Long-term relationships between environment and abundance in wheat of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola*

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Summary

• Relationships between weather, agronomic factors and wheat disease abundance were examined to determine possible causes of variability on century time scales.

• In archived samples of wheat grain and leaves obtained from the Rothamsted Broadbalk experiment archive (1844–2003), amounts of wheat, *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* DNA were determined by quantitative polymerase chain reaction (PCR). Relationships between amounts of pathogens and environmental and agronomic factors were examined by multiple regression.

• Wheat DNA decayed at approx. 1% yr^{-1} in stored grain. No *M. graminicola* DNA was detected in grain samples. Fluctuations in amounts of *P. nodorum* in grain were related to changes in spring rainfall, summer temperature and national SO_2 emission. Differences in amounts of *P. nodorum* between grain and leaf were related to summer temperature and spring rainfall. In leaves, annual variation in spring rainfall affected both pathogens similarly, but SO_2 had opposite effects. Previous summer temperature had a highly significant effect on *M. graminicola*. Cultivar effects were significant only at $P = 0.1$.

• Long-term variation in *P. nodorum* and *M. graminicola* DNA in leaf and grain over the period 1844–2003 was dominated by factors related to national SO_2 emissions. Annual variability was dominated by weather factors occurring over a period longer than the growing season.

Key words: environmental factors, host–pathogen–environment interactions, long-term patterns in agronomy, pathogen population dynamics, pollution, wheat septoria diseases.

Introduction

Man-made emissions of CO_2 and SO_2 are affecting plant–pathogen interactions in both natural and agricultural ecosystems worldwide, through climate change and pollution (Fitt *et al.*, 2006; Garrett *et al.*, 2006). It is often difficult to distinguish the long-term effects of climate change and pollution on disease epidemics in agricultural crops from short-term effects of seasonal changes in weather (e.g. temperature, rainfall) and agronomic practices. Such distinctions can be made only where long-term sets of data exist for both biological (e.g. pathogen DNA) and environmental (e.g. weather, pollutant) factors, as with the Rothamsted long-term experiment on wheat fertilization (Broadbalk) (Bearchell *et al.*, 2005). These data have been used to propose a new hypothesis to explain the unexpected change in the 1980s in the relative importance

of the two septoria diseases on leaves of winter wheat in the UK, which had been observed in national disease surveys (King, 1977, Polley & Thomas, 1991, Hardwick *et al.*, 2001).

These two septoria diseases of wheat, caused by *Phaeosphaeria nodorum* and *Mycosphaerella graminicola*, are important constraints to production worldwide. Both cause yield loss when they decrease the green leaf area of upper leaves that fill grain before harvest (Eyal, 1999). Both diseases are greatly influenced by weather factors such as rainfall and temperature. For example, both pathogens are spread within crops by rainsplash-dispersed asexual spores (conidia), and require moisture to infect leaves. *Mycosphaerella graminicola* also reproduces sexually, and its wind-dispersed ascospores are produced throughout the year (Eriksen & Munk, 2003). However, in Europe their major role is to disperse the pathogen to new fields from the debris of old crops at the start of the winter wheat-growing season in autumn (Eriksen *et al.*, 2001). *Phaeosphaeria nodorum* also produces ascospores, but these appear to be less universally important in its epidemiology (Cunfer, 1998); the pathogen is spread efficiently to new crops because seed infection is common (Cunfer, 1978, Bennett *et al.*, 2005). It is not clear how climatic factors affect the long-term pattern in amounts of *P. nodorum* on wheat grain, in relation to the interaction between *P. nodorum* and *M. graminicola* on leaves.

Polymerase chain reaction (PCR) testing of Broadbalk archive samples in a previous study showed that the long-term pattern in the ratio of *P. nodorum* to *M. graminicola* DNA, with *M. graminicola* predominant in the mid-19th century and after 1985, and *P. nodorum* predominant in the mid-20th century, could not be explained by fluctuations in weather and agronomic factors (Bearchell *et al.*, 2005). Hypotheses about the change in relative importance of the two pathogens in the 1980s, relating it to factors such as changes in cultivar (from long-strawed to short-strawed), introduction of fungicides, May–June rainfall or November–December temperature did not explain the long-term pattern in the data over the 160-yr period. By contrast, there was an unexpected but excellent correlation between the ratio of DNA of the two pathogens at Broadbalk and UK SO_2 emission over the period, which we hypothesize to be causal. In further work, we have obtained a similar PCR series for amount of *P. nodorum* in grain and, as a reference, a series for the survival of amplifiable wheat DNA in grain over the 160-yr period.

The aim of the work reported here was to analyse relationships over this period between abundance of the pathogens and environmental variables, and to compare relationships for DNA series from grain samples with those from leaf/stem (vegetative) samples.

