October 2021

Project Report No. 637

Managing resistance evolving concurrently against two or more modes of action to extend the effective life of new fungicides

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This is the final report of a 54-month project (21120058) that started in April 2017. The work was funded by Adama, BASF, Bayer, Corteva, Syngenta and a contract for £196,500 from AHDB. The contract funded the research partners RSK ADAS Ltd, NIAB, SRUC and Rothamsted Research. Teagasc contributed field experiments at no cost to the project.

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1. Abstract

In many crop-treatment programmes, at least two single-site acting fungicides are applied. This creates selection pressure for resistance to evolve concurrently against more than one mode of action (MoA). The project objectives were to:

1. Test tactics for the management of concurrent resistance using mixtures.
2. Test if there are circumstances when alternation may be a more effective resistance management tactic than mixtures.
3. Test the efficacy and economics of implementing resistance management tactics.

The effect of resistance management tactics was measured by their effect on the frequency of resistance mutations in *Zymoseptoria tritici* in the target sites for SDHI and DMI fungicides. Key messages from the work were:

- Integrated pest management (IPM) is the basis for resistance management.
- Reduced availability of multi-site acting fungicides will increase concurrent resistance evolution and the need for effective resistance management.
- Mixtures, alternation and limiting number of treatments are all effective resistance management strategies.
- Limiting treatments may limit use of mixtures, where there are few effective MoA available relative to the number of treatments required per season.
- Evidence from this project and the literature suggests there are many circumstances where alternation is as effective as mixtures at reducing selection. Therefore, the choice between adopting a mixture or alternation strategy can be guided by efficacy and practical considerations.
- Total dose of a MoA applied in a season is a key driver of resistance selection.
- Limiting total dose to manage resistance can be achieved by:
    - Limiting the number of treatments, or
    - Limiting total dose and allowing farmers flexibility in how that total dose is split
- Allowing flexibility in how a total dose is used, as part of an effective mixture strategy, is unlikely to have a substantial effect on selection. Nevertheless, the following limitations should apply:
    - The mixture partner should be effective.
    - The increase in the number of treatments allowed by dose splitting should be limited.
    - There should be clear evidence of benefits from allowing more flexibility, to justify the resulting small increase in resistance risk.
- The benefits could arise from improved efficacy, economics or protection of mixture partners. Such benefits were not demonstrated for septoria tritici management in wheat.
- There may be benefits from flexibility in other pathogen-crop systems, particularly where the number of treatments required is high and there are few MoA available.

2. Introduction

Much has been learnt about fungicide resistance management in the years since the premature loss of strobilurin (QoI) efficacy against key pathogens. Practical guidance developed through collaborative research in the UK between academia and industry has been underpinned by governing principles of fungicide resistance evolution, derived from a global analysis of research (van den Bosch et al., 2014a). As a result, guidance implemented from the introduction of the new generation SDHIs has created more effective resistance management than achieved previously. However, although considerable progress has been made, there were significant knowledge gaps at the start of the current work. In particular, there was limited evidence based on field experiments for the development of resistance to two fungicide modes of action (MoA) concurrently, and whether mixture or alternation is the more effective anti-resistance strategy.

Concurrent selection for resistance against two or more modes of action is likely to occur whenever two or more single-site acting modes of action (MOA) are being used in a spray programme. This is normally the case in cereals, potatoes and horticultural crops. Resistance will evolve at some level to both MoA concurrently, even if resistance does not become detectable for many years. Yet the available global experimental evidence is from studies where selection for resistance against only one MoA was measured (evidence reviewed by van den Bosch *et al*., 2014). Modelling conducted in an AHDB PhD studentship is starting to provide predictions of the effects of management strategies on concurrent selection, but field evidence was limited. It is the field evidence which is needed to persuade industry of the need to follow current guidelines or to change practice.

Mixtures and alternation are strategies expected to delay the development of resistant pathogens. At the same time, a restriction on the maximum number of treatments per crop with the same MoA is also a good resistance management strategy. For example, SDHIs are restricted to two applications (Anon., 2015) in the UK (typically applied at T2 and/or T1) and in France to one application. Repeated applications of fluazinam to potatoes in the Netherlands resulted in resistance in late blight and many blight fungicides are now limited by number of treatments. Therefore, there is a trade-off between strategies: it is beneficial to use fewer applications of a MoA, but if the number of applications is restricted and there are insufficient MoA available, this then results in another MoA applied on its own if additional spray timing treatments are needed.

For wheat in the UK, one or two sprays are often insufficient to achieve effective disease control, so growers are reliant on alternation of MoA or the use of less effective mixtures at T0 and T3. However, the results of LINK project LK09133 (‘Improved tools to rationalise and support stewardship programmes for SDHI fungicides to control cereal diseases in the UK’) suggest that this can expose one partner to excessive selection pressure. Though the governing principles (van

den Bosch, 2014a) have identified the circumstances under which mixtures or alternation should be most effective, this has never been tested in the field under concurrent selection (Hobbelen et al., 2013; van den Bosch et al., 2014b). These knowledge gaps leave room for different interpretations of the most effective anti-resistance strategies, which is reflected, for example, in contrasting approaches adopted in the UK and France.

The experimental system selected for the current work was a natural pathogen population known to be in the process of undergoing selection for resistance to fungicides, specifically, *Zymoseptoria tritici* (cause of septoria leaf blotch) in wheat. The work focused on two key fungicide MoA: SDHI and DMI. This provided an opportunity to look at concurrent selection for insensitivity to both MoA, because insensitivity levels detectable at the start of the work were sufficiently low, particularly for SDHI fungicides, to provide a window of opportunity to look at changes in selection following different treatment programmes designed to address the knowledge gaps. When new mutant strains of pathogens are detected, there is a 'window of opportunity' when treatments comparing resistance management tactics can be applied, and the relative success of those tactics measured by the rate of selection for insensitive pathogen strains. This window is when the frequency of the insensitive stains is above the level of detection (1-4%) but sufficiently low to allow differentiation of treatment effects. This period can last only a few years with rapidly shifting resistance status, as has happened with the development of resistance to QoIs in septoria. Experiments conducted on these naturally shifting populations have generated most of the current knowledge on which resistance management is based. The rate of selection (combined with the degree of insensitivity) determines how many years it will take for the new strains to erode efficacy. Mathematical modelling can combine these variables and convert selection rates into years of effective life, so that the practical costs and benefits of resistance management can be compared over the short, medium and long-term, and informed judgements made.

Insensitivity to SDHI fungicides was in a relatively early stage of development in field populations of septoria in the UK before the start of this project. In 2015 a high level of SDHI insensitivity conferred by new mutations in *Zymoseptoria tritici*, identified in the UK and Ireland, was demonstrated in glasshouse tests on seedlings (Bart Fraaije, Rothamsted), and these results were then seen in the field. Therefore, field experiments provided a good opportunity to look at the development of insensitivity to SDHI fungicides during a relatively early phase of introduction, and furthermore look at the extent to which fitness penalties associated with insensitivity mutations (a decline in frequency over winter when no fungicides are applied) might constrain spread of these strains. AHDB-funded monitoring, through project 21120018, has provided information on the frequency and spread of these new mutants. The SDHI target site is polymorphic and complex, but in summary: low and moderately insensitive strains are most commonly associated with mutations C-T79N, C-N86S and D-D129E in the SDH subunit target sites. Mutations were found in over half

the fields sampled at the start of the current work. Where present, the percentage of isolates carrying one or more mutations varied between 2% and 30%, but highly resistant isolates with the C-H152R mutation were rare.

Insensitivity against DMI fungicides is further developed compared to SDHIs but continues to evolve, as seen both in data from Fungicide Performance trials (21120013) and AHDB-funded resistance sampling in 2016 which preceded this project. The latter showed that particular mutant variants were changing frequency, especially variants containing CYP51 target site alteration S524T and/or a CYP51 120 base pair (b.p.) promoter insert resulting in CYP51 overexpression (Cools et al., 2012). Sensitivity to both SDHI and DMI fungicides is also modified by efflux pump mechanisms, of which the predominant type is overexpression of MgMFS1 due to a 519 b.p. promoter insert (Omrane et al., 2015). There was a further shift in the DMI sensitivity of field populations between 2015 and 2016, and no evidence that the rate of evolution of resistance would slow. The specific genetic changes listed above can be quantified in field populations and the mutations provide an experimental tool to quantify and compare rates of selection under different experimental treatments.

The field experiments in this study were designed to quantify:

- selection for pathogen resistance evolving concurrently against two or more MoAs.
- the trade-offs between mixtures, alternation and number of treatments of the MoAs.

The differences between findings reported in the literature and some of the experimental field results in this study were investigated by mathematical modelling. Further mathematical modelling work (funded separately through an AHDB PhD studentship (21120062)) will quantify increases in the effective life of fungicides from resistance management tactics against concurrent selection of insensitive strains; this modelling work is summarised within this report and will be reported more in-depth as part of the associated AHDB-funded PhD.

3. Project aims and objectives

Aims:

Develop methods to determine how any two or more MoAs should best be deployed in fungicide treatment programmes to combine robust, cost-effective control and slow down the development of resistant fungal pathogens.

Objectives:

1. Test tactics for managing concurrent resistance using mixtures.
2. Test circumstances when alternation may be a more effective resistance management tactic than mixtures.
3. Test the efficacy and economics of implementing resistance management tactics.

Objectives 1 and 2 were addressed by field experiments conducted by ADAS, SRUC, NIAB and Teagasc, with genotyping of strains by Rothamsted (latterly NIAB).
Objective 3 was addressed by field experiments conducted in each year by industry partners, Adama, BASF, Bayer, Corteva and Syngenta. These experiments looked at the economics of the strategies being tested by the academic partners.

4. Materials and methods

4.1. Field sites and experiment design

4.1.1. Testing mixture tactics for managing concurrent resistance (objective 1)

Rationale and treatments

Field experiments using natural infection of septoria (*Zymoseptoria tritici*) were set up to investigate selection for resistance evolving to two modes of action (MoA), SDHIs and DMIs from 2017 – 2020 (Table 1). The selection effect of different fungicide treatments was determined by measuring the frequency of SDH and DMI mutations, at the start and end of each season. Isopyrazam was chosen as the 'test case' SDHI fungicide because it was available as a solo SDHI product, with an efficacy expected to allow some septoria to develop in treated plots, ensuring sufficient infected leaf samples for reliable genotyping tests across all treatments. The DMI fungicide prothioconazole (a triazolinthione) was selected as an example DMI.

Table 1. Field experiment sites 2017-2020 to investigate development of concurrent resistance to SDHI and DMI fungicides in *Zymoseptoria tritici*

Year	Partner	Location name & Region	SDHI	DMI	Multi-site	Genotyping tests
2017	ADAS NIAB SRUC Teagasc	Murder field, Herefordshire Sutton Scotney, Kent Cauldshiel, Edinburgh Oak Park, Duck field, Cork	isopyrazam	prothioconazole	chlorothalonil	SDH + S524T
2018	ADAS NIAB SRUC Teagasc	Big Camp, Herefordshire Callow, Herefordshire Cauldshiel, Edinburgh Oak Park, Cork	isopyrazam	prothioconazole	None	SDH + S524T
2019	ADAS NIAB SRUC Teagasc	Benty Bear, Herefordshire Callow, Herefordshire Cauldshiel, Edinburgh Oak Park, Cork	isopyrazam	prothioconazole	None	SDH + S524T
2020	SRUC	Cauldshiel, Edinburgh	isopyrazam	prothioconazole	None	SDH + S524T

The 2017 experiment treatments were designed to investigate the effect of splitting the SDHI dose, with and without a DMI in mixtures, and the effect of including a multi-site fungicide (Table 2).

Table 2. 2017 field experiment treatments to investigate effects of SDHI, DMI and multi-site fungicides on development of mutations for SDHI and DMI resistance in *Zymoseptoria tritici*

	T0 – GS 25-30			T1 – GS 32			T2 – GS 39			T3 - GS 59			Total no. applications		
Trt	**[2]DMI**	**[3]SDHI**	**[4]MS**	**DMI**	**SDHI**	**MS**	**DMI**	**SDHI**	**MS**	**DMI**	**SDHI**	**MS**	**DMI**	**SDHI**	**MS**
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	[1]1	-	-	1	-	-	1	-	-	1	-	-	4	-	-
3	-	0.5	-	-	0.5	-	-	0.5	-	-	0.5	-	-	4	-
4	-	-	0.5	1	-	0.5	-	0.5	0.5	1	-	0.5	2	1	4
5	-	-		1	-	-	1	0.5	-	1	-	-	3	1	-
6	-	-	0.5	1	-	0.5	1	0.5	0.5	1	-	0.5	3	1	4
7	-	-	-	1	0.5	0.5	1	0.5	0.5	-	-	-	2	2	-
8	1	-	-	1	0.5	-	1	0.5	-	1	-	-	4	2	-
9	1	0.25	-	1	0.25	-	1	0.25	-	1	0.25	-	4	4	-
10	-	-	-	1	0.5	0.5	1	0.5	0.5	-	-	-	2	2	2
11	1	-	0.5	1	0.5	0.5	1	0.5	0.5	1	-	0.5	4	2	4
12	1	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1	0.25	0.5	4	4	4

[1] = numbers are fungicide rates as proportion of maximum permitted individual dose each application time (- = 0)
[2] = prothioconazole: Proline, Bayer (max. permitted indiv. dose = 0.72 L/ha; max. permitted total dose = 2.16 L/ha)
[3] = isopyrazam: Zulu, Adama (max. permitted indiv. dose = 1.0 L/ha; max permitted total dose = 2.0 L/ha)
[4]= chlorothalonil: Bravo, Syngenta (max. permitted indiv. dose = 2.0 L/ha; max permitted total dose = 2.0 L/ha)

The 2018 field experiment treatments were designed to investigate the effect of total dose and dose splitting of the SDHI fungicide, with DMI fungicides included at 2, 3 or 4 timings (Table 3).

Table 3. 2018 field experiment treatments to investigate effects of SDHI and DMI fungicides on development of mutations for SDHI and DMI resistance in *Zymoseptoria tritici*

	T0 – GS 25-30		T1 – GS 32		T2 – GS 39		T3 - GS 59		Total no. applications		Total dose
Trt	**[2]DMI**	**[3]SDHI**	**DMI**	**SDHI**	**DMI**	**SDHI**	**DMI**	**SDHI**	**DMI**	**SDHI**	**SDHI**
1	-	-	-	-	-	-	-	-	-	-	-
2	[1]1	-	1	-	1	-	1	-	4	-	-
3	-	0.5	-	0.5	-	0.5	-	0.5	-	4	2
4	1	-	1	1	1	1	1	-	4	2	2
5	1	-	1	0.67	1	0.67	1	0.67	4	3	2
6	1	0.5	1	0.5	1	0.5	1	0.5	4	4	2
7	-	-	1	-	1	0.75	-	-	2	1	0.75
8	-	-	1	0.75	1	0.75	-	-	2	2	1.5
9	-	-	1	0.75	1	0.75	1	-	3	2	1.5
10	-	-	1	0.5	1	0.5	1	0.5	3	3	1.5
11	1	-	1	0.75	1	0.75	1		4	2	1.5
12	1	-	1	0.5	1	0.5	1	0.5	4	3	1.5
13	1	0.38	1	0.38	1	0.38	1	0.38	4	4	1.5

[1] = numbers are fungicide rates as proportion of maximum permitted individual dose each application time (- = 0)
[2] = prothioconazole: Proline, Bayer (max. permitted indiv. dose = 0.72 L/ha; max. permitted total dose = 2.16 L/ha)
[3] = isopyrazam: Zulu, Adama (max. permitted indiv. dose = 1.0 L/ha; max permitted total dose = 2.0 L/ha)

The 2019 and 2020 experiments were designed to focus in more detail on the effects of splitting the dose of SDHI, at two different total doses of SDHI, 1.5 and 2.0, with DMIs included at all four timings or not at all (Table 4).

Table 4. 2019 and 2020 field experiment treatments to investigate effects of SDHI and DMI fungicides on development of mutations for SDH and DMI resistance in *Zymoseptoria tritici*

	T0 – GS 25-30		T1 – GS 32		T2 – GS 39		T3 - GS 59		Total no. applications		Total dose
Trt	**[2]DMI**	**[3]SDHI**	**DMI**	**SDHI**	**DMI**	**SDHI**	**DMI**	**SDHI**	**DMI**	**SDHI**	**SDHI**
1	-	-	-	-	-	-	-	-	-	-	-
2	[1]1	-	1	-	1	-	1	-	4	-	-
3	1	-	1	-	1	2.0	1	-	-	1	2
4	1	-	1	1.0	1	1.0	1	-	4	2	2
5	1	-	1	0.67	1	0.67	1	0.67	4	3	2
6	1	0.5	1	0.5	1	0.5	1	0.5	4	4	2
7	-	0.5	-	0.5	-	0.5	-	0.5	-	4	2
8	1	-	1	-	1	1.5	1	-	4	1	1.5
9	1	-	1	0.75	1	0.75	1	-	4	2	1.5
10	1	-	1	0.5	1	0.5	1	0.5	4	3	1.5
11	1	0.38	1	0.38	1	0.38	1	0.38	4	4	1.5
12	-	0.38	-	0.38	-	0.38	-	0.38	-	4	1.5

[1] = numbers are fungicide rates as proportion of maximum permitted individual dose each application time (- = 0)
[2] = prothioconazole: Proline, Bayer (max. permitted indiv. dose = 0.72 L/ha; max. permitted total dose = 2.16 L/ha)
[3] = isopyrazam: Zulu, Adama (max. permitted indiv. dose = 1.0 L/ha; max permitted total dose = 2.0 L/ha)

4.1.2. Testing alternation vs mixture tactics (objective 2)

Rationale and fungicide treatments

Alternation and mixtures are both good resistance management tactics. The evidence is divided in the literature (reviewed by van den Bosch et al., 2014) on which is the better of the two strategies for reducing selection. There is a theoretical rationale for why, in some cases mixtures may be better or *vice versa*. Consider two options for the simple case of a two-spray programme. The first option is to alternate fungicide A followed by fungicide B (where A and B are different MoA), each at the maximum permitted individual dose. The second option is to apply A at both spray timings, mixed with B. If both fungicides are of similar efficacy and there is no antagonism, then a mixture containing half the maximum individual dose of A and B will provide at least the same efficacy as the alternation programme and will apply the same total dose. Effectively, the mixture programme is dose-splitting (half the dose of A is applied twice as often). If dose splitting has no effect on selection, then the mixture strategy should be more effective at reducing selection than alternation. This is because the increase in exposure time is counteracted completely by the decreased dose per application, and the effect of mixture partner B reducing the *per capita* growth rates of strains resistant and sensitive to mixture partner A will reduce selection further. If dose splitting increases selection, then mixtures could be less effective than alternation at reducing selection (in cases

where the efficacy of the mixture partner is insufficiently effective) or mixtures could be more effective than alternation at reducing selection (in cases where the efficacy of the mixture partner is strong enough to compensate for the increased selection from dose splitting).

The experiments were designed to test this theoretical rationale by quantifying the effect of mixing versus alternating SDHI and DMI fungicides on the development of resistance. Dose was used as a means to vary the efficacy of the two fungicides. Different combinations of total dose were selected to cover high and low efficacy of the SDHI (at-risk fungicide) and of the DMI (mixing partner), with expected outcomes of reducing selection for resistant mutation shown in Figure 1.

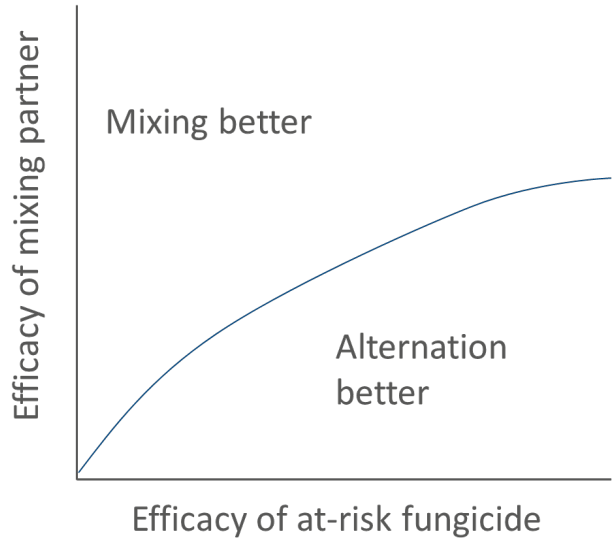

Figure 1. Rationale for fungicide doses in mixture and alternation treatments

The experimental system described above was used to investigate selection for septoria resistance evolving to SDHI fungicides, using a two-spray programme, T1 at GS 32 and T2 at GS 39. In 2018 there were three experiment sites: ADAS, Herefordshire; NIAB, Herefordshire; Teagasc, Cork. In 2019 there were three sites, NIAB, Herefordshire; SRUC, Edinburgh; Teagasc, Cork. In 2020 there were four sites, ADAS, NIAB, SRUC and Teagasc, using the same four regional locations as above.

In the 2018 experiments, two total doses of SDHI (1.0 or 2.0), and a low and a high total dose of DMI (0.4 and 2.0, respectively) were selected to test the effect of mixing and alternation strategies (Table 5).

Table 5. 2018 field experiment treatments to investigate effects of mixtures vs. alternations of SDHI (isopyrazam) and DMI (prothioconazole) fungicides on development of mutations for SDHI and DMI resistance in *Zymoseptoria tritici*

	T1 – GS 32		T2 – GS 39		Total dose	
Trt	**[2]SDHI**	**[3]DMI**	**SDHI**	**DMI**	**SDHI**	**DMI**
1	-	-	-	-	-	-
2	[1]1.0	0.5	1.0	0.5	2.0	1.0
3	0.5	1.0	0.5	1.0	1.0	2.0
4	1.0	0.2	1.0	0.2	2.0	0.4
5	0.5	0.1	0.5	0.1	1.0	0.2
6	2.0	-	-	1.0	2.0	1.0
7	1.0	-	-	1.0	1.0	2.0
8	2.0	-	-	0.2	2.0	0.4
9	1.0	-	-	0.4	1.0	0.2
10	-	1.0	2.0	-	2.0	1.0
11	-	1.0	1.0	-	1.0	2.0
12	-	0.2	2.0	-	2.0	0.4
13	-	0.4	1.0	-	1.0	0.2

[1] = numbers are fungicide rates as proportion of maximum permitted individual dose each application time (- = 0)
[2]= isopyrazam: Zulu, Adama (max. permitted indiv. dose = 1.0 L/ha; max permitted total dose = 2.0 L/ha)
[3]= prothioconazole: Proline, Bayer (max. permitted indiv. dose = 0.72 L/ha; max. permitted total dose = 2.16 L/ha)

In the experiments which followed in 2019 and 2020, a wider range of DMI doses were tested, and at two of the sites a different fungicide mixture was tested (Table 6). The aims were to (i) focus all treatments on the highest SDHI dose, to maximise the range of DMI doses where alternation might be better, (ii) to give an exponential series of DMI total doses, which approximated to linear steps in efficacy, and (iii) to include very low DMI doses to maximise the likelihood of detecting an 'alternation is better' region, if this exists.

Table 6. 2019 & 2020 field experiment treatments to investigate effects of mixtures or alternations of SDHI and DMI fungicides on development of mutations for SDHI resistance in *Zymoseptoria tritici*: isopyrazam & prothioconazole were used in all experiments except two sites in 2020 which used fluxapyroxad & mefentrifluconazole.

	T1 – GS 32		T2 – GS 39		Total dose	
Trt	**[2]SDHI**	**[3]DMI**	**SDHI**	**DMI**	**SDHI**	**DMI**
1	-	-	-	-	-	-
2	[1]1.0	0.5	1.0	0.5	2.0	1.0
3	1.0	0.25	1.0	0.25	2.0	0.5
4	1.0	0.125	1.0	0.125	2.0	0.25
5	1.0	0.0625	1.0	0.0625	2.0	0.125
6	2.0	-	-	1.0	2.0	1.0
7	2.0	-	-	0.5	2.0	0.5
8	2.0	-	-	0.25	2.0	0.25
9	2.0	-	-	0.125	2.0	0.125
10	-	1.0	2.0	-	2.0	1.0
11	-	0.5	2.0	-	2.0	0.5
12	-	0.25	2.0	-	2.0	0.25
13	-	0.125	2.0	-	2.0	0.125

[1] = numbers are fungicide rates as proportion of maximum permitted individual dose each application time (- = 0)
[2] = isopyrazam: Zulu, Adama (max. permitted indiv. dose = 1.0 L/ha; max permitted total dose = 2.0 L/ha)
[3] = prothioconazole: Proline, Bayer (max. permitted indiv. dose = 0.72 L/ha; max. permitted total dose = 2.16 L/ha) or mefentrifluconazole: Myresa, BASF (max. permitted indiv. dose = 1.5 L/ha; max. permitted total dose = 3.0)

4.2. Assessments and sampling (from objective 1 and 2 experiments)

All assessments, samples and yield measurements were taken from individual plots. Disease and % green leaf area were assessed by individual leaf layer, at T2 + 3 weeks, T2 + 6 weeks, with a final assessment no later than GS75.

Leaves infected with septoria were sampled from all field sites for the purpose of genotyping tests conducted by Rothamsted research in 2017-2019 and NIAB in 2020. Leaves were sampled early in the week to minimise delays with delivery for genotyping. In each year, an early season (12 February to 30 March) pooled sample of 100 infected leaves from across one individual field was taken from three to five candidate fields by each research partner, and samples tested promptly for the % frequency of SDH mutations (see genotyping test methods below). Based on this test, one field per partner was selected for experiments, aiming to choose a field with a low but detectable frequency of SDH mutations ($\geq$5%, the threshold of detection). In the fields selected in 2019 and 2020, additional samples were taken from each untreated plot, after marking out plots and immediately prior to applying the first fungicide treatment, keeping samples separate by plot. Only leaves with sufficient infection to ensure

good genotyping results were sampled, aiming to sample 10-15 leaves per plot. Therefore, leaves were not sampled randomly; the objective was to take samples from across each plot with sufficient infection to allow reliable genotyping (≥10% septoria severity, ideally 10-25%).

Samples were also taken by individual plot post-treatment, aiming for two latent periods (approximately 5-6 weeks) after the GS39 T2 spray. The most suitable uppermost leaf layer with clear septoria infection was selected for sampling, so that the same leaf layer was sampled across all plots within one experiment. Accordingly, before sampling, infection severities in the most intensively treated plots were compared with severities in untreated plots, to confirm the uppermost leaf layer that could be sampled across all treatments. In most experiments, leaf 1 (flag) or leaf 2 was selected, because lower leaves in untreated plots had >50% senescence which did not provide suitable material for reliable genotyping tests. Crops were monitored closely, but at some sites, senescence progressed very quickly and the leaf layer sampled from untreated plots was one leaf layer above all the treated plots.

Any excess water was removed from leaf samples before dispatch, using paper towels. Leaves were kept separate by plot, and packed in paper envelopes, labelled clearly to identify them (partner, site, date, plot) and posted first class to the laboratory for genotyping tests.

In the 2020 experiments, a fourth sample timing was added, aiming for one latent period after the GS39 T2 spray, approximately 3 weeks after the T2 spray. The sampling procedure was the same as described above for the 6 weeks post-T2 spray.

4.3. Genotyping tests (from objective 1 and 2 experiments)

4.3.1. DNA extractions and quantification

DNA was extracted directly from 10-15 Septoria-infected leaves by powdering samples in liquid nitrogen using a pestle and a mortar or, alternatively, crushing the leaves with a Pohlähne roller press whilst adding DNA extraction buffer. To each powdered sample, DNA extraction buffer consisting of 40 µl 1% (v/v) β-mercapthoethanol, 400 µl TEN buffer (500 mM NaCl, 400 mM Tris-HCl, 50 mM EDTA, 5 mM 1,10-phenanthroline monohydrate, 2% (w/v) polyvinylpyrrolidone; pH 8.0) and 400 µl 2% (w/v) SDS was added. For larger wheat leaf samples, the amount of DNA extraction buffer added was increased until the mixture could be poured. After incubating the mixture for 30 min at 70°C, 400 µl ice-cold ammonium acetate (7.5 M) was mixed with the heat-treated sample and the total suspension kept on ice for 30 min. After centrifugation at 10,000 rpm for 10 min, an equal volume of cold (-20°C) isopropanol was added to the supernatant and the extract shaken at room temperature for 15 min. After centrifugation at 6,000 rpm for 5 min, DNA pellets were washed with ice-cold 70% (v/v) ethanol, centrifuged again, and dissolved in 500 µl

sterile distilled water. DNA concentrations were measured via nanodrop spectrophotometer and diluted to the required concentration (20 ng/µl) using 1 x Tris-EDTA (TE) buffer.

4.3.2. SNP detection Pyrosequencing assays to measure selection by SDHIs

Four key SdhC alterations that are most frequently occurring in the UK population of *Z. tritici*, C-T79N, C-W80S, C-N86S and C-H152R, were targeted using three SNP detection Pyrosequencing assays. A nested PCR approach was carried out for the assays. For the first PCR, primers ZTCF1 (3'-AACGAAATCCTCGCCAAACA-5') and ZTCR1 (3'-CGCAACACTCAACCCCACA-5') were used to generate a 375 bp PCR product covering the DNA sequence encoding for SdhC codons 55-179. Twenty µl reactions were carried out, consisting of 2.5 µl of DNA sample (50 ng of DNA), 2 µl of 10X Red Hot Taq buffer, 0.1 µl of each primer (100 µM), 0.4 µl of dNTP solution (10 mM of each dNTP), 1.2 µl magnesium chloride (25 mM), 0.04 µl Red Hot DNA polymerase (5 U μl^{-1}) and 13.66 µl of sterile distilled water. PCR was carried out in a Biometra T3 thermocycler under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 40 cycles of 94°C for 10 s, 65°C for 30 s, 72°C for 45 s with a final extension at 72°C for 5 minutes.

For the second round PCR, primers (forward, reverse and sequence primers) were designed with Pyrosequencing Assay Design Software (Version 1.0.6; Biotage) taking also into account the overall *Z. tritici* sequence diversity at other nucleotide positions by using degenerate primers (Table 7). Reverse primers were designed with a biotin label at the 5'-end. PCR reactions were carried out with the One Taq DNA polymerase kit (New England Biolabs) in 40 µl reaction volumes, consisting of 2 µl of DNA sample (first round PCR product 1:500 diluted), 8 µl of 5X One *Taq* standard buffer, 0.2 µl of each primer (100 µM), 0.8 µl of dNTP solution (10 mM of each dNTP), 0.2 µl One *Taq* DNA polymerase (5 U μl^{-1}) and 28.6 µl of sterile distilled water. PCR reactions were run in a Biometra T3 thermocycler under the following conditions: initial denaturation at 94°C for 30 s, followed by 40 cycles of 94°C for 10 s, 54°C for 20 s, 68°C for 30 s with a final extension at 68°C for 4 minutes and 30 s.

Table 7. Targets, primers and assay nucleotide dispensation order for SdhC SNP detection pyrosequencing assays

SdhC target	Oligonucleotide sets[1]	Sequence to analyse	Nucleotide dispensation order
T79N, W80S	F: CCTCGCAATCTACAAACCGG R: CCGAAGGCGTAGAAGGCT* S: TCTACAAACCGCAAATAA	A/CCTG/CGTACCTC/GTCG/CGC	GACATCGCTACTCGATCG
N86S	F: CCTCGCAATCTACAAACCG R: CCGAAGGCGTAGAAGGCT* S: ACCTSTCSGCYCTCA	G/ACCGCGTGACCGG	TGATCGCGT
H152R	F: CGGTGACGTTTCATTCGTT R: GTCTGCACCTGCTTATTCGTAATC* S: TGAATGGAGTGASKC	A/GTTTGGTT/GTGGGATACGGCGAGTATG	CAGCTGTGTGCATA

[1] F, R and S indicate forward, reverse and sequence primers, respectively; primer 5' biotin label are marked with star.

Presence of first and second round PCR products was confirmed on ethidium bromide-stained 1.3 % (w/v) agarose gels run in 1X Tris-borate-EDTA buffer and exposed to UV light to visualise DNA fragments. The amplicon sizes of the PCR products were: 93 bp (SdhC T79N + W80S), 93 bp (SdhC N86S) and 82 bp (SdhC H152R). Single-stranded biotinylated PCR products were prepared for sequencing using the Pyrosequencing Vacuum Prep Tool (Biotage). Three µl Streptavidin Sepharose HP beads (Amersham Biosciences) were added to 40 µl binding buffer (10 mM Tris-HCl pH 7.6, 2 M NaCl, 1 mM EDTA, 0.1 % Tween 20) and mixed with 20 µl PCR product and 20 µl of sterile distilled water for 10 minutes at room temperature using an Orbis plate shaker (Mikura). Beads containing the captured templates were aspirated onto filters after applying the vacuum, washed with 70% (v/v) ethanol for 5 s, rendered single-stranded with denaturation solution (0.2 M NaOH) for 10 s and neutralised with washing buffer (10 mM Tris-Acetate, pH 7.6) for 5 s. The vacuum pressure was released, and beads transferred into a PSQ 96-well plate (Biotage) containing 45 µl annealing buffer (20 mM Tris-Acetate, 2 mM Mg Acetate, pH 7.6) and 0.5 mM sequence primer. Pyrosequencing reactions were performed according to the manufacturer's instructions using the PSQ 96 SNP Reagent kit (Biotage). Assays were performed on the PSQ MA96 (Biotage) using the nucleotide dispensation orders shown in Table 7. The allele frequencies (dynamic range between 5 and 95 %) were determined using the PyroMark ID SNP run software.

4.3.3. Quantitative allele-specific real-time PCR assays using CYP51 S524T as marker to measure selection by azoles

Sensitivity to the main azoles used for control of Septoria leaf blotch, epoxiconazole and prothioconazole, has shifted further since 2014, as can be seen from the annual monitoring studies at Rothamsted, with increasing numbers of isolates showing elevated EC_{50} values (≥0.2 ppm) for prothioconazole-desthio (Figure 2). Figure 3 shows the emergence of complex S524T variants in early season Rothamsted populations over time. S524T was first detected in 2015 at frequencies exceeding 5 % in variant [L50S, D134G, V136A, I381V, Y461H, S524T], but variant [L50S, V136C,

S188N, A379G, I381V, Δ, S524T] became the most detected S524T-harbouring CYP51 variant since 2019. This variant was also identified as the most common CYP51 variant in 2020 with a detection frequency of 32.3% in the overall field population. Complex CYP51 variants carrying S524T (e.g., [L50S, V136A, S188N, A379G, I381V, Δ, S524T], [L50S, V136C, S188N, I381V, Y461H, S524T], [L50S, V136C, S188N, A379G, I381V, Δ, S524T] and [L50S, D134G, V136A, S188N, I381V, Δ, N513K, S524T] are most insensitive to both epoxiconazole and prothioconazole-desthio and underly these sensitivity shifts (Table 8).

