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Relationship between the incidences of ear and spikelet infection of *Fusarium* ear blight in wheat

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Abstract

There is a urgent need to develop a rational strategy for managing *Fusarium* ear blight in order to reduce current reliance on routine fungicide applications, based on an objective assessment of disease risks. One of important components for such a management strategy is a fast, easy, accurate and reliable method for disease assessment. The relationship between incidence of *Fusarium* ear blight ear infection and number of spikelets infected on an ear (or incidence of spikelet infection) were investigated during three seasons and in four countries in order to derive a simple relationship for predicting disease at the spikelet level using ear incidence. More than half of the data sets of the number of infected spikelets on an ear could not be fitted satisfactorily by a Poisson distribution. Three two-parameter discrete distributions (negative binominal, Neyman type A and Polya-Aeppli) provided a significantly better fit than the Poisson distribution, indicating a degree of aggregation of number of infected spikelets on an ear. Taylor's power-law satisfactorily described the observed variance–mean relationship for the number of infected spikelets on an ear; this relationship was generally consistent over years and countries. A robust relationship between incidence of ear infection and average number of infected spikelets per ear was obtained assuming a fixed variance–mean relationship and a negative binomial distribution for the number of infected spikelets. A relationship between incidences of spikelet and ear infection was also obtained based on the complementary log–log or logit transformation of ear and spikelet infection incidence. These models appeared to be consistent over years and countries and thus may be used in making practical disease management decisions involving fungicide applications.

Introduction

Fusarium ear blight of wheat (scab) (FEB) is a significant but sporadic threat to wheat production world-wide (Bai and Shaner, 1994; Parry et al., 1995; McMullen et al., 1997). In addition to causing yield losses, *Fusarium* ear blight is of greater importance under certain conditions because of associated mycotoxin accumulation which can occur in infected grain. Contaminated grain is

unsuitable for animal and human consumption due to the adverse health effects of these toxins (Bhat et al., 1989; Huang, 1992; Li et al., 1999).

The main causal agents of FEB in the EU are *Fusarium culmorum*, *F. graminearum*, *Microdochium nivale* var *nivale* and var *majus* (formerly *Fusarium nivale*), *F. avenaceum* and *F. poae*. The toxigenic capabilities of the pathogens causing FEB varies. The distribution and predominance of these pathogens is, to a large extent, determined by climatic parameters, particularly temperature and moisture. Combinations of these pathogens can often occur on infected wheat ears and the impact of the environment on such disease complexes is poorly understood. There is a general consensus that FEB is most severe in cereals where cool/warm and wet conditions occur, especially during anthesis and the early seed development period (Bai and Shaner, 1994; Parry et al., 1995; Dubin et al., 1997; McMullen et al., 1997; Xu, 2003).

In countries where there are histories of severe epidemics of FEB, a routine fungicide application is adopted to manage the disease. However, a prophylactic approach to manage FEB is undesirable both in terms of cost and environmental concerns. For example, severe epidemics occur about 1 year in 10 in the UK and thus there is a potential reduction in fungicide application of 90% over 10 years if a reliable forecasting system could be developed. In areas where FEB occurs more frequently, significant savings could be made by targeting applications to high risk crops. In countries where fungicides are not routinely used for FEB control, there is no rational basis for fungicide application. Clearly there is a need to develop a more rational strategy for managing FEB, based on an objective assessment of disease risks in individual crops or areas.

A fast, easy, accurate and reliable method for assessing disease is crucial to an effective management system based on disease risk. Usually, disease intensity is measured as incidence (the number or proportion of diseased plant units), or severity (the relative area or volume of diseased plant tissue, sometimes as number of colonies per plant unit – a density measurement). It can be measured on different scales such as individual leaves, shoots or plants (Madden and Hughes, 1995). In general, incidence is easier and quicker to assess than severity, but severity may be a more useful measurement for practical disease management, and similarly incidence at a higher scale (e.g., plants) is easier and quicker to assess than incidence at a lower scale (e.g. leaves, shoots). Much effort has been devoted to study relationships between incidence and severity measures, and between incidences assessed at different scales (Seem, 1984; McRoberts et al., 2003) with the aim to predict severity or incidence at low scales from easily recorded incidence data at high scales. Incidence–severity and incidence–incidence relationships have usually been studied using correlation and regression analysis and on the basis of the frequency distribution of density or incidence data (Seem, 1984; Hughes et al., 1997; Turechek and Madden, 2001; McRoberts et al., 2003). In an artificial inoculation study of FEB, incidence of ear infection was significantly related to severity (% spikelets infected on infected ears) although such a relationship varied considerably between years (Groth et al., 1999).

The objective of this study was to develop a quantitative assessment model to predict the risk of FEB and the associated production of mycotoxins. We report results from investigating the relationship between the incidence of ear infection and the number of spikelets infected (severity) with FEB, with the aim of finding a simple and robust relationship between incidence and severity, hence predicting infection of spikelets using the incidence of infected ears. To achieve this objective, we first characterised the heterogeneity of the number of infected spikelets and then developed models for accurately predicting disease at the spikelet scale from the ear incidence based on empirical regression models and statistical distributions.

Materials and methods

Disease assessment

A number of sites were chosen from four countries: UK, Ireland, Italy and Hungary. The sites were chosen to represent a range of climatic conditions in Europe where FEB is likely to be a serious problem on wheat. Fungicides effective against FEB were not applied during anthesis or early seed development in the UK, Hungary and Italy, whereas about 50% of the sites in Ireland received fungicides. At each site, a sample of 200 ears was collected using a random W-shape

sampling strategy at the growth stage (GS)69 (end of flowering) and GS77 (milky ripe) (Zadoks et al., 1974). The ears were visually assessed for ear blight by recording the number of spikelets infected on each ear. In 2003, FEB was only assessed at GS77 as the results in 2001 and 2002 indicated a low incidence at GS69 (Table 1).

