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ORIGINAL ARTICLE

Weather-based logistic models to estimate total fumonisin levels in maize kernels at export terminals in Argentina

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Abstract Maize fumonisin (FB) contamination is strongly driven by the weather conditions and crop resistance. Logistic regression techniques were used to quantify the effect of weather-based variables on total FB content in kernel samples coming from many locations of the Argentinean Pampas region (over three growing seasons), and collected immediately after arriving at export terminals. The samples were analyzed by the HPLC method and grouped according to their proximity to the available weather stations (n = 52). The highest correlations between binary and ordinal FB levels and weather variables were found in an early critical period (17 December to 15 January) where maize silking phase (Si) frequently occurs and in a late period (15 February to 2 April) around physiological maturity (PM). The best-fitted models included variables calculated around Si that would meet the requirements of infection of *F. verticillioides* (precipitation-induced wetness events, high humidity and warm temperatures). Around PM, the effect of the number of days with precipitation combined with lower temperatures (13.3° to 25 °C) that would slow the kernel drying process was included, increasing the FB accumulation. An integrated system for FB management in the maize value chain should use validated weather-based models as tools for estimating seasonal kernel FB contamination levels in the Pampas region, being able to improve kernel sampling efficiency at export terminals and mills.

Keywords *Fusarium verticillioides* · Logistic regression models · Mycotoxins

Introduction

Maize (*Zea mays* L.) is one of the major crops in Argentina, together with soybean and wheat. Maize yields have increased rapidly (128 kg $ha^{-1}y^{-1}$) over the last 20 years driven by a wide adoption of no-till systems, increasing amount of fertilizers, and sowing herbicide- and insect-resistant maize hybrids with high yield potential (Aramburu Merlos et al. 2015). Nevertheless, both maize yield and kernel quality are affected by pre-harvest infection of toxigenic fungi, producing losses to farmers and processors. In Argentina, *Fusarium verticillioides* causes Fusarium ear rot in maize and also produces kernel contamination, with fumonisins (FB) the most prominent mycotoxins (Chulze et al. 1996; Presello et al. 2008). High genotypic correlations exist between Fusarium ear rot and FB concentrations in kernel (Presello et al. 2011). This mycotoxin is a probable human carcinogen, associated with increased incidence of esophageal cancers in South Africa and China (IARC 2002). *F. verticillioides* is ubiquitous in nature. Exposed silks are the main pathway for this fungus to naturally enter into the ear and reach the kernels, although kernel wounds made by insects or other biotic or abiotic agents could also favour kernel infection. Disease transmission from infected stalks seems to be less important than the other two pathways (Munkvold et al. 1997).

F. verticillioides growth and FB accumulation in maize have been found to depend on several interacting factors,

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mainly weather conditions during the crop growing season and varietal resistance to contamination (Santiago et al. 2015). One way to reduce FB levels in kernel is to prevent fungal infections in the field by using less susceptible host plant genotypes (Iglesias et al. 2010). Based on published reports, Munkvold (2003) concluded that available maize hybrids with transgenic insect protection (Bt hybrids) have a lower risk for FB contamination than conventional hybrids. Folcher et al. (2010) showed that Bt maize decreased concentrations of FB by 90% based on analyses of maize fields in Southwestern France. Maize cultivar characteristics, such us precocity, husk coverage, silks duration, or the chemical composition of the kernels may influence fungal infection and subsequent FB production (Santiago et al. 2015). In this regard, Butron et al. (2006) noted differences among maize genotypes in FB contamination that could be related to husk tightness and exposition of ear tips. Failure of the husk to protect the tip permits easier entry of insects and consequent fungal contamination according to Warfield and Davis (1996). Many studies showed that the application of appropriate cultural practices associated to sowing and harvest date, fertilization, irrigation, insecticide and fungicide treatments (Parsons and Munkvold 2010; De Curtis et al. 2011) could affect both fungal infection and FB production. Compared to the application of single practices, Blandino et al. (2009) found that the implementation of an integrated field programme involving factors such as plant maturation, nutrition, and insect control, has proved to lead to a more effective and constant reduction in FB contaminations.

