INNOVATIVE OPTICAL SENSORS FOR DIAGNOSIS, MAPPING AND REAL-TIME MANAGEMENT OF ROW CROPS: THE USE OF POLYPHENOLICS AND FLUORESCENCE.

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ABSTRACT

Force-A's Dualex® leaf-clips and Multiplex® proximal optical sensors give rapid and quantitative estimations of chlorophyll and polyphenolics of crops by measuring the fluorescence and absorption properties of these molecules. The in vivo and real-time assessments of these plant compounds allow us to define new indicators of crop nitrogen status, health and quality. The measurements of these indicators allow consultants and farmers to monitor the nitrogen status of row crops, for the management of the third nitrogen application on winter wheat, or to provide early estimation, at flowering, of future wheat grain quality, but also, to assess the canopy density of crops that can be used to adjust inputs of pesticides and fertilizers. We will illustrate these applications on row crops by showing results obtained both with the Dualex leaf-clip and an embedded Multiplex sensor, in wheat fields with variations in the nitrogen status, and in rapeseed fields with different seeding density. Force-A has also created recently a new data management tool. This system aims at acquisition, calculations, transformation and mapping of data. Thanks to these new optical sensors and associated models, the management of agricultural technical operations can be optimized and achieved for the whole field with a high spatial resolution, in real time or off-line.

Keywords: optical sensors, polyphenolics, chlorophyll, mapping, management tools, nitrogen status

INTRODUCTION

Crop management has shown large evolution since the past ten years thanks to the development of farming systems, the increase of cultivated surfaces and new environmental regulations.

One way to develop farming while protecting environment and farmers earnings is to adjust precisely quantity of products to the real nutrition and protection needs of crops. Crops health monitoring is a key part of a whole system allowing farmers to manage precisely their crops in the field:

- crop health status monitoring
- crop modelling
- treatments management (in real-time or off-line)

Force-A company (Orsay, France) provides the optical sensors Dualex® and Multiplex® for real time and in vivo measurements of polyphenols (PHEN) content (Agati et al.[1], 2008; Cerovic et al.[7], 2007; Cerovic et al.[6], 2008) and chlorophyll (CHL) content in plants. Chlorophyll and polyphenols contents are linked to the crop nitrogen status (Cerovic et al. [8], 2005; Cartelat et al.[5], 2005). Dualex Ferti® sensor allows farmers to manage third nitrogen treatment on wheat and Dualex Quali® forecasts at flowering grain protein content. Multiplex® assesses chlorophyll and polyphenolics content and also crop canopy density.

With these sensors, one measurement takes less than a second, is non destructive and does not require any plant sample preparation. Multiplex® can be embedded on farming machines.

The aim of this work is to illustrate the use of both Dualex® and Multiplex® sensor as diagnostic tools 1) for crop nitrogen status assessment; 2) for crop canopy density assessment. Furthermore, Multiplex can be used for crop mapping and in-field real-time crop management. Multiplex sensor can be used and integrated in many different farming systems. We will illustrate different possibilities of Multiplex uses.

MATERIALS AND METHODS

The Dualex® Sensor

Dualex leaf-clip (FORCE-A, France) is a quick non-destructive sensor dedicated to leaf chlorophyll and flavonols content assessment in every crop.

The measurement of the UV optical absorbance of the epidermis is based on the fluorescence emitted by the chlorophyll located in the mesophyll. It is well known that chlorophyll emits fluorescence when illuminated (Cerovic et al.[9], 2002). This fluorescence is induced by Dualex in two excitation wavelengths: UV and red. In a leaf, the epidermis absorbs the UV while it transmits the red. Then by comparing the fluorescence induced by UV and induced by red, the absorbance of the epidermis can be determined (Bilger et al.[2], 2001; Bilger et al.[3], 1997). For example in the case of flavonols, the UV radiation is highly absorbed by the epidermis, whereas in the red range the absorption is very low. Thanks to its unique patented technology, the Dualex® Scientific leaf-clip measures quantitatively the optical absorption of the leaf epidermis in UV. The epidermal absorption is directly linked to the polyphenols concentration in the leaf. It is important to note that the chlorophyll acts, in the case of Dualex® Scientific, as an internal detector (sensor) of photons. This method is known as the LogFER method.

Dualex leaf-clip is also able to assess chlorophyll content. The measurement of the leaf chlorophyll content is based on the measurement of the difference in transmission of two wavelengths, both in the near infra red (NIR).

A chlorophyll-specific absorption index is calculated as a difference of the optical transmission at two different wavelengths in the NIR.

Fig. 1. Picture of Dualex leaf-clip while assessment on wheat.

