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Derivation and testing of a model to predict selection for fungicide resistance

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A mathematical model was derived to predict selection for fungicide resistance in foliar pathogens of cereal crops. The model was tested against independent data from four field experiments quantifying selection for the G143A mutation conferring resistance to a quinone outside inhibitor (QoI) fungicide in powdery mildew (*Blumeria graminis* f.sp. *hordei*) on spring barley (*Hordeum vulgare*). Fungicide treatments with azoxystrobin differed in the total applied dose and spray number. For each treatment, we calculated the observed selection ratio as the ratio of the frequency of the resistant strain after the last and before the first spray. The model accurately predicted the variation in observed selection ratios with total applied fungicide dose and number of sprays for three of the four experiments. Underprediction of selection ratios in one experiment was attributed to the particularly late epidemic onset in that experiment. When the equation representing epidemic development was modified to account for the late epidemic, predicted and observed selection ratios at that site were in close agreement. On a scatter plot of observed selection ratios on predicted selection ratios, for all four experiments, the 1:1 line explained 89–92% of the variance in the mean of observed selection ratios. To our knowledge, this is the first fungicide resistance model for plant pathogens to be rigorously tested against field data. The model can be used with some degree of confidence, to identify anti-resistance treatment strategies which are likely to be effective and would justify the resources required for experimental testing.

Keywords: azoxystrobin, barley powdery mildew, *Blumeria graminis* f.sp. *hordei, Hordeum vulgare,* quinone-outside inhibitor fungicides

Introduction

Fungal pathogens have the potential to significantly reduce the yield of food and cash crops worldwide when untreated (Oerke & Dehne, 2004) and the control of fungal pathogens is therefore important. Fungicides are an essential part of (integrated) control strategies for fungal pathogens (Brent & Hollomon, 2007). However, the development of resistance against fungicides reduces their effectiveness and may eventually result in the loss of their efficacy. The loss of a fungicide as a pathogen control option is a problem, because most crop diseases are typically controlled by only three or four different fungicides (Brent & Hollomon, 2007) and the discovery of new active molecules has become increasingly difficult over the last decade (Russell, 2005).

Fungicide resistance management strategies aim to prevent or delay the invasion of resistant pathogen strains into a sensitive pathogen population and thereby preserve fungicide efficacy. The rate of invasion of a resistant strain depends on the difference in fitness between the resistant and sensitive pathogen strains. Resistance management strategies aim to reduce this difference without increasing the fitness of the sensitive strain (Milgroom *et al.*, 1989). Such strategies include choices about dose, mixing of fungicides, alternation of fungicides and spatial restrictions and heterogeneity in the use of fungicides (Shaw, 2006; Brent & Hollomon, 2007).

To determine the usefulness of proposed resistance management strategies, mathematical models could usefully complement field experiments. Many fungicide resistance models have been developed (van den Bosch & Gilligan, 2008), but, to the best of our knowledge, none of the published models have been formally tested against experimental datasets in order to demonstrate their predictive power. In general, model testing involves comparison of an output variable from an experiment with predictions by a model that is parameterized using an independent dataset (Zadoks & Rabbinge, 1985). This

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process is distinct from model fitting, which involves the estimation of model parameters from a dataset and proves only that the model can represent the data. To be of value a model should at least reproduce the qualitative trends observed. Ideally, the model predictions should also match the data quantitatively.

The testing of a model is only possible when fungicide resistance models are able to realistically simulate the experiments that produced the dataset. This requires models that account for seasonal patterns in the growth of hosts and pathogens, and are able to simulate the application of fungicides at specific times in this seasonal cycle. Accounting for seasonal growth and loss of green canopy area also incorporates density dependence effects arising from the finite supply of healthy host tissue. Most of the published fungicide resistance models do not account for seasonality in the growth of host and pathogen (van den Bosch & Gilligan, 2008).

In the work reported here, we therefore: (i) constructed a mathematical model that predicts selection for resistance in a cereal foliar pathogen population, accounting for seasonal patterns in the growth of the host and stages in the lifecycle of pathogen and (ii) tested the model using an independent dataset on the development of resistance in a fungal pathogen of a cereal crop in response to different treatments with a fungicide.

Methods

Dataset for model testing

For model testing we used data from experiments on the development of resistance in powdery mildew (*Blumeria graminis* f. sp. *hordei*) on spring barley (*Hordeum vulgare*) in response to different treatments of the quinone outside inhibitor (QoI) fungicide azoxystrobin (Fraaije *et al.*, 2006). To increase the likelihood of mildew infection the susceptible barley cv. Golden Promise was sown in replicated plots. Experiments were conducted in the UK at sites near ADAS Terrington, Norfolk (52°45′N), Edinburgh (55°57′N) and Inverness (57°30′N) in 2002 and repeated at sites near Terrington and Edinburgh in 2003. At these five site/year combinations, total doses of either 1, 2 or 3 L ha^{-1} of the commercial product Amistar (suspension concentrate containing 250 g azoxystrobin L^{-1}; Syngenta) were applied in one, two or three sprays (Table 1). For QoI fungicides, the substitution of glycine by alanine at codon 143 of the mitochondrial cytochrome *b* gene (G143A) correlates with resistance development in cereal mildews (Fraaije *et al.*, 2002; Baumler *et al.*, 2003). The frequency of the G143A mutation was determined before the application of the first spray and at the end of the growing season using quantitative allele-specific PCR assays (Fraaije *et al.*, 2006). For each site/year, treatment and treatment replicate, we calculated the 'selection ratio', defined here according to the following equation:

