Modeling the Effects of Environmental Conditions on HT2 and T2 Toxin Accumulation in Field Oat Grains

Xiangming Xu, Laurence V. Madden, and Simon G. Edwards

First author: State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China, and Genetics and Crop Improvement, East Malling Research, New Road, East Malling, ME19 6BJ, UK; second author: Department of Plant Pathology, Ohio State University, Wooster 44691; and third author: Harper Adams University, TF10 8NB, Newport, Shropshire, UK.

Accepted for publication 12 July 2013.

ABSTRACT

Xu, X., Madden, L. V., and Edwards, S. G. 2014. Modeling the effects of environmental conditions on HT2 and T2 toxin accumulation in field oat grains. *Phytopathology* 104:57–66.

Fusarium head blight (FHB) of wheat and barley has been extensively researched worldwide; in contrast, there is limited information on the effects of environmental conditions on *Fusarium* toxin accumulation in oat grains. More than 300 samples of oat grain from various regions of the United Kingdom from 2006 to 2008 were analyzed for mycotoxin contamination due to infection by *Fusarium* spp. HT2 and T2 toxins were the two most commonly detected, and their concentrations in individual samples were highly correlated. Hourly weather data were obtained from meteorological recording stations near most of the sampling sites. Statistical modeling was applied to both the original toxin (HT2 plus T2) data and the toxin data adjusted for oat cultivars and number of cereal crops in

the previous four seasons. Accumulation of HT2 and T2 toxin was positively correlated with warm and wet conditions during early May and dry conditions thereafter. Using a collection of 51 environmental variables summarized over three lengths (10, 15, and 20 days) of time periods encompassing early May, late May, and early July, all-subsets regression showed that many models, consisting of three to six predictor variables, could be identified with similar explanatory strength for the effect of environmental conditions on toxin accumulation. Most important predictor variables were related to wet conditions during the early-May period, which was before anthesis. These results suggest that the predominant period for *Fusarium langsethiae* infection of oat is likely to be before rather than during anthesis, as for other head blight pathogens. These empirical models may be further improved by using quantified pathogen biomass within the grains and weather predictor variables summarized in relation to plant growth stages (instead of calendar times).

Fusarium head blight (FHB) of wheat and barley has been extensively researched worldwide (2,27,34,48). The general consensus is that FHB development in wheat is favored by wet and warm conditions around anthesis (5,7,17,22,35,47,49). This may be interpreted in terms of the effect of environmental conditions on several disease components (e.g., spore production, infection, and subsequent colonization of grain). Several species, including *Fusarium graminearum*, *F. culmorum*, and *F. poae*, are known to produce mycotoxins, especially deoxynivalenol (DON) and nivalenol (NIV), contaminating the harvested grain. The economic importance of FHB is primarily due to reduction in yield and contamination of grain by mycotoxins (32).

There is limited information on occurrence of *Fusarium* mycotoxins in U.K. cereals prior to 2001. A survey conducted in 1999 found the greatest amounts of DON on wheat, with lower levels on barley and oat. Edwards (11) quantified *Fusarium* mycotoxins in barley and oat in 2002 to 2005 in the United Kingdom. The levels of DON and zearalenone (ZON) were much lower in barley and oat compared with wheat but the levels of HT2 and T2 were higher in oat than in barley and wheat. HT2 and T2 are type A trichothecenes, which are produced predominantly by *F. langsethiae* in U.K. oat (13). These type A trichothecenes

were implicated in Alimentary Toxic Aluekia caused by the consumption of cereals which had overwintered in fields in Russia in the 1940s (10). There are currently few legislative or guideline limits for HT2 and T2 worldwide (44). A combined tolerable daily intake for HT2 and T2 was recently set by the European Food Safety Authority at 0.1 µg kg⁻¹ body weight day⁻¹ (1). The European Commission subsequently published a recommendation for the continued monitoring of these mycotoxins and for investigations to identify factors that may cause excess contamination of grains by these toxins (1). The indicative level of the combined concentration of HT2 and T2 in unprocessed oat is 1,000 µg kg⁻¹.

There were large differences in HT2 and T2 levels between organic and conventional oat in the United Kingdom (11,12). Organic oat had approximately four times lower concentrations compared with samples from the conventional production system. This difference was partly due to the tendency for organic growers to use cultivars less susceptible to HT2 and T2 accumulation (11) and to adopt longer, less cereal-intense rotations (12). These results have been confirmed in similar studies in Norway (3,4) and, to a lesser extent, Poland (24).

In this article, our objective was to assess whether it is possible to develop models that can predict the accumulation of HT2 and T2 in oat grain using the data collected in the United Kingdom during the period of 2006 to 2008. Specifically, we focused on (i) evaluating the general correlation pattern of weather variables with toxin concentration using window-pane analysis, (ii) evaluating model consistencies using resampling methodology, (iii) identifying environmental variables most consistently associated with toxins in predictive models, and (iv) identifying one or more models with similar predictive abilities.

Corresponding author: X. Xu; E-mail address: Xiangming.Xu@emr.ac.uk

* The e-Xtra logo stands for “electronic extra” and indicates that the online version contains one supplemental text file, two supplemental tables, and one supplemental figure.

MATERIALS AND METHODS

Grain sample collection. More than 100 samples of oat grain and related agronomic data were collected by crop consultants Association of Independent Crop Consultants (AICC) members, Agrovista, Department of Agriculture and Rural Development for Northern Ireland (DARD), and Scottish Agronomy in each of the 3 years (2006, 2007, and 2008). Samples were collected at harvest from specific fields from either the combine or trailers leaving the field. Ten subsamples of ≈ 300 g were taken from arbitrary points around the field and combined to provide a 3-kg sample. Consultants sent these samples in cotton bags by overnight courier along with agronomic data pertaining to that field sample. A similar number of samples were requested from each region: South, East, Midlands, North, Scotland, and Northern Ireland. These regions were based on the U.K. cereal levy board (Home-Grown Cereals Authority, Agriculture & Horticulture Development Board (HGCA-AHDB)) corn-return regions. Many aspects of agronomy details were requested, including location, variety, and previous crops for the last 4 years. However, information for one or more of these details was not available for nearly a third of the samples.

On receipt of samples, their moisture content was determined. A 500-g subsample of grain was removed using a riffle divider, dried to 12% moisture content, and stored at room temperature for subsequent visual and quality assessment if required. The remaining sample was milled with a 1-mm screen and mixed in a tumbler mixer before two 200-g subsamples were collected. One sample was sent to Campden BRI (Chipping Campden, England) for mycotoxin analysis, and the remaining sample was held at -20°C at Harper Adams as an archive sample.

