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Delaying Selection for Fungicide Insensitivity by Mixing Fungicides at a Low and High Risk of Resistance Development: A Modeling Analysis

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ABSTRACT

Hobbelen, P. H. F., Paveley, N. D., and van den Bosch, F. 2011. Delaying selection for fungicide insensitivity by mixing fungicides at a low and high risk of resistance development: A modeling analysis. *Phytopathology* 101:1224-1233.

This study used mathematical modeling to predict whether mixtures of a high-resistance-risk and a low-risk fungicide delay selection for resistance against the high-risk fungicide. We used the winter wheat and *Mycosphaerella graminicola* host-pathogen system as an example, with a quinone outside inhibitor fungicide as the high-risk and chlorothalonil as the low-risk fungicide. The usefulness of the mixing strategy was measured as the “effective life”: the number of seasons that the disease-

induced reduction of the integral of canopy green area index during the yield forming period could be kept <5%. We determined effective lives for strategies in which the dose rate (i) was constant for both the low-risk and high-risk fungicides, (ii) was constant for the low-risk fungicide but could increase for the high-risk fungicide, and (iii) was adjusted for both fungicides but their ratio in the mixture was fixed. The effective life was highest when applying the full label-recommended dose of the low-risk fungicide and adjusting the dose of the high-risk fungicide each season to the level required to maintain effective control. This strategy resulted in a predicted effective life of ≤ 12 years compared with 3 to 4 years when using the high risk fungicide alone.

The rate of selection for a fungicide-resistant pathogen strain depends on the difference in fitness compared with a sensitive pathogen strain. Resistance management strategies aim to reduce this difference without increasing the fitness of the sensitive strain, because this would lead to increased disease pressure (28). Strategies proposed include (i) choice of dose, (ii) constraints on the maximum number of applications, (iii) avoiding unnecessary fungicide applications, (iv) mixing of fungicides with different modes of action, (v) alternation of fungicides with different modes of action, and (vi) spatial or temporal heterogeneity in the use of fungicides (1,2,5,7,36).

The risk of resistance development varies between fungicides (6). For the analysis presented in this article, we define a “high-risk” fungicide as a fungicide for which a less sensitive strain is present in the pathogen population at the start of the period under consideration. As is commonly the case in practice, we assume that the less sensitive strain cannot be controlled adequately by the high-risk fungicide alone, when its frequency in the pathogen population has increased due to selection. We define a “low-risk” fungicide as a fungicide to which no resistant strain emerges in the pathogen population during the period under consideration. Low-risk fungicides are usually of multi-site mode of action. However, mixing a low-risk fungicide with one that is high-risk may make it possible to reduce the dose of the high-risk fungicide and, therefore, the selection for resistance against this fungicide, while obtaining good disease control (40).

Fungicide resistance management strategies are only useful if they both delay the selection for resistance and give sufficient

disease control. A number of field studies (13,22,26,30,31,34,46) has been published for a variety of host-pathogen systems that compare the selection for resistance against a high-risk fungicide between treatments either when mixed or not mixed with a low-risk fungicide. Although some of these field studies also considered disease control provided by the fungicides within one or more seasons, there has been no rigorous test of whether or not mixing increases the effective life of fungicides before resistance increases to such levels that effective disease control is no longer possible at or below the maximum permitted dose. Such a test would be difficult or impossible to achieve by experimentation but mathematical modeling may provide insight.

A number of modeling studies have been published on the usefulness of mixtures as a resistance management strategy (16–18,21,27,35,39). However, most of these models assumed exponential growth of fungal pathogen strains and did not account for the influence of seasonality on the dynamics of host and pathogen density. Therefore, they could not ensure that all the strategies compared provided a commercially acceptable level of disease control and, therefore, were not very relevant to practice. In addition, to the best of our knowledge, none of these models has been tested against independent experimental or observational data.

Hobbelen et al. (15) developed a mathematical model to predict the dynamics of fungicide resistance in cereal pathogens, which accounts for the seasonal dynamics of both the host and pathogen. It is also able to predict the effect of fungicide treatments on green canopy duration and, therefore, allows the use of a criterion for the usefulness of resistance management strategies which is relevant to commercial practice. This model was successfully tested by comparing model predictions with independent data on the development of fungicide resistance in powdery mildew (*Blumeria graminis* f. sp. *hordeii*) on spring barley (*Hordeum vulgare*) that were not used for model parameterization (15). In the present study, the model was parameterized for *Mycosphaerella graminicola*.

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sphaerella graminicola on winter wheat (*Triticum aestivum*). *M. graminicola* (causal organism of Septoria blotch) is an economically important pathogen in wheat-growing areas around the world (38). Fungicides play an important role in the control of *M. graminicola*, but the evolution and spread of resistance has reduced the efficacy of methyl benzimidazole carbamate (MBC) (14), strobilurin (43), and azole (12) fungicides. Given the length of the development and registration process of new fungicides (33), resistance management is necessary to preserve the efficacy of the fungicides that are currently on the market. This study used the model to determine whether mixing of a low-risk and high-risk fungicide increases the number of years that an acceptable level of disease control can be maintained in comparison with the high-risk fungicide applied alone. Different application strategies of the mixture and doses of fungicides in the mixture were explored.

MATERIALS AND METHODS

Model structure. The ordinary differential equation (ODE) model developed and tested by Hobbelen et al. (15) was used to describe selection for resistance against a high-risk fungicide in an *M. graminicola* population on winter wheat in response to applications of a mixture consisting of a high-risk and a low-risk fungicide.

The model describes the seasonal growth and senescence of the winter wheat canopy in order to account for the effect of the availability of host tissue on the growth of the pathogen population and for the effect of the pathogen on healthy host tissue. The model describes the growth of the combined area of leaves 1 to 3 (counting down from the flag leaf) in square meters of leaf area per square meter of ground during each growing season. Hereafter, we refer to these leaves as the “upper leaves” and use the term “density” to indicate the amount of square meters of leaf area per square meters of ground. The density of the total leaf area (A), which consists of the sum of the density of the healthy, dead, and infected leaf area, is assumed to increase according to the monomolecular equation (42) and reaches its maximum value (A_{\max}) at growth stage (GS) 39 on Zadoks’ scale (45):

$$dA/dt = \gamma(A_{\max} - A) \quad (1)$$

The growth of the density of the total leaf area is not affected by disease within the range of severities that is realistic in agronomic practice (11).

The development of the healthy leaf area density (H) consists of a growth phase, a plateau, and a senescence stage. The end of the growth, plateau and senescence phases correspond to GS 39, 61, and 87, respectively (4). In the absence of disease, the equation for healthy leaf area density is

$$dH/dt = \gamma(A_{\max} - A) - \sigma(t)H \quad (2)$$

where σ represents the senescence rate.

