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Research Paper

Laboratory *vs.* in-field spectral proximal sensing for early detection of *Fusarium* head blight infection in durum wheat

Paolo Menesatti [a], *Francesca Antonucci* [a,*], *Federico Pallottino* [a], *Stefano Giorgi* [a], *Antonio Matere* [b], *Francesca Nocente* [b], *Marina Pasquini* [b], *Maria G. D'Egidio* [b], *Corrado Costa* [a]

[a] *Consiglio per la ricerca e la sperimentazione in agricoltura, Unità di ricerca per l'ingegneria agraria, Via della Pascolare 16, 00015 Monterotondo scalo, Rome, Italy*

[b] *Consiglio per la ricerca e la sperimentazione in agricoltura, Unità di ricerca per la valorizzazione qualitativa dei cereali, Via Cassia 176, 00191 Rome, Italy*

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A comparison between two VIS–NIR spectral based systems performed in laboratory *vs.* in-field for the early detection of Fusarium head blight infection in two cultivars of durum wheat (Creso and Simeto) was carried out. The VIS–NIR spectrophotometric data were analysed with multivariate statistical tools. For both, laboratory and in-field experiments two analytical conditions were tested for two cultivars: diseased plants (artificial infection without fungicide treatment) and healthy plants (treatment with Folicur SE). Spectral measurements were performed at the different sampling times 6, 8, 12 and 15 d after artificial infection which correspond to the following Zadoks' scale growth stages: GS70, GS71, GS73 and GS75. The infection visible onset (VO) was then evaluated by an expert at the soft dough growth stage GS85. Since the growth stages revealed a great influence on spectral reflectance data, separated PLSDA models were adopted to differentiate diseased and healthy plants at the different sampling times. Using the Euclidean distance matrix cladogram results for the laboratory, three models were used considering spectral data from GS70, GS71 + GS73, GS75, while for in-field data from GS70 + GS71 and GS73 + GS75. In the laboratory good performance of classification (86%) was observed at GS71 + GS73 *i.e.*, only 8–10 days after the infection. The in-field measurement showed a lower percentage of correct classification at the same growth stages. Finally the VIS–NIR spectral analysis could facilitate detection of *Fusarium* disease anticipating visual assessment.

1. Introduction

Fusarium head blight (FHB) is considered one of the most serious fungal diseases of wheat. FHB can be associated with different fungal species in the genus *Fusarium* (Edwards, Pirgozliev, Hare, & Jenkinson, 2001) resulting in yield losses and contamination of harvested grains with mycotoxins (Miedaner, Schneider, & Geiger, 2003). This disease is becoming of great importance in Italy, where it represents a problem in years with frequent rainfall during flowering (Pasquini,

* *Corresponding author*. Tel.: +390690675213; fax: +39069062559.
E-mail address: francescaantonucci@hotmail.it (F. Antonucci).

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Nomenclature

FHB	*Fusarium* Head Blight
VIS–NIR	Visible–Near Infrared
GS	Growth Stage
DON	Deoxynivalenol
D	Diseased Plant
H	Healthy
VO	Visible Onset
MANOVA	Multivariate Analysis Of Variance
PCA	Principal Component Analysis
PLSDA	Partial Least Square Discriminant Analysis

Pancaldi, Iori, Gazza, & Riccardi, 2003). Different optical (mainly spectral analysis) methods have been used in order to early detect FHB (Bauriegel, Giebel, Geyer, Schmidt, & Herppich, 2011) being fast and non-invasive applications (Menesatti, Antonucci et al., 2010; Sankaran, Mishra, Ehsani, & Davis, 2010) in both field and laboratory. Under field conditions, many environmental factors do affect the measurements (*i.e.*, illumination) making analysis much more complicated. Moreover, wheat ear spectral variations due to *Fusarium* infection could be masked by their maturation development (Bauriegel et al., 2011).