Dualex leaf-clip calculates automatically the Nitrogen Balance Index (NBI). It is based on the work of the Biospectroscopy team in Orsay on wheat (Cartelat et al.[5], 2005) and several ligneous species (Demotes-Mainard et al.[10], 2008; Meyer et al.[11], 2006). NBI has been shown to respond to nitrogen nutrition of the plant. It can be used directly in comparison studies, provided the light and the developmental stage are kept constant among samples.

The Multiplex® Sensor

Multiplex (FORCE-A, France) is a multi-parametric fluorescence sensor based on light-emitting-diodes (LED) excitation and filtered-photodiodes detection, dedicated to work in the field under daylight, on leaves, fruits and vegetables. The present version of the sensor has six UV-A (UV) LED-matrices light sources and three Red-Blue-Green LED-matrices (RGB). There are three synchronised detectors for fluorescence recording: yellow (YF), red (RF) and far-red (FRF). The sensor illuminates an 8-cm diameter surface at a 10-cm distance from the sources. The sensor records fluorescence signals and calculates a set of chosen ratios after each sequence of four-colour flashes (Table 1).

Fig. 2. Front view of Multiplex optical head with LED sources (6 UV & 3 RGB) and three detectors in the middle (YF, FRF, RF).

Table 1. Nomenclature of the Multiplex signals. The fluorescence excitation-emission matrix: emission abbreviation_excitation abbreviation.

	Excitation			
Emission	UV	Blue (B)	Green (G)	Red-Orange (R)
YF	**YF_UV**	**YF_B**	**YF_G**	**YF_R**
RF	**RF_UV**	**RF_B**	**RF_G**	**RF_R**
FRF	**FRF_UV**	**FRF_B**	**FRF_G**	**FRF_R**

Multiplex signals and ratios

The Multiplex sensor provides twelve signals (table 1). In the framework of precision farming, the Multiplex indices based on signal ratios related to epidermal content of flavonols and chlorophylls are of first importance for nitrogen management. We will concentrate on the chlorophyll-fluorescence signals that are the basis of Multiplex indexes, mainly FRF_UV, FRF_R, (FRF excited by UV and R light, respectively) and RF_R (RF excited by R) (table 1).

These single signals are influenced by the quantity of crops seen by the sensor. The more leaves, the higher the signals.

Two types of fluorescence excitation ratios (FER) are analysed here. First, the FER_RUV that is linked to shielding of leaves by flavonols according to Bilger et al. (Bilger et al.[2], 2001; Bilger et al.[3], 1997); second, the decadic logarithm of the R to UV excitation ratio of far-red chlorophyll fluorescence (FRF) that is proportional to the flavonol content of the leaf (Cerovic et al.[9],

2002) expressed on a surface basis, according to the Beer-Lambert law (Ounis et al.[12], 2001):

FLAV = logFER_RUV = log(FRF_R/FRF_UV)

The emission ratio SFR_R is linked to the chlorophyll content of the sample (Buschmann[4], 2007). It is a Simple Chlorophyll Fluorescence ratio (SFR) of far-red emission (FRF) divided by red emission (FR), with red-orange (R) excitation. Due to the overlap of the chlorophyll absorption and emission spectrum, re-absorption occurs at shorter wavelengths (RF) but not at longer wavelengths (FRF) (Buschmann[4], 2007). Therefore, the SFR=FRF/RF ratio increases with the increase of the chlorophyll content of the sample.

The NBI_G = FRF_UV/FR_G and NBI_R = FRF_UV/RF_R are both complex excitation-emission ratios that depend both on epidermal phenolics and chlorophyll contents.

Signal ratios are not influenced by variations of the sampling surface.

Sampling and Geolocalizing Multiplex Data

Multiplex can be used in single shot mode: it can save point by point and write on the sensor's screen means of many flashes and signals ratios. The number of flashes needed for one measurement can be changed by the user according to his specific needs of acquisition frequency or measurement speed. Multiplex can also be used in continuous mode: in this mode, each flash is saved continuously but is not written on the screen; ratios are not calculated in order to save time in data acquisition. So Multiplex can be integrated in different types of data acquisition systems and protocols as we will illustrate it in the following experiments.

Internal GPS operation.

The Multiplex sensor receives its own geo-localization data thanks to its internal GPS. Every data can be localized in space. This mode can be used for single shot measurements and embedded measurements. The user can record a large amount of data. In this case, the data acquisition frequency depends on both the number of flashes for one measurement and on the GPS frequency. The accuracy of the Multiplex's internal GPS is 1 to 3 meters.

The number of flashes and the tractor speed influence the data frequency and consequently the accuracy of maps. In order to acquire high data densities, the moving speed in the field can be adapted to the number of flashes and to the GPS frequency.

External GPS operation.