The *M. graminicola* population in the model consists of two strains. One strain is sensitive to both the high-risk and low-risk fungicide. The other strain is completely resistant to the high-risk fungicide but sensitive to the low-risk fungicide. Hereafter, subscript s denotes the fungal strain that is sensitive to both fungicides, and subscript r denotes the strain that is resistant to the high-risk fungicide. The life cycle of each strain is divided into a latent stage (L) and, subsequently, an infectious stage (I). Leaf tissue occupied by latent lesions remains green. The mean latent period is $1/\delta$. The leaf tissue dies when latent lesions become infectious (19). Therefore, leaf senescence decreases the density of latent lesions but not the density of infectious lesions. The length of the infectious period is $1/\mu$.

The healthy area of the upper leaves initially becomes infected with *M. graminicola* as a result of deposition of spores produced by infectious lesions on lower leaves. The density (F) and, therefore, spore production rate of these lesions is assumed to decline according to an exponential function

$$F(t) = F_0 e^{-\lambda t} \quad (3)$$

In this equation, λ represents the loss rate of infectious lesions on lower leaves due to reaching the end of the infectious period. We assume that a fraction θ of the infectious lesions on lower leaves (F) consists of the resistant strain. Parameter θ is kept constant during the growing season, because leaves 1 to 3 are assumed to intercept most of the fungicides applied. During the first simulated season, the fraction of resistant spores is set to an initial value θ_0 .

The rate at which an infectious lesion generates new infections, the transmission rate, is determined by the product of (i) the sporulation rate of infectious lesions; (ii) the probability that spores land on the upper leaves; (iii) the probability that a spore lands on healthy leaf tissue, given that it lands on these leaves; and (iv) the infection efficiency of spores. Points i, ii, and iv are combined in the compound parameter ρ . We account for point iii by multiplying parameter ρ by the fraction of the total area of leaves that consists of healthy leaf tissue, H/A . This makes the growth of the sensitive and resistant strain dependent on the availability of healthy host tissue.

Combining these assumptions and functional forms for the model components leads to the following equations describing the density of the healthy leaf area in the presence of disease and the densities of the latent and infectious leaf areas of the sensitive and resistant strains

$$dH/dt = \gamma(A_{\max} - A) - \rho_s(H/A)[I_s + (1 - \theta)F] - \rho_r(H/A)(I_r + \theta F) - \sigma(t)H \quad (4)$$

$$dL_s/dt = \rho_s(H/A)[I_s + (1 - \theta)F] - \delta_s L_s - \sigma(t)L_s \quad (5)$$

$$dI_s/dt = \delta_s L_s - \mu_s I_s \quad (6)$$

$$dL_r/dt = \rho_r(H/A)(I_r + \theta F) - \delta_r L_r - \sigma(t)L_r \quad (7)$$

$$dI_r/dt = \delta_r L_r - \mu_r I_r \quad (8)$$

The frequency of the resistant strain between growing seasons. Each growing season, the epidemic on the upper leaves is initiated by spores from a population on lower leaves. The fraction of resistant spores at the start of a growing season is assumed to be equal to the fraction of spores that is produced by the resistant strain at the end of the previous growing season (GS 87). This is similar to the fraction of infectious lesions that is resistant at the end of the previous season, because spore production rates by infectious lesions at the end of a growing season are not substantially affected by fungicides due to their decay. Denoting the leaf areas occupied by infectious lesions of the sensitive and resistant strain at the end of the previous season as I_s^- and I_r^- gives

$$\theta = \frac{I_r^-}{I_s^- + I_r^-} \quad (9)$$

The impact of fungicides on the pathogen life-cycle. Fungicide treatments affect the density of the strains by changing the values of pathogen life-cycle parameters. We assumed that the high-risk fungicide affects the infection efficiency (included in ρ_s) and the length of the latent period ($1/\delta_s$) of strain s and that the low-risk fungicide affects only the infection efficiencies of both strain s and r (included in ρ_s and ρ_r). These assumptions were based on the low-risk fungicide representing a protectant (e.g., chlorothalo-

nil) (32), while the high-risk fungicide represents a systemic material with eradicator and protectant activity (e.g., quinone outside inhibitor [QoI] fungicides) (22). The dependence of the infection efficiency of both strains and the length of the latent stage of the sensitive strain on the dose of the low-risk and/or high-risk fungicide is described by the functions

$$\rho_s = \rho[1 - \alpha_A(C_A)][1 - \alpha_B(C_B)] \quad (10)$$

$$\rho_r = \rho[1 - \alpha_A(C_A)] \quad (11)$$

$$1/\delta_s = 1/(\delta[1 - \alpha_B(C_B)]) \quad (12)$$

$$1/\delta_r = 1/\delta \quad (13)$$

In these equations, ρ represents the infection efficiency of the sensitive and resistant strain in the absence of fungicides. Parameter $1/\delta$ represents the length of the latent stage of the sensitive and resistant strain in the absence of fungicides. The term $\alpha_A(C_A)$ is the fraction by which parameters are reduced by the low-risk fungicide at dose C_A in absence of the high-risk fungicide. Similarly, $\alpha_B(C_B)$ is the fraction by which parameters are reduced by the high-risk fungicide at dose C_A in absence of the low-risk fungicide. We assumed that the low-risk and high-risk fungicides have independent modes of actions in a mixture and we multiplied the effect of the low- and high-risk fungicides on the infection efficiency of the sensitive strain (20). The length of the latent stage of the resistant strain is not affected by fungicides, because the low-risk fungicide does not target this stage in the life-cycle and resistance to the high-risk fungicide was assumed to be complete.

Dose-response curves. The fractions $\alpha_A(C_A)$ and $\alpha_B(C_B)$ depend on the fungicide concentrations according to the functions

$$\alpha_A = \alpha_{A,\max}(1 - e^{-\beta_A C_A}) \quad (14)$$

$$\alpha_B = \alpha_{B,\max}(1 - e^{-\beta_B C_B}) \quad (15)$$

In these equations, parameters $\alpha_{A,\max}$ and $\alpha_{B,\max}$ are the maximum reductions of the target parameters for the low-risk and the high-risk fungicides, respectively. Parameters β_A and β_B determine the curvature of the dose-response curves.

Decay of fungicides. The decay of the fungicide concentrations is modeled as

$$dC_A/dt = -v_A C_A \quad (16)$$

$$dC_B/dt = -v_B C_B \quad (17)$$

with decay rates v_A and v_B for the high-risk and the low-risk fungicides, respectively.

A degree-day scale was used to easily incorporate temperature effects on the growth of the host and the pathogen.

Parameter estimation. A summary of the definitions, values, and dimensions of parameters is given in Table 1. Parameters that were reported in the literature on a time scale of days were converted to a degree-day scale using a lower threshold temperature of 0°C and the average temperature during the growing season in Cambridgeshire in the United Kingdom during the years 1984 to 2003 (Met Office, United Kingdom, published online), 15.2°C.