Mycotoxin analysis of commercial samples. All mycotoxin analysis of the oat samples was performed by Campden BRI using the United Kingdom Accreditation Service (UKAS) accredited procedures. The trichothecenes DON, NIV, 3-acetyl DON, 15-acetyl DON, fusarenone X, T2 toxin, HT2 toxin, diacetoxyscirpenol (DAS), and neosolaniol, and the nontrichothecene ZON, were analyzed by liquid chromatography tandem mass spectrometry. Spiked samples were included in each batch to determine extraction recovery. The method had acceptable re-

covery range (60 to 120%) for all trichothecenes analyzed. Results were corrected for the extraction recovery.

The expanded measurement of uncertainty was calculated using a standard coverage factor of 2, equivalent to a confidence of $\approx 95\%$ that the actual level of the mycotoxin being measured lies within the quoted range. The expanded measurement of uncertainty was calculated to be 41% for T2 and 17% for HT2. The limit of quantification (LoQ) for the trichothecenes was $10\text{ }\mu\text{g kg}^{-1}$. Samples below the LoQ were assigned with a value of 50% of the LoQ (i.e., $5\text{ }\mu\text{g kg}^{-1}$) instead of zero.

Weather data collection. Weather data were obtained from a network of weather-recording instruments managed by the U.K. Meteorological Office (U.K. Met Office Integrated Data Archive System Land and Marine Surface Stations Data, 1853 to current) (http://badc.nerc.ac.uk/view/badc.nerc.ac.uk_ATOM_dataent_ukmo_midas). Hourly temperature ($^{\circ}\text{C}$), relative humidity (%), and rainfall (mm) data from the meteorological station closest (distance ≤ 23 km) to each sampling site were used. For nearly 20% of the sampling sites, it was not possible to find a meteorological station within a radius of 23 km and, hence, these samples were omitted from modeling. There were also sites for which it was not possible to obtain relevant agronomic information and which, therefore, were omitted from modeling analysis when the relevant agronomic information was needed. Thus, there were 273 sites (85, 96, and 92 in 2006, 2007, and 2008, respectively) with weather data available and 220 sites (84, 80, and 56 in 2006, 2007, and 2008, respectively) with both agronomic and weather data available. Based on previous studies on FHB of wheat (22,23), 17 daily summary variables (Table 1) were obtained from the hourly data. For any chronological series of hourly weather data, missing data within an interval not longer than 3 h were estimated with the simple linear interpolation. For any given day (defined as from 9:00 GMT to 8:59 GMT next day), if more than three consecutive hourly data were missing, the whole day was treated as a day with missing weather data.

Statistical analysis. In all analyses, the combined amount of HT2 and T2 was logarithm transformed before analysis. Previous studies suggested that accumulation of HT2 and T2 is significantly affected by oat cultivars and number of cereal crops in the previous seasons (12). Thus, we applied statistical modeling to

TABLE 1. Description of environmental variables used in window pane analysis and all-subsets (leaps and bounds) regression analysis for HT2 and T2 mycotoxins in oat grain in the United Kingdom

Name ^a	Description
T	Average temperature ($^{\circ}\text{C}$)
RH	Average relative humidity (%)
VPD	Average vapor pressure deficit (mbar), derived from T and RH
Rain	Average daily total rainfall (mm)
RD	Daily number of hours with rain (h)
OT90	Daily number of hours when temperature in $15^{\circ}\text{C} < T \leq 30^{\circ}\text{C}$ and $\text{RH} \geq 90$ (h)
OT80	Daily number of hours when temperature in $15^{\circ}\text{C} < T \leq 30^{\circ}\text{C}$ and $\text{RH} \geq 80$ (h)
OT70	Daily number of hours when temperature in $15^{\circ}\text{C} < T \leq 30^{\circ}\text{C}$ and $\text{RH} \geq 70$ (h)
RH90	Daily number of hours when $\text{RH} \geq 90$ (h)
WT90	Daily total of weighted temperature (T^*) with $\text{RH} \geq 90\%$ (WT90); T^* is calculated as
	$T^* = \begin{cases} \ln(T - 8) \cdot [\ln(17)]^{-1} & \text{when } 8 \leq T < 25^{\circ}\text{C} \\ 1 & \text{when } 25 \leq T < 30^{\circ}\text{C} \\ 0 & \text{otherwise, where } T \text{ is the hourly temperature.} \end{cases}$
RH80	Daily number of hours when $\text{RH} \geq 80$ (h)
WT80	As WT90 but with relative humidity $\geq 80\%$
RH70	Daily number of hours when $\text{RH} \geq 70$ (h)
WT70	As WT90 but with relative humidity $\geq 70\%$
V17	Daily number of hours when $\text{VPD} \leq 1.7$ mbar (h)
V34	Daily number of hours when $\text{VPD} \leq 3.4$ mbar (h)
V51	Daily number of hours when $\text{VPD} \leq 5.1$ mbar (h)

^a These 17 weather variables were calculated for each of the six window lengths (5, 10, 15, 20, 25, and 30 days) for window-pane analysis. For all-subsets regression analysis, each variable was summarized over three periods—early and late May and early July—with created variables designated by the prefix of 1_, 2_, and 3_, respectively. Because of similarity of results for different window lengths, summarized data for just three window frames (10, 15, and 20 days) were used in all-subsets regression analysis.

both the original (transformed) toxin data and the transformed toxin data that had been adjusted for the cultivar and previous crops (both were treated as a factor).

We used window-pane analysis (22,23) to conduct initial analyses on the relationship of HT2 and T2 accumulation with weather conditions and to select appropriate time periods over which weather variables were summarized for use in all-subsets regression, which was combined with resampling to model the relationship of toxin accumulation with weather conditions.

Window-pane analysis. The concept underlying window-pane analysis is the specification of a defined length for which environmental variables from hourly recorded data were summarized and correlated with a response variable. This time window (e.g., 15 days) is progressed along the total time frame of interest 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 one day of data. With a defined starting time and window length, the ending time of the window is automatically determined.

Ideally, time windows of environmental data should begin with a biologically relevant date, such as anthesis date, which would partially account for variability among samples, particularly if they came from geographically distant regions. For the present study, it was not possible to obtain anthesis dates for sampling sites. Instead, we constructed the window-pane data from 1 April to the end of September to approximate the oat growing season in the United Kingdom: panicle emergence and anthesis are normally in May to June and harvest in late August to September, though there is considerable variability in the growth stages among sampling sites. 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 limited results shown to 5, 15, and 25 days. Summary variables were not calculated if there were missing data (i) within the window length of 5 days, (ii) for more than 1 day for window lengths of 10 and 15 days, (iii) for more than 2 days for window lengths of 20 and 25 days, or (iv) for more than 3 days for the window length of 30 days. We wrote a specific computer program using the Delphi programming language (version XE3; Embarcadero Inc., San Francisco) to calculate daily average of each of the 17 weather variables (Table 1) for the different window lengths and starting times.