The aim of this work is to compare two VIS–NIR spectral based methods performed in laboratory *vs.* in-field for the early detection of FHB infection in durum wheat.

2. Materials and methods

For both laboratory and in-field experiments, durum wheat plants (cultivars Creso and Simeto) were grown in the "Consiglio per la ricerca e la sperimentazione in agricoltura" experimental field (Montelibretti, Rome, Central Italy) (plot sized 3 m^2 with two replicates). All the experiments referred to the Zadoks' scale, regarding the identification of wheat growth stages (GS; Zadoks, Chang, & Konzak, 1974). Plants were isolated with a plastic film and artificially inoculated in the late afternoon with a deoxynivalenol (DON) producing isolate of *Fusarium graminearum* with a spore concentration of 2.5×10^5 spores ml^{-1} (30–40 ml m^{-2} of conidial suspension for each plot) at half-way anthesis (GS65). The plastic film was removed 12 h after the inoculation. Control plants were grown in the same experimental field and treated with Folicur SE, (Bayer Crop Science, Monheim am Rhein, Germany) a 43.1 g [tebuconazole] l^{-1} formulation with a broad spectrum of activity against different epigeous fungal diseases. The identification of *Fusarium* species present on inoculated wheat kernels was based on conidial morphology according to Balmas, Vitale, Marcello, and Corazza (2000) and Leslie and Summerell (2006). Two conditions were tested for each cultivar: diseased plants (artificial infection without fungicide treatment – D) and healthy plants (treatment with Folicur SE – H) with two replications. A total of 8 plots were analysed for each cultivars (Creso and Simeto). The time and rate of application of Folicur SE were optimised. The fungicide was applied at flowering (GS65) at a dose of 1 l ha^{-1}, with a hand sprayer operated at low pressure to ensure an effective coverage. For the laboratory experiments about 13 durum wheat ears were sampled in the experimental field for each sampling time (104 total ears). Spectral measurements were performed at the different sampling times 6, 8, 12 and 15 days after artificial infection which correspond to the following Zadoks' scale growth stages: GS70, GS71, GS73 and GS75. The infection visible onset (VO) was then evaluated by an expert at the growth stage of soft dough (GS85). The incidence and severity of FHB were recorded for each plot, carrying out visual evaluations of the disease from the time immediately after artificial infection until soft dough (GS85). FHB incidence was calculated by visually estimating the percentage of ears with symptoms present in each plot. FHB severity was calculated as the percentage of ear surface with symptoms according to the modified Parry's scale (Parry, Bayles, & Priestley, 1984). The presence of infection caused by other fungi was also investigated for each analysed plot following the modified Cobb's scale (0–100%) (Pasquini & Delogu, 2003). Immediately after sampling, for the laboratory spectral measurements, whole ears were scanned by a hyperspectral system. The imaging spectrometer of the system (Spectral Scanner, v. 1.4.1, DV Optics, Padua, Italy) was used to acquire images ranging from 400 to 790 nm (step 5 nm) following details and settings exposed by Menesatti, Costa, and Aguzzi (2010). Each frame contained the line pixels in one dimension (spatial axis) and the spectral pixels in the other dimension (spectral axis), providing full spectral information for each line pixel. The reconstruction of the entire hyperspectral image of the sample was performed by scanning the sample line by line as the transportation plate moved it through the field of view. The resolution of the line image was 700 pixels per line for 500 lines. The system was operated in a dark laboratory to minimise interference from ambient light.

For the in-field experiments, 10 labelled durum wheat ears were measured directly in the experimental field for each sampling time (100 total ears). Sampling times were the same of the laboratory experimental setup. A punctual spectrometer (the portable Ocean Optics Jaz Modular Optical Sensing Suite (Dunedin, FL, USA)) with a modular structure (450–700 nm step 5 nm) has been used to acquire spectral information 3 times for each ear. The acquisition was carried out just using the natural sun light. This was done using a tripod and pointing the optical fibre towards the sun for the light acquisition, and using the measure optical fibre directly upon the ear to be evaluated. The probe was not in direct contact with the ear but 10 mm away and held in position using a metal support.