Materials and Methods

The methods for DNA extraction and quantitative real-time PCR detection of each target template DNA using fluorescent minor groove binder-conjugated TaqMan probes were described by Bearchell *et al.* (2005). For the grain series, 8 g harvested grain from each season were ground to a powder within two sealed polyethylene bags, then decanted into a 50-ml centrifuge tube for extraction. The total amount of DNA extracted from each sample was measured with the fluorescent dye thiazole orange (Fraaije *et al.*, 2005). Quantitative PCR was carried out with each sample using 50 ng total DNA, and the amplification cycle at which the increase of fluorescence exceeded the background (Ct) was determined. Separate reactions were carried out for each DNA target sequence. Regression equations for each target were estimated using the Ct values obtained from known amounts of DNA prepared from fresh extractions of each target species. These regression equations were used to convert Ct values to equivalent DNA concentrations. Thus six basic 160-yr series of DNA abundance data were obtained, one for each of the two pathogens, and wheat from both grain and leaf/stem samples.

No septoria disease severity data were collected from the Broadbalk experiment over most of the 160-yr period. Both Broadbalk DNA measurements and national wheat disease survey severity measurements (King, 1977, Polley & Thomas, 1991, Hardwick *et al.*, 2001) must contain large random components, making regression an unreliable guide to the slope of this relationship. To relate the amount of Broadbalk DNA to national visual disease severity (assessed on leaves at GS72, milky-ripe) over the period 1970–2003, we therefore calculated the first principal component of national visual severity on Broadbalk DNA measurements (Kendall, 1975). The significance of this relationship was judged from the correlation coefficient between the two series after log_e-transformation of the data. We did this for *P. nodorum* and *M. graminicola* separately, and for the ratio of national visual severities of the two septoria diseases against the ratio of the Broadbalk DNA measurements for the two pathogens.

The Broadbalk winter wheat experiment was sown in autumn (generally October) and harvested the following summer (August). For the analyses, the following environmental and host factors were considered. Average July–August (summer) temperature (for the year of harvest and the previous year) and average December–February (winter) temperature were obtained from Rothamsted data from 1879 onwards, and from the Central England Temperature (CET) series from 1844–99. When both Rothamsted and CET data were available (1879–99) they were very well correlated ($r = 0.98$), but Rothamsted data differed systematically from the CET series by $-1.8°C$ for December–February and by $+0.3°C$ for July–August temperatures. These differences were used to produce a single combined temperature series from 1844 onwards. Total rainfall in May and June (spring) from 1853 onwards and wind-run in April and May from 1947 onwards were obtained from Rothamsted records. Cultivar information was available from Rothamsted records. Approximate height of cultivar was estimated from crops of these cultivars or

closely related ones grown at the University of Reading in each of the 1998, 1999 and 2000 seasons. The area of land sown to wheat in England from 1866 onwards was obtained from UK government statistics. Data on harvest method (hand-cutting, stooking and carting or plot combine), seed fungicide treatment, sowing date and foliar fungicide use on Broadbalk were available from the experimental records; changes on the Broadbalk plots broadly coincided with changes in general farming practices. Annual UK emissions of SO_2 were obtained by third-order polynomial interpolation of 10-yr averages published in government reports (United Kingdom Review Group on Acid Rain, 1983, National Expert Group on Trans-boundary Air Pollution, 2001) using MATHEMATICA (Wolfram Research, Champaign, IL, USA).

Relationships were examined by multiple regression of the logarithms of the various pathogen DNA abundance series on the environmental and agronomic factors. The best models were selected by examining all subsets of the possible independent variables for which data were available during a given time period. The residuals from models including all variables selected were tested for autocorrelations or cyclical changes by standard time-series methods.

Results

DNA preservation during storage

The amount of amplifiable wheat DNA in the stored leaf/stem samples was a very small fraction of the total DNA quantified by thiazole orange binding, and showed no obvious time trend (Bearchell *et al.*, 2005; Fig. 1). In comparison with vegetative samples, much larger amounts of amplifiable wheat DNA were detected in the grain samples. This is not surprising as, at the end of the growing season, vegetative samples were taken from senesced tissues, whereas the grain samples were fresh. Interestingly, recent studies have shown that *M. graminicola* can cause degradation of wheat DNA during infection of leaves (Keon *et al.*, 2007). By contrast with vegetative samples, the amount of amplifiable wheat DNA in grain samples declined regularly with time from an amount similar to that
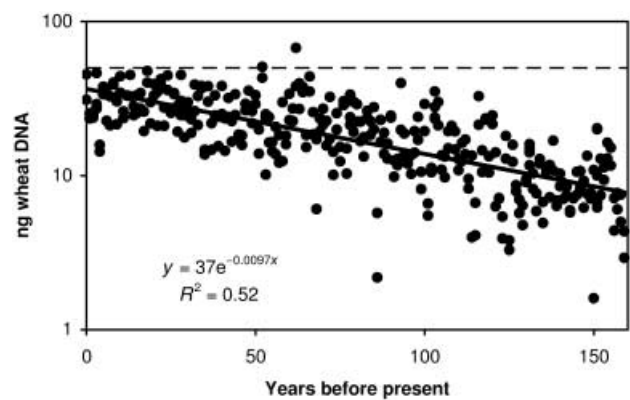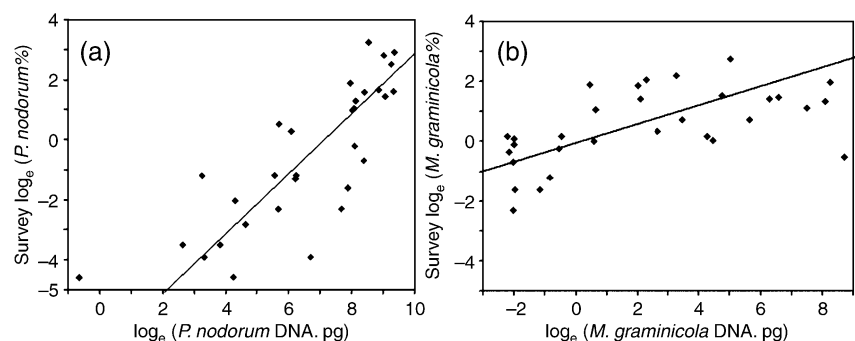