Table 1 Azoxystrobin treatments (Amistar; suspension concentrate of 250 g azoxystrobin L^{-1}) applied at each site/year experiment included in the dataset for model testing to determine the development of resistance to azoxystrobin in powdery mildew (*Blumeria graminis* f. sp. *hordei*) on spring barley (*Hordeum vulgare*). The three-spray programme was not applied at Edinburgh in 2002

Treatment number	Number of sprays	Dose per spray (L ha^{-1})	Total applied dose (L ha^{-1})
1	0	0	0
2	1	1	1
3	1	2	2
4	1	3	3
5	2	0·5	1
6	2	1	2
7	2	1·5	3
8	3	0·33	1
9	3	0·66	2
10	3	1	3

$$selection\ ratio = \frac{\begin{array}{c}frequency\ of\ the\ resistant\ strain\\ at\ the\ end\ of\ the\ growing\ season\end{array}}{\begin{array}{c}frequency\ of\ the\ resistant\ strain\\ before\ the\ first\ spray\end{array}}. \quad (1)$$

The selection ratio was thus the factor by which the frequency of the resistant powdery mildew strain changed over one growing season.

Data on the three-spray programme at Edinburgh 2002 were missing. Data on the three-spray programme at Terrington 2002 were excluded from the model testing dataset because the selection ratio increased according to an exponential curve with the total applied dose. This was inconsistent with the data from other site/years and incompatible with the asymptotic shape of dose-response curves of fungicides in general (Lockley & Clark, 2005; Oxley & Hunter, 2005).

When the effects of the different azoxystrobin treatments on the selection ratio were analysed statistically, the selection ratio was shown to increase significantly ($P < 0·05$) with increasing total applied dose at all site/year combinations. Given a total applied dose, the selection ratio ($P < 0·05$) increased significantly with spray number at all site/year combinations, except Edinburgh 2002 (PHF, S. Powers, BF, JL, unpublished data).

From the five site/year combinations, data collected from Edinburgh 2003 were randomly selected to be used for parameter estimation, leaving four site/year combinations for model testing.

Model structure

An ordinary differential equation (ODE) model was constructed to describe the development of resistance against azoxystrobin in a powdery mildew population growing on the leaves of a spring barley crop in response to appli-

cations of fungicide during one growing season. We assumed that resistance develops in a similar way for all plants in the crop. Definitions and dimensions of state variables and parameters in the model are given in Tables 2 and 3, respectively.

Table 2 Definitions and dimensions of the state variables in the model of azoxystrobin fungicide resistance in *Blumeria graminis* f. sp. *hordei* derived in this paper

State variable	Definition	Dimension
A	Total leaf area[a]	cm^2
H	Healthy leaf area	
L_s	Leaf area occupied by latent lesions of the sensitive pathogen strain	cm^2
I_s	Leaf area occupied by infectious lesions of the sensitive pathogen strain	cm^2
L_r	Leaf area occupied by latent lesions of the resistant pathogen strain	cm^2
I_r	Leaf area occupied by infectious lesions of the resistant pathogen strain	cm^2
F	Area of lower leaves[b] occupied by infectious lesions of both the sensitive and resistant pathogen strain	cm^2
C	Azoxystrobin concentration	L ha^{-1}

[a]Throughout the table, 'leaf area' represents the area of leaves one to four of spring barley (*Hordeum vulgare*) counting down from the flag leaf.
[b]Lower leaves are leaves that emerged before leaf four, when counting down from the flag leaf.

The model describes the seasonal development of the spring barley canopy in order to account for the effect of the availability of susceptible host tissue on the growth of the powdery mildew population. The model describes the development of the combined area of leaves one to four during a growing season, because we assume that most of the sprayed fungicides will be intercepted by leaves one to four (leaf one being the flag leaf) and because pathogen samples for mutation analysis were taken from upper leaves. The total area of leaves one to four, hereafter called total leaf area (A), is assumed to increase according to the monomolecular equation (Thornley & Johnson, 1990) and reaches its maximum value (A_{max}) at growth stage (GS) 39 on Zadoks' scale (Zadoks *et al.*, 1974):

$$\frac{dA}{dt} = \gamma(A_{\max} - A). \qquad (2)$$

We used the monomolecular equation since it predicts an approximately constant growth of the total leaf area during the emergence of leaves two to four on a time scale in degree-days. This is in agreement with the approximately constant length of phyllochrons of leaves two to four of spring barley in degree-days (Anonymous, 2006) and the approximately similar size of these leaves (NP, unpublished data). The growth of the total leaf area is not affected by disease and consists of the sum of healthy, dead and infected leaf tissue.

