t) = \begin{cases} 0.005\left(\frac{t - T_{GS61}}{T_{GS87} - T_{GS61}}\right) + 0.1\mathrm{e}^{-0.02(T_{GS87} - t)}, & \text{if } t \geq T_{GS61}, \\ 0, & \text{if } t < T_{GS61}. \end{cases} \tag{10}$$

This function is inherited from the models by ***Hobbelen et al.*** (***2013***) and ***Elderfield et al.*** (***2018***), and it tracks the senescence of healthy tissue. Senescence begins at growth stage

61, $t = T_{GS61}$ (where we use Zadok's growth scale for the growth of wheat (***Zadoks et al., 1974***)). Senescence is assumed to only affect tissue from the S and E compartments, but the disease is assumed to disrupt this process meaning that there is no senescence of tissue in the I compartment. By harvesting time at $t = T_{GS87}$ we get (almost) complete senescence of the healthy tissue.

Effect of fungicides

We denote the response of a particular pathogen strain to the application of a fungicide i by $\delta_i(t)$. The time dependency arises because the chemical concentration changes in the season. The fungicides decrease the rate of transition from healthy to latent, and from latent to infected. Both transition rates are assumed to decrease by the same amount. The fungicide response δ_i lies in the interval $[0, 1]$, and multiplies each respective transition rate.

For a dose C_F of a fungicide F, we use dose responses of the type

$$\delta_{F_s}(C_F) = 1 - \epsilon_F(C_F). \tag{11}$$

where δ_{F_s} is the effect on any strains sensitive to it, and

$$\epsilon_F(C_F) = \omega_F\left(1 - \mathrm{e}^{-\theta_F C_F}\right). \tag{12}$$

Appendix 3 Figure 1. TO UPDATE
Dose response curve for pyraclostrobin (***Hobbelen et al., 2011a***; ***Elderfield et al., 2018***). We will predominantly focus on dose responses comparable to pyraclostrobin, since it is a high risk fungicide (in particular it belong to the family of strobilurins).
This particular dose response curve is parameterised with a maximum efficacy of 1, and a curvature of $\theta = 9.6\mathrm{labeldose}^{-1}$. If the y-asymptote is denoted $\tilde{y}$, the maximum efficacy corresponds to 1-$\tilde{y}$.

We assume that resistant strains are completely unaffected by an application of fungicide, in the same way as ***Elderfield et al.*** (***2018***). This means that $\delta = 1$.

The concentration of fungicide i is assumed to decay exponentially with rate Δ_i.

Primary and secondary infection

Infection for any strains mn is given by the sum of primary infections and secondary infections. The rate of primary infections is given by $\delta_{A_m}\delta_{B_n}P_{mn}$, and the rate of secondary infection is given by $\delta_{A_m}\delta_{B_n}I_{mn}$. The rate of new infections of strain mn are given by the sum of their rates:

$$G_{mn} = \delta_{A_m}\delta_{B_n}(I_{mn} + P_{mn}). \tag{13}$$

Within-season model equations

We summarise the within-season dynamics as follows:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = g - \Gamma S - \frac{\beta S}{A}\sum_{mn\in\{rr,sr,rs,ss\}} G_{mn}, \tag{14}$$

$$\frac{\mathrm{d}E_{mn}}{\mathrm{d}t} = \frac{\beta S}{A}\left[\delta_{A_m}\delta_{B_n}(I_{mn} + P_{mn})\right] - \Gamma E_{mn} - \gamma\delta_{A_m}\delta_{B_n}E_{mn}, \tag{15}$$

$$\frac{\mathrm{d}I_{mn}}{\mathrm{d}t} = \gamma\delta_{A_m}\delta_{B_n}E_{mn} - \mu I_{mn}, \tag{16}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \mu\sum_{mn\in\{rr,sr,rs,ss\}} I_{mn} + \Gamma\left[S + \sum_{mn\in\{rr,sr,rs,ss\}} E_{mn}\right]. \tag{17}$$

Here m, n denote resistant (r) or sensitive (s) strain to fungicide A and B respectively. The fungicide function δ_{A_n} depends on the dose response of fungicide A and whether n denotes a resistant or a sensitive strain. Resistant strains are assumed to be completely unaffected by an application of fungicide (so that $\delta_{Ar} = \delta_{Br} = 1$).

Between-season dynamics

Any remaining primary inoculum from the previous winter is assumed to have gone by the end of the growing season. We assume a constant total initial amount of inoculum (denoted ψ_0), as is used by (REF). This inoculum is caused by spores remaining in the environment after harvest.

We keep the proportion of sexual reproduction as a free parameter denoted p_X (see Appendix 5) for a scan over various values). Then the remaining proportion $1 - p_X$ of the population is assumed to be clonal offspring. Initially we consider $p_X = 0$ as in (***Hobbelen et al., 2013***). We scan over all possible values of p_X in Appendix 5 to demonstrate the effect of alternative parameter choices.