Fig. 1 Abundance of amplifiable wheat target DNA (*y*) ($\log_e$-transformed) extracted from grain of different ages sampled from the Broadbalk archive plotted against age of sample (*x*). Dashed line, amount of target wheat DNA amplified from 50 ng total DNA extracted from fresh leaves (determined fluorimetrically). The linear regression of $\log_e$ (*y*) on *x* explained 52% of the variance.

in green wheat leaves freshly sampled in 2003 (Fig. 1). These data were fitted well by an exponential decay curve with a decay rate of 0.0097 yr^{-1} (±2 SE 0.0010 yr^{-1}). The DNA series for the individual pathogens must have been affected by damage to the DNA during storage accumulating with time. We assumed that this degradation occurred at a rate similar to that incurred by wheat DNA in grain, and used this wheat DNA decay rate to calculate corrected 160-yr series of DNA abundances for each pathogen in leaf/stem and grain samples. None of the environmental associations observed depends qualitatively on the correction.

Mycosphaerella graminicola

No *M. graminicola* DNA was detected in grain samples. The correlation between the national septoria tritici blotch severity estimates and the Broadbalk *M. graminicola* DNA abundance over the period 1970–2003 was 0.59 ($P < 0.001$, 33 df; Fig. 2b), with the relative change in disease severity being proportional to the 0.28 power of the change in DNA, as

Fig. 2 Relationships between pathogen DNA abundance (corrected) in harvest leaf/stem samples from the Broadbalk experiment and septoria national disease severity measurements at GS72 (grain milky ripe) on leaf 2 of wheat crops in England and Wales in each year from 1970 to 2003 (including crops with different cultivars, agronomic regimes and geographical variation in soil types and weather). (a) *Phaeosphaeria nodorum* (correlation coefficient 0.59, $P < 0.001$, 33 df). (b) *Mycosphaerella graminicola* (correlation coefficient 0.79, $P < 0.001$, 33 df). Both variates were $\log_e$-transformed.
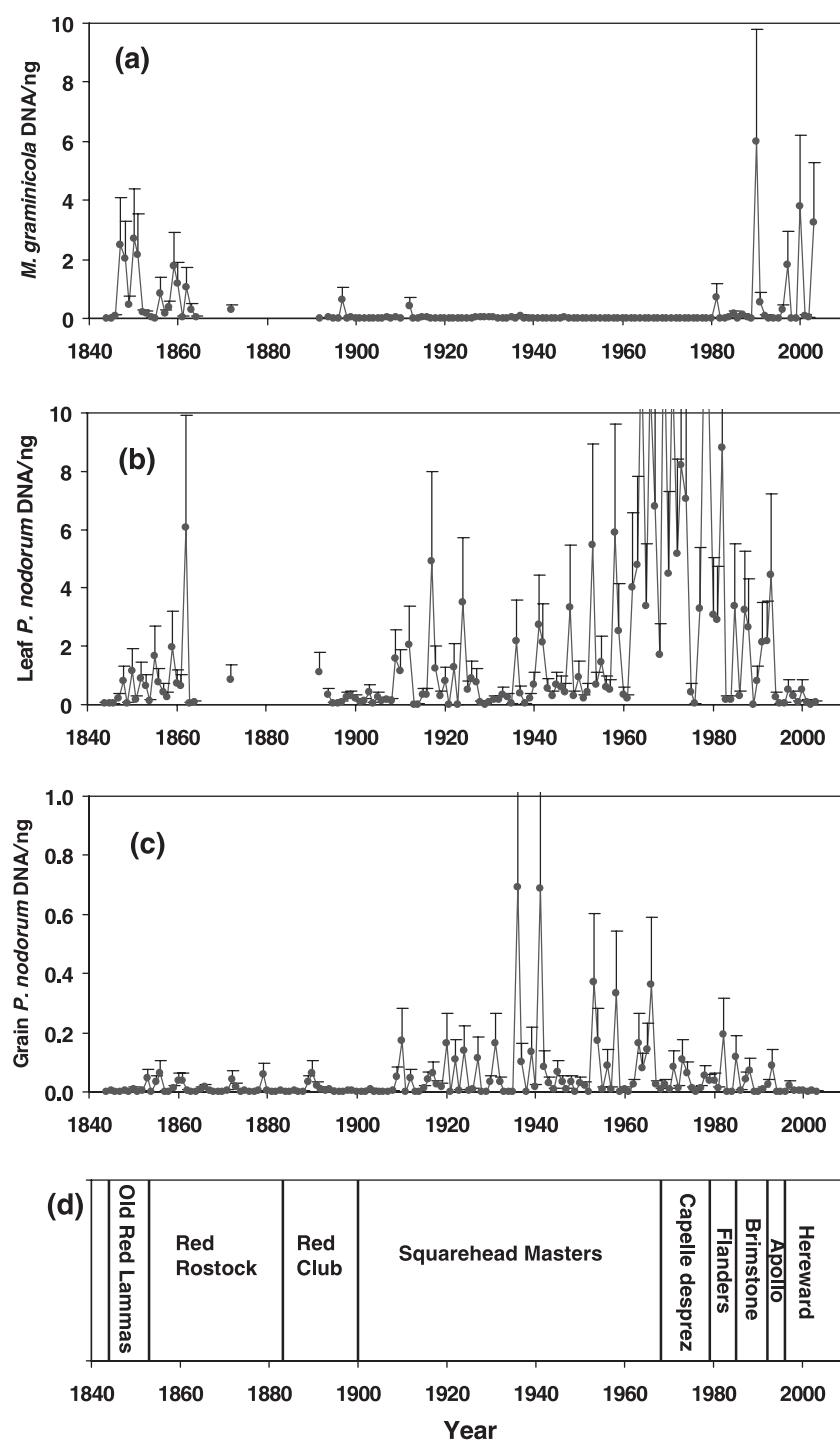
Fig. 3 Time-series data for corrected pathogen abundance in the Broadbalk experiment from 1844 to 2003. Panels show amplifiable DNA of *Mycosphaerella graminicola* in 50 ng total DNA extracted from harvest leaf/stem samples (a); *Phaeosphaeria nodorum* in leaf/stem (b) and grain (c) samples; and the main cultivars grown (d, cultivars grown within these runs for periods of 1 or 2 yr are omitted for clarity). Cappelle Desprez had straw approx. 40 cm shorter than cultivars that preceded it and approx. 20 cm taller than cultivars that followed it. Seed treatments were introduced in 1923 and foliar fungicides in 1979. Crops were harvested by hand (1844–1900), self-binder (1901–56) and combine harvester (1959–2003). Error bars are 1 SEM, based on the variance between five independent resamplings of archival material from each of 1852, 1872, 1900, 1925, 1950, 1975 and 2000.

