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Association of *Fusarium* species in the wheat stem rot complex

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Abstract

Data from a national survey were analysed to investigate whether there was interdependence among the *Fusarium* species, which cause the stem rot complex of wheat. About 25 wheat stems were sampled from each of 260 sites over the main wheat growing areas in the UK. Occurrence of each *Fusarium* species on individual stems was determined. *Fusarium culmorum*, *F. avenaceum* and *Microdochium nivale* were the three dominant species, detected in 248, 185 and 239 out of the 260 sites. There were no interactions among species in the distribution of the three species over the 260 sites. Several statistical tests were used to determine whether there was interdependence among the three species on the same stem within each site. Of the three species, there was only limited evidence of competition between *F. culmorum* and *F. avenaceum*.

Introduction

Fusarium foot rot of wheat is caused by a number of species which can either infect stem bases individually or as part of a disease complex (Parry et al., 1994). The predominant species present in UK wheat crops are *Fusarium culmorum*, *Microdochium nivale* and, to a lesser extent, *F. avenaceum* (Parry, 1990; Pettitt et al., 1993). Colonisation of wheat stem bases by these pathogens appears to follow a seasonal succession (Duben and Fehrman, 1979; Parry, 1990), which may in part be mediated by temperature (Pettitt and Parry, 2001). The predominant species in any one year is important in determining the severity of foot rot symptoms seen and appears to change from year to year (Pettitt et al., 1996). However, the importance of competition between these species for infection sites in determining predominance has not been explored in detail. In this paper the interdependence of the three main members of the UK *Fusarium* foot rot complex of wheat is considered by various statistical methods and by using isolation data collected from over 260 fields distributed across eastern England.

Materials and methods

Field samples

As part of an annual cereal disease survey of England and Wales carried out by Central Science Laboratory in Harpenden in collaboration with the Agricultural Development and Advisory Service, winter wheat stem-bases were collected in 1993 from between 350 and 400 crops, at growth stage 73–75 ('milky ripe', Zadoks et al., 1974), following the procedure described by Polley and Thomas (1991). From each site sampled, 50 fertile tillers were taken at random from along a diagonal traverse of the field. Detailed isolations were carried out on samples from 260 of these sites, mostly from the eastern counties of England (Figure 1). Subsamples of 25 tillers were taken for isolations once information on disease incidence and severity in each tiller sample had been recorded. At each site, monthly average temperatures were recorded.

Isolation and identification of pathogens

Isolations were made following the procedures described previously (Pettitt et al., 1993; 1996). Stem

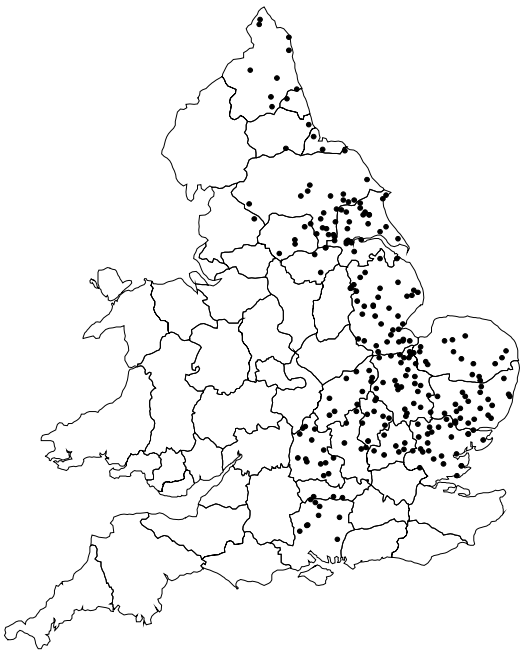

Figure 1. Map of England and Wales showing the distribution of the field sites (•) sampled in this study.

