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Abstract Measurements of local environmental conditions, intensity of Fusarium head blight (FHB) in wheat spikes, biomass of *Fusarium graminearum*, *F. culmorum*, and *F. poae* (pathogens causing FHB) and concentration of the mycotoxins deoxynivalenol (DON) and nivalenol (NIV) in harvested wheat grain were obtained in a total of 150 location-years, originating in three European countries (Hungary, Ireland, United Kingdom) from 2001 to 2004. Through window-pane methodology, the length and starting time of temporal windows where the environmental variables were significantly associated with the biological variables were identified. Window lengths of 5 to 30 days were evaluated, with starting times from 18 days before anthesis to harvest. Associations were quantified with nonparametric Spearman correlation coefficients. All biological variables were significantly associated with at least one evaluated environmental variable ($P \leq 0.05$). Moisture-related variables (e.g., average relative humidity, hours of relative humidity

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above 80%) had the highest positive correlations with the biological variables, but there also was a significant negative correlation between average temperature and several biological variables. When significant correlations were found, they were generally for all window lengths, but for a limited number of window start times (generally before anthesis for disease index and after anthesis for the toxins and late-season fungal biomasses). Semi-partial Spearman correlation coefficients were used to evaluate the relationship between the environmental variables and the concentration of DON and NIV after the effects of FHB intensity and fungal biomass on the mycotoxins were removed. Significant semi-partial correlations were found between relative humidity variables and DON, and between temperature and relative humidity variables and NIV for time windows that started after anthesis (and not for any earlier time windows). Results confirm that the environment influences disease, fungal biomass, and mycotoxin production, and help refine the time windows where the association is greatest. However, variability in the relationships was high, indicating that no single environmental variable is sufficient for prediction of disease or mycotoxin contamination.

Keywords Disease forecasting · *Gibberella zeae* · Multiple testing · Nonparametric correlations · Wheat scab · Window-pane

Introduction

Fusarium head blight (FHB) of wheat (*Triticum aestivum* L.) is a disease of foremost importance in many parts of the world, including North America, Asia, and Europe. FHB can be caused by many different pathogens worldwide but the most important are *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*, which can produce a range of mycotoxins (Bottalico and Perrone 2002), and *Microdochium nivale* and *M. majus* (Glynn et al. 2005), which do not produce any known mycotoxins. In the US, *F. graminearum* is the predominant pathogen causing FHB on wheat, but in Europe, a complex of pathogens can cause the disease (Doohan et al. 1998). In a recent study by Xu et al. (2005) of the prevalence of FHB-causing pathogens in four countries over 2 years, *F. poae* was found to have the highest overall frequency, followed by *F. graminearum*. However in recent years, prevalence of *F. graminearum* appears to be increasing in Europe, especially in the cooler regions (Waalwijk et al. 2003; Xu et al. 2005).

The economic importance of FHB is due to reduction in yield and contamination of grain with mycotoxins (Nganje et al. 2004). Yield reduction results from the production of small, shrivelled, light-weight kernels with corresponding reduction in test weight of the harvested grain (Arseniuk et al. 1999; Bai and Shaner 1996; Xu et al. 2004). Moreover, *F. graminearum*, *F. culmorum*, and *F. poae* produce mycotoxins, especially deoxynivalenol (DON) and nivalenol (NIV), which may contaminate the harvested grain (Hart et al. 1984). In a study by Xu et al. (2008b) where several FHB-causing pathogens and their associated mycotoxins were identified, DON was most strongly associated with the presence of *F. graminearum*, and NIV was most significantly associated with the presence of *F. culmorum*. However, isolates of both *F. graminearum* and *F. culmorum* can produce DON or NIV, while *F. poae* can produce only NIV (Jennings et al. 2004; Ward et al. 2002; Xu et al. 2007).

Kriss et al. (2010) explored the annual variability in FHB intensity and its impact on yield in four US states over periods of 23 to 44 years as a function of variability in environmental conditions. Using a 'window-pane' analysis (Coakley et al. 1985), they found moisture- or wetness-related variables (determined from weather-station measurements) to be positively correlated with FHB intensity at multiple time periods over the growing season, but especially during the last 2 months of the season. Significant correlations were found for a range of time-duration intervals (windows), and the highest correlations were generally evident for the shorter time intervals. Xu et al. (2008a) investigated the relationship between environmental conditions and the presence/abundance of the complex of FHB-causing species in several European countries over 4 years. They considered environmental summary variables for a very limited number of time windows, from the start of anthesis to 7 days after the end of anthesis and from the start of anthesis to harvest. They also found that the presence/abundance on wheat heads and/or grain of pathogens that cause FHB were related to moisture variables. Results from the European and US analyses are consistent with other published results on environmental effects on this disease and the components of the life cycles of the pathogens, such as moisture effects on spore

production and infection and colonization of wheat spikes (Birzele et al. 2002; Cowger et al. 2009; Gilbert et al. 2008; Paul et al. 2007; Trail and Common 2000).

Quantification of the inter-relationships among environment and FHB intensity, pathogen abundance (as measured by fungal biomass), and mycotoxin contamination can shed light on the epidemiology of this disease and could lead to improved forecasting systems (De Wolf et al. 2003) for disease intensity or for mycotoxin levels in harvested grain. For instance, there is considerable interest currently in elucidating the effects of post-flowering environment (especially moisture) on fungal colonization and production of DON and other mycotoxins (Cowger et al. 2009; Culler et al. 2007), although there is no consensus on the direction or magnitude of the effects. The dataset described in Xu et al. (2008a) provides an opportunity to quantify some of the relationships for European winter-wheat cropping systems. We expanded on the earlier work of Xu et al. (2008a) in several ways. First, we explored a wider range of window start times and durations of time windows. Second, we considered a larger set of environmental-summary variables that could, in some cases, potentially capture nonlinear relationships or more complex temperature/wetness effects on the biological responses. Third, we considered environmental effects on disease intensity, fungal biomass, and mycotoxin concentrations, focusing on DON and NIV and the biomass of the predominant fungal species known to produce these mycotoxins.

We utilized window-pane methodology, a form of data mining, for the current study to quantify the strength of the empirical associations within the European dataset. Since the original development by Coakley et al. (1985), this protocol has been generalized and used in several investigations (Calvero et al. 1996; Pietravalle et al. 2003; Te Beest et al. 2008). In a recent study with FHB data in the US (Kriss et al. 2010), the window-pane methodology was expanded by formally addressing the multiple-testing problem that is common with data-mining techniques. In the current investigation, besides focusing on biological and environmental data from a different continent, we extend this approach to consider multiple biological variables (disease intensity, fungal biomass, and mycotoxins), and within-field environmental measurements. The specific objectives of this research were to: (i) determine environmental variables most correlated with disease, fungal biomass, and DON and NIV in wheat grain; (ii) determine time-window lengths and start/end times—relative to the onset of anthesis—during the last 2 months of the season when disease and mycotoxins were most highly correlated with environmental variables; and (iii) adjust correlations involving mycotoxins and environment for confounding effects of disease and fungal biomass on the mycotoxins. Because DON and NIV were of ultimate interest, we generally restricted analyses to the predominant FHB fungal species that produce these mycotoxins.

Materials and methods

Field data

Numerous winter wheat fields in four countries across Europe (Hungary, Ireland, Italy, and the United Kingdom) were assessed for FHB, fungal biomass, and mycotoxin accumulation from 2001 to 2004 (Xu et al. 2008a). These sites represent a wide range of climatic conditions in important winter wheat production areas in Europe where FHB can cause serious damage. In addition to climatic conditions, these sites also differed in other agronomic characteristics, such as cultivar, tillage, cropping history, and soil type. All cultivars used were susceptible to FHB. The data from Italy was not included in this investigation because in-field environmental data were not collected for most of the sites. Any other specific location-years with missing environmental data due to malfunctioning loggers were also not included. A total of 150 location-years were used here (47 in 2001, 46 in 2002, 29 in 2003, and 28 in 2004). Details on the field sampling protocols are described in Xu et al. (2008a). Sampling was conducted at growth stage (GS)77 (milky ripe) and GS92 (harvest) (Zadoks et al. 1974). Intensity of FHB (percent of spikelets with FHB symptoms) was determined at GS77 at all location-years, except for fields in Hungary in 2001 and two fields in the UK in 2003. There were no missing samples at harvest from the selected 150 location-years.