estimated by principal components analysis. Because relationships between amount of *M. graminicola* DNA (Fig. 3a) and all environmental and agronomic factors were quantitatively similar for the corrected and uncorrected DNA data series, only relationships obtained for corrected data series are presented (Table 1). UK SO_2 emissions explained 42% of the interannual variation in the data series. Cultivar, spring rainfall and previous summer temperature (for the period before the start of the growing season in autumn) were then significantly related to the remaining variation and were not mutually confounded. Wind-run data were available only for 57 yr; there was no significant relationship with the DNA abundance data either before or after fitting SO_2 emissions. If rain and previous summer temperature were fitted, the introduction

Table 1 Relationships between *Mycosphaerella graminicola* abundance (Broadbalk, DNA) (corrected, $\log_e$-transformed) in leaf/stem samples at harvest and environmental and agronomic factors examined by multiple regression

Variate or factor level	Cultivar replication*	Change in SS	Marginal P	Parameter†	SE
SO_2		454.7	< 0.001	−1.12 SO_2	0.26
Rainfall in May and June§		53.1	< 0.001	+0.017 Rain	0.005
Temperature previous Jul–Aug¶		43.9	0.002	+0.29 T_{prev}	0.10
Cultivar**		76.2	0.097		
Giant Red	1		> 0.1‡	−2.4	2.1
Apollo	5		–	0.35	0.96
Stand Up	1		> 0.1	0.37	2.1
Cappelle Desprez	11		> 0.1	1.0	0.85
Hereward	7		> 0.1	1.1	1.0
Red Club	17		> 0.1	1.3	0.82
Squarehead Masters	63		> 0.1	1.3	0.30
Browick Red	1		> 0.1	1.6	2.0
Red Rostock	29		> 0.1	1.9	0.93
Flanders	6		> 0.1	2.3	0.87
Brimstone	6		0.008	3.9	0.84
Little Joss	2		0.09	4.3	2.1

The model including all factors shown explained 53% of the variance (121 df).
*Number of growing seasons for which the cultivar was grown.
†Regression coefficients for the continuous variables; for each cultivar the value shown is at the mean of all the continuous variables (SO_2, 3.91; rain, 110; T_{prev}, 10.6).
‡Only Brimstone was significantly different from the reference cultivar, Apollo. SED for comparisons between two cultivars varied between 0.9 and 2.9, depending on replication and details of fit.
§Total rainfall in May and June of harvest year (may affect secondary disease spread).
¶Average temperature July–August before start of winter wheat-growing season in autumn (may affect production of primary inoculum).
**Cultivars are ordered by increasing value of the regression coefficient.

of foliar fungicide in the late 1970s explained a significant, though very small, proportion of the remaining variation; however, fungicide was associated with increased amounts of *M. graminicola* DNA. Cultivar (Fig. 3d) explained a somewhat larger proportion of the variation, but with less significance because of the large number of associated degrees of freedom. Assuming that cultivar is more likely to be causally connected to *M. graminicola* DNA abundance, almost all its effect is caused by Brimstone (grown 1985–90 inclusive), which appears to have been especially susceptible to *M. graminicola* or grown in a period of severe epidemics for some other reason; differences among other cultivars were not significant (Table 1). Other factors were unrelated to the DNA measurements. No temporal autocorrelation or cyclical change patterns were found in the residuals from the final model.