FB biosynthesis by *F. verticillioides* is mainly associated with abiotic factors like water activity (aw) and temperature. Based on in vitro studies, higher water availability resulted in higher FB production and higher fungal growth. For *F. verticillioides*, and temperatures between 20 °C and 30 °C and aw in the range 0.97–0.98 were the optimal conditions for FB production (Marin et al. 1999and Samapundo et al. 2005). Under field conditions, Shelby et al. (1994) found FB contents in maize hybrid kernels inversely related to June rainfall in different regions of the United States. Similarly, other studies observed greater FB contaminations associated to warmer or drier years (Abbas et al. 2002; Abbas et al. 2006; Goertz et al. 2010). Since drought stress results in greater insect herbivory on maize (Miller 2001), it is difficult to separate the effect of both factors. Actually, the relationship between dryness-temperature and kernel contamination with FB varies in different environments. Ono et al. (1999) found higher FB levels in maize samples from the Northern and Central-Western regions of the State of Paraná (Brazil) compared to those from the South. This could be explained by the differences in precipitation levels during the month preceding harvest (92.8 mm in South, 202 mm in North). In Poland, Pascale et al. (2002) recorded the highest FB contamination in three seasons (over seven) that were characterized by the highest temperatures at pollination, but not necessarily by the lowest precipitations. In Arkansas, Abbas et al. (2007) could not explain lower FB contamination levels in maize kernels (mid-April planting date) by any measure of heat stress during the kernel-filling period. In a three-year evaluation in northern Italy, Maiorano et al. (2009a) found a lower amount of FB in maize kernels in the year with the driest and warmest conditions during flowering and ripening months, while higher contamination occurred in the year with higher rainfall during the same period. In northeastern Spain, Herrera et al. (2010) related warm temperature during maize flowering and wet weather in the last pre-harvest months, with higher kernel FB concentrations. In Argentina, in a 2-year field study, higher FB_1 contents in maize kernels were obtained during the growing season in which rainfall was higher from flowering to maturity (Pereira et al. 2010).

Because environmental conditions are the driving force of both fungal growth and FB accumulation, mechanistic or empirical weather-based models were developed to evaluate the risk of exceeding thresholds in kernel mycotoxin contents. Maiorano et al. (2009b) developed a mechanistic risk assessment model of FB production in maize kernels. The model not only considers the effect of the weather conditions at silking and head insect damage, but also simulates the growth of *F. verticillioides* and FB synthesis during grain maturation taking into account its weather (temperature) and physical (aw) conditions. De la Campa et al. (2005) adjusted multiple regression models for predicting FB contamination in maize kernels at harvest, based on simple weather variables (days with maximum temperatures >34 °C, minimum temperatures <15 °C and rainfall >2 mm) and their interactions calculated around silking. In Argentina, Martínez et al. (2010) developed ordinal response logistic regression models for predicting maize kernel contamination with FB_1 and FB_2 at harvest. The best predictor was associated with combined occurrence of precipitation and high air humidity around silking. In Spain, using factorial regression techniques, Cao et al. (2014) found that wetter and cooler conditions around silking limited *F. verticillioides* infection and growth, and high temperatures (> = 30 °C) increased FB contents. During the kernel drying critical period, an increase in damaged kernels favored fungal growth, and higher ear damage by maize borers and days with rainfall > = 2 mm favored FB concentration.

The aim of the present research was to develop logistic regression models based on weather variables calculated in pre-harvest maize critical periods, in order to estimate levels of maize kernel contaminated with FB at export terminals of the Argentinean Pampas region.

Materials and methods

Total fumonisin (FB) content (µg/kg) in maize kernel samples

Maize kernel samples (*n* = 300) were collected from trucks immediately after arriving at export terminals in Argentina during three growing seasons (2004/2005–2006/2007). Once the geographic origin was identified, each truck was sampled separately by a cylindrical sampler, which was inserted in five points, one at each corner of the truck and the other in the center. From these primary samples, a composite sample (up to three kg) was formed, placed in a plastic bag, sealed and identified. In laboratory, using a Waters 2695 Alliance HPLC equipment with a quaternary pump, autosampler and fluorometric detector (Waters 2475), final one kg samples were analyzed for total fumonisin (FB) content according to AOAC Official Method 995.15(2005) (Sydenham et al. 1996). The sample volume injected was 20 µl. A reverse-phase XBridge (Waters) C18 3.5 µm 3.0x100mm column protected with a guard precolumn packed with the same phase was used for the analysis. The mobile phase was solvent A (acetic acid 1% in acetonitrile) and solvent B (water, acetonitrile, acetic acid; 59:40:1) with a flow rate of 0.3 ml/min. Fumonisin-OPA derivatives were performed by a pre-column reaction and fluorescence was recorded at excitation and emission wavelengths of 335 and 440 nm.

Total kernel samples (already analyzed for FB contamination) were grouped according to their proximity to the available weather stations and the median of the FB contamination values per group was calculated (*n* = 52). From this original data set, 42 median FB concentration values (80%) were binary coded as 0 (FB < 594 µg/kg; L: moderate to light level) or 1 (FB > =594 µg/kg; S: Moderate to Severe level) and ordinally coded as 0 (FB < 467 µg/kg; L: light level), 1 (FB > =467 *µ*g/kg and <816 *µ*g/kg; M: moderate) or 2 (FB > =816 µg/kg; S: severe). Such thresholds were defined following a statistical criterion and corresponded to 60% (FB = 816 µg/kg), 50% (FB = 594 *µ*g/kg and 45% (FB = 467 *µ*g/kg) percentiles regarding the 42 kernel FB contents.