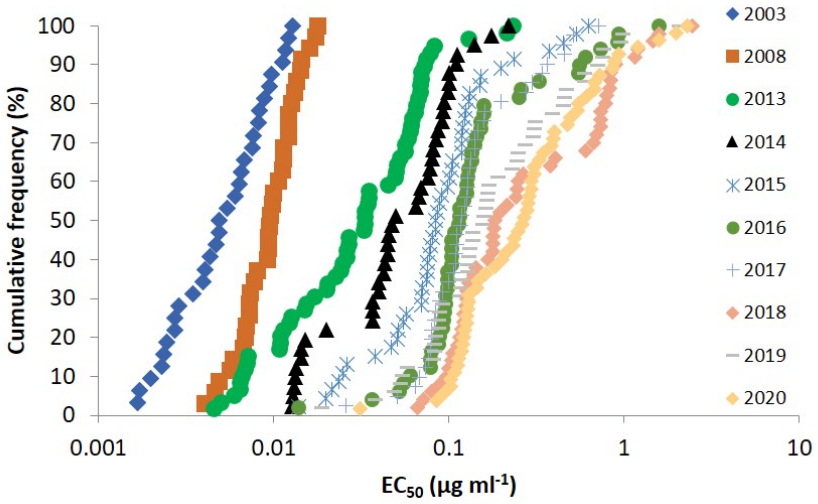

Figure 2. Prothioconazole-desthio sensitivity profiles of field populations of *Zymoseptoria tritici* sampled at Rothamsted during 2003 (n=32), 2008 (n=35), 2013 (n=59), 2014 (n=41), 2015 (n=46), 2016 (n=49), 2017 (n=41), 2018 (n=50), 2019 (n=49) and 2020 (n=55)

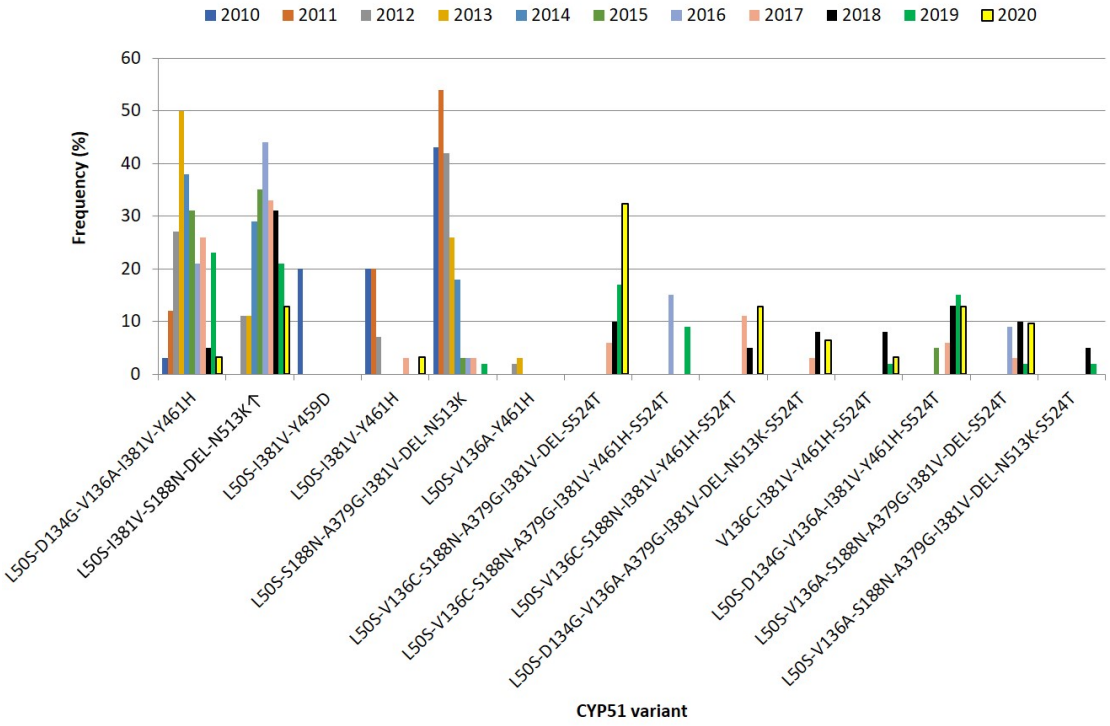

Figure 3. Dynamics of frequently occurring CYP51 variants (≥ 5 % in at least one year) in untreated Septoria field populations at Rothamsted during 2010-2020.

Table 8. Average azole resistance factors of the most commonly occurring CYP51 variants in Rothamsted field populations of *Zymoseptoria tritici* during 2010-2018.

CYP51 variant	Resistance Factor (RF)[1]			
	Epoxiconazole	Prochloraz	Tebuconazole	Prothio-desthio
[L50S, I381V, Y461H]	81	3.5	36	39
[L50S, S188N, A379G, I381V, Δ, N513K]	149	1.2	82	21
[L50S, D134G, V136A, I381V, Y461H]	196	11	5.0	102
[L50S, S188N, I381V, Δ, N513K] + CYP51↑	389	17	235	92
[L50S, D134G, V136A, I381V, Y461H, S524T]	809	18	11	336
[L50S, V136C, S188N, A379G, I381V, Δ, S524T]	1486	3.3	242	162

[1]Resistance factors of strains belonging to different CYP51 variants were calculated as the fold change in mean EC_{50} compared to the mean EC_{50} of four wildtype CYP51 strains carrying no amino acid substitutions. Mean EC_{50} values of the wild-type CYP51 variants were 0.0029, 0.0164, 0.0720 and 0.0014 ppm for epoxiconazole, prochloraz, tebuconazole and prothioconazole-desthio, respectively.

Based on the phenotype-to-genotype association, S524T was chosen as DNA marker for selection of azole resistance in field trials with spray applications of prothioconazole as mixing partner.

The quantitative allele-specific real-time PCR assay for detection of CYP51 S524T was developed according to a triple probe assay described by Fraaije *et al*. (2005) using a nested PCR approach. For the first PCR, primers CPF1 (3'-GACGACTGCCCTAGGAAGCAT-5') and ST51R2 (3'-TCAGTTCTTCTCCTCCTTCTCCTC-5') were used to generate a 295 or 301 bp PCR product covering the DNA sequence encoding for CYP51 amino acid codon positions 447-544. Twenty µl reactions were carried out, consisting of 2.5 µl of DNA sample (50 ng of DNA), 2 µl of 10X Red Hot Taq buffer, 0.1 µl of each primer (100 µM), 0.4 µl of dNTP solution (10 mM of each dNTP), 1.2 µl magnesium chloride (25 mM), 0.04 µl Red Hot DNA polymerase (5 U μl^{-1}) and 13.66 µl of sterile distilled water. PCR was carried out in a Biometra T3 thermocycler under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 40 cycles of 94°C for 10 s, 60°C for 30 s, 72°C for 45 s with a final extension at 72°C for 5 minutes. For the second PCR, the final

concentrations of the primers and probes were 0.3 µM forward primer 524F2new (5'-AATTCGCGTACGTGCAATTG-3'), 0.3 µM reverse primer 524R (5'-CCTCCTCTCCCACTTCAC TACTG-3'), 0.067 µM 5' CY5/3' BHQ2-labeled universal probe (5'-CATTACAGCGACGATGGT TCGCGA-3'), 0.1 µM 5' 6-carboxy-fluorescein (FAM)-labelled MGB probe (5'-CCGGCTGAACAAA-3') and 0.2 µM 5' VIC-labelled MGB probe (5'-CCGGGTGAACAAA-3'). PCR reactions were carried out in 15 µl reaction volumes (capped 96-well PCR plates) consisting of 2 µl DNA template (1:1000 diluted PCR product from 1st round), 7.5 µl Kapa Force Universal master mix, 0.03 µl ROX reference dye (Sigma) and 12.97 µl of sterile distilled water containing the primers and probes. The conditions for the reactions, performed in the Aria Mx real-time PCR system (Agilent), were 3 minutes at 98°C, followed by 50 cycles of 10 s at 95°C and 30 s at 60°C. The increase in fluorescence from probes was recorded at 60°C during every cycle. For each sample, the threshold cycle (cycle at which the increase of fluorescence exceeded the background (Ct) for the CY5-labeled probe was determined. Cleavage of this probe correlated with the total amount of alleles because of its binding to both azole-sensitive (S524) and -resistant CYP51 alleles (T524). Plotting known amounts of alleles against Ct values generated standard curves. Based on this, calibration samples were generated with similar amounts of total alleles but with varying levels of azole-resistant alleles (0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 98 and 100 %). For each sample, the ratio of VIC and FAM signals, indicators for R and S alleles, respectively, was measured five cycles after detection with the CY5-labeled probe. Plotting known R-allele frequencies against signal ratios generated standard curves with a dynamic range of 5 to 95 %. Resulting regression equations were used to determine R-allele frequencies in unknown samples. For each real-time PCR run, a series of standards were included.

4.4. Testing efficacy & economics of resistance management tactics (objective 3)

Field experiments were set up to investigate if resistance management strategies identified within objectives 1 can be applied to commercial fungicide programmes, and if these strategies would be economically viable. Trials were set up annually in four to seven locations across England and Wales. Trial site locations were chosen to encompass a range of septoria disease pressure, ranging from high pressure sites in Herefordshire and Wales to lower disease pressure sites in Nottinghamshire. Varieties of winter wheat were also selected with differing degrees of septoria susceptibility, with susceptible varieties such as KWS Santiago used at some trial sites, but varieties with lower levels of septoria susceptibility such as Skyfall used as well (Table 9). In 2017, a subset of treatments from the objective 1 trials were also compatible with treatments in the industry partner trials and were included within the cross-site analysis.

Table 9. Industry partner field experiments 2017–2020, location and variety information.

Year	Partner	Location	Variety	[1]Septoria tritici RL rating
2017	Corteva	Exeter	JB Diego	5.3
2017	Syngenta	Nottinghamshire	Skyfall	6.0
2017	Syngenta	Wales	KWS Santiago	[2]4.4
2017	Adama	Herefordshire	KWS Santiago	[2]4.4
2017	ADAS	Herefordshire	KWS Santiago	[2]4.3
2017	NIAB	Kent	KWS Santiago	[2]4.8
2017	SRUC	Edinburgh	Viscount	4.6
2017	Teagasc	Cork	KWS Lilli	5.9
2018	Bayer	Berwickshire	KWS Barrel	4.7
2018	Bayer	Cheshire	Leeds	4.6
2018	Corteva	Exeter	JB Diego	5.3
2018	Syngenta	Nottinghamshire	Skyfall	6.0
2018	Syngenta	Wales	KWS Santiago	[2]4.3
2019	Bayer	Hertfordshire	KWS Santiago	[2]4.3
2019	Bayer	Wiltshire	Grafton	[2]5.4
2019	Corteva	Exeter	KWS Santiago	[2]5.3
2019	Adama	Herefordshire	KWS Santiago	[2]4.3
2019	Adama	Herefordshire	KWS Santiago	[2]4.3
2019	Syngenta	Nottinghamshire	Skyfall	5.9
2019	Syngenta	Wales	[2]KWS Santiago	[2]4.3
2020	Bayer	Northamptonshire	KWS Siskin	6.5
2020	Bayer	Lincolnshire	Graham	6.8
2020	Corteva	Exeter	Elation	4.1
2020	Adama	Herefordshire	Elation	4.1
2020	Syngenta	Nottinghamshire	Skyfall	5.9
2020	Syngenta	Wales	Elation	4.1

[1] = AHDB resistance rating in recommended lists (RL) by year
[2] = variety not featured in recent AHDB RL trials so the latest available rating is shown

Fungicide treatments each year contained products with comparable modes of action at prescribed proportions of label rate (Table 10, Table 11 & Table 12). Changes to the treatment list between trial years were agreed between the industry partners at the start of each season. The specific products selected to represent the mode of action in each of the trials varied between trials and trial years depending on trial sponsor choice and changes in product availability. The exception to this was the 2020 trials where the choice of multi-site product and dose was selected early in 2020 and was the same for all trial sites

Table 10. Industry partner field experiment treatment list for trials conducted in 2017.

Trt	T0 (2-3 weeks before T1, GS25-30)	T1 (Leaf 3 emerged GS32)	T2 (Flag Leaf emerged GS39)	T3 (Ear Spray GS59)
1	UT	UT	UT	UT
2	MS 0.5	Azole 1.0+MS 0.5	SDHI 0.5+MS 0.5	Azole 1.0+MS 0.5
3	UT	Azole 1.0	Azole 1.0+SDHI 0.5	Azole 1.0
4	MS 0.5	Azole 1.0+MS 0.5	Azole 1.0+SDHI 0.5 +MS 0.5	Azole 1.0+MS 0.5
5	Azole 1.0	Azole 1.0+SDHI 0.5	Azole 1.0+SDHI 0.5	Azole 1.0
6	Azole 1.0 + MS 0.5	Azole 1.0+SDHI 0.5+MS 0.5	Azole 1.0+SDHI 0.5 +MS 0.5	Azole 1.0+MS 0.5

Table 11. Industry partner field experiment treatment list for trials conducted in 2018 and 2019.

Trt	T0 (2-3 weeks before T1, GS25-30)	T1 (Leaf 3 emerged GS32)	T2 (Flag Leaf emerged GS39)	T3 (Ear Spray GS59)
1	UT	UT	UT	UT
2	MS 0.5	Azole 1.0+MS 0.5	SDHI 0.75 + MS 0.5	Azole 1.0 + MS 0.5
3	UT	Azole 1.0	Azole 1.0+SDHI 0.75	Azole 1.0
4	MS 0.5	Azole 1.0+MS 0.5	Azole 1.0+SDHI 0.75+MS 0.5	Azole 1.0 + MS 0.5
5	Azole 1.0	Azole 1.0+SDHI 0.75	Azole 1.0+SDHI 0.75	Azole 1.0
6	Azole 1.0 +MS 0.5	Azole 1.0+SDHI 0.75+MS 0.5	Azole 1.0+SDHI 0.75+MS 0.5	Azole 1.0 + MS 0.5
7	MS 0.5	Azole 1.0+SDHI 0.375+MS 0.5	Azole 1.0+SDHI 0.75+MS 0.5	Azole 1.0+SDHI 0.375+MS 0.5

Table 12. Industry partner field experiment treatment list for trials conducted in 2020

Trt	T0 (2-3 weeks before T1, GS25-30)*	T1 (Leaf 3 emerged GS32)*	T2 (Flag Leaf emerged GS39)*	T3 (Ear Spray GS59)**
1	UT	UT	UT	UT
2	MS 0.67	Azole 1.0 + MS 0.67	SDHI 0.75 + MS 0.67	Azole 1.0 + MS 0.5
3	UT	Azole 1.0	Azole 1.0 + SDHI 0.75	Azole 1.0
4	MS 0.67	Azole 1.0 + MS 0.67	Azole 1.0 + SDHI 0.75 + MS 0.67	Azole 1.0 + MS 0.5
5	Azole 1.0	Azole 1.0 + SDHI 0.75	Azole 1.0 + SDHI 0.75	Azole 1.0
6	Azole 1.0 + MS 0.67	Azole 1.0 + SDHI 0.75 + MS 0.67	Azole 1.0 + SDHI 0.75 + MS 0.67	Azole 1.0 + MS 0.5
7	MS 0.67	Azole 1.0 + SDHI 0.375 + MS 0.67	Azole 1.0 + SDHI 0.75 + MS 0.67	Azole 1.0 + SDHI 0.375 + MS 0.5

* Multi-site at T0,T1, T2 was folpet (Arizona, Adama), at 0.66 proportion of label rate (1.0 L/ha)
** Multi-site at T3 was mancozeb (Unizeb Gold, UPL Europe), at 0.5 proportion of label rate (1.5 L/ha)

4.5. Statistical analyses

Mutation frequency data, septoria severity and yield data were logit transformed where necessary to ensure normality of data for analysis. Individual site data was analysed using ANOVA. Where treatment was the only factor, the ANOVA result was reported in figure legends, showing F df treatment & df residual, F statistic (variance ratio), p value, and SED if analysing non-transformed data, e.g., Treatment $F_{11,33}$ =15.7, p<0.001, SED 5.59. Where analysis was conducted on transformed data, the 95% confidence limits were calculated from back-transformed data.

For the experiments to test mixture tactics to manage concurrent resistance (Objective 1), ANOVA models were designed to focus on analysing the effects on SDH mutation frequency of**:** dose of SDHI, dose splitting of SDHI, mixing with DMI, and for 2017 data only, including a multi-site. The combinations of treatments included in each contrast within ANOVA models differed by year because of different treatment structures used in 2017, 2018, and 2019, with 2020 the same as 2019.

In 2017 and 2018, combinations of selected treatments as appropriate were analysed to show the effects of multi-site inclusion, mixing with DMI, SDHI dose and SDHI dose splitting. For 2019 and 2020 data, the ANOVA model to test the effect of SDHI dose and of splitting the SDHI dose was: *UT/DMI solo/SDHIsolo/SDHIsolodose/(SDHIdose*SDHIsplit).* The ANOVA model used to test the effect of mixing with DMI was *UT/DMIsolo/TestEffectMixtureDose*SDHIsolo).*

In general, where there were consistent effects of treatments between sites within one year, REML (linear mixed model and residual or restricted maximum likelihood) was also used to analyse data across sites, using fixed (site and treatment as appropriate) and random (block and plot) effects. In 2017, the fixed models used to analyse the key questions were as follows: for dose splitting, *Site*(SDHI dose/SDHI dose split);* for multi-site effects, *Site*(treatment pattern* MS),* where treatment pattern included only pairs of treatments differing by inclusion of MS or not. In 2018, 2019 and 2020, the fixed model used to analyse dose splitting was: *Site*(SDHI dose/SDHI dose split)*.

For the mixtures versus alternation field experiments (Objective 2), in 2018, where there were differing doses of both DMI and SDHI, the ANOVA model was: *UT/SDHI dose/(DMI dose*programme),* where the programmes were [1] mixture, [2] alternation SDHI T1 & DMI T2 or [3] alternation DMI T1 & SDHI T2. In 2019 and 2020, where only the DMI doses differed, the ANOVA model was *UT/(DMI dose*programme).*

4.6. Model simulations of field experiments

An updated parameterisation of the Hobbelen *et al.* (2011b) model of fungicide resistance (Figure 4.) has been developed as part of AHDB PhD studentship 21120062 (to be reported separately), with changes to the parameter values of the crop canopy and septoria infection sub-models. The updated parameterisation of the model was used to simulate the 2019 and 2020 field experiment treatments (Table 4) investigating the effects of splitting the dose of SDHI on selection for mutations for SDHI and DMI resistance, to test whether the ability to detect an effect of splitting the dose relative to the background level of variability in the experimental system is likely to be affected by i) high mutant frequencies early in the season (before treatments started) which may not leave enough 'headroom' for further selection to detect treatment differences, (ii) the effects of mixtures reducing the effect of dose splitting on selection, and/or (iii) bias caused by the timing of sampling, relative to the timing of treatment applications.

The model simulates a typical UK epidemic of *Zymoseptoria tritici* (septoria), describing the seasonal growth and senescence of the upper crop canopy (upper three leaves) of winter wheat under average temperature conditions in the UK, key processes in the pathogen life cycle (sporulation, infection and growth) and their interaction with fungicides. The dynamics of the epidemic are driven by the growth of the crop, in terms of leaf area available for infection. The leaf area passes sequentially through healthy, latent (infected but not yet sporulating), infectious (sporulating) and post-infectious stages. The infectious leaf area generates spores that cause new infections on healthy leaf area. The model simulates the densities of both the latent and infectious stages of a strain sensitive to the fungicide and a strain resistant to the fungicide.

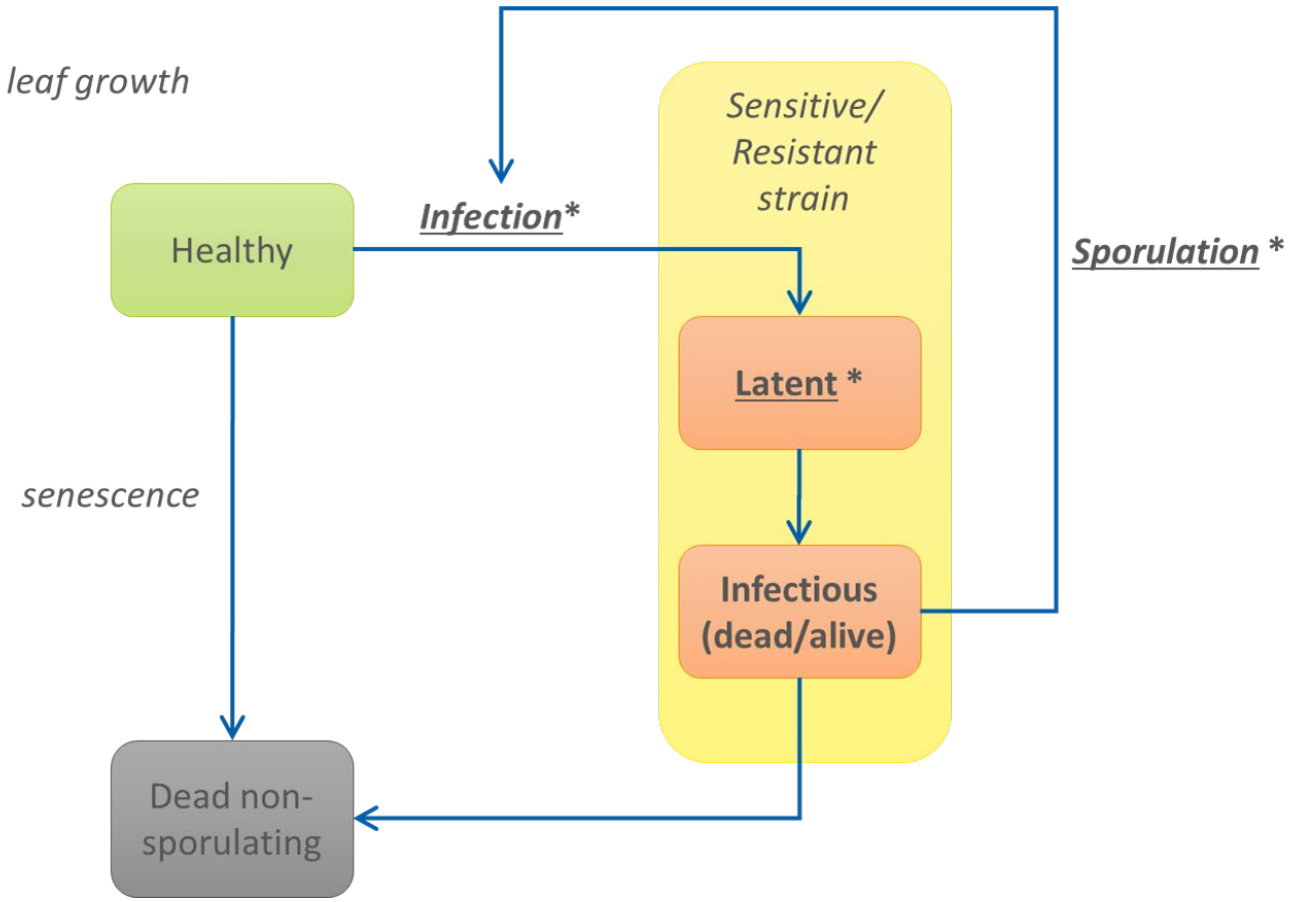

Figure 4. The model in schematic form (adapted from Hobbelen *et al.* (2011b)).

4.6.1. Simulating application timings

The model simulation of upper canopy growth and septoria disease runs on a degree days (base 0°C) timescale. To enable model simulation of the field experiments, it was therefore necessary to map the field experiment treatment timings to the relevant degree days timing in the model (Figure 5). The model degree days were mapped to winter wheat growth stages as part of the model reparameterization, so the approximate growth stages for each application (T0 - GS31; T1 - GS32; T2 - GS39; T3 – GS59) were used to estimate the timing of each application for the model simulations.

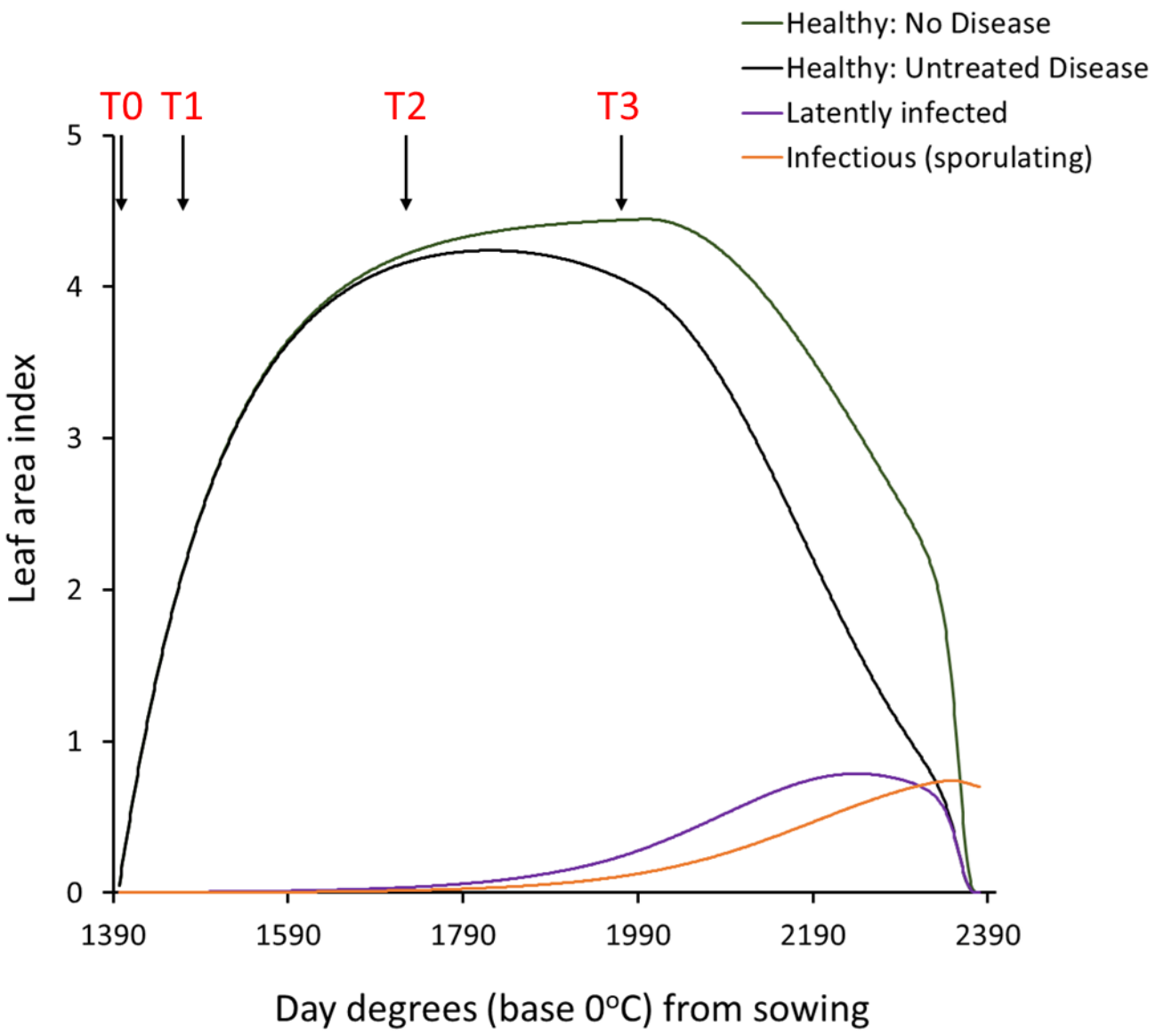

Figure 5. Estimated application timings in day degrees (base 0°C) from sowing, model simulation of healthy canopy leaf area index in absence of disease, and model simulation of healthy, latently infected and infectious (sporulating) leaf area index for an untreated septoria epidemic.

4.6.2. Fungicide dose response parameterisation

In the model (Hobbelen *et al.*, 2011b), a combination of four parameters describes the effect of a systemic fungicide in reducing septoria infection efficiency and increasing the length of the latent period i) the fungicide decay rate, which describes how quickly the concentration of a fungicide decays over time; ii) the asymptote, which describes the maximum fractional reductional in pathogen life cycle parameters at an infinite fungicide concentration; iii) the curvature, which describes how quickly the fractional reduction in pathogen life cycle parameters decreases as the concentration of the fungicide decreases; iv) the asymptote shift, which describes the proportional reduction in the asymptote parameter for a pathogen strain with resistance to the fungicide

Site-specific data from AHDB Fungicide Performance trials from the years 2011-2012 were available to parameterise the fungicide dose response for isopyrazam-sensitive strains. Data on isopyrazam fungicide performance was not available from later years, but data for fluxapyroxad were available for both 2012 and 2016-2018 and was used to estimate a realistic range of asymptote shift parameter values for SDHI-resistant strains. Data were available from the years 2016-2018 to parameterise the fungicide dose response for prothioconazole as a mixture partner in the field experiments.

Some trials had data from two separate assessment timings. For each trial, assessment timing and fungicide dose rate, the recorded growth stage at fungicide application was used to set the model degree days at which the application was made. As growth stage assessments later in the season are less accurate, the model degree days at disease assessment were instead calculated based on the number of days between fungicide application and disease assessment, multiplied by 12.1 (the average number of degree days in a day in the model). For each leaf, the logit severity on each leaf from all replicates was averaged for each fungicide dose rate and back-transformed to an average percentage severity on each leaf. These were weighted by a model estimate of the proportional contribution of each leaf at the disease assessment timing to calculate the overall percentage severity on the upper canopy. Trials/assessment timings where the average severity on untreated plots was ≤5% or ≥95% were excluded from the parameterisation, as were any trials/assessment timings where there was not data available for all of leaves 1, 2 and 3.

For each fungicide, least squares optimisation was used to fit the dose response curve and therewith estimate the asymptote and curvature parameter values (Table 13). We fitted dose response curves for isopyrazam (2011-2012) (Figure 6), prothioconazole (2016-2018) (Figure 7) and fluxapyroxad (2012) (Figure 8). In a population that is partly made up of sensitive strains and partly made up of resistant strains, as was the case for SDHI-resistant strains at the time the field experiments were carried out, the observed fungicide dose response will lie somewhere between the actual dose response of the sensitive and resistant strains respectively, depending on the fraction of the population that is resistant. Least squares optimisation was used to fit the asymptote shift that best predicted the observed fungicide dose response for fluxapyroxad trials in 2016-2018 (Figure 8) for four different starting frequencies of the resistant strain (25%, 33%, 50% and 100%) (Table 14). 25%-50% was assumed to be representative of the frequency of SDHI-resistant strains in the UK in 2016-2018, whilst the asymptote shift assuming a 100% starting frequency indicated the average effectiveness of an SDHI fungicide as a mixture partner to a DMI fungicide.

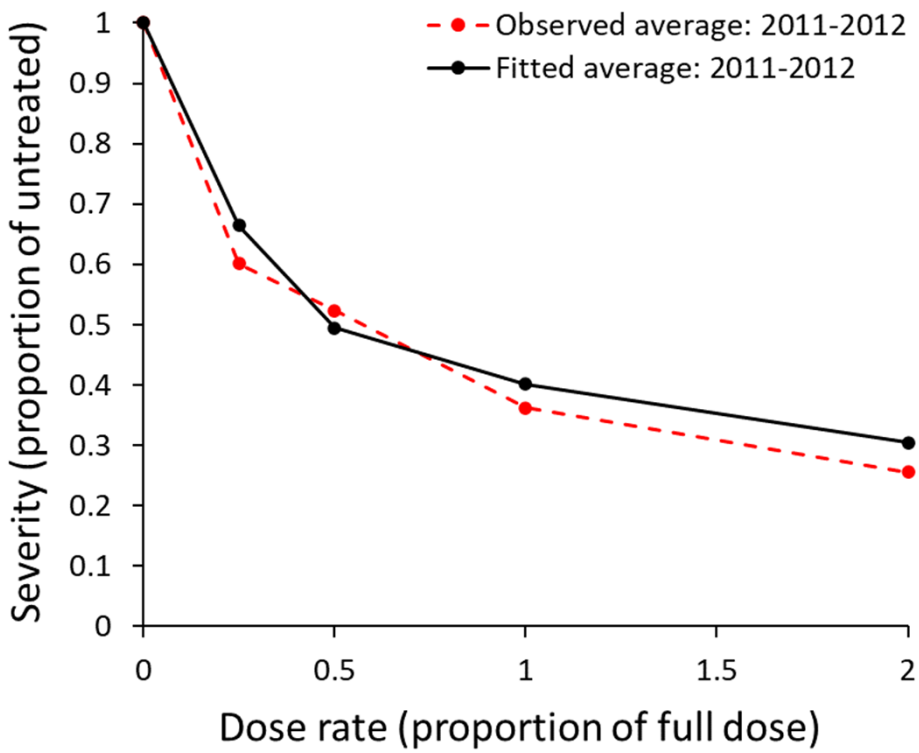

Figure 6. Fitted and observed average proportional control of septoria disease at a range of fungicide dose rates, isopyrazam 2011-2012.

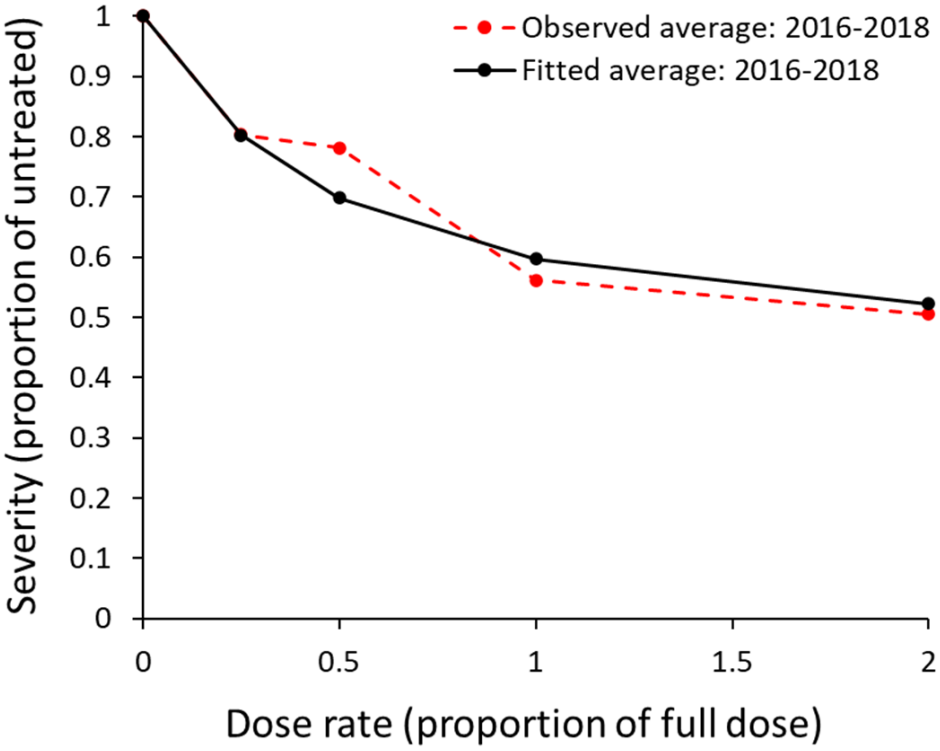

Figure 7. Fitted and observed average proportional control of septoria disease at a range of fungicide dose rates, prothioconazole 2016-2018.