The development of the healthy leaf area (H) consists of a growth stage, a plateau with no leaf growth or senescence followed by a senescence stage. The end of the

Table 3 Definitions, values and dimensions of the parameters in the fungicide resistance model derived in this paper

Parameter	Definition	Value	Dimension	Reference[e]
Host – Spring barley				
γ	Growth rate of leaf area	1·22E–02	t^{-1}[a]	1, 2
A_{max}	Maximum leaf area	88·8	cm^2	2
σ	Senescence rate	Eqn 14 in text	t^{-1}	1, 2
Disease – Powdery mildew				
F_0	Initial area of infectious lesions on lower leaves[b]	0·82	cm^2	3
λ	Rate of decrease of area of infectious lesions on lower leaves[b]	5·5E–03	t^{-1}	2
θ	Initial frequency of resistant mildew strain	Variable, see text	[c]	3
ρ	Transmission rate[d]	1·17E–02	t^{-1}	3
$1/\delta$	Length of latent stage	99	t	4
$1/\mu$	Length of infectious stage	262	t	5
Fungicide – Azoxystrobin				
ν	Decay rate of azoxystrobin	Variable, see text	t^{-1}	3
α	Proportional reduction of infection efficiency by azoxystrobin	Eqn 12 in text	–	–
α_{max}	Maximum proportional reduction of infection efficiency by azoxystrobin	1	–	6
β	Shape parameter of dose-response curve	9·5	–	3

[a]'t' represents degree-days.
[b]Lower leaves are leaves that emerged before leaf four, when counting down from the flag leaf.
[c]Dimensionless.
[d]A compound parameter that is the product of the sporulation rate per area of infectious lesion, the chance that a spore lands on leaves one to four (flag leaf = leaf 1) and the infection efficiency.
[e]1 = Anonymous, 2006; 2 = NP, unpublished data; 3 = Fraaije *et al.*, 2006; 4 = Eckhardt *et al.*, 1984; 5 = Asher & Thomas, 1984; 6 = it was assumed that the infection efficacy of the sensitive strain when exposed to an infinite dose would approximate to zero.

growth, plateau and senescence stages correspond to GS 39, 61 and 87 (Zadoks *et al.*, 1974), respectively. In the absence of disease, the equation for healthy leaf area is:

$$\frac{dH}{dt} = \gamma(A_{\max -}A) - \sigma(t)H. \tag{3}$$

In this equation, parameter σ represents the senescence rate.

The powdery mildew population on leaves one to four of spring barley consists of strains that are sensitive to azoxystrobin and those that are assumed to be completely resistant to this fungicide (with the range of doses applied) as a result of the presence of the G143A mutation. The life cycle of each powdery mildew strain is divided into a latent (L) and subsequently an infectious stage (I). Leaf tissue occupied by latent lesions of powdery mildew stays green and is still capable of photosynthesis. The length of the latent period is $1/\delta$. The length of the infectious period is $1/\mu$. Because powdery mildew is a biotroph, leaf senescence also kills the pathogen and thus senescence decreases the density of both latent and infectious lesions (Carver & Griffiths, 1981; Asher & Thomas, 1984). Subscripts s and r are used to distinguish between lesions of the sensitive and resistant powdery mildew strains.

At the start of the growing season, healthy leaf area becomes infected with powdery mildew as a result of deposition of spores produced by infectious lesions on lower leaves. The size (F) and therefore spore production rate of these lesions is assumed to decline according to an exponential function:

$$F = F_0 e^{-\lambda t}. \tag{4}$$

In this equation, λ represents the loss rate of infectious lesions on lower leaves as a result of both senescence and reaching the end of the infectious period. For the reasons outlined herein, we also modelled the size of infectious lesions on lower leaves for site/year Edinburgh 2002 according to the function:

$$F = 20 + 20\sin\left(\frac{t-950}{650}2\pi - 0{\cdot}5\pi\right). \tag{5}$$

We assume that a fraction θ of the infectious lesions at lower leaves (F) consists of the resistant powdery mildew strain. Parameter θ is kept constant during the growing season, because higher leaves intercept most of the sprayed azoxystrobin.

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 leaves one to four, (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 with 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 powdery mildew strains dependent on the availability of healthy host tissue.