Explanatory weather variables

Readings of daily maximum (MxT; °C) and minimum (MnT; °C) temperatures, precipitation (Prec; mm) and relative humidity (RH; %) were collected from meteorological stations of the National Weather Service (SMN) and the National Institute of Agricultural Technology (INTA). Daily average temperature (Td) was calculated as half the sum of MxT and MnT. Based on these daily weather data, a series of secondary weather variables were calculated in an early critical period including the silking phenological phase (Si: Silking critical period) (December to mid-January) and in a late critical period including the end of grain filling and kernel drying (PM: Physiological maturity critical period) (mid-February to early April) and are described in Table 1.

Statistical analysis

The temperature and moisture thresholds of the described weather variables and the critical period lengths were defined by iteratively maximizing their correlations with FB contamination levels. A computer program using SAS (Statistical Analysis Systems, version 8.0; SAS Institute, Inc., Cary, NC, USA) was written to calculate the different weather variables and to perform the iterative process (details of this preliminary analysis were not presented). The Freq procedure in SAS was used to calculate Kendall Tau-b nonparametric correlation coefficients (r_k) between binary (S and L) and ordinal (S, M and L) FB contamination levels and weather variables. Using the SAS Logistic procedure, weather-based logistic regression models were fit (maximum likelihood method) to estimate the probabilities of occurrence of the binary and ordinal mycotoxin contamination levels. Each logistic model fit one equation for estimating $\ln[pS/(1\text{-}pS)] = \beta_0 + \beta_1 X$, where pS is the probability of observing a severe (S) FB contamination level, β_0 and β_1 are the parameters estimators and X is a weather predictor. The logit function [ln(pS/(1-pS)], where ln is the natural logarithm, establishes the connection between the stochastic component and weather variables. The probability pS was obtained by solving: $\text{Exp}\{\ln[pS/(1\text{-}pS)]\}/\{1 + \text{Exp}\{\ln[pS/(1\text{-}pS)]\}\}$. For binary response data, the probability of having a moderate to light FB contamination level (pL) results from subtracting pS from 1. For final model assessment, the critical Pc value (probability value to classify a case as severe FB contamination that provides the most accurate prediction) was taken into consideration.

For ordinal response data, one of the assumptions underlying ordinal logistic regression is that the relationship between each pair of outcome categories is the same. A chi-squared score statistic was calculated to test the parallel regression assumption. Besides fitting $\ln[pS/(1\text{-}pS)] = \beta_0 + \beta_1 X$, the model fits the equation $\ln[pMc/(1\text{-}pMc)] = \beta_0 + \beta_1 X$, where pMc is the cumulative probability of reaching a FB contamination level = > to moderate (M). Solving the expression $\text{Exp}\{\ln[pMc/(1\text{-}pMc)]\}/\{1 + \text{Exp}\{\ln[pMc/(1\text{-}pMc)]\}\}$, pMc is obtained. The probabilities of observing a moderate (M) and light (L) FB contamination levels result from the next two differences: pM = pMc-pS and pL = 1-(pS + pM). The probability function that gave the highest probability value was considered to be the forecasted FB contamination level (S, M or L) for that growing season/site analyzed.

Stepwise logistic regression was used to select the most appropriate weather-based model (significance level of 0.01 was used as entry (SLE) and retention (SLS) criteria for the

Table 1 Definition of weather variables calculated in the silking critical period (from December 17th to January 15th) and physiological maturity critical period (from February 15th to April 2nd)