bases and first nodes were excised from each sampled tiller, surface sterilised in sodium hypochlorite solution (5% available chlorine) and washed three times in sterile distilled water. Tissue pieces were blotted on sterile tissue paper and split longitudinally in two. One piece was placed on potato dextrose agar containing streptomycin sulphate ($100\,\mu g\,ml^{-1}$), neomycin ($50\,\mu g\,ml^{-1}$) and chloramphenicol ($50\,\mu g\,ml^{-1}$), and the other on the same medium supplemented with $10\,\mu g\,ml^{-1}$ benomyl (Benlate fungicide, DuPont UK Ltd, 50% w/w a.i.). Since *M. nivale* populations are largely resistant to benomyl (Locke et al., 1987) and *F. avenaceum* is slightly more 'tolerant' to the fungicide than the faster-growing *F. culmorum*, this process allows the separation of the species present in isolations from multiple infections. Isolated fungi were identified by colony colour and morphology and by macroconidium morphology according to Booth (1971) with additional guidelines from D. Brayford (International Mycological Institute, Kew, UK, personal communication). The numbers of tillers with single infections by each of the three target pathogen species, *F. culmorum, F. avenaceum* and *M. nivale*, were recorded for each sample. Also recorded were the numbers of each combination of double infection and the numbers of tillers infected with all three species.

Statistical methods

Two levels of association between the three species were investigated. The first was at the site level, i.e., whether the occurrence of one species was geographically related to the other two species. The second was at the stem level, i.e., whether the occurrence of one species was related to the presence/absence of the other two species on the same stem on a site where more than one species was found. Chi-square test was used to determine whether there was significant association/interaction between species at the first level.

Log-linear models were used to test the independence of the three *Fusarium* species in the stem rot complex within each sampling site at the stem level assuming a Poisson sampling. In this context, Poisson sampling means that a fixed amount of effort was used to sample the wheat stems, which were then categorised into cells in the table depending on the presence/absence of three species. Specifically, it assumed that neither the total number of individual species nor the total occurrence of all species was known before the sampling took place. The present sampling indeed met these two criteria.

In the log-linear model, each count in the individual cell was treated as a Poisson response variable, which was then regressed on independent variables/factors. In the present study, there were three factors, i.e., three species (*F. culmorum, F. avenaceum* and *M. nivale*), each with two levels: present or absent. The full model, with all the interactions (one three-way and three two-way interactions), was the saturated model. The test statistic for independence of two or three species equals the sum of squared Pearson residuals from the log-linear model without the specific interaction term to be tested. This test statistic has a chi-square distribution with one degree of freedom. This test was done for all four interaction terms in turn. This process of fitting log-linear models for testing each interaction was done for all those sample sites where there were at least two species present.

Since the results from fitting the log-linear model showed that at no sites were there more than one significant interaction term and that for all the sites the three-way interaction was not significant, the Chi-square test and Fisher's exact test were also applied to determine whether two species interactions were significant. Chi-square test is an approximate test and its accuracy increases with the sample size. Fisher's exact test, as the name indicates, provides an exact probability for

the observed data, but its application is restricted to two-way interactions.

Logistic regression analysis (Cox and Snell, 1989), which is based on the logit transformation of the proportion (p) of stems infected ($\ln(p/(1-p))$) at each site, was used to assess the relationship between temperature and the incidence of each fungal species. In this analysis, the number of stems with each species at each site was assumed to be distributed binomially. All the statistical analyses were done using Genstat™ (Payne et al., 1993).

Results

General results

Figure 2 shows the histogram of the disease incidence over all the samples for the three species. Of three species, *F. culmorum* had the highest incidence with an average of 50.5% sampled stems infected, whereas *F. avenaceum* had the lowest incidence with average 12.5% stems infected: incidence for *M. nivale* was 37.1%. There were 12, 20 and 71 samples that did not contain *F. culmorum*, *M. nivale* or *F. avenaceum*. The frequency data, the number of stems infected with each species at individual sites, cannot be fitted satisfactorily to Poisson distributions for all three species. The variance and mean ratios were 5.7, 4.9 and 4.5 (for a Poisson distribution, the ratio is expected to be 1; a Poisson distribution is expected for random distributed counts data) for *F. culmorum*, *M. nivale* and *F. avenaceum*, respectively, indicating extreme aggregation of diseased stems within a sample site. A negative binomial distribution fitted satisfactorily only to the data for *F. avenaceum*, whereas none of common discrete distributions fitted *F. culmorum* and *M. nivale* data satisfactorily because of excess of observed infected stems in the upper level of incidence (Figure 2).