This leads to the following equations for the development of the healthy leaf area in the presence of disease and the development of latent and infectious leaf areas of the sensitive and resistant strains:

$$\begin{aligned}\frac{dH}{dt} = {} & \gamma(A_{\max -}A) - \rho\left(\frac{H}{A}\right)(I_s + (1-\theta)F) \\ & - \rho\left(\frac{H}{A}\right)(I_r + \theta F) - \sigma(t)H\end{aligned} \tag{6}$$

$$\frac{dL_s}{dt} = \rho\left(\frac{H}{A}\right)(I_s + (1-\theta)F) - \delta L_s - \sigma(t)L_s \tag{7}$$

$$\frac{dI_s}{dt} = \delta L_s - \mu I_s \tag{8}$$

$$\frac{dL_r}{dt} = \rho\left(\frac{H}{A}\right)(I_r + \theta F) - \delta L_r - \sigma(t)L_r \tag{9}$$

$$\frac{dI_r}{dt} = \delta L_r - \mu I_r \tag{10}$$

The decay of the azoxystrobin concentration is modelled as

$$\frac{dC}{dt} = -\nu C \tag{11}$$

with decay rate ν. Azoxystrobin reduces the infection efficiency of sensitive powdery mildew strains (Bartlett *et al.*, 2002). We model the dependence of the infection efficiency on the azoxystrobin concentration (C) by multiplying this parameter with a factor $(1 - \alpha(C))$, in which $\alpha(C)$ is the fraction by which the infection efficiency is reduced at fungicide dose C. This fraction depends on the azoxystrobin concentration according to the asymptotic function:

$$\alpha = \alpha_{\max}(1 - e^{-\beta C}) \tag{12}$$

In this equation, parameter α_{max} is the maximum reduction of infection efficiency and β determines the curvature of the dose-response curve.

All state variables are expressed in cm^2 leaf area per degree-day. A degree-day scale was used to incorporate temperature effects on the development of spring barley and powdery mildew. The number of degree-days (t_{stage}) that it takes to complete a developmental stage was calculated as

$$t_{stage} = l(T_{av} - T_{threshold}) \tag{13}$$

in which l is the length of a developmental stage in days, T_{av} is the average temperature and $T_{threshold}$ is the minimum temperature required for development (taken as approximating to 0°C for all developmental processes). Hereafter, t represents the number of

degree-days accumulated since the start of a model simulation.

Parameter estimation

All parameters were estimated from datasets independent from model testing. The only exception was the decay rate of azoxystrobin, which had to be estimated from the dataset used for model testing, for the reasons outlined herein.

Canopy growth and senescence

Using a phyllochron of 97 degree-days (Anonymous, 2006), it was estimated to take 388 degree-days from the emergence of leaf four to GS 39. The numbers of accumulated degree-days from GS 39 to 61 and from GS 61 to 87 were estimated from data on development of spring barley and the average daily temperature during one season in the UK (NP, unpublished data). Using a temperature threshold of 0°C, accumulated degree-days between GS 39 and 61 and GS 61 and 87 were calculated by summing the average daily temperatures between the dates that corresponded to these growth stages (149 and 454, respectively). A_{max} was estimated from repeated measurements of the area of leaves one to four of replicated plots of spring barley cv. Pallas during 2008 at ADAS Rosemaund, Herefordshire, UK (NP, unpublished data). The growth rate of leaves (γ) was estimated such that the leaf area at GS 39 was 99% of the maximum leaf area. Senescence was assumed to start at GS 61 at a very slow rate and to remain slow until GS 71, after which the senescence rate rapidly increased until complete senescence at GS 87 (Anonymous, 2006). This was achieved by modelling the senescence rate according to the function

$$\sigma(t) = 0{\cdot}005\left(\frac{t - t_{GS61}}{t_{GS87} - t_{GS61}}\right) + 0{\cdot}1e^{0{\cdot}02(t - t_{GS87})} \quad (14)$$

This reduced the healthy leaf area at GS 87 to <1% of the maximum leaf area, which approximated complete senescence. In all model simulations, 1 cm^2 was used as an initial value for the total (A) and healthy leaf area (H).

The predicted growth and senescence of the healthy area of leaves one to four of spring barley in the absence of powdery mildew are shown in Fig. 1a.

Powdery mildew

The initial size of the infectious lesions (F_0) on lower leaves and compound parameter ρ were estimated by fitting the model to disease severity data from Edinburgh 2003 in nontreated plots. The number of degree-days between the emergence of leaf four and complete senescence of the lower leaves was estimated from the same dataset as used for the estimation of parameter A_{max}. Using a temperature threshold of 0°C, accumulated degree-days between the emergence of leaf four and complete senescence of leaf five were calculated by summing the average daily temperatures between the dates that corresponded to these developmental stages. Parameter λ was solved from Eqn 4 by substituting the estimate for F_0 and $F = 0{\cdot}99F_0$ and setting the value of t in this equation to the number of accumulated degree-days between the emergence of leaf four and complete senescence of leaf five. The number of accumulated degree-days necessary for the completion of the latent stage ($1/\delta$) was taken from Eckhardt *et al.* (1984). The length of the infectious period ($1/\mu$) was taken from Asher & Thomas (1984). The predicted growth and loss of the leaf area infected by powdery mildew in the absence of fungicide treatments is shown in Fig. 1b.

