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Assessing the resistance risks associated with systemic fungicide seed treatments and the effectiveness of risk modifiers

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1. Abstract

Systemic seed treatments with efficacy against foliar crop diseases have the potential to improve disease control and raise yields. However, there is a potential risk that when the same mode of action is used for both seed and foliar treatments, fungicide-resistant pathogens will develop more quickly. This would lead to loss of control and the shortening of the effective life of a fungicide mode of action. The aim of this project was to produce evidence and assess the resistance risk associated with systemic fungicide seed treatments. The objectives were to: [1] quantify the effect of foliar-acting seed treatments on selection for fungicide resistance, [2] relate the effective life of a fungicide to disease control through its effects on resistance selection, so that the resistance risk associated with future seed treatments can be assessed using efficacy data, and [3] test the extent to which risk 'modifiers' (anti-resistance strategies) are effective for combined seed-treatment and foliar-fungicide programmes.

Mathematical models of fungicide resistance in the pathogen *Zymoseptoria tritici* (septoria leaf blotch), developed previously, were extended to incorporate seed treatment effects. The models were used to explore the resistance effects of a wide range of seed treatment foliar fungicide combinations. Model runs were made for different doses and combinations of seed, T1 and T2 treatments, to determine the influence of treatment programme on effective life (the number of years that a fungicide mode of action continued to provide effective septoria control). The key findings were (i) Maximum effective lives achieved from two foliar sprays or a seed treatment and one foliar spray (of similar efficacy) were equal, and (ii) Adding a seed treatment containing one mode of action to a two foliar spray programme reduced the effective life of a mode of action. Adding an effective foliar-acting mixture partner, of a different mode of action, to the seed treatment reduced the negative impact on effective life, as did constraining the total dose of the mode of action applied to the crop (across seed and foliar applications).

As an experimental test system, field experiments measured selection for MBC fungicide insensitive pathogen strains in the presence or absence of foliar-acting MBC seed treatments, with or without risk modifiers. In one experiment, the proportion of the pathogen population which was insensitive increased significantly, approximately four-fold, in the presence of the seed treatment.

Key practical messages from this work are: (i) Foliar-acting systemic seed treatments cause resistance selection, (ii) The strength of selection will be positively, but non-linearly, related to efficacy, with the precise relation depending on the pathogen, fungicide and application program, (iii) Seed treatments with nil or very low levels of foliar efficacy do not pose a material resistance risk and should not count as one of the permitted treatments, (iv) For effective foliar-acting seed SDHI treatments, the current FRAC guidance (two SDHI treatments in total) is supported, and (v) Modelling work has identified some combinations which could allow use of a foliar-acting seed treatment and up to two foliar treatments: if the total dose of a mode of action applied by seed and foliar treatments is limited, and if effective mixtures are used in seed and foliar treatments.

2. Introduction

Systemic seed treatments with efficacy against foliar diseases have the potential to improve disease control and raise yields. However, there is a potential risk of accelerated development of fungicide resistance in pathogens when the same mode of action is used for both foliar-acting seed treatment and foliar treatments. There is strong evidence that increasing the number of foliar applications of the same mode of action leads to an increased strength of selection for resistant strains of pathogens, which then dominate the pathogen populations more quickly and therefore reduce the effectiveness of control by fungicides (van den Bosch *et al.*, 2014). In principle, adding a foliar-acting seed treatment could have a similar effect. However, there has been little research to assess the effects of seed treatments on resistance selection. One experimental study from the 1980s (Brent *et al.*, 1989) tested selection for fungicide resistant strains of powdery mildew and concluded that the selection resulting from systemic seed treatments was similar to that by foliar treatments.

For succinate dehydrogenase inhibitor (SDHI) fungicides, the Fungicide Resistance Action Committee (FRAC), which represents the crop protection industry, initially advocated that a foliar acting seed treatment need not be counted as one of the restricted number of applications permitted per crop, provided that certain risk modifiers were in place, e.g. the use of mixing partners with different modes of action, or alternating modes of action. However, subsequent guidelines (Fungicide Resistance Action Committee statement, December 2015) were made more stringent, stating that if an SDHI seed treatment with efficacy against foliar pathogens gains approval, then this should count as one of the statutory limit of two SDHI applications per crop. This guidance makes the development of foliar-acting seed treatments commercially unattractive as a manufacturer would risk forgoing sales of foliar treatments if a seed treatment was introduced. Growers would be unlikely to sacrifice flexibility of foliar treatments in order to use a seed treatment.

For SDHI seed treatments with no efficacy against foliar pathogens, these need not be counted toward the statutory limit of two foliar SDHI applications, but the SDHI seed treatment should be co-formulated with a fungicide with an alternative mode of action.

Currently, there is insufficient evidence to assess whether the guidance is more stringent than needed, or whether modifiers might be sufficiently effective to allow more flexible use than the current guidelines allow. The issue of the degree of resistance risk from seed treatments is likely to arise again for new modes of action in future. In the absence of hard evidence, industry and regulatory authorities are likely to continue to take a precautionary approach, resulting in constraints on fungicide use which could be unnecessary.

The aim of this project was to produce evidence and assess the resistance risk associated with systemic fungicide seed treatments. The specific objectives were:

1. Quantify the effect of foliar-acting seed treatments on selection for fungicide resistance.
2. Relate effective life to disease control through its effects on resistance selection, so that the resistance risk associated with future seed treatments can be assessed using efficacy data.
3. Test the extent to which risk 'modifiers' (anti-resistance strategies) are effective for combined seed-treatment and foliar-fungicide programmes.

The objectives were addressed by a combination of field experiments and modelling, focussing on *Zymoseptoria tritici* (causal organism for septoria leaf blotch). Field experiments were devised to measure selection for fungicide insensitive pathogen strains in the presence or absence of foliar-acting seed treatments, and with or without risk modifiers.

Mathematical models of fungicide resistance, developed and tested previously in CRD/Defra-funded work, were extended to incorporate seed treatment effects. The models were used to: (i) explore the resistance effects of a wider range of seed treatment foliar fungicide combinations than can be tested experimentally, and (ii) quantify the rate of selection for fungicide insensitivity, and the resulting effect on effective lives for modes of action.

3. Materials and methods

3.1. Modelling

3.1.1. Model description

The model simulates a population of the pathogen *Zymoseptoria tritici*, comprised of a fungicide-sensitive and a fungicide-resistant strain on winter wheat under selection pressure from a fungicide treatment program comprising foliar-acting seed treatment and/or foliar applied treatments. The size of the pathogen population of each strain is quantified as the area of crop canopy occupied by latent (pre-symptomatic) or infectious (sporulating) lesions.

The canopy

The wheat crop canopy growth model developed is an extension of the model described by Van den Berg *et al.,* (2013) and simulates the growth and senescence of 11 leaf layers (between 9 and 14 layers can be produced by wheat depending on sowing date and environment). The flag leaf is leaf 1 and the bottom leaf is leaf 11. The leaf area of each leaf layer is measured in units of area index, which is defined as the ratio of the total planar leaf area to ground area. The life cycle of each leaf layer contains three phases: a growth phase, where leaf area increases according to a monomolecular function to a maximum; a lag phase, where leaf area remains constant; and a

senescent phase, where healthy leaf area decreases from necrosis (Fig. 1). Dead leaf layers are removed from the simulation.

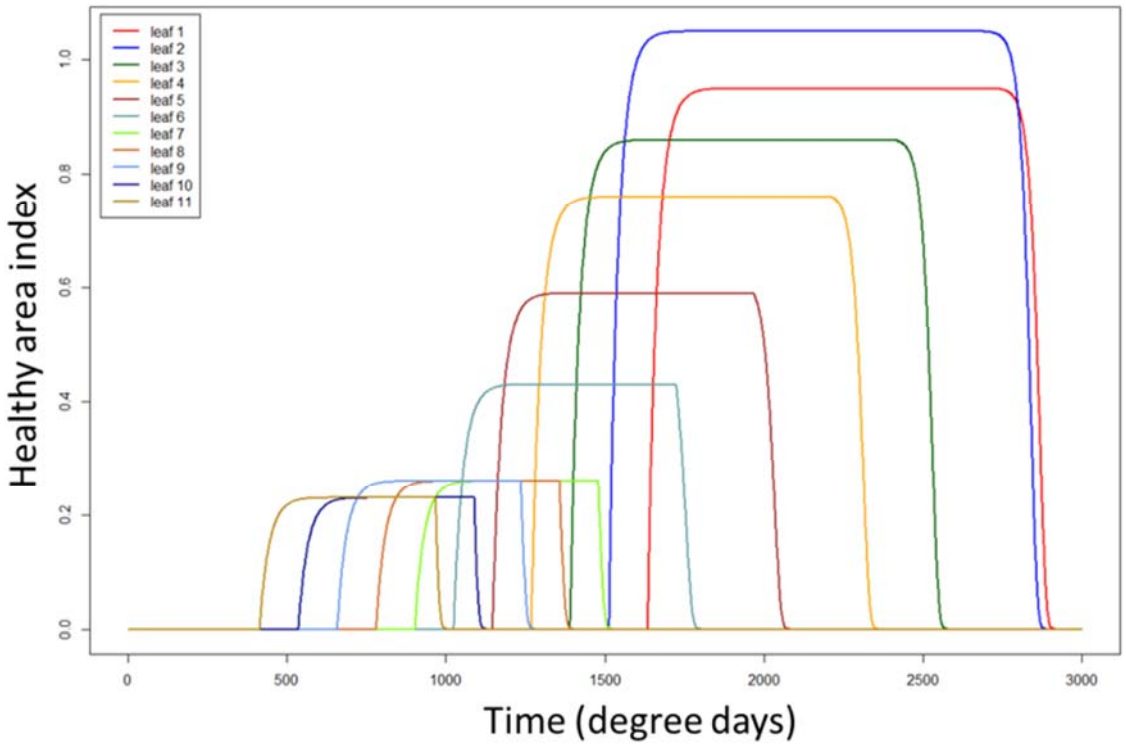

Figure 1. Graphical representation of the 11 leaves represented in the crop canopy growth model in the absence of disease. Leaf lives overlap and are comprised of a monomolecular growth phase, a lag phase and a logistic senescence phase.

The pathogen

The pathogen is modelled using compartments of latently infected tissue, comprised of non-sporulating mycelium, and infectious tissue, comprised of sporulating fruiting bodies (Figure 2). At the start of the crop growing season the epidemic is started by primary inoculum, the ascospores, produced by ascii on dead stubble left in the field after harvest of the previous season. The primary inoculum gives rise to lesions producing pycnidiospores. During the rest of the crop growing season the pycnidiospores produce a series of asexual generations that forms the secondary inoculum of the epidemic. This secondary inoculum is dispersed by rain splash. Transmission of inoculum between leaf layers is reduced according to their vertical separation through stem extension.

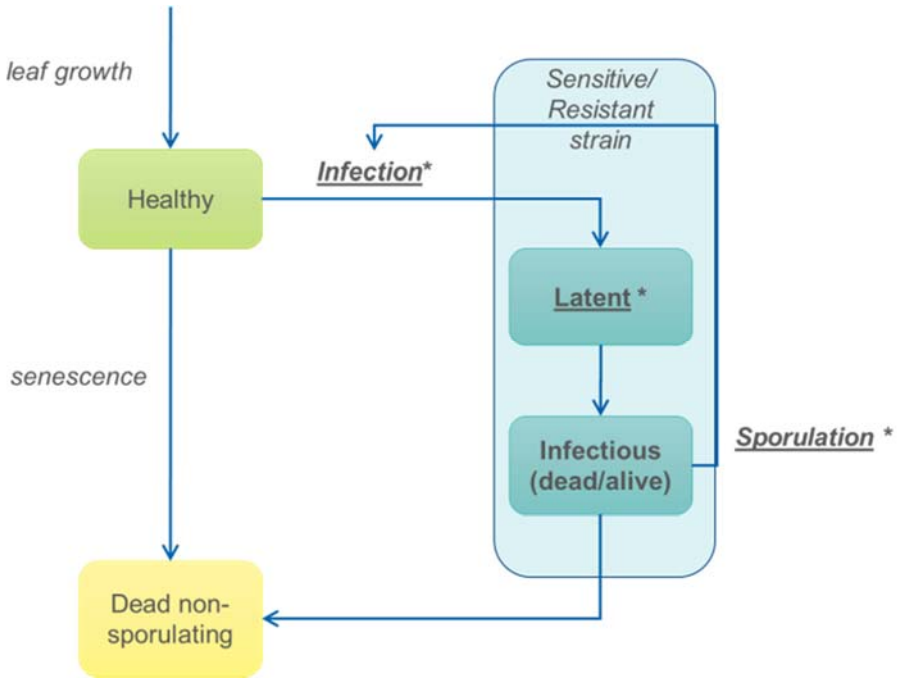

Figure 2. Flow diagram of the disease sub-model for a leaf layer. *Z. tritici* is a hemi-biotroph; it kills the leaf tissue when it starts sporulation. This implies that scensecence does not remove sporulating leaf tissue from the process, it remains sporulating until the leaf is fully scenesced and drops to the ground. The pathogen model includes two strains; a fungicide sensitive strain, *S*, and a fungicide resistant strain, *R*. The ***** mark the life-cycle components of the pathogen that can be affected by the fungicide.