Table 13. Fitted asymptote and curvature fungicide parameter values, and the number of trials used to fit each fungicide dose response.

Fungicide	Number of trials	Asymptote	Curvature
Isopyrazam (2011-2012)	6	0.4778	6.988
Prothioconazole (2016-2018)	10	0.3024	4.584
Fluxapyroxad (2012)	5	0.5507	6.055

Table 14. Fitted asymptote shift parameter values for a range of initial resistant strain starting frequencies (n=10).

Initial starting frequency of resistant strain (%)	Asymptote shift
25	0.70
33	0.59
50	0.44
100	0.25

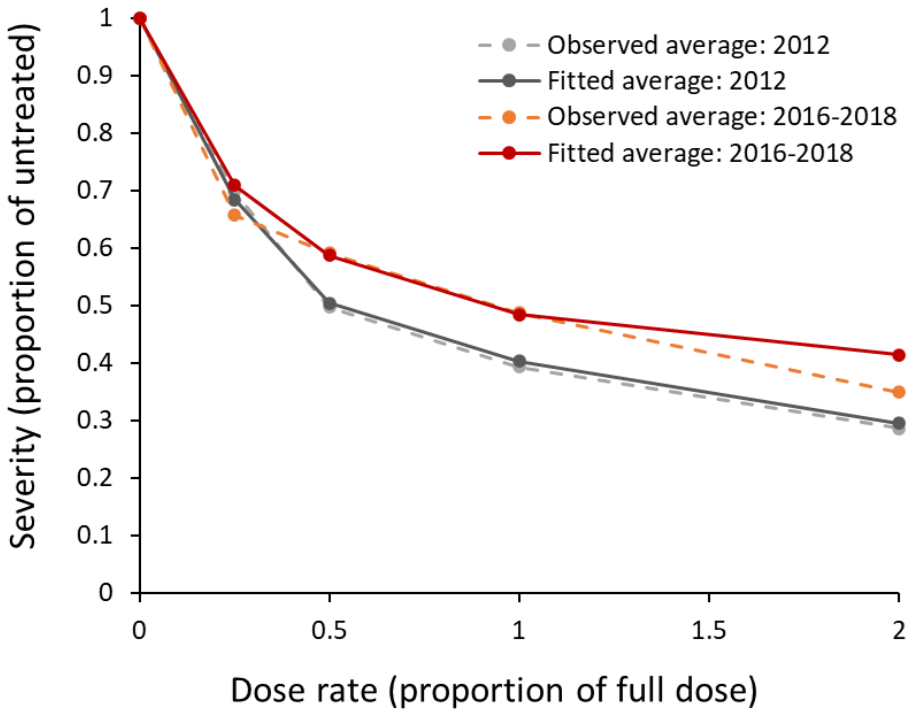

Figure 8. Fitted and observed average proportional control of septoria disease at a range of fungicide dose rates, fluxapyroxad 2012 (assuming completely sensitive population) and fluxapyroxad 2016-2018 (assuming 33% starting frequency of the resistant strain).

4.6.3. Testing if dose splitting effects were obscured by high starting mutant frequencies in the field experiments

To test whether the ability to detect an effect of splitting the dose relative to the background level of variability in the experimental system is likely to be affected by high mutant frequencies early in the season (before treatments started) which may not leave enough 'headroom' for further selection to detect treatment differences, the model simulations of the field experiment treatments in 2019 and 2020 were run for multiple resistant strain starting frequencies and asymptote shift parameters. Different values of the asymptote shift parameter were investigated for DMI fungicides and SDHI fungicides. For a given fungicide, all combinations of starting frequencies and asymptote shift parameters listed in Table 15 were simulated.

Table 15. Mutation starting frequencies (%) and asymptote shift parameter values (%) simulated.

Model parameter	Fungicide	
	SDHI	DMI
Resistant strain starting frequency (%)	0.01, 0.1, 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90	0.01, 0.1, 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90
Asymptote shift (%)	25, 40, 50, 70, 100	10, 25, 33, 50, 100

4.6.4. Testing if dose splitting effects were reduced below detectable levels by the effects of mixture partners

To test whether the effects of mixtures reducing the effect of dose splitting on selection may have reduced dose splitting effects below detectable levels, relative to the background level of variability in the experimental system, the predicted change in resistant strain frequency was compared for the treatments with and without the effects of a mixture partner. In the field experiments, treatments 6 & 7 and 11 & 12 (Table 4, 2019 and 2020 treatments) allow this comparison for the SDHI fungicide, for two total SDHI doses split over four applications, either applied solo (treatments 7 and 12) or in mixture with a DMI (treatments 6 and 11). Model simulations of the SDHI application patterns for treatments 3, 4, 5, 8, 9 and 10 without a DMI mixture partner were also simulated to allow comparison of model predictions for a wider range of splitting patterns. For both the field trial and modelling results, a comparison of treatment 2 (solo DMI) with treatments 3, 4, 5, 6, 8, 9, 10 and 11 enables investigation of the effect of an SDHI mixture partner on the ability to detect changes in DMI mutation frequency, for all SDHI splitting patterns investigated.

4.6.5. Testing if dose splitting effects were obscured by bias caused by the timing of sampling

To test whether the timing of sampling could have introduced bias that obscured the effects of dose splitting, the model predictions of resistant strain frequencies at a number of sample timings

(GS66, GS70, GS73, GS75, GS77, GS79, GS81, GS85) were output for each of the simulations described in sections 4.6.3 and 4.6.4, to enable comparison of sample timings and investigate whether suboptimal sample timing could lead to underestimation of dose splitting effects.

5. Results

5.1. Pre-season SDH mutation frequency, 2017-2020

5.1.1. SDH mutations

The baseline pre-season levels of total SDH mutations generally increased each year when testing samples from untreated plots at the start of experiments (Figure 9). There was variation between locations and years for the proportion of the four individual SDH mutations that were tested for, but all results showed a clear increase in total mutation frequency from 2017-2019. In 2018, the W80S mutation was below detection threshold in all samples, and in 2019 the W80S test results were invalid, but allowing for possible zero W80S levels, there was an increase in total mutations during the four years of the project. The H152R mutation was not seen in the 2017 or 2018 pre-season samples, but H152R started to appear pre-season in 2019 and 2020 (Figure 9).

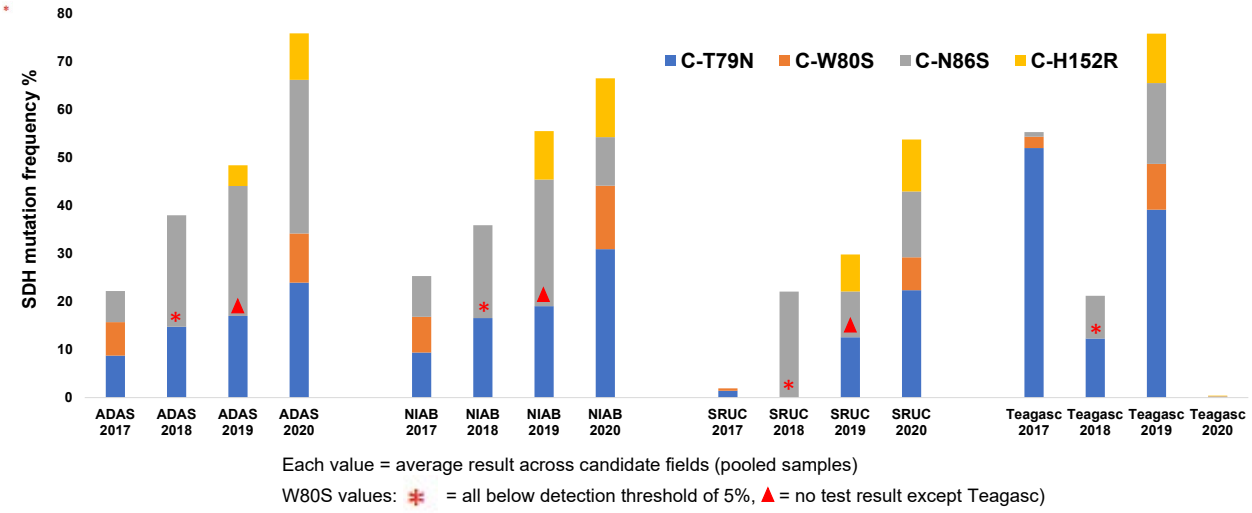

Figure 9. Pre-season SDH mutation frequency in untreated plots, 2017-2020.

5.2. Testing mixture tactics for managing concurrent resistance (objective 1)

The key findings are presented, illustrated by example data from individual sites and years as appropriate, with analysis by individual site or cross-site as appropriate. See appendices for charts and analyses of data for each site and year.

5.2.1. Timing of fungicide applications and leaf sampling

The dates of fungicide applications and dates for the target sampling time of T2 + 6 weeks for infected leaves varied by site and year (Table 16), with sample timing in particular influenced by the rate of development of septoria and of leaf senescence in untreated plots. The shortest and longest time intervals between T2 fungicide treatment and the leaf sampling were 58 and 98 days respectively, with intervals for all other sites ranging from 77 to 90 days. In 8 out of 13 sites, leaf layer two was sampled. Leaf layer 1 was sampled at 3 out of 13 sites, often where septoria infection progressed quickly (e.g., NIAB 2018, Teagasc 2019). At two sites, leaf layer 3 was sampled, where septoria infection was low, e.g., SRUC 2018.

Table 16. Objective 1 field experiments 2017-2020, fungicide application, main assessments and sample dates.

	Partner and site	Variety	Drilled prev year	T0 sample UT	T0	T1	T2	T3	T2+6 wk sample	T2+6 wk Septoria assess	Harv.	GS T0	GS T1	GS T2	GS T3	T2+6wk Leaf layer sample	Days from T0 to T2+6wk
2017	**ADAS Herefords**	Santiago	17-Oct	-	03-Apr	21-Apr	18-May	11-Jun	26-Jun	30-Jun	24-Aug	25-30	32	39	59	2	**84**
	NIAB Kent	Santiago	11-Oct	-	11-Apr	24-Apr	24-May	02-Jun	10-Jul	22-Jul	16-Aug	30	32	39	65	1	**90**
	SRUC Edinburgh	Viscount	30-Sep	-	13-Apr	03-May	24-May	07-Jun	29-Jun	06-Jul	07-Sep	26	31	39	59	2&3	**77**
	Teagasc Cork	Lilli	24 Oct	-	13-Apr	27-Apr	26-May	13-Jun	30-Jun	10-Jul	27-Aug	30	31	39	65	2	**78**
2018	**[a]ADAS Herefords**	Santiago	28-Oct	-	06-Apr	29-Apr	22-May	10-Jun	26-Jun	04-Jul	03-Aug	30	32	39	63	1	**81**
	NIAB Herefords	JB Diego	07-Oct	-	13-Apr	03-May	23-May	06-Jun	02-Jul	04-Jul	17-Aug	30	32	43	61	2	**80**
	SRUC Edinburgh	Viscount	30-Sep	-	19-Apr	11-May	30-May	22-Jun	18-Jul	10-Jul	25-Aug	27	32	42	65	3	**90**
	Teagasc Cork	Lumos	03-Nov	-	30-Apr	16-May	01-Jun	13-Jun	27-Jun	27-Jun	08-Aug	30	32	39	65	2	**58**
2019	**[b]ADAS Herefords**	Santiago	9-Oct	15-Apr	11-Apr	28-Apr	20-May	17-Jun	02-Jul	03-Jul	23-Aug	30	32	39	65	2	**82**
	NIAB Herefords	Elation	07-Oct	10-Apr	06-Apr	22-Apr	23-May	06-Jun	03-Jul	28-Jun	26-Aug	30	32	41	59	2	**88**
	SRUC Edinburgh	Motown	28-Sep	15-Apr	11-Apr	30-Apr	28-May	19-Jun	10-Jul	11-Jul	28-Aug	30	32	39	59	2	**90**
	Teagasc Cork	Lumos	20-Oct	not done	18-Apr	03-May	27-May	20-Jun	12-Jul	28-Jun	29-Aug	30	32	39	65	1	**85**
2020	**SRUC Edinburgh**	Viscount	02-Oct	27-Apr	13-Apr	05-May	27-May	18-Jun	20-Jul	15-Jul	05-Sep	25-30	32	39-45	65	2	**98**

[a] additional sprays for yellow rust control:19 June fenpropimorph (Clayton Spigot, Clayton Plant protection Ltd., 0.5L/ha) and cyflufenamid (Cyflamid Certis, 0.4 L/ha)

[b] additional sprays for yellow rust control: 31 May fenpropimorph (Corbel, BASF, 0.5 L/ha)

5.2.2. Effect of solo use of fungicide and fungicide dose

In general, across all sites and years, solo use and higher doses of SDHI fungicides resulted in a significant increase in total SDH mutation frequency, compared to no SDHI application. For example, at the ADAS Herefordshire site in 2017, total SDH mutation frequency was significantly increased with solo SDHI treatment compared to no SDHI (e.g., Figure 10, treatments 1 or 2 vs Trt 3, F prob <0.001). When increased total doses of SDHI were applied, e.g., in ADAS 2018 where a dose of 1.5 was compared to a dose of 2.0 (Figure 13), there was a significant increase (F prob <0.001) in total SDH mutation frequency.

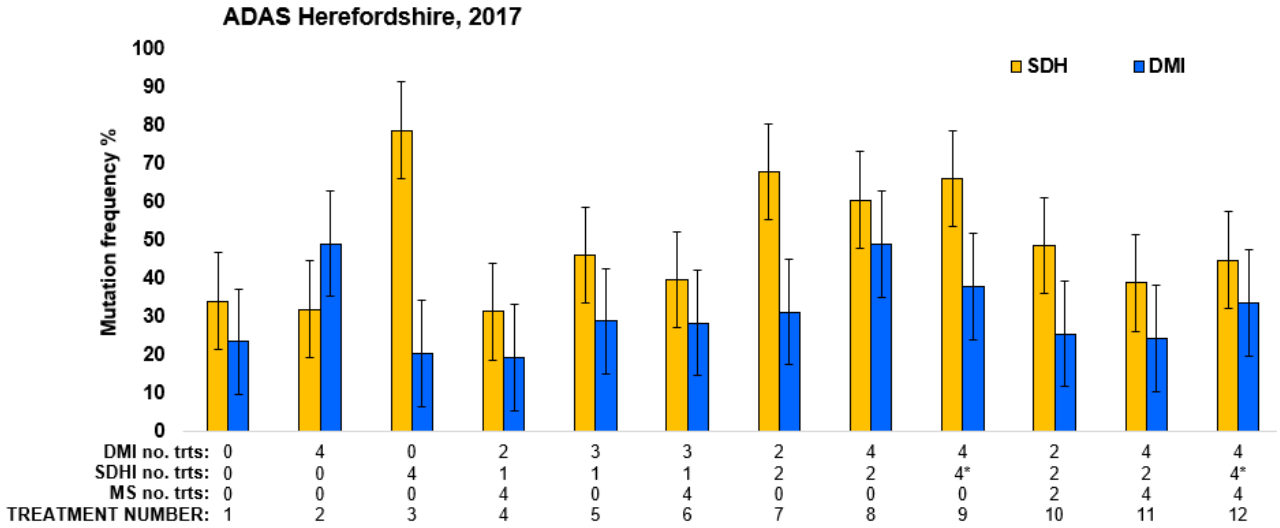

Figure 10. Effect of application frequency and dose of SDHI, DMI and multi-site (MS) fungicides on the total SDH and S524T DMI mutation frequency, ADAS Herefordshire 2017. Values are averages of four replicate plots per site, two genotyping tests per plot. SDHI = isopyrazam, dose 0.5 (except * = 0.25); DMI = prothioconazole dose 1.0; MS = chlorothalonil, dose rate 0.5 (doses are proportion of maximum permitted dose at each application) (See Table 2 for treatments). Error bars are 95% confidence limits. **SDH mutations, ANOVA:** Trt $F_{11,33}$ =15.7, p<0.001, SED 5.59. **DMI mutations, ANOVA:** Trt $F_{11,33}$ =5.3, p<0.001, SED 6.15.

5.2.3. Effect of including a multi-site fungicide in the mixture

A multi-site fungicide included in mixture treatments in 2017 significantly reduced the frequency of both SDH (REML cross-site, F prob = 0.004) and DMI mutations (REML cross-site, F prob = 0.043), for all pairs of treatments in which the only difference was the inclusion or not of the multi-site (Figure 11). This was a consistent effect seen at each of the four experiment sites in 2017.

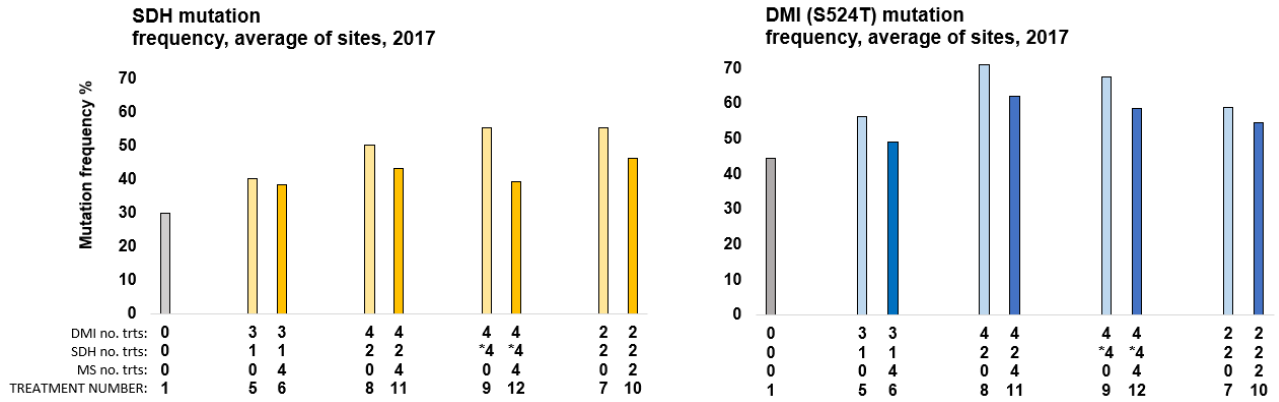

Figure 11. Effect of including a multi-site (MS) fungicide in treatment mixtures on the total SDH and S524T DMI mutation frequency in 2017 field experiments. Values are averages of four replicate plots (except three plots at NIAB), two genotyping tests per plot, across four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam, dose 0.5 (except * = 0.25); DMI = prothioconazole dose 1.0; MS = chlorothalonil, dose rate 0.5 (doses are proportion of maximum permitted dose at each application) (See Table 2 for treatments). **SDH mutations REML:** logit SDH for MS effect, $F_{3,51}$=4.93, p= 0.004, Max SED = 0.176. **DMI mutations REML:** logit DMI for MS effect, $F_{3,35}$ 3.02, p= 0.043.

5.2.4. Effect of SDHI total dose and splitting the dose

Overall, across the four years of the project, splitting the same SDHI total dose across different application times did not significantly increase selection for total SDH, nor did it affect S524T DMI mutation frequency. SDHI dose-splitting effects in individual experiments were generally non-significant or small, and there were some inconsistencies between different sites and years. In the 2017 experiments, the effect on total SDH mutations and on S524T DMI mutations of splitting the SDHI dose was non-significant across the four experiment sites (Figure 12). The effect of splitting the SDHI dose was non-significant for each site analysed individually; for SDH mutations, F prob = NS at each site, and for DMI mutations, F prob = NS at each site. This result was based on analysing a sub-set of treatments using the same SDHI total dose of 1.0, split across two or four application times. In summary, the fungicide treatments gave a significant increase in total SDH and S524T DMI frequency compared to untreated controls; a multi-site in the mixtures significantly decreased the mutation frequencies compared to equivalent treatments without a multi-site; splitting the dose of SDHI two ways or four ways had a non-significant effect.

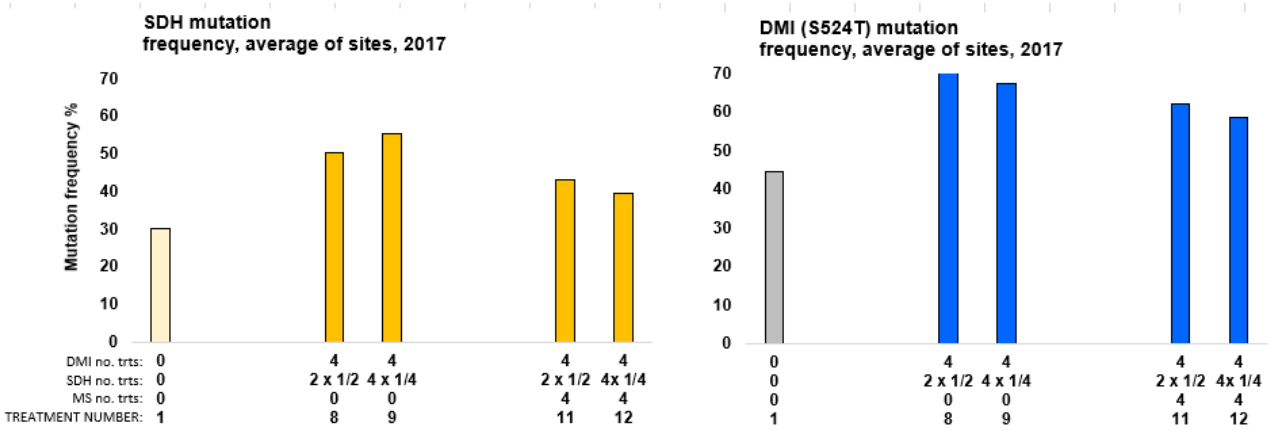

Figure 12. Effect of splitting the same total SDHI dose across two or four application times, on the total SDH and S524T DMI mutation frequency in 2017 field experiments. Values are averages of four replicate plots (except four plots at NIAB) with two genotyping tests per plot for SDH and one for DMI), across four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam, doses 0.5 or 0.25; DMI = prothioconazole, dose 1.0; MS = chlorothalonil, dose rate 0.5 (doses are proportion of maximum permitted full dose at each application) (see Table 2 for treatments). **SDH mutations, REML:** site effect, $F_{3,78}$=283.5, p<0.001; SDHI dose effect $F_{3,78}$=34.5, p<0.001; SDHI dose*SDHI split = NS; Max SED = 6.25. **DMI mutations, REML:** site effect, $F_{3,75}$=148.8, p<0.001; SDHI dose effect $F_{3,75}$=3.83, p=0.012, SDHI dose* SDHI split = NS; Max SED = 7.18.

In the 2018 field experiments, treatments were focused on programmes using SDHI and DMI only. SDHI dose alone had a significant effect of increasing the total SDH mutation frequency (REML F prob <0.001) (e.g., ADAS 2018, Trts 1 and 2 vs Trt 3 in Figure 13). Splitting the same total SDHI dose across 2, 3 or 4 application times had no significant effect on SDH mutation frequency, across the four sites, ADAS, NIAB, SRUC and Teagasc (REML, F prob NS). For example, in the ADAS 2018 field experiment, a total SDHI dose of 2.0 resulted in a slightly higher SDH mutation frequency overall than a dose of 1.5, however, the effects of dose splitting were not significant, for either the dose of 1.5 or the dose of 2.0.

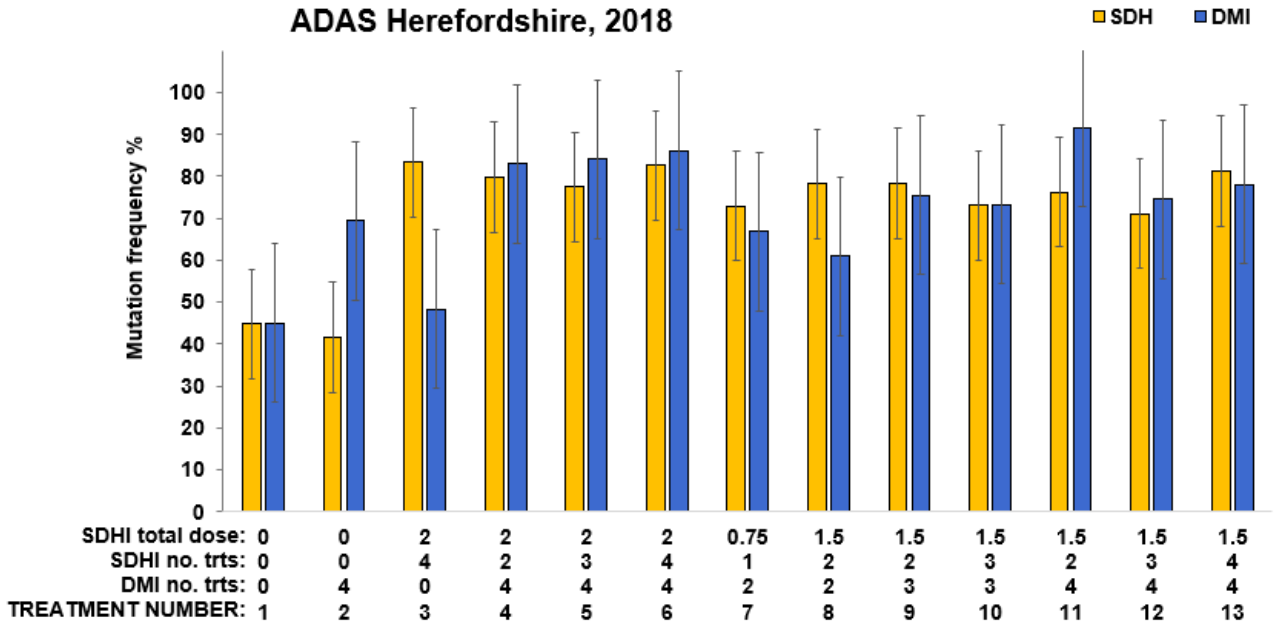

Figure 13. Effect of dose of SDH and DMI fungicides on the total SDH and S524T DMI mutation frequency, ADAS Herefordshire 2018. Each mutation value is an average across four replicate plots, with two genotyping tests per plot for SDH and one for DMI. SDHI = isopyrazam, with doses shown as proportion of maximum permitted dose at each application. DMI = prothioconazole, full dose each application (see Table 3 for treatments)Error bars are 95% confidence limits. **SDH mutations, ANOVA:** Trt $F_{12,36}$ =10.8, p<0.001, SED 5.81. **DMI mutations, ANOVA:** Trt $F_{12,36}$ =5.6, p<0.001, SED 8.39.

The 2019 and 2020 field experiments focused further on the effect of splitting the SDHI dose, using two SDHI doses (2.0 and 1.5), splitting the same total dose across 1, 2, 3, or 4 application times. The DMI treatments were either four sprays, or none (controls). There was no significant difference in SDH mutation frequency between splitting the SDHI dose across 1, 2, 3 or 4 application times, for a total SDHI dose of 2.0, or a total dose of 1.5, e.g., as seen at the SRUC Edinburgh site in 2020 (Figure 14).

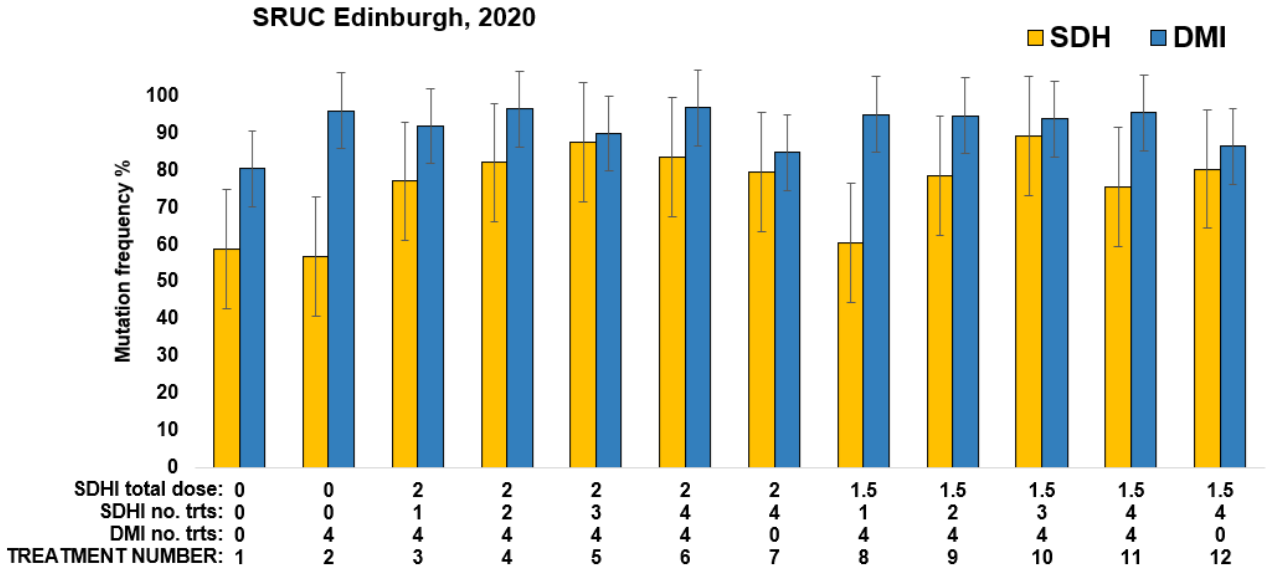

Figure 14. Effect of dose of SDH and DMI fungicides on the total SDH and S524T DMI mutation frequency, SRUC Edinburgh 2020. Values are averages of 4 replicate plots Each mutation value is an average across four replicate plots and two genotypying tests per plot. Analysis is based on logit transformed total mutation values. SDHI = isopyrazam, with doses shown as proportion of maximum permitted dose at each application. DMI = prothioconazole, full dose each application. Error bars are 95% confidence limits. **SDH mutations, ANOVA:** Trt $F_{11,33}$=4.7, p<0.001, SED 5.81. **DMI mutations, ANOVA:** Trt $F_{11,33}$=2.8, p=0.012, SED 8.39.

The effect of dose splitting of SDHI fungicides was analysed further in the five field experiments conducted over 2019 and 2020, and was significant in two out or five sites (2019 NIAB: F prob = 0.042, and 2019 Teagasc: F prob = 0.03) but the effects were small (Table 17 for 2019 analysis and Table 18 for 2020 analysis). See section below for selected results of individual SDH mutations and effects of treatments on control of septoria.

Table 17. Objective 1 field experiment 2019, analysis of effect of splitting the SDHI dose, for % total SDH mutations, % DMI (S524T) mutation and % septoria, ADAS Herefordshire, NIAB Herefordshire, SRUC Edinburgh, Teagasc Cork

[c]ANOVA model	F prob, % total SDH mutations (T79N, W80S, N86S, H152R)				F prob, % C-H152R				F prob, % DMI mutation (S524T)				F prob, % septoria severity (average top two leaves)			
	ADAS	**NIAB**	**SRUC**	**Tea-gasc**	**ADAS**	**NIAB**	**SRUC**	**Tea-gasc**	**ADAS**	**NIAB**	**SRUC**	**Tea-gasc**	**ADAS**	**NIAB**	**SRUC**	**Tea-gasc**
UT vs all other trts	<.001	<.001	<.001	<.001	<.001	0.458	0.033	0.302	0.914	<.001	<.001	0.677	<.001	<.001	<.001	<.001
UT.[a]DMI solo	<.001	<.001	0.001	0.915	<.001	0.064	0.319	0.259	0.76	0.203	0.419	0.815	<.001	0.075	0.082	0.078
UT.DMI solo. [b]SDHI solo	0.959	0.159	0.212	0.093	0.401	0.053	0.828	0.617	0.825	<.001	<.001	0.646	<.001	<.001	<.001	<.001
UT.DMI solo. SDHI solo. SDHI solo dose	0.953	0.703	0.479	0.844	0.961	0.188	0.998	0.829	0.937	0.1	0.582	0.464	0.356	1	0.959	0.103
UT.DMI solo. SDHI solo. SDHI solo dose. SDHI dose	0.233	0.079	0.932	0.029	0.007	0.07	0.538	0.106	0.91	0.109	0.993	0.84	0.072	0.029	0.05	0.214
UT.DMI solo. SDHI solo. SDHI solo dose. SDHI split	0.131	0.262	<.001	0.014	0.462	0.767	0.008	0.081	0.956	0.378	0.628	0.563	0.437	0.515	0.702	0.153
UT.DMI solo. SDHI solo. SDHI solo dose. SDHI dose. SDHI split	0.919	0.042	0.62	0.03	0.056	0.816	0.923	0.006	0.931	0.914	0.635	0.757	0.847	0.427	0.231	0.217
SED	4.828	5.10	7.580	4.413	2.537	6.008	4.665	1.549	8.5	3.614	4.464	8.27	0.224	0.113	0.18	0.256
df resid	33	22	32	33	33	22	33	33	33	22	32	33	33	22	33	33

[a] prothioconazole (Proline, Bayer), dose per application = full rate

[b] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[c] septoria analysis reported on logit transformed data

5.2.5. Effects on individual SDH mutations and effects of treatments on septoria

The proportions of the four SDH mutations varied widely between sites and years (see Appendices 1 to 4 for data by individual sites and years).
In 2017, C-H152R was not seen in control treatments and generally only appeared following the higher doses of SDHI. W80S was present in much larger proportions at the ADAS site than at the other three sites. At Teagasc, at least 80% of SDH mutations were C-T97N (Figure 35 in Appendix 1). In 2018, C-H152R appeared in some of the control treatments which received no SDHI fungicide. The proportions of the four mutations were more even across sites in 2018 than in 2017, again with the Teagasc site differing from the others, but this time with a large proportion of N86S (Figure 38 in Appendix 2). In 2019, the mutation pattern was similar to 2018 but with an obvious increase in the proportion of the C-H152R mutation across all sites in all treatments including controls, except Teagasc (Figure 41 in Appendix 3). In 2020, when there was one field experiment (SRUC), the C-H152R mutation was present in all treatments including untreated (Figure 15).

Control of septoria was lower in general across sites and years with solo DMI or solo SDHI treatments compared with using mixtures of the two fungicides. Including a multi-site increased the efficacy further. In the 2019 and 2020 experiments it was clear that the DMI played an important role in efficacy. The three SDHI-only treatments gave significantly poorer control at each site compared with the nine treatments which included a four spray DMI programme (e.g., 2019 Figure 43 in Appendix 3, and 2020 Figure 16).

By 2019 and 2020 the early season frequencies of SDH and DMI mutations were high across sites, e.g., 60% and 72% SDH and DMI, respectively, ADAS 2019) and the end-of season frequencies approaching 100% for some of the DMI treatments (ADAS 2019) were not necessarily associated with lower efficacy. For example, in 2020 the results show that inclusion of the DMI fungicide in a programme gave significantly improved control of septoria compared with programmes using SDHI only (Figure 16). There was also little difference in efficacy between the two total doses of SDHI used, 1.5 or 2.0 (Figure 16).

