A neural network model for prediction of deoxynivalenol content in wheat grain based on weather data and preceding crop

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ABSTRACT

Deoxynivalenol (DON) is the most prevalent *Fusarium* toxin in Czech wheat samples and therefore forecasting this mycotoxin is a potentially useful tool to prevent it from entering into food chain. The data about DON content in wheat grain, weather conditions during the growing season and cultivation practices from two field experiments conducted in 2002–2005 were used for the development of neural network model designed for DON content prediction. The winning neural network is based on five input variables: a categorial variable – preceding crop, and continuous variables – average April temperature, sum of April precipitation, average temperature 5 days prior to anthesis, sum of precipitation 5 days prior to anthesis. The most important input parameters are the preceding crop and sum of precipitation 5 days prior to anthesis. The weather conditions in April, which are important for inoculum formation on crop debris are also of important contribution to the model. The weather conditions during May and 5 days after anthesis play only an insignificant role for the DON content in grain. The effect of soil cultivation was found inferior for model function as well. The correlation between observed and predicted data using the neural network model reached the coefficient R^2 = 0.87.

Keywords: Fusarium head blight; mycotoxins; forecast; crop rotation; soil cultivation; temperature; rainfall; epidemiology

Importance of Fusarium head blight

Epidemics of Fusarium head blight (FHB) result in severe losses through direct reduction in grain yield and increased grain cleaning costs (McMullen et al. 1997). Mycotoxins caused by the disease often render the grain unfit for human consumption. The most important *Fusarium* mycotoxin worldwide is the trichothecene deoxynivalenol (DON) (Parry et al. 1995), which is formed by species such as *F. graminearum* Schw. and *F. culmorum* (W.G. Smith) Sacc. (Abramson et al. 1993). The maximum level set for DON in unprocessed cereals is 1250 μg/kg (European Commission 2005).

Host and environmental factors influence the growth, survival, dissemination and hence the incidence of *Fusarium* fungi and the disease severity. McMullen et al. (1997) concluded that while there is still very much to be learned about FHB, the effect of the climate plays a significant role. In wheat, environmental effects accounted for 48% of the variation in DON content, followed by variety (27%) and preceding crop (14 to 28%) (Schaafsma et al. 2005).

Two key periods for FHB infection could be indicated during the spring vegetation. The first one is important for the production and dispersal of asexual conidia and sexual ascospores of *F. graminearum*, and includes the early spring period up to anthesis initiation. The second one is much shorter and covers the time of flowering. This period is important for spike infection. In both cases, higher temperatures and higher relative humidity or rainfall are favourable for final disease severity.

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Production of inoculum

Gibberella zeae (teleomorph of *F. graminearum*) is a facultative saprophyte with an important part of its cycle occurring in crop residue, which serves as the main reservoir for inoculum that leads to infection (Sutton 1982). Perithecia of *G. zeae* develop on aboveground residues, and on maize and wheat kernels, at temperatures between 15 and 25°C (Gilbert and Fernando 2004). Moisture levels may also influence the perithecia development (Sung and Cook 1981). High humidity is required for the initial release of ascospores, although dry periods may be required for their forceful discharge into the air from perithecia (Parry et al. 1995).

Infection process and mycotoxin production

The FHB is best known as a flowering disease with anthers as the primary infection site, where fungus spores land and then grow into the kernels, glumes or other spike tissues (Sutton 1982). Germination is influenced by water availability and temperature. FHB infection is favoured by extended periods (48 to 72 hours) at > 90% relative humidity with temperatures between 15 and 30°C (Bai and Shaner 1991).

In Germany, Obst et al. (1997) defined the critical conditions for high risk of this disease and concomitant production of DON: the wheat must be at 50% heading to the end of flowering, a mean temperature, for at least 24 hours, higher than 18°C, and rainfall must be greater than or equal to 5 mm on the day, or on the day just preceding it.

Mycotoxin production in grain depends mainly on both well defined ranges of temperature and water availability. The significance of these parameters is probably not entirely direct but rather a function of their influence on fungal growth. Koch et al. (2006) concluded that a reliable estimation of the DON concentration from FHB infection values is not possible without adaptation to complex factors such as year, site, cultivar etc.