Dose-response curve

The maximum reduction in infection efficiency of the sensitive powdery mildew strain (α_{max}) was assumed to be one. The shape parameter of the dose-response curve

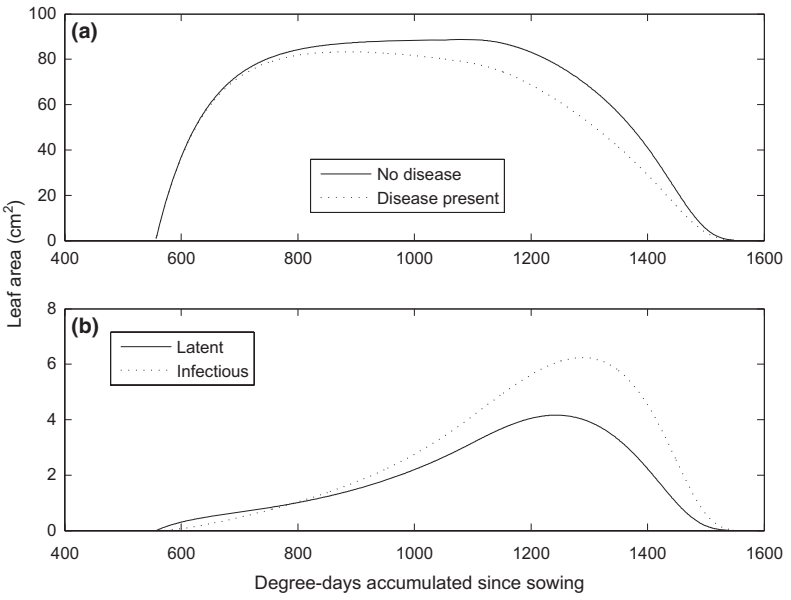

Figure 1 Predicted (a) growth and senescence of healthy leaf area (leaves one to four counting down from the flag leaf) and (b) development of latent and infectious leaf area of spring barley (*Hordeum vulgare*) during one season in the absence and presence of powdery mildew (*Blumeria graminis* f. sp. *hordei*).

(β) was determined by fitting the model to data from Edinburgh 2003, using the decay rate as a free parameter.

Decay rate of azoxystrobin

The decay rate of azoxystrobin (ν) is influenced by UV degradation (sunlight) (Garau *et al.*, 2002; Ghosh & Singh, 2009), other environmental factors (Bartlett *et al.*, 2002) and degradation by the biochemical processes within the plant (Joseph, 1999). In the absence of detailed data on the effect of these factors on the decay rate of azoxystrobin, we had to consider this to be a free parameter estimated using least-squares model fitting. The decay rate can be converted to a half-life time of azoxystrobin ($\tau_{0\cdot5}$) in degree-days according to the function

$$\tau_{0.5} = \frac{-In(0{\cdot}5)}{\nu} \quad (15)$$

Using an average daily temperature of 16°C during the period from the emergence of leaf four to complete senescence (GS 87) for all site/year combinations, the fitted values of the decay rate of azoxystrobin corresponded to half-life times of 6·9, 3·6, 4·8 and 1·2 days for Edinburgh 2002, Inverness 2002 and Terrington 2002 and 2003, respectively.

Simulations

Model testing

Having parameterized the model, we used it to simulate the development of resistance in powdery mildew of spring barley in response to the azoxystrobin treatments that were applied in the experiments. Simulations were performed for each treatment and site/year combination, to calculate the selection ratios. Each predicted selection ratio corresponds to the mean observed selection ratio for a given combination of treatment and site/year. Hereafter, we refer to the latter as the observed selection ratio. The initial frequency of the resistant strain (θ), the spray times of azoxystrobin and the sample times of leaves were set to values specific for each site/year combination.

Elasticity analysis

To determine how sensitive the predicted selection ratios were to changes in the values of parameters, we performed an elasticity analysis (Caswell, 2001) of the life cycle parameters of powdery mildew, the dose-response curve parameters and the decay rate of azoxystrobin. The elasticity of a parameter is an indicator of the factor by which a certain model prediction changes relative to the factor by which this parameter value changes, so the elasticity of each model parameter was calculated as

$$elasticity = \frac{\frac{dY}{Y_0}}{\frac{dP}{P_0}} \quad (16)$$

in which dY is the change in the predicted selection ratio when parameter P is changed by dP. P_0 is the default value of the parameter and Y_0 is the predicted selection ratio when using the default parameter value. Parameters were varied from 75% to 125% of their default value, giving $dP = 1{\cdot}25P_0 - 0{\cdot}75P_0 = 0{\cdot}5P_0$. Parameter α_{max} was varied from 75 to 100% of a default value since this parameter is a fraction and cannot exceed a default value of one. Separate elasticity analyses were performed for one-, two- and three-spray programmes with a total applied dose of 2 L commercial product ha^{-1}. In all analyses, the initial frequency of the resistant powdery mildew strain (θ) was set to the average across all site/year combinations, 0·091. Application timings of azoxystrobin and leaf sampling times were set to their average across all site/year combinations, excluding Edinburgh 2002.