The canopy of winter wheat. Using a phyllochron of 122 degree-days (4), it takes 366 degree-days from the emergence of leaf 3 to GS39. The number of accumulated degree-days from GS 39 to GS 61 and from GS 61 to GS 87 was estimated from data on the average development of winter wheat in the United Kingdom (4) and the average pattern in daily temperatures during the growing season in Cambridgeshire in the United Kingdom during

TABLE 1. Definitions and values of model parameters

Parameters	Definition	Value	Dimension ^a	Literature citation ^b
Host				
γ	Growth rate of leaf area	1.26E-2	t ⁻¹	4
A_{\max}	Maximum density of leaf area	4.1	Density	4
σ	Senescence rate	Equation 18	t ⁻¹	4, MO
Pathogen strain				
Sensitive and resistant				
F_0	Combined initial density of infectious lesions of the sensitive and resistant strain on lower leaves ^c	1.09E-2	Density	NP
λ	Rate at which F_0 decreases	8.5E-3	t ⁻¹	4,29
Sensitive				
ρ, ρ_s	Transmission rate in the absence (ρ) and presence (ρ_s) of fungicides ^d	2.08E-2, equation 10	t ⁻¹	NP, NA
$1/\delta, 1/\delta_s$	Length of the latent stage in the absence ($1/\delta$) and presence ($1/\delta_s$) of fungicides	266, equation 12	t	4, NA
$1/\mu_s$	Length of the infectious stage	456	t	10
Resistant				
θ_0, θ	Frequency of the resistant strain at the start of the first (θ_0) or later seasons (θ)	1E-5, equation 9	...	NA
ρ, ρ_r	Transmission rate in the absence (ρ) and presence (ρ_r) of fungicides ^d	2.08E-2, equation 11	t ⁻¹	NP, NA
$1/\delta, 1/\delta_r$	Length of the latent stage in the absence ($1/\delta$) and presence ($1/\delta_r$) of fungicides	266, equation 13	t	4
$1/\mu_r$	Length of the infectious stage	456	t	10
Fungicide parameters				
Low-risk				
v_A	Decay rate	6.91E-3	t ⁻¹	MO, NP
α_A	Reduction of the infection efficiency	Equation 14	...	NA
$\alpha_{A,\max}$	Maximum reduction of the infection efficiency	0.48	...	32
β_A	Shape parameter of the dose-response curve (see text)	9.9	...	32
High-risk				
v_B	Decay rate	1.11E-2	t ⁻¹	15
α_B	Reduction of the target parameters	Equation 15	...	NA
$\alpha_{B,\max}$	Maximum reduction of the target parameters	1	...	22
β_B	Shape parameter of the dose-response curve (see text)	9.6	...	22

^a Parameters: t = degree-days, Area = square meters of leaf area per square meter of ground, and ... = dimensionless.

^b MO = Met Office, United Kingdom, published online; NP = unpublished data, N. Paveley (see text for description); and NA = not applicable.

^c Lower leaves are leaves that emerged before leaf 3, when counting down from the flag leaf (flag leaf = 1).

^d A compound parameter.

the years 1984 to 2003 (Met Office, United Kingdom, published online). Accumulated degree-days from GS 39 to 61 and from GS 61 to 87 were calculated by summing the average daily temperatures between the dates that corresponded to these growth stages (343 and 849, respectively). The value of A_{\max} was derived from data on the density of green area at GS 39 during an average growing season in the United Kingdom (4) using the estimation that 85% of the green area at GS 39 consists of leaf area and that leaves 1 to 3 constitute 74% of this leaf area (4). We chose the growth rate of leaves (γ) such that the leaf area at GS 39 was 99% of the maximum leaf area. The senescence rate increases exponentially from ≈ 0 ($<1E-7$) at GS 61 to a maximum value of 0.1050 at GS 87 according to the function

$$\sigma(t) = \begin{cases} 0, & t < t_{GS61} \\ 0.005 \left(\frac{t - t_{GS61}}{t_{GS87} - t_{GS61}} \right) + 0.1e^{-0.02(t_{GS87} - t)}, & t \geq t_{GS61} \end{cases} \quad (18)$$

This reduces the healthy leaf area at GS 87 to $<1\%$ of the maximum leaf area, which approximates complete senescence. In all model simulations, we used $0.05 \text{ m}^2/\text{m}^2$ of ground as an initial density for the total (A) and healthy (H) leaf area. The predicted development of the healthy area of upper leaves of winter wheat in the absence of disease is shown in Figure 1A.

Disease density. The development rate of the latent stage of the sensitive strain in the absence of fungicides (δ_s) was calculated as the inverse of the length of the latent stage, which was taken from Lovell et al. (23). The mortality rate of infectious lesions of the sensitive strain (μ_s) was calculated as the inverse of the length of the infectious period, which was assumed to be 30 days (10). The development rate of the latent stage of the resistant (δ_r) and sensitive strain (δ_s) and the mortality rate of both strains (μ_r and μ_s) are equal in the absence of fungicides, because we do not assume fitness costs of resistance. The initial density of infectious lesions (F_0) at lower leaves and the transmission rate of the

sensitive strain in the absence of fungicides (ρ_s) were estimated by fitting the model to disease severity data (41) for leaves 1 to 3 of winter wheat crops, that were not treated with fungicides (Fig. 2). The data set contained disease severities for 35 site-year-cultivar experiments, including 10 sites across the United Kingdom, 8 different years within the period 1994 to 2002, and 12 susceptible cultivars. Only site-year-cultivar combinations with maximum disease severities $\geq 5\%$ and susceptible cultivars with host resistance ratings for Septoria blotch ≤ 5 were included in the data set. In order to obtain a data set representing disease severity through time, the growing season was divided into five equal intervals. Only site-year-cultivar combinations with data points in all intervals were included in the data set. The transmission rate of the resistant (ρ_r) and sensitive (ρ_s) strain are equal in the absence of fungicides, because we do not assume fitness costs of resistance. The number of degree-days between the emergence of leaf 3 and complete senescence of the lower leaves was estimated from data on the emergence of leaf 3 and 4 (4) and the life time of leaf 4 in degree-days (29), and amounted to 544. Parameter λ was then calculated by substitution of the estimate for F_0 , $t = 544$, and $F = 0.01F_0$ into the equation for F . The predicted development of the density of healthy and infected leaf area in the absence of fungicide treatments is shown in Figure 1B.