Mixed reproduction

Here we describe the general scenario for the between-season dynamics. We define the 'resistance frequency' as the proportion of the pathogen population that is resistant to a particular fungicide. The proportion of sexual offspring of strain mn is denoted by X_{mn}, and the proportion of asexual offspring is denoted by Y_{mn}. The calculation of these quantities is described in the sections to follow. The resistance frequencies at the start of the next season are given by:

$$P_{rr} = \psi_0\Big(p_X X_{rr} + (1 - p_X)Y_{rr}\Big), \tag{18}$$

$$P_{rs} = \psi_0\Big(p_X X_{rs} + (1 - p_X)Y_{rs}\Big), \tag{19}$$

$$P_{sr} = \psi_0\Big(p_X X_{sr} + (1 - p_X)Y_{sr}\Big), \tag{20}$$

$$P_{ss} = \psi_0\Big(p_X X_{ss} + (1 - p_X)Y_{ss}\Big). \tag{21}$$

Sexual reproduction

Here we explain how the proportions of sexual offsping, X_{mn}, are calculated. Let I^*_{mn} be the level of infection for strain mn at the end of the previous modelled season. Then define the sum of all disease strains:

$$D_S = \sum_{m,n} I^*_{mn}. \tag{22}$$

The proportion of primary inoculum resistant to fungicide i is denoted by ϕ_i:

$$\phi_1 = \frac{I^*_{rs} + I^*_{rr}}{D_S}, \tag{23}$$

$$\phi_2 = \frac{I^*_{sr} + I^*_{rr}}{D_S}, \tag{24}$$

Appendix 3 Table 1. Parameters and state variables used in the HRHR model.

Symbol	Meaning	Type	Default value/range/equation	Source
S	Susceptible tissue	Variable		
E_{mn}	Latently infected (exposed) tissue, strain mn	Variable		
I_{mn}	Infectious tissue, strain mn	Variable		
R	Removed tissue	Variable		
A	Total tissue ($= S + R + \sum_{m,n}[E_{mn} + I_{mn}]$)	Variable		
P_{mn}	Primary inoculum, strain mn	Variable		
C_i	Fungicide i concentration	Variable		
g	Host growth function	Function		
Γ	Senescence function	Function		
δ_i	Fungicide i function	Function		
β	Infection rate	Parameter		
γ^{-1}	Latent period	Parameter		
μ^{-1}	Infectious period	Parameter		
ν	Inoculum decay rate	Parameter		
Δ_i	Fungicide i decay rate	Parameter		

Source: https://www.sedl.org/afterschool/toolkits/science/pdf/ast_sci_data_tables_sample.pdf

Table 1–source data 1. This is a description of a data source.

Table 1–source code 1. This is a description of a source code.

We assume perfectly random mating of unlinked resistance genes. This means the resistance frequencies multiply each other, and we obtain:

$$X_{rr} = \phi_1\phi_2, \tag{25}$$

$$X_{rs} = \phi_1(1-\phi_2), \tag{26}$$

$$X_{sr} = (1-\phi_1)\phi_2, \tag{27}$$

$$X_{ss} = (1-\phi_1)(1-\phi_2). \tag{28}$$

Asexual reproduction

The proportions of asexual offsping, Y_{mn}, are calculated as follows:

$$Y_{rr} = \frac{I^*_{rr}}{D_S}, \tag{29}$$

$$Y_{rs} = \frac{I^*_{rs}}{D_S}, \tag{30}$$

$$Y_{sr} = \frac{I^*_{sr}}{D_S}, \tag{31}$$

$$Y_{ss} = \frac{I^*_{ss}}{D_S}. \tag{32}$$

Appendix 4

Link between curvature and dose

Maths to show dose D with curvature θ is equivalent to dose KD with curvature θ/K.

Dose response curves and description of parameterisation.

Appendix 5

Parameter Scan

There is a very rare case where the unmixed restriction might not guarantee that ERFB is possible. This case is best illustrated by example. Suppose we start with resistance frequencies of $(10^{-2}, 10^{-8})$, and we breakdown at $\approx (10^{-2}, 10^{-4})$ after applying $(0, 1)$ every year. Then we cannot achieve ERFB despite the fact that the unmixed fungicides were acceptable. However it is extremely unlikely that the chemical cannot handle a resistance frequency as low as 10^{-4}, but can handle lower frequencies.

However this case did not occur in the 1024 runs we tested, and we sought to show that when ERFB is possible it is the best strategy, so finding that there are occasional extra cases where it ERFB is not possible does not refute the idea that is it the best strategy when it is possible.

Appendix 6

Mathematical explanation of the sexual reproduction effect

Set up simple model.

When SR, can ignore double resistant since small amounts.

Now consider the case when $\delta = \delta_1 = \delta_2$.

The difference in growth rates is what determines the level of selection, and can be approximately given by:

$$\Delta_{single,double} = \delta - \delta^2 \tag{33}$$

This function is shown in Figure 1. It has minima for low applied doses and high applied doses, suggesting that doses in the middle are poor options.

Appendix 6 Figure 1. Delta curve