For samples from Italy, only the overall incidence of ear and spikelet infection was available for each sample and for samples from Hungary and Italy, average number of spikelets per ear for each sample was not taken. For samples from UK and Ireland, the average number of spikelets per ear ranged from 16 to 25, based on a sub-sample of 20 ears, with an overall average of 20. Hence, whenever needed, the number of spikelets per ear was assumed to be 20 for samples from Italy and Hungary.

Statistical analysis

Understanding heterogeneity in the density of spikelet infection (i.e. number of spikelets infected per ear), and how heterogeneity changes with disease level is important to developing more reliable incidence–density models, which may be easier to interpret than empirical regression models. In this study, we treated the number of spikelets infected as a discrete count variable and investigated the incidence of ear infection in relation to this density measurement. Next we calculated the incidence of spikelet infection and then investigated the incidence–incidence (ear-spikelet) relationship.

We first determined whether the number of spikelets infected on an ear was randomly distributed by fitting several discrete distributions to the observed data for each individual sample. Next, Taylor's power law (Taylor, 1961) was used to describe the relationship between variance and mean, and the observed heterogeneity in the number of spikelets infected. For Italian samples, distribution and variance–mean analyses were not possible as there was no information on number of infected spikelets on each individual ear. Several models were explored relating disease incidence to density based on the results from the distribution and power law analyses and also based on empirical curve fitting. Finally, we investigated whether it was possible to relate incidences at the two levels (ear and spikelet). All statistical analyses were done using Genstat™ (Payne et al., 1993).

Distribution of disease density

The Genstat™ 'DISTRIBUTION' directive was used to fit four commonly used discrete probability distributions (Poisson, negative binomial, Polya-Aeppli and Neyman type A) to the observed numbers of infected spikelets per ear (i.e., disease density) in each sample of 200 ears. The Poisson distribution is appropriate when the number of infected spikelets on an ear is randomly distributed. The other three are two-parameter generalised Poisson distributions (Ord, 1972; Freeman, 1980) and they can be used to describe over-dispersed or aggregated (clustered) data. Parameters were estimated using maximum likelihood and optimised using an iterative Gauss–Newton method. The sampling units in the statistical analysis were the individual ears at each sampling time. Goodness of fit was determined with a χ^2 test based on the deviance, which is the sum of squared so-called 'deviance residuals'.

Variance–mean relationship of disease density

Taylor's power law (Taylor, 1961) was used to describe the relationship between the sample mean

Table 1. Number of samples assessed visually for symptoms of FEB

	2001		2002		2003	
	GS69[a]	GS77	GS69	GS77	GS69	GS77
UK	5/20[b]	20/20	13/20	19/20		10/10
Hungary			15/15	15/15		9/10
Ireland	0/12	12/12	0/13	13/13		10/10
Italy	10/17	11/19	10/19	10/19		2/9

[a] Growth stage according to Zadoks et al. (1974): GS69 – anthesis; GS77 – milky stage.
[b] Number of samples with visual symptoms/total number of samples.

(m) and variance (v) of disease density on all the assessed ears for each sample

$$v = am^b. \quad (1)$$

When $a = b = 1$, there is no aggregation or over-dispersion over the full range of means. For parameter estimation, a linearised form of Equation (1) was used, i.e.,

$$\ln(v) = \ln(a) + b\ln(m), \quad (2)$$

and the parameters $\ln(a)$ and b were estimated by least squares. Differences in the two parameters between countries, years and sampling times were tested.

Relating incidence to density

In all analyses relating incidence to density there were 140 data points, i.e., the mean number of infected spikelets on an ear and the incidence of ear infection for each sample where visual symptoms were observed. In this study, our aim is to predict disease level at the spikelet level using ear incidence. Nevertheless, in developing models we used ear incidence as the dependent rather than as an independent variable in order to achieve consistency among the models to be developed, for some of which based on distribution theory it is easy and natural to express ear incidence as the function of the spikelet data. An inverse relationship can then be obtained to predict disease level at the spikelet level using ear incidence.

(1) Based on Poisson distributions. An incidence–density relationship was derived by assuming that the number of infected spikelets on an ear followed a Poisson distribution. The probability of an ear being infected (p) is then related to the mean number (m) of spikelets infected on an ear by the equation

$$p = 1 - P(0) = 1 - \exp(-m), \quad (3)$$

where $P(0)$ is the theoretical probability of ears with no infected spikelets. Thus, given an estimate of p, m can be estimated by rearrangement of Equation (3) as $-\ln(1-p)$.

(2) Based on negative binomial distributions. Preliminary results from fitting the four distributions to the observed data indicated that many data sets cannot be fitted by a Poisson distribution and the three two-parameter distributions fitted to these data sets similarly well (see Results). Hence, we used the negative binomial distribution for further investigation since this distribution is widely used in plant pathology. Thus, the incidence of ear infection was related to the two parameters (m, k) of a negative binomial distribution by the equation

$$p = 1 - P(0) = 1 - \left(\frac{m}{k} + 1\right)^{-k}. \quad (4)$$

This was, essentially, a regression problem, i.e., estimating parameter k from the observed data (p and m) with a constraint based on an assumption that a negative binomial distribution provided adequate fit to the data. The Genstat™ 'FIT-NONLINEAR' directive was used to fit Equation (4) to the observed data. Thus, given an estimate of p, m can be estimated by rearrangement of Equation (4) using the fitted parameter k value as

$$m = k((1-p)^{-1/k} - 1). \quad (5)$$

Equation (4) assumes a constant negative binomial aggregation index (k). However, it is known that disease aggregation characteristics tend to vary with changes in means (Madden and Hughes, 1995; Xu and Ridout, 1998; Ridout and Xu, 2000; Xu et al., 2001). Here we took into account this varying aggregation characteristic by assuming a common variance-to-mean relationship of Equation (1) for heterogeneity across all data sets as well as a negative binomial distribution for the data, so

$$p = 1 - e^{-m(am^{b-1}-1)^{-1}\ln(am^{b-1})}, \quad (6)$$

where a and b are parameters of Equation (1) (McRoberts et al., 2003). The Genstat™ 'FIT-NONLINEAR' directive was used to fit Equation (6) directly to the observed data to estimate a and b, instead of using the parameters estimated using Equation (2). This is because of the expected differences in parameter estimates due to the fact that in fitting Equation (2) the objective is to minimise the residual variance in $\ln(v)$ whereas in Equation (6) it is the residual variance in p that needs to be minimised. Instead, There is no explicit solution for m in terms of p, and only iterative solutions are possible.