Acronym	Period/ Description of the weather variables[a]
Si	***Silking critical period***
DT	Days with MxT < 32.9 °C and MnT > 18 °C
DDMxT	Sum of the exceeding amounts of daily MxT from 32.9 °C when MxT > =32.9 °C
DDMnT	Accumulated differences between 18 °C and MnT when daily MnT < =18 °C
MMnT	Mean of daily MnT
MMxT	Mean of daily MxT
DPrec	Days with precipitations (Prec > 0.2 mm)
TPrec	Total accumulated millimeters of daily Prec > 0.2 mm
DPrecT	Total days with simultaneous occurrence of Prec > 0.2 mm, and MxT < 32.9 °C and MnT > 18 °C
TPrecT	Total accumulated millimeters of daily Prec > 0.2 mm, when MxT < 32.9 °C and MnT > 18 °C
DPrecTRH	Days with occurrence of Prec > 0.2 mm and RH > =70, when daily MxT < 32.9 °C and MnT > 18 °C
PPrecTRH	Total two day periods with simultaneous occurrence of Prec > 0.2 mm, RH > =70%, and MxT < 32.9 °C and MnT > 18 °C in the first day and RH > 65% in the second day
Tot	Sum of DPrecTRH and PPrecTRH
DRH	Total days with RH > =70%
DryD	Days without precipitations (Prec < =0.2 mm)
$\mathrm{Int1}_{\mathrm{Si}}$	DryD * DPrecT
$\mathrm{Int2}_{\mathrm{Si}}$	DryD * DPrecTRH
$\mathrm{Int3}_{\mathrm{Si}}$	DryD * PPrecTRH
PM	***Physiological maturity critical period (PM)***
DT	Days with MxT < 25 °C and MnT > 13.3 °C
DDMxT	Sum of the exceeding amounts of daily MxT from 28.5 °C when MxT > 28.5 °C
DDMnT	Accumulated differences between 20 °C and MnT when daily MinT < =20 °C
MMnT	Mean of daily MnT
MMxT	Mean of daily MxT
DPrec	Days with precipitations (Prec > 0.2 mm)
DPrecT	Total days with simultaneous occurrence of Prec > 1 mm, and MxT < 25 °C and MnT > 13.3 °C
TPrecT	Total accumulated millimeters of daily Prec > 1 mm, when MxT < 25 °C and MnT > 13.3 °C
DPrecTHR	Days with occurrence of Prec > 0.2 mm and RH > =81, when daily MxT < 25 °C and MnT > 13.3 °C
PPrecTHR	Total two day periods with simultaneous occurrence of Prec > 0.2 mm, RH > =81%, and MxT < 25 °C and MnT > 13.3 °C in the first day and RH > 70% in the second day
Tot	Sum of DPrecTRH and PPrecTRH
DRH	Total days with RH > =70%
$\mathrm{Int1}_{\mathrm{Si\text{-}PM}}$	$\mathrm{Int1}_{\mathrm{Si}}$ * $\mathrm{DPrecT}_{\mathrm{PM}}$

[a] *Prec* precipitation, *MxT* maximum temperature, *MnT* minimum temperature, *RH* relative humidity

variables). The predictive ability of the models was calculated based on the number of pairs of observations (t) with different outcome category (551 pairs). A pair of input observations with different responses is said to be concordant (or discordant) if the larger ordered value of the response has a higher (or lower) predicted event probability than the smaller response. If the pair is neither concordant nor discordant, it is a tie. Somers'D and Gamma correlation indices were calculated from the number of concordant (nc) and discordant (nd) pairs of observations. The values of both indices range from −1.0 (all pairs disagree, no association) to 1.0 (all pairs agree, perfect association). The prediction accuracy of the models selected was also calculated as the percentage of cases analyzed (n = 42) in which there was agreement between the observed FB content level and that predicted by the logistic equations.

Validation Total FB contamination levels predicted by selected binary and ordinal response weather-based models were validated against observed FB levels. For this purpose, from the original data set (n = 52), ten median FB concentration values (20%) were used to validate the performance of the best-fitted logistic regression models.

Cross validation Cross validated probabilties were derived from the leave-one-out principle, dropping at each step one observation from the original data set ($n = 52$) and reestimating the parameters estimates of the best fitted binary and ordinal logistic regression models. Once the first observation was returned, the process was repeated for all the other data points. Accuracies of the regression models for predicting the response probabilities of each of these n (52) deleted observations were calculated.

Results

The highest Kendall *Tau-b* correlation coefficients (r_K) between binary and ordinal kernel FB contamination levels and weather variables were found when these variables were calculated in early and late critical periods extended from December 17th to January 15th (Si critical period) and from February 15th to April 2nd (PM critical period), respectively (Table 2). For the Si critical period, the variable $DPrecTRH_{Si}$ reached the maximum r_K values for binary ($r_K = 0.66$) and ordinal ($rk = 0.62$) response data. $DPrec_{Si}$, which only considers the effect of daily precipitations exceeding 0.2 mm on FB contamination, showed much lower and negative r_K correlations ($r_K = -0.12$ for both response data). According to Table 2, among the temperature-related variables, DT_{Si} was the most highly correlated with FB concentrations levels ($r_K = 0.60$ and 0.47 for binary and ordinal response data, respectively). During the PM critical period, the variable $DPrecT_{PM}$ (number of days with precipitation combined with temperatures between 13.3° and 25 °C) showed the highest correlations ($r_K = 0.62$ and $r_K = 0.65$ for both binary and ordinal response data respectively) with kernel FB contamination at harvest. The product between the interaction term $Int1_{Si}$ (product between $DryD_{Si}$: days with $Prec < =0.2$ mm and $DPrecT_{Si}$: total days with simultaneous occurrence of $Prec > 0.2$ mm, and $MxT < 32.9$ °C and $MnT > 18$ °C) and the simple variable $DPrecT_{PM}$ increased the correlation with the FB content levels ($Int1_{Si\text{-}PM,}$ $r_k = 0.68$ and 0.70 for binary and ordinal response data respectively).