Soil cultivation and crop rotation

Soil cultivation systems that leave most residues on the soil surface are expected to produce the greatest amount of inoculum and cause the highest severity of FHB. Regarding the DON concentration in wheat grain, the tillage system applied is less important compared to the influence of preceding crop and the susceptibility of the wheat cultivar (Dill-Macky and Jones 2000, Koch et al. 2006).

Krebs et al. (2000) examined the degree of contamination of harvested grain with *Fusarium* and the content of DON from field experiments with mouldboard plough, cultivator and no-tillage. The highest incidence of the disease and mycotoxin content was observed in the no-tillage system with maize or wheat as preceding crops. The effect of crop rotation on FHB infection was studied by Fernandez et al. (2001), but the results did not demonstrate differences in FHB severity due to rotations even when wheat follows maize as a part of the rotation. The report of a 3-year survey concluded that the principal factor in FHB development is the weather. These data confirm the importance of crop rotation and soil management to reduce the risk of contamination with *Fusarium* and DON under moist and warm weather conditions during the flowering period in wheat, but they have only marginal effect under disadvantageous weather conditions.

Prediction models

Several groups have tested prediction models based on meteorological observations to manage FHB (Hooker et al. 2002, De Wolf et al. 2003). The model used for FHB risk forecasting by Moschini and Fortugno (1996) reported how different combinations of temperature, relative humidity and precipitation could be used to predict FHB severity based on linear equations. De Wolf et al. (2003) developed logistic regression models using combinations of temperature, relative humidity and rainfall or durations of specified weather conditions, for 7 days prior to anthesis and 10 first days of crop anthesis, as potential predictor variables. Prediction accuracy of developed logistic regression models ranged from 62 to 85%. The most useful predictor variables were duration (h) of the precipitation 7 days prior to anthesis; time (h) when temperature was between 15 to 30°C and relative humidity was greater than or equal to 90%.

Hooker et al. (2002) based their model on empirical relationships between weather variables and concentrations of DON. An early prediction of DON in mature grain was developed using rain and temperature data from 4 to 7 days prior to heading. However, the full predictive model used data from three periods taken from 7 days before heading to 10 days after heading. Hooker et al. (2002) hypothesized that the concentration of DON in mature grain is closely associated with environmental factors influencing both inoculum production and infection in wheat at heading.

MATERIAL AND METHODS

Field trials

The grain samples from winter wheat field experiments, conducted in 2002–2005 in order to assess the effect of soil cultivation in combination with the effect of the preceding crop on FHB infection, were analyzed for the DON content. Three soil cultivation systems were used prior to sowing: tillage to the depth of 22 cm, shallow cultivation with discs (10 cm) and no-till sowing. The experiments were sown following two preceding crops: lucerne as a non-host crop, and maize as a host crop. In both experiments, sowing took place during the first 10 days of October. The plots were 20 m^2 and replicated four times. Each soil cultivation and preceding crop variant was separated by 3-m preventive strips to protect the plots from neighbouring primary infection source. Average daily weather data (temperature, rainfall, relative humidity) were collected using an automated weather station and analyzed separately for April, May and a 5-day period prior to the beginning of flowering, and 5 days after the beginning of flowering. The weather data were correlated to DON content in grain separately for each parameter. To analyze a multiple effect of several weather parameters on DON content, the artificial neural network was trained on the dataset using neural network procedure in Statistica 7 software. The neural networks were trained using back propagation learning algorithms, which use the data to adjust the weights and thresholds of the network so as to minimize the error in its predictions on the training set. The winning neural network was selected on the basis of minimized sum of square deviations between predicted and observed data.

Analytical method for DON determination

All the grain samples collected during the period 2002–2005 were analyzed for DON levels using the method of gas chromatography coupled with electron capture detector (GC/ECD) (Radová et al. 1998). Briefly, the homogenized ground cereal sample (10 g) was extracted with 100 ml acetonitrile: water mixture (84:16, v/v) by shaking for an hour, columns MycoSep 225 were used for purification of extract, DON was determined by GC/ECD after derivatization with trifluoroacetic anhydride (TFAA). The limit of detection was 5 μg/kg, the limit of quantification was 15 μg/kg and average recovery (n = 5, DON level in spike samples was 500 μg/kg) was 86.3% ± 4.9%.

