The status of research on application of NIRS to prediction of *Fusarium* Head Blight (“Scab”) in terms of DON

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Introduction

Fusarium head blight, called “Scab” in the USA, is a disease of cereals caused by species of fungus of the genus *Fusarium*. It has become an important cause of reduction in grain yield and value in western Canada, and on the eastern Prairie area in particular. Of the four species that commonly occur across the Canadian wheat-growing area, and in other parts of the world, *Fusarium graminearum* has emerged as the most important scourge of the wheat crop. The disease annually causes losses in yield worth millions of dollars, and still further losses due to grain being degraded to lower grades.

The fungus produces a mycotoxin that is a tricothecene called deoxynivalenol (DON). This causes gastric disorders and low weight gain in domestic monogastric animals and birds. The incidence of *Fusarium*-damaged kernels (% FDK) is a grade factor in Canada. Damaged kernels are identified by a manual/visual method (Fig. 1), which is time-consuming, and rather subjective.

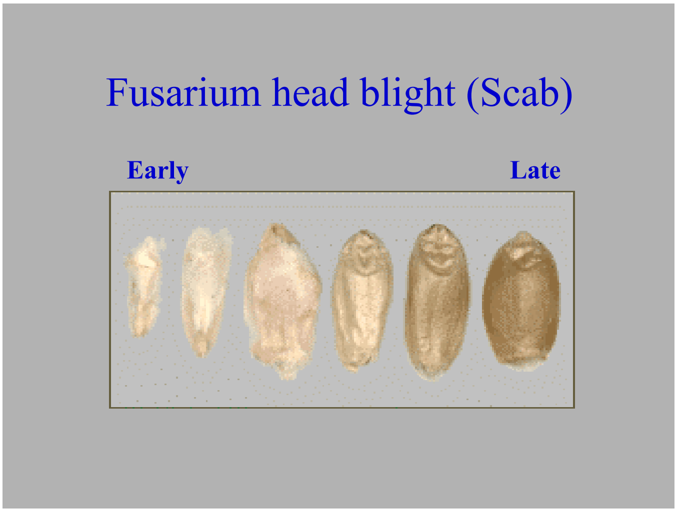

Figure 1. Influence of degree of infestation of Hard Red Spring wheat with *Fusarium graminearum.*

Research has been conducted in western Canada (and elsewhere in the world) to develop a rapid instrumental method for predicting the incidence and severity of *Fusarium* infection particularly of wheat, but also of other cereals. The paper summarizes the status of research using near-infrared spectroscopy (NIRS) at the Canadian Grain Commission up to and including 2002.

Methods

Conventional methods

Deoxynivalenol can be determined by gas-liquid chromatography and by ELISA kits. The correlation between % FDK and DON is fairly high, but differs significantly depending on the level of DON. This is because it becomes progressively easier to detect *Fusarium*-damaged kernels at higher levels of infection (and higher levels of DON).

Table 1. Correlation between visual grading (% damaged kernels) and ppm DON (ELISA)

Up to x ppm DON	2.0	5.0	10.0	19.7
Coefficient of Correlation	0.72	0.89	0.98	0.91
Regression Coefficient	0.547	0.702	0.870	1.078
Intercept	0.24	0.23	0.15	-0.16
Number of Observations	69	96	110	119

As a grade factor *Fusarium* is determined manually and visually at the Canadian Grain Commission (CGC) by sub-dividing an automatically-withdrawn sample accurately to about 10 - 20 g and inspecting each individual kernel. The degree of damage is expressed as % *Fusarium*-damaged kernels (% FDK). Determination of % FDK by visual hand-picking is laborious, and takes about 15 min. This is too slow for on-the-spot grading of carloads of wheat as they are being unloaded into terminal elevators, or receiving farmers' truckloads at country delivery points.

Tolerances for FDK in CWRS wheat (Canada's highest volume wheat commodity) are 0.25 % for grade 1 CWRS, 1.0 % for Grade 2 CWRS and 2 % for grade 3 CWRS wheat. Up to 5 % FDK is allowed in Canada Feed wheat. Above this level the wheat cannot be used as a feed ingredient.

Both gas-liquid chromatography and ELISA are time-consuming and too slow for use at delivery. An NIR method would be very attractive to the industry.