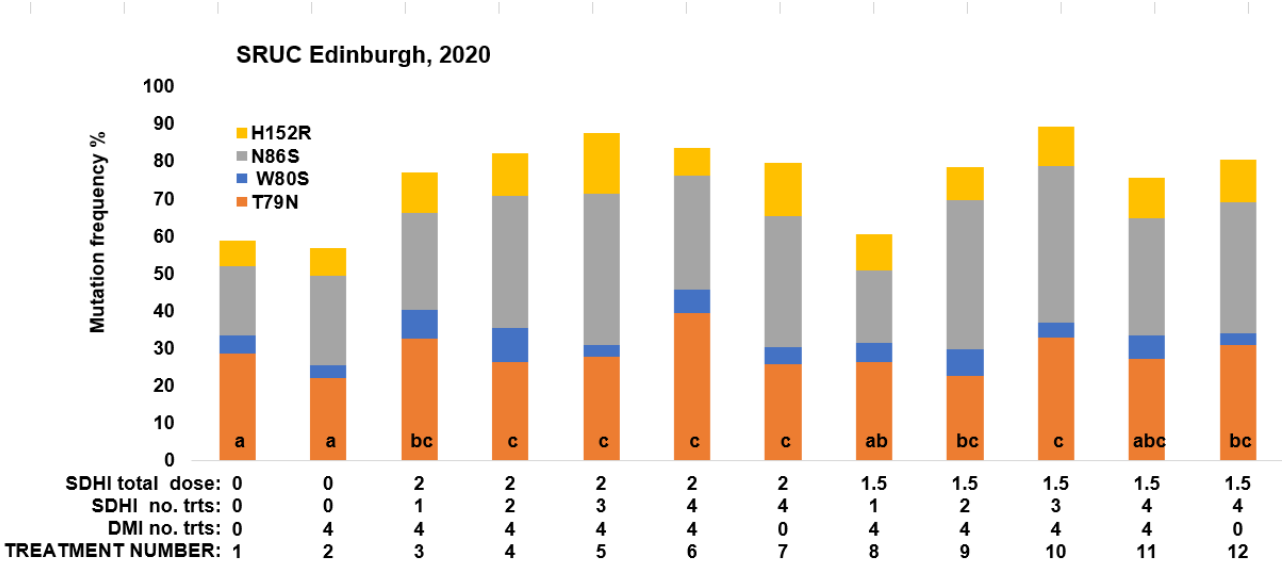

Figure 15. Selection for SDH mutations in *Zymoseptoria tritici* strains, 2020 SRUC Edinburgh field experiment, 20 July. Each individual mutation value is an average across four replicate plots and two genotyping tests per plot. SDHI = isopyrazam, with doses shown as proportion of maximum permitted dose at each application. DMI = prothioconazole, full dose each application. Letters denote Tukey comparisons. **SDH total mutations, ANOVA:** Trt $F_{11,33}$=4.7, p<0.001, SED 5.81.

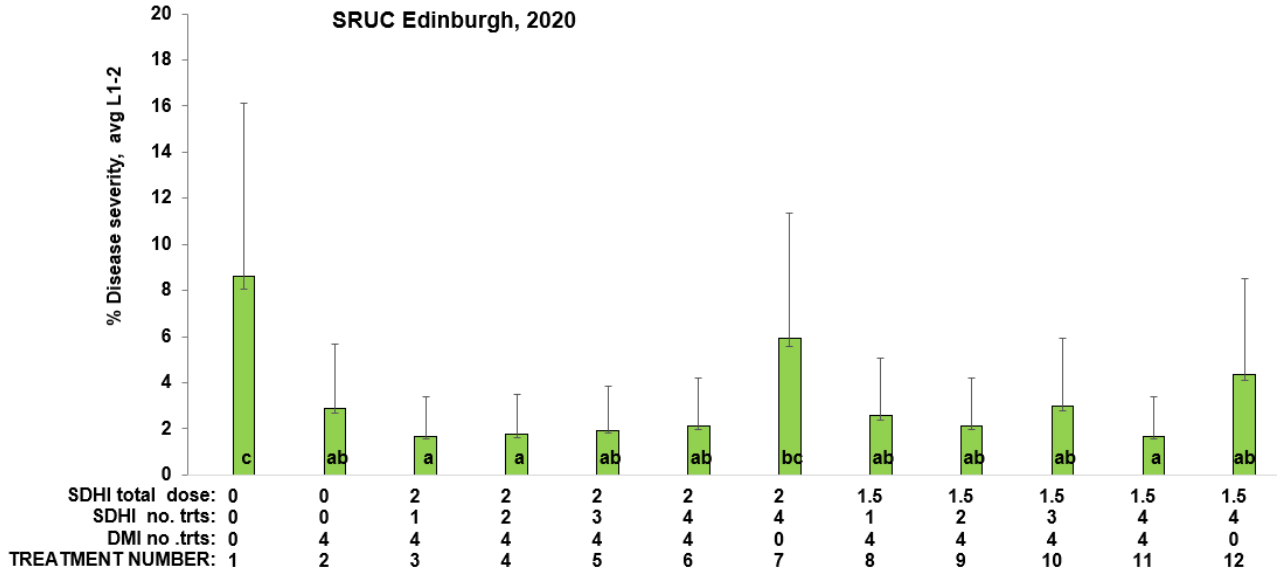

Figure 16. Septoria (*Zymoseptoria tritici*) severity on the upper two leaves, 2020 SRUC Edinburgh field experiment, 15 July (same experiment as in Figure 15). Each severity value is an average of four replicate plot values with analysis based on logit transformed values. SDHI = isopyrazam, with doses shown as proportion of maximum permitted dose at each application. DMI = prothioconazole, full dose each application. Error bars are 95% confidence limits calculated from back-transformed data. Letters denote Tukey comparisons. **Septoria severity, ANOVA:** Trt $F_{11,33}$ =6.33, p<0.001, SED 5.81.

Table 18. Objective 1 field experiment 2020, analysis of % total SDH mutation, % DMI (S524T) mutation and % septoria, SRUC Edinburgh

ANOVA model	F prob, Effect of mixing SDHI with [a]DMI				ANOVA model	[c]F prob, Effect of splitting [b]SDHI dose, (two doses)			
	% total SDH mutation	**% C-H152R**	**% DMI mutation (S524T)**	**[c]% septoria severity (average top two leaves)**		**% total SDH mutation**	**% C-H152R**	**% DMI mutation (S524T)**	**% septoria severity (average top two leaves)**
UT	0.005	0.111	≤ 0.001	≤ 0.001	UT	0.001	0.108	≤ 0.001	≤ 0.001
UT. DMI solo	≤ 0.001	0.133	0.286	0.347	UT.DMI solo	≤ 0.001	0.129	0.302	0.351
UT.DMI solo. Mixture effect	0.887	0.862	0.175	0.009	UT.DMI solo. SDHI solo	0.804	0.240	0.002	≤ 0.001
UT.DMI solo. Mixture effect. Mixture dose effect	0.593	0.928	0.946	0.264	UT.DMI solo. SDHI solo. SDHI solo dose	0.917	0.364	0.713	0.451
UT.DMI solo. Mixture effect. SDHI solo	0.876	0.111	0.002	≤ 0.001	UT.DMI solo. SDHI solo. SDHI solo dose. SDHI dose	0.071	0.406	0.663	0.299
UT.DMI solo. Mixture effect. Mixture dose effect. SDHI solo	0.510	0.177	0.640	0.96	UT.DMI solo. SDHI solo. SDHI solo dose. SDHI split	0.007	0.318	0.524	0.621
df resid	38	38	38	38	UT.DMI only. SDHI solo. SDHI solo dose. SDHI dose. SDHI split	0.377	0.282	0.743	0.464
					df resid	33	33	33	33

[a] prothioconazole (Proline, Bayer), dose per application = full rate

[b] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[c] septoria analysis reported on logit transformed data

5.3. Testing alternation vs. mixture tactics (objective 2)

5.3.1. Timing of fungicide applications and leaf sampling

Fungicide application dates and sample dates varied by site and year (Table 19). The shortest and longest time intervals between the T1 fungicide treatment and the leaf sampling at T2 + 6 weeks were 26 and 57 days respectively, with intervals for all other sites ranging from 41 to 54 days.

Table 19. Objective 2 field experiments 2018- 2020, fungicide application, main assessments and sample dates

	Partner and site	Variety	Drilled prev year	T1	T2	T2+3 wk sample	T2+6 wk sample	T2+6 wk STB assess	Harv.	GS T1	GS T2	T2+3wk Leaf layer sampled	T2+6wk Leaf layer sampled	Days from T1 to T2+6wk
2018	**[a]ADAS Herefords.**	Santiago	28-Oct	29-Apr	22-May	None	04-Jul	04-Jul	03-Aug	32	39	None	1	43
	NIAB Kent	Santiago	07-Oct	03-May	23-May	None	04-Jul	04-Jul	17-Aug	32	43	None	2	42
	Teagasc Cork	LILLI	30-Sep	16-May	01-Jun	None	27 Jun	27-Jun	08-Aug	32	39	None	1	26
2019	**NIAB Kent**	JB Diego	09-Oct	03-May	23-May	None	28-Jun	28-Jun	26-Aug	32	41	None	2	41
	SRUC Edinburgh	Viscount	07-Oct	11-May	30-May	None	11-Jul	11-Jul	28-Aug	32	39	None	2	43
	Teagasc Cork	Lumos	22-Oct	16-May	01-Jun	None	28-Jun	28-Jun	29-Aug	32	39	None	1	46
2020	**[b]ADAS Herefords.**	Santiago	09-Oct	26-Apr	25-May	16-Jun	07-Jul	07-Jul	09-Aug	32	43-45	4	2	43
	NIAB Herefords.	Gravity	23-Oct	27-Apr	12-May	02-Jun	22-Jun	29-Jun	07-Aug	32	39	3	2	41
	SRUC Edinburgh	Viscount	02-Oct	05-May	27-May	06-Jul	20-Jul	24-Jul	05-Sep	32	39-45	3	2	54
	Teagasc Cork	Costello	14-Nov	06-May	27-May	29-Jun	23-Jul	15-Jul	14-Aug	32	39	3	1	57

[a] additional sprays for yellow rust control:19 June fenpropimorph (Clayton Spigot, Clayton Plant protection Ltd., 0.5L/ha) and cyflufenamid (Cyflamid, Certis, 0.4 L/ha)

[b] additional sprays for yellow rust control: 11 May azoxystrobin (Amistar, Syngenta, 0.5 L/ha) and 29 May fenpropimorph (Corbel, BASF, 0.5 L/ha)

5.3.2. Effect of alternation vs. mixtures: varying SDHI and DMI dose

In 2018 experiments, across all four sites, there was little difference in the total SDHI mutation frequency between the three treatment strategies: DMI-SDH mixture, and alternation DMI then SDHI, or alternation SDHI then DMI (e.g., ADAS Herefordshire, Figure 17). All three strategies resulted in significantly higher SDH mutation frequencies compared with the control (F prob <0.001, UT in Figure 17). There was no significant difference between mutation frequencies resulting from the two 'extreme' fungicide dose combinations used, i.e., SDHI 2.0 & DMI 0.4 (high:low), and SDHI 1.0 & DMI 2.0 (low:high). There was no significant difference between the three treatment strategies for H152R frequency.

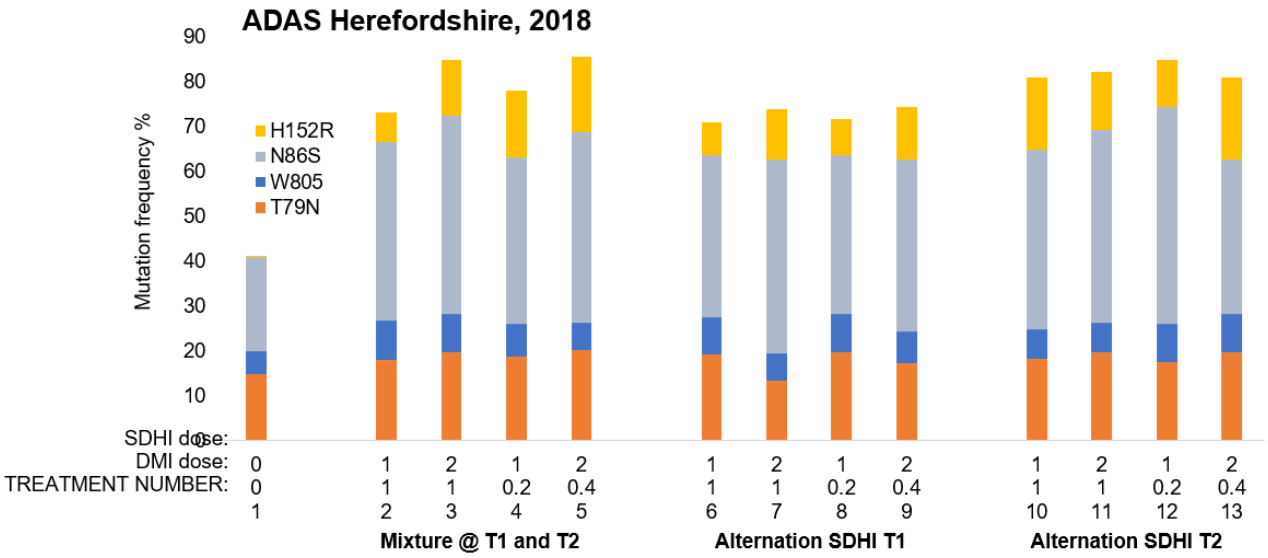

Figure 17. Selection for SDH mutations in *Zymoseptoria tritici* strains, 2018 ADAS Herefordshire field experiment, using a two-spray treatment programme, T1, GS 32 and T2, GS 39. Each individual mutation value is an average across four replicate plots and two genotypying tests per plot. SDHI = isopyrazam, DMI = prothioconazole. Doses are proportion of maximum permitted dose at each application. Total SDH mutations, ANOVA: UT $F_{1,36} = 66.5$, $p<0.001$; UT*DMI dose* Trt programme $F_{4,36} = 2.1$, $p=0.105$; Max SED = 6.02. H152R, ANOVA: UT $F_{1,36} = 16.3$, $p<0.001$; UT*DMI dose* Trt programme $F_{6,36} = 1.77$, $p=0.157$, Max SED = 3.98.

5.3.3. Effect of alternation vs. mixtures: constant SDHI dose, varying DMI dose

A greater dose range of DMI fungicide was used in the 2019 and 2020 experiments, to increase the chance of detecting differences in strategies for effects on selection for SDH mutations. As in 2018, there was little difference in mutation frequency between the three strategies: mixture, alteration one way or alternation the other, and little difference between the DMI dose rates, despite the total dose of DMI ranging from 1.0 to 0.125 (Figure 18). However, there were significant increases in septoria severity with decreasing doses of DMI (Figure 19).

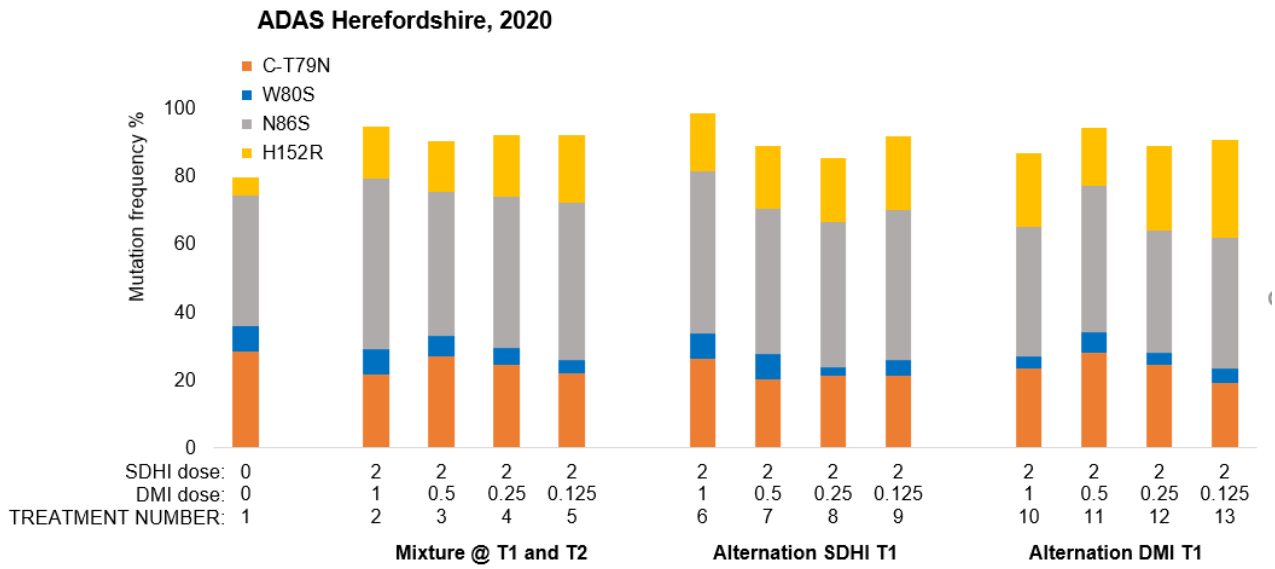

Figure 18.Selection for SDHI mutations in *Zymoseptoria tritici* strains, 2020 ADAS Herefordshire field experiment. Each individual mutation value is an average across four replicate plots and two genotypying tests per plot, from second of two sample times. SDHI = fluxapyroxad, all treatments 2-13 same total dose **2.0**. DMI = mefentrifluconazole. Doses are proportion of maximum permitted dose at each application. Total SDH mutations, ANOVA: UT $F_{1,36}$ = 10.1, p=0.003; UT*DMI dose* Trt programme $F_{6,36}$ = 0.78, p=0.592; Max SED = 5.52. H152R, ANOVA: UT $F_{1,36}$ = 20.0, p<0.001; UT*DMI dose* Trt programme $F_{6,36}$ = 0.41, p=0.868, Max SED = 4.34.

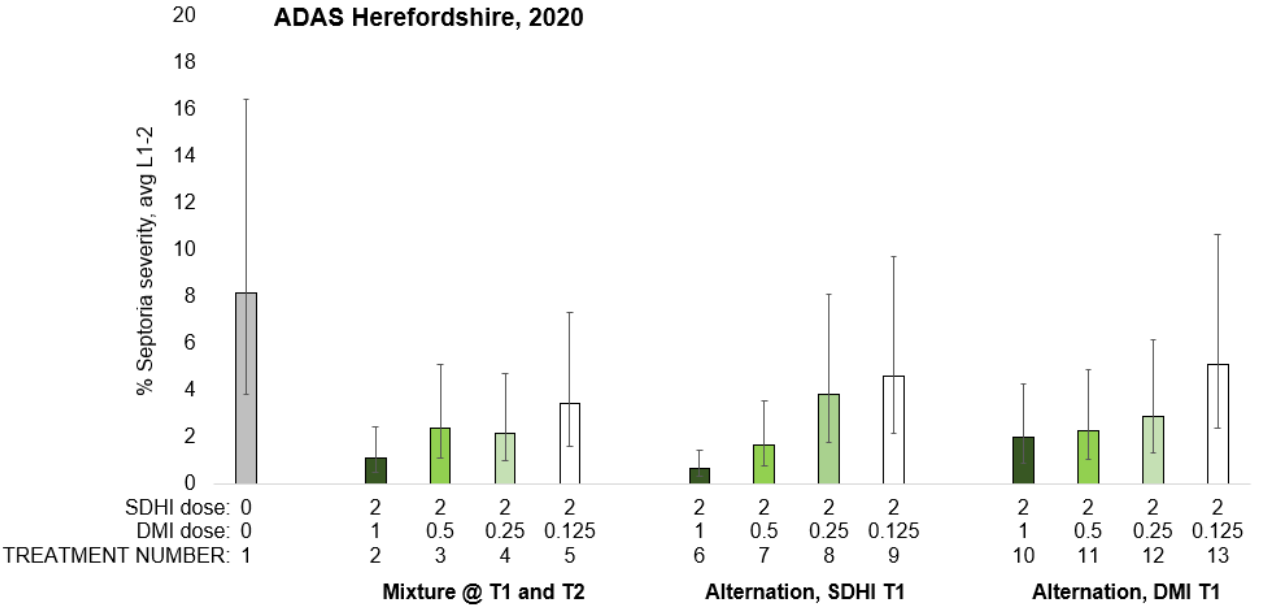

Figure 19. Septoria (*Zymoseptoria tritici*) severity on the upper two leaves, 2020 ADAS Herefordshire field experiment (same experiment as in Figure 18 above). Each severity value is an average of four replicate plot values with analysis is based on logit transformed values. Error bars are back-transformed 95% confidence limits. Logit disease, ANOVA: UT $F_{1,36}$ = 25.5, p<0.001; UT*DMI dose* Trt programme $F_{6,36}$ = 1.9, p=0.107, Max SED = 0.354.

5.4. Investigating differences between findings reported in the literature and the experimental results

The results from experiments addressing objective 1 showed that there was no significant effect of dose splitting on selection. This differs from work published previously (reviewed by van den Bosch et al., 2014), which typically showed a modest increase in selection due to dose splitting. An important question is therefore whether these experimental results are generalisable or whether they were the specific result of the particular set of characteristics of the *Zymoseptoria tritici* and fungicides in the experimental system used in this study. The effect of dose splitting is of practical importance for two reasons: Firstly, because choosing to use a mixture strategy, rather than alternation, is effectively dose splitting. Secondly, because if dose splitting has little effect on selection, it opens the opportunity to loosen restrictions on maximum number of applications, provided a limit on total dose is adhered to.

The following hypotheses were explored:

1. The extent of the dose splitting effect depends on the shapes of the dose response curves of the sensitive and resistant mutants (see Paveley et al., 2019 for rationale).
2. Dose splitting effects were smaller than the detection limits of the field experiments, due to: (i) spore movement between plots biasing the quantification of selection for resistant mutants in the field experiments (ii) variability in the mutation frequency data, (iii) high mutant frequencies early in the season (before treatments started) which did not leave enough ‘headroom’ for further selection to detect treatment differences, and/or (iv) the effects of mixtures reducing the effect of dose splitting on selection.
3. Dose splitting effects were obscured by bias caused by the timing of sampling, relative to the timing of treatment applications.

5.4.1. Testing whether the dose splitting effect depends on the shapes of dose response curves

Simulation modelling of resistance in the AHDB PhD studentship (to be reported separately) explored the parameter space for the effects of resistance mutations on the shape of the dose response. These simulations were run without fitness costs and without mixture partners. Particular parameter combinations were found which resulted in small dose splitting effects. There were no practically plausible parameter combinations which resulted in zero or negative effects of dose splitting on selection. In general, the range of dose splitting effects predicted by modelling were in agreement with the range of effects reported in the literature.

5.4.2. Testing whether spore movement between plots biased quantification of selection

It is possible that spore movement between plots could bias the quantification of selection for resistant mutants in the field experiments. To test whether this may have occurred, the percent frequency of total SDHI resistant mutations from the early season samples was compared with the total SDHI mutations at the end of the season, for untreated plots across all four sites in 2019. The histogram of differences (Figure 20) and a T-test indicated that there is no reason to assume that on average the difference is different from zero (P=0.18, and test for normality of data did not show any significant deviation).

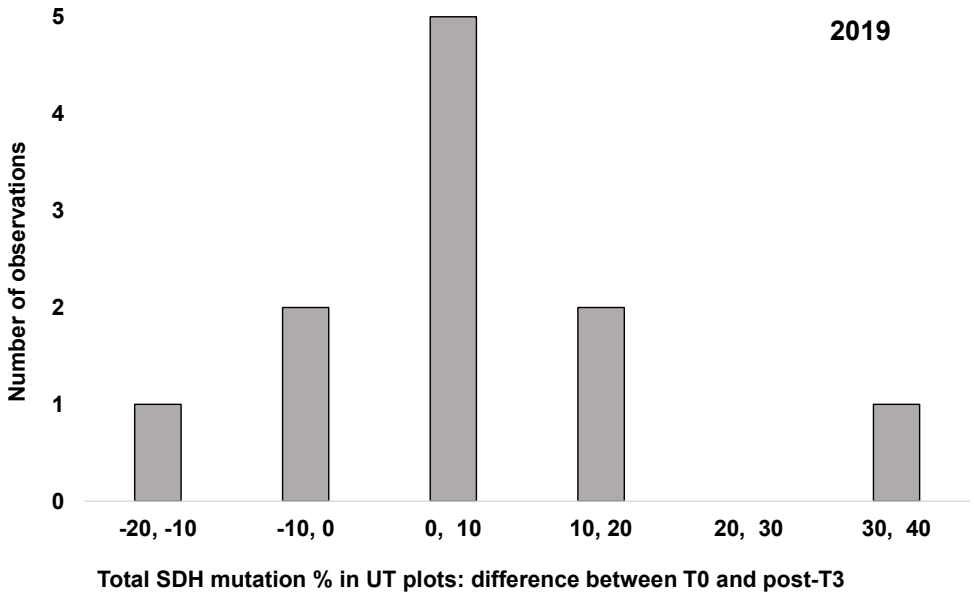

Figure 20. Distribution of differences between GS30 and end of season, for total SDH mutation frequencies in *Zymoseptoria tritici* from untreated field plots across four sites in 2019.

The conclusion is that there is no systematic change from start to end of season in the % total SDHI mutation frequencies in untreated plots during the experiment. This indicates that it is not necessary to take account of spore flow between plots when analysing the mutation frequency data. However, the differences between the total SDHI mutation frequencies between the start and end of season were often large, with 10 of the 15 observations differing more than 10%. However, there is large variation from zero in some cases, likely to be caused by accumulating variability from a number of processes during the experiment, such as variability in the leaf sampling or the genotyping test procedures. The implications of this large variation in mutation frequency results are that it is easy for a small difference in treatment effect to disappear in this variability. Therefore, we are likely to detect significant differences in treatment strategies only where the effects are substantial.

5.4.3. Testing if dose splitting effects were obscured by high starting mutant frequencies in the field experiments

Splitting a total SDHI dose over a greater number of applications was predicted to increase selection for SDHI-resistant strains. The predicted difference in mutant frequencies at GS75 between treatments depended on both the starting frequency of the resistant strain and the level of asymptote shift (Figure 21):

- Resistant strains with a higher level of asymptote shift were predicted to give the largest difference in mutation frequencies between different dose splitting treatments.
- The largest differences between treatments were predicted for starting mutation frequencies of approximately 20-25%. Overall, starting mutation frequencies of approximately 10-50% are predicted to give the best chance of detecting differences between treatments. At high starting frequencies, differences between treatments decrease due to running out of 'headroom', whilst low starting frequencies result in small absolute percentage differences relative to background experimental variation e.g., due to spore movement.
- The higher the asymptote shift, the lower the resistant strain starting frequency at which the greatest difference in final mutation frequencies between treatments was predicted: within the optimum range, lower starting frequencies are optimal for detecting treatment differences when resistance shifts are large and higher frequencies are optimal for detecting treatment differences when resistance shifts are small.
- Depending on which treatments were compared, for partially resistant strains with asymptote shifts of 40%-70%, absolute predicted differences in mutation frequencies between treatments ranged from approximately 3%-20%. In many cases the predicted differences are similar to, or smaller than, the average LSD (approx. 10%) in the field experiments. It may therefore be difficult to detect differences between dose splitting treatments in selection for partially resistant strains using field experiments.
- Starting mutation frequencies in some of the field experiments were within the range in which running out of 'headroom' may have reduced the ability to detect dose splitting effects.

Comparing selection for SDHI resistant strains between all dose splitting treatments with the same total SDHI dose and mixture partner use, based on 2019 and 2020 experiments (treatment list in Table 4) the biggest difference between treatments was predicted between treatments 3 and 6 (total SDHI dose of 2, either as a single application (treatment 3) or split into four applications at half dose (treatment 6)) (Figure 21); predicted differences between the 3-way split (treatment 5) and the single application (treatment 3) were slightly smaller, and differences between a 2-way split (treatment 4) and the single application were smaller again (Figure 22). Predicted differences between different split-dose treatments (4-way split (treatment 6) vs. 2-way split (treatment 4), 4-

way split vs. 3-way split (treatment 5), and 3-way split vs. 2-way split) were similar to or smaller than the predicted difference between a 2-way split and a single application (Figure 23). Predicted differences between treatments for a total SDHI dose of 1.5 followed the same pattern, but were smaller than the predicted differences for a total SDHI dose of 2 (Figure 24), as selection for resistant strains is less strong at the lower total SDHI dose.

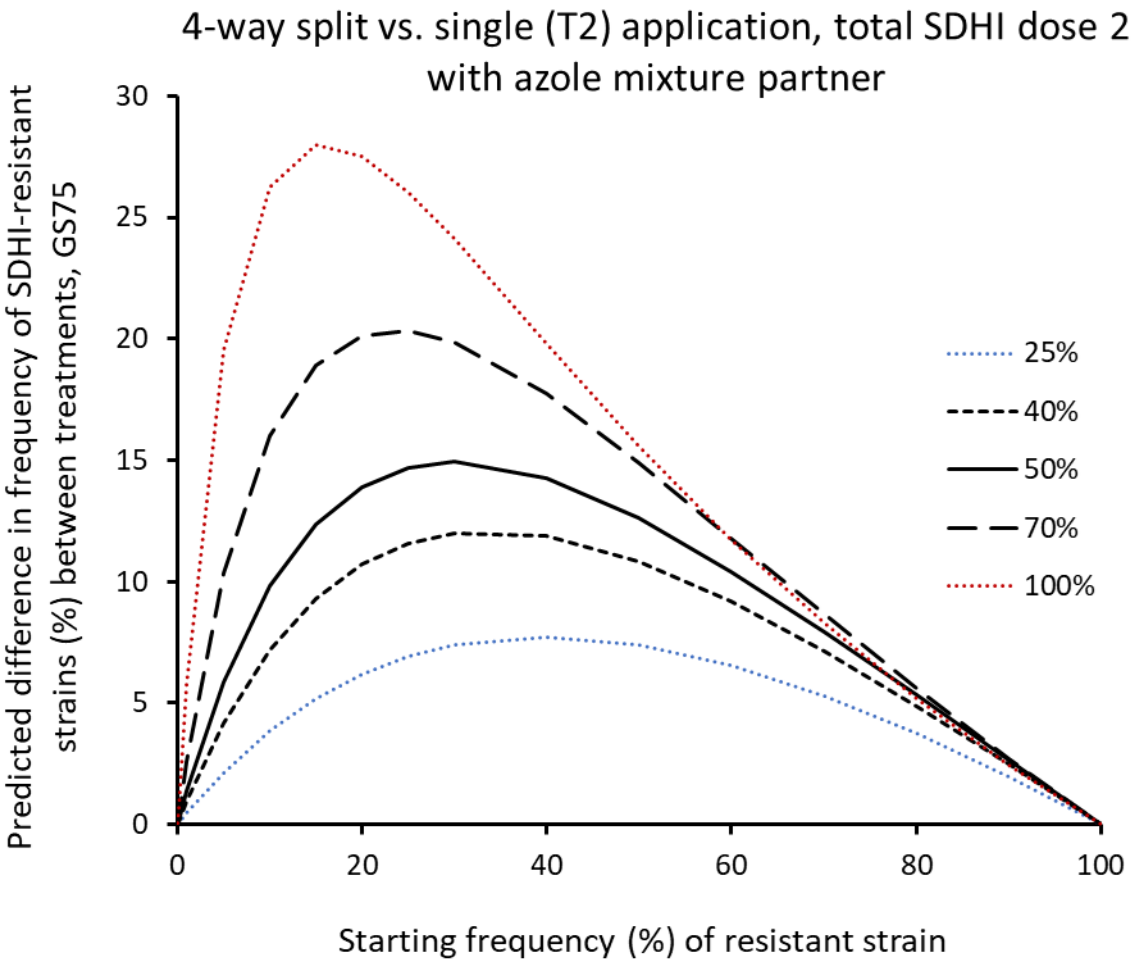

Figure 21. Predicted differences in the frequency of an SDHI-resistant strain at GS75 following Treatments 6 and 3 (a 4-way split application vs. a single application of a total isopyrazam dose of 2 x recommended label dose in mixture with prothioconazole), for a range of starting mutation frequencies and asymptote shifts.

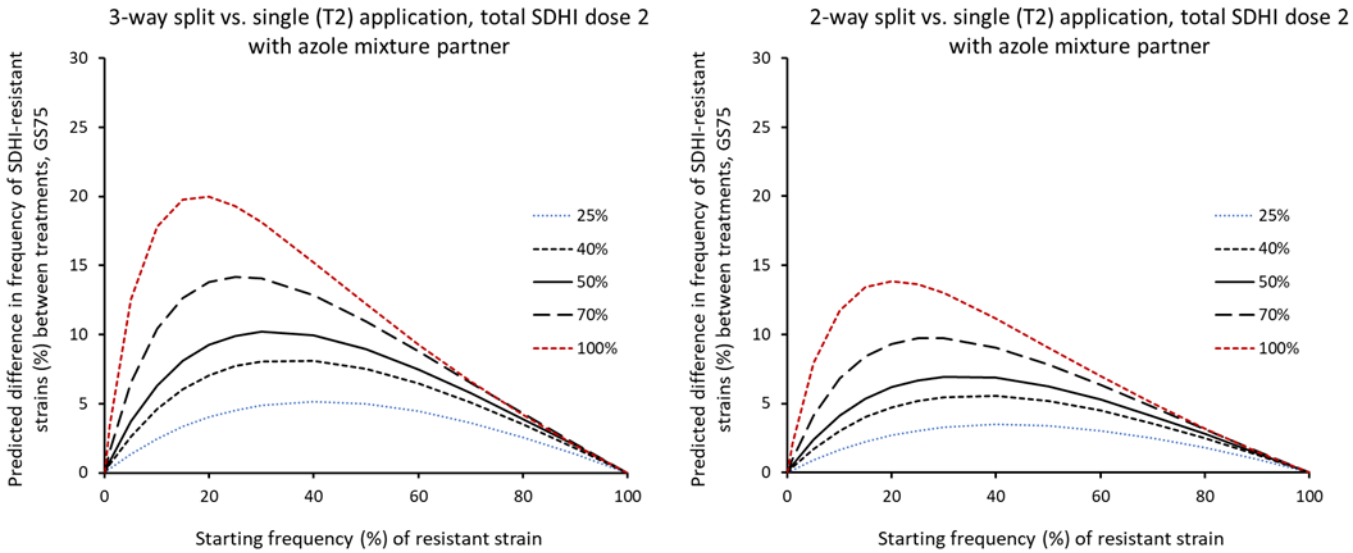

Figure 22. Predicted differences in the frequency of an SDHI-resistant strain at GS75 between treatments 5 and 3 (3-way split application vs. single application), and treatments 4 and 3 (2-way split application vs. single application).