The Multiplex sensor can also be linked to a differential GPS or RTK-GPS in order to improve its positioning accuracy to a few centimeters. The link with an external GPS can be achieved directly in the Multiplex or thanks to a lap-top and a specific software developed by Force-A in order to store, in one file, Multiplex and GPS data. Thanks to this architecture, Multiplex data acquisition frequency can be up to 240 Hz. It means that every flash can be monitored. The GPS frequency is 1 Hz or 10Hz. In the case of a 1Hz GPS frequency, 240 Multiplex flashes can be acquired within one single GPS

acquisition. Thus high data frequency allows the Multiplex users to analyze very precisely the signal in order to set up proper data filtering algorithms.
The use of a lap-top will shortly allow to achieve other calculations and modelling in real-time in order to manage crop treatments in real-time while crop health status monitoring.

Sites and Sampling parameters

Two different fields were studied with two different architectures for data acquisition with Multiplex and GPS.

Experimental field #1

The field#1 was seeded with rapeseed. It is located in France near Evreux (48°52'42"N, 01°06'26" E) (figure 2). The field was divided in two areas with different soil properties: on one part a deep and heavy soil, and on the other part a light and shallow soil. Two cultivars were seeded at two different dates: *cv.* Catalina and *cv.* Adriana in September 2nd, 2008 and *cv.* Catalina in September 20th, 2008. Temperature observed during autumn 2008 didn't allow a good sprouting to rapeseed seeded lately. So the part of field seeded on September, 20th didn't sprout intensively before winter.

For each cultivar at each seeding date, crops were seeded according to six densities: 20 / 40 / 60 / 80 / 100 /120 g/m². The field is 700 meters long and 250 meters large. The Multiplex was embedded on a quad (figure 3). The quad was operated through the field in the length way every 10 meters, at a constant speed of 12 km/h.

GPS measurements were achieved at every u-turns of the quad (with a Garmin GPS, GPSMap76). At each u-turn, white papers were measured with Multiplex in order to localize u-turn in the data files. With this method, Multiplex measurements were correctly geo-localized. Data were acquired in continuous mode in order to store data with high frequency.

Table 2. Data acquisition frequency for Multiplex in continuous mode

Number of flashes	Acquisition frequency -Hz-
50	5
250	1
550	0,5

Multiplex measurements were performed on December 20th, 2008. Biomass measurements were also achieved for each seeding density and each soil by weighing crops harvested on a 1 m² area, corresponding to 34 samples.

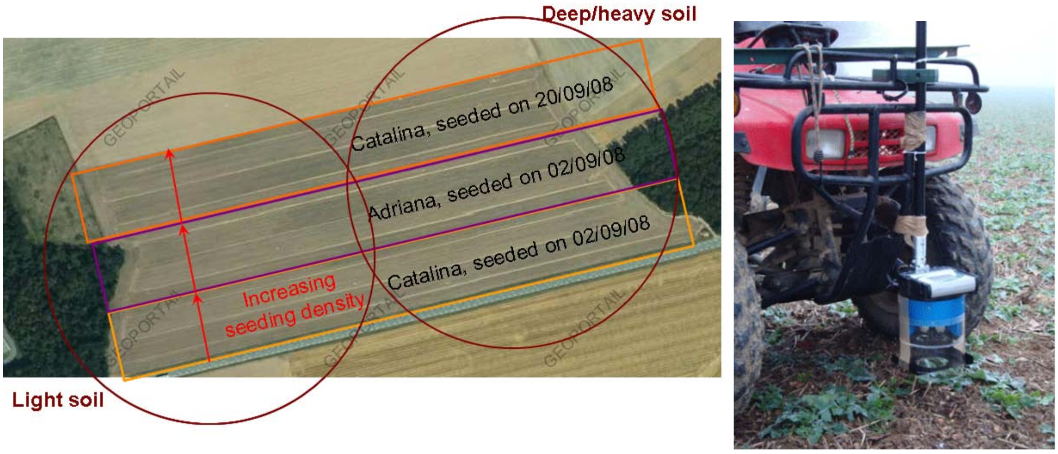

Fig. 3. Description of rapeseed experimental plot and picture of Multiplex embedded on quad

Experimental fields #2

We studied two fields of a farm seeded with wheat and located in France near Saint-Illiers-la-ville (48°58'31"N, 01°32'08" E) (figure 4). The 2 fields were seeded with Caphorn cultivar. Each field measured up to 10 ha. Northern field is called “Boulinets” and southern one “Grand Pré”.

Two Multiplex sensors were embedded on a sprayer boom on each side of the tractor (figure 5). The GPS used is the farmer’s differential GPS (EZ-Guide 500, Trimble). GPS data and Multiplex data were saved in a lap-top (Panasonic Toughbook) thanks to a proprietary Force-A software. A serial cable was used between the GPS and the lap-top, and serial and Ethernet links were used between the Multiplex and the lap-top.