Quality assurance in DON analysis

Mycotoxin standard was purchased from the Sigma-Aldrich (Germany). Prepared stock solution of DON was stored at –18°C. Working standard solution was prepared prior to analysis and its concentration was verified by UV absorbance measurements on a spectrophotometer.

Certificate reference materials (CRM), DON in wheat flour (< 0.05 μg/kg, BCR 396, Belgium) and DON in naturally contaminated wheat (0.7 ± 0.1 μg/kg, R-Biopharm Rhone, UK) were used for quality assurance in mycotoxin analysis.

The analytical method used was accredited and every year it successfully passed Food Analysis Performance Assessment Scheme (FAPAS) organized by the Central Science Laboratory (York, UK).

RESULTS AND DISCUSSION

Over the four experimental years, the highest level of DON content was found in 2005 (Figures 1 and 2). The differences between soil cultivation methods and preceding crops increased with the overall infestation level and were highest in 2005, whereas the highest DON content was determined in the no-till sowing and the lowest DON content in tillage in wheat following maize. Using maize as a preceding crop, the DON content increased up to 7 times compared to lucerne. In wheat following lucerne, the differences between soil cultivation methods were low and the highest DON level was determined in years 2004 and 2005 at shallow cultivation. The years 2002 and 2003 were characterized by generally very low infection level in all treatments.

The correlation of DON content to weather data was performed for monthly data prior to heading, and for 5-day data prior to and after the beginning of anthesis (Table 1). The highest positive correlation coefficients were found for sum of precipitation in April, average temperature in April

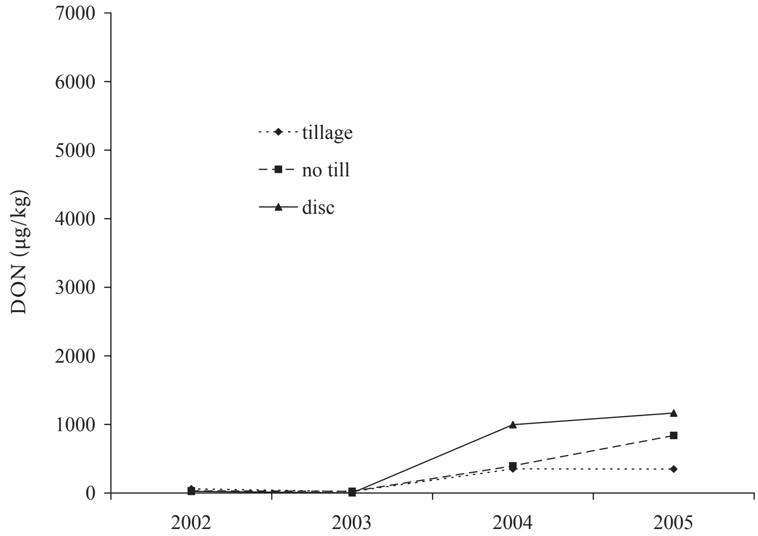

Figure 1. Interactions between the effect of soil cultivation and growing season on DON content (preceding crop – lucerne)

and sum of precipitation 5 days prior to anthesis. A significant negative correlation was found for average temperature in May and average relative humidity 5 days prior to anthesis.

Using the data from this experiment, we trained neural networks for prediction of DON content on the basis of weather data as a continuous input variable and preceding crop as categorial input variable. The winning neural network with architecture ZRNS 5:5-12-2-1:1 works with five input variables: the categorial variable (preceding crop) and continuous variables (average April temperature, sum of April precipitation, average temperature 5 days prior to anthesis, sum of precipitation 5 days prior to anthesis). Reliability of prediction using neural network can be seen in Figure 3, representing fit of observed and predicted data. Table 2 shows how individual input variables contribute to prediction in winning neural network. One of the most important input parameters is preceding crop (categorial variable) and sum of precipitation 5 days prior to anthesis (continuous variable). From a response graph (Figure 4), it is evident that the effect of sum of rainfall 5 days prior to anthesis starts from the limit of 15 mm. Other two parameters critical for perithecia formation are average temperature and sum of rainfall in April. The response of DON content to these