The fungicide

Latent or infectious tissue belongs to a fungicide-sensitive or a fungicide-resistant strain of the pathogen. The latter strain is assumed to be unaffected by the fungicide within the range of doses permitted and may infect leaf area at the same rate in the presence or absence of the fungicide. We model a fungicide that is causing selection for the fungicide-resistant strain. The fungicide is applied as a T1 (GS 31-32) and/or a T2 (GS 39) foliar spray or as a foliar-acting seed treatment. The fungicide is systemic and exhibits both protectant (transmission rate limiting) and eradicant (latent period prolonging) activity toward the fungicide-sensitive strain. The fungicide from the systemic seed treatment is initially present on, or around, the seed, and then gradually translocates to each leaf.

Little quantitative information is published about how fungicides from seed coatings are taken up. We, therefore, developed two variants of the model:

1. The fungicide from the seed coating is taken up at a constant rate by the plant.
2. The fungicide is taken up at a rate relating to the transpiration rate of the plant.

We compare outputs from the model for each of these mechanisms.

The fungicide concentration in each leaf from the foliar and seed treatments decays exponentially over time due to breakdown from exposure to light and plant catabolism.

Dose response curves: The model is parameterised for a generic SDHI fungicide and for fluquinconazole. In Figure 3 we show that data as well as the model fit to the dose response data. The 'generic SDHI' fungicide data are the mean values for the dose response curves of isopyrazam, fluxapyroxad, penthiopyrad as measured in the AHDB Fungicide Performance trials in 2012 and 2014 (Bounds *et al*., 2016; Anonymous, 2014).

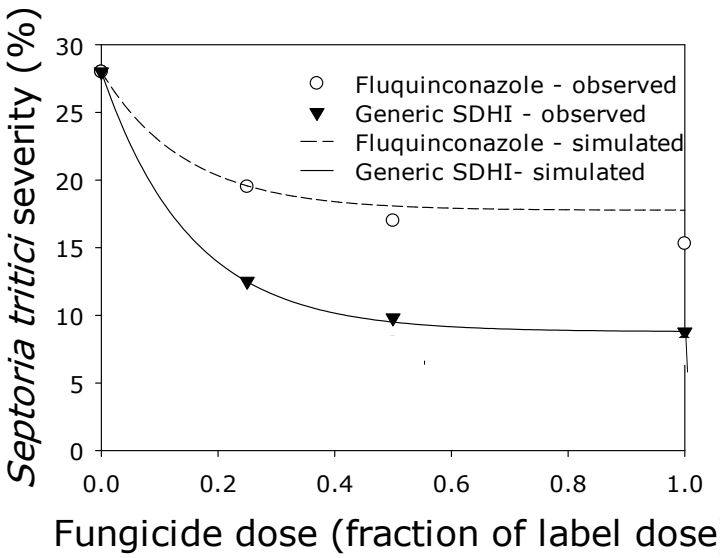

Figure 3. Dose response curves. Data and fitted model output for the high-risk fungicide for which we used a generic SDHI, and the low-risk fungicide for which we used fluquinconazole.

The fungicide effective life

We use the effective life to compare application programmes. The effective life is the number of consecutive years the fungicide treatment programme is able to maintain effective disease control. Good resistance management strategies provide effective control over many years. We define loss of effective disease control as a greater than 5% reduction in green canopy healthy area duration (HAD), as measured on leaves 1 – 3. This definition is used by national (FRAG) and European crop protection committees (EPPO) and all the major crop protection companies, and has provided the basis for many peer reviewed papers (e.g. Hobbelen et *al., 2011,* Hobbelen et *al., 2014*, van den Berg *et a.,* 2013). Because the fraction resistant is increasing non-linearly, changing the threshold from, 5% to 10% or to 20%, makes relatively little difference to the effective life. We use this quantification of effective disease control because HAD is closely correlated with yield.

The mathematics and programming details

In this report we do not discuss the details of the mathematical equations nor the coding of the model in a computer program. These details will be published in a scientific journal (available from the authors).

3.1.2. Parameter values

Parameter values were derived from published data and data available to the research group. Here we only discuss the parameterisation of the seed treatment. Further details are in the above mentioned publication manuscript. Very few data exist measuring the efficacy of seed treatments, and public domain data are non-existent on SDHI seed treatments. We therefore parameterised our seed treatment model in two contrasting ways. Comparing the outcomes using the two parameterisations will show to what extend outcomes are dependent on these parameter values. The initial amount of fungicide in the seed coating in units of mg seed^{-1} was estimated by searching for values that resulted in a specific level of disease control. In our simulations, we used two different parameterisations:

Parameterisation 1:

The initial seed treatment dose was set to provide the same disease control effect as a T1 spray. This was achieved by adjusting the initial amount of fungicide in the seed treatment until the calculated HAD at the end of the first growing season equalled the HAD value obtained from a T1 spray. This parameterisation provided the upper bound for the disease control provided by a systemic seed treatment, as it is unlikely that a systemic seed treatment could provide a stronger reduction in disease severity than a T1 spray. The estimated doses varied according to the fungicide breakdown rate and the seed treatment uptake model in use.

Parameterisation 2:

We referred to the literature to obtain data on disease control between seed treated and untreated plots. A dataset was obtained from Parker and Lovell (2001), which contained spore-washing data in spores ml^{-1} on leaves of winter wheat that were infected by septoria leaf spot, between untreated plots and plots as treated by fluquinconazole seed treatment (product 'Jockey'). The area under disease progress curves (AUDPC) for the spore washing data were calculated. The percentage AUDPC remaining after treatment was observed and recorded for each data point from both sets. Averaging over all data points for both sets yielded average reductions in AUDPC values of 60% after treatment. Values of the initial amount of fungicide in the seed treatment were then adjusted in the model to obtain this 60% reduction in AUDPC.

3.2. Field experiments, seed treatments

3.2.1. Azole and MBC seed treatments Year 1

Experimental approach

Field experiments with azole and methyl benzimidazole carbamate (MBC) fungicides were set up to measure selection for fungicide-insensitive strains of *Zymoseptoria tritici*, in the presence or

absence of seed treatments, and with or without risk modifiers. Azole and MBC mode of action fungicides were chosen for the studies because systemic seed treatments are available and the two modes of action cause 'slow-shifting' and large 'single-step' resistance, respectively. The risk modifiers tested here were: reduced dose of foliar sprays to account for inclusion of a seed treatment in the fungicide programme, alternation of mode of action within the fungicide programme, reduced seed treatment dose and mixture of mode of action in the seed treatments. Small (1 m^2) field plots of winter wheat variety Scout were inoculated with selected strains of *Z. tritici*. The plots were covered with vapour permeable fabric 'tents' which allowed air flow but excluded the influx of external ascospores. The tents remained in place during the main period for ascospore dispersal, i.e. from crop emergence to stem extension. Scout was selected because it is relatively susceptible to septoria leaf blotch (RL rating 5) but resistant to yellow rust (RL 9), brown rust (RL 8), mildew (RL 7) and *Septoria nodorum* (RL 6). Disease development was assessed at intervals, and samples of infected leaves were collected from each treatment. Samples of lesions were sent to Rothamsted Research for pathogen genotyping and determination of the proportion of samples carrying the insensitive mutation.

Spore-proof tents

'Tents' were made at ADAS Boxworth and ADAS Rosemaund, constructed from wood frames with a waterproof polyurethane top cover stapled closely in place, and breathable waterproof fabric (KBTRPMZ-F15-B16, from Fabric UK) covering each side, also stapled closely in place such that there were no gaps or openings (Figure 4). The frames were 1m^2 and 80 cm height above ground level, with at least 20 cm additional length of wooden leg at each corner to sink into the ground and secure the tent. Typical pore size in the membrane of a breathable fabric is 1.4 billion pores per cm^2 with average pore size < 10µm, typically 0.1 to 10 µm. The pores in the polymers used in breathable membranes are linked together in complex pathways (Fuller and Taylor, 2012) and as such they act as a filter and will trap or exclude *Z. tritici* spores, which on average are 2.5 x 11 µm in size. Tents were placed over plots prior to crop emergence, and apart from inoculation and irrigation procedures, stayed closed and on plots until the start of stem extension at the latest, after which the tents were removed. This was after the end of the external spore release from natural infections, which typically finish by late January or early February (Fraaije, 2005). Openings for inoculation, sampling or irrigation purposes were created by cutting a slit in one corner of the top cover, which was then sealed immediately after use with waterproof sticky tape.

Figure 4. Wood-framed tents with breathable fabric sides and waterproof polyurethane top, ADAS Boxworth, February 2014. Small opening in top cover is used for inoculation with *Z. tritici* spores and for irrigation purposes. Frames in 2015 had additional cross-pieces under top cover to reduce rain water collecting on top.

Z. tritici inoculum and field inoculation procedures

All strains of *Z. tritici* were supplied by Bart Fraaije at Rothamsted Research. In year 1, field experiments used azole sensitive and insensitive strains which were CYP51 variants, with mutations in the CYP51 enzyme which is essential for ergosterol synthesis. Normally, azoles bind to CYP51 and block ergosterol synthesis. In this experiment, three strains were used which had an insert causing over expression of CYP51 (azole-insensitive) and three strains had no insert (azole-sensitive). All isolates had been tested for sensitivity against various azole fungicides. The three azole-sensitive strains were R3-29, Opus 6 and R3-54. The three azole-insensitive strains were TAG74-3, Opus 7 and R13-36.

In year 1, additional field experiments used MBC sensitive and insensitive strains: two MBC sensitive and triadimenol resistant strains (4417 and NZ75) and two MBC insensitive and triadimenol resistant strains (4414 and Rd9). This experiment was not repeated in year 2.

Cultures on nutrient agar were sent to ADAS High Mowthorpe where they were used to initiate multiple new cultures on nutrient agar. From these new cultures, after 3 days growth at 16.5–17^{o}C,

spores were scraped off the agar under sterile conditions to produce pots of spores which were then frozen ready for thawing, counting and dilution at each field site. Pots were sent frozen on dry ice to each site, then kept frozen until thawing immediately prior to use on the day of field inoculation (thawed for at least 1 hour at room temperature, out of direct sunlight). For the azole experiment, on the inoculation day, one conidial suspension was prepared which contained roughly equal proportions by volume of spore concentrate of three azole-sensitive strains, and another conidial suspension was prepared which contained roughly equal proportions of three azole insensitive strains. The spores/ml were counted in the mixed suspensions, using a haemocytometer, and diluted to 10^6 spores/ml in distilled water (not sterile) with 1 drop of Tween 20 mixed in. These two conidial suspensions, sensitive and insensitive, were used to prepare the two inoculum suspensions required for the field plots, [1] a 95% azole sensitive and 5% azole insensitive mix and [2] 100% azole sensitive. For the MBC experiments, sensitive and insensitive conidial suspensions were prepared in the same way.

The day for the field inoculation was selected to be when wind speed was low and average temperatures forecast to stay above 4^{o}C for at least the following week. Suitable temperatures are those between 4^{o}C and 19^{o}C. A slit was cut in the top of each tent through which the spray nozzle was inserted, and the opening was sealed immediately after inoculation with waterproof tape. Inoculum was sprayed at the rate of 50 ml/m^2 onto all appropriate plots for each treatment (dates in Table 1, treatment lists in Tables 2 and 3). The sprayer was agitated every 10 seconds or so to keep the spores evenly suspended because they tended to settle quickly.

The first inoculation was planned for when the crop reached the 2–4 leaf stage and when temperatures were suitable, and a second inoculation was made a week or so later, depending on temperatures (suitable temperatures for *Z. tritici* infection are from 4 to 19^{o}C). Disease development was monitored by opening slits in the tent tops and checking the plants. Plots were irrigated by hand-held sprayers at intervals, using the openings in the top covers, to simulate rain splash which promotes spore dispersal up plants.

Assessments, leaf sampling and genotyping

Assessments were made just prior to, or at each sample time. Disease and % green leaf area were assessed by individual leaf layer. Disease (including any other disease as well as septoria leaf blotch) was assessed as the % area of sporulating lesions per leaf, on ten randomly selected shoots per plot, in all plots, by individual leaf layer. The assessment timings were: at first sample i.e. when first symptoms appear; just before the GS 32 spray; just before the GS 39 spray and four weeks after the GS 39 spray.