(3) Empirical regression analysis. The complementary log–log transformation of the disease

incidence ($\mathrm{CLL}(p) = \ln(-\ln(1-p))$) was directly regressed on the logarithmic transformation of the average number of infected spikelets on an ear:

$$\mathrm{CLL}(p) = \ln(\gamma) + \delta \ln(m), \tag{7}$$

which corresponds to the relationship: $p = 1 - \exp(-\gamma m^{\delta})$. This represents a two-parameter generalisation of the Poisson model (Nachman, 1981); the Poisson model is a special case with $\gamma = 1$, $\delta = 1$.

In addition, the number of ears infected was assumed to be binomially distributed with an index equal to the total number of ears in each sample (i.e., 200) and hence the incidence (proportion) of ear infection (p) was related to the logarithmic transformation of the average number of infected spikelets on an ear ($\ln(m)$) by the logistic equation

$$\ln\left(\frac{p}{1-p}\right) = d \ln(m). \tag{8}$$

Preliminary analysis indicated that the constant term in Equation (8) is not significant and hence was omitted.

Relating incidences at two levels

Assuming the frequency distribution of the number of diseased spikelets on an ear follows a binomial distribution, the proportion of ears with at least one diseased spikelet is given by

$$p_e = 1 - (1 - p_s)^n \tag{9}$$

where p_e and p_s are the respective incidence of ear and spikelet infection and n is the number of spikelets per ear.

The $\mathrm{CLL}(p_e)$ was directly regressed on $\mathrm{CLL}(p_s)$:

$$\mathrm{CLL}(p_e) = f + g\mathrm{CLL}(p_s) \tag{10}$$

In addition, as before the number of ears infected was assumed to be binomially distributed and hence incidence of ear infection (p_e) was related to the logit transformation of p_s (i.e., $\ln(\frac{p_s}{1-p_s})$).

Results

General disease development

Disease incidence varied considerably between countries as well as between sites within each country within each year. Over the 3 years, Ireland had the highest incidence of *Fusarium* ear blight and Italy had the lowest (Figures 1 and 2). Across all the samples, the overall incidence of ear infection was about 35% in Ireland compared to 22% in

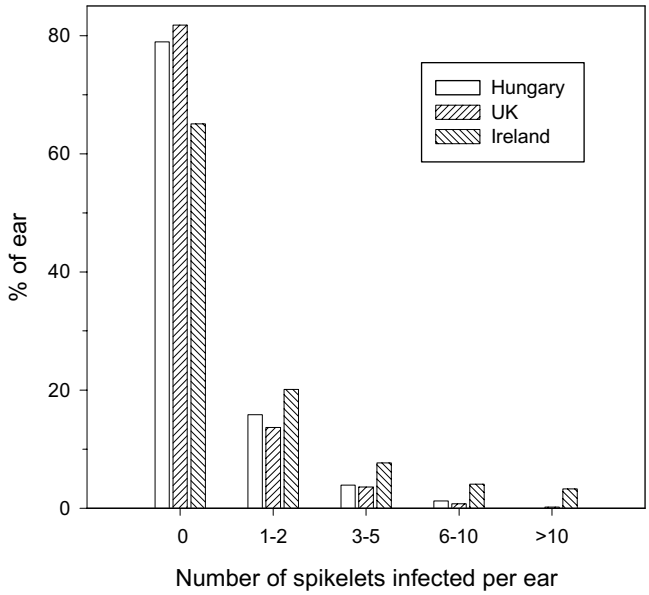

Figure 1. Frequency distributions of numbers of FEB-infected spikelets on an ear pooled over all the samples over the 3 years in Hungary, UK and Ireland. About 4% ears were infected across all the samples from Italy.