Phaeosphaeria nodorum

Corrected series of leaf/stem and grain *P. nodorum* DNA data were calculated (Fig. 3b,c). The correlation between the national septoria nodorum blotch severity estimates and the Broadbalk *P. nodorum* leaf/stem DNA abundance over the period 1970–2003 was 0.79 ($P < 0.001$, 33 df; Fig. 2a). Corrected DNA abundance ($\log_e$-transformed) was approximately proportional to national disease severity ($\log_e$-transformed), with the slope based on the first principal component between

them being 1.02 (Fig. 2a). UK SO_2 emissions explained 15% of the interannual variation in these data. Spring rainfall explained 22% of the variance. These figures were almost unchanged in whichever order the fitting was done. Together, the two variates explained 39% of the variance. Winter temperature then explained a further 3.6% of the variance. The best model also included cultivar and explained 47% of the variance (Table 2). As with *M. graminicola*, the relationships were qualitatively similar for the corrected and uncorrected data series. No temporal autocorrelation or cyclical change patterns were found in the residuals from the final model. There was no effect of other variates tested.

With the exceptions of 1936 and 1941, when relatively large amounts of *P. nodorum* DNA were measured, the pattern of changes in *P. nodorum* DNA in grain was similar to the pattern in the DNA in leaf/stem samples (Fig. 3), but the absolute amounts of DNA were less. The smaller amounts of pathogen DNA detected in grains in comparison with leaf/stem material can be explained by increased preservation of host plant DNA. The pattern during the period for which leaf DNA data are missing (most of 1860–90) is consistent with that before and afterwards. Temperature in July and August of the harvest year was the single variable that explained the greatest proportion (21%) of the variance in abundance of *P. nodorum* DNA in grain, followed by rainfall in May and June (19% of variance explained). UK SO_2 emissions

Table 2 Relationships between *Phaeosphaeria nodorum* abundance (Broadbalk DNA) (corrected, $\log_e$-transformed) in leaf/stem samples at harvest and environmental and agronomic factors, examined by multiple regression

Variate or factor level	Cultivar replication*	Change in SS	Marginal P	Parameter†	SE
Rainfall in May and June§		110	< 0.001	+0.021 Rain	0.0036
SO$_2$		82	< 0.001	+0.89 SO$_2$	0.19
Temperature previous Dec–Feb¶		19	0.003	–0.10 T_{win}	0.036
Cultivar**		42	0.06		
Red Rostock	29		– ‡	8.1	0.70
Little Joss	2		> 0.1	7.9	1.5
Hereward	7		> 0.1	7.5	0.72
Apollo	5		> 0.1	7.2	0.68
Stand Up	1		> 0.1	7.0	1.5
Browick Red	1		> 0.1	7.0	1.5
Red Club	17		> 0.1	6.4	0.58
Brimstone	6		0.03	6.1	0.60
Cappelle Desprez	11		0.09	6.2	0.61
Flanders	6		0.03	5.9	0.62
Squarehead Masters	63		0.002	5.6	0.21
Giant Red	1		0.1	5.5	1.5

The model including all factors shown explained 47% of the variance (121 df).
*Number of growing seasons for which the cultivar was grown.
†Regression coefficients for the continuous variables; for each cultivar the mean value shown is at the mean of all the continuous variables (SO$_2$, 3.91; rain, 110; T_{win}, 10.6).
‡Significance value refers to difference between the cultivar and the reference cultivar, Red Rostock. SED for comparisons between two cultivars varied between 0.6 and 2.1, depending on replication and details of fit.
§Total rainfall in May and June of harvest year (may affect secondary disease spread).
¶Average temperature during December–February of winter wheat-growing season (may affect latent periods of pathogens).
**Cultivars are ordered by decreasing value of the regression coefficient.

explained 10% of the variance. Once these three variates were fitted, no other variate or factor, including cultivar, had any significant relationship with the amount of *P. nodorum* DNA in grain. There was a good correlation between the amount of *P. nodorum* DNA in grain and leaf/stem samples in the same year ($r = 0.7$, $P < 0.001$; Fig. 4). Since infection of wheat grain by *P. nodorum* must arise from disease already present on leaves and stems, we fitted a model including leaf *P. nodorum* DNA abundance as an independent variable. UK SO$_2$ emissions did not explain a significant proportion of the remaining variance ($P = 0.8$), but summer temperatures explained a further 6% of the variance. Rainfall in May and June had an additional effect on the amount of *P. nodorum* in grain ($P = 0.009$), beyond the effect mediated through the amounts in leaf samples (Table 3). There was no relationship between the amounts in leaves in a given year and the amount in grain in the previous year. No temporal autocorrelation or cyclical change patterns were found in the residuals from the final models for either leaf/stem or grain samples. There was no effect of other factors tested.