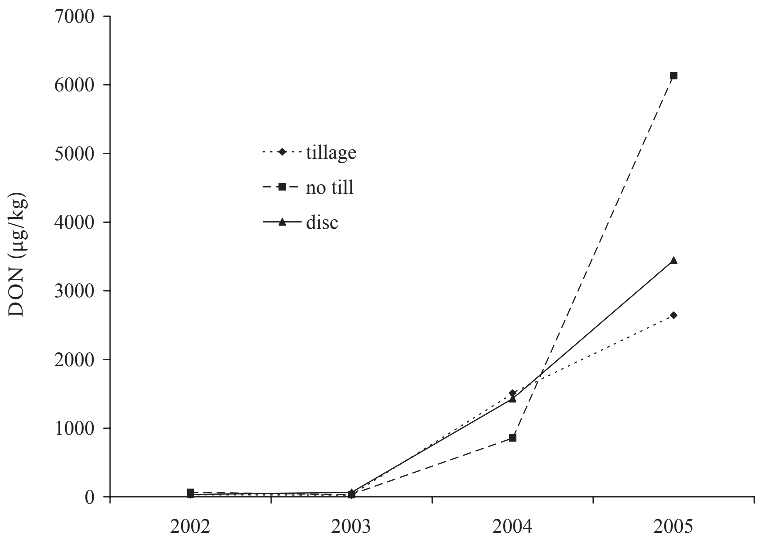

Figure 2. Interactions between the effect of soil cultivation and growing season on DON content (preceding crop – maize)

Table 1. Correlation coefficients for individual weather variables and DON content in grain (experiment with soil cultivation)

Weather variable	P	$^*P < 0.05$
Average temperature (April)	0.53	*
Average temperature (May)	–0.42	*
Sum of precipitation (April)	0.66	*
Sum of precipitation (May)	0.40	
Average relative humidity (April)	–0.01	
Average relative humidity (May)	–0.23	
Average temperature 5 days prior to anthesis	0.27	
Sum of precipitation 5 days prior to anthesis	0.52	*
Average relative humidity 5 days prior to anthesis	–0.57	*
Average temperature 5 days after anthesis	–0.31	
Sum of precipitation 5 days after anthesis	0.31	
Average relative humidity 5 days after anthesis	0.25	

two parameters is shown in Figures 5 and 6. In general, the DON content is increasing with temperature and precipitation rise. The production of DON is suppressed at average April temperatures below 9.5°C with continuous increase in DON content up to average temperatures around 11°C. The response of DON content to average April temperatures shows sigmoidal relationship. The effect of rainfall in April on DON content has exponential course.

Deoxynivalenol content in wheat grain was mainly influenced by the year and preceding crop. This corresponds with Schaafsma et al. (2005) who reported that environmental effects accounted for 48% of the variation in DON content in wheat grain, followed by variety (27%) and preceding crop (14 to 28 %). The effect of soil cultivation was very small in most experimental years. These results show that the burial of crop residues plays a significant role only if the infection level is generally high. Koch et al. (2006) concluded from their on-farm large-scale experiments that the impact of annual weather conditions affects Fusarium infection and DON concentration to the same extent as the preceding crop and cultivar susceptibility, and in comparison with these factors, applied tillage sys-