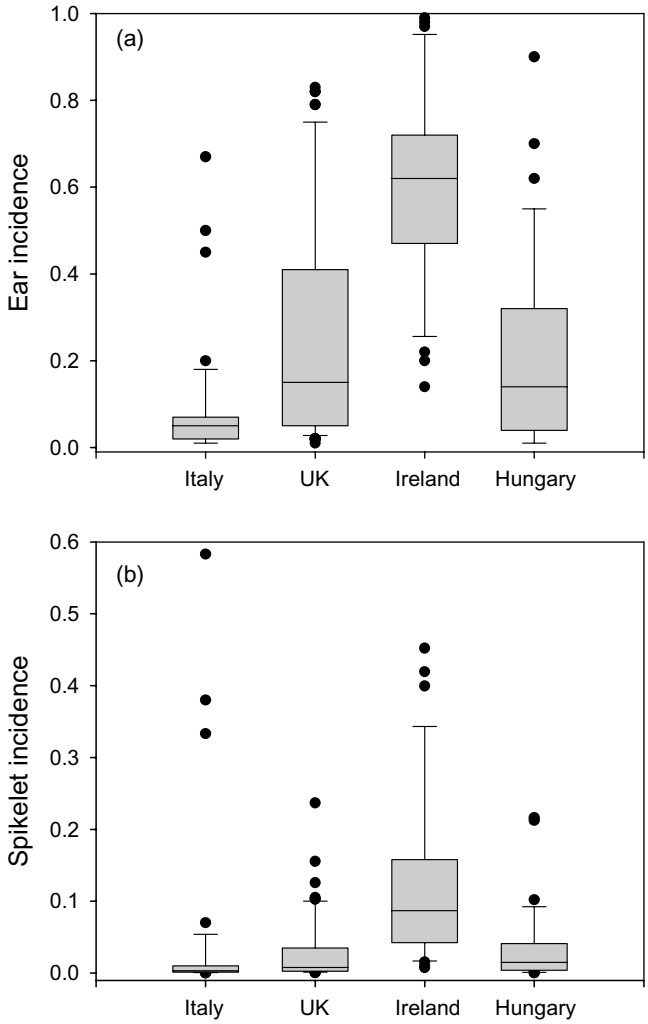

Figure 2. Boxplots of the incidence of visible ear (a) and spikelet (b) infections caused by FEB pathogens in four countries in Europe. The upper and lower limits of the box indicate the upper and lower quartiles of the distribution and the horizontal line through the box indicates the median. The 'whiskers' extending beyond the box indicate the range of 10th and 90th percentiles; 'outlying' points are shown individually as open circles.

Hungary, 19% in UK and 4% in Italy (Figure 1). Of the samples with visual symptoms, the median incidence of ear infection was 0.05, 0.14, 0.15 and 0.62 for Italy, Hungary, UK and Ireland, respectively (Figure 2a); the corresponding median incidence of spikelet infection was 0.004, 0.027, 0.009 and 0.087 (Figure 2b), and on average, about 3.1%, 4.9% and 12.4% of spikelets were infected at GS77 in UK, Hungary and Ireland, respectively. In Italy, all samples had <3% spikelets infected except in four samples from two sites at GS69 and GS77 in 2002 (Figure 2b). There was no visual disease at the anthesis sampling for most of the sites and disease increased considerably at the second sampling time (milky stage) and most sites at GS77 had disease (Table 1).

Distribution of disease density

Nearly half (63 out of 140 samples from UK, Ireland and Hungary) the data sets of the numbers of spikelets infected on an ear could not be adequately described by a Poisson distribution (Table 2). The lack of fit is primarily due to the excessive number of ears either with no infected spikelets or with almost all spikelets infected in the

Table 2. Results of fitting four different probability distributions to the 140 data sets of numbers of infected spikelets on an ear of *Fusarium* ear blight in wheat

	Poisson	Negative binomial	Neyman Type A	Polya-Aeppli
UK (total 66 samples with visual disease symptoms)				
Not optimised[a]	0	0	0	0
χ^2 significant[b]	23	6	4	3
Hungary (total 39 samples with visual disease symptoms)				
Not optimised	0	0	0	0
χ^2 significant	15	2	1	1
Ireland (total 35 samples with visual disease symptoms)				
Not optimised	0	4	4	4
χ^2 significant	25	15	14	14

It was not possible to include Italian samples for this analysis, as there were no detailed data available on number of spikelets infected on each individual ear.

[a] Number of data sets in which optimisation failed with no clear reasons.

[b] Number of data sets in which the χ^2 goodness of fit test was significant at $P = 0.01$. Significance means lack of fit; thus, a small number indicates that most data sets were well fitted by the corresponding distribution.

observed data. For these 63 data sets, the three two-parameter discrete distributions significantly improved the fit over the Poisson distribution on the basis of the difference in the deviance. Nevertheless, there were still 27, 23, and 22 of the 63 data sets that cannot be satisfactorily fitted by the negative binomial, Neyman type A and Polya-Aeppli distributions, respectively. There were large variations in the estimated aggregation parameter of the negative binomial distributions (Figure 3). Most of the data sets that failed to fit the two-parameter distributions were from Ireland

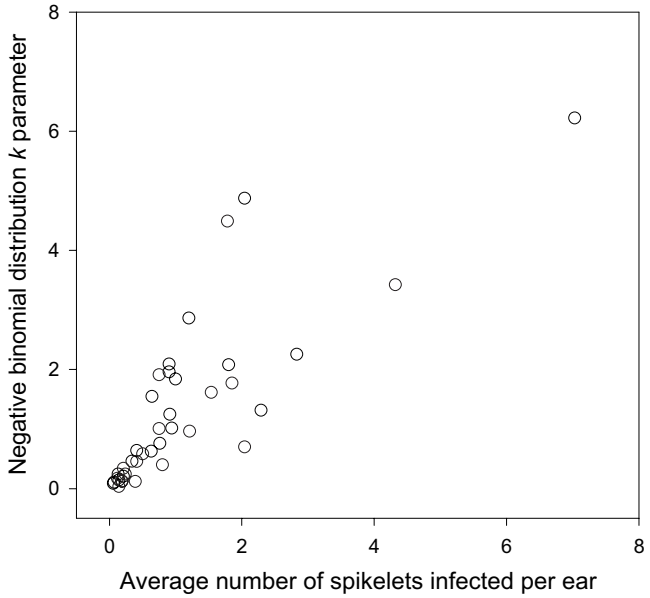

Figure 3. Observed average number of FEB-infected spikelets on an ear and the estimated k parameters of the negative binomial distributions. Only those data sets that a Poisson distribution failed to fit but a negative binomial distribution fits adequately are shown.