Analysis was based on the pooled data across all 3 years. The association between variables was quantified with the usual parametric correlation (r), which was calculated within the program written in Delphi language that was used to calculate window-pane summary weather data. Because of missing weather data for certain combinations of weather variables, location, and year, each correlation was determined from a varying number of data points (at least 244 location-years). Because window-pane analysis was only used as a first step for further regression analysis, we used only a single critical value ($\alpha = 0.001$) for assessing statistical significance without considering other, more complex corrections for multiple hypothesis testing (23).

All-subsets regression. In the present study, many potential explanatory variables are possible, including agronomic practices, host resistance, and climatic conditions; and, particularly for oat, there is little prior epidemiological information on variable selection for predicting toxin accumulation. Which variable is selected for inclusion in a model is an important issue in applied regression analysis. In spite of wide recognition of the limitations of stepwise multiple regression analysis (19,21,42,43,45,46), this variable selection methodology is usually used to select predictor variables for inclusion in models in plant pathology. In addition to several statistical drawbacks, stepwise regression places an inappropriate focus or reliance on a single best model when there are two or more models that have almost equal explanatory strength.

One alternative to stepwise variable selection is the all-subsets regression approach (28). In the present study, we used the leaps-and-bounds algorithm (15) in the all-subsets regression analysis. The leaps-and-bounds algorithm organizes all the possible models into tree structures and scans through them, skipping (or leaping) over those that are definitely not optimal. The leaps-and-bounds algorithm can give a number of optimal models for a given number of predictors in a model. The R “subselect” package was used, which implements the leaps-and-bounds algorithm for variable selection (40,41). In the present study, a simple linear regression was used to relate the original or adjusted toxin data to weather predictors; thus, model selection in all-subsets regression is based on the standard coefficient of determination (R^2) based on the sum of squares.

The window-pane analysis identified three periods during which there was significant and consistent correlation between toxin concentration and weather variables. All 17 weather variables were summarized for each of the three periods: early and late May and early July (see Results). The start time of each of the three periods was chosen to coincide with the starting time of a window frame with the maximum correlation during the corresponding period. All-subsets leaps-and-bounds regression was applied to weather data of each window length separately; thus, there were 51 variables (17 from each period) for a given window-pane length.

Resampling was used to assess model consistency, mimicking the situation where an investigator develops an empirical model from only a sample of the population of possible observations. First, a random set of data samples (65% of the total set) was obtained without replacement and subjected to all-subsets regression analysis; the best model with three, four, five, or six predictors was saved. This resampling process was repeated 100 times. Next, these best-resample models were analyzed for model uniqueness and frequencies of each predictor variable included. Then, the all-subset regression was applied to the whole data set to generate the top 10 models (with three, four, five, or six predictors). These whole-data set models were then compared with those 100 best-resample models. Finally, several models with similar predictive power (but different combinations of predictor variables) were selected for further evaluation.

RESULTS

General results. In total, 303 samples were analyzed for toxin: 100, 103, and 100 for 2006, 2007, and 2008, respectively. Numbers of samples collected from all regions were reasonably balanced for oat, although fewer samples were supplied from Scotland in 2006. This was compensated for by more samples from Scotland in later years. Low sample numbers were received from Northern Ireland in all years.

HT2 was the most frequently detected *Fusarium* mycotoxin and usually present at the highest concentration. HT2 was highly correlated with T2 ($r = 0.91$ on the logarithm scale) (Fig. 1). Summary information for HT2 and T2 is given in Table 2. The number of samples exceeding $1,000 \mu\text{g kg}^{-1}$ of combined HT2 and T2 was 21, 8, and 1 for 2006, 2007, and 2008, respectively ($1,000 \mu\text{g kg}^{-1}$ is the European Commission indicative limit for unprocessed oat).

Window-pane analysis. Multiple environmental variables were significantly correlated with the total HT2 and T2 toxin. Two clear patterns emerged from all the correlations, as illustrated by a few typical examples in Figure 2. First, there was large positive correlation between rain or moisture-related variables and toxin concentrations during the early- to mid-May period. Second, from late May onward until early September, there was generally a consistent positive correlation between toxin concentrations and warm- or dry-related variables (such as temperature and vapor pressure deficit). The second pattern was mirrored by the con-

sistent negative correlation in the same, latter period between toxin and moisture-related variables such as V17, V34, and V51.

In all, ≈16% of the total variation in the toxin was accounted for by the cultivar and the number of cereal crops in the previous four seasons (both variables were treated as a factor in linear regression analysis). The overall pattern of the correlation between weather variables and the toxin adjusted for the cultivar and the number of cereal crops in the previous four seasons was very similar to that of the unadjusted toxin data (Figs. 2 and 3). Namely, the pattern was characterized by positive correlation of toxin with rain-related variables in the early-to-mid-May period, and with dry or warm conditions from late May onward. Based on these results, three time periods were chosen over which weather variables were summarized for use in the regression analysis: early May, late May, and early July.

All-subsets regression. For each of the three chosen periods, three window lengths (10, 15, and 20 days) were selected based on significant correlations as identified in the window-pane analysis. Window start times for 10-day windows were 13 and 25 May and 8 July for the three periods, respectively; the corresponding dates were 11 and 27 May and 4 July for 15-day windows and

5 and 25 May and 4 July for the 20-day windows. Because one or more weather variables was missing for various lengths of times, there were only 244 samples for which all summary variables were available for the entire sampling period. Furthermore, there were also sites where information on cultivar or previous crops was not available. Thus, all-subsets regression analysis was applied to 244 and 203 samples for the original and adjusted toxin data, respectively.

Models from resample data. Of the 100 resamplings, there were many models selected with different subsets of predictors (Table 3). Even for those models with only three predictors, on average, there were 44 and 36 different models from the 100 resampled data sets for the original and adjusted toxin data, respectively. As expected, the number of unique models generally increased with the number of predictors (from three to six) included in the model, regardless of whether the original or adjusted toxin data were used (Table 3). On average, there were 85 and 74 different models selected with six predictors for the original and adjusted toxin data, respectively. The number of unique models appeared to be fewer for 20-day window (mean = 54) than 10-day (mean = 60) and 15-day (mean = 61) windows. There were also fewer unique models for the adjusted toxin data (mean = 53) than for the original toxin data (mean = 63). As expected, the percentage of variability (R^2) explained by the models gradually increased with increasing number of predictors included. However, the rate of increase in R^2 did not change appreciably with increasing number of predictors (from three to six) (Supplemental Figure 1). Models fitted to the 10-day window data, on average, accounted for ≈4% more variability in toxin concentrations than the models fitted to the data summarized over longer windows.