Ratio between *P. nodorum* and *M. graminicola*

The ratio of the amounts of DNA of the two pathogens should not be influenced by factors equally affecting the preservation of DNA of both, and is therefore the series in
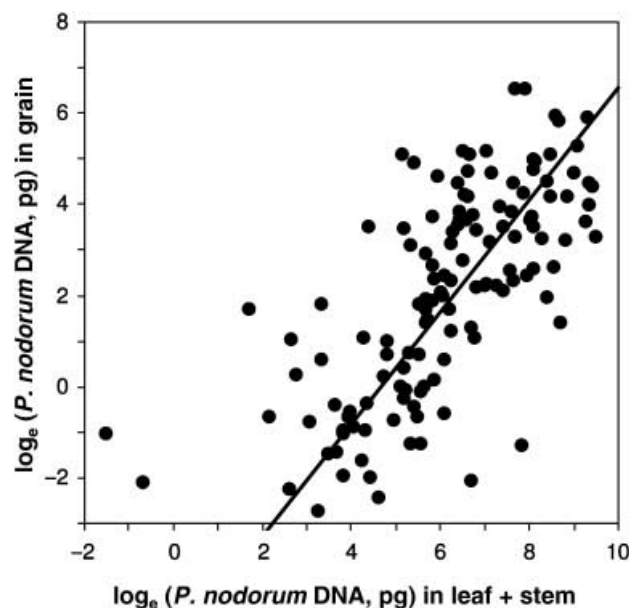

Fig. 4 Relationship between the abundance of *Phaeosphaeria nodorum* DNA in wheat grain and the abundance in wheat leaf/stem samples at harvest of the Broadbalk experiment, for years between 1844 and 2003 where both samples are available, examined by linear regression (data corrected then $\log_e$-transformed). Axes refer to amplifiable *P. nodorum* DNA in 50 ng total DNA.

Table 3 Relationships between the *Phaeosphaeria nodorum* abundance (DNA; corrected, $\log_e$-transformed) in grain harvested from the Broadbalk experiment and explanatory factors, examined by multiple regression

Variate	Change in SS	Marginal P	Parameter	SE
Model with environmental variables only*				
SO$_2$	94	< 0.001	+0.45 SO$_2$	0.091
Rainfall in May and June	153	< 0.001	+0.020 Rain	0.0037
Temperature in July and August (harvest year)	77	< 0.001	−0.36 T_{summ}	0.072
Model including leaf disease†				
Corrected leaf DNA abundance	305	< 0.001	+0.60	0.083
Temperature in July and August (harvest year)‡	38	< 0.001	−0.26 T_{summ}	0.071
Rainfall in May and June§	17	0.009	0.011 Rain	0.040

*Model including all variates explained 40% of the variance (149 df).
†Model including all variates explained 55% of the variance (121 df).
‡Average temperature in July–August of harvest year (may affect pathogen spread to grain).
§Total rainfall in May–June of harvest year (may affect secondary disease spread).

Table 4 Relationships between the ratio of *Phaeosphaeria nodorum* DNA abundance to *Mycosphaerella graminicola* DNA abundance (in Broadbalk leaf/stem samples) and environmental and agronomic factors examined by multiple regression

Variate or factor level	Change in SS	Marginal P	Parameter*	SE	Resistance to *P. nodorum*†	Resistance to *M. graminicola*†
SO$_2$	894.6	< 0.001	+2.04 SO$_2$	0.19		
Temperature Dec–Feb§	34.4	0.03	−0.12 T_{win}	0.054		
Temperature previous Jul–Aug¶	15.0	0.08	−0.18 T_{prev}	0.10		
Cultivar**	103.5	0.07				
Giant Red		> 0.1	1.9	2.3		
Apollo		> 0.1	0.9	1.2	5	7
Stand Up		> 0.1	0.5	2.5		
Red Rostock		> 0.1	0.46	0.63		
Hereward		> 0.1	0.3	0.91	6	6
Browick Red		> 0.1	−0.3	2.4		
Red Club		> 0.1	−0.8	1.0		
Cappelle Desprez		> 0.1	−0.9	1.6	4	
Old Red Lammas		> 0.1	−1.4	1.1		
Squarehead Masters		0.03‡	−1.6	1.0		
Flanders		0.03	−2.3	1.4	6	
Little Joss		> 0.1	−2.8	2.4		
Brimstone		0.001	−3.7	1.3	6	5

The model including all factors shown explained 60% of the variance (121 df).
*Regression coefficients for the continuous variables; for each cultivar the value shown is at the mean of all the temperature variables and an SO$_2$ emission of 1 Mt yr^{-1}.
†Published cultivar resistance rating (Anonymous., 1965–98); for older cultivars these were estimated at a time when only ratings for resistance to *P. nodorum* were published.
‡*P* value refers to a comparison with the reference cultivar, Apollo.
§Average temperature during December–February of winter wheat-growing season (may affect latent periods of pathogens).
¶Average temperature before start of winter wheat-growing season in autumn (may affect production of primary inoculum).
**Cultivars are ordered by decreasing value of the regression coefficient.

which we can have the most confidence. UK SO$_2$ emissions explained nearly 60% of the total interannual variation in this series (Bearchell *et al.*, 2005), more than any other factor. High concentrations of SO$_2$ were strongly correlated with increased relative amounts of *P. nodorum* DNA. This correlation remained very significant over the period during which the cultivar grown was mainly Squarehead Masters (1900–67).