Including all the explanatory weather variables (simple and interaction terms), the stepwise logistic regression selected model I (Table 3) as the most adequate binary response model. This model only included the effect of the interacction term $Int1_{Si\text{-}PM}$, which classified correctly 39 out of 42 observed cases (prediction accuracy = 92.9%). Two of the three misclassified cases were overestimated (observed as a moderate to light FB level and predicted as

Table 2 Kendall Tau-b correlation coefficients (r_k) between binary-ordinal levels of fumonisin (FB) contamination of kernels and weather variables calculated at two different maize critical periods (Si: Silking critical period and PM: Physiological Maturity critical period)

Weather Variables[a]	Kendall Tau-b correlation coefficients (r_k)[b]			
	Maize critical period			
	Silking (Si)		Physiological Maturity (PM)	
	Binary response	Ordinal response	Binary response	Ordinal response
DT	0.60	0.47	0.54	0.50
DDMxT	0.07	0.06	−0.11	−0.14
DDMnT	−0.56	−0.46	−0.55	−0.44
MMxT	0.00	−0.02	−0.02	−0.07
MMnT	0.57	0.47	0.54	0.43
DPrec	−0.12	−0.12	0.35	0.44
TPrec	0.17	0.13		
DPrecT	0.60	0.60	0.62	0.65
TPrecT	0.38	0.40	0.39	0.43
DPrecTRH	0.66	0.62	0.53	0.56
PPrecTRH	0.65	0.60	0.55	0.59
Tot	0.64	0.60	0.52	0.56
DRH	0.33	0.25	0.49	0.46
DryD	0.10	0.08		
$Int1_{si}$	0.62	0.60		
$Int2_{si}$	0.65	0.62		
$Int3_{si}$	0.64	0.60		

[a] Definitions in Table 1

[b] **Si-PM: $\mathbf{Int1_{Si\text{-}PM:}}$** $Int1_{Si}$ * $DPrecT_{PM,}$ $\mathbf{r_k} = 0.68$ and 0.70 for binary and ordinal response data respectively

Table 3 Parameter estimates of the binary logistic regression models for estimating the probability of occurrence of each level of fumonisin (FB) contamination of kernels: severe (S) and moderate to light (ML), based on simple and interactive weather variables calculated at maize Silking (Si) and Physiological Maturity (PM) critical periods

Model[a]	Variable[b]	Parameter estimate	P[c]	Predictive ability		
				Prediction Accuracy (%)	Somers'D	Gamma
I	Intercept S	−4.5431	0.57	92.9 (39/42*100)	0.943	0.945
	Int1 $_{\text{Si-PM}}$	0.02				
II	Intercept S	−10.0729	0.55	92.9 (39/42*100)	0.943	0.950
	DPrectT $_{\text{Si}}$	1.8676				
	DPrecT$_{\text{PM}}$	1.2020				
III	Intercept S	−10.4813	0.50	95.2 (40/42*100)	0.950	0.950
	Int1 $_{\text{Si}}$	0.1017				
	DPrecT$_{\text{PM}}$	1.0832				
IV	Intercept S	−15.2097	0.55	97.6 (41/42*100)	0.975	0.977
	DPrecTRH$_{\text{Si}}$	3.5190				
	DPrecT$_{\text{PM}}$	1.9771				

[a] Model I: concordant(%) = 97.1, discordant (%)2.7, tied (%) = 0.2; Model II: concordant (%) = 96.8, discordant (%) 2.5, tied (%) = 0.7; Model III: concordant(%) = 97.5, discordant (%)2.5, tied (%) = 0; Model IV: concordant (%) = 98.6, discordant (%) 1.1, tied (%) = 0.2. LogitPrS = ln(PrS/1-PrS). Solving the expression Exp(LogitPrS)/(1 + Exp(LogitPrS)), PrS values (probability of observing a severe FB content level (S) are obtained. Ln: natural logarithm. PrML = 1-PrS, being PrML the probability of observing a moderate to light FB content level (ML)

[b] *Prec* precipitation, *MaxT* maximum temperature, *MinT* minimum temperature, *RH* relative humidity. Si: Silking critical period: DryD$_{\text{Si}}$: days with Prec < =0.2 mm; DPrecT$_{\text{Si}}$: total days with simultaneous occurrence of Prec > 0.2 mm, and MxT < 32.9 °C and MnT > 18 °C; DPrecTRH$_{\text{Si}}$: days with simultaneous occurrence of Prec > 0.2 mm and RH > =70, when daily MxT < 32.9 °C and MnT > 18 °C (DPrecTRH $_{\text{Si}}$); Int1$_{\text{S}}$ = DryD$_{\text{Si}}$ * DPrecT$_{\text{Si}}$; PM: Physiological Maturity critical period: DPrecT$_{\text{PM}}$: total days with simultaneous occurrence of Prec > 1 mm, and MxT < 25 °C and MnT > 13.3 °C. Int1$_{\text{Si-PM}}$: Int1$_{\text{Si}}$ * DPrecT$_{\text{PM}}$