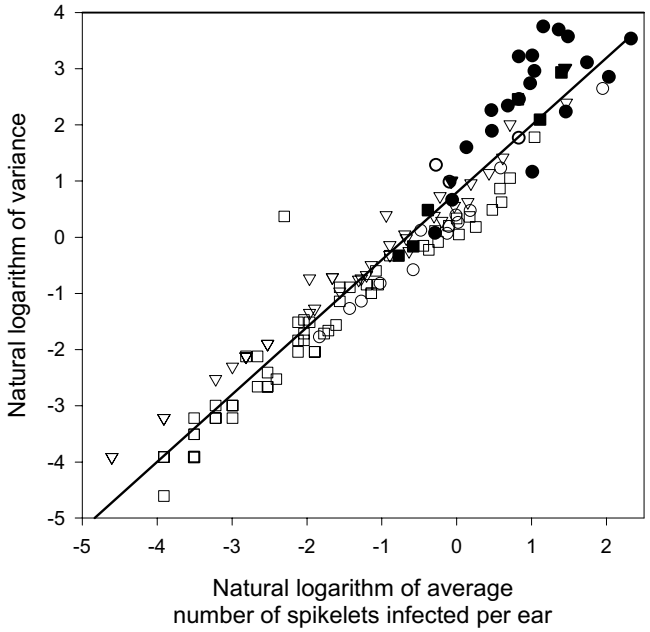

Figure 4. Observed and fitted power-law relationships between variance and mean of disease density (number of spikelets infected per ear) data of FEB in wheat. (○) – Ireland, (□) – UK, (▽) – Hungary. The solid line is the fitted log-linear model (Equation (11)). The filled symbols are for those samples that cannot be fitted by a negative binomial distribution.

(Table 2). There are no easy methods to test statistically which of the three distributions is most appropriate because they are not nested.

Variance–mean relationship of disease density

In general, $\ln(v)$ increased linearly with increasing $\ln(m)$ for numbers of infected spikelets on an ear (Figure 4). Most samples that failed to fit a negative binomial distribution were clustered in the top-right region of the graph. Although there were significant differences in the two model parameters (a and b in Equation (2)) among the four countries, particularly between Ireland and the others, these accounted for only an additional 3% of the total variation in $\ln(v)$. Thus a common regression model was fitted for all the data sets:

$$\ln(v) = 0.798 + 1.199\ln(m) \quad (0.054)\ (0.028) \qquad (11)$$

where the standard errors of parameter estimates are in brackets below the corresponding parameter. This equation accounted for 93% of total observed variation in $\ln(v)$. The estimate of a in Equation (1) is $\exp(0.798) = 2.155$. The parameter a was significantly greater than 1 ($\ln(a) > 0$); the estimate of the slope parameter b was also significantly different from 1 ($P < 0.01$).

Incidence–density and incidence–incidence relationship

In all the following analyses of incidence–density and incidence–incidence relationships, Italian samples were excluded for two reasons. First, there were only five out of 83 samples with % of infected spikelets >1.5%, of these five the four most severe ones were from the same two sites in 2002. Second, the relationship between ear and spikelet incidence (or density) for these five samples differed profoundly from the general pattern shown by all the samples from the other three countries (Figures 5 and 6b).

Relating incidence to density

When the number of infected spikelets on an ear was assumed to follow a Poisson distribution, predicted numbers of infected spikelets using ear incidence (Equation (3)) were generally greater than observed (Figure 5). About 87.7% of variation in the observed incidence was accounted for by Equation (3). This over-estimation was due to

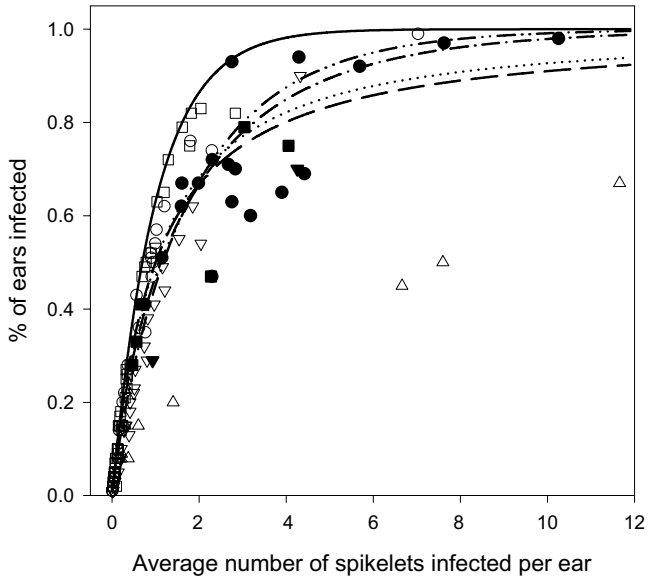

Figure 5. Observed and fitted relationships between disease density and incidence of ear infection of FEB in wheat. (○) – Ireland, (□) – UK, (▽) – Hungary, (△) – Italy. Solid line – fitted curve based on Poisson distributions, dot-dot-dashed line – regression model based on complementary log–log transformation of ear incidence, dashed line – directly fitted curve based on negative binomial distributions with a constant k, dot-dashed line – directly fitted curve based on negative binomial distributions with log-linear variance–mean relationship, dotted line – logistic curve. The data from Italy were excluded in deriving the relationships. The filled symbols are for those samples that cannot be fitted by a negative binomial distribution.

the aggregated patterns of the number of infected spikelets on an ear as shown by the distribution analysis.

The relationship between incidence of infected ears and number of infected spikelets on an ear was derived by assuming that the number of infected spikelets on an ear followed a negative binomial distribution with a constant k over all the samples. The derived model is

$$p = 1 - P(0) = 1 - \left(\frac{m}{k} + 1\right)^{-k} = 1 - \left(\frac{m}{1.007} + 1\right)^{-1.007} \quad (12)$$

and the standard error of estimated k is 0.077. About 92.9% of variation in the observed incidence was accounted for by Equation (13) and this model was also shown in Figure 5.