Fungicides. Chlorothalonil (chloronitriles, FRAC number M5) was used as an example of a low-risk fungicide and pyraclostrobin (QoIs, FRAC number 11) as a high-risk fungicide. The half-life time of chlorothalonil was set to 6.6 days (8). The half-life of pyraclostrobin was set to 4.1 days, as reported for the QoI fungicide azoxystrobin (15). Dose-response curves for chlorothalonil and pyraclostrobin reported in the literature describe the relationship between the disease severity at a certain time after spraying and the applied fungicide dose (22,32). The disease severity at a certain point in time after spraying depends on many factors, including the weather and the decay of fungicides. Therefore, these dose-response curves are different from those in our model, which describe the instantaneous relationship between a fungicide concentration and the values of pathogen life-cycle parameters. Therefore, to parameterize the dose-response curves of the pyraclostrobin and chlorothalonil, the model was fitted to data. For chlorothalonil, we used data on the severity of Septoria

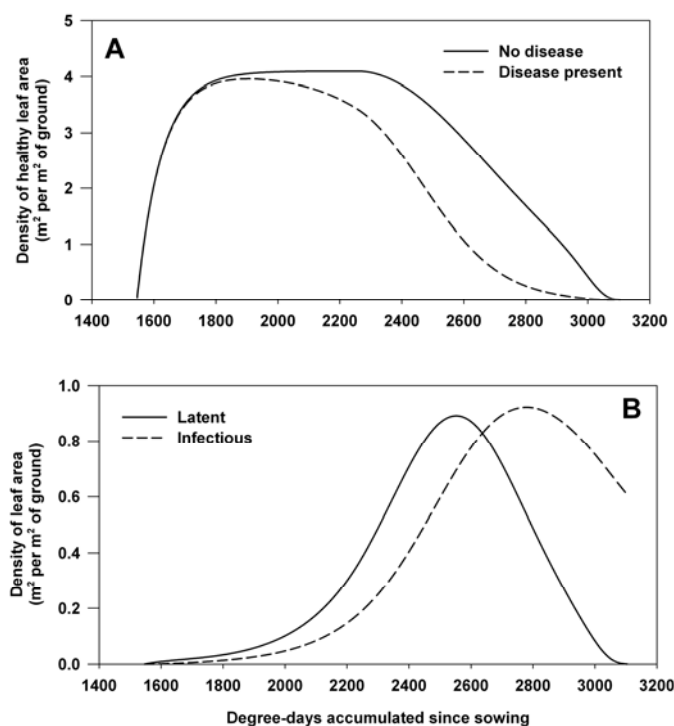


Fig. 1. Predicted seasonal development of leaves one to three (counting down from the flag leaf) of the winter wheat canopy in the absence and presence of *Mycosphaerella graminicola* without fungicide treatments. **A**, Development of the density of healthy leaf area and **B**, development of the density of latent and infectious leaf areas for the default epidemic.

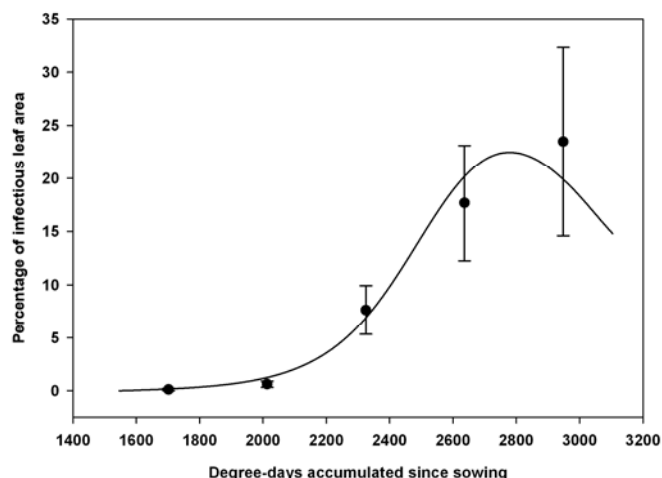


Fig. 2. Data on the progress of Septoria blotch on leaves one to three (counting down from the flag leaf) of winter wheat in the absence of fungicide treatments (dots) and the predicted disease progress (line) that was determined by fitting the model to the data. Data set contained disease severities for 35 site-year-cultivar experiments, including 10 sites across the United Kingdom, 8 different years within the period 1994 to 2002, and 12 susceptible cultivars. To show the temporal trend in the disease severity data, the growing season was subdivided into five equal intervals. Dots represent the mean disease severity and the vertical bars show the 95% confidence interval of the mean disease severity for each time interval.

blotch on leaves 1 and 2, and 3 and 4, of winter wheat as a function of the fungicide dose, averaged across several sites and years in the United Kingdom, and a number of wheat cultivars (32). Disease severities were assessed 4 and 6 weeks after a single spray at approximately GS 37 to 39, respectively. The average of the disease severities was used as an estimate of the disease severity on the upper leaves 5 weeks after a single spray. The transmission rate parameter was adjusted such that the predicted disease severity in the absence of fungicides became similar to the observed severity in absence of fungicides. The dose-response curve parameters of chlorothalonil were then estimated by fitting the model to the observed disease severities and the corresponding chlorothalonil doses. The dose-response curve parameters for pyraclostrobin were determined using both a data set (22) and methods similar to those described for chlorothalonil. The dose-response curve for this fungicide should be fitted to data on the impact of pyraclostrobin on a completely sensitive pathogen population, because pyraclostrobin only affects the sensitive strain. Therefore, we only used dose-response data for year 2001, when resistance against pyraclostrobin was at a very low frequency. The spray time in this data set was GS 32 and disease severities on leaves 3 and 4, and 1 and 2, were assessed 3 and 6 weeks after a single spray.

Criterion for the usefulness of a fungicide resistance management strategy. The success of a resistance management strategy was quantified here as the number of consecutive growing seasons that a treatment is able to keep the disease-induced loss of healthy area duration (HAD) (44) below a threshold value. Hereafter, this period will be denoted as the “effective life” of the high-risk fungicide when used in a particular resistance management strategy. According to the definition of HAD by Waggoner and Berger (44), the healthy area consists of green area available for photosynthesis. In the case reported here, HAD includes leaf tissue occupied by latent *Septoria* blotch lesions. A HAD loss of 5% was set as a default for this threshold and a sensitivity analysis was performed to determine the effect of deviations of the threshold value on the effective life. Hereafter, the threshold for disease-induced loss of HAD is denoted as the HAD threshold.

HAD was calculated as the area under the healthy (*H*) and latent (*L*) leaf area density curves between GS 61 (anthesis) and GS 87 (end of grain filling). Yield of winter wheat is approximately proportional to the HAD over this period (9,44). In order to calculate HAD on a time scale of days, we calculated the number of accumulated degree-days since sowing that corresponded to each day in the period GS 61 to 87 using data on the average pattern in daily temperatures in Cambridgeshire in the United Kingdom during the years 1984 to 2003 (Met Office, United Kingdom, published online).

Selection ratio. To explain the effect of the dose of the low-risk and high-risk fungicides in a mixture on effective life, we determined the selection that a mixture exerts on the resistant strain by calculating selection ratios (SR) (15) according to the equation

$$SR = f_{\text{end season}} / f_{\text{before spraying}} \quad (19)$$

where $f_{\text{before spraying}}$ and $f_{\text{end season}}$ stand for the frequency of the resistant strain in the pathogen population before the first fungicide application and at the end of the growing season, respectively. Thus, the selection ratio is the factor by which the frequency of the resistant strain is multiplied over one growing season.