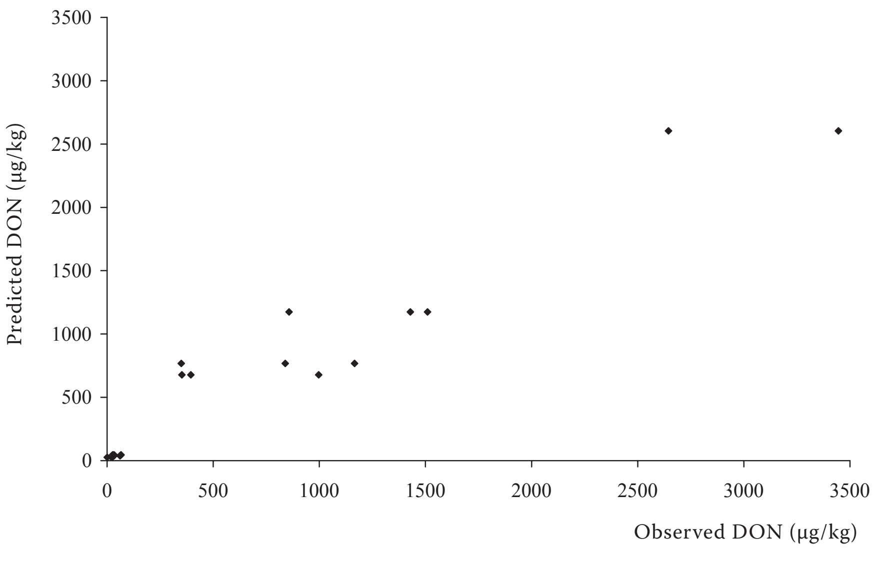

Figure 3. Graph of fit between observed and predicted (using neural network with weather input variables ZRNS 5:5-12-2-1:1) DON concentrations in grain

Table 2. Sensitivity analysis describing the importance of individual inputs in winning neural network ZRNS 5:5-12-2-1:1

	Preceding crop	Average temperature (April)	Sum of precipitation (April)	Average temperature 5 days prior to anthesis	Sum of precipitation 5 days prior to anthesis
Ratio	1.484	1.007	1.020	1.006	1.026
Ranking	1	4	3	5	2

tem is less important. Dill-Macky and Jones (2000) found out only small differences in DON content between mouldboard plough, chisel plough and no-till plots. If the conditions are less favourable for perithecia and ascospores formation (dry spring – 2004), the infection units could be formed on older crop residues and deeper soil cultivation would be preferable for this process. In this case, the effect of the preceding crop is small. Shallow burial of crop debris in dry years, such as 2004, forms better moisture conditions for perithecia maturity and ascospore release.

Although buried residue decomposed substantially faster than residue on the soil surface (Pereyra et al. 2004), the exhumation of crop residues from a deeper soil layer can restart the perithecia formation on crop debris, which is much lower than the first year, but it can be a reason for overlapping differences between preceding crops. Khonga and Sutton (1988) found out that maize stems and wheat spikelets left on the soil surface were able to form perithecia during the second year.

The correlation analysis shows that under relatively dry conditions of continental climate in the Czech Republic, one of very important factors for FHB infection and resulting DON formation, is the development of infection potential on preceding crop residues during early spring (April). Warm and humid early spring supports increasing infection and DON formation in winter wheat grain. Such conditions in spring favour perithecial development and maturation on plant debris in time to produce ascospores concomitantly with the flowering of cereal crops (Suty and Mauler-Machnik 1996). Some studies (e.g. Fernando et al. 1997) concluded that FHB infection results mainly from primary infection, and the secondary spread is of minor importance. Therefore, conditions favourable for perithecia development (relative humidity and temperature in April) could have a higher impact than weather conditions around anthesis.

High humidity is required for the initial release of ascospores, although dry periods may be re-