Logit regression showed that the percentage of variation in disease incidence within a sampling site accounted for by weather condition was very small, especially for *M. nivale* and *F. avenaceum*. Temperature was summarised either from 100 days before harvest or from February to harvest. There were no indications from these data that the incidences of the three species at the time of sampling were significantly differently affected by temperature.

Association/interaction between species at the site level

Table 1 gives the summary of species coexistence at the site level. Of the 260 sampling sites, *F. culmorum*, *M. nivale* and *F. avenaceum* were found in 248, 239

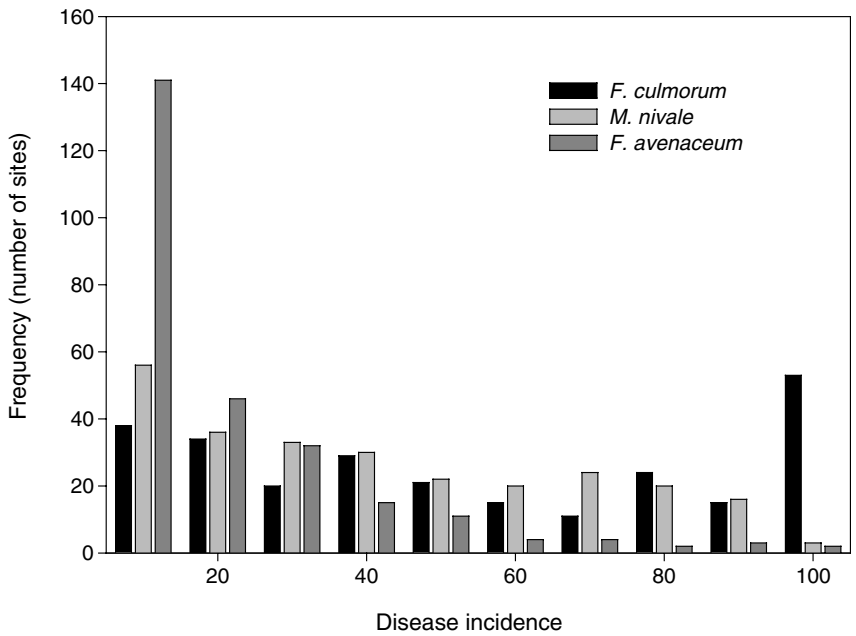

Figure 2. Histograms of the sampling sites with different incidence levels of *F. culmorum, M. nivale* and *F. avenaceum.*

Table 1. Summary of the coexistence of *F. culmorum, F. avenaceum* and *M. nivale* at site level

Species	Number of sites with the species (out of 260)	Species combination	Expected number of sites with the combination assuming no interactions	Observed
F. avenaceum (Fa)	185	Fa/Fc	176	178
F. culmorum (Fc)	248	Fc/Mn	228	228
M. nivale (Mn)	239	Fa/Mn	170	169
		Fa/Fc/Mn	162	163

Table 2. Summary of statistical analysis of species coexistence of *F. culmorum* (Fc), *F. avenaceum* (Fa) and *M. nivale* (Mn) on the individual stem-base of wheat at each site

Species	No of sites out of 260 with the species	Number of site with significant interaction		
		LL	Fisher	χ^2
Fa × Fc	178	25	10	25
Fc × Mn	228	13	5	14
Fa × Mn	169	5	0	8

LL – Log-linear method; Fisher – Fisher's exact test; χ^2 – Chi-square test.

and 185 sites, respectively. The observed number of sites with various combinations of three species was extremely close to the expected under the assumption of independence between species (Table 1). For example, assuming independence, both *F. culmorum* and *F. avenaceum* were expected to occur in 176 out of 260 sites, comparing the observed 178 sites. All three species were expected to occur in 162 out 260 sites under the assumption of independence, compared to the observed value of 163 sites.

Association between species on the same stem

Results from fitting log-linear models showed that at none of sites were there significant three-way interactions between the species. Furthermore, there was no more than one significant two-way interaction at any given site.

Table 2 gives the summary of analysis on association of species on the same stem. In 25 out of 178 sites, significant interactions between *F. culmorum* and *F. avenaceum* were detected by the log-linear model analysis, compared to 13 out of 228 sites and 5 out 169 sites for *F. culmorum* × *M. nivale* and *F. avenaceum* × *M. nivale*, respectively. Similar results were obtained from the Chi-square testing (Table 2). Almost all of the sites with significant interactions identified by log-linear modelling coincided with those identified by Chi-square testing. Most of significant interactions were of competition type, i.e., presence of one species inhibited the presence of another on the same stem.