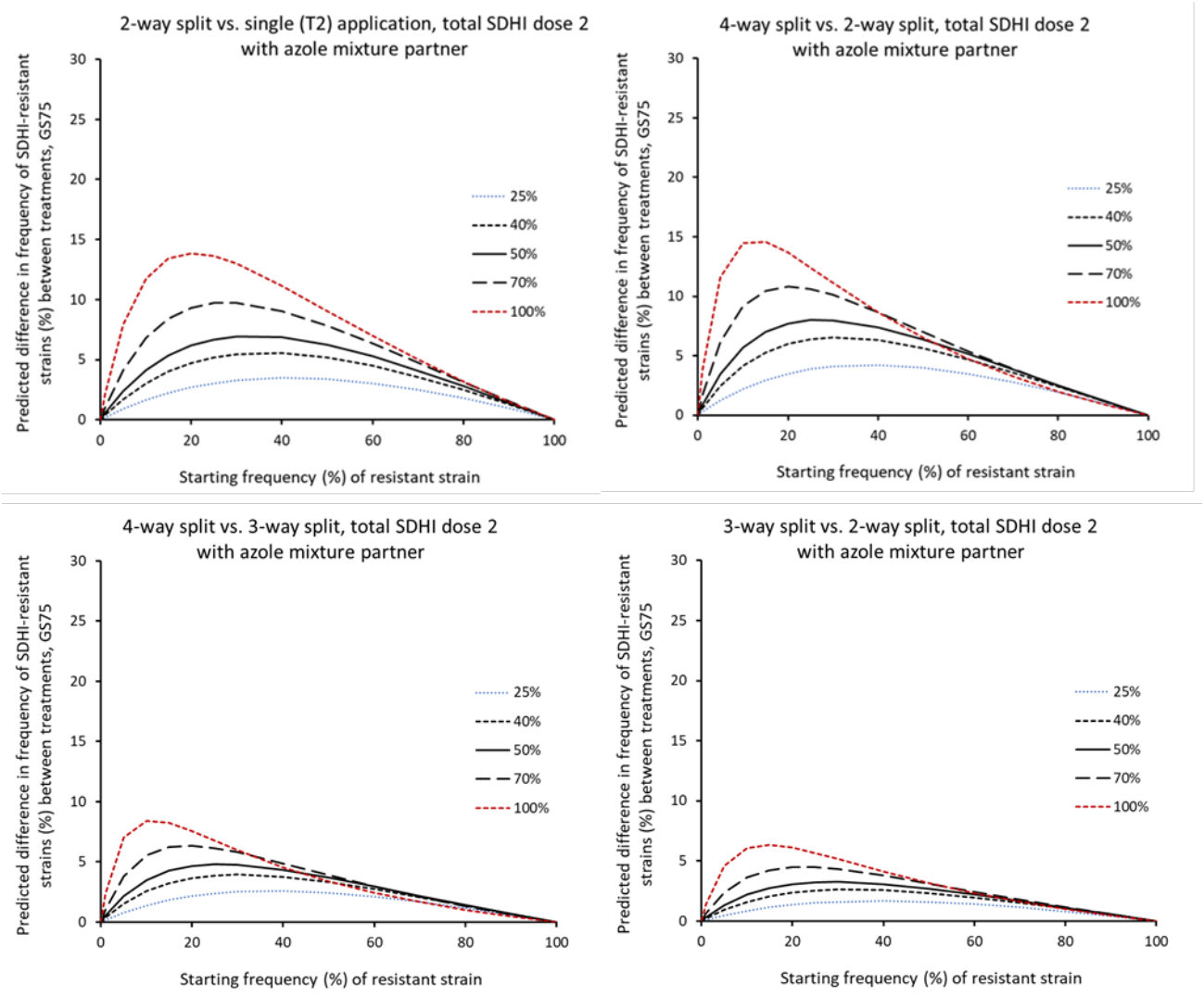

Figure 23. Predicted differences in the frequency of an SDHI-resistant strain at GS75 between treatments 4 and 3 (2-way split application vs. single application), treatments 6 and 4 (4-way split application vs. 2-way split application), treatments 6 and 5 (4-way split application vs. 3-way split application) and treatments 5 and 4 (3-way split application vs. 2-way split application).

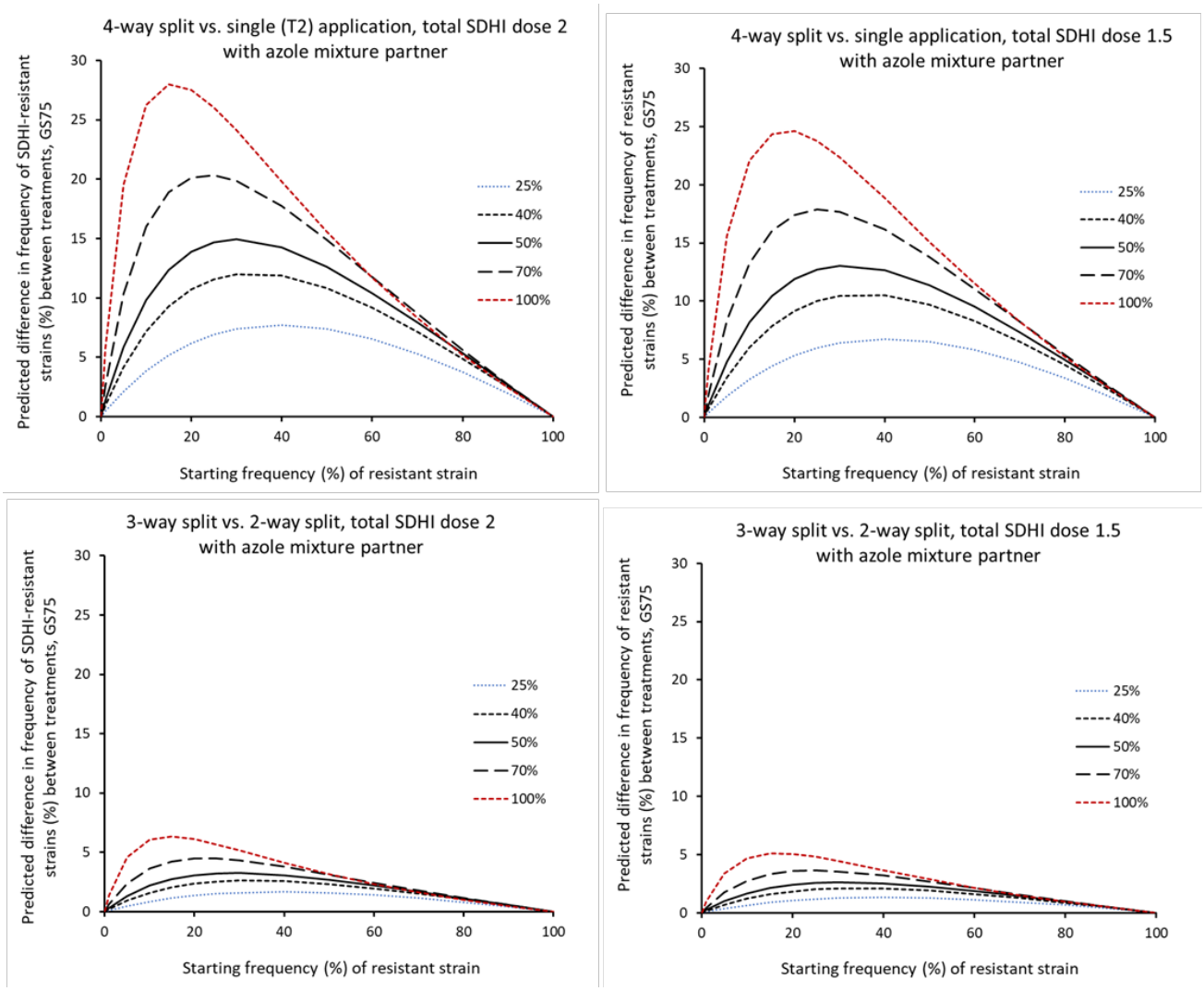

Figure 24. Predicted differences in the frequency of an SDHI-resistant strain at GS75 between treatments 6 and 3 (4-way split application vs. single application, total SDHI dose 2), treatments 11 and 8 (4-way split application vs. single application, total SDHI dose 1.5), treatments 5 and 4 (3-way split application vs. 2-way split application, total SDHI dose 2) and treatments 10 and 9 (3-way split application vs. 2-way split application, total SDHI dose 1.5).

Considering DMI-resistant strains, the use of isopyrazam as a mixture partner to the DMI (prothioconazole) was predicted by the model to reduce selection for these strains relative to solo DMI application at all four application timings, whether the SDHI was applied as a single or split-dose. For both total SDHI doses simulated (1.5x and 2x single dose label rate), a 3-way or 4-way split SDHI dose split provided the greatest reduction in selection for DMI-resistant strains, with a very small difference between these two SDHI-splitting patterns; the reduction in selection for DMI-resistant strains provided by a 2-way split SDHI dose was smaller, and that provided by a single SDHI application at the same total SDHI dose was smaller again (Figure 25). A total SDHI dose of 2x single dose label rate provided greater protection than a total SDHI dose of 1.5x single dose label rate, but, comparing across doses for the same SDHI-splitting pattern, the difference in the predicted frequency of DMI-resistant strains was small (Figure 26). The absolute reduction in predicted frequency of DMI-resistant strains as a result of mixture with isopyrazam was greatest for

strains that were more highly DMI-resistant (i.e. with a greater asymptote shift). The absolute reduction in frequency due to mixture was no more than approximately 5% within the highest level of partial resistance (asymptote shift of 50%) simulated for DMI fungicides, and the effects of splitting the SDHI dose on DMI mutation frequency were much smaller than the L.S.D. (approx. 12%) in the field experiments. The increase in protection against DMI-resistant strains due to SDHI dose-splitting was small relative to the predicted increase in selection for SDHI-resistant strains resulting from SDHI dose-splitting.

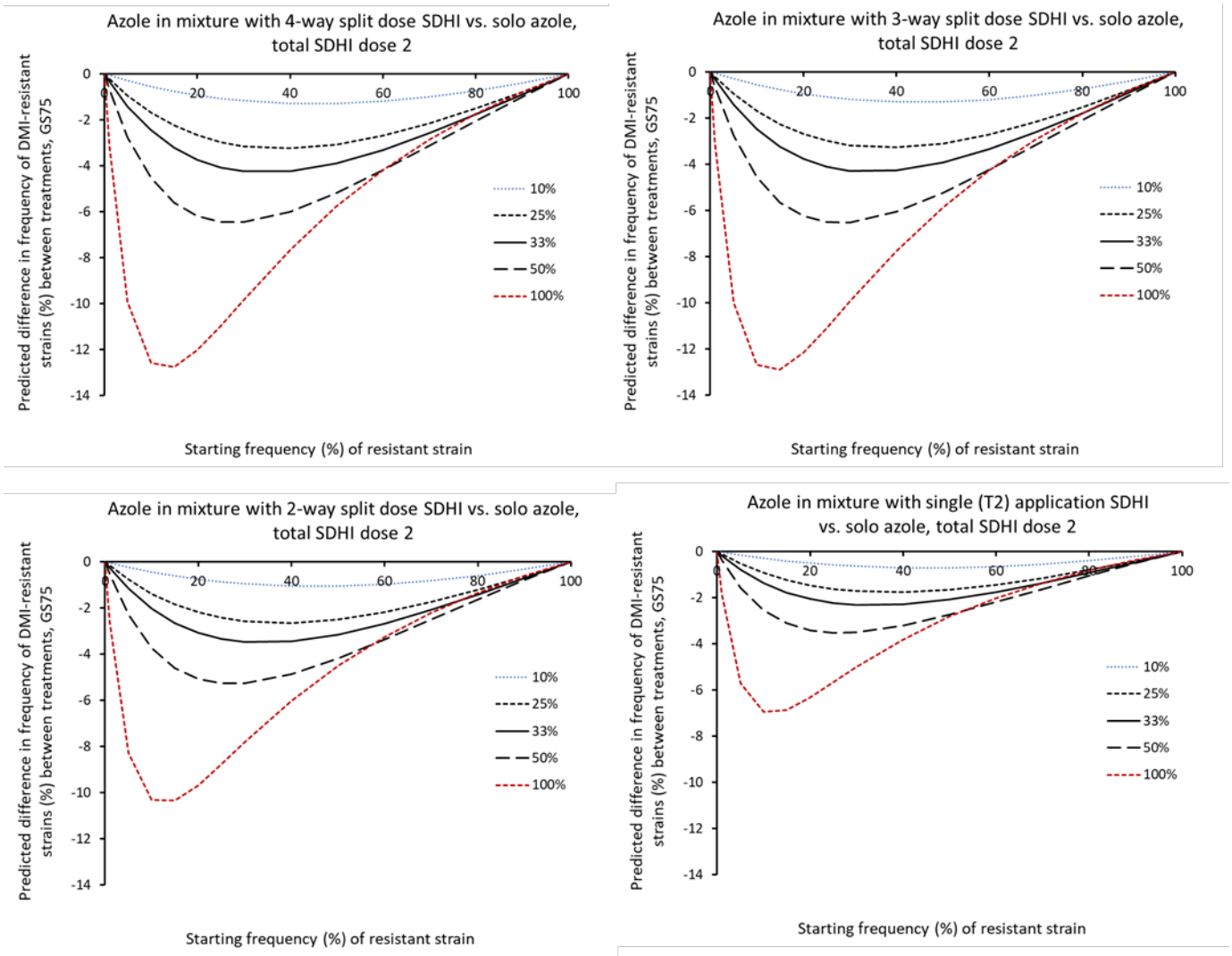

Figure 25. Predicted differences in the frequency of a DMI-resistant strain at GS75 between treatments 6 and 2 (DMI in mixture with a 4-way split SDHI total dose 2 vs. DMI solo at all four application timings), treatments 5 and 2 (DMI in mixture with a 3-way split SDHI total dose 2 vs. DMI solo at all four application timings), treatments 4 and 2 (DMI in mixture with a 2-way split SDHI total dose 2 vs. DMI solo at all four application timings) and treatments 3 and 2 (DMI in mixture with a single application of SDHI at total dose 2 vs. DMI solo at all four application timings).

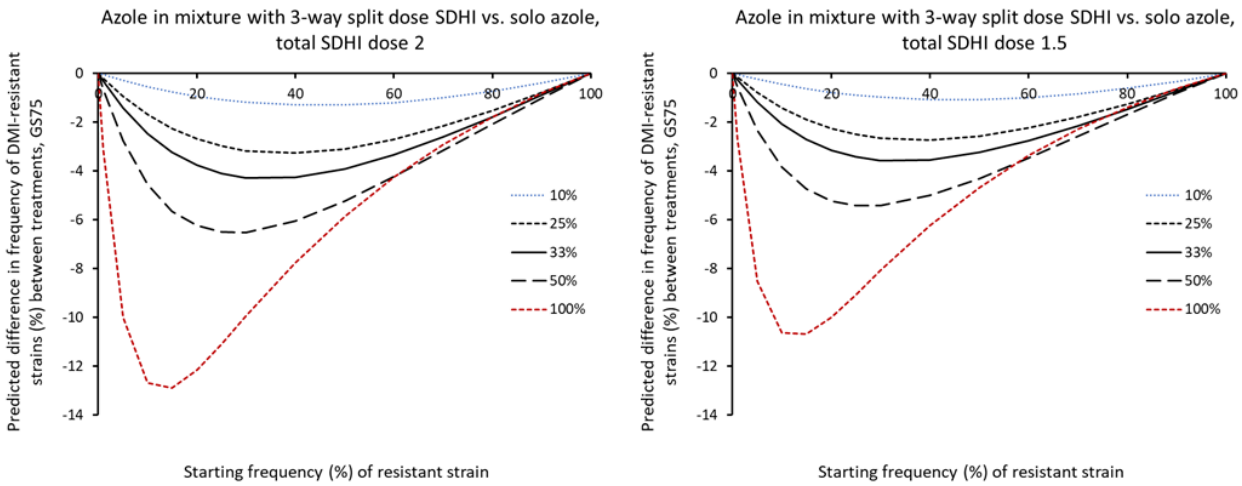

Figure 26. Predicted differences in the frequency of a DMI-resistant strain at GS75 between treatments 5 and 2 (DMI in mixture with a 3-way split SDHI total dose 2 vs. DMI solo at all four application timings) and between treatments 10 and 2 (DMI in mixture with a 3-way split SDHI total dose 1.5 vs. DMI solo at all four application timings).

5.4.4. Testing if dose splitting effects were reduced below detectable levels by the effects of mixture partners

The use of prothioconazole as a mixture partner to the SDHI (isopyrazam) was predicted by the model to reduce selection for SDHI-resistant strains. The use of a mixture partner also reduced the difference in mutation frequency between treatments, by a maximum of approx. 5% absolute change in resistant strain frequency for partially resistant strains (Figure 27). The optimum starting mutation frequency for detecting differences was slightly higher with the use of a mixture partner. The greater the asymptote shift, the larger the absolute reduction in the expected difference due to the effects of mixture partners, but differences between treatments are still larger and therefore easier to detect relative to background variability for strains with larger asymptote shifts, whether a mixture partner is used or not. Where absolute differences in predicted mutation frequency between treatments are small, such as between a 3-way split and a 2-way split application, and/or for small asymptote shift values, the inclusion or non-inclusion of a mixture partner makes very little difference to the absolute predicted differences between treatments of mutation frequencies or the ability to detect a difference between treatments.

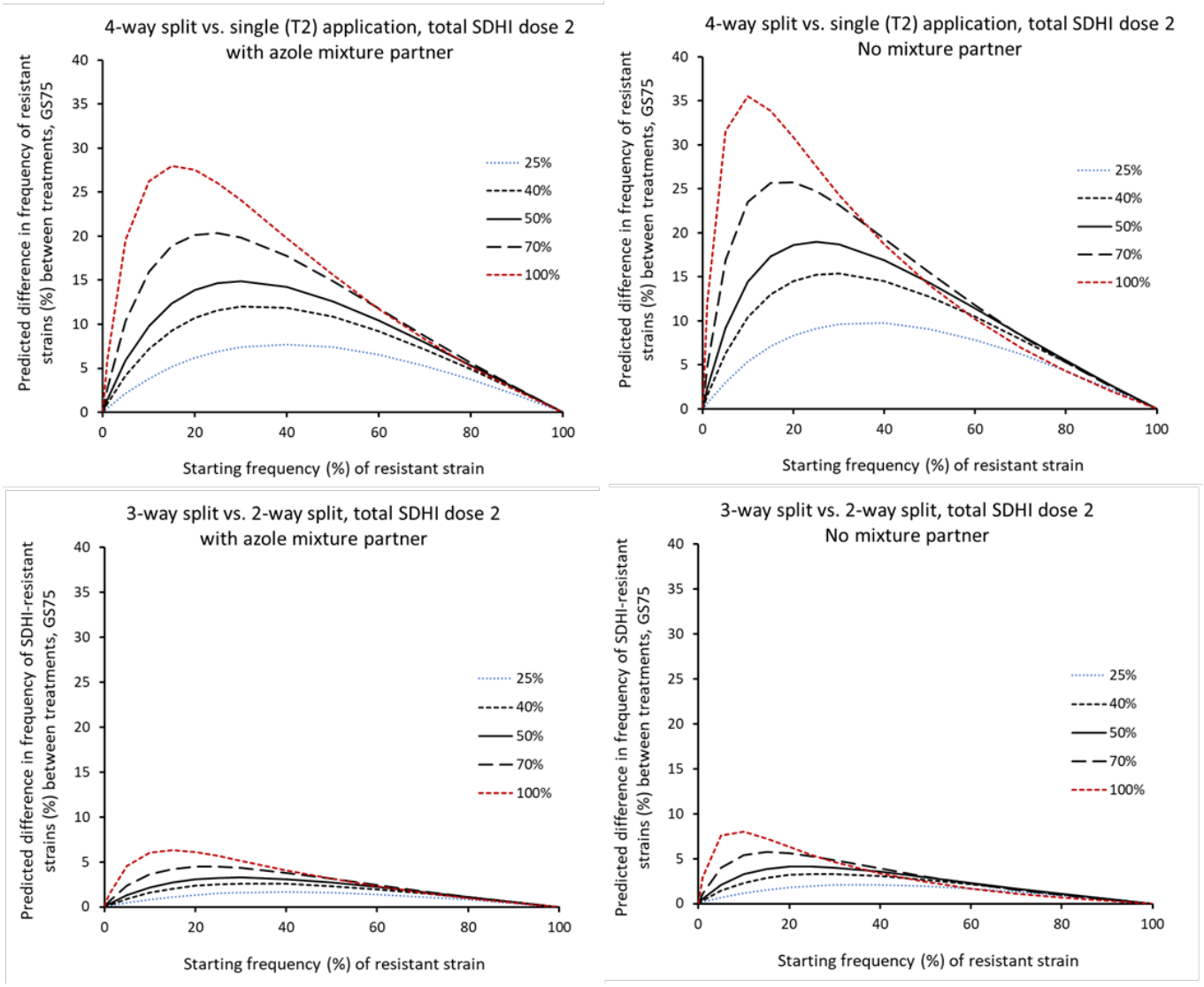

Figure 27. Predicted differences in the frequency of an SDHI-resistant strain at GS75 between 4-way split application & single application of an SDHI at a total dose of 2, and 3-way split application and 2-way split application of an SDHI at a total dose of 2, with and without an azole mixture partner (applied at all four application timings).

5.4.5. Testing if dose splitting effects were obscured by bias caused by the timing of sampling

The modelling suggests that an earlier sample timing slightly underpredicted the difference in mutation frequencies between treatments (Figure 28), as the fungicide continued to select for resistant strains after the sample timing. The underprediction was greatest for partially resistant strains (out of the asymptote shift parameter values simulated for SDHI-resistant strains, underprediction was greatest at the 70% asymptote shift). However, even at the earliest sample timing considered (GS66), the underprediction was no more than 3%; so was small relative to the other sources of variability in the experimental system. When sampling at GS75, the underprediction relative to sampling at the end of the season was less than 1%, and it is more practically feasible to sample before all of the leaf area has senesced. It is therefore unlikely that

bias caused by timing had a large influence on the detection, or otherwise, of dose splitting effects in the field experiments. Sampling at GS75 appears to be a good compromise timing to minimise underprediction of selection whilst working within practical constraints.

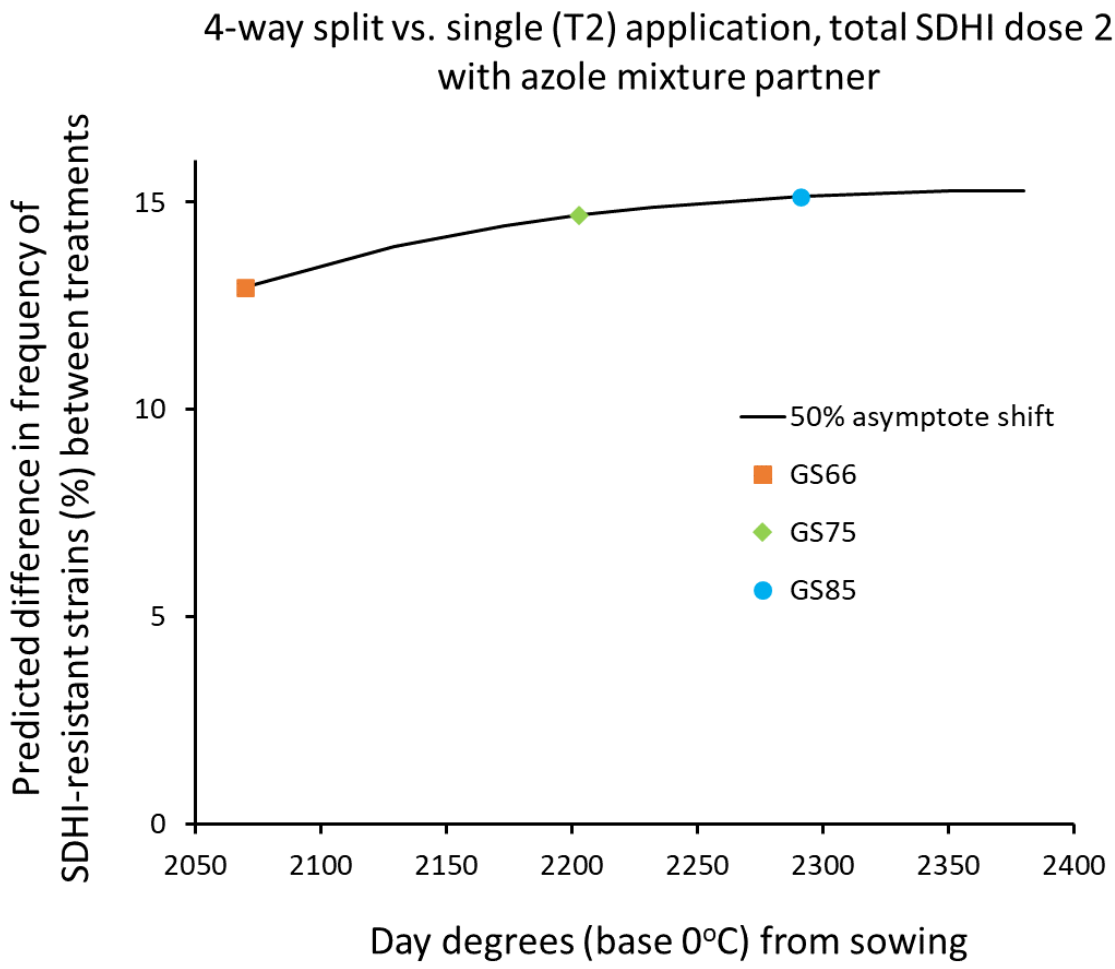

Figure 28. Effect of sample timing on the predicted detectable difference in frequency between treatments 6 and 3 (a 4-way split application vs. a single application of a total isopyrazam dose of 2x recommended label dose in mixture with prothioconazole), for a resistant-strain with a 50% asymptote and 25% starting frequency.

5.5. Testing efficacy & economics of resistance management tactics (objective 3)

Industry partners conducted trials in each of the four project years, enabling data collection to be completed across a range of growing seasons with differing septoria disease pressures. Trials run in 2017 and 2019 generally had high septoria pressure, with an average of 30% leaf area affected by septoria across untreated plots on leaf layers 1 and 2. Trials run in 2018 typically had moderate-low disease pressure, with 8% leaf area affected across untreated leaf layers 1 and 2. Trials run in 2020 had a low disease pressure across all trial sites, with 1% leaf area affected across untreated leaf layers 1 and 2. In all project years septoria severity was reduced by fungicide treatments, with control varying from between 55-88% (Figure 29).

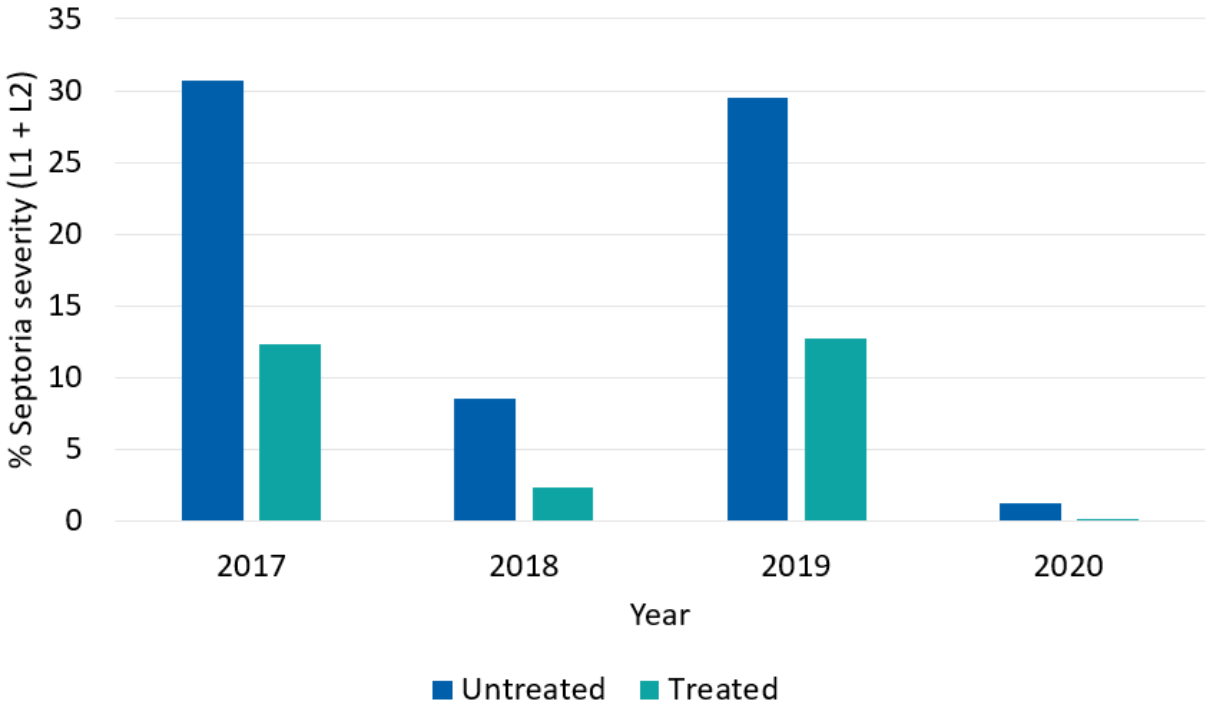

Figure 29. Septoria severity across fungicide treated and untreated plots from trials across all project years.

Yield responses to fungicides were consistent with disease data, with the highest yield responses observed in 2017 (2.03 t/ha) and 2019 (2.13 t/ha), with these years also having the highest total yield in fungicide treated plots (Figure 30). The lowest yield response to fungicide was seen in 2020 (0.46 t/ha), with lower overall yields than other trial years which reflects the dry spring that year depressing both yields and the septoria epidemic.

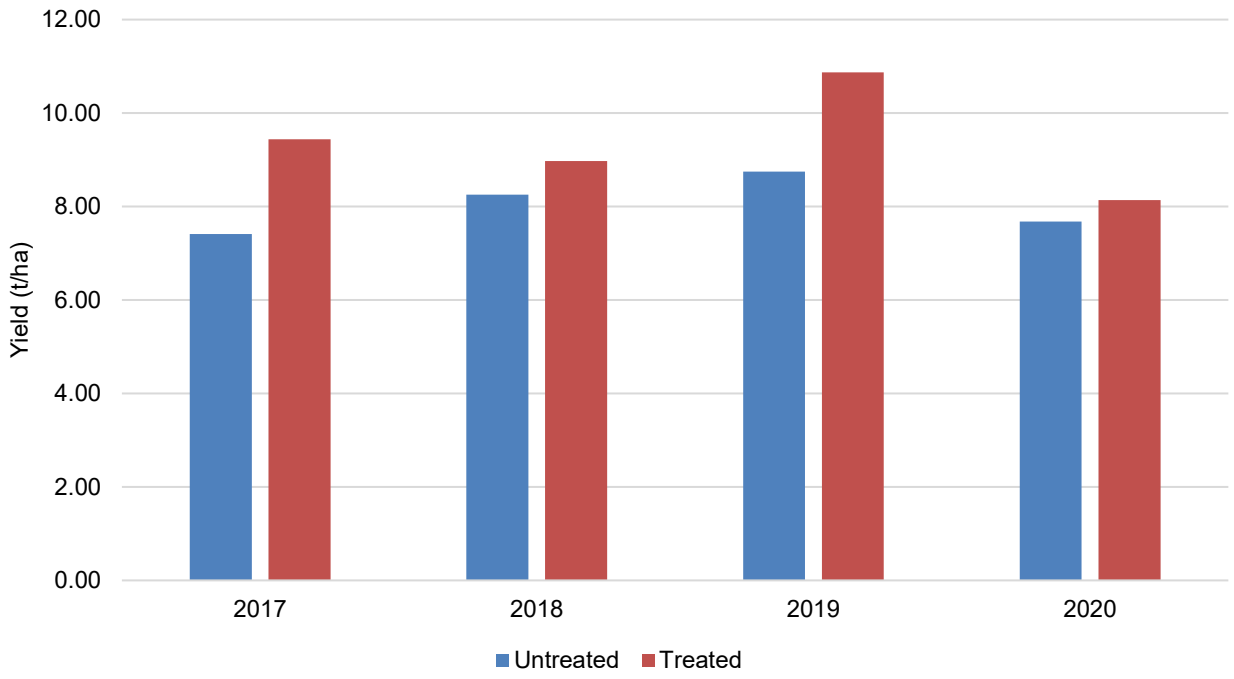

Figure 30. Average yields across fungicide treated and untreated plots from trials across all project years.

A cross site analysis of the yield data was completed separately for each of the four project years, with summaries by treatment shown in Figure 31, Figure 32, Figure 33 and Figure 34. Results are summarised below in relation to each treatment comparison:

- *Splitting the dose of SDHI: comparing application of 0.75 SDHI at T1 compared to 0.38 SDHI at T1 and T3. Treatment 6 compared with treatment 7 (see Tables 11 & 12) Trial years 2018, 2019 & 2020.*
  There were no significant yield differences observed from splitting the T1 SDHI dose between T1 and T3, compared to applying the same total dose at T1 in all three years this treatment comparison was included in trials.

- *Addition of multi-site protectant products to programmes. Comparing treatments 3 with 4, and treatments 5 with 6 (see Tables 10, 11 & 12). Trial years 2017, 2018, 2019 and 2020.*
  Significant yield improvements from the addition of multi-site fungicides were observed in 2017 and 2019 in both sets of multi-site treatment comparisons. A significant yield increase was observed in one set of treatments in 2018 (treatments 5 and 6) with a positive but not significant, yield increase observed between treatments 3 and 4 (0.17 t/ha). The lowest disease pressure year in this trial project was 2020, and this was the only year where there was little yield increase observed from the addition of multi-site fungicides to fungicide programmes (0.04 t/ha).

- *Alternation or mixture: Inclusion or exclusion of DMI at T2. Comparing treatments 2 with 4 (see Tables 10, 11 & 12). Trial years 2017, 2018, 2019 and 2020.*
  Treatment 2 explores the possibility of alternation by restricting the number of DMI applications by not including this mode of action at T2, leaving the SDHI as the only product with a single site mode of action applied at this timing, with a DMI product applied at T1 and T3. This is compared to treatment 4 where an SDHI and DMI mixture is applied at T2.
  No significant yield differences were seen between treatments 2 and 4 in 2018, 2019 and 2020 trials, with no consistent yield trends observed. A significant yield increase of 0.30 t/ha was observed in 2017 when a DMI was applied in mixture with an SDHI at T2.

- *Increasing program intensity:*
  Addition of T0 DMI and T1 SDHI. Comparing treatments 4 with 6, and 3 with 5 (see Table 10, Table 11 and Table 12). Trial years 2017, 2018, 2019 and 2020.
  Standard and reduced intensity fungicide programmes were compared both with and without the inclusion of multi-site protectants. In 2017 and 2019 (years of the highest disease pressure) there was a significant yield improvement from using more intense fungicide programmes. In lower disease pressure years of 2018 and 2020 there were trends for higher yields from more intense fungicide programmes however these were not significant. The additional cost of the more intense fungicide programme was only financially justified in 2019, where yield responses were 0.37 t/ha where multi-sites were also included in programmes, and 0.65 t/ha where multi-sites were not included in the fungicide programme.