Similarly, the relationship between ear incidence and number of infected spikelets on an ear was derived by assuming that the number of infected spikelets on an ear followed a negative binomial distribution and assuming a common variance–mean relationship (i.e. Equation (6). This model is

$$p = 1 - \mathrm{e}^{-m(2.344m^{0.357}-1)^{-1}\ln(2.344m^{0.357})} \quad (13)$$

This relationship between p and m was very close to the model based on the constant k (i.e. Equation 12) (Figure 5), accounting for about 92.6% of total variation in p.

The complementary log–log transformation of the ear incidence was well related to logarithm of the average number of infected spikelets per ear:

$$\underset{(0.034)\ (0.018)}{\mathrm{CLL}(p) = -0.509 + 0.896\ln(m)} \quad (14)$$

This relationship (Figure 5) accounted for about 92.0% of variation in p. There were significant differences between the countries in the two parameter estimates, but only accounted for <2% of the variation in CLL(p), hence a common model (Equation (16)) was used.

The logit of ear incidence (i.e. $\ln(\frac{p}{1-p})$) was linearly related to $\ln(m)$:

$$\ln\left(\frac{p}{1-p}\right) = 1.103\ln(m) \quad (15)$$

with a standard error of the parameter estimate of 0.189. This model accounted for 93.2% of total variation in p.

These five models (Equations (3) and (12)–(15)) are all shown in Figure 5 together with the observed data. It is clear that Equations (12) and (15) are nearly identical as are Equations (13) and (14). The Poisson based model (Equation (3)) gave the worst predictions among the five models. The other four models differed very little and all gave very accurate predictions when $P < 0.7$. Correlation between the predicted and observed incidence was >0.96.

Relating incidences of infected ear and spikelets

Assuming a binomial distribution of diseased spikelets on an ear, the relationship between these two incidences is shown in Figure 6a, using the estimated average number of total spikelets per ear for each sample. Similar to the results based on the density, predicted incidence of infected spikelets using ear incidence (Equation (9)) was generally greater than observed. About 86.8% of total variation in the observed ear incidence was accounted for by Equation (10).

The complementary log–log transformation of the ear incidence related well to the complementary log–log transformation of incidence of infected spikelets:

$$\begin{aligned} \mathrm{CLL}(p_e) &= 2.001 + 0.865\mathrm{CLL}(p_s) \\ &\quad (0.075)\ (0.017) \end{aligned} \tag{16}$$

This relationship (Figure 6b) accounted for 91.7% of total variation in p_e. There were significant differences between the countries in the two parameter estimates, but these only accounted for <2% of the total variation in CLL(p_e), hence a common model (Equation (16)) was used.

The logit of ear incidence (logit(p_e)) was linearly related to logit(p_s):

$$\begin{aligned} \ln\left(\frac{p_e}{1-p_e}\right) &= 2.911 + 1.024\ln\left(\frac{p_s}{1-p_s}\right) \\ &\quad (0.639)\quad (0.184) \end{aligned} \tag{17}$$

This model accounted for 93.3% of total variation in p_e. These two models (Equations (16) and (17)) were very similar for $p_e < 0.7$ (Figure 6b). Correlation between predicted and observed p_e was >0.96 for both Equations (16) and (17).

Discussion

In this study, we explored the relationship between incidence of ear infection and number of spikelets infected on an ear (and incidence of spikelet infection) for *Fusarium* ear blight, with the aim of predicting disease at the spikelet level using incidence of infected ears. When relating incidence to disease density (number of spikelets infected) using a mechanistic approach, a critical basis is the type of distribution that the disease density follows: the disease incidence can then be estimated using the zero term of this probability distribution (Hughes et al., 1997). The present study showed that numbers of spikelets infected by the FEB pathogens on an ear generally did not follow a Poisson distribution; this lack of fit is mainly due to the excessive number of ears either with no infected spikelets or with almost all spikelets infected. This is not surprising given that most plant diseases are not randomly distributed (Madden and Hughes, 1995; Hughes et al., 1997). The three generalised two-parameter distributions (Neyman type A, negative binomial and Poly-Aeppli distributions) generally provided a similarly good fit to the majority of data sets, indicating a certain degree of aggregation of infected spikelets on an ear. This is further confirmed by the variance–mean analysis, which indicated that the variance generally increased linearly with colony density on a log–log scale. Moreover, it appeared that the variance–mean relationship is generally consistent over the three countries. An aggregated pattern of density data is often observed for plant diseases, e.g., tobacco blue mould (Waggoner, 1981), apple powdery mildew (Barlow, 1977; Xu and Madden, 2002), leaf blight of carrot caused by *Cercospora carotae* (Boivin et al., 1990), and leek rust (de Jong, 1995).

There are two likely explanations for the observed aggregation of FEB-infected spikelets on an ear. First, the observed aggregation may have resulted from aggregation of the inoculum that landed on individual ears. Second, number of spikelets initially infected may follow a Poisson distribution (i.e., random), but subsequent colonisation of previously healthy spikelets may lead to apparent aggregations of infected spikelets. The rate of colonisation may depend on weather conditions, host susceptibility, and FEB species. Of course, it is also possible that the observed

aggregation was the combined results of aggregated initial inoculum and subsequent colonisation. However, distinguishing these probable causes is not a trivial matter.