In contrast, there were fewer sites with significant interactions from Fisher's exact testing (Table 2): 10 out of 178 and 5 out of 228, and zero were significant for *F. culmorum* × *F. avenaceum*, *F. culmorum* × *M. nivale* and *F. avenaceum* × *M. nivale*, respectively. The sites with significant interactions identified by the Fisher's testing were a subset of those identified by log-linear modelling or Chi-square test.

Discussion

This study showed that the conclusions on the independence of species, causing the *Fusarium* stem rot complex, critically depended on the analytical method used. Applying both log-linear model and Chi-squared analyses resulted in similar conclusions, indicating a much greater number of sites with significant interactions than the Fisher's exact test. Since the Fisher's test gives the exact probability, its results might be expected to be more reliable than the other two methods. The main reason for this difference between three analytical methods is likely to be the relatively restricted number of stems sampled per site.

The survey results strongly indicate that *F. culmorum*, *F. avenaceum* and *M. nivale* are independently distributed across the sampling sites, with logistic regression analysis showing no differential effects of temperature sum on the incidence of the three species at the time of sampling. If there were differential effects of temperature on the three species, it would be expected that the species would not be independently distributed across the sites. It may also be possible that the temperature range across the sites in the survey year was not great enough to detect differential

effects on the three pathogen species. Alternatively, other factors such as local agronomy and/or fungicide treatments may have confounded some individual site data. When data from a number of sites in a particular region (e.g. a county) have been pooled, weak correlations between incidence and temperature sum were obtained for *M. nivale* (Pettitt et al., 1996) and for *F. graminearum* (Smiley and Patterson, 1996).

This study showed no evidence of competition or codependence between *F. culmorum* and *M. nivale.* However, this may be the result of a combination of the normal temporal sequence of infections leading to *M. nivale* infections becoming established and reaching their peak before significant activity of *F. culmorum*, and the inability of *M. nivale* to exclude *F. culmorum* from infected tissues. It may be that these two species are able to colonise the same host tissues because of differing requirements from the colonised tissues. However, when *F. culmorum* is the primary coloniser of new substrates, including winter wheat stems, it is usually extremely efficient at excluding subsequent potential colonisers (Cook, 1970). It would therefore be useful to carry out sequential inoculations of wheat plants with *F. culmorum* and *M. nivale* to determine the effects of each species as a primary coloniser on the incidence of subsequent infections, before finally discounting the existence of competition between these two species in wheat stem-bases.

The only evidence of competition in this study was between *F. culmorum* and *F. avenaceum*, with significant competition, as identified by Fisher's test, occurring in 10 (ca. 5.6%) out of the 178 sites were both of these species are present. This may reflect the fact that *F. avenaceum* and *F. culmorum* are more closely related than *F. culmorum* and *M. nivale* (Booth, 1971) and are closer together in terms of temperature and moisture requirements for infection and pathogenicity (Colhoun and Park, 1964; Pettitt and Parry, 2001). Whilst competition between *F. culmorum* and *F. avenaceum* probably results from their similar infection and colonisation requirements, *F. avenaceum* is generally a far weaker pathogen of UK winter wheat crops (Colhoun et al. 1968). The techniques used in this first investigation of the interdependence of the main *Fusarium* species involved in the stem base rot complex of wheat were limited to confirming the presence or absence of individual species on individual tillers. Further work is now needed to confirm the tentative conclusions of this study and this will probably be best achieved using new molecular techniques that will allow quantification of individual pathogen biomass as well as confirmation of their presence. Competitive PCR and, more recently, 'real-time' PCR assays have been developed for quantifiable detection of *F. graminearum* and *F. culmorum* (Nicholson et al., 1998; Schilling et al., 1996), *M. nivale* var. *majus* and var. *nivale* (Nicholson et al., 1996), and *F. avenaceum* (Turner et al., 1998; Schilling et al., 1996). These assays should provide the means to dissect the *Fusarium* stem rot complex into its component parts and determine the prevalence and role of each component in disease development and under various conditions.

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