  Additional SDHI applied at T1 and T3, treatment 4 compared to treatment 7 (see Tables 11 & 12) . Trial years 2018, 2019 & 2020.
  The benefit of applying SDHI products at three timings (T1, T2 & T3, treatment 7) were compared to the application of SDHI products at a one single timing (T2, treatment 4) to see if there was a significant yield improvement from the more intense fungicide programme. In 2019 a significant yield increase of 0.39 t/ha was observed, with treatment 7 yielding 11.35 t/ha compared to treatment 4 yielding 10.96 t/ha. A similar trend was observed in 2018 and 2020, however the yield increases in these years were not significant.

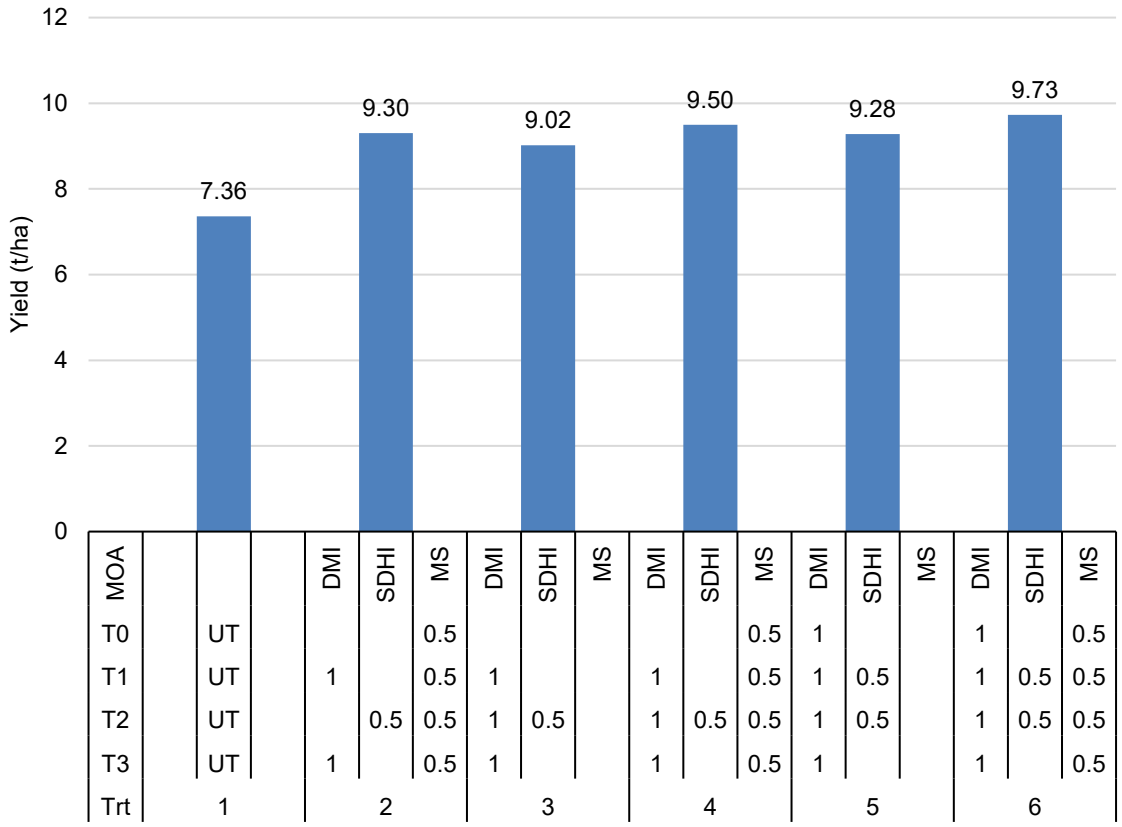

Figure 31. Cross site analysis of yield results from industry and research partners trials (n=8), 2017. Site Fpr <0.001, Site LSD 0.32; Treatment Fpr <0.001, Treatment LSD 0.20; Site.Treatment Fpr <0.001, Site.Treatment LSD 0.62.

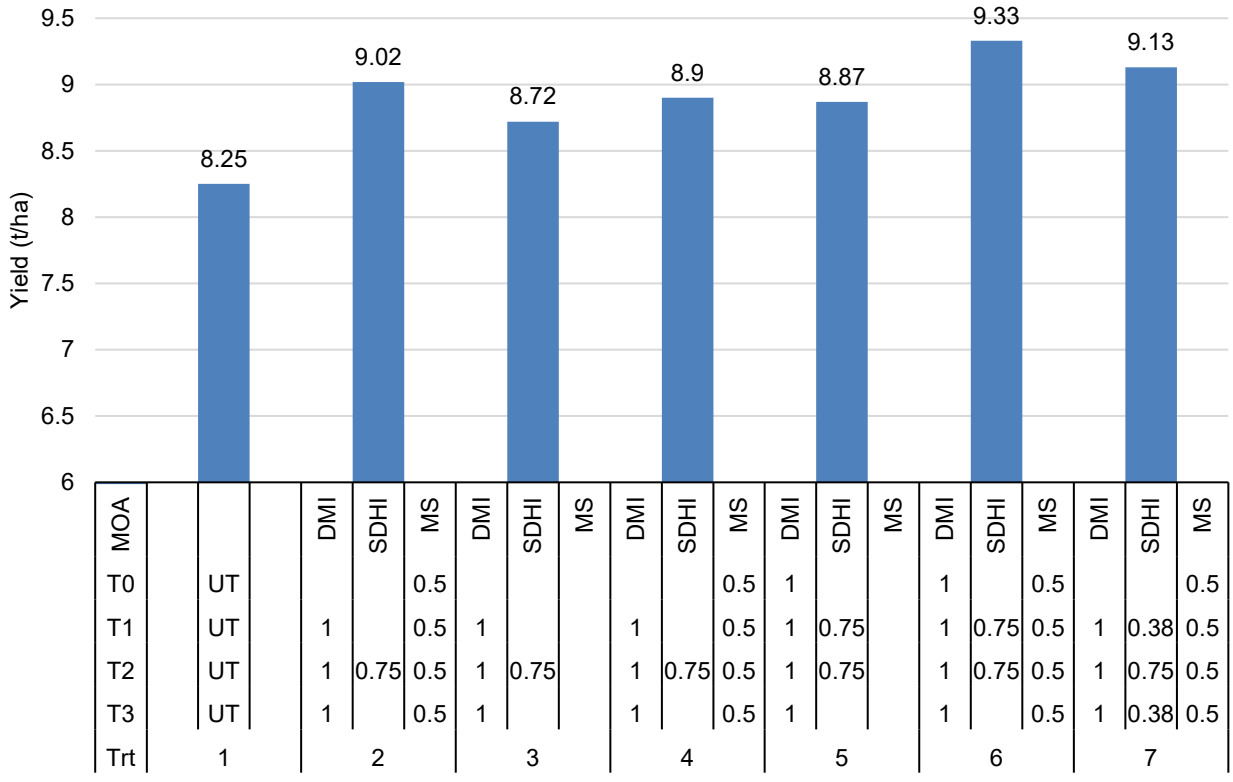

Figure 32. Cross site analysis of yield results from industry and research partners trials (n=5), 2018. Site Fpr <0.001, Site LSD 0.59; Treatment Fpr <0.001, Treatment LSD 0.31; Site.Treatment Fpr 0.004, Site.Treatment LSD 0.85.

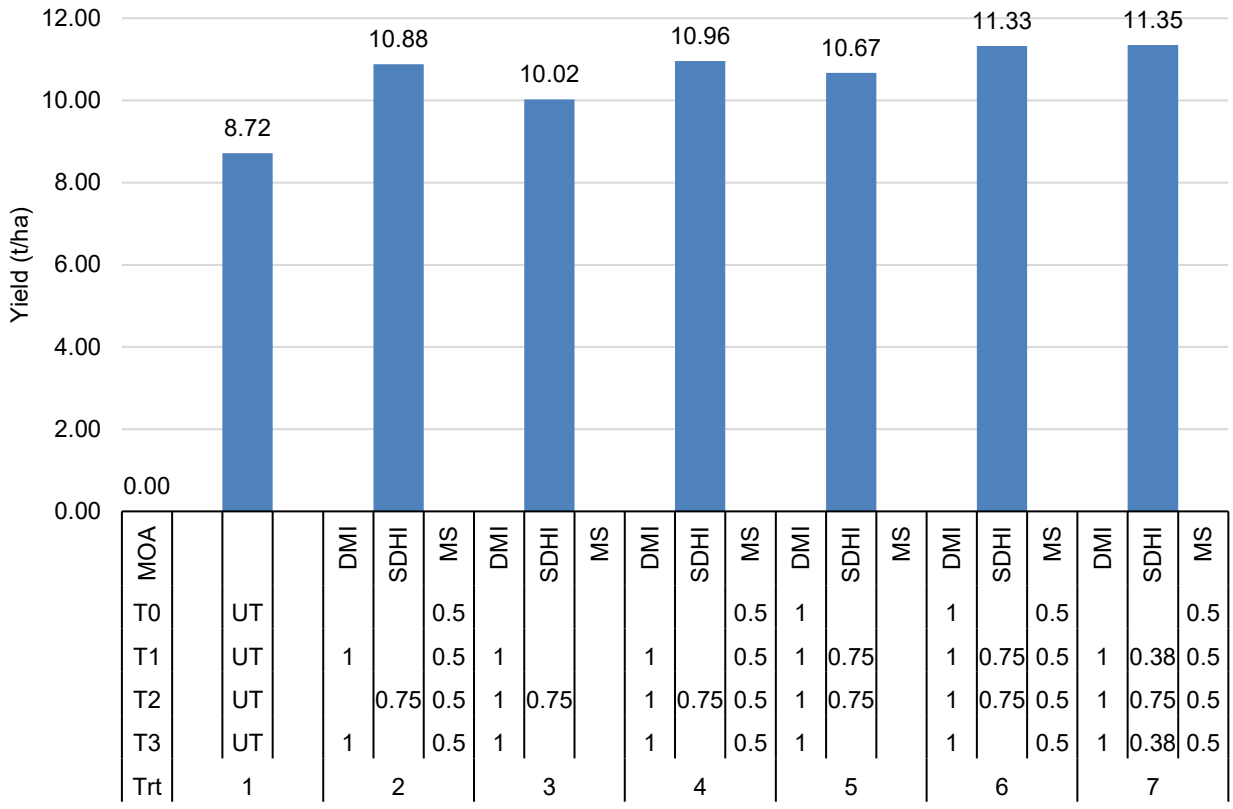

Figure 33. Cross site analysis of yield results from industry and research partners trials (n=7), 2019. Site Fpr <0.001, Site LSD 0.71; Treatment Fpr <0.001, Treatment LSD 0.26; Site.Treatment Fpr <0.001, Site.Treatment LSD 0.93.

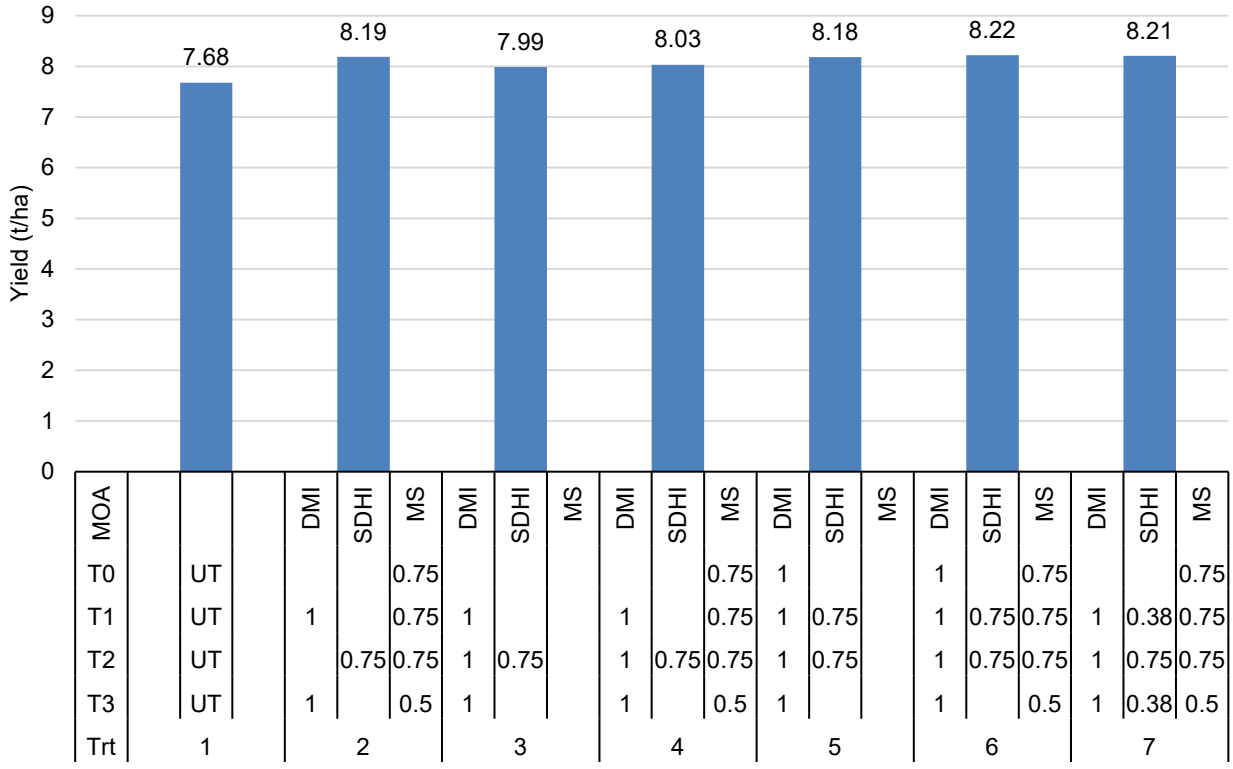

Figure 34. Cross site analysis of yield results from industry and research partners trials (n=6), 2020. Site Fpr <0.001, Site LSD 0.35; Treatment Fpr 0.053, Treatment LSD 0.38; Site.Treatment Fpr 0.529, Site.Treatment LSD 0.93.

6. Discussion

6.1. Conclusions

6.1.1. Conclusions on methods to test resistance management tactics

1. This project has created a unique set of field experimental data quantifying the effect of resistance management tactics on resistance occurring concurrently against two MoA.
2. The experimental method, using *Zymoseptoria tritici* as the test pathosystem, was effective.
3. There was no evidence that bias was being caused by cross-flow of spores between plots. Strong and significant treatment effects on selection were found when MoA were used repeatedly solo. Increased selection was associated with higher doses and where a higher total dose was applied by an increase in the number of applications (thus increasing exposure time). Lower selection was found in mixtures containing an effective multi-site mixture partner. These findings are in agreement with expectations from previous work and provide confidence that the experimental methods were appropriate.
4. There is a limited 'window of opportunity' for field experiments to measure the effect of resistance management tactics on selection, because:
    i. Experiments can only be conducted when the frequency of resistant mutants reaches a level quantifiable by high throughput assays.
    ii. There is an optimum pre-treatment frequency (determined by the size of resistance shift) which maximises the ability to discriminate treatment effects.
    iii. Unless mutants are unfit in the absence of treatment, the frequencies of mutations increase through time to levels which constrain the ability to discriminate between treatments.
5. Modelling, based on the field experiments, suggests that pre-season frequencies of mutants in the range 10%–50% give the best chance of detecting differences between treatments. High starting frequencies lead to running out of 'headroom' to detect differences. Low starting frequencies result in small absolute percentage differences. Within the optimum range, lower starting frequencies are optimal for detecting treatment differences where there are larger resistance shifts and higher frequencies where there are smaller resistance shifts.
6. Evidence from the field experiments and modelling suggests that the effect of dose splitting, within a fixed total dose, was small relative to background variability in the experimental system. Modelling suggests that the size of the splitting effect was limited by a combination of the incomplete resistance of 'moderately resistant' sdh mutants and the use of mixtures reducing selection. The dose splitting effects predicted by modelling were generally smaller than the detection limits of the experiments.
7. The timing of sampling for genotyping is a compromise between allowing sufficient time post-treatment for selection effects to be expressed, whilst not delaying sampling to a point

where leaves and lesions have deteriorated. Modelling suggests that sampling around GS75 appears to be a good compromise timing which results in only a very small underestimation of selection.

8. Current high-throughput genotyping methods quantify frequencies of individual mutations not haplotypes:
    i. This limitation was of less consequence for quantifying sdh mutants, as the majority of isolates carry a single mutation (during the period of the experiments).
    ii. CYP51 haplotypes carry multiple mutations. Nevertheless, S524T provided a good marker for new, less sensitive, strains increasing in the pathogen population.
9. Revisiting the DNA samples using new high-throughput sequencing methods to quantify haplotypes would increase the ability to discriminate the effects of treatments on selection.
10. The fungicides used in this project were selected according to the situation at the outset of the work. Isopyrazam was selected to be sufficiently effective to create selection, but to leave sufficient lesions present to be able to sample for genotyping. Newer, more effective products are now available or in the pipeline but the project results remain of relevance to other actives with the same MoA and to new MoA.

6.1.2. Conclusions for resistance management

1. There are many examples of concurrent evolution of resistance in many pathogens worldwide. Concurrent evolution should be considered as the likely outcome, where two or more single-site acting MoA are used against a pathogen.
2. An effective multi-site acting fungicide as a mixture partner slows selection. Removal of the widely used multi-sites chlorothalonil and mancozeb will increase dependence on single-site acting fungicides to control key pathogens in a range of crops, resulting in higher selection for concurrent resistance.
3. Unnecessary fungicide applications or unnecessarily high doses create resistance selection, without providing economic benefit:
    i. Resistance selection occurred similarly in low and high disease years of the experiments.
    ii. The intensity of fungicide treatment that maximised gross margin differed substantially with seasonal disease pressure.
    iii. Unnecessary treatment can only be identified with certainty in retrospect.
    iv. Decision support can guide treatments according to need.
4. Useful control of septoria was obtained from DMI and SDHI fungicides in the field experiments, despite high mutation frequencies:
    i. Inclusion of DMI fungicide in a programme gave significantly improved control of septoria compared with programmes using SDHI only.

ii. In the case of the SDHIs, efficacy reflects the predominance of moderately insensitive mutants in the population and the low frequency of highly resistant mutants, such as H152R.

5. Despite providing useful efficacy, the effect of older azoles protecting SDHIs was difficult to detect.
6. The frequency of H152R increased during the project. However, the frequencies at the start of each season were lower than the frequencies at the end of the preceding season, indicating that a fitness penalty associated with the mutation was causing H152R strains to be selected against in the absence of SDHI treatment.
7. There was some indication that the frequencies of moderately SDHI resistant strains were also decreasing over-winter, but the evidence was unclear. This indicates that such strains are generally fit.
8. Changes in the relative frequencies of different moderately resistant mutants between and within seasons indicates relative differences in fitness, in the presence and/or absence of SDHI.
9. Evidence from the field experiments and from modelling show that the effect of dose splitting (within a fixed total dose) on selection for sdh mutants was small.
10. Evidence from the field experiments and from modelling show that the benefit of splitting the dose of SDHI, to allow more of the DMI treatments to be protected by an SDHI mixture partner, was small and below the detection limits of the experiments.
11. The field experiment data show that dose splitting was generally neutral for efficacy of disease control and for the economics of fungicide programmes.
12. In principle, alternation is only likely to be better than mixtures for reducing selection where dose splitting increases selection, and the mixture partner is relatively ineffective. This rationale was not corroborated experimentally and will be explored further by modelling.
13. Alternation and mixtures were similarly effective at reducing selection and for disease control.
14. There was no consistent evidence that the relative efficacy of the two components of the alternation or mixture programmes affected whether one strategy was better than the other.

6.2. Messages for practice

Key messages:

- The project results provide field evidence that supports current FRAG guidelines (FRAG 2021).
- IPM is the basis for resistance management and resistance management is a key component of IPM.

- Reduced availability of multi-site acting fungicides, due to regulation, will increase dependence on single-site actives, increasing concurrent resistance evolution and the need for effective resistance management.
- Mixtures, alternation and limiting number of treatments are all effective resistance management strategies.
- Limiting treatments may limit use of mixtures, where there are few effective MoA available relative to the number of treatments required per season.
- Evidence from this project and the literature suggests there are many circumstances where alternation is as effective as mixtures at reducing selection.
- Therefore, the choice between adopting a mixture or alternation strategy can be determined by efficacy and practical considerations.
- Total dose of a MoA applied in a season is a key driver of resistance selection.
- Limiting total dose to manage resistance can be achieved by:
    - Limiting the number of treatments (and maximum individual dose), or
    - Limiting total dose (and maximum individual dose) and allowing farmers flexibility in how that total dose is split
- Allowing flexibility in how a total dose is used, as part of an effective mixture strategy, is unlikely to have a substantial effect on selection. Nevertheless, the following limitations should apply:
    - The mixture partner should be effective.
    - The increase in the number of treatments allowed by dose splitting should be limited.
    - There should be clear evidence of benefits from allowing more flexibility, to justify the resulting small increase in resistance risk.
- The benefits could arise from improved efficacy, economics or protection of mixture partners.
- Such benefits were not demonstrated for septoria tritici in wheat.
- There may be benefits from flexibility in other pathogen-crop systems, particularly where the number of treatments required is high and there are few MoA available

6.3. Recommendations for uptake of findings

It takes time for messages about resistance management to be disseminated and accepted into practice, so debates about potential changes to guidance should be treated with caution to ensure consensus and consistent messaging. The findings should be debated at FRAG, FRAC and though EPPO. The work should be subjected to peer review by publication.

6.4. Research gaps

- Further insights into concurrent resistance management are likely to result from the ongoing AHDB PhD associated with this project – to identify fruitful approaches which would justify experimental corroboration.
- Work to increase implementation of IPM, including improving 'treatment according to need' through decision support, would benefit resistance management.
- High-throughput genotyping methods should be exploited to improve testing of resistance management tactics.

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8. Appendix 1

8.1. Objective 1, 2017 sites: mutation frequency, septoria severity and yield

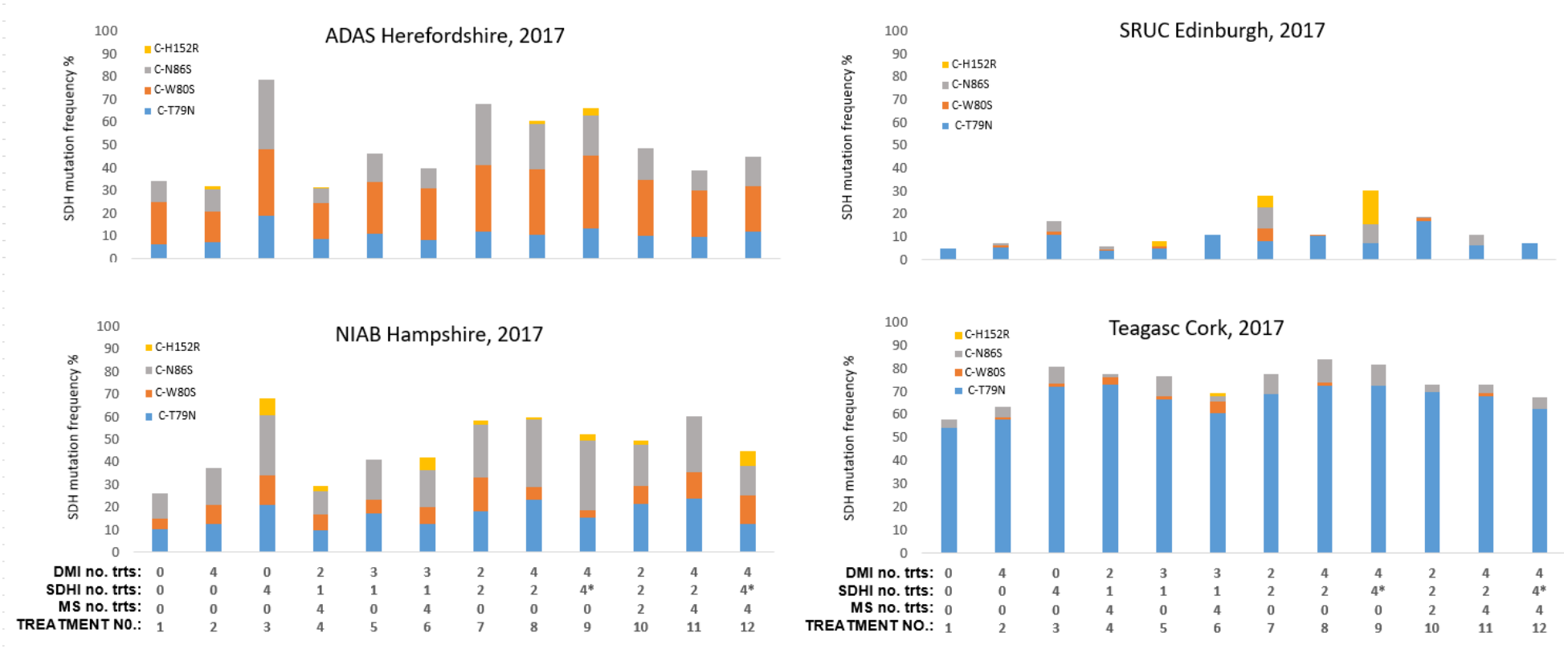

Figure 35. Effect of dose of SDH, DMI and multi-site fungicides on SDH mutations in septoria, in 2017 field experiments. Each individual mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. Numbers on X-axis refer to number of application times. Dose rates (proportion of full label rate per application) = SDHI ½ (except * = ¼), isopyrazam (Zulu, Adama); DMI **1.0**, prothioconazole (Proline, Bayer); MS ½, chlorothalonil (Bravo, Syngenta).

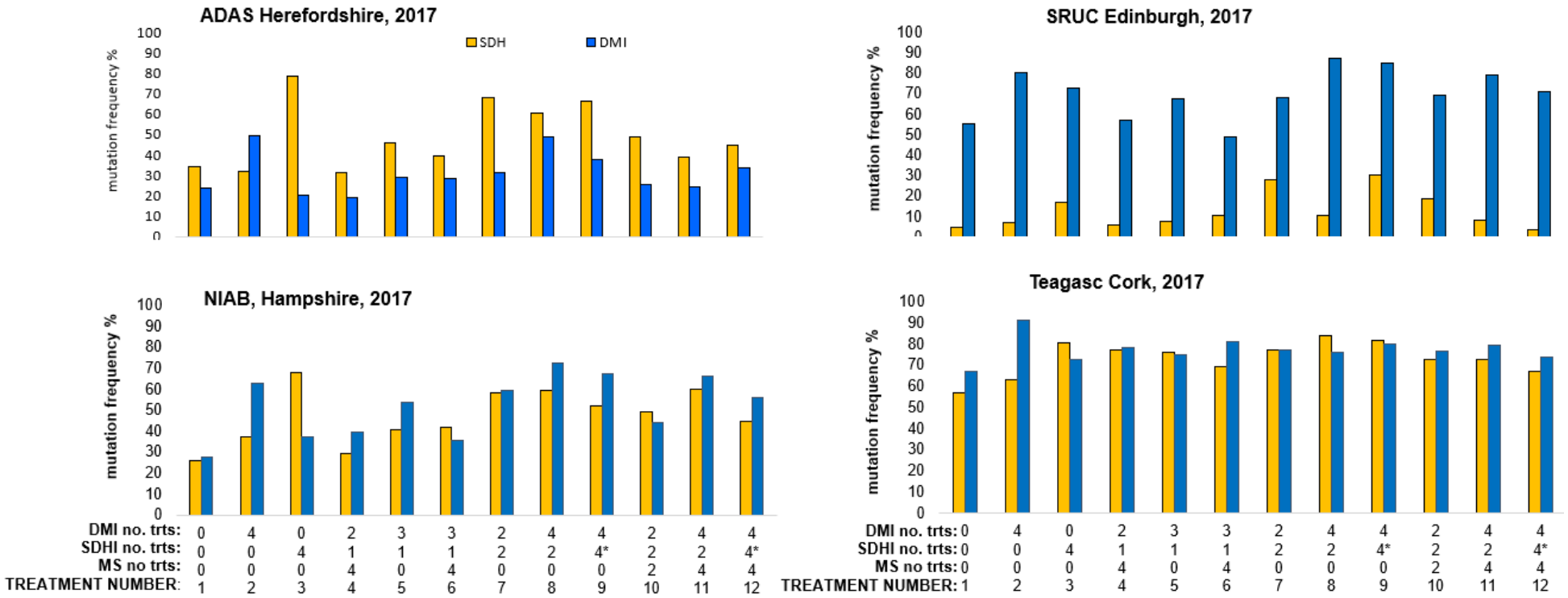

Figure 36. Effect of dose of SDH, DMI and multi-site fungicides on total SDH and S524T DMI mutations in septoria, in 2017 field experiments. Each value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. Numbers on X-axis refer to number of application times. Dose rates (proportion of full label rate per application) = SDHI ½ (except * = ¼), isopyrazam (Zulu, Adama); DMI **1.0**, prothioconazole (Proline, Bayer); MS ½, chlorothalonil (Bravo, Syngenta).

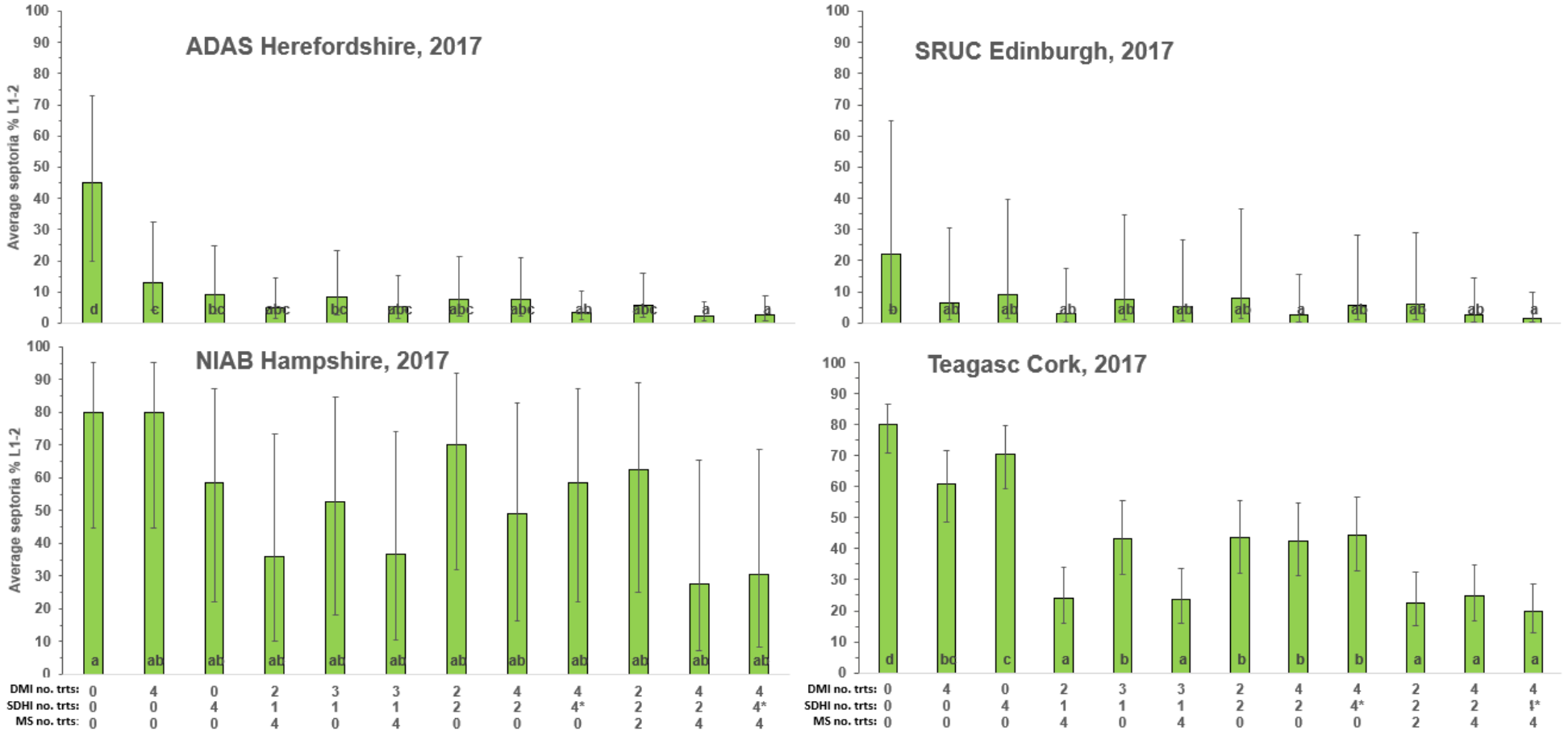

Figure 37. Effect of dose of SDH, DMI and multi-site fungicides on % septoria severity on top two leaves, in 2017 field experiments. Values are averages of four replicate plots (except three at NIAB) at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. Numbers on X-axis refer to number of application times. Dose rates (proportion of full label rate per application) = SDHI ½ (except * = ¼), isopyrazam (Zulu, Adama); DMI **1.0**, prothioconazole (Proline, Bayer); MS ½, chlorothalonil (Bravo, Syngenta).