In the literature, disease density data often are explicitly fitted by the negative binomial distribution, for reasons discussed by Xu and Madden (2002), but mainly for its ease of use and its integration with variance–mean relationships, though Xu and Madden (2002) have also demonstrated that this is also possible for the Neyman type A distribution. In this study, we have adopted

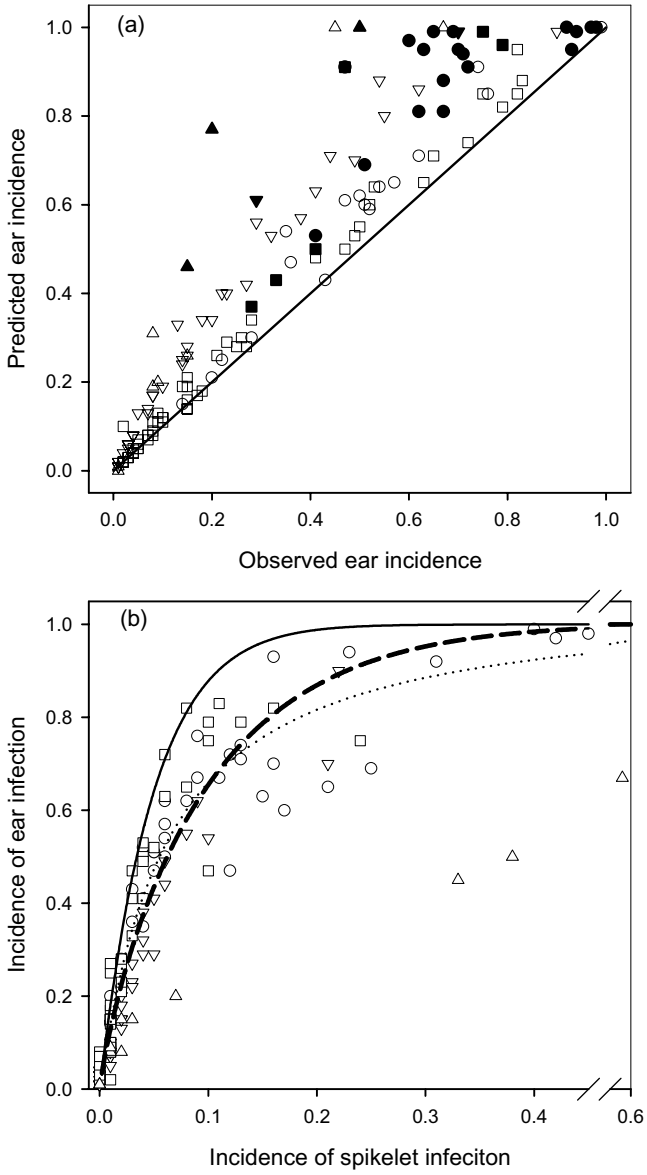

Figure 6. Observed and fitted relationships between incidence of FEB on ears and spikelets in wheat: (a) assuming the number of infected spikelets on an ear is binomially distributed and (b) other models. (○) – Ireland, (□) – UK, (▽) – Hungary, (△) – Italy. Solid line – fitted curve assuming the number of infected spikelets on an ear is binomially distributed (an average 20 spikelets per ear was used), dashed line – regression model based on complementary log–log transformation of ear and spikelet incidences, dotted line – logit model. The data from Italy were excluded in deriving the relationships. The filled symbols are for those samples that cannot be fitted by a negative binomial distribution.

the Negative binomial distribution as a basis for developing incidence–density relationships. Despite the significant improvement in the goodness of fit over the Poisson distribution, there are still 27 data sets that cannot be adequately described by a negative binomial distribution. The failure to obtain a successful fit is mainly due to fewer ears with no infected spikelets and a greater number of ears with almost all spikelets infected than predicted by the negative binomial distribution. Most of these data sets were from Ireland, which could be due to two reasons. First, during the critical stage the weather was wetter in Ireland than in the other countries, leading to fast colonisation of the ears from initially infected spikelets, which might be random or aggregated due to aggregated inoculum and/or microclimate on an ear. Second, this may also be due to the different colonisation characteristics between FEB pathogens. Species-specific PCR research of the same samples have shown that *F. culmorum*, *F. graminearum* and *M. nivale* were the dominant species in Ireland, whereas in UK and Hungary the dominant species is *F. poae* (results not shown). *Fusarium poae* is generally believed to be less pathogenic and aggressive than other FEB pathogens (Pettersson and Olvång, 1995; Brennan et al., 2003). The four samples from two Italian sites showed even more extreme aggregation of infected spikelets within an ear, though we do not have detailed information to quantify the degree of aggregation for these samples.

Several approaches were used to relate ear incidence (p) to the number of spikelets infected (m) based on the relationship: $p = 1 - \exp(-f(m))$. The methods differ in the precise form of $f(m)$. In method 1, $f(m)$ is given simply by m (i.e., assuming that number of infected spikelets per ear follows a Poisson distribution); in the other methods, it is assumed that the number of spikelets infected per ear follows an alternative discrete distribution. Two models were developed on the basis of a negative binomial distribution assuming that the aggregation characteristic follows a power-law variance–mean relationship or that the aggregation parameter k of the negative binomial distribution is constant. Finally, a two-parameter generalised Poisson model was also fitted to the data. Not surprisingly, the Poisson-based model provided the worst predictions among the four models, as it did not take the aggregation of infected spikelets on an ear into account. Unexpectedly, the two models based on negative binomial distributions performed similarly well despite the fact that the one assumed a power-law variance–mean relationship and the other assumed a constant k. However, we must remember that constant k does not necessarily mean constant aggregation and in fact it implies a quadratic relationship between variance and mean since $k = \frac{m^2}{v-m}$. An empirical logistic model also provided accurate predictions and indeed it is very close to the model based on constant k.

Similarly, several models were also derived to relate ear incidence to spikelet incidence. The incidence–incidence relationship should be the same as the incidence–density relationship if the average number of spikelets per ear is the same for all the samples. Given the relatively small amount of variation in the average number of spikelets per ear between samples, it is expected that the general pattern in these two relationships should be similar and, indeed, this is the case (Figures 5 and 6). Similar results were also reported in the USA on FEB caused by *F. graminearum* (McRoberts et al., 2003), using a subset of the data from Groth et al. (1999), though the relationship has less scatter than observed here. There are several possible explanations for this difference. First, our data were collected from more than 60 sites over four European countries, where their data were from a single region. Second, our samples were from field wheat naturally infected whereas their data were from artificially infected plots. Finally, there are several FEB pathogens present in our samples as shown by the detective PCR study (results not shown here) while *F. graminearum* is the only species in USA causing FEB on wheat.