[c] Probability value for classifying a FB content level as severe (S) that reaches the greatest prediction accuracy

moderate to severe FB contamination), whereas the other case was observed as moderate to severe FB level and predicted as moderate to light. The number of concordant pairs was very high (97.1%). Accordingly, values of Somers'D (0.943) and Gamma (0.945) correlation indices were also high. Nevertheless, the best predictive ability indices were presented by the models III (included the variables Int1$_{\text{Si}}$ and DPrecT$_{\text{PM}}$) and IV (DPrecTRH$_{\text{Si}}$ and DPrecT$_{\text{PM}}$), which misclassified only two and one observed case, respectively (Table 3).

Model V (Table 4) which included the interaction term Int1$_{\text{Si-PM}}$ resulted the most adequate stepwise ordinal response regression model and was also appropriate for the data according to the chi-squared test (chi-square = 1.8247 with one degree of freedom, Pr > chi-square = 0.1768). Both models V and VI (which included DPrecT$_{\text{Si}}$ and DPrecT$_{\text{PM}}$ as weather-driven variables) misclassified five out of 42 observed FB concentration levels (prediction accuracy = 88.1%). Model VI, which overestimated three cases out of five misclassified cases, showed higher predictive ability indices such as the number of concordant pairs (93.8%) and both Somers'D (0.886) and Gamma (0.894) correlation indices. Squared score test for the parallel line assumption indicated that the model VI was also appropriate for the data (chi-square = 4.1734 with two degrees of freedom, Pr > chi-square = 0.1241).

Validation

According to Table 5, eight out of ten observed FB contamination levels (samples collected at export terminal from ten site/growing season combinations) agreed with those predicted by binary response logistic regression models (models III and IV). For ordinal response data, only one case (site of origin: Bolívar; growing season: 2006/2007) was misclassified by both models V and VI.

Cross validation (leave-one-out principle)

Using this procedure to the original data set ($n = 52$), both binary response models III and IV (Table 3) misclassifed six (prediction accuracy = 88.5%) and five (prediction accuracy = 90.4%) out of 52 FB content levels, respectively. Both ordinal response regression models V and VI (Table 4) classified correctly 46 (prediction accuracy = 88.5%) and 45 (prediction accuracy = 86.5%) out of 52 FB contamination levels, respectively.

Discussion

Logistic regression has been widely used for developing models to predict levels of crop fungal diseases and associated mycotoxins. For example, De Wolf et al. (2003)

Table 4 Parameter estimates of ordinal logistic regression models for estimating the probability of occurrence of each level of fumonisin (FB) contamination of kernels: severe (S), moderate (M) and light (L), based on simple and interactive weather variables calculated at maize silking (**Si**) and physiological maturity (**PM**) critical periods

Model[a]	Variable[b]	Parameter estimate	Predictive ability		
			Prediction accuracy (%)	Somer's D	Gamma
V	Intercept S	−5.548	88.1	0.869	0.876
	Intercept SM	−3.2171	(37/42*100)		
	Int1$_{\mathrm{Si\text{-}PM}}$	0.0165			
VI	Intercept S	−9.0506	88.1	0.886	0.894
	Intercept SM	−6.762	(37/42*100)		
	DPrecT$_{\mathrm{Si}}$	1.3652			
	DPrecT$_{\mathrm{PM}}$	0.9116			

[a] Model V: concordant(%) = 93.1, discordant (%) 6.2, tied (%) = 0.7; Model VI: concordant (%) = 93.8, discordant (%) 5.3, tied (%) = 0.9. LogitPrS = ln(PrS/1-PrS); LogitPrSM = ln(PrSM/1-PrSM). Solving the expressions Exp (LogitPrS)/ (1 + Exp (LogitPrS)) and Exp(LogitPrSM)/(1 + Exp (LogitPrMS)), PrS values (probability of observing a severe FB content level (S) and PrSM (cumulative probability of a FB content level = > to moderate (M)) are obtained. Ln: natural logarithm. PrM = PrSM-PrS. PrL = 1-(PrS + PrM), being PrL the probability of observing a light to nil FB content level (L)