Sampling started one latent period after inoculation, i.e. as soon as first symptoms appear, in order to quantify the initial percentage of strains that were sensitive and also the percentage insensitive. Leaves with lesions were also sampled just before the GS 32 spray, just before the GS 39 spray and 4 weeks after the GS 39 spray or when new symptoms appeared following the application (sampling dates in Table 1). All plots were sampled at each sample time, with 10 leaves with lesions collected per plot, selecting the same leaf layer across all plots. The leaves had surface water removed by pressing them gently between paper hand towels, and were then wrapped in clean paper towels (all 10 leaves together), placed in a paper envelope (no plastic bags), and posted first class to Rothamsted, ensuring that leaves arrived early or mid-week and were thus processed without delay for genotyping tests.

At Rothamsted, leaves were washed, surface sterilised and incubated overnight. Spores were harvested suspended in buffer and plated onto nutrient agar for colony screening and testing. DNA was isolated from 25 lesions per plot, and tested for the presence of mutations for fungicide insensitivity. In the MBC experiment, isolates were tested for presence of mutation E198A. In the azole experiment, tests were planned for the D134G and V136A mutations.

Sites and treatments

In 2013, Scout was drilled at two sites, ADAS Rosemaund and ADAS Boxworth, in strips one Oyjord drill width by 4m, after pre-emergence herbicide application to the field. Plots, each 1 m^2, were created by burning off surrounding plants, such that there was at least a 2 m gap around each plot. There were two experiments at each site, (1) azole seed treated and (2) MBC seed treated. Table 1 shows the dates for all operations from drilling to final sampling for the year 1 experiments. Seed treatments (done at ADAS Boxworth) and foliar fungicide treatments were applied to *Z. tritici* inoculated and non-inoculated plots as appropriate, for the azole experiment (Table 2) and the MBC experiment (Table 3).

Table 1. Azole and MBC seed treatments field experiment diaries, year 1.

	Timing	MBC		AZOLE	
		BOXWORTH	**ROSEMAUND**	**BOXWORTH**	**ROSEMAUND**
Drill plots		26-Sep-13	10-Oct-13	26-Sep-13	10-Oct-13
Spore tents onto plots		08-Oct-13	18-Oct-13	07-Oct-13	18-Oct-13
First inoculation	4 leaf	15-Nov-13	20-Nov-13	15-Nov-13	20-Nov-13
Second inoculation	5–6 leaf	11-Dec-13	10-Dec-13	11-Dec-13	10-Dec-13
First sample*	first symptoms	15-17 Jan-14	22-Jan-14	15-17 Jan-14	22-Jan-14
First disease assess	First symptoms	28 Jan-14	22-Jan-14	15-17 Jan-14	22-Jan-14
Irrigation under tents	if little disease	02-Feb-14	-	02-Feb-14	19-Feb-14
TENTS REMOVED		17-Feb-14	03-Mar-14	17-Feb-14	03-Mar-14
Second sample + disease assess	before GS 30 spray	18-20 Feb-14	04-Mar-14	19-21 Feb -14	04-05 Mar-14
GS 30 spray		04-Mar-14	10-Mar-14	TRIAL STOPPED	10-Mar-14
PGR spray	early GS 37	01-Apr-14	11-Mar-14		11-Mar-14
GS39 sample	before GS 39 spray	16-Apr-14	14-May-14		15-May-14
PGR spray			12-May-14		12-May-14
GS 39 spray	GS 37–39	17-Apr-14	16-May-14		16-May-14
Third sample + disease assess	GS 39	25-Apr-14	14-May-14		15-May-14
Galileo overspray for YR	GS 39	30-Apr-14			
Fourth sample	GS 55-65	20-May-14	19-Jun-14		21-May-14
Fourth disease assess	GS 59	28-May-14	19-Jun-14		21-May-14

*winter wheat variety Scout

Table 2. Azole field experiments Year 1: *Z. tritici* inoculation treatments with seed and/or foliar fungicide treatments.

Trt.	**Tent in place, emergence to GS 30**	**95% sensitive & 5% insensitive**	**Sensitive only**	**Seed treatment (proportion of full dose)**	***Foliar treatment (proportion of full dose), total**
1	Yes	✓		Azole 1.0	Azole 1.0
2	Yes	✓		Azole 1.0	Azole 0.5
3	Yes	✓		Azole 1.0	CTL 0.5 at GS 32, Azole 0.5 at GS 39
4	Yes	✓		Azole 0.5	Azole 1.0
5	Yes	✓		Azole 0.5 + SDHI 0.5	Azole 1.0
6	Yes	✓		Azole 1.0	-
7	Yes	✓		-	Azole 0.25
8	Yes	✓		-	Azole 0.5
9	Yes	✓		-	Azole 1.0
10	Yes		✓	-	Azole 1.0
11	Yes	✓		-	-
12	Yes		✓	-	-
13	Yes	Un-inoculated		-	-
14	No	Un-inoculated		-	-

- Wheat variety = Scout
- Seed treatments: azole = fluquinconazole (Jockey Solo); SDHI = development product
- *Foliar treatments: azole = epoxiconazole (Ignite); CTL = chlorothalonil (Bravo 500). Dose was split between a GS 32 and a GS 39 application (except Trt 3).
- *Z. tritici*: 3 isolates azole sensitive, and 3 isolates azole insensitive

Table 3. MBC field experiments Year 1: *Z. tritici* inoculation treatments with seed and/or foliar fungicide treatments.

Trt.	Tent in place, emergence to GS 30	95% sensitive & 5% insensitive	Sensitive only	Seed treatment (proportion of full dose)	*Foliar treatment (proportion of full dose), total
1	Yes	✓		MBC 1.0	MBC 1.0
2	Yes	✓		MBC 1.0	MBC 0.5
3	Yes	✓		MBC 1.0	CTL 0.5 + MBC 0.5
4	Yes	✓		MBC 0.5	MBC 1.0
5	Yes	✓		MBC 0.5 + azole 0.5	MBC 1.0
6	Yes		✓	MBC 0.25	-
7	Yes		✓	MBC 0.5	-
8	Yes	✓		MBC 1.0	-
9	Yes		✓	MBC 1.0	-
10	Yes		✓	-	MBC 0.25
11	Yes		✓	-	MBC 0.5
12	Yes		✓	-	MBC 1.0
13	Yes	✓		-	-
14	Yes		✓	-	-
15	Yes	Un-inoculated		-	-
16	No	Un-inoculated		-	-

- Wheat variety = Scout
- Seed trts: MBC = fuberidazole + triadimenol (Tripod), azole = fluquinconazole (Jockey Solo)
- *Foliar trts: MBC = carbendazim (Delsene 50 Flo), CTL = chlorothalonil (Bravo 500). Dose was split between a GS 32 and a GS 39 application (except Trt 3).
- *Z. tritici*: 2 isolates MBC sensitive and triadimenol resistant, and 2 isolates MBC insensitive and triadimenol resistant

3.2.2. Azole and SDHI seed treatments Year 2

Experimental approach

Field experiments were set up to repeat the azole-septoria experiments in year 1 (described above). In addition, field experiments were set up to measure selection for SDHI fungicide insensitive strains of the pathogen *Pyrenophora teres* (cause of net blotch in barley) in the presence or absence of seed treatments, and with or without risk modifiers. Results of tests on air samples from the preceding season indicated the presence of SDHI insensitive *P. teres* in the UK. The risk modifiers tested were the same as in year 1, i.e. reduced foliar dose, alternation of mode

of action, reduced seed treatment dose and mixture of mode of action in the seed treatments. Standard size plots of the winter barley variety Flagon were drilled and net blotch disease development relied on natural infection. Flagon was selected because it is susceptible to net blotch (RL rating 4) but relatively resistant to other diseases, i.e. yellow rust (RL 8), brown rust (RL 7), mildew (RL 6) and rhynchosporium (RL 6). Disease development was assessed at intervals, with the aim of collecting samples of infected leaves from each treatment. Samples of lesions were sent to Rothamsted Research for pathogen genotyping and determination of the proportion of samples carrying the insensitive mutation.

Sites and treatments

In 2014, winter what variety Scout was drilled at one site, ADAS Boxworth, and the same azole seed treatment experiment as in 2013 was repeated. An azole seed treatment site in mid-Wales was planned but conditions were too wet to allow drilling in autumn, in time to allow inoculation with *Z. tritici* before average temperatures fell below 4^{o}C. Table 4 shows the dates for all operations from drilling to final sampling, for the 2015–15 azole-septoria experiment at ADAS Boxworth.

Spore-proof tents used in year 2 for the azole seed treatment experiment were the same construction as in year 1, except for a reinforcing cross-piece of wood under the top waterproof cover to reduce sagging with rainwater accumulation.

Also in 2014, Flagon was drilled for net blotch-SDHI experiments at high-risk sites at ADAS Rosemaund (one field) and at ADAS High Mowthorpe (two fields). The plots were standard size, 12 x 3 m and disease development relied on natural infection from the pathogen *Pyrenophora teres*. Table 6 shows the dates for all operations from drilling to final assessments.

Seed treatments (done at ADAS Boxworth) and foliar fungicide treatments were applied to *Z. tritici* inoculated and non-inoculated plots as appropriate, for the azole experiment (Table 7) and seed and foliar treatments were applied to appropriate plots for the SDHI experiment (Table 8).

Z. tritici inoculum and field inoculation procedures

Year 2 experiments used the same procedures for inoculum preparation and inoculation as in year 1, except that different isolates with known mutations (Table 5) were supplied by Rothamsted. The aim was to increase the difference in resistance factor between the sensitive and insensitive isolates, i.e. to increase the contrast in sensitivity between the two isolate types. Prior to making and adjusting the final field-ready inoculum suspension, all six sensitive or relatively sensitive isolates were mixed in equal volumes of spores, produced from bulking up initial cultures. All three insensitive isolates were similarly mixed to create the ‘insensitive’ inoculum for field use.

Table 4. Azole seed treatments Year 2: field experiment diaries.

Wheat Var. Scout	**Boxworth BX**	**GS**	**Aberystwyth**	**GS**
Drill	30-Sep-14		Too wet, trial drilling abandoned	NA
Spore tents on plots	21-Oct-14	11		
1st inoculation	13-Nov-14	13		
2nd inoculation	28-Nov-14	15		
Inspect	05-Jan-15	20		
Mildew & YR spray	04-Feb-15	23-30		
Assess	03-Feb-15	23-30		
Irrigate plots	11-Feb-15	23-30		
Irrigate plots	19-Feb-15	23-30		
Irrigate plots	24-Feb-15	23-30		
Irrigate plots	03-Mar-15	30-31		
Inspect	03-Mar-15	30-31		
Inspect	16-Mar-15	31		
No septoria	07-Apr-15	32		
Stop trial and tents removed	07-Apr-15	32		

Table 5. *Z. tritici* isolates year 2: for inoculating field plots of wheat var. Scout, autumn 2014.

Sensitivity	**Relative resistance factor**	**Isolate label**	**Percentage of inoculum suspension**	***Mutation***
Sensitive	0	CTRL01-01	15.8	*CYP51 Y137F+S524T*
	0	R6-31	15.8	"
	0	R3-45	15.8	"
	16.6	T2	15.8	*CYP51 D107V+I381V+N513K+S524T*
	16.6	R11-16	15.8	"
	16.6	R14-31	15.8	"
Insensitive	100	R12-9	1.67	*CYP51 L50S+D134G+V136A+I381V+Y461H*
	100	R14-4	1.67	"
	100	R14-18	1.67	"

Assessments, leaf sampling and genotyping

These were the same as in year 1 for the azole seed treatment experiment (dates in Table 4). The SHDI seed treatment experiments did not develop disease and the experiments were stopped (Table 6).

Table 6. SDHI seed treatments year 2: field experiment diaries.

Barley var. Flagon	**High Mowthorpe WB01**	**GS**	**High Mowthorpe WB03**	**GS**	**Rosemaund**	**GS**
Drill	25-Sep-14		01-Oct-14		02-Oct-14	
NB infected straw					21-Jan-15	
Assess - NB<1.0%	29-Jan-15		29-Jan-15	31		
T1 spray	10-May-15	32	10-May-15	32	08-Apr-15	31
Assess - no NB	01-May-15	31	11-May-15	31	08-Apr-15	31
T2 spray	None (Late T1)		None (Late T1)		07-May-15	39-45
Assess - no NB	18-Jun-15	32	18-Jun-15	32	12-May-15	39-45
Trial stopped	01-May-15		02-Jul-15		19-May-15	

Table 7. Azole field experiments Year 2: *Z. tritici* inoculation treatments with seed and/or foliar fungicide treatments.