Spectral data from both laboratory and in-field were separately analysed with a 50–50 MANOVA procedure (Langsrud, 2002): a generalised multivariate ANOVA method based on principal component analysis (PCA) on standardised data. The Euclidean distance matrix was clustered in a cladogram using the complete linkage algorithm for both laboratory and in-field analysis.

For the laboratory and in-field experiments different PLSDA models were developed for the early pathogen prediction on the base of these extracted cladograms and of the influence of the phenological growth stage, demonstrated in the spectral reflectance value trends. For the laboratory, three

models were performed considering spectral data from: 1) GS70, 2) GS71 + GS73, 3) GS75, while two models were considered for the in-field: 1) GS70 + GS71 and 2) GS73 + GS75. This allowed spectra measured on plants that were in very similar phenological stage to be compared.

The multivariate statistical PLSDA was considered in order to find out the stage of grain development. The PLSDA consists of a classical partial least squares (PLS) regression analysis where the response variable is categorical (Y-block; sampling times or treatment strategies expressed as dummy variables; Sabatier, Vivein, & Amenta, 2003). The dataset of each of the four models has been subdivided into two groups: (1) 70% of observations for the class modelling and validation set, and (2) 30% of observations for the independent test set, optimally chosen with the Euclidean distances based on the Kennard and Stone (1969) algorithm. The model includes a calibration phase and a cross-validation phase (Antonucci et al., 2012). All the statistical analyses were performed using Matlab (rel. 7.1, PLSToolbox Eigenvector rel. 4.0) (Mathworks, Natick, MA, USA).

3. Results

The FHB symptoms were evident at the beginning of dough development stage, but the infection measurement reference was performed at soft dough (GS85). The fungal species isolated in the ear samples taken from the field in the soft dough stage (GS85) was *F. graminearum*, used for the artificial infections. The FHB incidence and severity were calculated for H and D plots. The infection pressure was high in the inoculated plots with a percentage of ears affected by FHB of 50% for Creso and Simeto respectively and a FHB severity of 50% for Simeto and 30% for Creso. The control samples treated with the fungicide resulted completely free from infection with a complete reduction of the incidence and severity of FHB.

The application of the fungicide (Folicur SE) reduced other fungal infections on the plots. The level of infection in the diseased plot by other diseases like powdery mildew, leaf rust and septoria complex was very low.

For the laboratory analysis, MANOVA showed as all the observed factors (cultivar, treatments, sampling times) and their interactions resulted to be significant apart from the interactions cultivar*treatments and cultivar*-treatments*sampling times. Figure 1 shows the cladograms extracted from the average laboratory VIS–NIR spectral reflectance at different sampling times for both cultivars (Fig. 1A Creso and B Simeto).

It is possible to observe as both H and D samples at the same sampling time clustered together for the two cultivars. Cutting the cladogram at the linkage distance 0.7, three groups were identified for both cultivars excluding the infection