Fungal DNA and mycotoxin quantification

Xu et al. (2008a) quantified fungal biomass for six FHB-causing species (*Fusarium graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *Microdochium nivale*,

and *M. majus*) at GS77 and GS92 using quantitative PCR (qPCR) as described by Simpson et al. (2000). Here biomass results for *F. graminearum*, *F. culmorum*, and *F. poae* were used since they produce the mycotoxins deoxynivalenol (DON) and/or nivalenol (NIV). DON (ppb) and NIV (ppb) were also quantified at harvest as discussed in detail by Xu et al. (2008a).

Environmental variables

Temperature (°C), rainfall (mm), relative humidity (%) and surface wetness were recorded electronically at intervals of 2 h or less using electronic data loggers at each location (Xu et al. 2008a). All locations had environmental data available around anthesis, but the total time span of data available at each location differed. The average number of days with available environmental data in Hungary, the UK, and Ireland was 55, 70, and 127 days, respectively. However, the extreme time windows before or after anthesis were not used so that there would be a sufficient number of observations for analysis (see below) and so data from a single country did not dominate the results. Due to differences in overall temperatures, anthesis lasts approximately 1 week in Hungary and approximately 2 weeks in Ireland and the UK (Xu et al. 2008a).

Window-pane analysis

As described by Kriss et al. (2010) and Pietravalle et al. (2003), the concept underlying window-pane analysis is the specification of a time window of defined length, and the construction of summary environmental variables (e.g., means) for the specified window. This time window (e.g., 30 days) is progressed (or slid) along the total time frame of interest (e.g., a year or a growing season), in daily steps, so that environmental data from the entire time frame is ultimately considered in the data analysis. Two successive time windows (of the same length) share all but 1 day of data. With a defined starting time and window length, the ending time of the window is automatically determined. Time windows of environmental data were constructed separately for each year and location. The date when anthesis (GS60) began was recorded for each location-year; this growth stage was used to align all of the location-years and, for convenience, was set as day "0". Positive numbers indicate days after the beginning of anthesis and negative numbers indicate days prior to the beginning of anthesis. The presented day is the edge of the window that is closest to the beginning of the season. For example, a listed time window at day −10 that is 30 days in length covers the period from 10 days before anthesis to 19 days after anthesis (day 19). This labelling is opposite of what was done in Kriss et al. (2010) where we referred to the edge of the window closest to harvest as the "starting" time of the window.

Summary environmental variables were calculated for windows of lengths 5 to 30 days (increments of 5 days), but because of high similarity of results for windows of similar lengths, we limit results shown to 5, 15, and 30 days. Table 1 lists all the summary environmental variables considered within the present study. Summary variables were not calculated if there were any missing data within the window. The construction of summary variables for the different window lengths and starting times was completed with a macro written for SAS (SAS, Inc., Cary, NC). The summary variables were averages of daily values (e.g., average daily temperature [AT]) or summations of hours where certain conditions were met (e.g., number of hours with measureable precipitation [IP]). Construction of most of the summary variables was based, in part, on the variables used in other studies (Lu et al. 2001; Paul et al. 2007). However, LTRH80 and LTRH90 (Table 1) are unique summary variables; relative to THRH80 and THRH90, they give more weight to hours where the temperature is closer to the optimum for *F. graminearum* growth and development as compared to hours when temperature is farther from the optimum. For instance, THRH80 can be viewed as a variable where each hour in the summation receives a weight of 0 or 1 (the latter only occurring when temperature is in the prescribed 15–30°C range and RH is greater than 80%). In contrast, the hourly weights for LTRH80 can be 0, 1, or a continuous value between 0 and 1 (depending on the closeness of temperature to 25°C) (Table 1). This variable is consistent with work showing that infection or sporulation rate increases with temperature (roughly) between 9 and 25°C (Parry et al. 1995; Rossi et al. 2001).

The relationship between each summary environmental variable and disease intensity, fungal biomass, and mycotoxin concentration was determined for each of the window lengths and starting times. Analysis was based on the pooled location-year data (a separate

Table 1 Description of environmental variables, with units in parentheses, used in window-pane analysis of Fusarium head blight and associated mycotoxins in Europe

Weather variables	Description
AT	Average mean daily temperature (°C)
ARH	Average mean daily relative humidity (%)
AP	Average mean daily precipitation (mm)
IP	Number of hours with measurable precipitation
AW	Average mean daily surface wetness
IW	Number of hours with surface wetness >612[a]
HRH90	Number of hours with relative humidity >90%
THRH90	Number of hours with relative humidity >90% and temperature between 15 and 30°C
LTRH90	Sum of T^{*}[b] for each hour with relative humidity >90%
HRH80	Number of hours with relative humidity >80%
THRH80	Number of hours with relative humidity >80% and temperature between 15 and 30°C
LTRH80	Sum of T^{*} for each hour with relative humidity >80%

[a] Surface wetness ranged from 0 to 1023. Threshold of 612 indicated wet or dry

[b] $T^{*} = \begin{cases} [ln(17)]^{-1} \cdot ln(T-8) & if\ 9°C \leq T < 25°C \\ 1 & if\ 25°C \leq T < 30°C \\ 0 & otherwise \end{cases}$ where T is temperature for each hour

analysis for each biological variable and each window of each environmental variable). The association between variables was quantified with the nonparametric Spearman rank correlation (r) (Spearman 1904) using the CORR procedure of SAS (SAS, Inc., Cary, NC). The Spearman correlation measures the strength of the monotonic relation between two variables, is robust to the presence of outliers, especially when there is sufficient sample size, and is applicable for any continuous or ordinal scale (Sheskin 2007). Correlations were only considered if at least 35 location-years had non-missing data for a given window (of given length and starting time). Therefore, each correlation was determined from a possible range of between 35 and 150 location-years. Due to the varying number of observations for each window starting time, there is not a single critical value for significance.

Because of differences among location-years in the start and end of environmental monitoring, the smallest number of location-year observations was generally for windows that were the greatest time before or after anthesis. Windows used in the analysis had start days that began 23 days before anthesis. This was the first day where at least 35 location-years had environmental data available. Until day −12, these correlations are mostly derived from data for Ireland and Hungary, although at least some observations from all three countries comprised the data set. However, by starting day −8, there were at least 10 location-years within each of the 3 countries with environmental data available for the analysis. For biological variables recorded at GS77, windows extended forward to 25 days post-anthesis. For biological variables recorded at GS92, windows extended forward to 60 days post-anthesis. Results for windows late in the season were generally from the UK and Ireland, since there was limited environmental data available from Hungary for this period because grain had already been harvested by then.

Semi-partial correlations

Semi-partial Spearman correlation coefficients were calculated to quantify the monotonic association between the mycotoxins and environment after adjusting for the monotonic effect of other biological variables on the mycotoxins. A semi-partial correlation coefficient quantifies the strength of the relationship between two variables while the influence of one or several other variables is removed from only one of the two variables in the pair that are being correlated (Sheskin 2007). Specifically, semi-partial Spearman rank correlation coefficients were calculated between each environmental variable (Table 1), for each

window length and starting time, and the concentration of a mycotoxin (DON or NIV) after any association that the mycotoxin had with disease intensity or fungal biomass was removed. As an extreme example, suppose that the quantity of DON was correlated perfectly with disease intensity, but that disease intensity was a function of the environment. Although there would be a nonzero correlation between DON and environment (because disease is related to the environment), the semi-partial correlation between DON and environment (adjusting for disease effects on DON) would be zero. Semi-partial correlations allow one to assess the environmental effects on mycotoxin concentrations due to effects other than those expected towards the biological variables that affect mycotoxin concentrations.

Semi-partial Spearman correlations were calculated with specialized programs written in SAS using the formulas in Sheskin (2007) after first adjusting for disease intensity alone, then adjusting for disease intensity and fungal biomass quantified at GS77, and then adjusting for disease intensity and fungal biomass quantified at GS77 and at GS92. This sequence represents adjustments for increasing numbers of variables that could be influencing mycotoxin accumulation.