Once SO$_2$ emission was fitted, winter temperature and then cultivar explained a significant proportion of the remaining variation, each improving the fit of the other alone. If both winter temperature and cultivar were fitted, the influence of previous summer temperature was nearly significant without altering parameter estimates for other factors (Table 4). Thus warmer winters tended to increase the proportion of *M.*

graminicola; Brimstone, Flanders and Squarehead Masters tended to have more *M. graminicola* than other cultivars, and Apollo or Giant Red appeared to have the least *M. graminicola*. These results are supported by the published cultivar resistance-rating data (Table 4). No temporal autocorrelation or cyclical change patterns were found in the residuals from the final model. There was no effect of other variates tested.

Discussion

Over the period 1844–2003, annual weather conditions (spring rainfall and summer temperature) were the most important factors affecting short-term variation in the amount of *P. nodorum* DNA in wheat tissues. Spring rainfall was much the more important, and affected *M. graminicola* in the same way. Long-term variation in each pathogen, after correction for DNA decay, remained well but oppositely correlated to atmospheric SO_2 emission. The ratio of *P. nodorum* to *M. graminicola* DNA in vegetative tissues (Bearchell *et al.*, 2005) was therefore less sensitive to short-term weather variation and correspondingly better correlated to long-term SO_2 emission. A positive relationship between spring rainfall and disease severity was expected from much other data (Tyldesley & Thompson, 1981, Coakley *et al.*, 1985, Daamen & Stol, 1992, Gladders *et al.*, 2001; Shah & Bergstrom, 2002) and presumably arises because rain during the stem-extension period transports asexual spores onto new tissues (Shaw, 1987, Shaw & Royle, 1993) and provides wetness for infection and, for *P. nodorum*, sporulation (Shearer & Zadoks, 1972, Eyal *et al.*, 1977, Shaw, 1991). The absence of correlation between spring rainfall and the ratio of the DNA of the two pathogens in leaf/stem tissues suggests that both diseases are affected similarly by rain in spring. The positive effect of rainfall in May–June on *P. nodorum* abundance in grain, after allowing for the effect on abundance in leaf/stem tissues, may have occurred because air humidity near the ear is less than that lower in the canopy, so that the ear dries more quickly after rain, making infection and fungal growth in ears more sensitive to rainfall.

There was a negative relationship between amount of *P. nodorum* DNA in grain and July/August temperature, suggesting that hot weather (also likely to be dry) in July and August impedes the growth of *P. nodorum* in grain. While there were no long-term trends in spring rainfall and summer temperature observed over the 160-yr period 1844–2003, it is predicted that climate change will decrease UK spring rainfall and increase both summer and winter temperatures over the next 50 yr (Hulme *et al.*, 2002). The effect of climate change on these two diseases will depend on the balance of the changes in these factors.

The amount of *P. nodorum* DNA in grain was much less than in leaf/stem material, and varied more (interquartile range between years, 3.7 in grain, 2.5 in leaf/stem, in natural logarithms) but in approximately the same pattern. A very large proportion of the variation in amount of *P. nodorum* DNA in grain was accounted for by variation in the amount in leaf/stem material. This is to be expected, as the disease is polycyclic and ear infection is initiated by conidia produced lower in the crop (King *et al.*, 1983). Although the grain *P. nodorum* series was correlated with SO_2 emission, this was entirely accounted for by its relationship to the leaf/stem series; no independent relationship remains after regression on leaf/stem abundance.

If *P. nodorum* is mainly seedborne, one might predict a relationship between the amount of *P. nodorum* in grain in 1 yr and the amount in the following summer. No such relationship was found. This presumably means that there is density-dependent compensation in the multiplication within a growing season sufficient to disguise any year-to-year correlation.

This work confirms the association of *P. nodorum* and *M. graminicola* DNA abundances with SO_2 emission over the period 1844–2003 (Bearchell *et al.*, 2005). Changes in UK SO_2 emission have been directly related to changes in the ratio of stable sulphur isotopes ($\delta^{34}S$) in Broadbalk grain and straw over the 160-yr period, suggesting that national and local SO_2 values are correlated (Zhao *et al.*, 2003). Effects of SO_2 on *P. nodorum* in grain presumably reflect the more closely correlated effects on vegetative tissues. The data reinforce the unexpected correlation between SO_2 and the ratio of the two pathogens in leaf/stem tissues (Bearchell *et al.*, 2005). The influence of SO_2 on *M. graminicola* appears to be larger than that on *P. nodorum*, and opposite to it. The much closer association of SO_2 with the ratio of the two pathogens than with either individually is accounted for by the opposite sign of the two correlations, which makes the change in the ratio larger than the change in either pathogen individually, and the similar relationship of both series to May–June rainfall, which therefore does not contribute to variation in the ratio (Table 4). Assuming the link is not coincidental, the biological explanation could be either that the two pathogens are, oppositely, directly or indirectly (phytotoxicity or host resistance) affected by SO_2 pollution. At low SO_2 concentration, *M. graminicola* may be competitively superior to *P. nodorum*, with the apparent beneficial effect of increased SO_2 concentration on *P. nodorum* caused by release from this competition.