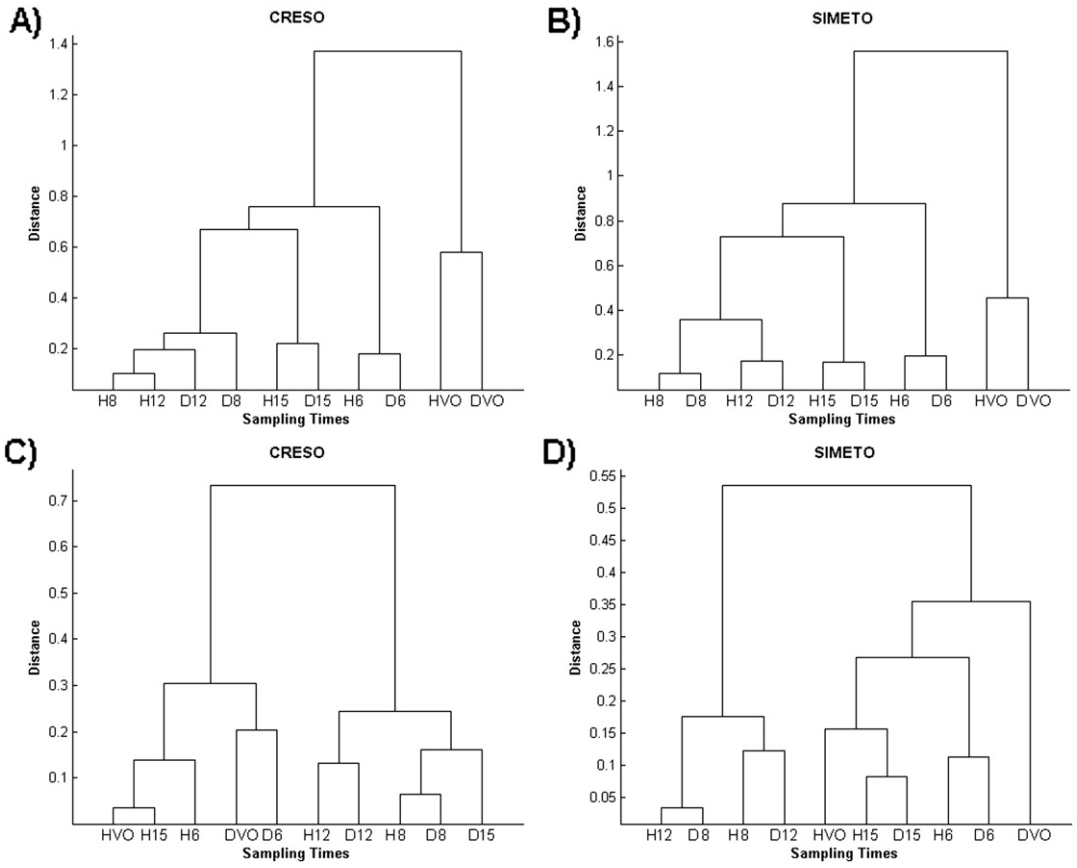

Fig. 1 – Cladograms based on the Euclidean distances, built using the complete linkage algorithm extracted from the average VIS–NIR spectral reflectance at different sampling times (6, 8, 12, 15 d after artificial infection) and at the visible onset (VO) and the two conditions H = healthy and D = diseased for both cultivars in laboratory (1A = Creso and 1B = Simeto) and in-field (1C = Creso and 1D = Simeto).

visible onset (H GS85 and D GS85): GS75, GS71 + 73 and GS70. Moreover, for both cultivars, the H GS85 and D GS85 samples are more distant from the other groups, among which H GS70 and D GS70 ones are more distant from the rest. Table 1 reports the results of the 4 PLSDA models for the laboratory prediction of *Fusarium* infection based on the VIS–NIR spectrophotometric analysis. Although the sampled ears were around 13 *per* time the last sampling time was not used for the PLS analysis, which theoretically makes the total number equal to 104. However since during the transportation some of the samples appeared to be not in perfect shape were eliminated making the total effective number equal to 96, as shown in Table 1. Good classification performance (86%) could be observed at GS71 + GS73 *i.e.*, only 8–10 days after the infection.

For the in-field analysis, MANOVA showed as all the observed variables resulted to be significant apart the interactions cultivar*treatments, treatments*sampling times and cultivar*treatments*sampling times. The cladograms in Fig. 1 showed for both cultivars (1C = Creso and 1D = Simeto) the sampling times and treatments are confused not allowing an interpretation of the clusters as in the laboratory.

The PLSDA models revealed that the prediction of *Fusarium* infection based on the VIS–NIR spectrophotometric analysis in test resulted to be less performing with respect to the laboratory one for all sampling times and groups.