Model simulations. Hereafter, the term “dose” indicates the number of liters of a fungicide applied per hectare per spray and the term “total dose” indicates the total number of liters of fungicide applied per hectare during one growing season. For the model simulations, the maximum dose of the low-risk and high-risk fungicides were set to their respective label recommended doses (which is the maximum permitted dose, referred to hereafter as the “label dose”). All simulated fungicide programs con-

sisted of two sprays per growing season. The first spray was applied at the full emergence of leaf 3 (GS 32) and the second spray was applied at complete emergence of leaf 1 (GS 39), counting down from the flag leaf (designated leaf 1). These spray times correspond to the T1 and T2 spray that are commonly used in spray programs for the control of *M. graminicola* (3).

The effect of the dose of the high-risk and low-risk fungicides in a mixture on effective life. The effective life of mixtures of the high-risk and low-risk fungicides was determined for three application strategies.

Strategy 1: constant doses of the low-risk fungicide and the high-risk fungicide. In this strategy, the doses of both fungicides in the mixture were constant throughout consecutive growing seasons. Simulations were performed to determine effective lives for concentrations of the low-risk fungicide that varied from nil to the label dose in steps of 10%. For each dose of the low-risk fungicide, we varied the dose of the high-risk fungicide from nil to the label dose in steps of 1%.

Strategy 2: constant dose of the low-risk fungicide, variable dose of the high-risk fungicide. In this strategy, the dose of the low-risk fungicide was kept constant throughout consecutive growing seasons. At the start of the first growing season, the dose of the high-risk fungicide was adjusted in steps of 1% of the label dose to the lowest amount of this fungicide that was needed to keep the disease-induced HAD loss below the HAD threshold during this growing season. This procedure was repeated for all subsequent growing seasons. This strategy represented one approach to the deployment of a tank mixture of two active substances. Model simulations were performed to determine the effective life of mixtures with concentrations of the low-risk fungicide that varied from nil to the label dose in steps of 10%. Simulations ended when the disease-induced HAD loss exceeded the threshold and disease control by the mixture could not be increased further without exceeding the label dose of the high-risk fungicide.

Strategy 3: variable dose with a constant ratio of the low-risk fungicide and high-risk fungicide in the mixture. In this strategy, the ratio of the dose of the low-risk fungicide and high-risk fungicide in the mixture was kept constant but the applied dose of the mixture was allowed to increase between seasons. This strategy represented the use of a formulated mixture of two active substances. Simulations were performed with initial doses of the low-risk fungicide varying from nil to the label dose in steps of 10%. The initial dose of the high-risk fungicide was set at the lowest amount of this fungicide that had to be added to the low-risk fungicide in order to keep the disease-induced HAD loss $\leq 5\%$. This was determined by increasing the dose of the high-risk fungicide in the model simulations in steps of 1% of the label dose, starting at nil. For each pair of initial doses, we subsequently performed model simulations to determine the effective life. During the simulations, if the disease-induced HAD loss was $>5\%$, the dose of both fungicides was increased in between seasons by multiplying the dose of both fungicides by the same factor. The value of this multiplication factor was calculated such that the average of the increase in the dose of the low-risk fungicide and the increase of the dose of the high-risk fungicide, as a percentage of their label doses, was 1%. This was done to improve the comparison of effective lives between strategy 3 and strategies 1 and 2, where the minimum increase in the dose of high-risk fungicide was 1% of the label dose. If one multiplication step was not enough to reduce the disease-induced HAD loss below the 5% threshold, the dose increase was repeated until the disease-induced HAD became $\leq 5\%$. Simulations ended when the disease-induced HAD loss became $>5\%$ and disease control by the mixture could not be further increased without exceeding the label dose of one or both of the fungicides.

The optimum dose of the high-risk fungicide in mixtures as a function of the control and severity of the epidemic. The maxi-

num loss of HAD that may be acceptable for a grower will vary depending on the costs of spraying fungicides and the expected gain in income due to increased yield. Changes in the HAD threshold and, therefore, in the required level of disease control, will affect the dose of the high-risk fungicide that results in the maximum effective life of the mixture. This was explored by performing simulations to determine the range of optimum doses of the high-risk fungicide for HAD-loss thresholds of 2 to 10%. Simulations were performed using application strategy 1 with the low-risk fungicide at the label dose.

Changes in the severity of epidemics are likely to affect the doses of the high-risk fungicide which maximize the effective life of the mixture. To study this, we created a number of epidemics with different severities by multiplying the transmission rates (ρ) by a common factor of 0.8 to 1.5 in steps of 0.1. For values of the multiplication factor <0.8 , the low-risk fungicide alone was able to control the *M. graminicola* epidemic. For values of the multiplication factor >1.5 , the epidemic was too severe to be sufficiently controlled by a mixture of the two fungicides at their label doses. In the absence of fungicides, this resulted in epidemics with maximum disease severities of 13.1 to 36.9% and disease-induced HAD loss of 15.3 to 57.2%. Simulations were performed to determine the range of optimum doses of the high-risk fungicide for severity of epidemic, using application strategy 1 with the low-risk fungicide at the label dose.

RESULTS

Selection ratios. The selection ratio remained approximately constant during the effective life of fungicide mixtures for all application strategies, except during the last 1 or 2 years of the effective life, when the selection ratio sometimes sharply decreased as the frequency of the resistant strain at the start of the season increased. High selection ratios cannot occur with high initial frequencies.

For a given dose of the low-risk fungicide, the median selection ratio during the effective life of a fungicide mixture increased with increasing dose of the high-risk fungicide for strategy 1 (Fig. 3). For a given dose of the high-risk fungicide, increases in the dose of the low-risk resulted in a decrease of the median selection ratio during the effective life of a fungicide mixture (Fig. 3). The dose of the high-risk and low-risk fungicides could not be varied separately in strategies 2 and 3, because the dose of the high-risk fungicide was adjusted to the lowest dose that gave sufficient

disease control. For these strategies, a combination of low doses of the high-risk fungicide with high doses of the low-risk fungicide resulted in the lowest selection ratios, whereas a combination of high doses of the high-risk fungicide with low doses of the low-risk fungicide resulted in the highest selection ratios.

The optimum dose of the low-risk fungicide in mixtures with the high-risk fungicide, to maximize effective life. For all strategies, the effective life of a mixture of the high-risk with the low-risk fungicide was higher than spray programs consisting of the high-risk fungicide alone. For strategies 1 and 2, the gain in the effective life of the high-risk fungicide due to mixing with the low-risk fungicide increased with the dose of the low-risk fungicide and was longest for mixtures with levels of the low-risk fungicide equal to the label dose (Table 2). For strategy 3, the gain in effective life of the high-risk fungicide due to mixing with the low-risk fungicide first increased with an increasing initial dose of the low-risk fungicide, then stabilized and subsequently decreased (Table 2). This was because the maximum factor by which the fungicide doses in the mixture could be increased in order to maintain disease control became smaller when initial doses of the low-risk fungicide were closer to the label dose.

The optimum dose of the high-risk fungicide in mixtures with the low-risk fungicide, to maximize effective life. For strategy 1 at all doses of the low-risk fungicide, the effective life decreased sharply to zero when the dose of the high-risk fungicide was decreased below its optimum range, because effective control could not be achieved even with a sensitive population. For doses of the high-risk fungicide above the optimum range, the effective life decreased more gradually with increasing dose of the high-risk fungicide (Fig. 4).