Trt.	**Tent**	**inoculation: 95% sens. & 5% insens.**	**inoculum: Sensitive only**	**inoculum: Insensitive only**	**Seed treatment (dose)**	**Foliar treatment (proportion of full dose) GS31**	**Foliar treatment (proportion of full dose) GS39**
1	*Y	✓			Azole (1.0)	Azole (0.5)	Azole (0.5)
2	Y	✓			Azole (1.0)	Azole (0.25)	Azole (0.25)
3	Y	✓			Azole (1.0)	CTL (0.5)	0
						0	Azole (0.5)
4	Y	✓			Azole (0.5)	Azole (0.5)	Azole (0.5)
5	Y	✓			Azole (0.5) +SDHI(0.5)	Azole (0.5)	Azole (0.5)
6	Y	✓			Azole (1.0)	-	-
7	Y	✓			-	Azole (0.5)	Azole (0.5)
8				✓	-		-
9	Y	✓			-		-
10	Y		✓		-		-
11	Y	None			-		-
12	N	None			-		-

- Wheat variety = Scout
- Seed treatments: azole = fluquinconazole (Jockey Solo); SDHI = development product
- Foliar treatments: azole = epoxiconazole (Ignite); CTL = chlorothalonil (Bravo 500)
- *Z. tritici*: 3 isolates azole sensitive, and 3 isolates azole insensitive

Table 8. SDHI field experiments Year 2: seed and/or foliar fungicide treatments.

Trt.	Seed treatment (proportion of full dose)	Foliar treatment (proportion of full dose) GS 31	Foliar treatment (proportion of full dose) GS 39
1	SDHI (1.0)	SDHI (0.5)	SDHI (0.5)
2	SDHI (1.0)	SDHI (0.25)	SDHI (0.25)
3	SDHI (1.0)	CTL (0.5)	0
		0	SDHI (0.5)
4	SDHI (0.5)	SDHI (0.5)	SDHI (0.5)
5	SDHI (0.5) + azole (0.5)	SDHI (0.5)	SDHI (0.5)
6	SDHI (0)		
7	SDHI (0.25)	-	
8	SDHI (0.5)	-	
9	SDHI (1.0)	-	
10	SDHI (2.0)	-	
11	-	SDHI (0.5)	SDHI (0.5)

- Barley variety = Flagon
- Seed treatments: SDHI = BASF development product; azole = Fluquinconazole (Jockey solo)
- Foliar treatments: SDHI = Fluxapyroxad (Imtrex); multi-site = Chlorothalonil (Bravo)
- Natural infection with net blotch (*Pyrenophora teres)*; no pathogen inoculations

4. Results

4.1. Modelling

4.1.1. Selection for fungicide resistance

The model was used to calculate fungicide-sensitive and fungicide-resistant infectious leaf tissue over ten growing seasons on leaf layers 1, 5 and 11 (representing the upper, mid- and lower canopy, respectively) after applying either a solo T1 foliar spray or a solo systemic seed treatment (Figures 5 and 6, respectively). To provide an appropriate comparison between both treatments, the input seed treatment dose was parameterised to approximate the HAD gain of a T1 spray. As expected, fungicide-sensitive infectious leaf tissue after applying a seed treatment was more greatly reduced at the earlier emerging leaf layers (Figure 6) than after applying a foliar treatment (Figure 5), particularly on leaf layer 11. However, on leaf layers 1 and 5 the remaining fungicide-sensitive infectious leaf tissue after applying either a systemic seed treatment or a T1 foliar spray were similar. There were no substantial differences in the growth of the fungicide-resistant strain after either treatment, indicating that the selection pressure for fungicide-resistance was similar in both cases.

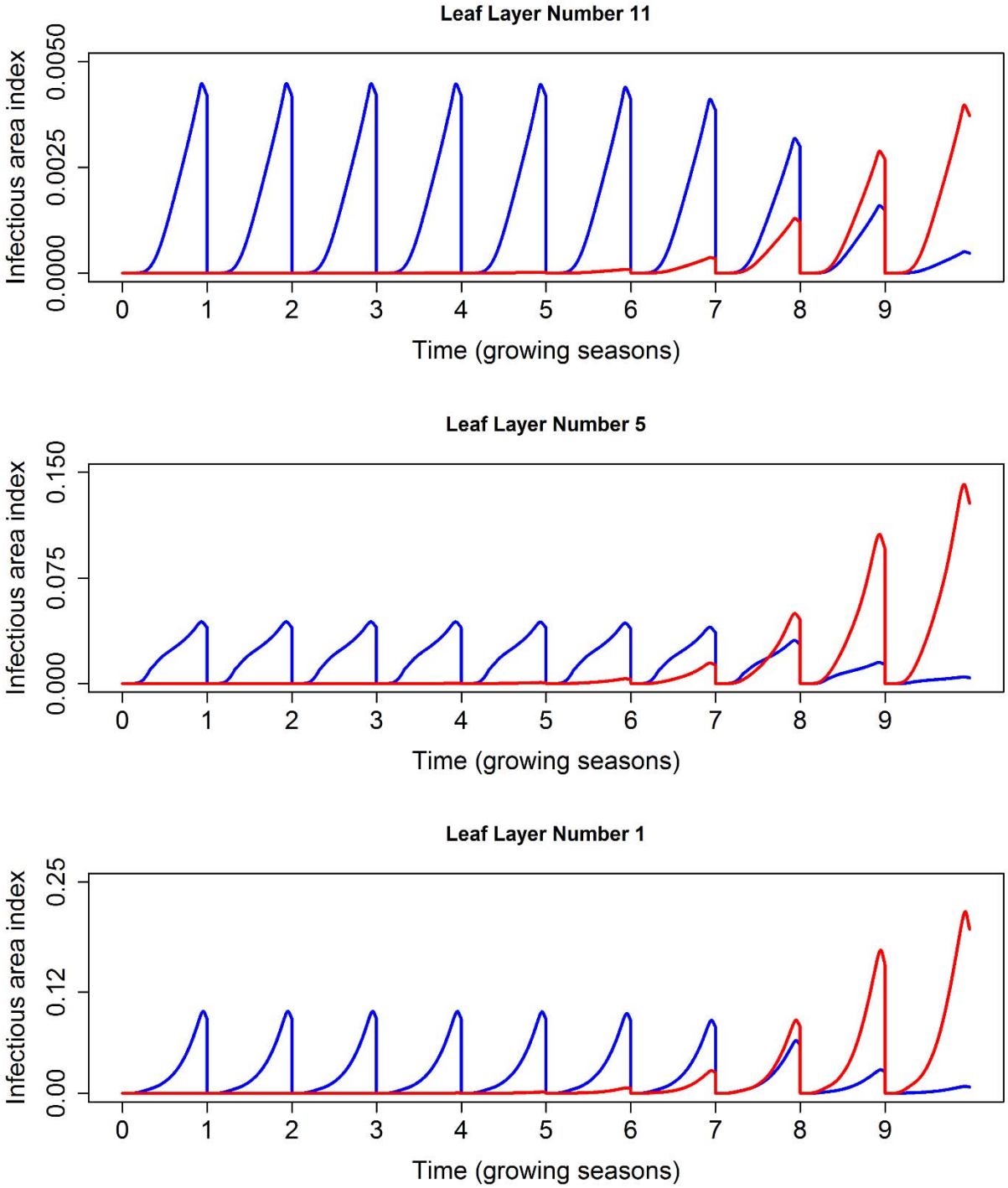

Figure 5. Time-course of the sensitive and resistant pathogen strain. T1 spray only. Blue and red lines indicate fungicide-sensitive and fungicide-resistant area index, respectively. Top panel: leaf layer 11. Middle panel: leaf layer 5. Bottom panel: leaf layer 1. A T1 spray was applied at 20 mg/m^2 and the simulation was run for 10 growing seasons.

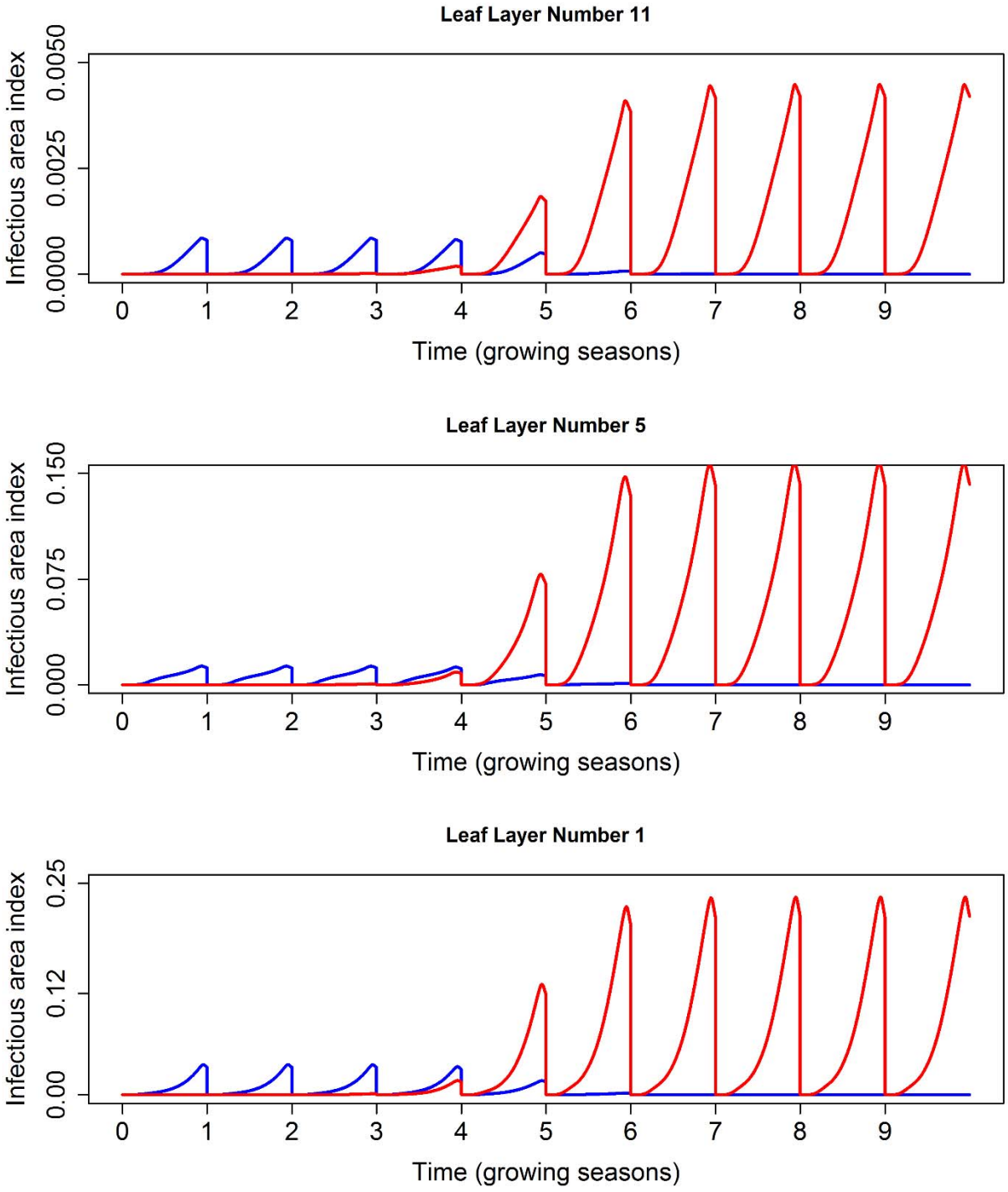

Figure 6. Time course of the sensitive and resistant pathogen strain. Seed treatment only. Blue and red lines indicate fungicide-sensitive and fungicide-resistance area index, respectively. Top panel: leaf layer 11. Middle panel: leaf layer 5. Bottom panel: leaf layer 1. A seed treatment was applied at 4.5 mg/m^2, which provides a HAD gain that approximates that of a T1 spray, and the simulation was run for 10 growing seasons.

4.1.2. Effective fungicide lives

Solo fungicide applications

Effective lives were compared between four treatment programmes over a range of doses. The regimens are labelled in Table 9 as the following:

ST + T1: A seed treatment and a foliar spray at T1 were applied each growing season.

ST + T2: A seed treatment and a foliar spray at T2 were applied each growing season.

T1 + T2: Two foliar sprays at T1 and T2 were applied each growing season.

ST + T1 + T2: A seed treatment and two foliar sprays at T1 and T2 were applied every growing season.

The doses of the foliar treatments at T1 and T2 were equal.

First consider the left-hand side of Table 9. The table leads to three key conclusions for this set of simulations. Firstly, the ST+T1+T2 column shows that adding a seed treatment to a two foliar spray programme shortens the effective life of the fungicide. Secondly, the effective life of all spray programmes that include a seed treatment is equal to or smaller than a spray programme with two foliar sprays. Thirdly, comparing spray programmes with equal total fungicide dose used, the effective life of the spray programme including a seed treatments is equal to or smaller than that of a programme without a seed treatment.

Now consider the right-hand side of Table 9. Even though this table is constructed using a widely different model parameterisation for the uptake of the fungicide from the seed coating, the results are qualitatively similar. (i) Adding a seed treatment to a spray programme with two foliar sprays reduces the effective life of the fungicide, (ii) the effective life of all spray programmes that includes a seed treatment is equal to or smaller than a spray programme of only two foliar applications, and (iii) comparing spray programmes with equal total dose the effective life of the programme including a seed treatment is equal to or smaller than that of the programme without a seed treatment.