Comparison of the predicted selection ratio and observed selection ratio

To quantify how well the model described the observed data for each site/year combination, the percentage of variance in the observed data that was accounted for by the model predictions was calculated as:

$$\%\ explained\ variance = 100\left(\frac{SS_{tot} - SS_{res}}{SS_{tot}}\right) \quad (17)$$

In this equation, SS_{tot} is the total sum of squares of the observed data for a certain site/year combination and was calculated as $SS_{tot} = \sum_{i=1}^{m}\sum_{j=1}^{n}(x_{ij}-\bar{x})^2$ with m representing the total number of treatments per site/year combination, n representing the number of replicates per treatment, x_{ij} representing the observed selection ratio for treatment i and replicate j, and $\bar{x}$ representing the average observed selection ratio across all treatments and replicates. SS_{res} is the residual sum of squares for the same site/year combination, calculated as $SS_{res} = \sum_{i=1}^{m}\sum_{j=1}^{n}(x_{ij}-\hat{x}_i)^2$ with $\hat{x}_i$ representing the predicted selection ratio for treatment i.

To quantify how well the model described the data over all site/year combinations, we pooled the predicted and mean observed selection ratios for all treatments and site/year combinations. When the predicted selection ratios exactly match the observed ones, the relationship between these two variables is described by the 1:1 line. We calculated the percentage of the variance in the observed selection ratios that was explained by this 1:1 line as a quantitative measure of the predictive power of the model.

Results

Model testing

The model predicted the observed significant increase of the selection ratio with increasing total applied azoxystrobin dose for all site/year combinations and the significant increase of the selection ratio with increasing spray number, given a certain total applied dose, for site/years Inverness 2002 and Terrington 2002 and 2003 (Fig. 2).

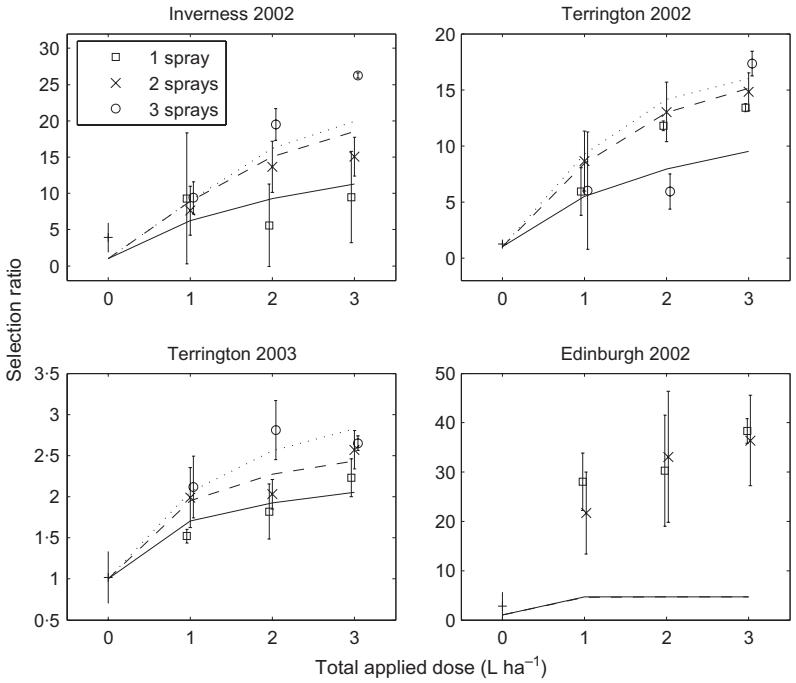

Figure 2 Observed and predicted response of the selection ratio for resistant powdery mildew (*Blumeria graminis* f. sp. *hordei*) to variation in total applied dose and number of sprays of azoxystrobin applied to spring barley (*Hordeum vulgare*), for four site/year combinations used for model testing. Vertical bars indicate 95% confidence intervals around the mean of observed selection ratios. Solid, dashed and dotted lines indicate predicted one-, two- and three-spray programmes, respectively. Only the one- and two-spray programmes were applied at Edinburgh in 2002. Only the predicted selection ratios for the one- and two-spray programmes are shown for Terrington 2002 (see text). The predicted selection ratios for spray programmes for Edinburgh 2002 overlay each other.

The model also predicted that spray number had a negligible effect on the selection ratio for Edinburgh 2002 (Fig. 2). This was in agreement with the statistical analysis that showed no significant effect of spray number for this site/year combination (PHF, S. Powers, BF, JL, unpublished data).

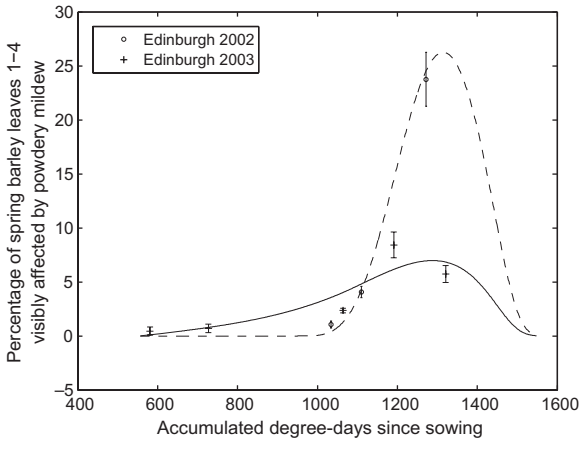

Figure 3 Progress curve of the percentage of spring barley (*Hordeum vulgare*) leaves one to four (counting down from the flag leaf) visually affected by powdery mildew (*Blumeria graminis* f. sp. *hordei*) in the absence of fungicide treatments for Edinburgh 2002 (late epidemic, dashed line) and Edinburgh 2003 (average epidemic, solid line). Vertical bars indicate 95% confidence intervals of observed mean disease levels.