The upper and lower boundary of the range of optimum doses of the high-risk fungicide decreased and the range became smaller at higher doses of the low-risk fungicide (Fig. 5). The lower boundary of the range of optimum doses was always close to the minimum dose needed to reduce the HAD loss to or below its threshold value during the first simulated year (Fig. 5).

Given a certain dose of the low-risk fungicide during the first season, the initial dose of the high-risk fungicide for strategies 2 and 3 was similar and set to the lowest dose needed to reduce the HAD loss to or below the threshold value. This initial dose of the high-risk fungicide decreased when the dose of the low-risk fungicide increased from nil to the label dose (Fig. 5).

For strategies 2 and 3, the doses of the fungicides in the mixture stayed at the initial level for several years (Figs. 6 and 7) until the frequency of the resistant strain in the fungal population reached levels of 1 to 5%. From that time, the dose of the high-

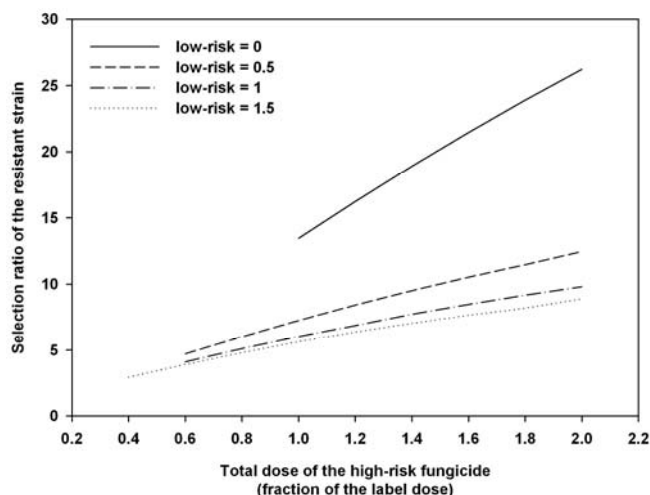


Fig. 3. Effect of the total dose of the high-risk fungicide on the median selection ratio during the effective life of a mixture of the high-risk and the low-risk fungicide. Simulations were performed for total doses of the low-risk fungicide of 0 (solid), 0.5 (dashed), 1 (dash-dot), and 1.5 (dotted) times the label dose. Doses of both fungicides were kept constant in time.

TABLE 2. Maximum effective life of mixtures of the high-risk and the low-risk fungicide for different total applied doses of the low-risk fungicide in the mixture^a

Dose	Effective life (years)		
	Strategy 1	Strategy 2	Strategy 3
0	3	4	4
0.2	4	5	6
0.4	5	6	7
0.6	6	7	8
0.8	7	8	9
1.0	7	9	9
1.2	8	10	10
1.4	8	11	10
1.6	9	11	10
1.8	9	12	10
2.0	10	12	7

^a Simulations were performed for three mixture strategies (see text). For strategy 3, the first column of the table represents the initial total applied dose of the low-risk fungicide during the first simulated year. The doses of high-risk fungicide in the mixture were optimized according to the different strategies. Total dose of low-risk fungicide (fraction of label dose).

risk fungicide (strategy 2) or both fungicides (strategy 3) needed to be increased sharply to maintain the HAD loss equal to or below the threshold value.

Comparison of strategies. A comparison of the model predictions for the different application strategies showed that strategy 2 resulted in the highest effective life for a mixture of a high-risk and a low-risk fungicide (and, therefore, for the high-risk fungicide), followed by strategy 3, then 1 (Table 2). For similar doses of the low-risk fungicide, the effective life of the high-risk fungicide for application strategy 2 was always higher than for the effective life for application strategy 1. The gain in effective life of the high-risk fungicide by using application strategy 2 instead of 1 increased with increasing dose of the low-risk fungicide (Table 2). The gain in effective life of the high-risk fungicide by using application strategy 3 instead of 1 initially increased and then decreased again with increasing initial dose of the low-risk fungicide in the mixture (Table 2).

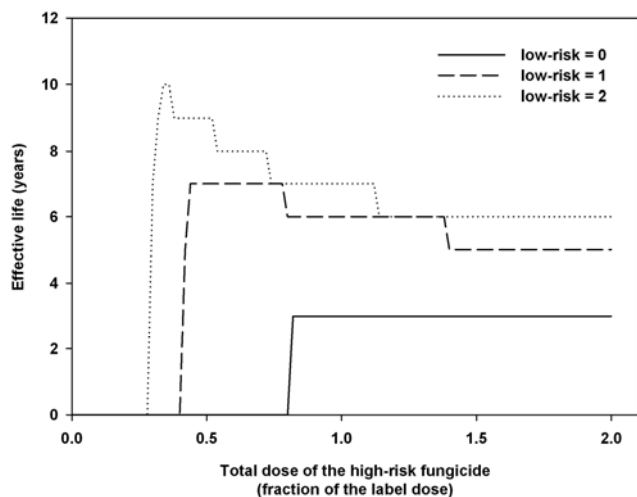


Fig. 4. Predicted effect of the total dose of the high-risk fungicide on the effective life of mixtures of the high-risk and the low-risk fungicide. Simulations were performed for total doses of the low-risk fungicide amounting to 0 (solid), 1 (dashed), and 2 (dotted) times the label (strategy 1, described in text) dose. Total doses of both fungicides were kept constant in time.

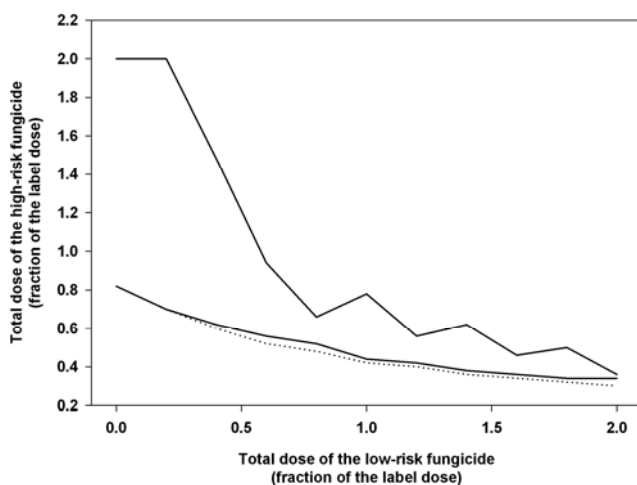


Fig. 5. Predicted effect of the total dose of the low-risk fungicide on the range of total doses of the high-risk fungicide for which the effective life of a mixture of both fungicides was highest. Lower and upper solid line represent the lower and upper boundary of this dose range, respectively. Total doses of both fungicides were kept constant in time. Dotted line indicates the minimum total dose of the high-risk fungicide that reduces the disease-induced healthy area duration loss to <5% during the first simulated season as a function of the total dose of the low-risk fungicide in the mixture.