We can thus conclude from Table 9 that the three qualitative conclusions are not sensitive to which of the two models for the uptake of the fungicide from the seed coating are used.

We have constructed the same tables for the model parameterised such that the seed treatment efficacy is such that it reduces the AUDPC value on leaf 11 to 6 by 60% after treatment (Appendix 2). The resulting tables again lead to the same qualitative conclusions as those derived from Table 9. Moreover we have done all these four combinations of uptake model and efficacy parameterisation for a faster and a slower fungicide decay rate (because these values are not well known for the fungicides used) and we again found the same qualitative outcomes, although some quantitative differences exist.

Our simulations thus show that the model output is stable over quite large differences in the exact model specification and parameterisation. This provides confidence in the use of the model to

derive guidance for the use of seed treatments even though some aspects of the model and its parameterisation lack experimental data.

Table 9. Calculated effective lives for two different models for seed treatment fungicide uptake. The left-hand table is for the constant systemic seed treatment uptake rate model, and the right-hand table is for the transpiration dependent uptake model. The seed treatment was parameterised such that the efficacy of the seed treatment was equal to that of a T1 spray. The foliar dose is the dose per application. ST_dose = seed treatment dose. Cells with '-' indicate that the effective life is zero.

ST_dose	foliar_dose	ST+T1	ST+T2	ST+T1+T2
0	0	-	-	-
0	0.2	-	-	-
0	0.4	-	-	5
0	0.6	-	-	5
0	0.8	-	-	5
0	1	-	-	4
0.6	0	-	-	-
0.6	0.2	4	5	4
0.6	0.4	4	4	3
0.6	0.6	4	4	3
0.6	0.8	4	4	3
0.6	1	3	4	3
1	0	-	-	-
1	0.2	4	4	4
1	0.4	4	4	3
1	0.6	3	4	3
1	0.8	3	4	3
1	1	3	3	3

ST_dose	foliar_dose	ST+T1	ST+T2	ST+T1+T2
0	0	-	-	-
0	0.2	-	-	-
0	0.4	-	-	5
0	0.6	-	-	5
0	0.8	-	-	5
0	1	-	-	4
0.6	0	-	-	-
0.6	0.2	-	-	5
0.6	0.4	4	-	4
0.6	0.6	5	5	4
0.6	0.8	4	5	4
0.6	1	4	5	3
1	0	-	-	-
1	0.2	-	-	5
1	0.4	5	5	4
1	0.6	4	5	4
1	0.8	4	5	3
1	1	4	5	3

Fungicide mixtures.

Next we explored the effect of fungicide mixtures on the effective life in the presence and the absence of a seed treatment. We used as mixing partner a dose-response parameterisation representing the systemic azole fungicide fluquinconazole. In the simulations, all the strains remained sensitive to fluquinconazole (referred to hereafter as the 'low risk' fungicide, in that resistance did not evolve during the period of simulation). Resistance was simulated to evolve to the other fungicide (referred to hereafter as the 'high risk' fungicide) as described for the previous simulations above for the SDHI example. Table 10 shows two sets of simulations, one for the constant uptake rate (10a) and one for the transpiration dependent uptake model (10b). The dose of the fluquinconazole is kept at a full dose per treatment (all our previous work has shown that the higher the dose of the mixing partner, the longer the effective life of the fungicide).

Table 10. Calculated effective lives for fungicide mixtures applied to foliar and seed treatments. ST = seed treatment, T1 = T1 spray, T2 = T2 spray, h = high-risk fungicide, l = low risk fungicide, l+h= a mixture of the two and – indicates no fungicide used. Table 10a shows results based on the constant uptake rate model. Table 10b shows results based on the uptake rate dependent on transpiration rate of the plant. The seed treatment is parameterised such that the efficacy of a single seed treatment equals the efficacy of a T1 spray. The first four rows show four different seed treatments, each with the same foliar treatment of one spray at T2 (mix of l+h), The next four rows show the same four seed treatments, each with the same foliar programme of two sprays, T1 plus T2.

Table 10a.

ST	T1	T2	effective_life	SDHI_dose	Total SDHI dose
-	-	l+h	7	1	1
l	-	l+h	9	1	1
h	-	l+h	7	0.5	1
l+h	-	l+h	8	0.5	1
-	l+h	l+h	7	0.5	1
l	l+h	l+h	7	0.5	1
h	l+h	l+h	6	0.33	1
l+h	l+h	l+h	7	0.33	1

Table 10b.

ST	T1	T2	Effective life	SDHI dose	Total SDHI dose
-	-	l+h	7	1	1
l	-	l+h	10	1	1
h	-	l+h	6	0.5	1
l+h	-	l+h	7	0.5	1
-	l+h	l+h	7	0.5	1
l	l+h	l+h	8	0.5	1
h	l+h	l+h	6	0.33	1
l+h	l+h	l+h	7	0.33	1

For application programs consisting of a single spray at T2, or a two spray programme with applications at T1 and T2, without a seed treatment the effective life of our fungicide is simulated as 7 years. This effective life is longer than the effective life of these spray programmes reported in Table 9 which shows the use of mixtures. If we add to these application programmes a seed treatment with the low-risk mixing partner only, then the effective life increases as would be expected. If a seed treatment is added with the high-risk fungicide only the effective life is equal to or smaller than the effective life of the application programme without the seed treatment. This reiterates our previous finding that adding a solo seed treatment will not increase the fungicide

effective life. If a seed treatment is added composed of a mixture of the low and the high-risk fungicide the effective life is between that of the solo low-risk and solo-high risk seed treatment.

In most cases, when adding a mixture seed treatment, the effective life is the same as for the application programme using foliar treatments only. There is an exception in the case of Table 10a where the effective life of the mixture seed treatment is one year longer than the effective life of the application programme with a single T2 application.

We have repeated these simulations for the other parameterisation of the seed treatment, as well as for other fungicide decay rates. The results of the simulations are consistent with those shown in Table 10. In most cases adding a seed treatment consisting of a mixture of the high-risk and the low-risk fungicide results in the same effective life as for a foliar treatments only programme. In a few cases, the programme where a seed treatment is added has an effective life that is one year longer than the foliar treatments only programme. However, note that in the simulations described in this section, the total dose of the high risk fungicide was held constant, i.e. dose per treatment was reduced as the number of seed and/or foliar applications was increased. This has important practical consequences which are covered in the discussion.

The relationship between efficacy and resistance risk

Is there a level of efficacy of a foliar-acting seed treatment that has no material effect on the effective life of the fungicide? This question is important because the FRAC guidance excludes non-foliar-acting seed treatments from the restriction on total number of treatments permitted. Proving complete absence of an effect potentially requires a large number of field trials (unless the product is clearly non-systemic). In reality, there is likely to be some low level of efficacy which has no material effect on resistance evolution. To answer this question we calculated the effective life for a range of foliar treatment programmes with a T1 and a T2 spray. For each foliar treatment programme seed treatments were added and the resulting effective life calculated. This was done for a range of seed treatments either with solo SDHI or a mixture of SDHI and fluquinconazole. The results are given in Figure 7.

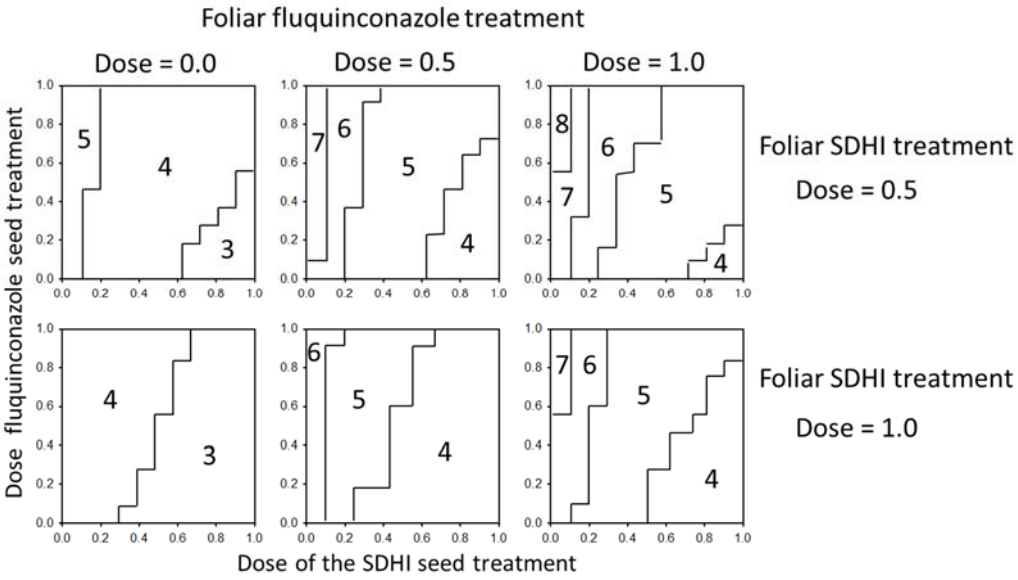

Figure 7. The effective life for a range of seed treatment dosages of the SDHI and the fluquinconazole fungicides, for different foliar treatments programs. Each foliar treatment programme consists of a T1 and a T2 spray, the dosages given in the figure are the dosages per spray. The SDHI seed treatment is parameterised as having the same efficacy as a T1 spray.

The highest number in each panel of Figure 7 is the effective life of the application programme when there is no SDHI seed treatment and the seed treatment is the highest dose of the not-at-risk fungicide. Thus, the no-effect seed treatment is all seed treatment dosages of SDHI and fluquinconazole that do not affect the effective life of the foliar treatment only programme. Figure 7 shows that there is a small SDHI dose range (relating to a small efficacy range) for which seed treatments can be applied that will not reduce the effective life of the fungicide, although this no-effect dose is different for each of the foliar treatment programmes. This implies that there is no general rule for what a no-effect dose (or level of efficacy) will be.

4.2. Field experiments

4.2.1. Azole seed treatments Year 1

At the Rosemaund azole site, initial (pre-GS 30) septoria leaf blotch development under the tents was low (<2%) on inoculated tented plants, but much higher (>20%) on non-inoculated plants which were not covered by tents. Therefore, the tents appeared to be excluding the natural *Z. tritici* spores arriving from surrounding fields. By the last assessment at GS 65, there were similar levels of septoria on inoculated and non-inoculated untreated plots (Figure 8), and it was not clear if all disease across the tented and untented plots had been initiated from external inoculum rather than the isolates used for inoculation. There was also no clear difference between the disease levels on seed-treated plants or on those with foliar and seed treatments, with the exception of the two foliar

treatments of 2 x full dose rate which gave the lowest disease. No genotyping tests were done due to concerns about external inoculum influx and lack of efficacy from the seed treatments.

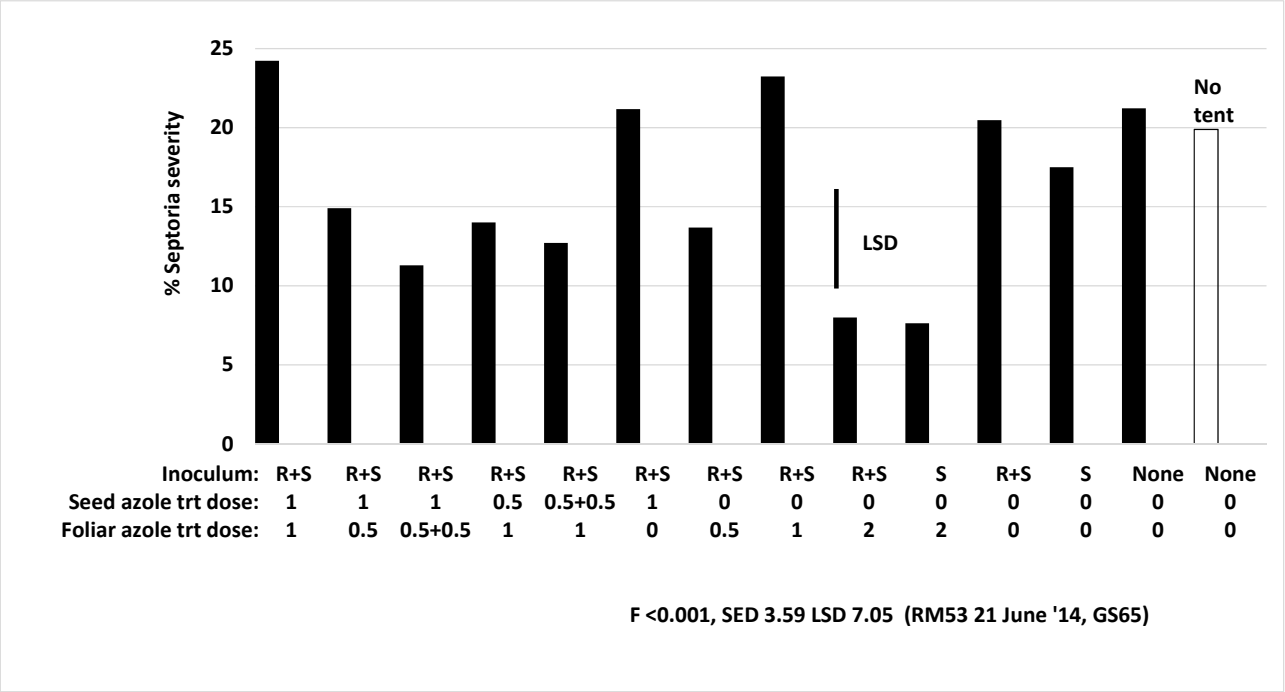

Figure 8. Septoria leaf blotch development at GS 65 in azole seed treatment field experiment, Herefordshire site, year 1. R = azole resistant (5%) and S = azole susceptible (95%) *Z. tritici* inoculum. Seed azole = fluquinconazole (Jockey Solo), foliar azole = epoxiconazole (Ignite). Seed 0.5+0.5 = azole + SDHI development product, and Foliar 0.5+0.5 = azole + chlorothalonil (Bravo). All doses are proportions of the label recommended dose.