In conclusion, this study showed that there is a degree of aggregation in the number of infected spikelets on an ear for FEB and that the degree of aggregation varied with the mean according to a power law. It was feasible to predict mean density of infected spikelets per ear (or incidence of spikelets infection) from the incidence of ear infection. Because the relationships were generally consistent over seasons and countries, it should be possible to use them in making efficient disease management decisions based on either incidence or colony-density thresholds. Of these models, the empirical logistic model is worth singling out as it has only one parameter and does not dependent on any

distribution assumptions. However, further validation of these models will ultimately be needed using independent data. In addition, these models may not be accurate when considerable secondary colonisation has taken place on infected ears after initial infections and this is especially likely to occur during anthesis and early seed development stages when conditions are wet. Under these circumstances, the model is likely to underestimate the disease severity.

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References

Bai G and Shaner G (1994) Scab of wheat: Prospects for control. Plant Disease 78: 760–766

Barlow GP (1977) Sampling and variation. East Malling Research Station Annual Report for 1976: 68

Bhat RV, Ramakrishna Y, Beedu SR and Munshi KL (1989) Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat products in Kashmir valley India. The Lancet 7: 35–37

Boivin G, Kushalappa AC and Brodeur L (1990) Spatial dispersal and binomial sequential sampling plan for *Cercospora carotae* on carrots. Canadian Journal of Plant Pathology 12: 209–212

Brennan JM, Fagan B, van Maanen A, Cooke BM and Doohan FM (2003) Studies on in vitro Growth and Pathogenicity of European *Fusarium* Fungi. European Journal of Plant Pathology 109: 577–588

de Jong PD (1995) Sampling for detection: Leek rust as an example. International Journal of Pest Management 41: 31–35

Dubin HJ, Gilchrist L, Reeves J and McNab A (eds) (1997) Fusarium head scab: Global status and prospects. CIMMYT, Mexico DF, Mexico 130 p

Freeman GH (1980) Fitting two-parameter discrete distributions to many data sets with one common parameter. The Journal of the Royal Statistical Society Series C (Applied Statistics) 29: 259–267

Groth JV, Ozmon EA and Busch RH (1999) Repeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries. Plant Disease 83: 1033–1038

Huang SX (1992) Mycotoxicoses occurring in flooded areas and preventive measures against them. In: Proceedings of Chinese Countermeasures for Anti-epidemic and Disaster Relief (pp 45–49) CMH, Beijing, China

Hughes G, McRoberts N, Madden LV and Gottwald TR (1997) Relationships between disease incidence at two levels in a spatial hierarchy. Phytopathology 87: 542–550

Li FQ, Luo XY and Yoshizawa T (1999) Mycotoxins (trichothecenes, zearalenone and fumonisins) in cereals associated with human red intoxications stored since 1989 and 1991 in China. Natural Toxins 7: 93–97

Madden LV and Hughes G (1995) Plant disease incidence: Distributions, heterogeneity, and temporal analysis. Annual Review of Phytopathology 33: 529–564

McMullen MP, Jones R and Gallenberg D (1997) Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease 81: 1340–1348

McRoberts N, Huges G and Madden LV (2003) The theoretical basis and practical application of relationship between different disease intensity measurements in plants. Annals of Applied Biology 142: 191–211

Nachman G (1981) A mathematical model of the functional relationship between density and spatial distribution of a population. Journal of Animal Ecology 50: 453–460

Ord JK (1972) Families of Frequency Distributions. Griffin, London, UK

Parry DW, Jenkinson P and McLeod L (1995) *Fusarium* ear blight (scab) in small grains – a review. Plant Pathology 44: 207–238

Payne RW, Lane PW, Todd AD, Digby PGN, Thompson R, Harding SA, Wilson TG, Leech PK, Welham SJ, Morgan GW and White RP (1993) Genstat^{TM} 5 Release 3: Reference Manual. Oxford University Press, Oxford, UK

Pettersson H and Olvång H (1995) Trichothecene production by *Fusarium poae* and its ecology. In: Book of Abstracts of International Seminar on Fusarium – mycotoxins, taxonomy and pathogenicity, Martina Franca 1995 (pp 178–179)

Ridout MS and Xu X-M (2000) Relationships between several quadrat-based statistical measures used to characterize spatial aspects of data on disease incidence. Phytopathology 90: 568–575

Seem RC (1984) Disease incidence and severity relationships. Annual Review of Phytopathology 22: 137–150

Taylor LR (1961) Aggregation, variance and the mean. Nature 189: 732–735

Turechek W and Madden L (2001) Effect of scale on plant disease incidence and heterogeneity in a spatial hierarchy. Ecological Modelling 144: 77–95

Waggoner PE (1981) Models of plant disease. Bioscience 31: 315–319

Xu X-M (2003) Effects of environmental conditions on the development of *Fusarium* ear blight. European Journal of Plant Pathology 109: 683–690

Xu X-M and Madden LV (2002) Incidence and density relationships of powdery mildew on apple. Phytopathology 92: 1005–1014

Xu X-M and Ridout MS (1998) Effects of initial epidemic conditions, sporulation rate, and spore dispersal gradient on the spatio-temporal dynamics of plant disease epidemics. Phytopathology 88: 1000–1012

Xu X-M, Robinson JD, Berrie AM and Harris DC (2001) Spatio-temporal dynamics of brown rot (*Monilinia fructigena*) on apple and pear. Plant Pathology 50: 569–578

Zadoks JC, Chang TT and Konzak CF (1974) Decimal code for growth stages of cereals. Weed Research 15: 415–421