Multiple hypothesis testing

With a single test result, a significant correlation is declared if $P \leq \alpha$, where α is the prespecified significance level for an individual test and P is the achieved significance level of the individual test. Because of the large number of test statistics calculated, by definition, in a window-pane analysis, adjustments to the simple hypothesis-testing problem are needed to avoid excessively large type I error rates and false positive proportions (Westfall et al. 1999). Because the test statistics are highly correlated, there is no simple solution to the multiple-testing problem; we dealt with the issue in three separate ways, as described in detail in Kriss et al. (2010). First, we performed a global test of significance across all window starting times for a given environmental variable by calculating the adjusted P value (P_g) using the Simes' method (Simes 1986). This is a test for the global null hypothesis ($H_0(g)$) that none of the individual correlations are significant, and the alternative ($H_a(g)$) that at least one is different from 0. Values of $P_g < \alpha_g$ (with $\alpha_g = 0.05$) are considered significant for the global test. Second, the individual estimated correlation coefficients were compared with critical values corresponding to individual prespecified significance levels (α values) of 0.005 (instead of 0.05). Third, the ad-hoc method of Pietravalle et al. (2003) was used to reduce the chances of declaring spurious results for a window as being true significant results. A significant relation during a given time period between an environmental variable and FHB intensity was declared only if there were *clusters* of five successive correlations that were individually significant (each at individual $P<0.005$). Unlike in Kriss et al. (2010), we performed two-sided tests for the correlations. These procedures were followed for the Spearman correlation coefficients and for the semi-partial Spearman correlation coefficients.

Results

Overall significance and magnitude of correlations

Multiple environmental variables were significantly correlated with disease intensity, fungal biomass, and mycotoxin levels. Tables 2 to 5 provide the adjusted global P value (P_g) and the highest individual Spearman correlation coefficient across all window starting times for each environmental variable and window length. The tables also indicate whether the presented correlation was significant at an individual $P \leq 0.005$, and if there was at least one cluster of five successive correlation coefficients that were significant. All environmental variables tested were significantly correlated ($P_g \leq 0.05$) with at least one of the FHB intensity, fungal biomasses, or mycotoxin variables. In general, when significant, temperature had negative relationships with the biological variables, and moisture or wetness variables had positive relationships.

For disease intensity, significant correlations were found for multiple environmental variables that included a measure of relative humidity (e.g., ARH, LTRH90, HRH80); for the environmental variables with the strongest associations, the general trend was for decreasing correlation with increasing window length (Table 2). Combining temperature with duration of relative humidity measurements in a simple way (THRH90, THRH80) did not improve the correlations compared to the duration measurements (HRH90, HRH80), and using the more complex

Table 2 Global significance levels based on Simes' method and the highest Spearman rank correlation coefficients between each of the listed environmental variables and Fusarium head blight (FHB) intensity, biomass of *Fusarium graminearum*, *F. culmorum*, and *F. poae*, for data collected at growth stage 77 (milky ripe)[a]

	Significance level for window length (days)											
	FHB intensity			*F. graminearum* (GS77)			*F. culmorum* (GS77)			*F. poae* (GS77)		
Variable[b]	5	15	30	5	15	30	5	15	30	5	15	30
P_g												
AT	0.012	0.090	0.005	0.003	0.001	<0.001	0.032	0.077	0.359	0.161	0.285	0.331
ARH	<0.001	<0.001	0.002	0.002	<0.001	<0.001	0.881	0.767	0.604	0.962	0.869	0.548
AP	0.018	0.011	<0.001	0.002	0.004	0.035	0.499	0.049	0.639	0.005	0.002	0.001
IP	0.006	0.049	0.015	0.002	0.001	0.002	0.803	0.695	0.954	<0.001	<0.001	<0.001
AW	<0.001	0.046	0.002	0.003	0.004	0.006	0.920	0.919	0.868	0.100	0.153	0.478
IW	<0.001	0.196	0.010	<0.001	<0.001	0.002	0.611	0.796	0.873	0.220	0.181	0.147
HRH90	<0.001	<0.001	<0.001	0.001	0.009	0.031	0.945	0.997	0.970	0.306	0.471	0.505
THRH90	0.189	0.134	0.139	0.146	0.037	0.002	0.194	0.175	0.231	0.670	0.514	0.025
LTRH90	<0.001	<0.001	<0.001	0.144	0.097	0.953	0.538	0.738	0.872	0.118	0.299	0.999
HRH80	<0.001	<0.001	<0.001	0.013	0.003	0.020	0.746	0.845	0.866	0.941	0.986	0.987
THRH80	<0.001	0.003	0.036	0.208	0.025	<0.001	0.163	0.135	0.530	0.199	0.914	0.190
LTRH80	<0.001	<0.001	<0.001	0.461	0.155	0.940	0.167	0.233	0.674	0.079	0.588	0.693
Spearman[c]												
AT	*−0.455*	−0.364	*−0.469*	**−0.514**	**−0.493**	**−0.536**	*−0.268*	−0.238	−0.201	0.391	0.402	0.402
ARH	**0.593**	**0.583**	**0.482**	**0.385**	**0.450**	**0.438**	0.215	0.113	0.161	−0.179	−0.218	−0.291
AP	*0.425*	**0.370**	**0.529**	**0.431**	**0.394**	0.345	0.246	*0.252*	0.176	**−0.355**	**−0.325**	**−0.370**
IP	*0.437*	0.346	*0.433*	**0.491**	**0.398**	**0.449**	0.183	0.175	0.093	**−0.378**	**−0.356**	**−0.469**
AW	**0.515**	0.343	**0.460**	*0.593*	**0.552**	*0.534*	−0.176	−0.202	−0.184	−0.299	−0.282	−0.217
IW	*0.472*	0.339	**0.358**	*0.631*	**0.630**	**0.591**	0.202	0.123	0.138	−0.249	−0.278	−0.298
HRH90	**0.555**	**0.483**	**0.390**	**0.380**	**0.390**	0.330	0.204	−0.117	−0.112	0.278	0.201	0.170
THRH90	0.233	0.222	−0.321	−0.260	−0.230	**−0.324**	*0.417*	−0.229	−0.246	0.189	0.197	0.448
LTRH90	**0.471**	**0.482**	**0.407**	0.245	0.305	0.143	0.302	0.245	−0.135	−0.280	−0.290	0.143
HRH80	**0.585**	**0.546**	**0.475**	**0.325**	**0.394**	0.354	0.315	0.108	0.122	0.176	0.117	−0.139
THRH80	**0.370**	**0.329**	−0.332	−0.214	−0.249	**−0.372**	0.329	0.288	0.237	−0.230	0.166	0.291
LTRH80	**0.551**	**0.571**	**0.431**	−0.229	0.289	−0.107	0.360	0.336	0.262	−0.295	−0.218	−0.238

[a] Adjustment for multiple correlated test statistics. Single adjusted global P value (P_g) for the collection of time windows, each with different starting and ending dates, of the listed window lengths. Values of $P_g<0.05$ (α_g) are considered significant

[b] Variables are defined in Table 1

[c] Highest Spearman correlation coefficient; italic font indicates individually significant correlations at $P\leq0.005$. Bold indicates there is at least one cluster of five contiguous partial correlation coefficients with individual P values≤0.005

weighted function (e.g., LTRH90, LTRH80) resulted in similar correlations to those without temperature (e.g., HRH80).

Fusarium graminearum and *F. culmorum* had significant (global) and negative correlations with mean daily temperature (AT) (Tables 2 and 3). Interestingly, for *F. graminearum*, the significant correlations with temperature were found when the fungus was isolated at milky ripe (GS77), but not at harvest (GS92). This is in contrast to *F. culmorum*, where the significant negative correlations were detected from the samples taken at GS92 and not at GS77.

Fusarium graminearum was the only fungal species investigated that had a significant relationship

Table 3 Global significance levels based on Simes' method and the highest Spearman rank correlation coefficients between each of the listed environmental variables and biomass of *Fusarium graminearum*, *F. culmorum*, and *F. poae*, for data collected at growth stage 92 (harvest)[a]