At the Boxworth azole site, there was less than 3% septoria development in all treatments by 16 Jan 2014. Mildew was the predominant disease although it was controlled. There was no overall difference in disease development in seed-treated plots compared with non-seed-treated plots. Septoria was low in the fungicide-untreated plots, for the sensitive and insensitive inoculum mix as well as for the sensitive-only inoculum, indicating that the infection from the inoculations was too low to promote sufficient disease development. The experiment was stopped and no foliar treatments or sampling was done.

4.2.2. MBC seed treatments Year 1

At the Rosemaund site by GS 30, there was 2–7% septoria severity across all treatments in tented plots (Figure 9). This included the non-inoculated plots as well as inoculated, which suggests that some of the disease in tented plots could have been initiated by external inoculum rather than the inoculated isolates. However, there was significantly more septoria (over 20%) in non-tented plots, indicating that tents excluded much of the external inoculum.

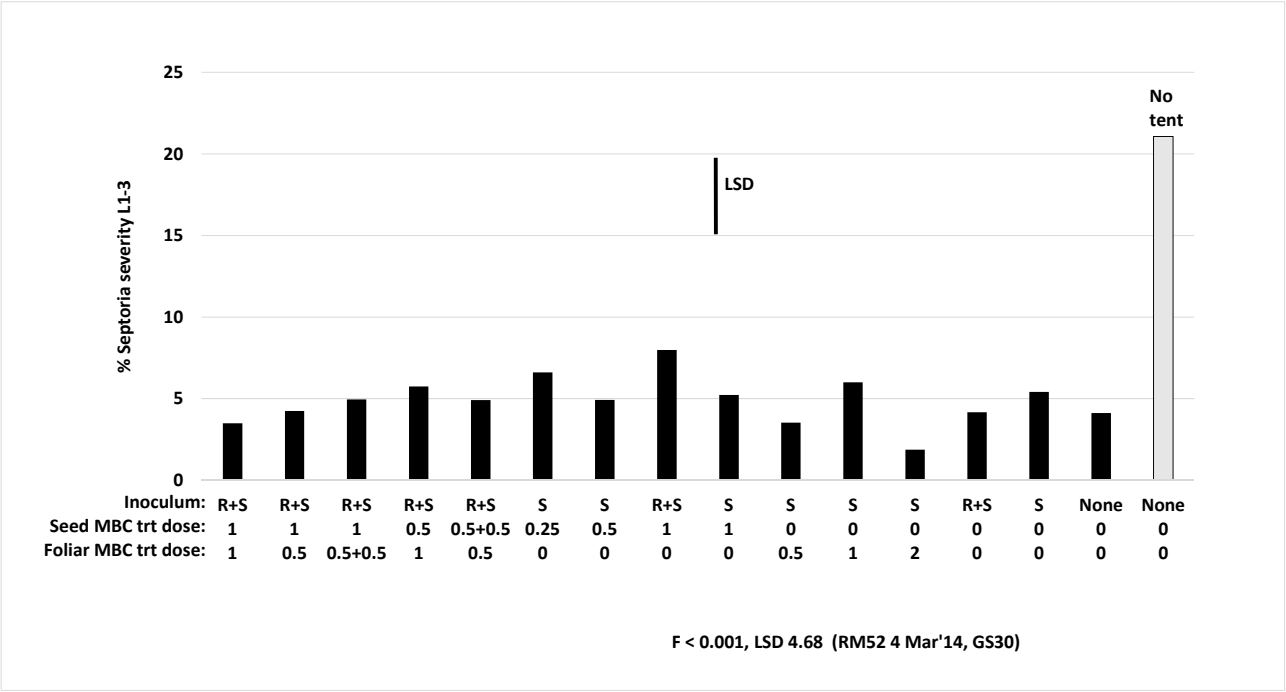

Figure 9. Septoria leaf blotch development at GS 30 in MBC seed treatment field experiment, Herefordshire site, year 1. **R** = MBC resistant and **S** = MBC susceptible *Z. tritici* inoculum, mixture 5% **R** and 95% **S**. Seed MBC = fuberidazole + triadimenol (Tripod). Foliar MBC = carbendazim (Delsene 50 Flo). Seed 0.5+0.5 = MBC + azole (fluquinconazole; Jockey Solo), and Foliar 0.5+0.5 = MBC + chlorothalonil (Bravo).

By GS 65 the disease levels were similar in tented and untented non-treated plots and there were no significant treatment differences.

The efficacy results did not rule out whether seed and/or foliar treatments had influenced selection for insensitive *Z. tritici* at Rosemaund, because lack of efficacy could have been due to rapid selection for the insensitive strain. Therefore, genotyping tests were done on isolates cultured from samples from each plot, at each sample time (Figure 10). For the early samples, all treatments had a low proportion of insensitive isolates compared to untreated. By the last two sample times at GS 66 and 75, the proportion of insensitive isolates was approaching 100% in all treatments, so there was no differentiation between treatments at this stage for selection for MBC-insensitive isolates.

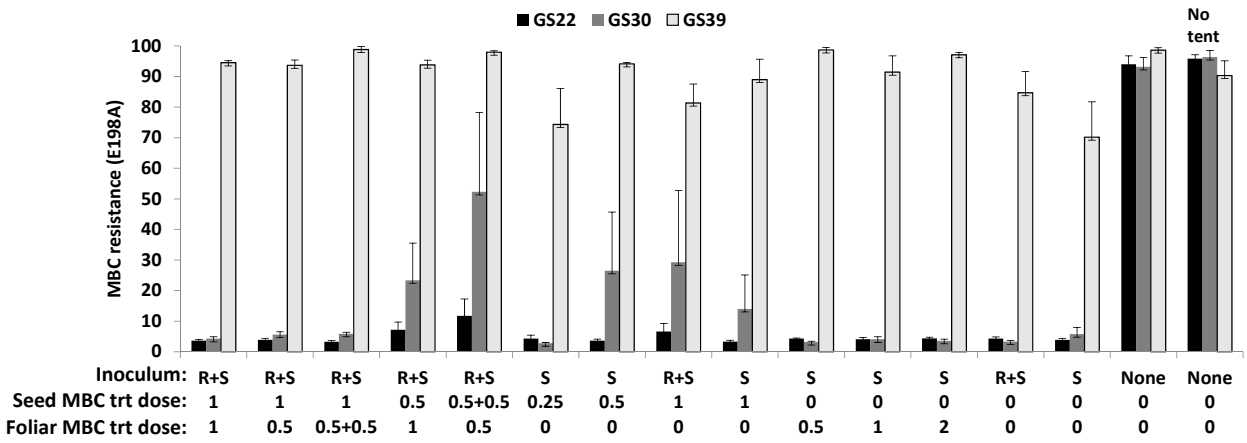

Figure 10. Percentage of isolates with MBC resistance (E198A mutation), Herefordshire site, year 1. **R** = MBC resistant and **S** = MBC susceptible *Z. tritici* inoculum, mixture 5% **R** and 95% **S**. Seed MBC = fuberidazole + triadimenol (Tripod). Foliar MBC = carbendazim (Delsene 50 Flo). Seed 0.5+0.5 = MBC + azole (fluquinconazole; Jockey Solo), and Foliar 0.5+0.5 = MBC + chlorothalonil (Bravo).

However, in the early samples prior to GS 30 there were some differences between treatments in the proportion of insensitive isolates (Figure 10), with indications that for the GS 30 samples, all plots which had received a seed treatment had a lower proportion of E198A than those which had no seed treatment. Prior to GS 30, no foliar treatments had yet been applied. At GS22, the insensitive fraction was still too low to discriminate treatment effects. By GS39, external influx, plus selection, drove the population close to the upper limit of resistance frequency and so at this stage we could cannot discriminate between treatment effects. Therefore, the data for % E198A mutations was grouped and averaged by inoculum type and seed treatment dose (i.e., including data as appropriate from plots which were destined to have foliar treatments which had not yet been applied), separately for GS 22 and for GS 30 data. The selection ratio for GS22–30 was calculated as % E198A GS 30 / % E198A GS 22 (ratios shown in Figure 11). Influx of *Z. tritici* from outside the trial area would have been that of MBC resistant strains (as these predominate the UK population), thus the plots inoculated with sensitive only strains were, in fact, infected with a mixture of sensitive and insensitive strains. So the 'R+S' and 'S' treatments in Figure 10 can be considered as similar.

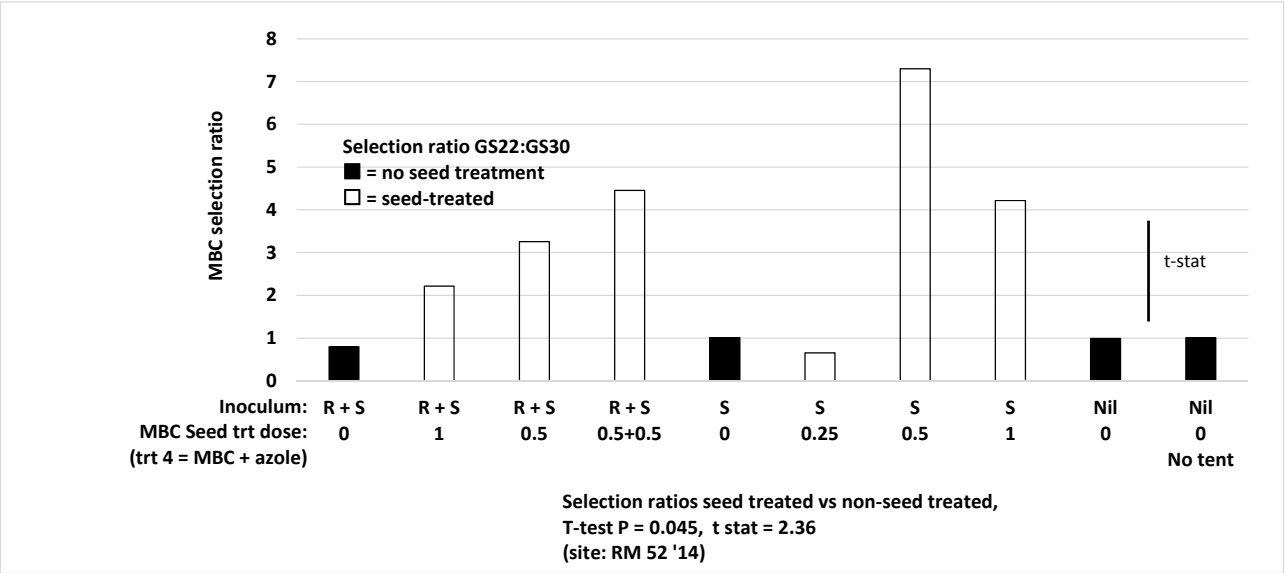

Figure 11. Selection ratios, based on % E1989A at GS 30 / % E1989A at GS 22, Herefordshire site, year 1. **R** = MBC resistant and **S** = MBC susceptible *Z. tritici* inoculum, mixture 5% **R** and 95% **S**. Seed MBC = fuberidazole + triadimenol (Tripod); 0.5+0.5 = MBC + azole (fluquinconazole; Jockey Solo).

In the absence of fungicide selection for resistance the selection ratio should be one (i.e. no change in the proportion of the resistant strain in the population). The selection ratio was close to one in all untreated plots, indicating there was little cross-flow of strains between treatments. There was a significantly greater selection (p = 0.045) for MBC-insensitive isolates of septoria with seed treatments compared to no seed treatments (Figure 11), for the GS 22–GS 30 phase. After this, the proportions of insensitive isolates became too high to allow differentiation between the seed and/or foliar treatments, indicating that most of the selection occurred early, resulting from seed treatments.