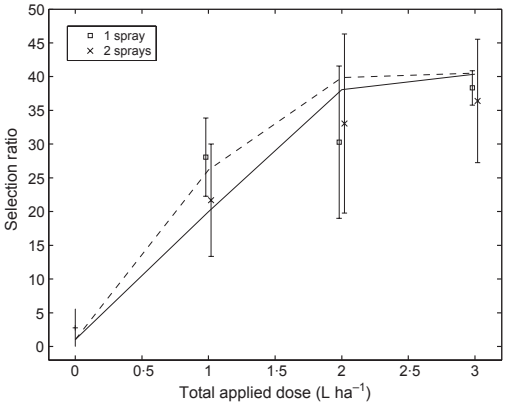

Figure 4 Observed and predicted effects of variation in total applied dose and number of sprays of azoxystrobin on the selection ratio for resistant powdery mildew (*Blumeria graminis* f. sp. *hordei*) on spring barley (*Hordeum vulgare*) at Edinburgh in 2002, when accounting for the late start of the powdery mildew epidemic. Vertical bars indicate 95% confidence intervals around mean of observed selection ratios for the resistant mildew strain. Solid and dashed lines indicate predicted values for the one- and two-spray programmes, respectively.

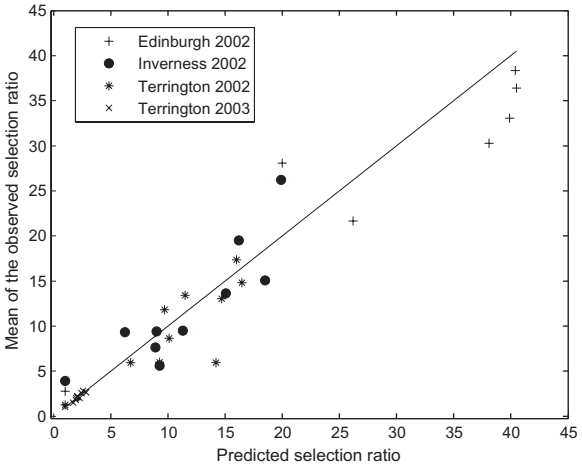

Figure 5 Scatter plot of predicted versus observed mean selection ratios of resistant powdery mildew (*Blumeria graminis* f. sp. *hordei*) on spring barley (*Hordeum vulgare*) for all azoxystrobin treatments and site/years in the dataset used for model testing. Solid line is the 1:1 line through the origin, which represents the perfect model fit. The three-spray programme was missing for Edinburgh 2002 and was omitted for Terrington 2002 (see text).

Quantitatively, the model predictions explained 75%, 89% and 90% of the variation in the mean selection ratio for site/year combinations Inverness 2002 and Terrington 2002 and 2003, respectively. The predicted selection ratios for Edinburgh 2002 were much lower than the mean of observed selection ratios and SS_{res} = 4443 was higher than SS_{tot} = 880 for Edinburgh 2002. For this specific year, the powdery mildew epidemic in the absence of fungicides started particularly late and peaked at a higher severity than the epidemic that the model parameterization produced (Fig. 3). A better description of the epidemic for Edinburgh 2002 was obtained by replacing the function used to describe the initial infection (Eqn 4) by Eqn 5. This adjustment resulted in a predicted epidemic which matched the limited observations available (Fig. 3). Recalculating the mean selection ratios for the different azoxystrobin treatments for site/year combination Edinburgh 2002 (Fig. 4) decreased SS_{res} to 217 and the model explained 75% of the variation in the mean selection ratios. Although the new model predictions show that the two-spray programme selected slightly more strongly for the resistant strain than the one-spray programme, this difference is small and decreases for higher total applied doses, in agreement with the observed data.

To illustrate how the predicted selection ratios compare to the observed selection ratio data over all site-year combinations, Figure 5 shows a scatter plot of the mean of observed selection ratios versus the predicted selection ratios for all treatments and for all site/years used for the model testing. The percentages of the variation in the mean observed selection ratios explained by the 1:1 line (Fig. 5) were 89% and 92% when excluding and including Edinburgh 2002, respectively.