Regardless of the toxin data type (adjusted or original) and the window lengths, the best 100 resample models included similar frequencies of weather variables from the early- and late-May periods (Table 4). In total, ≈41, 47, and 12% of the variables in the best resample models were from the early-May, late-May, and early-July period, respectively. The frequency of predictors from the early-July period included in the resample models increased gradually with increasing number of predictors and with increasing window frame length (Table 4), particularly for the adjusted toxin data. Thus, the frequency of predictors in the six-predictor resample models was similar for all the three periods when the adjusted toxin data were regressed on the 15- and 20-day window predictors.

Rain duration in the early-May and, to a lesser extent, rain in the late-May period were most frequently included in the resample models, regardless of the toxin data type, window length, and number of predictors in the models (Supplemental Table 1). Several other predictors, mostly related to rain, vapor pressure deficit, and relative humidity or its combination with temperature, were also frequently selected but with the inclusion frequency varying greatly among window lengths and numbers of predictors in the models. No predictors from the early-July period were consistently included in the resample models.

Fig. 1. Plot of T2 against HT2 toxin concentration for 303 oat samples in 2006 to 2008; both T2 and HT2 were logarithm-transformed (the limit of quantification [LoQ] for HT2 and T2 was $10 \mu\text{g kg}^{-1}$; samples <LoQ were assigned with a value of 50% of the LoQ instead of zero, giving $\ln(5) = 1.61$). The line is the fitted regression model [$\ln(\text{T2}) = 0.519 + 1.037 \cdot \ln(\text{HT2})$], explaining 82% of the total variability.

TABLE 2. Summary of quantified T2 and HT2 toxin concentrations ($\mu\text{g kg}^{-1}$) in U.K. oat in 2006 to 2008

Year, toxin	Concentration (%) ^a	Mean	Percentiles			Maximum
			Median (50%)	90%	95%	
2006 (100 samples)						
T2	95	251	135	552	865	2,321
HT2	97	544	272	1,271	2,361	3,940
2007 (103 samples)						
T2	77	104	38	183	346	1,919
HT2	88	331	123	532	1,275	6,480
2008 (100 samples)						
T2	65	41	21	120	189	257
HT2	68	79	25	198	371	1,005

^a Percentage of samples with $>10 \mu\text{g kg}^{-1}$ (the limit of quantification).

Weather variables in the early-July period were, on average, more likely to be selected in the models for the adjusted data than for the original data (Table 4). Such differences for five-predictor models based on the weather data summarized over a 20-day window are illustrated in Figure 4. The importance of individual variables also differed considerably between the original and adjusted data sets. In general, humidity-threshold-based predictors (e.g., RH70) and their combinations with temperature (e.g., OT70) in the late-May period were more frequently included in the models for the original toxin data than for the adjusted toxin data.

Models from the entire data set. The general pattern of the model fitting results was very similar between the original and adjusted toxin data. Within a given number of predictors, there were little differences in the explanatory strength (R^2) among the top 10 models (Table 5). Thus, it is difficult to select the “best” one from these possible models for the current data set. For example, the difference in the R^2 between the best and the worst of the top 10 models with three-predictors was only 0.006 for the original toxin data.

None of predictors from the July period was among the top three predictors that were most frequently included in the top 10 models (Table 5). When the number of predictors from each time period in the top three most frequently selected predictors (Table 5) was summarized over the three window lengths, predictors from the early-May period featured 152 times for the original toxin data, compared with 107 times from the late-May period. The corresponding values for the adjusted data were 172 and 102. In all cases, the three most frequently selected predictors were all directly related to wet or moist conditions.

Comparison of models from the entire data set and resample data sets. In general, the top 10 models from the entire data set were more likely to be represented in the best 100 resample models (Supplemental Table 2) when there were few (e.g., three) predictors in the model. For the original toxin data regardless of the window length, <50% of the 100 best resample models were among the top 10 models derived from the entire data set (i.e., using the same subset of predictor variables). This frequency decreased with the increasing number of

Fig. 2. Correlation coefficients for 5-, 15-, and 25-day windows of three environmental variables (average daily temperature, daily rain duration, and daily vapor pressure deficit) with the total concentrations of HT2 and T2 toxins (logarithm-transformed) in the U.K. oat samples obtained in 2006 to 2008. Horizontal axis represents the starting time of the each window length, with day 0 representing 1 April (heading around day 55 and anthesis around day 65). Two dotted lines represent the threshold correlation coefficients that are statistically significant at $\alpha = 0.001$.

TABLE 3. Number of unique (with the same subset of weather predictors) models among the best 100 models from resample data sets, describing the effects of environmental predictor variables on the concentration of HT2 and T2 toxins in oat grain (original data or adjusted for cultivar and previous crops)^a

Window length ^b	Original toxin				Adjusted toxin			
	3v	4v	5v	6v	3v	4v	5v	6v
10	52	59	59	83	50	30	63	83
15	42	59	82	91	31	50	57	72
20	39	48	63	81	26	44	64	66

^a Number of predictor variables in each model = three to six (3v to 6v). For each resampling (without replacement) from the entire data, all-subsets regression analysis was used to find the best model. Frequency of each selected model was recorded.

^b Weather data were summarized for the three periods (early and late May and early July) over 10-, 15- and 20-day windows. Separate analyses performed for each window length.

predictors in the models but increased when adjusted toxin data were used.

Because of the inter-correlations among the predictor variables, there are many combinations of predictors that can give the same predictive strength. Interpretation of estimated parameters is not direct because of the variable correlations—the value of a parameter for one variable depends on the other variables in the model. We give details of eight possible models (four from the original data and four from the adjusted data) that may be evaluated further for prediction of toxin concentrations (Table 6). As dis-

cussed above, these models were dominated by moisture or wetness predictors, especially for the first two time periods. For the original toxin data, the four models accounted for ≈ 32 to 36% of the total variation in the log-transformed toxin data; for the adjusted toxin data, the four models accounted for ≈ 44 to 50% of the total variability (including the contribution from cultivar and previous crops). The plot of fitted values against the observed toxin levels for two example models is presented in Figure 5. Similar graphs were found for all the selected models (X. Xu, unpublished data). Predicted toxin concentrations among these

Fig. 3. Correlation coefficients for 5-, 15-, and 25-day windows of three environmental variables (average daily temperature, daily rain duration, and daily vapor pressure deficit) with the total concentrations of HT2 and T2 toxins (logarithm-transformed), adjusted for cultivars and number of cereal crops in the previous four seasons, in the U.K. oat samples in 2006 to 2008. Horizontal axis represents the starting time of the each window length, with day 0 representing 1 April (heading around day 55 and anthesis around day 65). Two dotted lines represent the threshold correlation coefficients that are statistically significant at $\alpha = 0.001$.