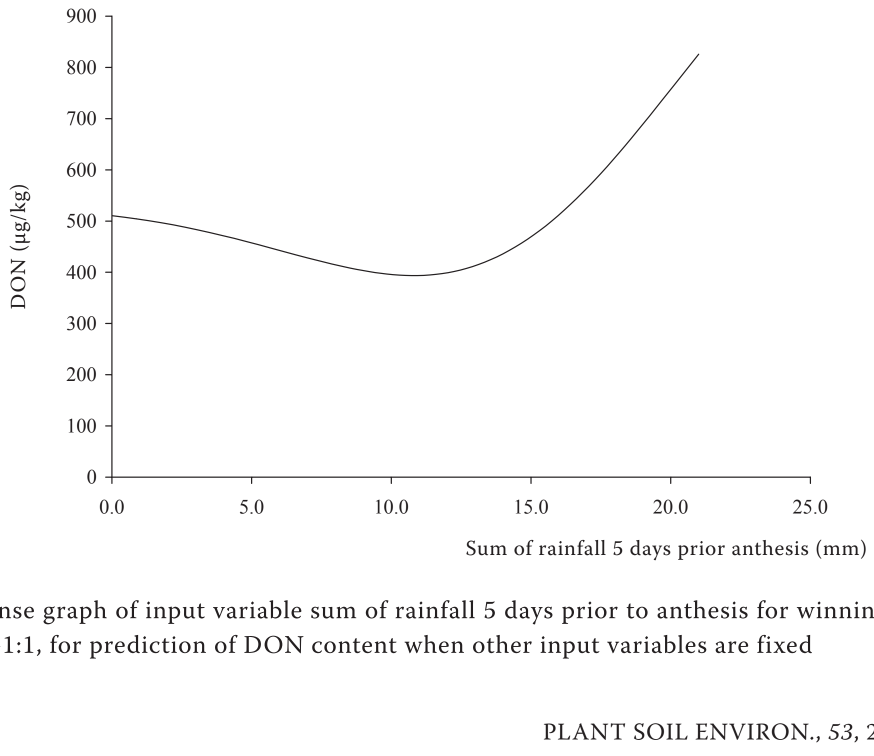

Figure 4. Response graph of input variable sum of rainfall 5 days prior to anthesis for winning neural network ZRNS 5:5-12-2-1:1, for prediction of DON content when other input variables are fixed

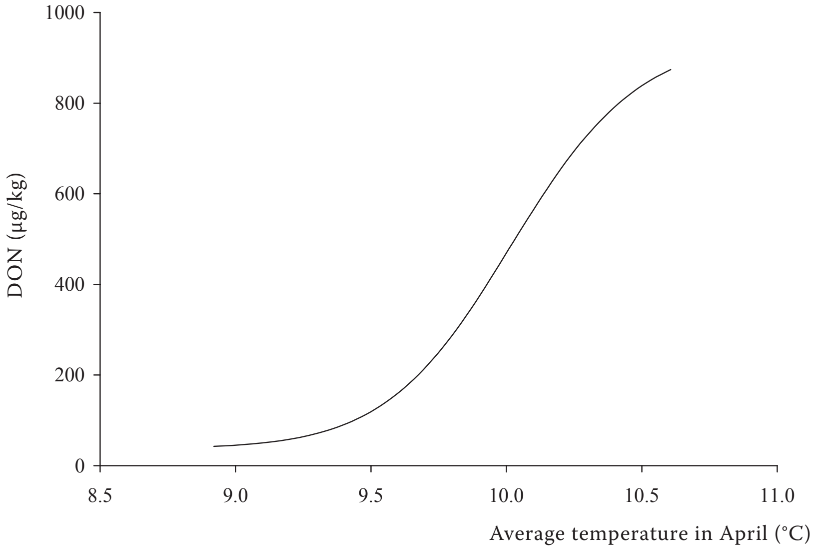

Figure 5. Response graph of input variable average temperature in April for winning neural network ZRNS 5:5-12-2-1:1, for prediction of DON content when other input variables are fixed

quired for their forceful discharge into the air from perithecia (Parry et al. 1995).

Since perithecia of *G. zeae* develop on above-ground residues at temperatures between 15 and 25°C, but not below 15°C (Gilbert and Fernando 2004), the April temperature effect is quite high. Andries et al. (2000) found that perithecium formation is limited by average daily temperatures below 9°C. April is one of the months with the most variable weather in the Czech Republic. The periods with temperatures below 10°C are very frequent and these delay the formation of perithecia on crop debris resulting in late ascospore release (after anthesis) and lower FHB infection.

There is an interesting paradox in the correlation coefficients for relative humidity and sum of precipitation 5 days prior to anthesis. For the first parameter, the correlation is positive and for the second negative. This can be easily explained by optimum conditions for ascospore release from perithecia, i.e alternately wetting and drying. Paulitz (1996) suggested that perithecial drying during the day followed by sharp increases in relative humidity may provide the stimulus for release of ascospores. The most important weather parameter directly influencing infection process was the sum of precipitation 5 days prior anthesis.