The optimum dose of the high-risk fungicide in mixture with the low-risk fungicide as a function of the severity of the *M. graminicola* epidemic and the level of disease control that is required. The range of doses of the high-risk fungicide that resulted in the highest effective life of mixtures of a high-risk and a low-risk fungicide were calculated for HAD thresholds of 2 to 10%. In all simulations, strategy 1 was used, with the low-risk fungicide at the label dose. The model predicted that the upper and lower boundary of the optimum range for dose of the high-risk fungicide increased when the HAD threshold was decreased from 10 to 2% (Fig. 8A). The range of optimum values sharply increased at HAD threshold values <5% (Fig. 8A).

Increasing the severity of the epidemic increased the optimum range for the dose of the high-risk fungicide (Fig. 8B). The range of optimum doses became larger when the severity of the epidemic to be controlled was increased but decreased again after the upper boundary of the range of optimum doses had reached its maximum value (Fig. 8B).

DISCUSSION

We used a mathematical model to explore whether the selection for resistance against a high-risk fungicide can be slowed by applying it in a mixture with a low-risk fungicide. As far as we know, this is the first study that addresses this question using (i) a model that has successfully been tested against independent data (15); (ii) a model that accounts for the seasonality in the development of the canopy and the pathogen and, thus, accounts for resource-dependent growth of the pathogen; and (iii) a measure for the usefulness of a resistance management strategy that accounts for the need to obtain and retain a commercially acceptable level of disease control.

The model simulations suggest that mixing a low-risk fungicide with a high-risk fungicide can substantially increase the effective life of the high-risk fungicide. For all application strategies, the gain in effective life of a high-risk fungicide increased with the dose of the low-risk fungicide in the mixture. Much of this effect is explained by higher doses of the low-risk fungicide reducing the dose of the high-risk fungicide that is necessary to obtain sufficient disease control. A reduced dose of the high-risk fungicide results in a smaller difference in the fitness between the sensitive and the resistant strain and, therefore, less selection for resistance. However, not all of the beneficial effect of the mixture was due to the reduced dose required of the high-risk component. The effective life of a mixture with both the low-risk and high-risk fungicides applied at the label dose was longer than the

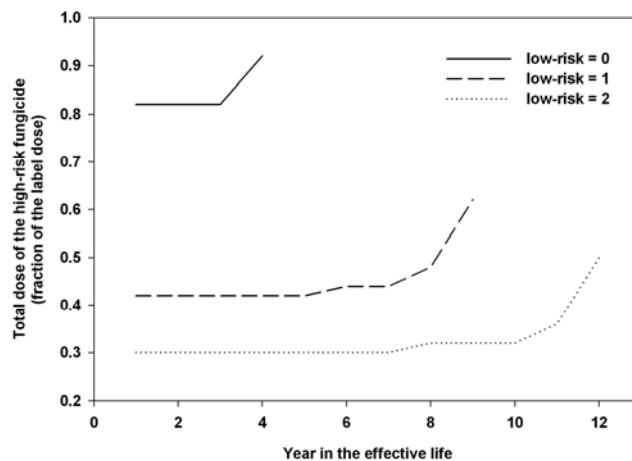


Fig. 6. Dynamics of the minimum total dose of the high-risk fungicide that is needed to reduce the disease-induced loss of healthy area duration to <5% for total doses of the low-risk fungicide amounting to 0 (solid), 1 (dashed), and 2 (dotted) times the label dose.

effective life of the high-risk fungicide when applied alone. This suggests that, even for constant doses of the high-risk fungicide, the selection for resistance decreases with increasing doses of the low-risk fungicide.

For similar (initial) doses of the low-risk fungicide, the results show that application strategies, which include adjustment of fungicide doses in time, result in a longer effective life than application strategies with constant doses. This is because strategies with adjustable doses enable the use of a low initial dose (to minimize selection) and a high dose later to adjust for loss of efficacy as the frequency of the resistant strain increases. The adjustment of fungicide doses in time can be achieved by using tank mixtures (strategy 2) or formulated mixtures (strategy 3). The advantage of using tank mixtures in comparison with formulated mixtures is the possibility to apply the low-risk fungicide at the label dose while adjusting the dose of the high-risk fungicide to the level needed to obtain sufficient disease control. This is the strategy with the highest effective life. When using a preformulated mixture, the possibility to increase the dose of both fungicides is limited at high doses of the low-risk fungicide. This explains the decrease in the effective life of the formulated mixture for dose rates of the low-risk fungicide >90% of the label dose. However, in addition to ease of use, an advantage of formulated mixtures in comparison with tank mixtures is the fact that they contain optimal concentrations and combinations of adjuvants, which may benefit efficacy and minimize the risk of phytotoxicity.

In practice, growers could adjust fungicide doses at the start of a growing season based on dose-response curves of fungicides in the previous growing season and on the decrease in the efficacy of fungicides between previous growing seasons. If dose-response curves are not determined frequently, our simulations suggest (Figs. 6 and 7) that growers could initially apply a low dose of the high-risk fungicide in a tank mixture or apply a low dose of the

preformulated mixture. When disease control becomes insufficient, growers could subsequently switch to the label-recommended dose of the high-risk fungicide in the tank mixture or the label-recommended dose of a preformulated mixture.

The results described above differ from current advice issued by FRAC (5), which suggests that both components of a fungicide mixture should be at a dose which would provide effective control if used alone. This advice may be appropriate during the adjustment phase of resistance development, when growers need to maintain control despite one of the components having lost efficacy. However, use of high doses of both components may, in some circumstances, accelerate selection.

Experimental studies have shown that selection for a resistant strain decreased when the dose of the high-risk fungicide decreased for a given dose of the low-risk fungicide in the mixture (13,25,34). A number of experimental studies compared the selection for resistance by a mixture of a high-risk and a low-risk fungicide with the selection for resistance by the high-risk fungicide applied alone at a similar dose to that applied in the mixture. These studies show that selection for resistance was approximately equal to (26,30) or lower than (13,31,34) the selection pressure by the high-risk fungicide alone. Hence, most results are in agreement with the model predictions and support the conclusion that mixtures increase the effective life of the high-risk fungicide.