	Significance level for window length (days)								
	F. graminearum (GS92)			*F. culmorum* (GS92)			*F. poae* (GS92)		
Variable[b]	5	15	30	5	15	30	5	15	30
P_g									
AT	0.598	0.636	0.350	0.021	0.013	0.012	0.041	0.611	0.382
ARH	0.006	0.011	0.009	0.014	0.057	0.066	0.365	0.618	0.643
AP	0.222	0.118	0.064	0.403	0.323	0.199	0.002	<0.001	<0.001
IP	0.210	0.059	0.167	0.054	0.195	0.160	0.003	<0.001	<0.001
AW	0.105	0.574	0.616	0.062	0.060	0.067	0.017	0.001	0.005
IW	0.348	0.527	0.441	0.111	0.104	0.035	0.039	0.013	0.024
HRH90	0.013	0.013	0.003	0.032	0.008	0.007	0.080	0.124	0.411
THRH90	<0.001	0.003	0.013	0.119	0.370	0.506	<0.001	<0.001	0.005
LTRH90	0.002	0.004	<0.001	0.311	0.272	0.081	0.012	0.175	0.158
HRH80	0.055	0.053	0.024	0.008	0.009	0.132	0.416	0.854	0.845
THRH80	0.006	0.011	0.029	0.298	0.954	0.242	<0.001	<0.001	0.070
LTRH80	0.012	0.055	0.007	0.345	0.202	0.109	0.003	0.132	0.056
Spearman[c]									
AT	−0.234	−0.273	−0.398	**−0.499**	**−0.505**	**−0.485**	0.464	0.409	0.388
ARH	**0.380**	**0.321**	**0.355**	*0.400*	0.358	0.386	0.241	−0.249	−0.312
AP	0.351	0.271	*0.283*	0.311	0.258	0.372	−0.406	**−0.397**	**−0.442**
IP	0.400	*0.342*	0.277	0.369	0.352	*0.418*	**−0.383**	**−0.352**	**−0.471**
AW	*0.334*	0.227	0.222	0.419	0.426	*0.489*	**0.347**	**0.406**	**0.486**
IW	*0.292*	0.297	0.250	*0.458*	0.443	*0.488*	*−0.306*	*−0.383*	−0.407
HRH90	*0.381*	**0.311**	**0.397**	*0.375*	**0.421**	**0.399**	0.280	0.265	0.215
THRH90	**0.454**	**0.386**	**0.357**	−0.282	−0.410	−0.475	**−0.614**	*−0.601*	*−0.475*
LTRH90	*0.419*	**0.398**	**0.441**	−0.280	0.262	*0.313*	*−0.464*	−0.317	−0.285
HRH80	*0.322*	*0.278*	*0.298*	**0.396**	**0.405**	0.342	0.238	0.173	−0.179
THRH80	**0.389**	**0.357**	*0.335*	−0.240	0.189	−0.318	*−0.573*	*−0.584*	*−0.384*
LTRH80	*0.359*	*0.337*	**0.379**	−0.274	0.254	*0.262*	*−0.499*	*−0.387*	−0.292

[a] Adjustment for multiple correlated test statistics. Single adjusted global P value (P_g) for the collection of time windows, each with different starting and ending dates, of the listed window lengths. Values of $P_g<0.05$ (α_g) are considered significant

[b] Variables are defined in Table 1

[c] Highest Spearman correlation coefficient; italic font indicates individually significant correlations at $P\leq0.005$. Bold indicates there is at least one cluster of five contiguous correlation coefficients with individual P values≤0.005

with mean daily relative humidity (ARH) (Tables 2 and 3). Other moisture and wetness-related variables had varying relationships with the different Fusarium pathogens. There were some significant, although not consistent, correlations between *F. graminearum* or *F. culmorum* and HRH90 and HRH80. When temperature was coupled with relative humidity variables to create composite variables (e.g., THRH90, LTRH80), significant correlations were found at multiple window lengths for *F. graminearum* (GS92) (Table 3). *Fusarium poae* did not have the same strong relationships with temperature and relative humidity as the other *Fusarium* pathogens, but it was also significantly correlated with mean daily surface wetness (AW)

(Table 3) and both mean daily precipitation (AP) and number of hours of precipitation (IP) (Tables 2 and 3). The relationship between *F. poae* and precipitation was unique among the three species in that significant negative correlations were identified.

Deoxynivalenol (DON) (Table 4) and nivalenol (NIV) (Table 5) had significant negative correlations with AT and significant positive correlations with ARH and other moisture and wetness-related variables (e.g., HRH80, AW). For DON, when temperature was coupled

Table 4 Global significance levels based on Simes' method and the highest Spearman rank correlation coefficient or semi-partial Spearman rank correlation coefficient between each of the listed environmental variables for three window lengths and DON[a]

	Adjustment for:											
	DON			FHB intensity			FHB intensity, Fungal biomass (GS77)[d]			FHB intensity, Fungal biomass (GS77,GS92)[e]		
Variable[b]	5	15	30	5	15	30	5	15	30	5	15	30
P_g												
AT	<0.001	0.003	0.043	0.002	0.035	0.261	0.004	0.081	0.794	0.003	0.059	0.205
ARH	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.003	0.002	0.003	0.010
AP	0.648	0.915	0.767	0.977	0.991	0.929	0.974	0.986	0.933	0.805	0.491	0.524
IP	0.176	0.696	0.765	0.560	0.963	0.900	0.788	0.990	0.853	0.612	0.658	0.368
AW	0.030	0.024	0.040	0.052	0.040	0.074	0.045	0.026	0.061	0.044	0.041	0.044
IW	0.047	0.198	0.291	0.146	0.200	0.226	0.966	0.998	0.994	0.160	0.307	0.262
HRH90	0.002	0.004	0.016	0.023	0.034	0.064	0.033	0.066	0.077	0.051	0.133	0.116
THRH90	0.149	0.342	0.721	0.394	0.441	0.810	0.274	0.290	0.872	0.287	0.304	0.867
LTRH90	0.018	0.026	0.020	0.093	0.114	0.125	0.056	0.073	0.079	0.065	0.115	0.176
HRH80	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.003	0.002	0.002	0.003	0.004	0.002
THRH80	0.335	0.415	0.363	0.359	0.407	0.716	0.273	0.245	0.420	0.195	0.219	0.755
LTRH80	0.030	0.014	0.002	0.163	0.104	0.052	0.095	0.062	0.020	0.095	0.111	0.050
Spearman[c]												
AT	**−0.423**	**−0.392**	**−0.368**	**−0.361**	**−0.302**	−0.242	**−0.349**	*−0.287*	−0.165	**−0.354**	*−0.297*	−0.182
ARH	**0.424**	**0.438**	**0.411**	**0.385**	**0.364**	**0.389**	**0.368**	**0.345**	**0.349**	**0.361**	**0.333**	**0.318**
AP	0.241	0.242	0.166	0.243	0.202	−0.142	0.245	0.219	−0.169	0.210	−0.263	−0.237
IP	*0.252*	0.202	0.224	0.216	−0.152	−0.154	0.209	−0.175	−0.186	0.204	−0.232	−0.234
AW	**0.314**	**0.291**	**0.289**	*0.280*	**0.291**	*0.275*	*0.285*	**0.303**	*0.278*	*0.285*	**0.298**	*0.298*
IW	*0.280*	0.209	0.207	*0.256*	0.254	0.244	0.215	0.151	0.157	*0.280*	0.250	0.239
HRH90	**0.336**	**0.308**	**0.286**	**0.294**	**0.274**	0.249	*0.280*	*0.256*	0.238	*0.273*	*0.251*	0.223
THRH90	−0.337	*−0.399*	0.261	−0.295	−0.391	0.237	−0.336	−0.382	0.226	−0.351	−0.399	0.245
LTRH90	*0.287*	**0.276**	**0.332**	*0.263*	0.255	0.293	*0.281*	*0.263*	*0.301*	*0.277*	0.265	0.268
HRH80	**0.380**	**0.405**	**0.421**	**0.367**	**0.365**	**0.364**	**0.349**	**0.345**	**0.342**	**0.344**	**0.337**	**0.333**
THRH80	−0.250	−0.264	0.243	−0.234	−0.260	0.229	−0.288	−0.298	0.238	−0.306	−0.336	0.221
LTRH80	**0.286**	**0.295**	**0.379**	*0.274*	*0.281*	*0.321*	*0.275*	*0.285*	**0.327**	*0.288*	0.261	*0.305*

[a] Adjustment for multiple correlated test statistics. Single adjusted global P value (P_g) for the collection of time windows, each with different starting and ending dates, of the listed window lengths. Values of $P_g<0.05$ (α_g) are considered significant

[b] Variables are defined in Table 1

[c] Highest semi-partial Spearman correlation coefficient; italic font indicates individually significant semi-partial correlation at $P\leq 0.005$. Bold indicates there is at least one cluster of five contiguous partial correlation coefficients with individual P values≤ 0.005

[d] Adjustments made for the fungal biomass of *Fusarium graminearum* and *F. culmorum* at growth stage (GS) 77-milky ripe

[e] Adjustments made for the fungal biomass of *F. graminearum* and *F. culmorum* at GS77 and GS92 (harvest)

Table 5 Global significance levels based on Simes' method and the highest Spearman rank correlation coefficient or semi-partial Spearman rank correlation coefficient between each of the listed environmental variables for three window lengths and NIV[a]