[b] Prec: precipitation, MxT: maximum temperature, MnT: minimum temperature; RH: relative humidity. **Si: silking critical period**: DryD$_{\mathrm{Si}}$: days with Prec < =0.2 mm; DPrecT$_{\mathrm{Si}}$: total days with simultaneous occurrence of Prec > 0.2 mm, and MxT < 32.9 °C and MnT > 18 °C; Int1$_{\mathrm{Si}}$ = DryD$_{\mathrm{Si}}$ * DPrecT$_{\mathrm{Si}}$; **PM: physiological maturity critical period**: DPrecT$_{\mathrm{PM}}$: total days with simultaneous occurrence of Prec > 1 mm, and MxT < 25 °C and MnT > 13.3 °C. Int1$_{\mathrm{Si\text{-}PM}}$: Int1$_{\mathrm{Si}}$ * DPrecT$_{\mathrm{PM}}$

used this technique to model binary epidemic levels of *Fusarium* head blight in wheat. In Italy, Battilani et al. (2008) used logistic regression techniques to predict FB contamination in maize by integrating information on previous crop, hybrid type, nitrogen fertilization, planting and harvesting dates and grain moisture content. Martínez et al. (2010) developed meteorological-based logistic regression models to estimate the probability of occurrence of ordinal levels of FB$_1$ and FB$_2$ in maize kernels at harvest. Similarly, in this study we also used logistic regression techniques to quantify the effect of weather variables on FB contamination in maize kernel samples coming from many locations of the Argentinean Pampas region, and collected immediately after arriving at export terminals.

In the current study, the highest correlations (r$_k$: Kendall Tau-b correlation coefficient) between binary-ordinal levels of

Table 5 Comparison between total fumonisin (FB) contamination levels observed in ten maize kernel samples collected from trucks arriving at export terminals and those predicted by binary and ordinal response weather-based models (Tables 3 and 4). Sites of origin of the maize kernels and the corresponding growing season are also specified

Site	Growing season	Observed FB level[a]		Predicted FB level			
		Binary	Ordinal	Binary response Model[b]		Ordinal response Model[c]	
				III	IV	V	VI
Laboulaye	2004/2005	L	L	Yes	Yes	Yes	Yes
Pergamino	2004/2005	L	L	Yes	No	Yes	Yes
Córdoba	2005/2006	S	S	Yes	Yes	Yes	Yes
Pehuajó	2005/2006	L	L	Yes	Yes	Yes	Yes
Río Cuarto	2005/2006	L	M	No	Yes	Yes	Yes
Tandil	2005/2006	L	L	Yes	Yes	Yes	Yes
Balcarce	2006/2007	S	M	Yes	Yes	Yes	Yes
Bolívar	2006/2007	S	S	No	No	No	No
Río Cuarto	2006/2007	S	S	Yes	Yes	Yes	Yes
Venado Tuerto	2006/2007	S	S	Yes	Yes	Yes	Yes

[a] FB binary levels: L: moderate to light: FB < 594 μg/Kg or S: moderate to severe: (FB > =594 μg/Kg; S: severe). FB ordinal levels L:light: (FB < 467 μg/Kg, 1; M: moderate: FB > =467 μg/Kg and <816 μg/Kg;) or S: severe: FB > =816 μg/Kg. Yes: agrees with the observed level; No: does not agree

[b] See Table 3

[c] See Table 4

FB contamination in kernels and weather-driven variables were found when those variables were calculated in an early pre-harvest critical period (17th December to 15th January) where maize silking phase (Si) (R1 phase according to Ritchie and Hanway 1982) frequently occurs and in a late critical period (15th February to 2nd April) around physiological maturity (PM) date. Coincidently, Cao et al. (2014) reported that flowering and kernel drying were the most critical periods throughout the maize growing season for *F. verticillioides* infection and FB contamination. De la Campa et al. (2005) and Martínez et al. (2010) found weather variables strongly associated with maize kernel FB contamination only on 24 and 17-day critical periods around silking, respectively.

According to our results, for the Si critical period, the variable $DPrecTRH_{Si}$ (days with simultaneous occurrence of Prec > 0.2 mm and RH > =70, when daily MxT < 32.9 °C and MnT > 18 °C) reached the maximum r_K value and would appear to have a direct effect on fungus infection and consequent kernel FB contamination. The variable $DPrecTRH_{Si}$ would meet the requirements for the infection of *F. verticillioides* (precipitation-induced wetness events, high humidity and warm temperatures). In this regard, Maiorano et al. (2009b) reported that precipitation during flowering can be favourable for FB contamination, but only when daily precipitation intensity is lower than 2 mm/h. Precipitation and humidity seem to be favourable for kernel infection by *F. verticillioides* and consequent contamination with FB, but hard precipitation could limit spore dispersal and wash off inoculum reservoirs counteracting the positive effect of wetness (Rossi et al. 2009). According to our results, the total accumulated precipitations (TPrec) during the Si critical period showed a weak correlation with FB contamination (r_K = 0.17 and 0.13 for binary and ordinal response data respectively).