Previous modeling studies, that did not account for density dependence and assumed exponential growth of pathogen strains, showed that the selection for resistance by the high-risk fungicide may decrease when mixed with a low-risk fungicide (27,39) but may also remain unchanged or even increase depending on assumptions about the spray coverage (18) and the type of inter-

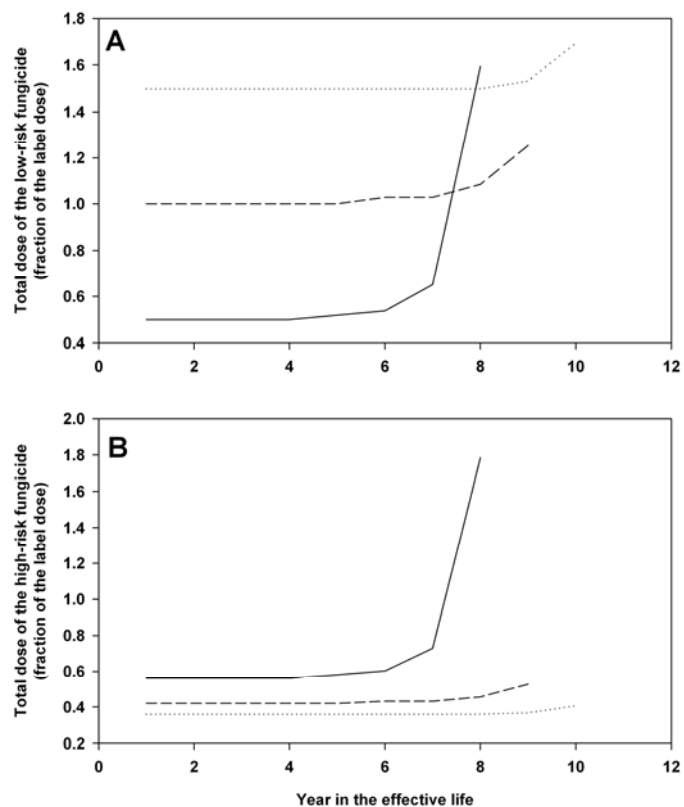


Fig. 7. Dynamics of the total dose of the **A**, low-risk and **B**, high-risk fungicide for strategy 3 (in which the ratio of the two fungicides in the mixture remains constant) for initial total doses of the low-risk fungicide of 0.5 (solid line), 1 (dashed line), and 1.5 (dotted line) times the label dose.

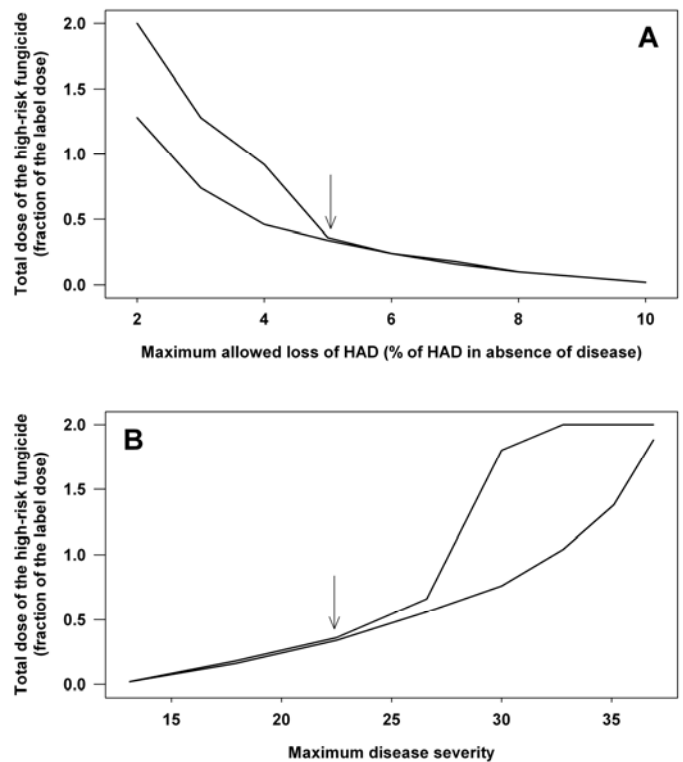


Fig. 8. Effect of **A**, the healthy area duration (HAD) threshold and **B**, the severity of the epidemic in the absence of fungicides on the range of total doses of the high-risk fungicide for which the effective life of a mixture of a high-risk and a low-risk fungicide is maximum. Upper and lower lines represent the upper and lower boundaries of the dose range. Total dose of the low-risk fungicide was kept at two times the label dose and the total doses of both fungicides were constant in time. Arrows indicate the default values of the acceptable HAD loss and the disease severity in the absence of fungicides that were used in the model simulations in this article.

action between the high-risk and the low-risk fungicide on the growth rate of the pathogen population (21). However, Shaw (35) shows that, for this type of model, the low-risk fungicide is unlikely to affect the selection pressure on the resistant strain when (i) the high-risk fungicide is systemic and (ii) the low-risk fungicide is protectant and the high-risk and low-risk fungicides target different stages in the life cycle of the pathogen. This differs from our model predictions for a mixture of a protectant and systemic fungicide. This difference may be accounted for by the model reported here accounting for the availability of healthy leaf area on the growth of the fungal pathogen and for differences in the sensitivity of different developmental stages of the fungal pathogen to the two types of fungicide.

The benefit to effective life from a high dose of a low-risk fungicide in a mixture needs to be balanced against any resulting increase in the environmental impact of the mixture (24,37). However, the benefit to effective life is most sensitive to changes in the dose of the low-risk fungicide from ≈ 0 to 120% (e.g., up to two applications, each of 60% of the maximum dose permitted per application). Such doses are commonly used currently in the United Kingdom (The Food and Environment Research Agency, United Kingdom, published online). Without such use, higher doses of high-risk fungicides would be required.

In the work reported, here we reparameterized a model reported previously (15) which used the same model structure to describe the development of resistance in powdery mildew on spring barley. This study focused on a host–pathogen system consisting of winter wheat and *M. graminicola*. However, the development of the canopy of different cereal species proceeds through similar stages (38) and the life cycle of most fungal foliar pathogens of cereal crops consists of similar developmental stages. Therefore, the basic model structure should be widely applicable to cereal crops and their fungal pathogens, given parameters appropriate to different cereal crops and pathogens. Hence, the qualitative conclusions about the benefits of mixtures of low-risk and high-risk fungicides to resistance management are likely apply to other cereal crops and their fungal pathogens. The findings may differ for indeterminate host species for which density dependence may be less influential. The stages in the life cycle of a fungal pathogen that are affected may differ between fungicides. In this study, we determined the effective life of the different mixture strategies assuming that the high-risk fungicide affects the infection efficiency and the length of the latent stage. Additional model simulations showed that the qualitative conclusions from the study do not change when the high-risk fungicide affected the sporulation rate and length of the infectious stage in addition to the life-cycle stages mentioned above. This suggests that the conclusions in this study are likely to hold for mixtures of low-risk fungicides with different types of high-risk fungicides.

In this study, a fungal strain resistant to the high-risk fungicide was assumed to be present in the fungal population at a very low frequency from the start of the simulations. In reality, when a new mode of action is introduced, a resistant fungal strain may still need to arise through mutation or may be present in such low densities that it is likely to die out due to stochastic processes. The effect of fungicide treatments on the dynamics of the resistant strain during this stochastic phase cannot be described using the model presented in this article. Therefore, the conclusions in this study apply to the selection phase, after the emergence of the resistant strain in the fungal population.

Mixing a high-risk fungicide with a low-risk fungicide should be a useful strategy to delay the development of resistance against the high-risk fungicide and increase its effective life. The results suggest that the effective life of a high-risk fungicide is highest for tank mixtures with the low-risk fungicide at the label recommended dose and with the dose of the high-risk fungicide adjusted each season as required to maintain a commercially acceptable level of disease control.

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