	Adjustment for:											
	NIV			FHB intensity			FHB intensity, Fungal biomass (GS77)[d]			FHB intensity, Fungal biomass (GS77,GS92)[e]		
Variable[b]	5	15	30	5	15	30	5	15	30	5	15	30
P_g												
AT	<0.001	<0.001	0.004	<0.001	0.002	0.009	<0.001	0.001	0.032	<0.001	0.002	0.033
ARH	0.001	<0.001	<0.001	0.005	0.003	0.005	0.006	0.002	0.004	0.015	0.006	0.010
AP	0.917	0.993	0.887	0.725	0.988	0.955	0.914	0.869	0.870	0.979	0.699	0.531
IP	0.928	0.635	0.959	0.656	0.814	0.903	0.885	0.968	0.555	0.955	0.822	0.344
AW	0.089	0.175	0.085	0.022	0.081	0.034	0.020	0.048	0.042	0.023	0.049	0.023
IW	0.139	0.264	0.227	0.026	0.016	0.006	0.033	0.027	0.004	0.046	0.036	0.005
HRH90	0.070	0.050	0.054	0.101	0.110	0.102	0.084	0.067	0.066	0.118	0.090	0.074
THRH90	0.131	0.071	0.220	0.092	0.082	0.293	0.088	0.084	0.524	0.276	0.381	0.693
LTRH90	0.206	0.541	0.327	0.516	0.844	0.979	0.595	0.796	0.782	0.936	0.930	0.994
HRH80	0.011	<0.001	<0.001	0.014	0.003	0.005	0.018	0.004	0.004	0.049	0.011	0.007
THRH80	0.094	0.028	0.123	0.070	0.035	0.230	0.061	0.041	0.403	0.132	0.092	0.557
LTRH80	0.352	0.394	0.058	0.520	0.485	0.444	0.561	0.592	0.303	0.732	0.818	0.781
Spearman[c]												
AT	**−0.433**	**−0.380**	**−0.333**	**−0.431**	**−0.370**	**−0.345**	**−0.437**	**−0.367**	**−0.285**	**−0.403**	**−0.355**	**−0.281**
ARH	**0.330**	**0.374**	**0.379**	**0.338**	**0.346**	**0.342**	**0.339**	**0.353**	**0.352**	**0.321**	**0.331**	**0.325**
AP	−0.296	−0.328	−0.205	−0.276	−0.305	−0.155	−0.256	−0.210	−0.247	−0.248	−0.234	−0.255
IP	−0.270	−0.286	−0.302	−0.322	−0.295	−0.154	−0.240	−0.229	−0.198	−0.255	−0.232	−0.229
AW	*0.255*	0.273	0.263	**0.317**	*0.280*	**0.290**	**0.316**	*0.303*	**0.281**	*0.322*	*0.299*	**0.304**
IW	*0.254*	0.264	0.229	*0.305*	**0.322**	**0.344**	*0.292*	**0.309**	**0.356**	*0.293*	*0.286*	**0.350**
HRH90	*0.277*	*0.249*	*0.253*	*0.286*	*0.262*	0.226	*0.264*	*0.284*	0.253	0.253	*0.274*	0.239
THRH90	*0.304*	*−0.260*	0.258	0.298	−0.298	0.229	0.292	−0.304	−0.205	0.283	−0.302	−0.201
LTRH90	*0.295*	0.234	0.231	0.275	0.196	0.189	0.271	0.199	0.202	0.219	0.155	0.161
HRH80	**0.309**	**0.347**	**0.377**	**0.320**	**0.331**	**0.319**	**0.314**	**0.335**	**0.323**	**0.291**	**0.303**	**0.315**
THRH80	0.321	**0.288**	0.327	0.347	*−0.307*	0.289	0.367	**−0.305**	0.215	0.353	*−0.274*	0.200
LTRH80	0.284	0.249	*0.308*	0.309	0.244	0.260	0.291	0.263	0.272	0.287	0.252	0.232

[a] Adjustment for multiple correlated test statistics. Single adjusted global P value (P_g) for the collection of time windows, each with different starting dates, of the listed window lengths. Values of $P_g<0.05$ (α_g) are considered significant

[b] Variables are defined in Table 1

[c] Highest semi-partial Spearman correlation coefficient; italic font indicates individually significant semi-partial correlation at $P\leq 0.005$. Bold indicates there is at least one cluster of five contiguous partial correlation coefficients with individual P values≤ 0.005

[d] Adjustments made for the fungal biomass of *Fusarium graminearum*, *F. culmorum*, and *F. poae* at growth stage (GS) 77-milky ripe

[e] djustments made for the fungal biomass of *F. graminearum*, *F. culmorum*, and *F. poae* at GS77 and GS92 (harvest)

with relative humidity (e.g., LTRH80, LTRH90), there were significant correlations for multiple window lengths, but the magnitude of these correlations was generally lower than for relative humidity alone.

Temporal patterns to correlations with environment—disease and fungal biomass

Although individual Spearman correlation coefficients were determined for each window length and starting

time for each environmental variable, only a subset of the individual correlations are displayed in order to save space. Specific examples of disease intensity and fungal biomasses of the three *Fusarium* species are shown in Figs. 1 and 2 for 15-day window lengths. The example graphs were selected to demonstrate some of the most highly significant correlations or interesting time patterns to the correlations. The horizontal axis represents the starting time of each window of a given length and each vertical bar represents the (estimated) Spearman correlation coefficient for the association between the biological variable and environmental variable.

The highest individual Spearman correlation between disease intensity and ARH was 0.583 at day −16 ($P<0.0001$) of window length 15. This indicates that the average daily relative humidity from day −16 to day −2 (i.e., from 2 to 16 days prior to the beginning of anthesis) was significantly positively associated with disease intensity. With all of the humidity-related variables, there were two main clusters of windows with significant correlations (Fig. 1). One

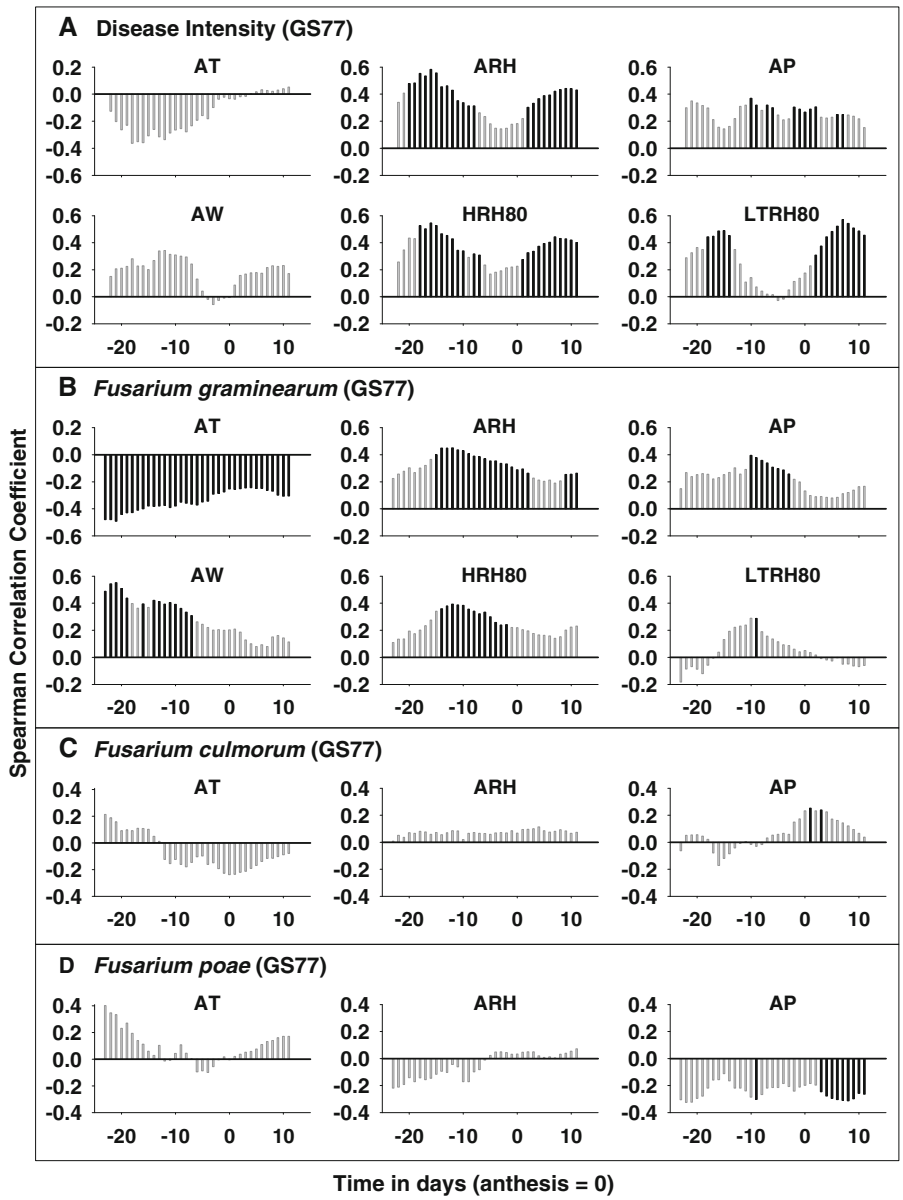

Fig. 1 Spearman rank correlation coefficients at 15-day windows for the association between environmental variables (chart title abbreviations defined in Table 1) and (**a**), Disease intensity; (**b**), *Fusarium graminearum* biomass; (**c**), *F. culmorum* biomass; and (**d**), *F. poae* biomass quantified at GS77 (*milky ripe*) across three European countries. Horizontal axis represents the starting time of the 15-day window, with day 0 representing the beginning of anthesis. Negative numbers represent days before anthesis began and positive numbers represent days after anthesis began. Bold vertical bars represent correlation coefficients for individual significance at $\alpha=0.005$