Under field conditions, many authors observed greater fungus infection and consequent FB contaminations associated with warmer or drier years (Shelby et al. 1994; Abbas et al. 2002; Abbas et al. 2006; Goertz et al. 2010). In agreement, in our study the variable $DPrec_{Si}$, which counts the total days with precipitations exceeding 0.2 mm on silking critical period, showed a low and negative r_K correlation ($r_K = -0.12$ for both binary and ordinal response data). Even though De la Campa et al. (2005) and Cao et al. (2014) found similar results, it is worth clarifying the controversy regarding the role of the precipitation frequency on *F. verticillioides* infection. This fungus, as every necrotrophic pathogen, requires long silk precipitation-induced wetness duration for infection, but not all days with precipitations can lead to that. In the current study, temperature and precipitation variables such as $DPrec_{Si}$ and DT_{Si} improved their predictive ability when both effects were combined and integrated with high air relative humidity records, as expressed by $DPrecT_{Si}$ and $DPrecTRH_{Si}$. From our results, the occurrence of periods of warm weather with persistent silk wetness was the key factor for maize ear rot epidemics. Martínez et al. (2010) found that levels of FB1 and FB2 in maize kernels at harvest were explained by the high frequency around silking of two day periods with occurrence of precipitation and high humidity. This combination of factors would be acting on fungal infection and would explain the different levels of toxin in kernels. According to Moschini and Fortugno (1996) the length of the wetness period on wheat heads following a precipitation depends on air evaporative demand. Low levels of air relative humidity lead to a greater demand of atmospheric water vapor, producing shorter wetness periods. During the PM critical period, the increase of precipitation days combined with lower temperatures (13.3° to 25 °C) (expressed by $DPrecT_{PM}$) would slow the kernel drying process. Precipitation probably maintains moisture conditions conducive to FB production inside the kernels because kernel drying is slowed down. Consequently, a high kernel water activity would remain for more time (Marin et al. 1999; Cao et al. 2014), stimulating the FB synthesis by the fungus already inside the kernels.

Some authors have pointed out that drought stress in maize during flowering increases susceptibility to fungal infection and insect attack (Miller 2001; Parsons and Munkvold 2010), increasing the probability of FB contamination. In our study, despite the fact that the frequency of dry days around silking ($DryD_{si}$) was poorly associated to kernel FB content (r_k = 0.10 and 0.08 for binary and ordinal response data respectively), the variable was included as component of an interaction term ($\mathbf{Int1_{Si}} = DryD_{si} * DPrecT_{Si}$) in the binary-ordinal logistic regression models selected by stepwise procedure.

Other authors (Butron et al. 2006; Nielsen 2012) found that husk length and ear husk tighness would be affected with the occurrence of extreme drought around silking. In theses cases, our hypothesis is that a new pathway for the fungus to naturally enter into the ear would make available during the PM critical period. Moschini et al. (2017), analyzing FB concentration data of kernel samples from a maize hybrid susceptible to ear rot, related very high kernel FB contaminations to the occurrence of extreme drought around Si phase and favorable conditions for infection/synthesis of FB around PM. These cases contradicted the general tendency expected around silking and were removed from the modelling process.

Because the method of collecting maize kernel samples from trucks immediately after arriving at export terminals, agronomical data like maize planting date, genotype type, phenological observations and insect damage, were not available. Data about the site of origin of maize kernel samples (*N* = 300) for the three growing seasons and daily meteorological records from near weather stations were only available for this study. It is worth pointing out that the total kernel samples were grouped according to their proximity to available meteorological stations, being finally available 52 median FB content values for the modelling process (42 FB values for model development and 10 for validation purposes). Even though the validation data set represented the 20% of the total original data set, its size was not big enough for making a robust validation of the fitted models. Using the cross validation (leave one out principle) procedure to the original data set (*n* = 52) for the best fitted binary and ordinal regression models, prediction accuracies values did not differ significantly from those obtained by the models adjusted to the 42 FB content values.

Both binary and ordinal models IV (included $\text{DPrecTRH}_{\text{Si}}$ and $\text{DPrecT}_{\text{PM}}$ as weather-based variables) and VI (included $\text{DPrecT}_{\text{Si}}$ and $\text{DPrecT}_{\text{PM}}$ as weather-driven variables) showed the highest predictive ability indices and the best performance when their predictions were contrasted with independent kernel FB contamination data. The variables calculated around Si ($\text{DPrecTRH}_{\text{Si}}$ and $\text{DPrecT}_{\text{Si}}$) would meet the requirements of infection of *F. verticillioides* (precipitation-induced wetness events, high humidity and warm temperatures). The increase in the number of days with precipitation combined with lower temperatures (13.3° to 25 °C) during the late PM window, and expressed by $\text{DPrecT}_{\text{PM}}$, would slow the kernel drying process. An integrated system for FB management in maize value chain should include the use of these validated weather-based models as tools for estimating seasonally kernel FB contamination levels on the Pampas region, being able to improve kernel sampling efficiency at export terminals and mills.

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