4. Discussion

In this study, laboratory results could promisingly early predict FHB infection on wheat ears with higher performance with respect to in-field ones. The problems relative to the in-field conditions rose partially from illumination that can change very rapidly, the selection of the same ear at different sampling times and manipulation of the same. Moreover within the context of the work, analysing the ears directly in field could lead to timely information but to an entire image in the laboratory. Generally, most of the optical methods for detecting fungal plant diseases have been used directly in field under uncontrolled factors (Dammer, Möller, Rodemann, & Heppner, 2011) while for the first time a comparison between in-field and laboratory approaches has been conducted. Laboratory analysis is simpler but it must be considered as a destructive approach with constraints *e.g.*, spectrophotometric analyses should be effectuated immediately after the sample collection. Spectral variations are largely influenced by the ear maturation (Broge & Mortensen, 2002) as confirmed by the MANOVA results (Table 1) but they are well distinguished only in laboratory. The proximal sensing approach used in this study, differently from the previously ones, considered the degree of infection of the single sample plot and not of the single ear. This kind of approach has been chosen because it is requested by field operators looking for a simpler process, but one that influences the prediction performance based on mean plot values. Our results are comparable with those obtained by Bauriegel et al. (2011), which used a 100% modelling approach (*i.e.*, without testing), with values comprises between 94% and 100% of correct infection prediction. The lower performances in the test suggest the use of partitioning methods, for more accurate data validation. As outlined by Bauriegel et al. (2011) VIS–NIR spectral analysis could facilitate detection of *Fusarium* disease anticipating visual assessment. The early detection of toxigenic fungi can be useful to prevent the spreading of contaminated materials and to ensure product quality and safety. Spectral variation could be linked with fungal onset in the first period after artificial infection (6 days; *i.e.*, GS70). This phenomenon is shown within the NIR range, which is invisible to human eye.

Table 1 – Results of partial least squares discriminant analysis (PLSDA) for the laboratory and in-field prediction of *Fusarium* infection obtained with VIS–NIR spectrophotometric analysis for all the sampling times (6, 8, 12 and 15 days after artificial infection) and the growth stages (GS75 – 15 d, GS71 + 73 – 12 + 8 d and GS70 – 6 d, for the laboratory; GS73/75 and GS70/71for the in-field) considering the two cultivars (Creso and Simeto) together. Table reports: pre-processing for X- and Y-blocks, number of records, of X variables, of units in the Y-blocks, of latent vector (LV), percentage of cumulated variance of X- and Y-block, of mean specificity, sensitivity, classification error, random probability and finally of the correct classification for model (70% of whole dataset) and test (30% of whole dataset).

	Laboratory				In-field		
	Sampling times	GS75	GS71 + GS73	GS70	Sampling times	GS73 + GS75	GS70 + 71
Pre-proc. X-block	None	Autoscale	Mean centre	Autoscale	None	None	None
Pre-proc. Y-block	None	None	None	None	None	None	None
No. records	96	24	48	24	154	78	76
No. of X variables	115	115	115	115	51	51	51
No. units (Y-block)	3	2	2	2	2	2	2
No. LV	5	9	18	6	11	11	9
% Cum. var. X-block	99.97	98.81	99.99	95.16	99.55	99.56	99.67
% Cum. var. Y-block	95.29	45.22	49.43	47.05	47.39	48.43	47.8
Mean sp. (%)	1	1	1	1	0.99	1	1
Mean sen. (%)	1	1	1	1	0.98	1	1
Class. error (%)	0	0	0	0	0.002	0	0
Ran. prob. (%)	33.33	50	50	50	50	50	50
% Corr. class. mod. (70%)	100	100	100	100	100	100	100
% Corr. class. test (30%)	100	100	85.71	66.67	84.44	63.64	77.27

5. Conclusions

These preliminary, but promising, results showed a potential degree of applicability in the FHB prediction. Both approaches, but particularly that in-field, will require repeated measurements over following years to validate the technique and increase the robustness of the approach.

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