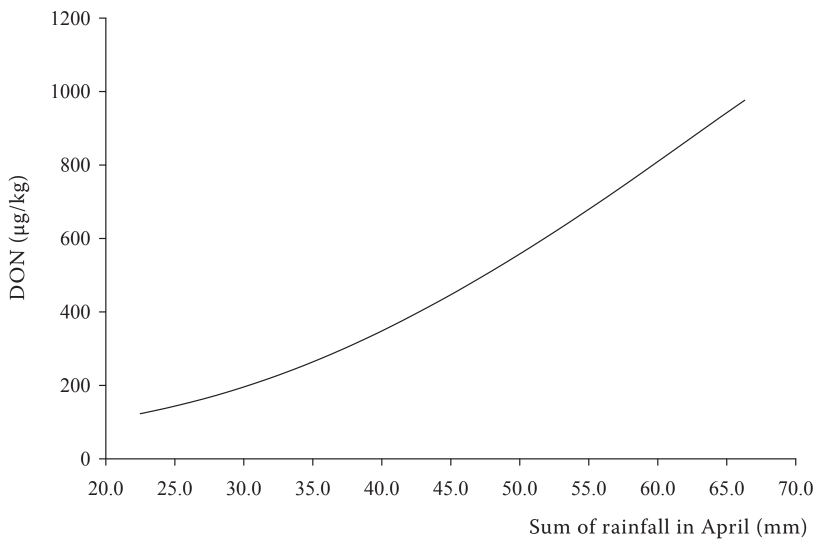

Figure 6. Response graph of input variable sum of rainfall in April for winning neural network ZRNS 5:5-12-2-1:1, for prediction of DON content when other input variables are fixed

The effect of temperature and rainfall after anthesis was not significant. According to Bai and Shaner (1991), FHB infection is favoured by extended periods (48 to 72 hours) at > 90% relative humidity with temperatures between 15 and 30°C. When wetness or high moisture events are discontinuous, infection can still occur but infection efficiency is reduced. Although temperature could have strong influence on infection process (Brennan et al. 2005), from practical view under Czech conditions the wetness is more often a limiting factor. Rainfall shortly before anthesis has a combined effect on ascospore maturity and release, macroconidia splash and infection allowance by wetting spikes. Paulitz (1996) determined that the highest numbers of *Fusarium* spores were captured from wheat plots from 2 to 4 days after rainfall event. Rossi et al. (2002) detected no or very few conidia before rainfall, but their number progressively increased during rainfall. With subsequent humid conditions, conidia continued to be produced for some hours after rainfall and usually reached their peak under these conditions. Artificial neural networks have recently gained popularity in plant pathology mostly in forecasting models (Yang et al. 1995, De Wolf and Francl 1997, Chakraborty et al. 2004). The developed neural network model is based on the categorial input variable (preceding crop) and the continuous variables (average April temperature, sum of April precipitation, average temperature 5 days prior to anthesis and sum of precipitation 5 days prior to anthesis). The main advantage of the model is that it integrates the effect of the preceding crop, weather conditions on inoculum formation and weather conditions on ascospore release and infection process.

The FHB epidemics forecasting model, based on hourly weather data was developed by De Wolf et al. (2004). Weather data used by the model include relative humidity, hours that air temperatures range from 9 to 30°C, hours of rainfall exceeding 0.3 mm and an interaction of high relative humidity and air temperatures favourable, collected during the 7-day interval prior to flowering. The model is estimated to accurately predict FHB epidemics and non-epidemics at about 80% of the time. By using stepwise logistic regression analysis De Wolf et al. (2000) identified three variables useful for the prediction of FHB epidemics. Two of the variables were summaries of the environment 7 days prior to crop anthesis, and included the duration of precipitation and the duration of temperature within the temperature range from 15 to 30°C. The third variable combined the temperature and relative humidity 10 days after the initiation of anthesis.

Based on our experimental results and indices in literature, we can summarize that variables representing environment prior to anthesis may provide models with information about potentially limiting factors for inoculum production simultaneously with the direct effect on spike infection. The construction of prediction models on the basis of weather data prior to anthesis has also practical implication for control measurements, because efficacy of fungicide treatments highly depends on proper time of application, which is limited by flowering stage.

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