Table 20. Concurrent resistance field experiments 2017, yields

Trt	Number of applications [a]DMI	[b]SDHI	[c]MS	Yield t/ha ADAS Hereford	NIAB Hampshire	SRUC Edinburgh	Teagasc Cork		Site average
1	0	0	0	8.37	8.82	9.50	6.01		8.14
2	4	0	0	10.32	10.47	11.51	7.71		9.97
3	0	4	0	10.19	11.15	10.67	6.65		9.56
4	2	1	4	10.70	11.36	11.09	7.92		10.20
5	3	1	0	10.38	11.19	11.25	7.40		9.98
6	3	1	4	10.78	11.88	11.22	8.44		10.49
7	2	2	0	10.05	11.53	10.97	7.62		9.94
8	4	2	0	10.84	12.10	11.40	7.39		10.32
9	4	*4	0	10.51	12.15	11.20	7.42		10.20
10	2	2	2	10.49	11.53	10.95	7.96		10.15
11	4	2	4	11.00	12.11	11.54	8.46		10.69
12	4	*4	4	10.98	12.19	11.28	8.18		10.56
				ANOVA	ANOVA	ANOVA	ANOVA		REML
			F prob UT vs Trt	<0.001	<0.001	<0.001	<0.001	F prob UT vs Trt	<0.001
			F prob Trt only	<0.001	0.017	0.019	<0.001	F prob Site	<0.001
			LSD UT vs Trt	0.251	0.686	0.341	0.179	F prob Trt	<0.001
			LSD Trt only	0.339	0.928	0.462	0.243	F prob UT. site	0.016
			df resid	33	22	33	33	F prob UT.Trt.site	0.004

[A] prothioconazole (Proline, Bayer) dose per application (proportion of full label rate) = 1.0

[B] isopyrazam (Zulu, Adama) dose per application (proportion of full label rate) = 1/2, except * indicates ¼ dose

[C] chlorothalonil (Bravo, Syngenta), dose per application (proportion of full label rate) = ½

9. Appendix 2

9.1. Objective 1, 2018 sites: mutation frequency, septoria severity and yield

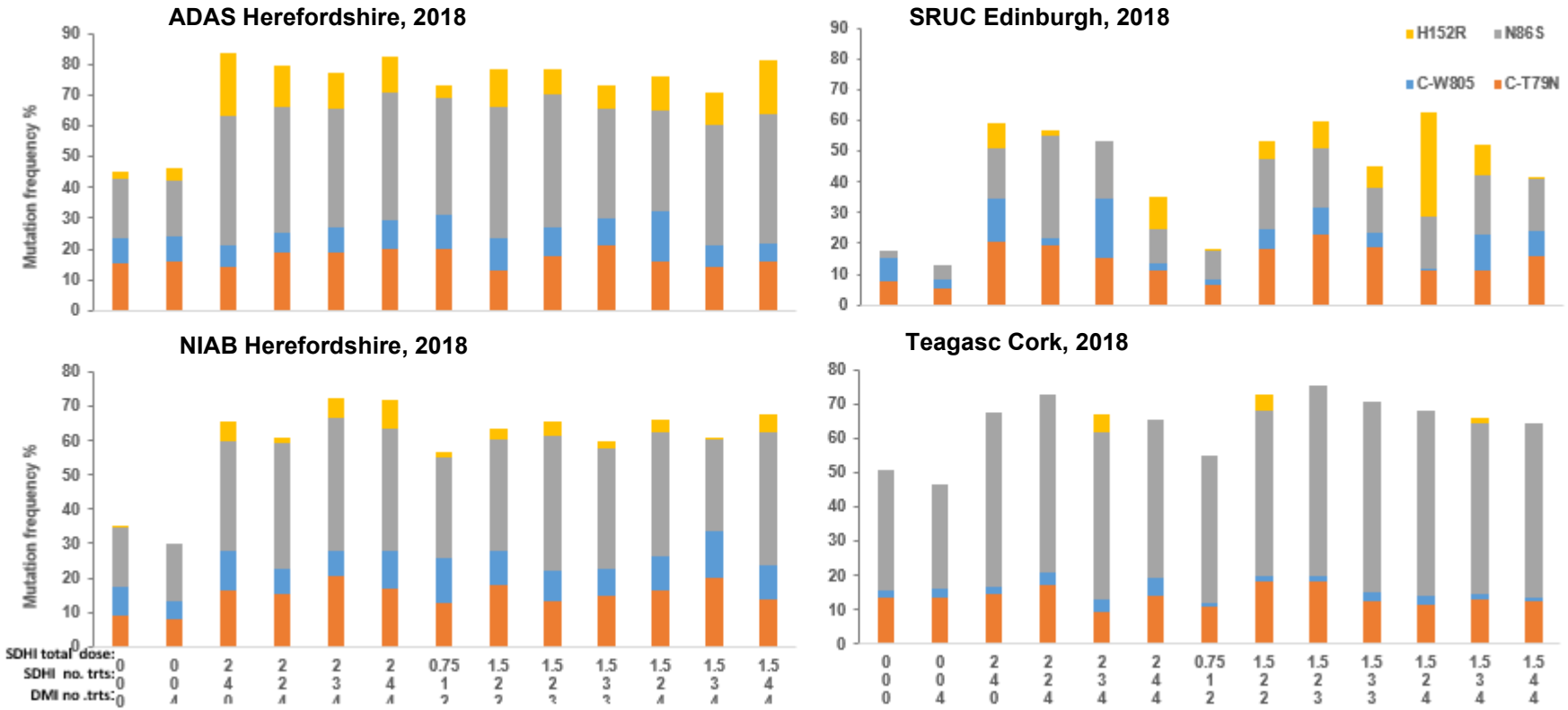

Figure 38. Effect of dose of SDH and DMI fungicides on SDH mutations in septoria, in 2018 field experiments. Each individual mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama); total dose across season is proportion of full label rate. DMI = prothioconazole (Proline, Bayer); dose per application is full rate.

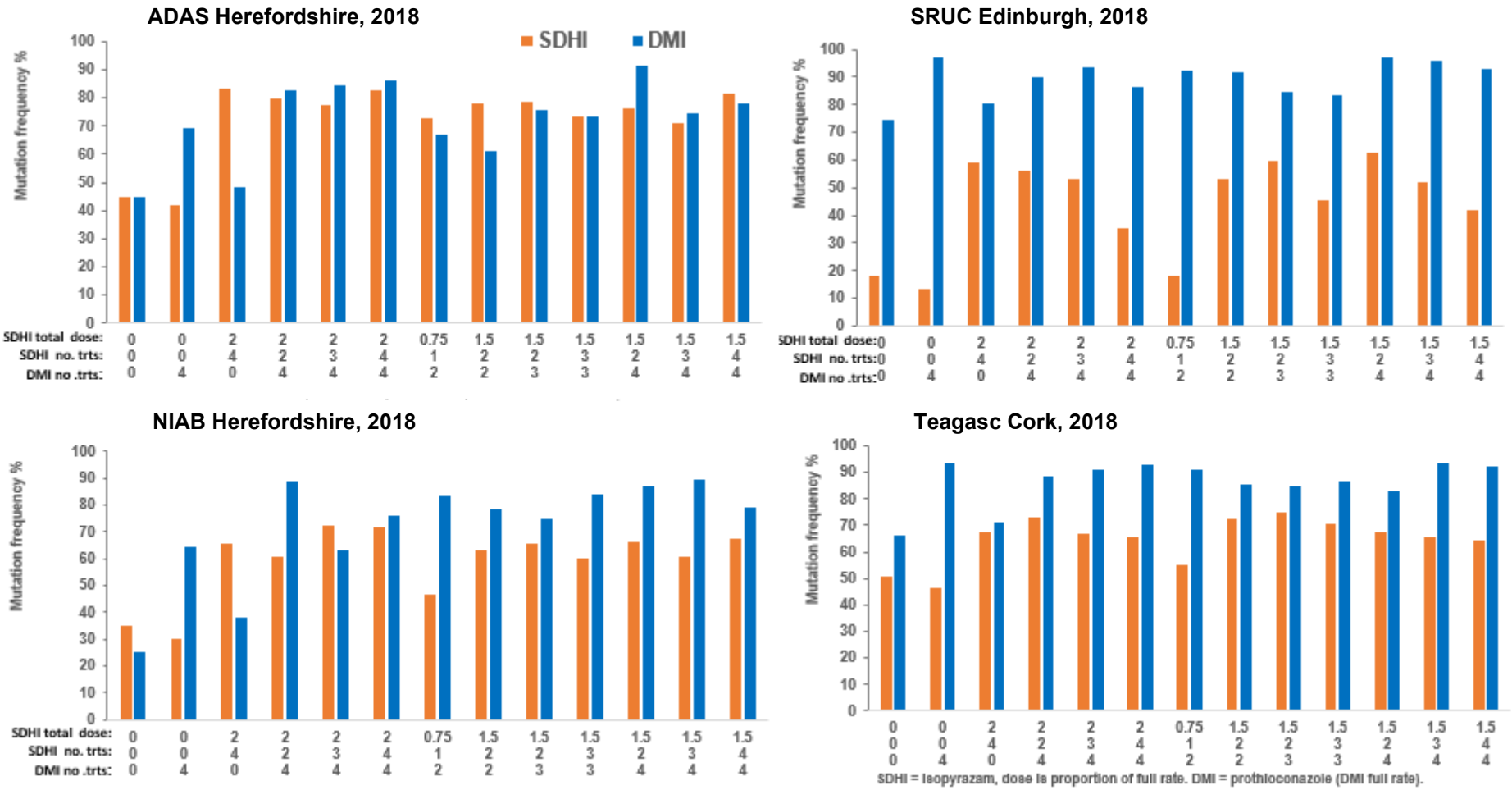

Figure 39. Effect of dose of SDH and DMI fungicides on total SDH and S524T DMI mutations in septoria, in 2018 field experiments. Each mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama), total dose across season is proportion of full label rate. DMI = prothioconazole (Proline, Bayer), dose per application is full rate.

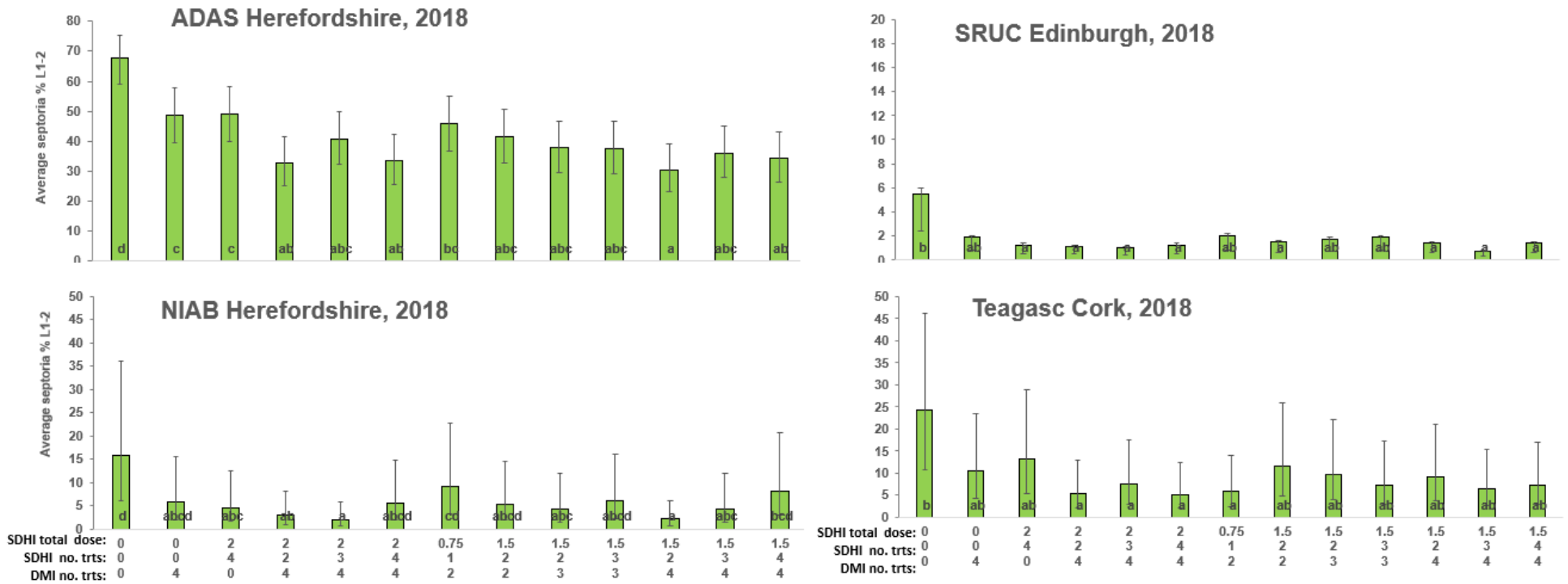

Figure 40. Effect of dose of SDHI and DMI fungicides on % septoria severity on top two leaves, in 2018 field experiments. Values are averages of four replicate plots (except three at NIAB) at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama), total dose across season is proportion of full label rate. DMI = prothioconazole (Proline, Bayer), dose per application is full rate.

Table 21. Concurrent resistance field experiment 2018, Cross-site analysis, effect of SDHI dose splitting

Trt	[a]SDHI total dose	Number of applications		[c]% total SDH mutations	% C-H152R	% DMI (S524T)
		SDHI	[b]DMI			
1	0	0	0	37.37	0.79	54.64
2	0	0	4	32.90	1.03	82.07
3	2	4	0	69.09	8.76	60.35
4	2	2	4	68.62	4.56	87.23
5	2	3	4	68.11	5.99	83.60
6	2	4	4	63.27	7.76	86.01
7	0.75	1	2	48.31	1.45	83.33
8	1.5	2	2	67.09	6.75	79.13
9	1.5	2	3	69.96	5.38	80.17
10	1.5	3	3	62.37	4.35	81.65
11	1.5	2	4	68.65	11.24	89.33
12	1.5	3	4	63.29	5.73	87.74
13	1.5	4	4	63.52	5.84	85.95
			F prob Site	$\leq$ 0.001	<0.001	<0.001
			F prob SDHI dose	$\leq$ 0.001	<0.001	<0.001
			F prob SDHI split	0.261	0.018	0.466
			F prob Site.SDHI dose	0.005	0.002	0.077
			F prob Site. SDHI split	0.127	0.076	0.575
			F prob SDHI dose.SDHI split	0.603	0.246	0.004
			F prob Site.SDHI dose.SDHI split	0.935	0.049	0.721

[a] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[b] prothioconazole (Proline, Bayer), dose per application = full rate

Values are averages of 4 sites (ADAS, NIAB, SRUC & Teagasc) and 4 plot reps per site

Table 22. Concurrent resistance field experiments 2018, yields

Trt	Number of applications			Yield t/ha					
	[a]SDHI total dose	SDHI number of trts	[b]DMI number of trts	ADAS Hereford	NIAB Hampshire	SRUC Edinburgh	Teagasc Cork		Sites average
1	0	0	0	8.77	10.23	9.15	8.67		9.14
2	0	0	4	10.23	11.24	10.02	10.02		10.32
3	2	4	0	10.11	10.69	9.02	9.48		9.77
4	2	2	4	11.04	11.14	9.38	10.40		10.45
5	2	3	4	10.98	11.66	9.40	10.44		10.55
6	2	4	4	10.57	11.54	9.74	10.72		10.58
7	0.75	1	2	10.45	11.06	9.16	10.20		10.16
8	1.5	2	2	10.66	11.32	9.41	10.43		10.40
9	1.5	2	3	11.03	11.27	9.84	10.19		10.53
10	1.5	3	3	10.94	11.06	9.71	10.18		10.43
11	1.5	2	4	11.07	11.50	9.74	10.48		10.64
12	1.5	3	4	11.00	11.38	10.08	10.37		10.66
13	1.5	4	4	10.95	10.98	9.73	10.23		10.44
				ANOVA	ANOVA	ANOVA	ANOVA		REML
			F prob UT vs Trt	<0.001	<0.001	NS	<0.001	F prob UT vs Trt	<0.001
			F prob Trt only	<0.001	0.017	NS	<0.001	F prob Site	<0.001
			SED UT vs Trt	0.176	0.128	0.260	0.115	F prob Trt	<0.001
			SED Trt only	0.239	0.174	0.353	0.156	F prob UT. site	<0.001
			df resid	36	38	36	36	F prob UT.Trt.site	<0.001

[a] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[b] prothioconazole (Proline, Bayer), dose per application = full rate

10. Appendix 3,

10.1. Objective 1, 2019 sites: mutation frequency, septoria severity and yield

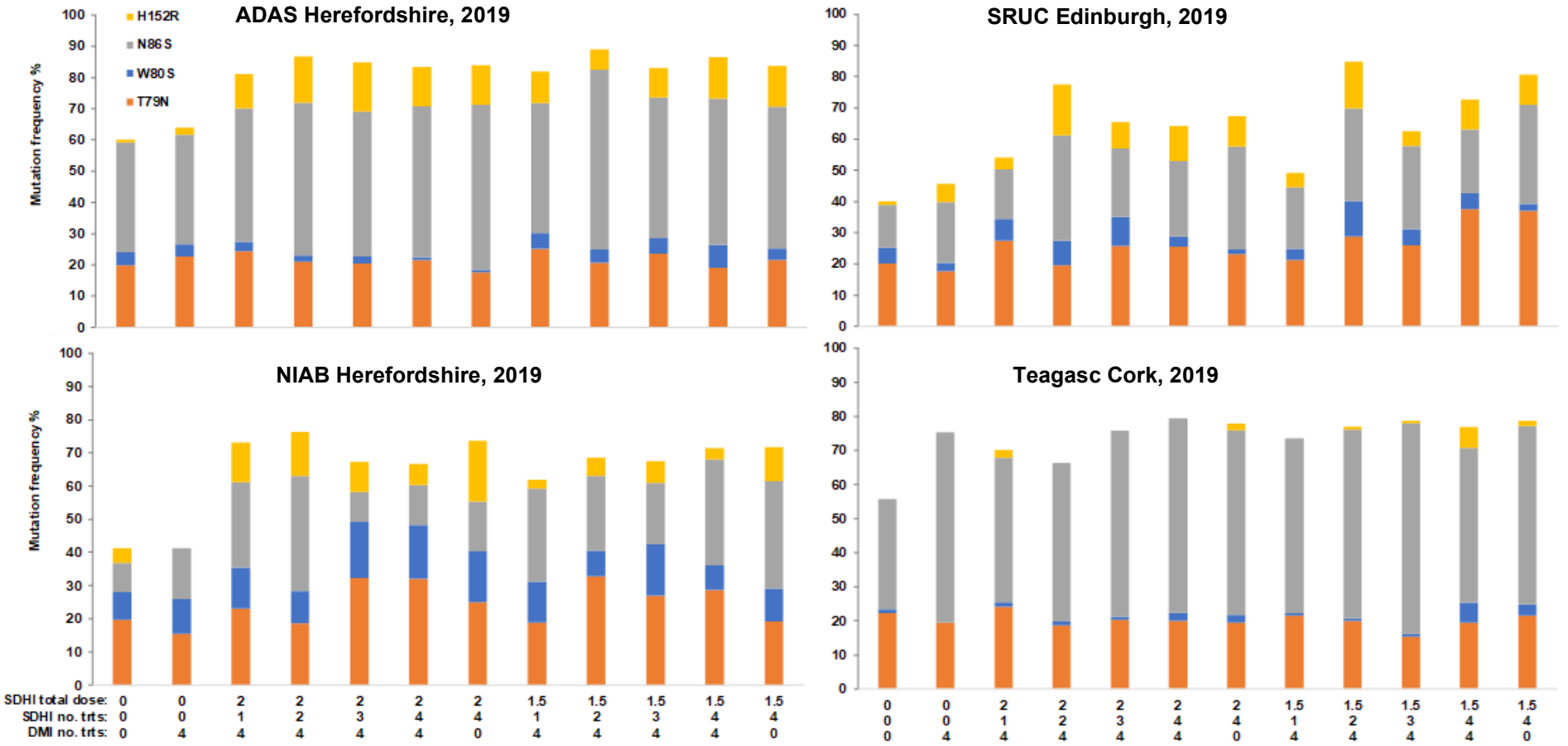

Figure 41. Effect of dose of SDH and DMI fungicides on SDH mutations in septoria, in 2019 field experiments. Each individual mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama); total dose across season is proportion of full label rate. DMI = prothioconazole (Proline, Bayer); dose per application is full rate.

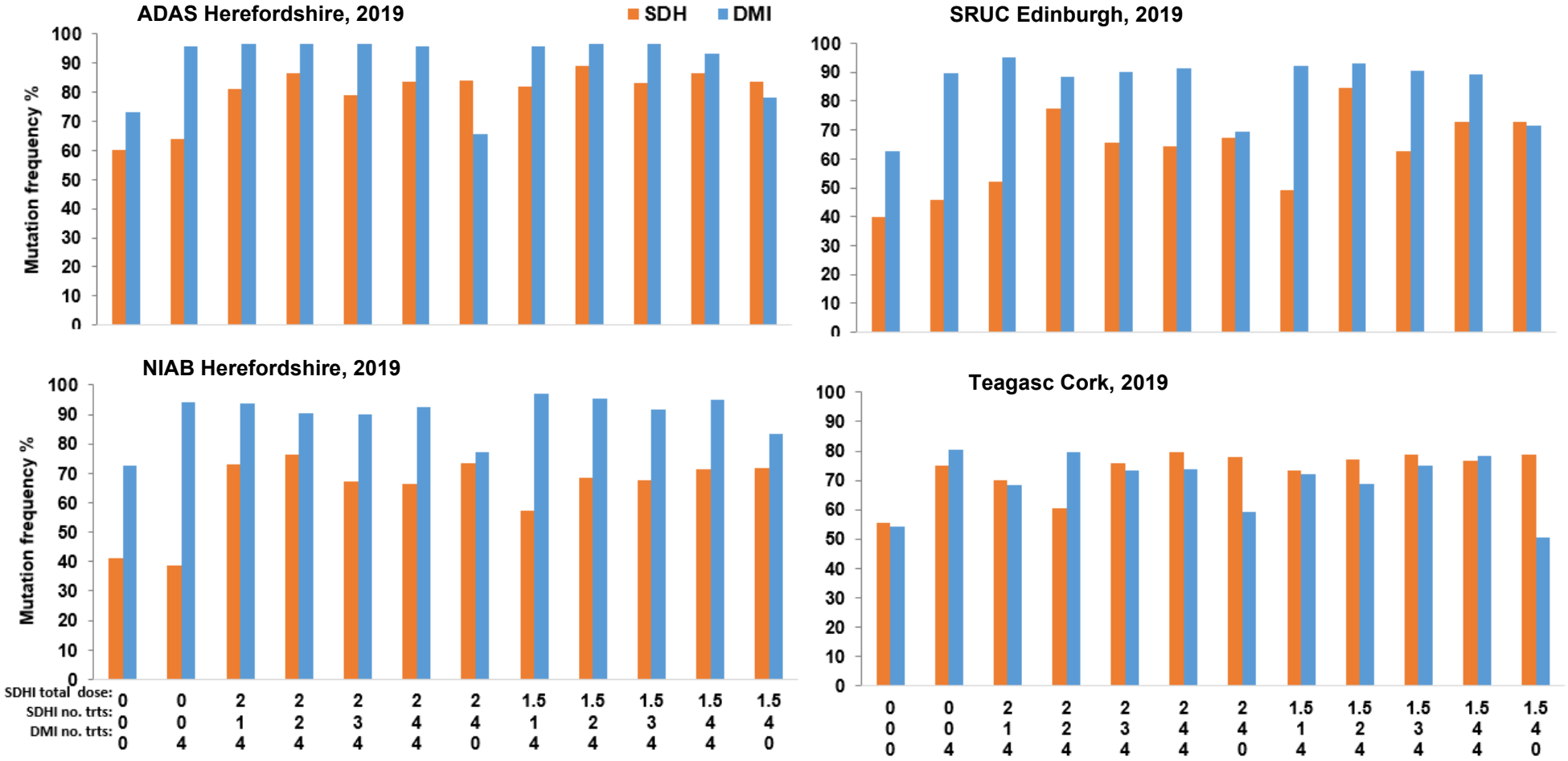

Figure 42. Effect of dose of SDH and DMI fungicides on total SDH and S524T DMI mutations in septoria, in 2019 field experiments. Each mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites: ADAS Herefordshire, NIAB Kent, SRUC Edinburgh and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama), total dose across season is proportion of full label rate. DMI = prothioconazole (Proline, Bayer), dose per application is full rate.

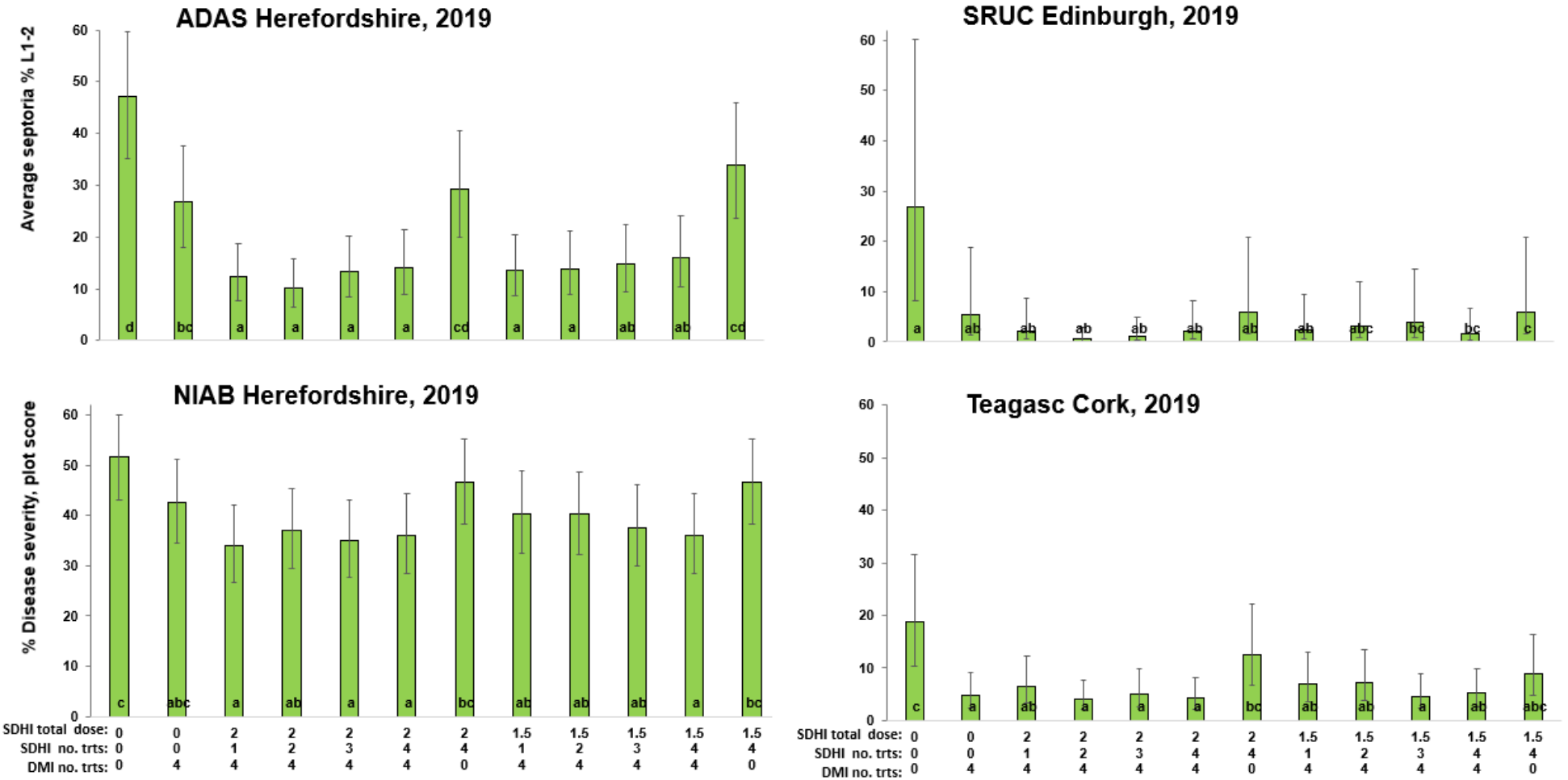

Figure 43. 2019 Septoria (*Zymoseptoria tritici*) severity on the upper two leaves, field experiments at ADAS Herefordshire, NIAB Herefordshire, SRUC Edinburgh and Teagasc Cork (same experiments as in Figure 41). Each severity value is an average of four replicate plot values. Analysis is based on logit transformed values.

Table 23. Concurrent resistance field experiment 2019, analysis of effect of mixing SDHI with DMI, for % total SDH mutations, % DMI (S524T) mutation and % septoria, ADAS Herefordshire, NIAB Herefordshire, SRUC Edinburgh, Teagasc Cork.

[d]ANOVA model	F prob, % total SDH mutations (T79N, W80S, N86S, H152R)				F prob, % C-H152R				F prob, % DMI mutation (S524T)				[c] F prob, % septoria severity (average top two leaves)			
	ADAS	**NIAB**	**SRUC**	**Tea-gasc**	**ADAS**	**NIAB**	**SRUC**	**Tea-gasc**	**ADAS**	**NIAB**	**SRUC**	**Tea-gasc**	**ADAS**	**NIAB**	**SRUC**	**Tea-gasc**
UT	≤ 0.001	≤ 0.001	≤ 0.001	≤0.001	≤ 0.001	0.454	0.053	0.292	0.908	<.001	<.001	0.67	<.001	<.001	<.001	<.001
UT.DMI solo	≤ 0.001	≤ 0.001	0.006	0.93	≤ 0.001	0.06	0.369	0.249	0.745	0.208	0.406	0.811	<.001	0.097	0.099	0.096
UT.DMI solo. Mixture effect	0.606	0.38	0.137	0.028	0.235	0.624	0.597	0.021	0.562	0.001	<.001	0.851	<.001	0.007	0.009	0.025
UT.DMI solo. Mixture effect. Mixture dose effect	0.687	0.749	0.288	0.817	0.853	0.192	0.824	0.013	0.831	0.117	0.941	0.518	0.187	1	0.924	0.586
UT.DMI solo. Mixture effect. SDHI only	0.737	0.40	0.8	0.972	0.995	0.035	0.871	0.236	0.879	<.001	<.001	0.652	<.001	<.001	0.006	<.001
UT.DMI solo. Mixture effect. Mixture dose effect. SDHI solo	0.628	0.442	0.815	0.645	0.9	0.557	0.829	0.006	0.925	0.453	0.467	0.677	0.986	1	0.869	0.104
SED	4.965	6.084	9.092	5.341	2.943	5.978	5.210	1.528	7.970	3.683	3.432	8.080	0.224	0.13	0.666	0.273
df resid	38	27	37	38	38	27	37	38	38	27	37	38	38	27	37	38

[a] prothioconazole (Proline, Bayer), dose per application = full rate

[b] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[c] septoria analysis reported on logit transformed data

[d] DMI solo (Trt 2); mixture effect compares same SDHI progs with and without DMI (Trts 6&11 vs 7&12); mixture dose effect compares SDHI dose 1.5 or 2.0 (Trts 6&7 vs 11&12); SDHI solo (Trt 7).

Table 24. Concurrent resistance field experiment 2019, analysis of effect of splitting the SDHI dose, for % total SDH mutations, % DMI (S524T) mutation and % septoria, ADAS Herefordshire, NIAB Herefordshire, SRUC Edinburgh, Teagasc Cork

[d]ANOVA model	F prob, % total SDH mutations (T79N, W80S, N86S, H152R)				F prob, % C-H152R				F prob, % DMI mutation (S524T)				[c] F prob, % septoria severity (average top two leaves)			
	ADAS	NIAB	SRUC	Tea-gasc	ADAS	NIAB	SRUC	Tea-gasc	ADAS	NIAB	SRUC	Tea-gasc	ADAS	NIAB	SRUC	Tea-gasc
UT	<.001	<.001	<.001	<.001	<.001	0.458	0.033	0.302	0.914	<.001	<.001	0.677	<.001	<.001	<.001	<.001
UT.DMI solo	<.001	<.001	0.001	0.915	<.001	0.064	0.319	0.259	0.76	0.203	0.419	0.815	<.001	0.075	0.082	0.078
UT.DMI solo. SDHI solo	0.959	0.159	0.212	0.093	0.401	0.053	0.828	0.617	0.825	<.001	<.001	0.646	<.001	<.001	<.001	<.001
UT.DMI solo. SDHI solo.	0.953	0.703	0.479	0.844	0.961	0.188	0.998	0.829	0.937	0.1	0.582	0.464	0.356	1	0.959	0.103
UT.DMI solo. SDHI solo. SDHI solo dose.	0.233	0.079	0.932	0.029	0.007	0.07	0.538	0.106	0.91	0.109	0.993	0.84	0.072	0.029	0.05	0.214
UT.DMI solo. SDHI solo. SDHI solo dose. SDHI solo	0.131	0.262	<.001	0.014	0.462	0.767	0.008	0.081	0.956	0.378	0.628	0.563	0.437	0.515	0.702	0.153
UT.DMI solo. SDHI solo. SDHI solo dose. SDHI dose. SDHI split	0.919	0.042	0.62	0.03	0.056	0.816	0.923	0.006	0.931	0.914	0.635	0.757	0.847	0.427	0.231	0.217
SED	4.828	5.10	7.580	4.413	2.537	6.008	4.665	1.549	8.5	3.614	4.464	8.27	0.224	0.113	0.18	0.256
df resid	33	22	32	33	33	22	33	33	33	22	32	33	33	22	33	33

[a] prothioconazole (Proline, Bayer), dose per application = full rate

[b] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[c] septoria analysis reported on logit transformed data

[d] DMI solo (Trt 2); SDHI solo (Trt 7); SDHI solo dose = solo SDHI at 1.5 (Trt 12) or 2.0 (Trt 7); SDHI dose = total 1.5 or 2; SDHI split = 1, 2, 3 or 4

Table 25. Concurrent resistance field experiments 2019, yields

Trt	Number of applications			ADAS Hereford	NIAB Hampshire	SRUC Edinburgh	Teagasc Cork
	[a]SDHI total dose	SDHI number of trts	[b]DMI number of trts				
1	0	0	0	8.41	8.12	8.53	8.20
2	0	0	4	10.59	9.52	9.89	10.35
3	2	1	4	11.01	10.00	10.25	9.84
4	2	2	4	11.22	10.04	10.19	10.24
5	2	3	4	11.08	10.10	10.18	10.21
6	2	4	4	11.22	10.20	9.97	10.24
7	2	4	0	9.81	8.88	9.28	8.81
8	1.5	1	4	11.06	9.87	10.15	10.17
9	1.5	2	4	11.11	9.94	10.65	10.09
10	1.5	3	4	10.92	10.03	10.16	10.33
11	1.5	4	4	10.84	10.26	10.37	10.26
12	1.5	4	0	9.56	8.95	9.10	9.12
				ANOVA	ANOVA	ANOVA	ANOVA
			F prob UT vs Trt	<0.001	<0.001	NS	<0.001
			F prob Trt only	<0.001	0.017	NS	<0.001
			SED UT vs Trt	0.16	0.10	0.130	0.14
			SED Trt only	0.21	0.13	0.18	0.19
			df resid	36	38	36	36

[a] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[b] prothioconazole (Proline, Bayer), dose per application = full rate

11. Appendix 4

11.1. Objective 1, 2020 SRUC, yield

Table 26. Concurrent resistance field experiment 2020, yields, SRUC Edinburgh

Trt	Number of applications			Yield t t/ha
	[a]SDHI total dose	SDHI number of trts	[b]DMI number of trts	SRUC Edinburgh
1	0	0	0	9.33
2	0	0	4	10.41
3	2	1	4	10.57
4	2	2	4	10.51
5	2	3	4	10.28
6	2	4	4	10.53
7	2	4	0	9.64
8	1.5	1	4	10.40
9	1.5	2	4	10.53
10	1.5	3	4	10.25
11	1.5	4	4	10.62
12	1.5	4	0	9.62
				ANOVA
			F prob UT vs Trt	≤ 0.001
			F prob Trt only	≤ 0.001
			LSD UT vs Trt	0.338
			LSD Trt only	0.458
			df resid	33

[a] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)
[b] prothioconazole (Proline, Bayer), dose per application = full rate

12. Appendix 5

12.1. Objective 2, 2018 sites: mutation frequency, septoria severity and yield

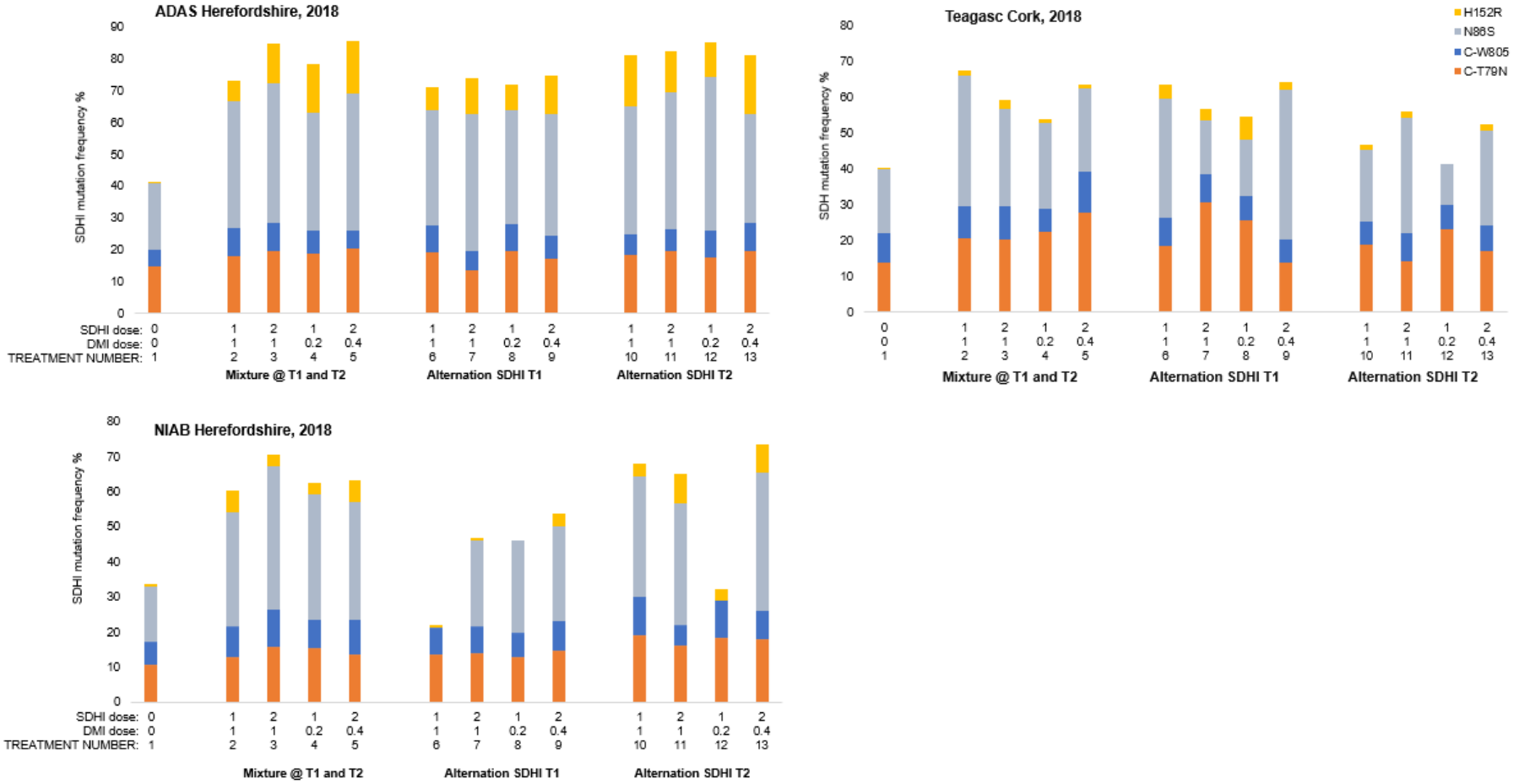

Figure 44. Effect of SDH and DMI fungicides when applied as mixtures or alternated, on % SDH mutations in septoria in 2018 field experiments. Each mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of three sites: ADAS Herefordshire, NIAB Kent and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama) and DMI = prothioconazole (Proline, Bayer). Numbers on x-axis are total doses (proportion of full rate) applied across a two-spray programme, T1 GS32 and T2 GS39.