At the Boxworth Cambridge site with the same septoria-MBC seed treatment experiment as at Rosemaund, the efficacy of treatments at GS 30 was similar to that at Rosemaund, i.e. there was low septoria (<1%) across all tented plots and treatments, with no difference in efficacy between seed treatment doses. By comparison there was just over 9% disease in the non-tented plots. By GS 61 there was no difference in efficacy across all treatments, with septoria severity ranging from approximately 3 to 5% across all treatments, with tents or without. Genotyping showed that the frequency of insensitivity was too high to discriminate any treatment effects.

4.2.3. Azole seed treatments Year 2

There was no visible septoria disease development on any of the inoculated plots at ADAS Boxworth in 2015 despite two inoculations of *Z. tritici* isolates in autumn 2014, and weekly irrigation

from early February to early March. Mildew infections were present but were controlled. No samples of septoria-diseased leaves were possible, therefore no genotyping tests were done.

4.2.4. SDHI seed treatments Year 2

Only trace levels of net blotch were observed in untreated plots at the two sites in Yorkshire or the Hereford site. Therefore, the trials were stopped, and no samples were taken for genotyping.

5. Discussion

Fungicide resistance evolution is driven by fungicide efficacy and we could find no plausible mechanistic scenario by which a foliar-acting seed treatment could be effective, but not create increased selection for resistant strains. The more effective the product, whether by increased dose or by higher inherent activity, the greater the selection. However, restricting the use of foliar-acting seed treatments in order to manage the resistance risk has, so far, precluded foliar-acting SDHI seed treatments from being brought to market. Similar restrictions are likely to prevent future modes of action being used in that way. This is a robust precautionary position, perhaps justified further by the recent finding of highly SDHI insensitive strains of *Z. tritici* in the UK and Ireland. The restrictions deprive growers of a useful disease management tool which can potentially increase yields and gross margins, so the restrictions on using foliar-acting seed treatments need to be proportionate. Here we discuss the modelling and limited experimental evidence, which underpin the practical guidance from the project.

5.1.1. Simulation modelling

We developed a model to simulate epidemics of *Zymoseptoria tritici* on winter wheat controlled by treatments of systemic fungicidal seed treatments and foliar sprays. We used the model to compare the selection pressures for fungicide-resistance between both fungicide treatment types. Unlike foliar sprays, for which information on dose response curves are readily available, there is little information on the efficacy and the uptake dynamics of systemic seed treatments. We therefore did a sensitivity analysis with respect to the parameter values for the systemic seed treatment model and combined them with a structural sensitivity analysis, using two different approaches to model the uptake dynamics of the fungicide from the seed treatment. Our results show that the qualitative trends in the model output are insensitive to: (i) the value of the parameter scaling the seed treatment efficacy, (ii) the half life time of the fungicide, and (iii) the model description of the fungicide uptake by the plant from the seed coating. As mentioned, a range of other parameters were explored by sensitivity analysis and also showed that the conclusions were very robust to these parameter changes. Hence, whilst we cannot predict with any confidence the actual number of years of effective life which will be obtained from a mode of action, the pattern of

modelling conclusions (i.e. the rank order of the different treatments compared) should be reliable across a wide range of plausible scenarios.

For solo (single mode of action) treatments for both seed and foliar treatments, the model outputs result in three key conclusions about the effect of seed treatment on fungicide resistance development as compared to foliar sprays:

(i) Adding a seed treatment to a spray programme with two foliar sprays reduces the effective life of the fungicide.

(ii) The effective life of all spray programmes that include a seed treatment is equal to or shorter than a spray programme of only two foliar applications.

(iii) Comparing spray programmes with equal total dose the effective life of the programme including a seed treatment is equal to or smaller than that of the programme without a seed treatment.

For *Z. tritici* on wheat, and for high risk fungicides such as SDHI treatments, the selection for fungicide resistance of a seed treatment is equal or at least comparable to that of a foliar spray, if the seed treatment efficacy is equal or comparable to that of a foliar spray. This finding is consistent with the current FRAC guideline concerning seed treatments of SDHI fungicides.
In the case of a simulated mixture of SDHI and fluquinconazole used as a seed treatment, there are exceptional cases where the effective life increased slightly with the addition of the seed treatment to the foliar spray programme. Whether this happens in our simulations depends on the uptake model used. This implies that for practical applications we need to know in detail how the fungicide is taken up from the seed coating by the plant. Only then will it be possible to determine whether the mixture seed treatment increases the effective life of the fungicide. An important point is that if a seed treatment is added to a foliar programme, effective life can only be maintained (or occasionally improved) so long as the total dose of the ‘high risk’ fungicide is kept constant (i.e. the dose per treatment is reduced as the number of treatments is increased). There are likely to be practical issues with this approach, such as, growers may be reluctant to use reduced foliar dose rates in order to allow a seed treatment to be included in a fungicide programme. These issues are likely to be relevant when making decisions about barley and wheat crop management, as these crops require a different emphasis in protection timing. Barley needs early tiller protection and the later leaves are less important in yield formation than in wheat, where later protection of the upper leaves is more important. Therefore, a seed treatment and one foliar treatment may be a useful approach for barley, whereas wheat is more likely to benefit from two foliar sprays.

Our results lead to the conclusion that in the majority of the cases simulated, adding a seed treatment reduces the effective fungicide life.

5.1.2. The relationship between efficacy and resistance risk

Our results show that seed treatments with very low efficacy against foliar pathogens are unlikely to add materially to the selection pressure for resistance. Such products should be excluded from the restriction on the maximum total number of SDHI treatments. It was not possible to generalise where the boundary of efficacy was below which the additional resistance risk was negligible. The modelling results suggested that there is no general rule as to the level of a no-effect dose, as the no-effect dose was different for each of the foliar treatment programmes. The no-effect dose is a small SDHI dose range (relating to a small efficacy range) for which seed treatments can be applied that do not reduce the effective life of the fungicide. However, it is probably unnecessary to require exhaustive proof of lack of efficacy from multiple field trials.

5.1.3. Field experiments and evidence for selection effects

It proved difficult to provide corroboration of the modelling results from field experiments. There is, however, previously published experimental evidence from a range of pathogens, crops and fungicide modes of action, to show that increasing the number of foliar applications of the same mode of action results in increased selection for insensitive strains of pathogens (van den Bosch *et al*., 2014, Hunter *et al.,* 1987, Sanders *et al.,* 1985). Systemic active ingredients can persist in plants for some time after seed treatment, therefore contributing to foliar disease control, and the selection effect from treatment continues as long as the treatment is constraining the epidemic (regardless of whether or not the active substance remains present). Treatment effects on *Z. tritici* epidemics were shown to persist in wheat up to leaf 4 emergence, following a fluquinconazole seed treatment (Parker & Lovell, 2001). It can be inferred from this combination of experimental evidence that any effective foliar-acting seed treatment should produce fungicide resistance selection if insensitive strains are present in the pathogen population.

In one field experiment in this project, we showed a significant selection effect for insensitive septoria from an MBC seed treatment. The selection effect found in our field experiment on seed treatments was substantial (a 4-fold increase in the frequency of the resistant strain in a short period). The only other example of field evidence is from work by Brent *et al*., (1989) on selection in powdery mildew, which concluded that selection from systemic seed treatments (which were highly effective against the pathogen) was similar to that by foliar treatments. This is consistent with our limited experimental data and the modelling work.

5.1.4. Practical messages and guidance

- Foliar-acting systemic seed treatments cause resistance selection

- The strength of selection will be related to the efficacy of the foliar treatment
- Seed treatments with nil or very low levels of foliar efficacy do not pose a material resistance risk. In the case of SDHIs, such treatments should not count as one of the two permitted treatments.
- For effective foliar-acting SDHI seed treatments, the current FRAC guidance (two SDHI treatments in total, seed and/or foliar) is supported.
- Modelling work has identified some combinations which could allow use of a foliar-acting seed treatment and up to two foliar treatments: (i) if the total dose of a mode of action applied by seed and foliar treatments is limited, and (ii) if effective mixtures of different modes of action are used in seed and foliar treatments.
- The strategy above could, in principle, be implemented by restricting the maximum total dose of a mode of action rather than the maximum number of treatments per crop. This would allow growers greater flexibility to use seed and foliar sprays. In practice, however, such a strategy would be limited by (i) the availability of sufficiently effective foliar-acting seed treatment mixture partners, (ii) grower reluctance to accept restricted foliar dose in order to use a seed treatment, and (iii) the challenge of enforcing guidance on total dose across seed and foliar treatments.

5.1.5. Future research

Field experiment evidence to validate the model findings remains limited. There is a window of opportunity to use naturally occurring resistance evolution to quantify resistance selection from different treatments in field experiments. This window of opportunity lasts from the point at which a new insensitive strain becomes reasonably widespread, to the time at which the proportion of the resistant strain becomes too high, thus limiting further selection. Such a window of opportunity is occurring now for SDHI insensitive *Pyrenophora teres* (net blotch of barley) and a similar window is likely to occur in the next few years for SDHI-insensitive *Z. tritici*.

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7. Appendix 1, Field experiment methods for *Z. tritici* inoculated plots

The main objective of the fabric tents used to cover the plots was to keep out *Z. tritici* spores arriving from external disease sources, such that disease developed only from inoculated isolates with known mutations and fungicide sensitivity or insensitivity. This novel experimental method was designed to determine the changes in proportions of sensitive and insensitive isolates following different fungicide treatments. In practice, the success rate of these experiments was low. This appendix reviews the methods and lessons for future research.

Across the experiments in year 1 and 2, disease lesions developed early within tented plots which had *Z. tritici* inoculations, but few lesions were seen in the non-inoculated tented plots, suggesting that the tents were effective in excluding external inoculum. By the final samples, the % disease in plots without tents was similar to that within tents, and it is likely that some of the infection within tented plots developed from external inoculum. Tent removal date was considered carefully, and the information available at the time of the experiment design showed that spore release was mainly over by the end of January (Fraaije *et al* 2005), and therefore keeping tents in place until mid-February was the appropriate strategy for excluding external septoria spore inoculum. The disease assessment data suggest that ascospore arrivals continued after mid-February, making it desirable to keep tents in position for longer. However, there are two practical limitations: Firstly, field experiments in other projects have showed that temperatures inside these spore-proof tents become too high for disease development in phases of warm weather, which are likely to occur from early April onwards. In particular, the internal temperature increases very rapidly in sunshine and can exceed 30 degrees even if the external air temperature is low. The second reason for removing tents in mid-February was that plants become too tall for the tents. This is exacerbated by plants inside tents growing taller than external plants, due to over-winter protection. Taller tents were considered, but this causes problems with access for sampling procedures. Tents would have to be removed completely from plots for considerable lengths of time to allow sampling, and this would partly negate the strategy of keeping them in place longer.

There were some concerns about possible changes to wheat plant development from covering them with fabric tents. The plants under tents tended to progress faster through growth stages and grew taller than plants not covered by tents. However, application of a PGR helped to prevent excessive growth and by the time of tent removal the plants were touching the top covers but were not unduly hindered by the covers.

Mildew was more prevalent on plants inside tents, but in practice it was possible to control this using specific mildewicides. The enhanced mildew levels indicated that conditions inside the tents were sufficiently humid for disease development.

In general, the tent construction was fit for purpose, but some modifications were made to year 2 tents to help improve the practicability of use. The robustness and anchoring of the frames was tested in year 1 by high winds, and some tent frames were damaged and could not be stored for re-use. Where new tents were built for year 2, they were made 20 cm taller so that they could be left on plots for longer (to delay the start of spore influx) and not hinder growth of plants. Cross-pieces of wood were added underneath the top cover to reduce the amount of water which collected with high rainfall, causing the covers in year 1 to sag. Anchor legs were made longer to make tents more secure in high wind. Originally, the tents were planned to be removable for inoculation and irrigation, but in practice they needed to be anchored too securely against wind to enable quick removal and replacement. Therefore the approach of cutting and re-sealing openings in the top of the tent was used, made possible by use of waterproof tape for re-sealing the top polythene cover between each operation, using fresh tape. Easy opening and re-sealing of tent tops proved to be important to enable proper monitoring of disease development and crop growth to ensure that the assessments, sampling, and all foliar fungicide and other treatments (e.g. PGR) were made at the correct timing.

Overall, the tent fabrics and frames appear to provide a viable method for excluding outside inoculum, while allowing the crop within to develop normally. The establishment of infection and disease from inoculated spores was more problematic. There was insufficient infection from the isolates of *Z. tritici* inoculum used, and subsequent low disease development in covered plots. This may be part of the reason why there were few differences in disease development between seed-treated and non-seed-treated plots, leading to lack of samples for genotyping.