TABLE 4. Percentage of times when an environmental predictor variable from each of the three periods (early and late May and early July) was in the best 100 resample models, describing the effects of environmental variables on the concentration of HT2 and T2 toxins in oat grain (original data or adjusted for cultivar and previous crops)^a

Window length, period ^b	Original toxin				Adjusted toxin			
	3v	4v	5v	6v	3v	4v	5v	6v
10								
Early May	40	48	45	45	48	50	47	46
Late May	46	39	40	40	47	44	40	38
Early July	14	13	15	15	5	6	13	16
15								
Early May	24	32	38	42	36	36	35	32
Late May	70	63	50	44	61	55	39	35
Early July	6	5	12	14	3	9	26	33
20								
Early May	37	34	39	45	40	43	52	39
Late May	61	63	54	42	57	52	29	26
Early July	2	3	7	13	3	5	19	35

^a Number of predictor variables in each model = three to six (3v to 6v). For each resampling (without replacement) from the entire data, all-subsets regression analysis was used to find the best model. Each resampling can potentially result in a different model (i.e., with different subsets of weather predictors) (Table 3).

^b Weather data were summarized for the three periods (early and late May and early July) over 10-, 15-, and 20-day windows. Separate analyses were performed for each window length.

models were highly correlated, especially for the adjusted toxin data, where the correlation coefficient was at least 0.9. For the original toxin data, the lowest correlation was still >0.83. This shows that the predictive values were strongly associated for the different models, even though they were based on different combinations of (correlated) predictor variables.

DISCUSSION

HT2 and T2 accumulation in oat grain in the United Kingdom was positively related to warm and wet conditions in early to mid-

May and dry conditions from late May through to harvest. Several agronomic factors, including oat cultivar and previous crops, have been previously shown to significantly affect the HT2 and T2 concentration in oat (11,12). For oat, following one or more seasons of cereals increased the level of HT2 and T2, indicating that the inoculum is largely present on cereal crop debris. The crop for the previous 2 years had been reported to have a significant positive effect on DON in wheat in 1 of 4 years studied (38). The overall window-pane correlation patterns for both original toxin data and those adjusted for cultivar and previous crops in this study suggested that the seasonal weather effects on

Fig. 4. Histogram of the number of times that each of the 51 variables was included in the 100 best models fitted to resample data sets, describing the effects of environmental predictors on total HT2 and T2 toxin concentrations (log transformed) in oat grain. Weather data (Table 1) were summarized for three periods (early and late May and early July, with the corresponding prefix of 1_, 2_, and 3_). Results are shown only for the 20-day window for models with five predictor variables.

TABLE 5. Summary of the top 10 models derived from the entire data set in all-subsets regression, describing the effects of environmental variables on the concentration of HT2 and T2 toxins in oat grain (original data or adjusted for cultivar and previous crops)

Predictors	Original toxin			Adjusted toxin		
	Window 10	Window 15	Window 20	Window 10	Window 15	Window 20
<i>R</i> ² range ^a						
Three	0.31–0.31	0.28–0.29	0.29–0.30	0.32–0.34 ^b	0.28–0.31	0.28–0.30
Four	0.33–0.34	0.30–0.31	0.31–0.32	0.36–0.38	0.32–0.33	0.31–0.32
Five	0.35–0.36	0.32–0.33	0.33–0.34	0.39–0.39	0.34–0.35	0.34–0.35
Six	0.36–0.37	0.33–0.34	0.35–0.36	0.40–0.40	0.36–0.38	0.36–0.37
Top three ^c						
1	2_Rain (30)	1_RD (37)	1_RD (40)	1_RH80 (34)	2_V34 (32)	1_RD (40)
2	1_RH80 (29)	2_RD (27)	2_RD (24)	1_RH (33)	2_RH (32)	1_RH (21)
3	1_RH (29)	2_RH (26)	1_RH (17)	2_Rain (21)	1_RD (27)	1_V51 (17), 2_RD (17) ^d

^a Range of coefficient of determination (*R*²) for three to six predictors.
^b This is the proportion of variation in the residuals after both cultivars and previous crops were fitted into the original data as factors, which accounted for ≈16% of the total variability in the original data.
^c Top three most frequently selected predictors in the top 10 models with three to six predictors. Numbers in parentheses are the number of times the particular predictor was included in the 40 models (top 10 models for each of three, four, five, and six predictors) fitted to the entire data set for each window length (see Table 1 for definition of the predictors).
^d These two predictors were equally frequently selected.

HT2 and T2 were not affected by the two agronomic factors. This is supported by the regression result that the difference in the R^2 between the models for the original and the adjusted toxin data was nearly equal to the percentage of variability accounted for by cultivar and previous crops (Table 6). This consistency gives us confidence in this overall correlation pattern showing the genuine effects of weather conditions on toxin accumulation.

Although epidemiology of *F. langsethiae* in oat is currently poorly understood (20), it could be speculated that the infection of oat grain most likely originates from the infection of spikelets during anthesis period, as for FHB in wheat (34,48). Thus, we may further speculate that, as for FHB of wheat (9,47), infection of oat panicles may be most severe under warm and wet conditions around anthesis. Accumulation of DON in spring barley is related to temperature and high humidity during the period shortly prior to heading (6); however, unlike wheat, anthesis in many barley varieties and types start during head emergence. A recent

study (33) showed that infection of oat by *F. langsethiae* appeared to occur before anthesis, and higher infection and mycotoxin levels appeared to be associated with warm, wet conditions before anthesis. In the present study, precise growth stage data were not available and, thus, it was not possible to exactly pinpoint the key growth stages across the sampling sites. There is a large spread in the timing of key growth stages in the United Kingdom from south to north and between autumn- and spring-sown cultivars. Individual cultivars of a given sowing type (autumn versus spring) also vary somewhat in growth stages, especially with differences in sowing dates. The majority of samples received were of autumn-sown cultivars from England and, as such, would typically be at panicle emergence in late May and anthesis in early June; in Scotland, anthesis is even later, usually in early July. The positive relationship of HT2 and T2 concentrations with “wet” weather predictors during May (especially early May) in this study supports the hypothesis that *F. langsethiae* infection of oat likely