There was evidence that cultivar affected the amounts of *P. nodorum* and *M. graminicola* DNA and the ratio between these two amounts in the vegetative tissue, but not the amount of *P. nodorum* DNA in the grain. In the few cases where there are published data on the susceptibility of cultivars grown on Broadbalk to both pathogens, they are consistent with the directions of effects fitted to the data (Table 4). In view of the dependence of *M. graminicola* on dispersal of ascospores from previous crops, it could be argued that what matters is not the cultivar grown in the Broadbalk field, but the cultivars grown regionally. Exact data are unavailable, but Bersee was grown widely in the 1930s to 1950s and has good

adult plant resistance to *M. graminicola* (Brown *et al.*, 2001; Arraiano & Brown, 2006), as has Cappelle Desprez, which was grown on most of the wheat area during the 1960s. Dwarfing genes were introduced into UK wheat cultivars in the 1970s along with susceptibility to *M. graminicola* (Brown *et al.*, 2001); of the recent cultivars, Apollo, which appears relatively resistant to *P. nodorum* in our data, is unusual in having no specific dwarfing genes.

An alternative hypothesis to the influence of SO_2 to account for the long-term pattern is therefore that the initial decline in *M. graminicola* abundance occurred because the reduced area of wheat in the agricultural depression of the later part of the 19th century decreased the production of ascospores, disadvantaging *M. graminicola* but not *P. nodorum*, which is spread on seed. The very high ratio of *P. nodorum* to *M. graminicola* in the mid-20th century then reflected the further (accidental) introduction of resistance to *M. graminicola* in cultivars Bersee and Cappelle Desprez. As the wheat area increased with increased profitability in the 1970s and thereafter, the use of susceptible cultivars caused a large increase in amount of *M. graminicola* inoculum arriving in the Broadbalk field. A feedback mechanism, in which early infection by *M. graminicola* because of severe epidemics in source crops leads to severe epidemics in the subsequent growing season, could then explain why it has remained the dominant pathogen, although more recent cultivars are more resistant than those of the mid-1980s. This scenario is not impossible, and can be modelled (Shaw, in press), but it depends crucially on the amount of summer disease caused by *M. graminicola* being regulated by the density of initial ascospore infections the previous autumn. However, the changes in DNA abundance in successive years cover several orders of magnitude (Fig. 2a), so initial autumn inoculum levels do not appear to limit the severity of epidemics in growing seasons with favourable weather, which does not support the hypothesis that changes in wheat area could explain the observed changes in pathogen abundance.

It is surprising at first that height of cultivar was not related to the measurements (Fig. 3d), in view of the postulated role of height as an escape mechanism from infection by *M. graminicola* (Shaw & Royle, 1993, Lovell *et al.*, 1997). However, the Broadbalk data come from the entire plant, and it is quite possible that in the 1840s *M. graminicola* did not spread to the uppermost leaves of the taller cultivars grown at the time.

It is not clear why there was an effect of temperature the previous summer (before the start of the winter wheat-growing season in autumn) on the amount of *M. graminicola* DNA in vegetative tissues. However, the data confirm the positive correlation observed between sunshine hours in the previous August (immediately postharvest) and the abundance of *M. graminicola* on wheat in the Netherlands (Daamen & Stol, 1992). This relationship may occur because saprotrophic organisms multiply slowly in years with long August sunshine hours and high temperatures, leaving more nutrients in the straw to support *M. graminicola* ascospore production. However, if this explanation is correct, one might have expected a correlation between the amount of *M. graminicola* in one season and the amount of *M. graminicola* the previous summer, which was not observed.

Pietravalle *et al.* (2003) found a correlation between wind-run in the spring and *M. graminicola* severity. This was entirely absent in this data set, even after correcting for SO_2, which suggests that the original correlation may have been coincidental.

The combined models here account for up to about half of the variation in log amount of *M. graminicola* DNA on wheat in these data, and rather more of the variation in the ratio of the amounts of DNA of the two pathogens. Such information cannot easily be used predictively to provide guidance to growers because it is based on DNA measurements that are well, but not perfectly, correlated with disease severity; the best of the correlations refers to events known only after the optimal time for intervention with fungicides; and the asymmetry of loss between applying an unnecessary fungicide and the potential loss from unexpected epidemic requires nearly 100% confidence in predictions. On the other hand, the work reported here also shows that we should always be cautious of forecasts of plant disease severity based on mechanistic models, as short-term observations or experiments on variation between years could not have revealed SO_2 as a driving variable in this system.

Acknowledgements

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