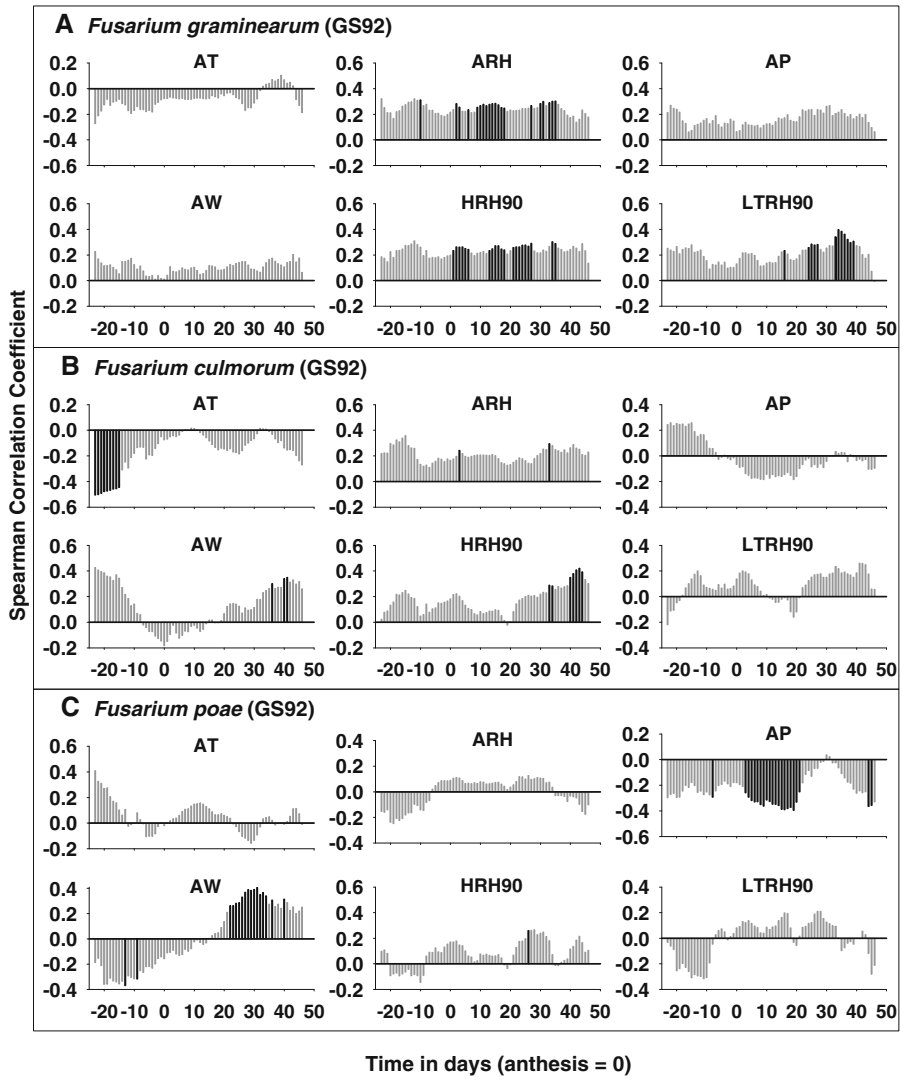

Fig. 2 Spearman rank correlation coefficients at 15-day windows for the association between environmental variables (chart title abbreviations defined in Table 1) and (**a**), *Fusarium graminearum* biomass; (**b**), *F. culmorum* biomass; and (**c**), *F. poae* biomass quantified at GS92 (*harvest*) across three European countries. Horizontal axis represents the starting time of the 15-day window, with day 0 representing the beginning of anthesis. Negative numbers represent days before anthesis began and positive numbers represent days after anthesis began. Bold vertical bars represent correlation coefficients for individual significance at α=0.005

was near anthesis (with starting times around days −20 to −10) and the other was after anthesis began (with starting times around days 0 to 10). The relative-humidity based variables that did not include temperature (e.g., ARH, HRH80, and HRH90) had the strongest correlations around anthesis, and the variables that coupled temperature with relative humidity (e.g., LTRH80) had the strongest correlations post-anthesis.

Fusarium graminearum biomass at GS77 was significantly associated with several different environmental variables for a range of window starting times (Fig. 1). For *F. graminearum* (GS77) and AT, all individual correlations were negative and significant ($P \leq 0.005$) for the 15-day windows (Fig. 1). This significance was also found for all the 30-day windows; correlations for several windows, but not all, were also significant for the 5-day windows (*data not shown*). Significant positive relationships were found for moisture or wetness-related variables (e.g., ARH, HRH80, AP) at window start days around 15 days before anthesis through slightly after anthesis began. In most cases, the correlations for windows prior to anthesis were not significant for the moisture-related variables.

For *F. graminearum* quantified at harvest (GS92), there were clusters of significant correlations with humidity-related variables (e.g., ARH, HRH90) approximately 5 to 25 days post-anthesis (Fig. 2). These higher correlations were later than the corresponding one for *F. graminearum* quantified at GS77. Combining temperature with humidity measurements (e.g.,

LTRH90) yielded significant positive correlations for windows that started around day 35.

Fusarium poae biomass was significantly correlated with surface wetness and rainfall variables, and the correlation pattern was generally consistent between GS77 and GS92 (Figs. 1 and 2). Clusters of negative correlations around anthesis to 10 days post-anthesis were found for the relationships between *F. poae* and precipitation (AP) (Figs. 1 and 2). In contrast, *F. poae* biomass at GS92 had positive relationships with surface wetness (AW) towards the end of the growing season (around days 20 to 35).

Significant negative correlations between *F. culmorum* biomass at GS92 and temperature were identified before anthesis (Fig. 2), and positive correlations with HRH90 and HRH80 were evident at the end of the season (approximately day 25). These correlations at the end of the season mostly reflected locations in Ireland and the UK. In general, individual correlations were only assessed when the Simes' global test was significant (Table 3). The correlations with AW in Fig. 2b demonstrate the situation where the global test was not significant (P=0.06) but some individual correlations were significant at the individual P value of 0.005. These small correlations were considered to be false positives.

Temporal patterns to correlations with environment—mycotoxins

DON concentrations and AT were negatively correlated at around 25 days after anthesis (Fig. 3). NIV was significantly negatively correlated with AT around the same time period as found for DON, but there was also an expanded period of significant correlations from approximately day −5 to 25 days post-anthesis (Fig. 3). At similar time periods, there were significant positive correlations between ARH and both DON and NIV. Other moisture and wetness-related variables were also significantly associated with DON and NIV for several window starting times (Tables 4 and 5, Fig. 3). In particular, hours with relative humidity >80% (HRH80) had significant correlations with the two mycotoxins approximately 5 days before anthesis to a month after anthesis for all window lengths tested. For DON, a similar result was found for hours with relative humidity >90% (HRH90), although these individual correlations were lower than those for HRH80 (*data not shown*). Other wetness or moisture variables were significantly correlated with DON over the same general time span, but for a much smaller number of window starting times than found for ARH and HRH80 (Fig. 3). The correlations for time windows substantially before anthesis were not significant for any of the environmental variables.

Semi-partial correlations

Tables 4 and 5 provide the adjusted global P value (P_g) and the individual Spearman correlation coefficient with the lowest P value for the relationship between the mycotoxin concentration and each environmental variable and window length after adjusting for one or more other biological variables. The semi-partial correlations usually decreased as adjustments were made for increasing numbers of biological variables that could be affecting the mycotoxins, but the decrease was often minor (e.g., LTRH80 and DON) and the outcome of the global tests of significance often did not change. In a few cases, the semi-partial correlations actually increased slightly (e.g., AW and NIV) compared with the Spearman correlations. This likely happened because of missing values in the data set. Some observations (location-years) were not used in determining the adjusted correlations that were used for the Spearman correlations; these dropped observations could move the statistics slightly up or down.