Elasticity analysis

In general, across the different spray programmes, the elasticity of parameters determining the impact of azoxystrobin on the sensitive powdery mildew strain was higher than the elasticity of pathogen life cycle parameters and parameters describing the size of the initial infection (Table 4). Selection ratio was always most sensitive to changes in parameter α_{max} which determined the maximum impact of azoxystrobin on the transmission rate (ρ). Three other parameters with particularly high elasticity for the different spray programmes were the decay rate of azoxystrobin (ν), the initial frequency of the resistant powdery mildew strain (θ) and the transmission rate (ρ). The rank order of parameters based on their elasticity was almost similar for the one-, two- and three-spray programmes and the elasticity of parameters decreased with the number of sprays. The initial frequency of the resistant powdery mildew strain (θ) was an exception; its elasticity increased with the number of sprays.

Discussion

The testing of the model demonstrated that it correctly predicted selection for resistance in response to fungicide treatments that varied in total applied dose and spray

Table 4 Elasticity of model parameters with respect to selection ratio for one-, two- and three-spray programmes and a total applied dose of 2 L commercial product (250 g azoxystrobin L^{-1}) ha^{-1}. Parameters were varied from 75% to 125% of default values (default values listed in Table 3). Parameter α_{max} was varied from 75% to 100% of its default value since this parameter is a fraction and cannot exceed a default value of one. Averages across site-year experiments were used for the value of the initial frequency of the resistant strain, and spray and sample times

Parameter	One-spray programme	Parameter	Two-spray programme	Parameter	Three-spray programme
α_{max}	+1·53	α_{max}	+1·39	α_{max}	+1·16
ν	+1·06	θ	−0·78	θ	−0·88
ρ	+0·54	ν	+0·72	ν	+0·53
θ	−0·52	ρ	+0·39	ρ	+0·31
β	+0·36	β	+0·32	β	+0·29
λ	+0·34	δ	+0·22	δ	+0·18
δ	+0·27	λ	+0·17	λ	+0·08
μ	−0·13	μ	−0·05	μ	−0·01
F_0	0·00	F_0	0·00	F_0	0·00

number, in both a qualitative and quantitative way, for all site/years. Overall, the results suggest strong predictive value. For a full validation of the model we would, however, need to test it against data from a contrasting host–pathogen–fungicide system.

Whilst the model was parameterized and tested using datasets on a specific host–pathogen–fungicide system, the structure and assumptions underlying the model would be readily applicable to many cereal foliar pathosystems and foliar-applied fungicides. For example, only parameter values would need to be changed to describe the development of the canopy of other cereal crops. Similarly, the model divides the life cycle of fungal pathogens into latent and infectious stages, which could equally be parameterized to represent most fungal pathogens of cereals.

The model is also flexible in other respects. Firstly, it can be applied to both biotrophic (e.g. powdery mildew) and necrotrophic or hemibiotrophic fungal pathogens (e.g. *Mycosphaerella graminicola*) by adding or omitting the senescence terms in the equations for infectious leaf areas. Secondly, as a result of the division of the lifecycle of the pathogen into two stages, the model can represent the effects of fungicides from contrasting groups, that target the fungal pathogen in different parts of its lifecycle (infection efficiency/spore production rate and length of the latent/infectious period). Thirdly, the healthy leaf area duration (AD) can be calculated from the model output. Given that yield is related to AD (Waggoner & Berger, 1987; Bryson *et al.*, 1997), the model could be used to assess the usefulness of resistance management strategies, using measures of the success of the strategies which relate to their cumulative benefit to yield over an extended period. Finally, the number of fungal strains representing different genotypes in the model could be increased to represent pathogen populations where, for example, strains carrying mutations conferring differing degrees of insensitivity coexist. For example, three different cytochrome *b* genotypes (F129L, G137R and G143A) were found in QoI-resistant isolates of *Pyrenophora tritici-repentis*, the causal agent for tan spot in wheat (Sierotski *et al.*, 2007).

Because the model describes the seasonal development of the canopy of cereal crops, it can account for the availability of host tissue on the growth of fungal pathogens in a realistic way. It also takes the effect of temperature into account as an important factor influencing the development of both the crop and the fungal pathogen. Whilst other fungicide resistance models have accounted for the availability of host resources on the growth of fungal pathogens (e.g. Gubbins & Gilligan, 1999; Parnell *et al.*, 2005; Hall *et al.*, 2007), the combination of density-dependent growth of fungal pathogens with a realistic seasonal development of the host is a novel approach.

The model assumes that resistant fungal strains are present in the fungal population at a low frequency from the start of the simulations. However, it is possible that the resistant fungal strain may still need to arise through mutation or may be present in such low densities that its survival depends on stochastic processes at the start of a fungicide treatment. The effect of the simulated fungicide treatments on the dynamics of the resistant fungal strain during this stochastic phase cannot be described using the model presented in this paper.

To the best of our knowledge, this is the first fungicide resistance model for crop pathogens that has been rigorously tested. The model can now be used, with some degree of confidence, to evaluate the effectiveness of anti-resistance strategies. One practical difficulty with studies on fungicide resistance is that there are too many combinations of active substance, mixtures, alternation, dose and number of treatments (and interactions thereof) to test their effects on selection experimentally. The model described here could be employed to identify promising strategies which justify experimental testing.

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