TABLE 6. Estimated parameters of several selected models for the effects of environmental variables on the concentration of HT2 and T2 toxins in oat grain (original data [models A to D] or adjusted for cultivar and previous crops [models E to H])^a

Model ^b	Parameters
Original toxin data	
10-day window	
A	$\ln(X) = 22.28 (4.70) - 0.292 \times 1\_RH (0.077) + 6.743 \times 1\_RD (1.589) + 9.548 \times 1\_RH80 (2.790) - 0.185 \times 2\_Rain (0.041) (R^2 = 34.1\%)$
B	$\ln(X) = 25.00 (4.78) - 0.344 \times 1\_RH (0.079) + 6.826 \times 1\_RD (1.572) + 11.56 \times 1\_RH80 (2.876) - 0.208 \times 2\_Rain (0.042) + 2.627 \times 3\_RD (1.055) (R^2 = 35.8\%)$
20-day window	
C	$\ln(X) = 19.47 (2.77) + 5.857 \times 1\_RD (1.376) - 0.262 \times 2\_RH (0.053) + 13.45 \times 2\_OT90 (3.92) + 7.510 \times 2\_V34 (2.254) (R^2 = 32.2\%)$
D	$\ln(X) = 16.81 (3.77) - 0.467 \times 1\_RH (0.077) + 8.06 \times 1\_RD (1.544) + 11.15 \times 1\_V17 (2.53) + 13.25 \times 1\_V51 (2.63) + 0.484 \times 2\_T (0.093) (R^2 = 33.4\%)$
Toxin data adjusted ^c	
10-day window	
E	$\ln(X) = 24.13 (4.86) - 0.344 \times 1\_RH (0.080) + 13.30 \times 1\_RH80 (2.77) - 0.154 \times 2\_Rain (0.043) - 3.669 \times 2\_OT70 (0.754) (R^2 = 48.6\%)$
F	$\ln(X) = 25.35 (4.90) - 0.369 \times 1\_RH (0.084) + 4.648 \times 1\_RD (1.713) + 12.78 \times 1\_RH80 (2.94) - 0.146 \times 2\_Rain (0.043) - 5.0 \times 2\_OT70 (1.457) (R^2 = 50.0\%)$
20-day window	
G	$\ln(X) = 13.02 (2.96) + 8.10 \times 1\_RD (1.444) - 0.21 \times 2\_RH (0.057) - 6.942 \times 2\_RD (2.851) + 9.344 \times 2\_V34 (2.463) (R^2 = 44.1\%)$
H	$\ln(X) = 20.88 (3.88) - 0.40 \times 1\_RH (0.078) + 8.819 \times 1\_RD (1.679) + 9.780 \times 1\_V17 (2.678) + 8.953 \times 1\_V51 (2.386) - 7.035 \times 2\_RD (1.960) (R^2 = 47.4\%)$

^a Weather data were summarized for the three periods (early and late May and early July) over 10- and 20-day windows. All subsets regression analysis was used to find the best model with four or five predictor variables for each window length.

^b Model labels (A to H) are arbitrary names. X represents toxin concentrations ($\mu\text{g kg}^{-1}$) and the predictor variables are described in Table 1. Variables from different time windows are identified with prefixes (1_ for the early May, 2_ for the late May, and 3_ for the early July). R^2 , coefficient of determination, is the percentage of variability explained by the model.

^c Toxin data adjusted for cultivar and previous crop (R^2 includes the contribution by cultivar and previous crops fitted as factors).

Fig. 5. Plot of observed toxin concentration (HT2 + T2: logarithm transformed) in oat grain against the fitted values by two models: A, model A and B, model E in Table 6.

occurs before anthesis. This is a critical difference compared with head blight infection of wheat by other *Fusarium* pathogens and will be key in the development of chemical control measures. The standard timing to control head blight is early anthesis (36) and control efficacy drops as the spray application is further in time from the infection period (37). A fungicide application during anthesis would be too late to control an infection occurring during oat panicle emergence.

There was a positive relationship of HT2 and T2 concentrations in harvested oat with “dry” weather variables from late May onward. Drier conditions post-panicle emergence may not be necessary for accumulation of HT2 and T2 but, rather, they may reduce the loss of HT2 and T2 from the host plant. Two recent studies (8,16) have demonstrated that DON can be removed from wheat between anthesis and harvest during irrigation events, resulting in DON detected in drainage water from head-blight-infested fields (6). Although HT2 and T2 toxins have a lower water solubility than DON, it is still plausible that these toxins can also be leached from the oat crop by rainfall over this time period. However, the relationship between toxins and late-season weather remains uncertain. For instance, in a window-pane correlational study, Kriss et al. (23) found a positive relation between DON in wheat and wet weather conditions during the period after anthesis.

Recently, HT2 accumulation in oat was shown to be positively correlated with warm temperature during the anthesis period in Norway (4) but also with high humidity near harvest, which differed from the present study. This difference may be partially due to the fact that only the weather conditions in two periods (anthesis and preharvest) were used in the Norwegian study. It may result as a consequence of the mycotoxins being in a state of flux, with toxin production and leaching occurring under similar environmental conditions, with high moisture promoting mycotoxin production and high rainfall promoting leaching. It may also have resulted from the fact that climatic factors that mostly constrain fungal development (hence, mycotoxin accumulation) differ between the two regions because of the differences in the general climatic conditions. Thus, more studies are required to elucidate the multiple environmental effects on mycotoxins in cereals near the end of the growing season.

This study has illustrated the difficulty in identifying a single “best” empirical model for predicting HT2 and T2 accumulation in oat grain based on environmental conditions. All-subsets regression revealed many possible models with similar prediction strength; weather variables related to the moist or wet conditions in May were most frequently selected in these models. Finding a best model for predicting disease development, yield loss, or toxin accumulation is often an objective in epidemiological research, because this model, once identified, can help growers to manage disease effectively through correct timing of appropriate control interventions. In reality, however, there is never a best-possible model, although there can be a “most appropriate” model, or a set of appropriate models (46,50), given the amount of available data and high correlation among predictors.