But the approach using inoculation and tented plots has the potential to provide valuable field evidence on the influence of risk modifiers on the effective life of fungicide active ingredients, and is worth pursuing. There are some possible modifications to the methods that could improve the efficiency of infection by spores and promote disease increase from these infections.

In the current work, we inoculated ahead of the cold temperatures (4^{o}C or below) in both years, but in practice this could have been a week or so earlier, and this could have made a difference. Inoculations should be started as early as possible in the autumn, to experience warmer temperatures (above 4^{o}C) for as long as possible. But the earliness of the first inoculation date is limited by the need for the crop to have 2–3 leaves or more developed, to provide a target for inoculation. Earlier drilling could be considered, but this needs to be balanced against the likelihood of plants reaching the top of the tents before suitable spring weather conditions for tent removal.

Additional resources to the field work in terms of funding and lead-time should be planned to include field pathogenicity tests of *Z. tritici* isolates that have been cultured from stored isolates. Ideally, a field test of pathogenicity should be done ahead of the field inoculation under tents, before committing to the full field experiment. In the current work, pathogenicity tests in year 1 were done in a greenhouse using potted wheat plants, prior to the field inoculations, but no field test was done before the tented field experiments were set up. In year 2, new isolates (selected to have greater differences between resistance factors) were cultured just prior to field inoculations, and there was no time for pathogenicity tests before the field inoculations.

The two seasons of field work coincided with exceptionally high and low septoria years. The high external inoculum in year 1 probably exacerbated spore influx. The poor conditions for septoria in year 2 probably reduced infection and symptom expression, despite irrigation. Planning for three field seasons of experiments would increase the likelihood of obtaining reliable field data (project resources were insufficient to enable this).

8. Appendix 2, additional results for effective life

Additional tables with results for the model parameterised so that the seed treatment efficacy reduces the AUDPC value on leaves 11 to 6 by 60% after treatment. Results are presented for four combinations of the uptake model and efficacy parameterisation, including a faster and a slower fungicide decay rate (because these values are not well quantified in the public domain for the fungicides used). The results show the same qualitative outcomes as given for Table 9, although some quantitative differences exist

Table 11. Effective lives for a seed treatment with the same efficacy as a T1 foliar treatment, the constant fungicide uptake model and a low fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	5
0	0.6	0	0	4
0	0.8	0	0	4
0	1	0	0	4
0.2	0	0	0	0
0.2	0.2	0	0	4
0.2	0.4	4	4	4
0.2	0.6	4	5	4
0.2	0.8	4	5	3
0.2	1	4	4	3
0.4	0	0	0	0
0.4	0.2	4	0	4
0.4	0.4	4	5	4
0.4	0.6	4	5	3
0.4	0.8	4	4	3
0.4	1	4	4	3
0.6	0	0	0	0
0.6	0.2	4	5	4
0.6	0.4	4	5	4
0.6	0.6	4	4	3
0.6	0.8	4	4	3
0.6	1	4	4	3
0.8	0	0	0	0
0.8	0.2	4	5	4
0.8	0.4	4	4	3
0.8	0.6	4	4	3
0.8	0.8	4	4	3
0.8	1	4	4	3
1	0	0	0	0
1	0.2	4	5	4
1	0.4	4	4	3
1	0.6	4	4	3
1	0.8	4	4	3
1	1	4	4	3

Table 12. Effective lives for a seed treatment with the same efficacy as a T1 foliar treatment, the constant fungicide uptake model and a high fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	0
0	0.6	0	0	5
0	0.8	0	0	5
0	1	0	0	5
0.2	0	0	0	0
0.2	0.2	0	0	5
0.2	0.4	0	0	4
0.2	0.6	0	0	4
0.2	0.8	4	0	4
0.2	1	4	5	4
0.4	0	0	0	0
0.4	0.2	0	0	5
0.4	0.4	4	0	4
0.4	0.6	4	5	4
0.4	0.8	4	5	4
0.4	1	4	5	4
0.6	0	0	0	0
0.6	0.2	0	0	4
0.6	0.4	4	5	4
0.6	0.6	4	5	4
0.6	0.8	4	5	4
0.6	1	4	5	3
0.8	0	0	0	0
0.8	0.2	4	0	4
0.8	0.4	4	5	4
0.8	0.6	4	5	4
0.8	0.8	4	5	3
0.8	1	4	4	3
1	0	0	0	0
1	0.2	4	4	4
1	0.4	4	5	4
1	0.6	4	5	4
1	0.8	4	4	3
1	1	4	4	3

Table 13. Effective lives for a seed treatment leading to a 60% reduction in AUDPC, a constant fungicide uptake rate and a high fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	0
0	0.6	0	0	5
0	0.8	0	0	5
0	1	0	0	5
0.2	0	0	0	0
0.2	0.2	0	0	0
0.2	0.4	0	0	5
0.2	0.6	0	0	5
0.2	0.8	0	0	4
0.2	1	0	0	4
0.4	0	0	0	0
0.4	0.2	0	0	0
0.4	0.4	0	0	5
0.4	0.6	0	0	4
0.4	0.8	0	0	4
0.4	1	0	0	4
0.6	0	0	0	0
0.6	0.2	0	0	5
0.6	0.4	0	0	5
0.6	0.6	0	0	4
0.6	0.8	0	0	4
0.6	1	4	4	4
0.8	0	0	0	0
0.8	0.2	0	0	5
0.8	0.4	0	0	4
0.8	0.6	3	0	4
0.8	0.8	4	4	4
0.8	1	4	5	4
1	0	0	0	0
1	0.2	0	0	5
1	0.4	0	0	4
1	0.6	4	0	4
1	0.8	4	5	4
1	1	4	5	4

Table 14. Effective lives for a seed treatment leading to a 60% reduction in AUDPC, a constant fungicide uptake rate and a low fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	5
0	0.6	0	0	4
0	0.8	0	0	4
0	1	0	0	4
0.2	0	0	0	0
0.2	0.2	0	0	5
0.2	0.4	0	0	4
0.2	0.6	0	0	4
0.2	0.8	0	0	4
0.2	1	0	4	4
0.4	0	0	0	0
0.4	0.2	0	0	5
0.4	0.4	0	0	4
0.4	0.6	0	0	4
0.4	0.8	0	5	4
0.4	1	3	5	4
0.6	0	0	0	0
0.6	0.2	0	0	5
0.6	0.4	0	0	4
0.6	0.6	4	5	4
0.6	0.8	4	5	4
0.6	1	4	5	3
0.8	0	0	0	0
0.8	0.2	0	0	4
0.8	0.4	4	4	4
0.8	0.6	4	5	4
0.8	0.8	4	5	4
0.8	1	4	4	3
1	0	0	0	0
1	0.2	0	0	4
1	0.4	4	5	4
1	0.6	4	5	4
1	0.8	4	5	3
1	1	4	4	3

Table 15. Effective lives for a seed treatment with the same efficacy as a T1 foliar treatment, the transpiration-based fungicide uptake model and a high fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	5
0	0.6	0	0	4
0	0.8	0	0	4
0	1	0	0	4
0.2	0	0	0	0
0.2	0.2	0	0	5
0.2	0.4	0	0	4
0.2	0.6	0	0	4
0.2	0.8	0	5	4
0.2	1	0	5	4
0.4	0	0	0	0
0.4	0.2	0	0	4
0.4	0.4	4	5	4
0.4	0.6	4	5	4
0.4	0.8	4	5	3
0.4	1	4	4	3
0.6	0	0	0	0
0.6	0.2	4	0	4
0.6	0.4	4	5	4
0.6	0.6	4	4	3
0.6	0.8	4	4	3
0.6	1	4	4	3
0.8	0	0	0	0
0.8	0.2	4	5	4
0.8	0.4	4	5	3
0.8	0.6	4	4	3
0.8	0.8	4	4	3
0.8	1	4	4	3
1	0	0	0	0
1	0.2	4	5	4
1	0.4	4	4	3
1	0.6	4	4	3
1	0.8	4	4	3
1	1	4	4	3

Table 16. Effective lives for a seed treatment with the same efficacy as a T1 foliar treatment, the transpiration-based fungicide uptake model and a low fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	0
0	0.6	0	0	5
0	0.8	0	0	5
0	1	0	0	5
0.2	0	0	0	0
0.2	0.2	0	0	0
0.2	0.4	0	0	5
0.2	0.6	0	0	4
0.2	0.8	0	0	4
0.2	1	0	0	4
0.4	0	0	0	0
0.4	0.2	0	0	5
0.4	0.4	0	0	4
0.4	0.6	4	0	4
0.4	0.8	4	5	4
0.4	1	4	5	4
0.6	0	0	0	0
0.6	0.2	0	0	5
0.6	0.4	4	4	4
0.6	0.6	4	5	4
0.6	0.8	4	5	4
0.6	1	4	5	4
0.8	0	0	0	0
0.8	0.2	4	0	4
0.8	0.4	4	5	4
0.8	0.6	4	5	4
0.8	0.8	4	5	3
0.8	1	4	4	3
1	0	0	0	0
1	0.2	4	5	4
1	0.4	4	5	4
1	0.6	4	4	4
1	0.8	4	4	3
1	1	4	4	3

Table 17. Effective lives for a seed treatment leading to a 60% reduction in AUDPC, the transpiration-based fungicide uptake model and a high fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	0
0	0.6	0	0	5
0	0.8	0	0	5
0	1	0	0	5
0.2	0	0	0	0
0.2	0.2	0	0	0
0.2	0.4	0	0	5
0.2	0.6	0	0	5
0.2	0.8	0	0	5
0.2	1	0	0	4
0.4	0	0	0	0
0.4	0.2	0	0	0
0.4	0.4	0	0	5
0.4	0.6	0	0	4
0.4	0.8	0	0	4
0.4	1	0	0	4
0.6	0	0	0	0
0.6	0.2	0	0	5
0.6	0.4	0	0	5
0.6	0.6	0	0	4
0.6	0.8	0	0	4
0.6	1	4	4	4
0.8	0	0	0	0
0.8	0.2	0	0	5
0.8	0.4	0	0	4
0.8	0.6	4	0	4
0.8	0.8	4	5	4
0.8	1	4	5	4
1	0	0	0	0
1	0.2	0	0	5
1	0.4	4	0	4
1	0.6	4	5	4
1	0.8	4	5	4
1	1	4	5	4

Table 18. Effective lives for a seed treatment leading to a 60% reduction in AUDPC, the transpiration-based fungicide uptake model and a low fungicide breakdown rate.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	5
0	0.6	0	0	4
0	0.8	0	0	4
0	1	0	0	4
0.2	0	0	0	0
0.2	0.2	0	0	4
0.2	0.4	0	0	4
0.2	0.6	0	0	4
0.2	0.8	0	0	4
0.2	1	0	0	4
0.4	0	0	0	0
0.4	0.2	0	0	5
0.4	0.4	0	0	4
0.4	0.6	0	0	4
0.4	0.8	0	5	4
0.4	1	0	5	4
0.6	0	0	0	0
0.6	0.2	0	0	5
0.6	0.4	0	0	4
0.6	0.6	4	5	4
0.6	0.8	4	5	4
0.6	1	4	5	4
0.8	0	0	0	0
0.8	0.2	0	0	4
0.8	0.4	4	4	4
0.8	0.6	4	5	4
0.8	0.8	4	5	3
0.8	1	4	4	3
1	0	0	0	0
1	0.2	0	0	4
1	0.4	4	5	4
1	0.6	4	5	4
1	0.8	4	4	3
1	1	4	4	3

Table 19. Effective lives for a seed treatment with the same efficacy as a T1 foliar treatment, the constant fungicide uptake model and a low fungicide breakdown rate. In this scenario the ascospores initiating the epidemic in the next growing season were related to the infectious tissues densities on all leaf layers rather than only the top 5 leaf layers.

ST_dose	foliar_dose	ST_T1	ST_T2	ST_T1_T2
0	0	0	0	0
0	0.2	0	0	0
0	0.4	0	0	5
0	0.6	0	0	5
0	0.8	0	0	5
0	1	0	0	5
0.2	0	0	0	0
0.2	0.2	0	0	5
0.2	0.4	4	4	4
0.2	0.6	4	5	4
0.2	0.8	4	5	4
0.2	1	4	5	4
0.4	0	0	0	0
0.4	0.2	4	0	4
0.4	0.4	4	5	4
0.4	0.6	4	5	4
0.4	0.8	4	5	4
0.4	1	4	4	4
0.6	0	0	0	0
0.6	0.2	4	5	4
0.6	0.4	4	5	4
0.6	0.6	4	5	4
0.6	0.8	4	4	3
0.6	1	4	4	3
0.8	0	0	0	0
0.8	0.2	4	5	4
0.8	0.4	4	5	4
0.8	0.6	4	4	3
0.8	0.8	4	4	3
0.8	1	4	4	3
1	0	0	0	0
1	0.2	4	5	4
1	0.4	4	4	4
1	0.6	4	4	3
1	0.8	4	4	3
1	1	4	4	3