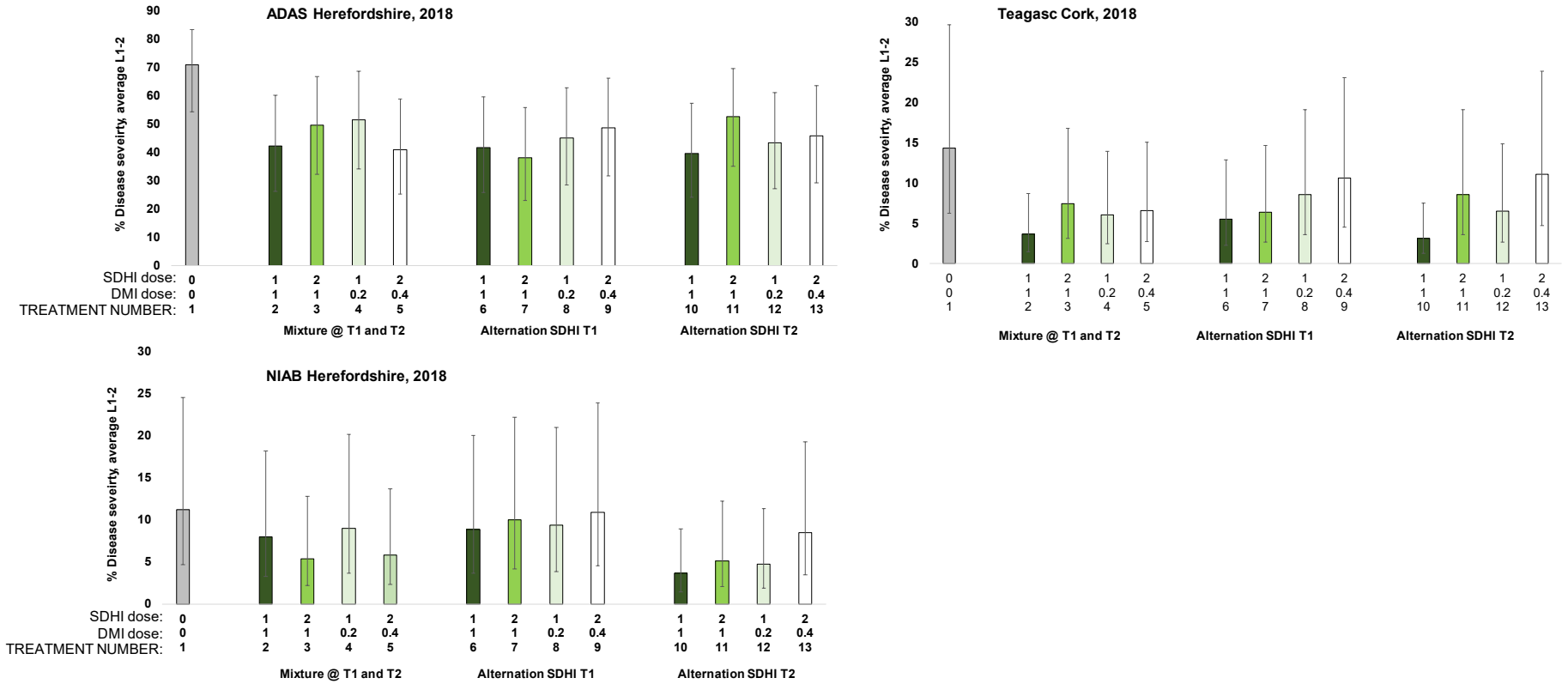

Figure 45. Effect of SDH and DMI fungicides when applied as mixtures or alternated, on % septoria severity on top two leaves in 2018 field experiments. Each severity value is an average of four replicate plot values (except NIAB, 3 reps.) at each of three sites: ADAS Herefordshire, NIAB Kent and Teagasc Cork. SDHI = isopyrazam (Zulu, Adama) and DMI = prothioconazole (Proline, Bayer). Numbers on x-axis are total doses (proportion of full rate) applied across a two-spray programme, T1 GS32 and T2 GS39.

Table 27. Mixtures vs alternation field experiment 2018 analysis of effect of treatment programme on % total SDH mutations, C-H152R mutation and % septoria severity, at ADAS Herefordshire, NIAB Herefordshire and Teagasc Cork.

	F prob, % total SDH mutations (T79N, W80S, N86S, H152R)			F prob, % C-H152R			[c]F prob, %septoria severity (average top two leaves)		
F prob	ADAS	NIAB	Tea-gasc	ADAS	NIAB	Tea-gasc	ADAS	NIAB	Tea-gasc
UT	<.001	<.001	0.428	<.001	0.016	0.428	<0.001	0.037	0.007
UT.DMI dose	0.125	0.001	0.852	0.045	0.005	0.852	0.529	0.609	0.007
UT.DMI dose. SDHI dose	0.821	0.62	0.828	0.301	0.076	0.828	0.466	0.308	0.038
UT.DMI dose. Trt prog	0.105	<.001	0.229	0.191	<.001	0.229	0.709	<0.001	0.357
UT.DMI dose.SDHI dose. Trt prog.	0.852	0.011	0.868	0.157	0.449	0.868	0.43	0.799	0.785
SED Trt prog	6.02	5.02	11.7	3.98	1.73	2.88	0.32	0.31	0.41
df resid	36	24	36	36	24	36	36	24	36

[a] prothioconazole (Proline, Bayer), dose per application = full rate

[b] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[c] septoria analysis reported on logit transformed data

Table 28. Mixtures vs Alternation field experiments 2018, yields

Trt	Strategy	[a]SDHI dose	[b]DMI dose	ADAS Hereford	NIAB Hampshire	Teagasc Cork		Sites average
1	UT	0	0	8.79	9.44	7.93		8.65
2	Mixture at T1 and T2	1	1	9.78	10.15	9.36		9.73
3	"	2	1	10.04	10.28	9.52		9.92
4	"	1	0.2	9.70	9.91	8.87		9.46
5	"	2	0.4	9.73	10.31	9.35		9.75
6	Alternation SDHI T1	1	1	9.90	9.52	9.13		9.52
7	"	2	1	9.96	10.30	9.37		9.84
8	"	1	0.2	9.65	10.01	8.91		9.48
9	"	2	0.4	9.95	9.79	9.22		9.64
10	Alternation DMI T1	1	1	10.00	10.39	9.68		9.99
11	"	2	1	7.66	10.17	9.78		9.11
12	"	1	0.2	9.27	10.00	8.83		9.31
13	"	2	0.4	9.70	10.16	9.17		9.63
				ANOVA	ANOVA	ANOVA		REML
			F prob UT vs Trt	0.256	0.014	<0.001	F prob UT vs Trt	<0.001
			F prob Trt only	0.548	0.363	<0.001	F prob Site	<0.001
			SED UT vs Trt	0.711	0.241	0.138	F prob Trt	<0.001
			SED Trt only	0.967	0.327	0.187	F prob UT. site	0.068
			df resid	36	24		F prob site.UT.Trt	0.712
							Max SED UT vs Trt	1.18

[a] isopyrazam (Zulu, Adama) (dose = proportion of max. indiv. application full label rate)
[b] prothioconazole (Proline, Bayer), (dose = proportion of max. indiv. application full label rate)

13. Appendix 6

13.1. Objective 2, 2019 sites: mutation frequency, septoria severity and yield

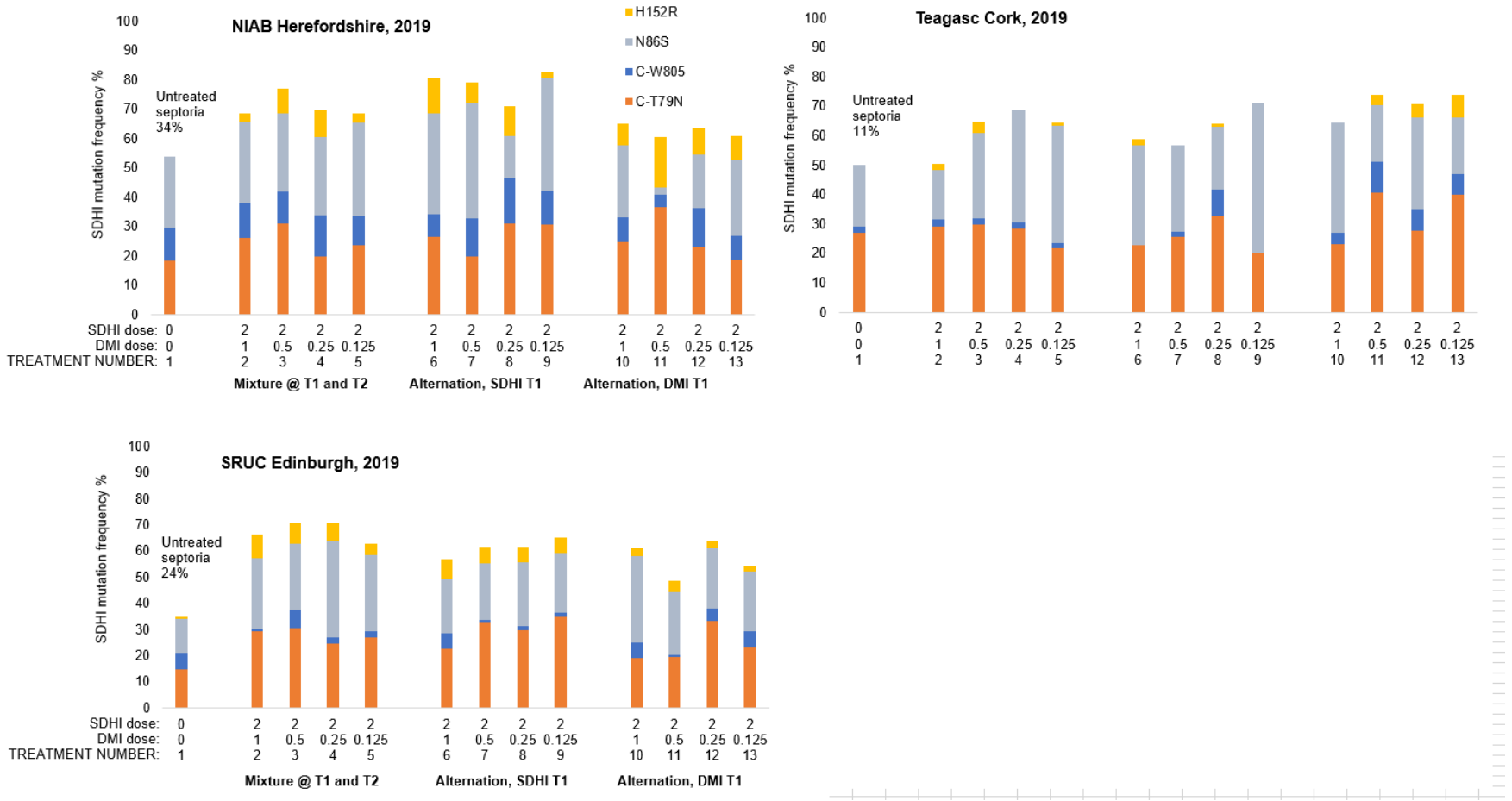

Figure 46. Effect of SDH and DMI fungicides when applied as mixtures or alternated, on % SDH mutations in septoria in 2019 field experiments. Each mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of three sites: ADAS Herefordshire, NIAB Kent and Teagasc Cork. Numbers on x-axis are total doses of DMI (mefentrifluconazole: Myresa, BASF), as proportion of full rate, applied across a two-spray programme, T1 GS32 and T2 GS39. SDHI (isopyrazam: Zulu, Adama) was applied at the **same total dose of 2.0 in all treatments.**

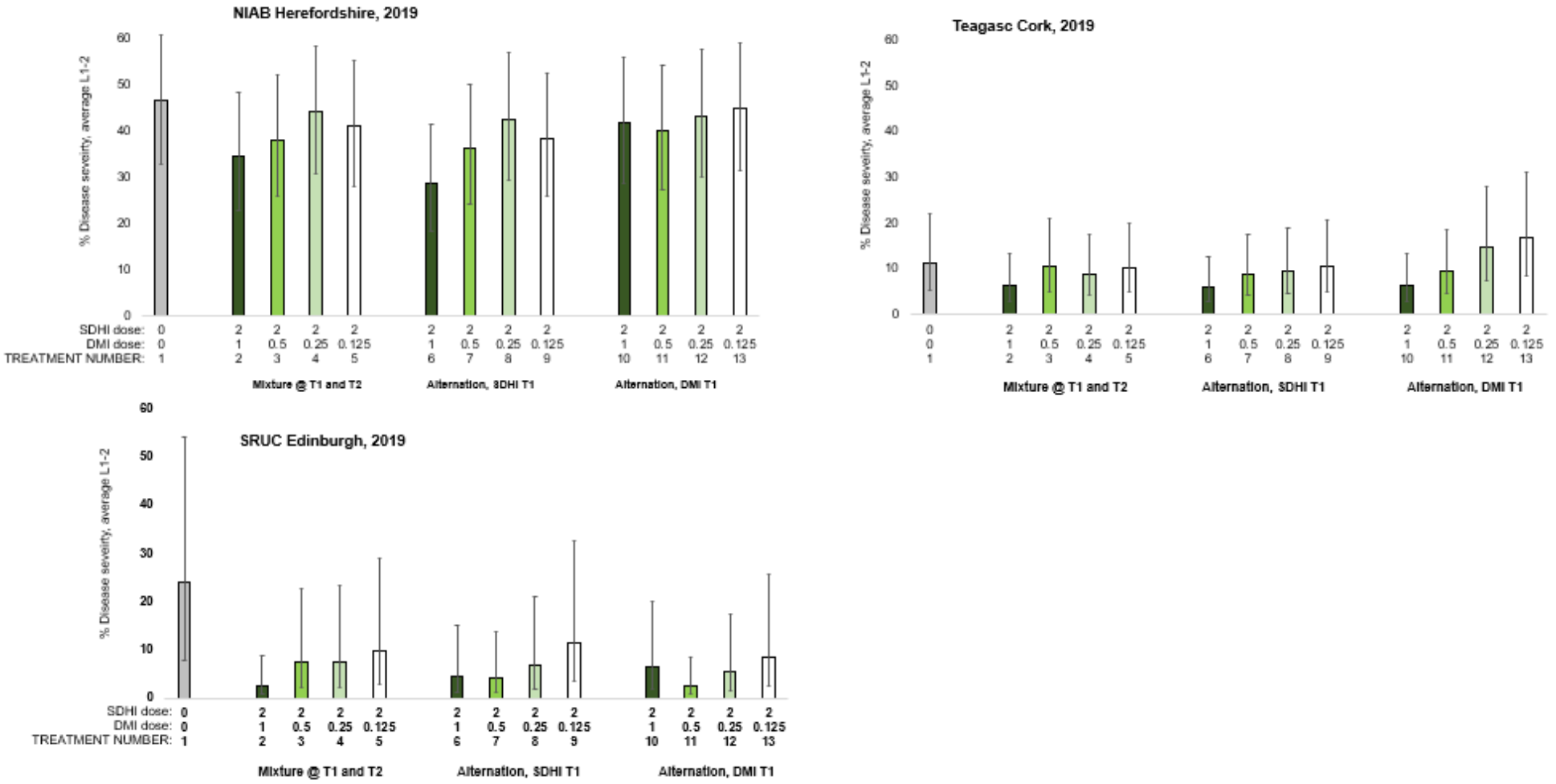

Figure 47. Effect of SDH and DMI fungicides when applied as mixtures or alternated, on % septoria on top two leaves in 2019 field experiments. Each severity value is an average of four replicate plot values (except NIAB, 3 reps.), at each of three sites: ADAS Herefordshire, NIAB Kent and Teagasc Cork. Numbers on x-axis are total doses of DMI (mefentrifluconazole: Myresa, BASF) as proportion of full rate, applied across a two-spray programme, T1 GS32 and T2 GS39. SDHI (isopyrazam: Zulu, Adama) was applied at the same total dose of 2.0 in all treatments.

Table 29. Mixtures vs alternation field experiment 2019, analysis of effect of treatment programme on % total SDH mutations, C-H152R mutation and % septoria severity, at NIAB Herefordshire, SRUC Edinburgh and Teagasc Cork.

[d]ANOVA model	F prob, % total SDH mutations (T79N, W80S, N86S, H152R)			F prob, % C-H152R			[c]F prob, % septoria severity (average top two leaves)		
	NIAB	SRUC	Tea-gasc	NIAB	SRUC	Tea-gasc	NIAB	SRUC	Tea-gasc
UT	0.427	<.001	0.025	0.058	0.117	0.224	0.042	<0.001	0.461
UT.DMI dose	0.727	0.408	0.12	0.193	0.716	0.713	0.014	0.027	0.008
UT.Trt prog	0.324	0.003	0.106	0.23	0.102	0.032	0.035	0.774	0.205
UT.DMI dose. Trt prog.	0.382	0.353	0.763	0.508	0.996	0.051	0.577	0.363	0.698
SED Trt prog	18.2	6.326	8.829	5.359	4.027	2.322	0.189	0.581	0.352
df resid	24	36	36	21	36	36	24	36	36

[a] mefentrifluconazole (Myresa, BASF), dose per application = full rate

[b] isopyrazam (Zulu, Adama) total dose across season (proportion of full label rate)

[c] septoria analysis reported on logit transformed data

Table 30. Mixtures vs Alternation field experiments 2019, yields

Trt	Strategy	[a]SDHI dose	[b]DMI dose	Yield t/ha NIAB Hampshire	SRUC Edinburgh	Teagasc Cork		Sites average
1	UT	0	0	8.49	8.25	7.58		8.07
2	Mixture at T1 and T2	2	1.0	9.84	9.61	9.15		9.50
3	“	2	0.5	9.84	9.53	9.65		9.66
4	“	2	0.25	9.22	9.09	9.17		9.16
5	“	2	0.125	9.34	9.18	8.79		9.08
6	Alternation SDHI T1	2	1.0	10.81	9.67	9.32		9.85
7	“	2	0.5	9.74	9.37	9.41		9.49
8	“	2	0.25	9.27	9.15	9.09		9.16
9	“	2	0.125	9.38	9.04	9.02		9.12
10	Alternation DMI T1	2	1.0	9.63	9.73	9.31		9.55
11	“	2	0.5	9.62	9.38	8.79		9.23
12	“	2	0.25	9.18	9.15	9.04		9.12
13	“	2	0.125	9.13	9.10	8.91		9.04
				ANOVA	ANOVA	ANOVA		REML
			UT	<.001	<.001	<.001	Site	0.244
			UT.DMI dose	<.001	<.001	0.179	DMI dose	0.011
			UT.Trt prog	0.025	0.816	0.424	Site* DMI dose	0.988
			UT.DMI dose.Trt prog	0.086	0.871	0.374	DMI dose* Trt prog	0.996
							Site* DMI dose* Trt prog	0.991
			SED Trt prog	0.282	0.159	0.327	Max SED site.dose.prog	1.528
			df resid	24	36	36		

[a] isopyrazam (Zulu, Adama) (dose = proportion of max. indiv. application full label rate)

[b] mefentrifluconazole (Myresa, BASF), (dose = proportion of max. indiv. application full label rate)

14. Appendix 7

14.1. Objective 2, 2020 sites: mutation frequency, septoria severity and yield

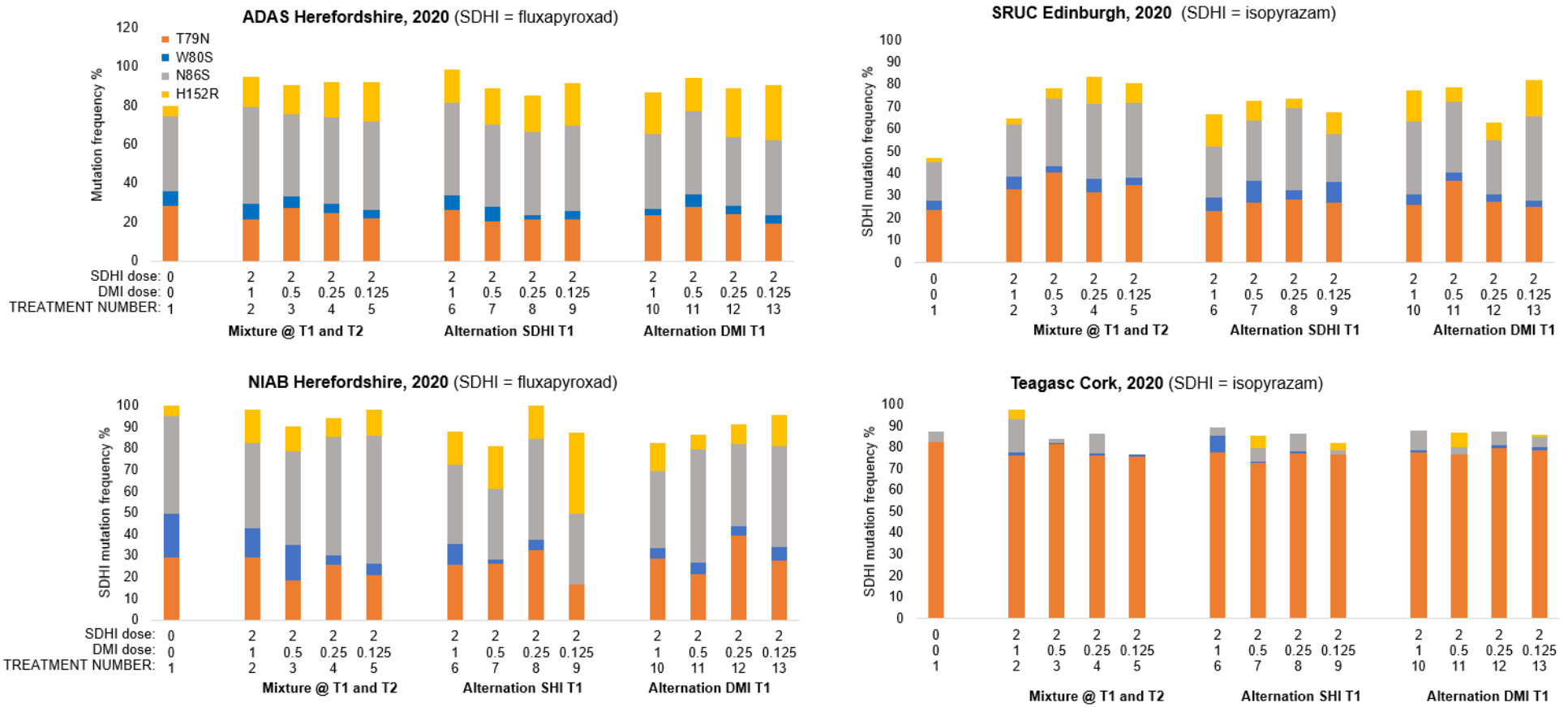

Figure 48. Effect of SDH and DMI fungicides when applied as mixtures or alternated, on % SDH mutations in septoria in 2020 field experiments. Each mutation value is an average across four replicate plots (except NIAB, 3 reps.) and two genotypying tests per plot, at each of four sites. Numbers on x-axis are total doses of DMI, mefentrifluconazole (Myresa, BASF), as proportion of full rate, applied across a two-spray programme T1 GS32 and T2 GS39. SDHI was applied at the same total dose of 2.0 in all treatments, using fluxapyroxad (Imtrex, BASF) at the ADAS Herefordshire and NIAB Herefordshire sites, and isopyrazam (Zulu, Adama) at the SRUC Edinburgh and Teagasc Cork sites.

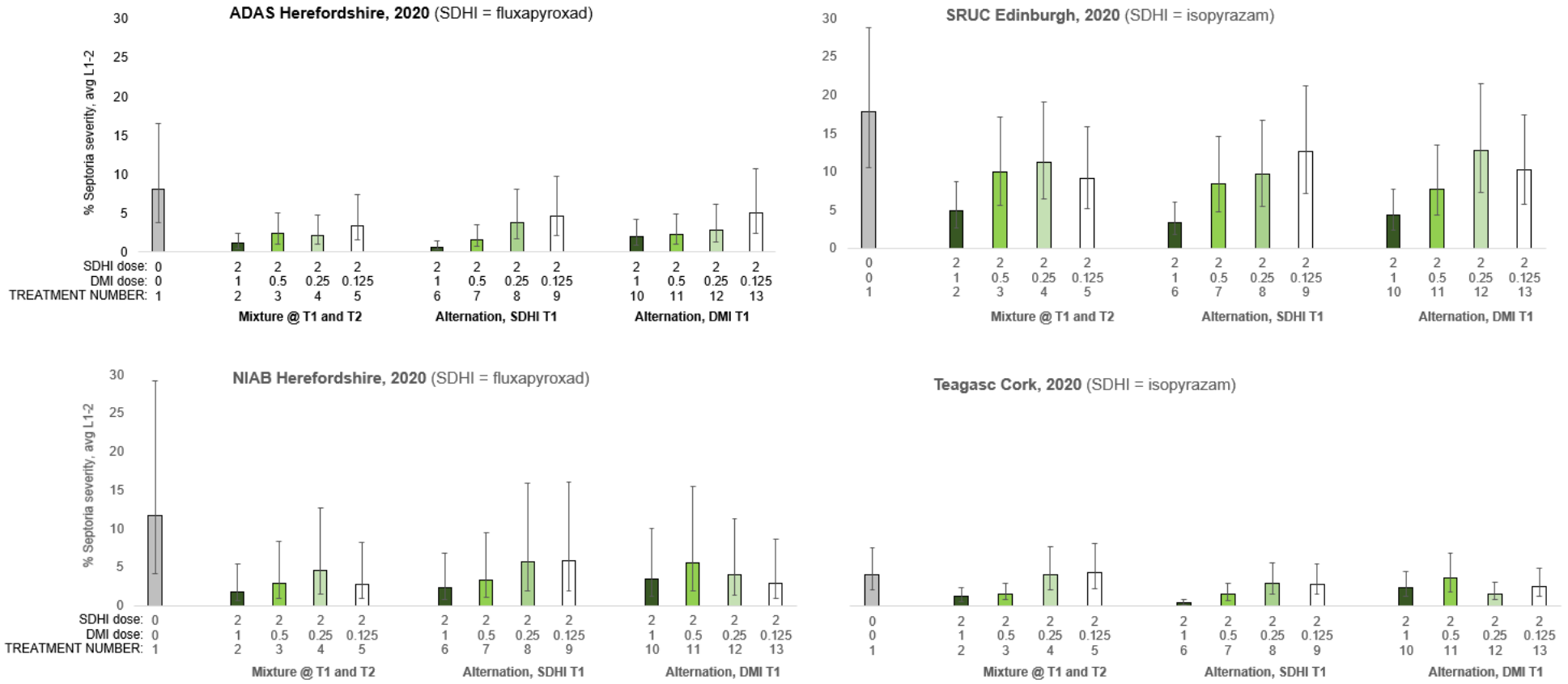

Figure 49. Effect of SDH and DMI fungicides when applied as mixtures or alternated, on % septoria on top two leaves in 2020 field experiments. Each severity value is an average of four replicate plot values (except NIAB, 3 reps.), at each of four sites. Numbers on x-axis are total doses of DMI, mefentrifluconazole (Myresa, BASF), as proportion of full rate, applied across a two-spray programme T1 GS32 and T2 GS39. SDHI was applied at the same total dose of 2.0 in all treatments, using fluxapyroxad (Imtrex, BASF) at the ADAS Herefordshire and NIAB Herefordshire sites, and isopyrazam (Zulu, Adama) at the SRUC Edinburgh and Teagasc Cork sites.

Table 31. Mixtures vs alternation field experiment 2020 analysis of effect of treatment programme on % total SDH mutations, C-H152R mutation and % septoria severity, at ADAS Herefordshire, NIAB Herefordshire, SRUC Edinburgh and Teagasc Cork.

[d]ANOVA model	F prob, % total SDH mutations (T79N, W80S, N86S, H152R)				F prob, % C-H152R				[b] F prob, % septoria severity (average top two leaves)			
	[a]Fluxapyroxad & mefentrifluconazole		Isopyrazam & mefentrifluconazole		Fluxapyroxad & mefentrifluconazole		Isopyrazam & mefentrifluconazole		Fluxapyroxad & mefentrifluconazole		Isopyrazam & mefentrifluconazole	
	ADAS	NIAB	SRUC	Teagasc	ADAS	NIAB	SRUC	Teagasc	ADAS	NIAB	SRUC	Teagasc
UT	0.003	0.14	<.001	0.894	<.001	0.249	0.022	0.409	<0.001	<0.001	<0.001	0.004
UT.DMI dose	0.523	0.477	0.416	0.11	0.062	0.187	0.164	0.106	<0.001	0.036	<0.001	<0.001
UT.Trt prog	0.766	0.218	0.376	0.986	0.024	0.021	0.184	0.631	0.141	0.133	0.783	0.004
UT.DMI dose. Trt prog.	0.592	0.742	0.022	0.625	0.868	0.444	0.054	0.212	0.107	0.21	0.479	<0.001
Max SED	5.52	9..39	6.64	7.06	4.374	8.41	4.209	2.904	0.354	0.372	0.276	0.292
df resid	36	24	36	35	36	24	36	34	36	24	36	36

[a] Fungicide products: fluxapyroxad (Imtrex, BASF), mefentrifluconazole (Myresa, BASF), isopyrazam (Zulu, Adama).

[b] septoria analysis reported on logit transformed data

[c] 'Trt prog' refers to three programmes tested: mixtures, alternation 1 (SDH T1 & DMI T2) and alternation 2 (DMI T1 & SDHI T2)

Table 32. Mixtures vs Alternation field experiments 2020, yields

				Yield t/ha					
				Fluxapyroxad & mefentrifluconazole		Isopyrazam & mefentrifluconazole			
Trt	**Strategy**	**[a]SDHI dose**	**[b]DMI dose**	**ADAS Hereford**	**NIAB Hampshire**	**SRUC Edinburgh**	**Teagasc Cork**		**Site average**
1	UT	0	0	8.50	11.39	8.67	10.53		9.67
2	Mixture at T1 and T2	2	1.0	8.91	11.65	9.76	10.60		10.13
3	"	2	0.5	8.58	11.16	9.36	10.72		9.97
4	"	2	0.25	8.34	11.89	9.32	10.59		10.02
5	"	2	0.125	8.76	11.23	9.00	10.46		9.77
6	Alternation SDHI T1	2	1.0	8.68	11.72	9.82	10.80		10.16
7	"	2	0.5	8.78	11.94	9.56	10.77		10.15
8	"	2	0.25	8.86	10.99	9.44	10.79		9.95
9	"	2	0.125	9.82	11.23	9.04	10.61		10.10
10	Alternation DMI T1	2	1.0	8.95	10.91	9.45	10.54		9.90
11	"	2	0.5	8.47	11.71	9.15	10.43		9.82
12	"	2	0.25	8.64	12.01	8.99	10.66		9.95
13	"	2	0.125	8.54	11.64	9.21	10.43		9.84
				ANOVA	ANOVA	ANOVA	ANOVA		REML
			F prob Trt	0.311	0.955	0.002	0.99	F prob sites	<0.001
			SED Trt	0.454	0.836	0.245	0.529	F prob Trt	0.071
			df resid	34	24	36	36	Max SED Trt	0.739
								df trt	93

[a] fluxapyroxad (Imtrex, BASF) or isopyrazam (Zulu, Adama), dose = proportion of max. indiv. application full label rate
[b] mefentrifluconazole (Myresa, BASF), dose = proportion of max. indiv. application full label rate