Modeling with resample data showed that many possible models could fit resample data but most of these models were not those that could be found with a single analysis of the entire data set. Because of the inter-correlation among predictors, small differences in the resampled data sets could result in different best models being identified. A recent study also demonstrated the difficulty in finding the single most appropriate model to predict FHB epidemic development on wheat (39). Recently, statisticians have become aware of ubiquity of multiple predictive models for a common response variable, and advocated the use of model averaging to improve predictions (14,29). There are also ample examples in published studies that several forms of forecasting systems for a given disease can be derived from different data sets, including FHB in wheat (9,30,31). Other examples include

the various modifications made to the Mills’ criteria for predicting apple scab infection (26) or the multiple forecasting systems developed for late blight of potato (18).

In conclusion, accumulation of HT2 and T2 toxin in oat grain was shown to be positively related to wet conditions during early May and dry conditions from late May until harvest. This provides evidence supporting the hypothesis that *F. langsethiae* infection of oat is likely to be before rather than during anthesis, which warrants further studies. Modeling showed the difficulty in identifying a single best empirical model from field data to predict toxin accumulation. Many possible models with different combinations of predictor variables had similar powers of predicting HT2 and T2 accumulation, with coefficients of determination ≈ 40 to 45%. Improving these models will likely require data sets with known dates for growth stages so that environmental data can be more tightly fixed to putative key stages in the disease cycle. A better understanding of the relationship between fungal development in the grain and toxin accumulation is also needed for more accurate prediction of toxin accumulation. However, given the lower predictability of DON and HT2 in oat in recent Norwegian studies (4,25), predictability of HT2 and T2 in oat may never be very high based on environmental and agronomic factors. Unlike fungal infection, which is primarily affected by external environmental conditions and host susceptibility at a particular growth stage, toxin accumulation can occur over a long period of time once the infection had taken place. Toxin accumulation is likely to be also influenced by other factors, such as host metabolic status, toxin translocation, and competition of the pathogen with other microorganisms.

ACKNOWLEDGMENTS

Generation of the mycotoxin and agronomy data used within this article was funded by the HGCA-AHDB project RD-2007-3332 (“Improving risk assessment to minimise *Fusarium* mycotoxins in harvested oats”). Weather data were supplied by the U.K. Meteorological Office. X. Xu carried out most of this research in the period of his Organisation for Economic Co-operation and Development fellowship at the Ohio Agricultural Research and Development Center (OARDC), Ohio State University, and was also partially supported by the 111 Project from Education Ministry of China (B07049). L. V. Madden was supported by state and U.S. federal funds appropriated to OARDC.

LITERATURE CITED

1. Anonymous. 2013. Commission recommendation on the presence of T-2 and HT-2 toxin in cereals and cereal products. Off. J. Eur. Union L91:12-15.
2. Bai, G. H., and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.
3. Bernhoft, A., Clasen, P. E., Kristoffersen, A. B., and Torp, M. 2010. Less *Fusarium* infestation and mycotoxin contamination in organic than in conventional cereals. Food Addit. Contam. 27:842-52.
4. Bernhoft, A., Torp, M., Clasen, P., Løes, A., and Kristoffersen, A. 2012. Influence of agronomic and climatic factors on *Fusarium* infestation and mycotoxin contamination of cereals in Norway. Food Addit. Contam. 29:1129-40.
5. Birzele, B., Meier, A., Hindorf, H., Kramer, J., and Dehne, H. W. 2002. Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. Eur. J. Plant Pathol. 108:667-673.
6. Bucheli, T. D., Wettstein, F. E., Hartmann, N., Erbs, M., Vogelgsang, S., Forrer, H. R., and Schwarzenbach, R. P. 2008. *Fusarium* mycotoxins: overlooked aquatic micropollutants? J. Agric. Food Chem. 56:1029-1034.
7. Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., and Perugini, L. 2009. Post-anthesis moisture increased *Fusarium* head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99:320-327.
8. Culler, M. D., Miller-Garvin, J. E., and Dill-Macky, R. 2007. Effect of extended irrigation and host resistance on deoxynivalenol accumulation in *Fusarium*-infected wheat. Plant Dis. 91:1464-1472.
9. Desjardins, A. E. 2006. *Fusarium* Mycotoxins: Chemistry, Genetics and Biology. American Phytopathological Society, St. Paul, MN.
10. De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment

- models for wheat *Fusarium* head blight epidemics based on within-season weather data. *Phytopathology* 93:428-435.
11. Edwards, S. 2007. Investigation of *Fusarium* mycotoxins in UK barley and oat production. HGCA Project Report. Stoneleigh, UK.
  12. Edwards, S. G. 2012. Improving risk assessment to minimise *Fusarium* mycotoxins in harvested oats and malting barley. HGCA-AHDB Proj. Rep. No. 500. Stoneleigh, UK.
  13. Edwards, S. G., Imathiu, S. M., Ray, R. V., Back, M., and Hare, M. C. 2012. Molecular studies to identify the *Fusarium* species responsible for HT-2 and T-2 mycotoxins in UK oats. *Int. J. Food Microbiol.* 156:168-175.
  14. Exbrayat, J. F., Viney, N. R., Frede, H. G., and Breuer, L. 2013. Using multi-model averaging to improve the reliability of catchment scale nitrogen predictions. *Geosci. Model Dev.* 6:117-125.
  15. Furnival, G. M., and Wilson, R. W. 1974. Regressions by leaps and bounds. *Technometrics* 16:499-511.
  16. Gautam, P., and Dill-Macky, R. 2012. Free water can leach mycotoxins from *Fusarium*-infected wheat heads. *J. Phytopathol.* 160:484-490.
  17. Gilbert, J., Woods, S. M., and Kromer, U. 2008. Germination of ascospores of *Gibberella zeae* after exposure to various levels of relative humidity and temperature. *Phytopathology* 98:504-508.
  18. Hardwick, N. V. 2006. Disease forecasting. In: *The Epidemiology of Plant Diseases*. B. Cooke, D. G. Jones, and B. Kaye, eds. Springer, Dordrecht, The Netherlands.
  19. Hurvich, C. M., and Tsai, C. L. 1990. The impact of model selection on inference in linear regression. *Am. Stat.* 44:214-217.
  20. Imathiu, S. M., Edwards, S. G., Ray, R. V., and Back, M. A. 2013. *Fusarium langsethiae*—a HT-2 and T-2 toxins producer that needs more attention. *J. Phytopathol.* 161:1-10.
  21. Johnson, C. J., Seip, D. R., and Boyce, M. S. 2004. A quantitative approach to conservation planning: Using resource selection functions to map the distribution of mountain caribou at multiple spatial scales. *J. Appl. Ecol.* 41:238-251.
  22. Kriss, A. B., Paul, P. A., and Madden, L. V. 2010. Relationship between yearly fluctuations in *Fusarium* head blight intensity and environmental variables: A window-pane analysis. *Phytopathology* 100:784-797.
  23. Kriss, A. B., Paul, P. A., Xu, X.-M., Nicholson, P., Doohan, F. M., Hornok, L., Rietini, A., Edwards, S. G., and Madden, L. V. 2012. Quantification of the relationship between the environment and *Fusarium* head blight, *Fusarium* pathogen density, and mycotoxins in winter wheat in Europe. *Eur. J. Plant Pathol.* 133:975-993.
  24. Kuzdraliński, A., Solarska, E., and Mazurkiewicz, J. 2013. Mycotoxin content of organic and conventional oats from southeastern Poland. *Food Control*. Online publication. <http://dx.doi.org/10.1016/j.foodcont.2013.02.014>
  25. Lindblad, M., Börjesson, T., Hietaniemi, V., and Elen, O. 2012. Statistical analysis of agronomical factors and weather conditions influencing deoxynivalenol levels in oats in Scandinavia. *Food Addit. Contam.* 29:1556-71.
  26. MacHardy, W. E. 1996. *Apple Scab: Biology, Epidemiology, and Management*. American Phytopathological Society, St. Paul, MN.
  27. McMullen, M. P., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Dis.* 81:1340-1348.
  28. Miller, A. 2002. *Subset Selection in Regression*. Chapman & Hall, Blackwell Publishing Ltd., London.
  29. Montgomery, J. M. 2012. Improving predictions using ensemble Bayesian model averaging. *Polit. Anal.* 20:271-291.
  30. Moschini, R. C., and Fortugno, C. 1996. Predicting wheat head blight incidence using models based in meteorological factors in Pergamino, Argentina. *Eur. J. Plant Pathol.* 102:211-218.
  31. Moschini, R. C., Pioli, R., Carmona, M., and Sacchi, O. 2001. Empirical predictions of wheat head blight in the northern Argentinean Pampas region. *Crop Sci.* 41:1541-1545.
  32. Nganje, W. E., Bangsund, D. A., Leistriz, F. L., Wilson, W. W., and Tiapo, N. M. 2004. Regional economic impacts of *Fusarium* head blight in wheat and barley. *Rev. Agric. Econ.* 26:332-347.
  33. Opoku, N., Edwards, S. G., and Back, M. A. 2013. Development of *Fusarium langsethiae* in commercial cereal production. *Eur. J. Plant Pathol.* Online publication. doi:10.1007/s10658-012-0151-x
  34. Parry, D. W., Jenkinson, P., and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grains—a review. *Plant Pathol.* 44:207-238.
  35. Paul, P. A., Lipps, P. E., De Wolf, E., Shaner, G., Buechley, G., Adhikari, T., Ali, S., Stein, J., Osborne, L., and Madden, L. V. 2007. A distributed lag analysis of the relationship between *Gibberella zeae* inoculum density on wheat spikes and weather variables. *Phytopathology* 97:1608-1624.
  36. Pirgozliev, S. R., Edwards, S. G., Hare, M. C., and Jenkinson, P. 2003. Strategies for the control of *Fusarium* head blight in cereals. *Eur. J. Plant Pathol.* 109:731-742.
  37. Pirgozliev, S. R., Ray, R. V., Edwards, S. G., Hare, M. C., and Jenkinson, P. 2008. Effect of timing of fungicide application on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in winter wheat grain. *Cereal Res. Commun.* 36:289-299.
  38. Schaafsma, A. W., Tamburic-Ilicic, L., and Hooker, D. C. 2005. Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of *Gibberella zeae*/*Fusarium graminearum*, *Fusarium* head blight severity, and deoxynivalenol accumulation in winter wheat. *Can. J. Plant Pathol.* 27:217-224.
  39. Shah, D. A., Molineros, J. E., Paul, P. A., Willyerd, K. T., Madden, L. V., and De Wolf, E. D. 2013. Predicting *Fusarium* head blight epidemics with weather-driven pre- and post-anthesis logistic regression models. *Phytopathology* 103:906-919.
  40. Silva, P. D. 2001. Efficient variable screening for multivariate analysis. *J. Multivar. Anal.* 76:35-62.
  41. Silva, P. D. 2002. Discarding variables in a principal component analysis: Algorithms for allsubsets comparisons. *Comput. Stat.* 17:251-271.
  42. Stephens, P. A., Buskirk, S. W., Hayward, G. D., and Martinez del Rio, C. 2005. Information theory and hypothesis testing: A call for pluralism. *J. Appl. Ecol.* 42:4-12.
  43. Steyerberg, E. W., Eijkemans, M. J. C., and Habbema, J. D. F. 1999. Stepwise selection in small data sets: A simulation study of bias in logistic regression analysis. *J. Clin. Epidemiol.* 52:935-942.
  44. Van Egmond, H. P., and Jonker, M. A. 2004. Worldwide regulations for mycotoxins in food and feed in 2003. *FAO Food Nutr. Pap.* 81. FAO, Rome.
  45. Whittingham, M. J., Stephens, P. A., Bradbury, R. B., and Freckleton, R. B. 2006. Why do we still use stepwise modelling in ecology and behaviour? *J. Anim. Ecol.* 75:1182-1189.
  46. Wintle, B. A., McCarthy, M. A., Volinsky, C. T., and Kavanagh, R. P. 2003. The use of Bayesian model averaging to better represent uncertainty in ecological models. *Conserv. Biol. Ser. (Cambridge)* 17:1579-1590.
  47. Xu, X.-M., Monger, W., Ritieni, A., and Nicholson, P. 2007. Effect of temperature and duration of wetness during initial infection periods on disease development, fungal biomass and mycotoxin concentrations on wheat inoculated with single, or combinations of, *Fusarium* species. *Plant Pathol.* 56:943-956.
  48. Xu, X.-M., and Nicholson, P. 2009. Community ecology of fungal pathogens causing wheat head blight. *Annu. Rev. Phytopathol.* 47:83-103.
  49. Xu, X.-M., Nicholson, P., Thomsett, M. A., Simpson, D., Cooke, B. M., Doohan, F. M., Brennan, J., Monaghan, S., Moretti, A., Mule, G., Homok, L., Beki, E., Tatnell, J., Ritieni, A., and Edwards, S. G. 2008. Relationship between the fungal complex causing *Fusarium* head blight of wheat and environmental conditions. *Phytopathology* 98:69-78.
  50. Yuan, Z., and Ghosh, D. 2008. Combining multiple biomarker models in logistic regression. *Biometrics* 64:431-439.