Near-infrared spectroscopy

The NIR research has been conducted using two types of NIR instrument, a "stand-alone" monchromator-based instrument, in wide use at grain-receiving points, and scanning monochromator, with a wide wavelength range. The concept of "double variance" is introduced as one reason for the difficulty in achieving reliable calibrations. Discriminant analysis is introduced as a possible method for screening wheat for possible use in animal feed production, where higher levels of DON (up to 5 ppm) are permissible for feed in beef cattle, sheep and poultry.

Research at the CGC on the prediction of DON in wheat has included development of calibrations for the prediction of DON (in ppm), and % FDK, using the Tecator Infratec Model 1241, and the Foss NIRSystems Model 6500 scanning spectrophotometer (Table 2). The percentage of *Fusarium*-damaged kernels (% FDK) was determined visually by grain inspectors of the Canadian Grain Commission (CGC). The concentration of DON was determined using ELISA kits. The NIR results were obtained using a Foss/NIRSystems Model 6500 instrument. Similar results were obtained using the Foss/Tecator Infratec Model 1241, over the wavelength range 700-1098 nm.

Results

Results for near-infrared prediction of % FDK and DON are summarized in Table 2

Table 2. Prediction of % FDK and ppm DON in CWRS wheat by NIR spectroscopy.

Wavelengths	% FDK			DON ppm		
	r^2	SEP	b	r^2	SEP	b
608-1092: 11041*	0.62	1.27	0.82	0.64	1.36	0.78
608-1692: 11041	0.69	1.11	0.88	0.72	1.14	0.87
* 11041 = first derivative, segment 4, "gap" 10.						

Figure 2 gives the second derivative spectra of wheat with low and high DON. The spectrum with the strong band at 778 nm is a sample with high DON content (20 ppm)

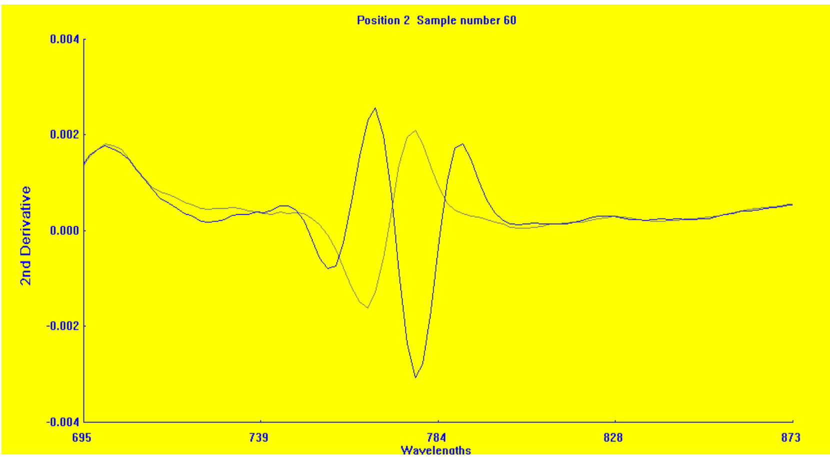

Figure 2. Spectra of two samples of wheat differing widely in ppm DON (695 to 873 nm).

Discussion

The main reason why NIR is capable of approximate predictions is believed to be due to prediction of small changes in the texture of the grain, caused by the invasion by the fungus. This would in turn change the way in which the radiant energy is diffusely reflected or transmitted by the grain.

A common feature of NIR calibration and prediction of DON or % *Fusarium*-damaged kernels is that, while acceptable calibrations have been developed for both parameters, for both types of instrument, they are generally not fully transferable to new data sets. The slopes (regression coefficients) are generally low until a range in DON is attained in the samples that is far beyond the level of DON allowed in even feed wheat (Table 3).

Table 3. Changes in slope and RPD of DON NIR predictions at different DON levels

Up to x ppm DON	2.0	5.0	10.0
Regression Coefficient	0.605	0.809	0.870
RPD	1.21	0.98	2.32

The concept of "double variance" is introduced as an important factor responsible for the low slopes and high standard error for prediction of DON in the affected kernels. Samples used in the NIR work ranged from 0.0 to 10 ppm DON (well above the level permitted in feed grain, to enable screening). The DON content of individual kernels has been shown to range from 0.0 to over 100 ppm (D.G. Gaba, Canadian Grain Commission, pers. comm.). These differences in the degree of infection of kernels ("double variance") can cause further changes in kernel texture that are not accounted for in the overall variance in the data used for calibration.