Significant semi-partial correlations ($P_g \leq 0.05$, individual $P \leq 0.005$) were found for the relationship between toxins and humidity-related variables (e.g., ARH, HRH80) when adjusted for disease intensity, and then for *F. graminearum* and *F. culmorum* (and *F. poae* for NIV) at GS77 and then at GS92 (Tables 4 and 5; Fig. 3). The largest correlations covered the period from around the time of anthesis to approximately 25 days after anthesis. Moreover, based on the global test, air temperature was negatively correlated with DON and NIV concentrations (Tables 4 and 5) for one or more window lengths after adjustments for disease, and disease and fungal biomasses at GS77. The correlation with AT was either significant or close to significant (P_g=0.059) when adjustment was made for all the biological variables (including fungal biomass at GS92). The highest individual semi-partial correlations with AT were at time windows around 15 to 20 days post-anthesis for DON and around 15 to 25 days post-anthesis for NIV.

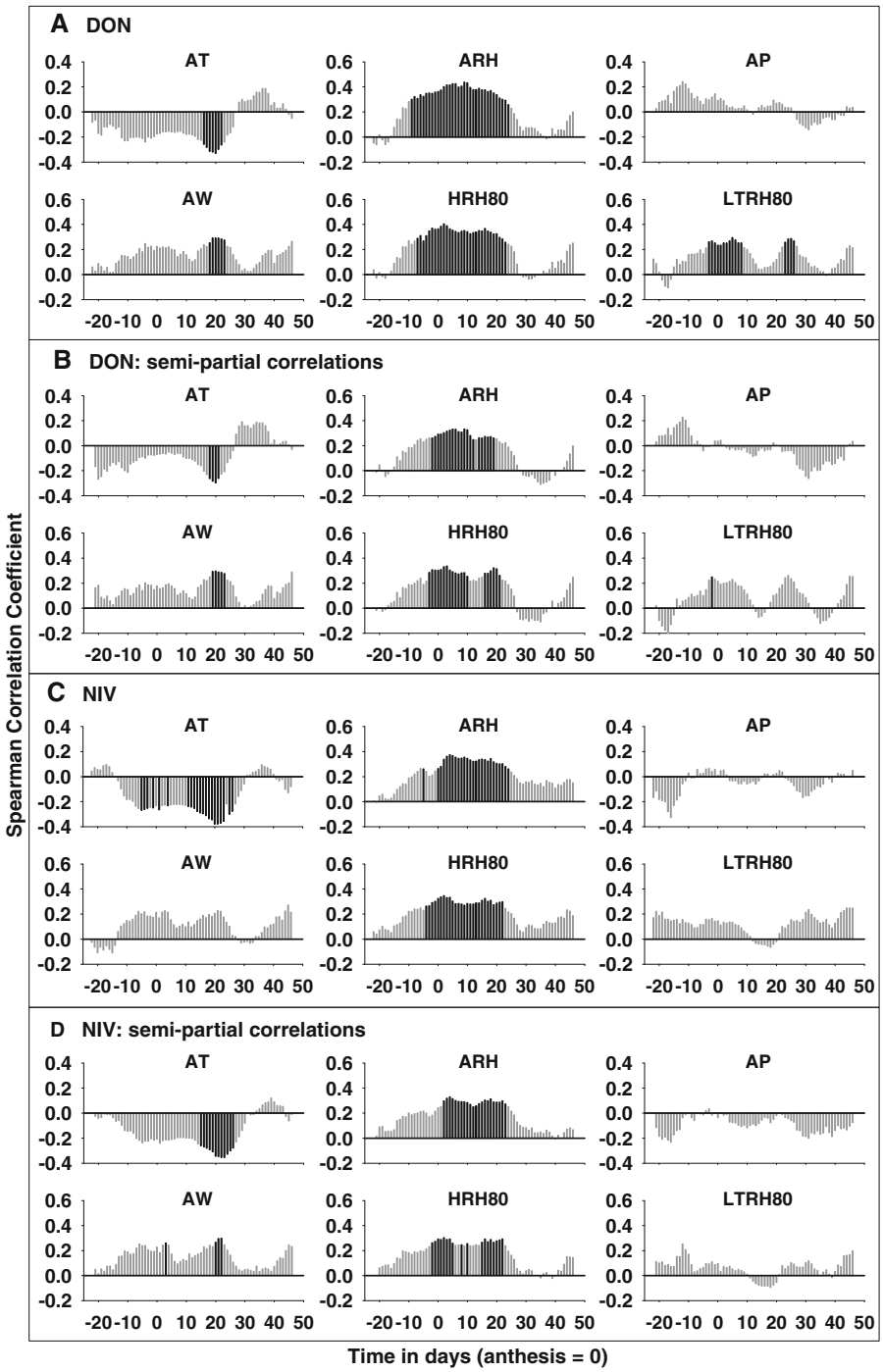

Fig. 3 Spearman rank correlation coefficients and semi-partial Spearman rank correlation coefficients at 15-day windows for the association between environmental variables (chart title abbreviations defined in Table 1) and (**a**), deoxynivalenol (DON) concentration; (**b**), DON adjusted for disease intensity, *Fusarium graminearum* biomass at GS77 and GS92, and *F. culmorum* biomass at GS77 and GS92; (**c**), nivalenol (NIV) concentration, and (**d**), NIV adjusted for disease intensity, *F. graminearum* biomass at GS77 and GS92, *F. culmorum* biomass at GS77 and GS92, and *F. poae* biomass at GS77 and GS92 across three European countries. Horizontal axis represents the starting time of the 15-day window, with day 0 representing the beginning of anthesis. Negative numbers represent days before anthesis began and positive numbers represent days after anthesis began. Bold vertical bars represent correlation coefficients for individual significance at α=0.005

Discussion

A window-pane analysis (Coakley et al. 1985) based on the use of the nonparametric Spearman correlation coefficient was used in the current investigation to identify environmental variables that were associated with disease intensity, fungal biomass of FHB-causing pathogens, and the mycotoxins DON and NIV across three European countries. All disease, pathogen, and mycotoxin variables were significantly associated with at least one evaluated environmental variable. Significant correlations and semi-partial correlations

with environmental variables were found for multiple windows that ranged from approximately a month before anthesis through to harvest. This was a generalization of the original work of Xu et al. (2008a), as they limited their analysis to two windows, one from anthesis to 7 days post-anthesis and the other from anthesis to harvest.

The current work extends a window-pane analysis conducted for four states in the US (Kriss et al. 2010) to Western Europe, and the work reported here also builds on the past analysis in several ways. New moisture-temperature-duration variables were considered that give differential weight to high-moisture hours based on closeness of temperature to the optimum (e.g., LTRH80). When these variables were correlated with the biological response variables, the correlations were higher than found for the cruder moisture-temperature variables based on (in effect) a 0/1 weighting (e.g., THRH80). The US analysis was based solely on disease intensity, but here we considered environmental effects on disease intensity, fungal biomass, and DON and NIV mycotoxin concentrations. Semi-partial Spearman rank correlation coefficients were used to quantify the association between the mycotoxin concentrations and environment after adjusting for the influence of disease intensity and toxigenic pathogen density on DON and NIV. This allowed a more direct appraisal of environmental effects on mycotoxins, rather than an indirect effect of environment (through the influence of environment on the other variables). The current analysis also exclusively used in-field measurements of environmental data rather than measurements from nearby regional stations that were used in the US analysis. Thus, the environmental measurements should be more representative of the microenvironment in and around the wheat fields. Moreover, because the date of anthesis was known for the European location-years (unlike with the US data sets), we were able to explicitly connect the windows to the time-distance from this important phenological stage known to be important for FHB infection (Pugh et al. 1933; Lacey et al. 1999).