A further significant factor in the use of the Tecator Infratec is the sample size of the individual increments. The path-length used for wheat is 18 mm. The area of the square "window" is about 1.0 cm. The total volume of grain per increment is 1.8 cm^2. This is equivalent to about 1.55 g of wheat (about 45 kernels). Yet another factor is that not all of the kernels are affected - the maximum number of visually detectable affected kernels is only 1 % even in Grade 2CW wheat - so it is possible for some increments (in an Infratec scan) to contain no affected kernels.

The standard deviation (SD) of *Fusarium*-damaged kernels in an Infratec scan of 10 - 12 increments can be very high relative to the mean % FDK, which contributes further to the error. The SD of protein content among the individual kernels in a given sample of wheat is only about 0.6 % protein. The SD of moisture in individual wheat kernels is even lower, at 0.35-0.40. As a result, at given protein or moisture contents most of the kernels will be of about the same average protein or moisture contents. This partly explains why the Infratec is so successful for the prediction of these constituents. Prediction of parameters such as DON or sprout damage is complicated by the fact that only a small percentage of the kernels are affected.

Because of the low slopes, errors in prediction of both % FDK and DON ppm incurred at low levels are too high for practical application. For example, if the NIR-predicted value was 0 for either factor it could not be **legally** stated that the grain was **free** of FDK or DON. The error is also too high to be of practical value in grading wheat for milling. But even at the relatively high errors in predictability of DON, there is some value in using NIR as a rough screening tool in the feed industry, where feed mills need to know if there is a possibility of using farmers' deliveries with fairly high levels of DON by blending it off with grain at lower levels to reach a "safe" level for a feed mix. Feed mills would benefit by being able to use the NIR guidelines in blending to secure a safe feed mix, while farmers would benefit by being able to market their feed wheat.

Discriminant analysis may be a useful tool in screening out wheat damaged to the extent of being unacceptable for animal feed. The training set would be developed to identify wheat that is apparently significantly higher than the acceptable level of 5 ppm DON for animal feed.

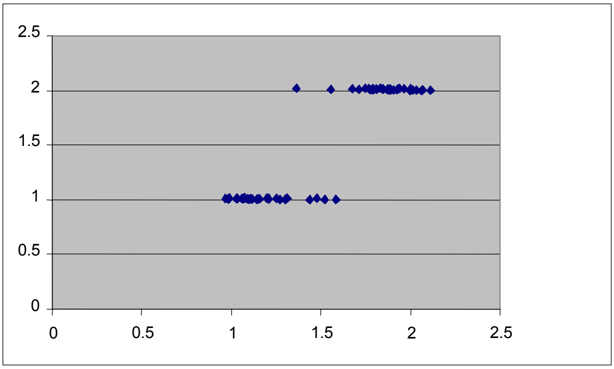

Figure 3. Discriminant analysis between high and low DON series.

In common with sprout damage, commonly assessed by the Falling Number (FN) and RVA tests, successful NIR calibrations could probably be developed for DON using a combination of large data bases and ANN (Artificial Neural Networks), together with more rigorously controlled reference testing. The standard errors of both the FN and ELISA tests inhibit the development of successful calibrations for these parameters.

Summary

The status of prediction of *Fusarium* damage to wheat is summarized in terms of the percentage of *Fusarium*-damaged kernels (% FDK) and ppm of deoxynivalenol (ppm DON). In general the results were not sufficiently accurate to enable official statements to be made as to freedom of deliveries of wheat from either % FDK or ppm DON. Spectra of whole wheat samples high and free of DON showed the presence of a strong band in the neighbourhood of 778 nm. The possibility of using NIR as a screening method to determine usefulness of wheat deliveries in animal feed-mix manufacture remains. The concept of "double variance" was introduced as a partial explanation for the only moderate success achieved with NIR predictions. The use of NIR Discriminant Analysis as a tool for rapid screening of farm deliveries of wheat was discussed.

Acknowledgments

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