Several moisture and/or wetness-related variables (e.g., ARH, HRH90, LHRH90) were significantly correlated with the biological response variables in the present analysis, which is consistent with the window-pane analysis for the US analysis (Kriss et al. 2010). Average daily relative humidity (ARH) and disease intensity in the US and in Europe had significant correlations around or slightly before anthesis. Precipitation and disease intensity had a positive relationship around 3 weeks post-anthesis in the US data, and similar results for this time period were found between several moisture-related variables and disease intensity in the Europe data. Biomass of *F. graminearum*, and concentration of DON and NIV, was also correlated with moisture variables for various window start times during the 4 weeks after anthesis. Others also have shown the positive influence of moisture and wetness duration on this disease and related biological variables (De Wolf et al. 2003; Paul et al. 2007). De Wolf et al. (2003) found that the number of hours that temperature was between 15 and 30°C and relative humidity was greater than or equal to 90% (THRH90) was related to disease in the US, where *F. graminearum* is the most prevalent FHB-causing pathogen. THRH90 and LTRH90 also had some of the highest correlations with late-season *F. graminearum* (GS92) in the Europe data for different windows. However, this was the only case where the two moisture-temperature-duration variables had similar estimated correlations. In other cases, the new variables exhibited the higher estimated correlations for DON and disease intensity. In addition, when the moisture-temperature-duration variables were significantly correlated with the biological variables, the highest correlations were at the same general times as found for the moisture-duration variables that did not include temperature (HRH80 and HRH90). The correlations for the moisture-duration variables that incorporated temperature also were not of greater magnitude than found for the duration variables that did not include temperature, indicating the dominating effect of moisture in the relationships. For disease intensity, DON, and NIV, correlations with HRH80 were generally higher in magnitude than correlations with HRH90, but for the biomass of the *Fusarium* species (when significant), correlations with HRH90 were generally higher in magnitude than correlations with HRH80.

For the data from the US states (Kriss et al. 2010), there was no evidence of a relationship between FHB intensity and any of the temperature variables that did not involve moisture (e.g., AT) for any time window. However, for the European data, significant negative correlations were found between AT and disease, pathogen [*F. graminearum* (GS77) and *F. culmorum* (GS92)], and mycotoxin (DON and NIV) variables

for several window lengths and starting times. However, the temperature effect is complex and uncertain. The result could be influenced by the common negative relationship between ambient temperature and RH in a particular location (over short time spans), although this relation would not necessarily transfer across locations and years. Moreover, based on standard temperature-response relations (Magan 2007), high ambient temperatures (above the optimum) can result in a negative relationship with a biological response, but daily temperatures in the European data set were seldom very high (relative to the optimum for the fungi). There could also be confounding effects of other nonmeasured variables on the temperature-biological variable relations, where regional temperature was decreasing as some unknown regional variable favorable for the disease was increasing. Interestingly, when temperature was combined with moisture in an environmental variable (e.g., LTRH80), the correlations were positive, in part due to the strong effect of moisture on the biological variables.

Our results identified several of the environmental variables that have been implicated in other research as having a relationship with disease, mycotoxin, or fungal biomass (Xu et al. 2008a; Hope et al. 2005; van der Fels-Klerv et al. 2010) but we were able to give more accurate definitions of when these associations were occurring. Interestingly, the environmental variables most associated with disease intensity, fungal biomass of the *Fusarium* species (not including *F. poae*), and the mycotoxins were similar, but in general, the length of significant windows and/or the window start times differed somewhat. For disease intensity, windows with the highest correlations were for moisture variables (e.g., ARH, HRH80), were relatively short in duration (e.g., 5-days), and started somewhere around 15 days before the beginning of anthesis.

For *F. graminearum*, Xu et al. (2008a) suggested that a longer duration of wetness was required for infection relative to the requirements for fungal colonization within the wheat spike. Some support for this suggestion is found in the current investigation by focusing on *F. graminearum* at GS77 and GS92. The highest correlations with *F. graminearum* at GS77 were for wetness variables (AP, AW), corresponding to windows with start times between 20 days to 10 days before anthesis. In contrast, the correlations for these wetness variables with *F. graminearum* at GS92 were considerably lower; rather, other moisture (but not wetness) variables had the highest correlations, and these were for several windows after anthesis (from about 5 days after anthesis to 40 days after anthesis). Therefore, our results suggest that *F. graminearum* is favoured by moisture throughout the anthesis and post-anthesis time window, but that the importance of surface wetness declines over time after infection, during the later colonization of the spikes (and production of the mycotoxins).

Fusarium culmorum biomass did not have many significant correlations with environmental variables; in general, it was most associated with cooler conditions and with high relative humidity before anthesis. Although generally not significant, the negative correlations with temperature and positive correlations with moisture/humidity remained during the time between anthesis and maturity. This is similar to the results of Xu et al. (2008a) and Turner and Jennings (1997). In contrast, *F. poae* at both GS77 and GS92 was associated with drier conditions for all window lengths and most window start times, and the highest of these negative correlations were around 10 to 20 days post anthesis. Similarly, Turner and Jennings (1997) found that wheat spikes inoculated with *F. poae* were less infected under high humidity than medium humidity. However, there is variability across studies, as others (Xu et al. 2007) have suggested there is a positive relationship between *F. poae* and moisture.

Most statistical models for FHB (or the mycotoxins) use environmental data around anthesis, since this is the time when wheat is most susceptible to infection and is about the latest time that a fungicide can be applied for effective FHB control (De Wolf et al. 2003; Lacey et al. 1999). However, predictions closer to crop maturity can be of benefit for estimating the magnitude of yield loss or contamination by mycotoxins before the crop is harvested or sold at grain elevators. As we found here with the nonparametric correlations, environmental conditions prior to anthesis were of little value in predicting mycotoxin concentration or late-season fungal biomass. Thus, for situations similar to those investigated here, we conclude that risk predictions for mycotoxins will need to be based on post-anthesis environmental conditions or forecasts of the environment for the period after anthesis (and possibly other factors not considered by us). Published studies so far are inconsistent in terms of the effects of moisture variables (including rainfall) late in the

growing season on DON production and accumulation in grain (Cowger et al. 2009; Culler et al. 2007). Here we show that moisture variables post-anthesis were positively correlated with DON and NIV levels in harvested grain. Unlike in the case with moisture, rainfall variables were not significantly associated with mycotoxin levels.

Using the same data set, Xu et al. (2008a) showed that the estimated probability of DON being present was significantly associated with *F. graminearum*, and the presence of NIV was mainly associated with *F. culmorum*. Other researchers have also found relationships between mycotoxins and fungal biomass, and especially between DON and disease intensity (Demeke et al. 2010; Oerke et al. 2010; Paul et al. 2005). This, and the significant correlations found here between environment and disease and fungal biomass, suggests that the significant (Spearman) correlations between the mycotoxins and environmental variables could be due just to the effects of the environment on the biomass of the fungal pathogens (which would then be affecting mycotoxin concentration), rather than to direct effects of environment on the mycotoxins. Through the use of semi-partial Spearman correlations, we showed that there were still significant associations between mycotoxins and several environmental variables (e.g., ARH, HRH80) after adjusting for monotonic effects of confounding biological variables. More complex (nonmonotonic [e.g., certain polynomial]) relations involving the confounding variables would not be removed by the semi-partial adjustment method; however, based on graphs of the data and of the residuals from fitting models to the ranked data, we saw no evidence of nonmonotonic relations (Kriss, *unpublished*). In general, the decrease in the semi-partial correlation (relative to the usual unadjusted correlations) was rather small, and the highest correlations continued to be found for windows about 5 days before anthesis to 20 days post-anthesis. No pre-anthesis time windows were found with large semi-partial correlations, demonstrating the difficulty in predicting mycotoxin risk based on conditions before anthesis.

Although several environmental variables were found to be associated with disease, fungal biomass, and mycotoxins, the magnitude of the correlations was not high. Thus, there remains a substantial amount of unexplained variability. This is not surprising, given the heterogeneity found in other studies or among studies (Cowger et al. 2009; Culler et al. 2007; Paul et al. 2007). There are several factors not directly considered in the present analysis, which also have been shown to affect both FHB and mycotoxin levels. Some of these factors are use of fungicides, tillage method, soil type, cropping history in the field or nearby fields. All cultivars used were susceptible to FHB, but their level of resistance to mycotoxin production and movement within the wheat spike is generally unknown. Moreover, inoculum density, diversity of fungal species, and diversity of strains (biotypes) of each species in the region within the location-years could vary greatly, and independently of the environmental variables during a fairly narrow time span. Differential effects of environment on fungal species and biotypes could ultimately result in differences in disease and mycotoxins. Given the magnitude of the correlations, no single environmental variable for a single window length and start time will be sufficient for prediction purposes. However, the window-pane results show the variables and windows that will likely be most useful in the development of risk models with multiple predictor variables. Future research will focus on the use of logistic modelling, regression tree, and recently developed machine learning methods for developing predictions of disease or mycotoxins based on the environment from the identified time windows.

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