Multiplex measurements were performed on April 27th, 2009. In the same time, Dualex measurements were achieved on each field.

Fig. 4. Localisation of the 2 wheat fields and picture of Multiplex embedded on sprayer boom.

On the fields picture, we localised the areas where Dualex measurements were realised. The yellow circle is the area where wheat was visually under nitrogen deficiency, the light green area where wheat looked like all over the field and the dark green area where wheat had visually more nitrogen content than all

over the field. This last area may be an area where nitrogen spreader went through two times during the second nitrogen treatment.
Yield has been monitored directly in the harvester through yield-sensor (New-Holland company).

Data treatment and geostatistical analysis

Experimental field #1

Multiplex stored the means of 100 flashes for each single recording and ratios. The Multiplex was used in continuous mode. The GPS acquisition frequency was 1Hz and the Multiplex frequency is 4Hz. The quad speed was 12 km/h. The distance between two GPS acquisitions was 3.3 meters.

In one second, 4 measurements were acquired on a 3.3 meter distance. Consequently, Multiplex integrated crops signals on a 83 cm distance.
The quad made one length acquisition per seeding density. So we acquired lines of data every 10 meters.

Data were mapped thanks to the 3DField mapping software, using the inverse distance interpolation method.

Experimental fields #2

In this case, Multiplex data frequency was 80Hz. GPS data frequency was 1Hz. Multiplex stored 80 flashes for one GPS point. The tractor speed was 10 km/h. So, one measure was stored every 4 centimetres. We achieved the means of the 80 measurements around the GPS data. Data were filtered in order to delete saturated signals.

Data were mapped thanks to the 3DField mapping software, using the inverse distance interpolation method.

Dualex leaf-clip measurements were performed in visually nitrogen-deficiency areas, in over-fertilised areas, and in medium nitrogen areas, on “Boulinets” and “Grand Pré”. Leaf-clip measurements have to be achieved in the middle of thirty flag-leaves in each area.

RESULTS AND DISCUSSION

Experimental field #1

We mapped Multiplex FRF_R signals and NBI ratios monitored on rapeseed crops (figures 6 and 7).

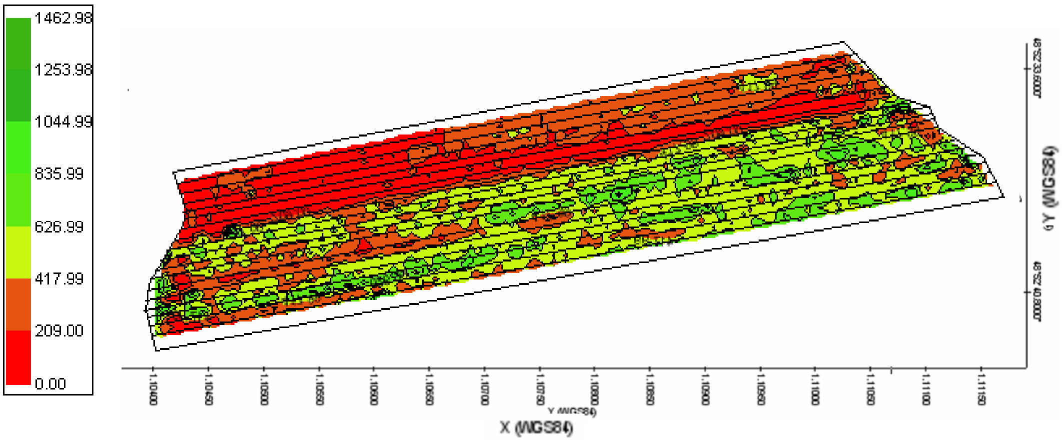

Fig. 5. Map of FRF_R signal (mV, millivolt unit) on rapeseed experimental plot on December 20th, 2008. Inverse distance interpolation method. WGS 84.

FRF_R signal varies from 0 to 1500 mV. FRF_R signal is lower for Catalina seeded on September 20th, 2008. FRF_R is higher for Adriana and Catalina seeded on September 2nd, 2008. Areas corresponding to FRF-R lower to 500 mV appear mainly in light soil and for low grain density. In deep and heavy soil, FRF_R is higher for every cultivar. For low seeding density, FRF_R is lower in light soil than in heavy and deep soil.

The mapped data are means of Multiplex signals integrated on a 83 cm distance. So Multiplex data is the mean of the plant optical signature (fluorescence signature) and of the soil optical signature, which is null. The more crops the higher the signal. The mean of FRF_R on 83 cm is higher in deep soil and for early seeding data. It means that the crop density is higher in these areas.

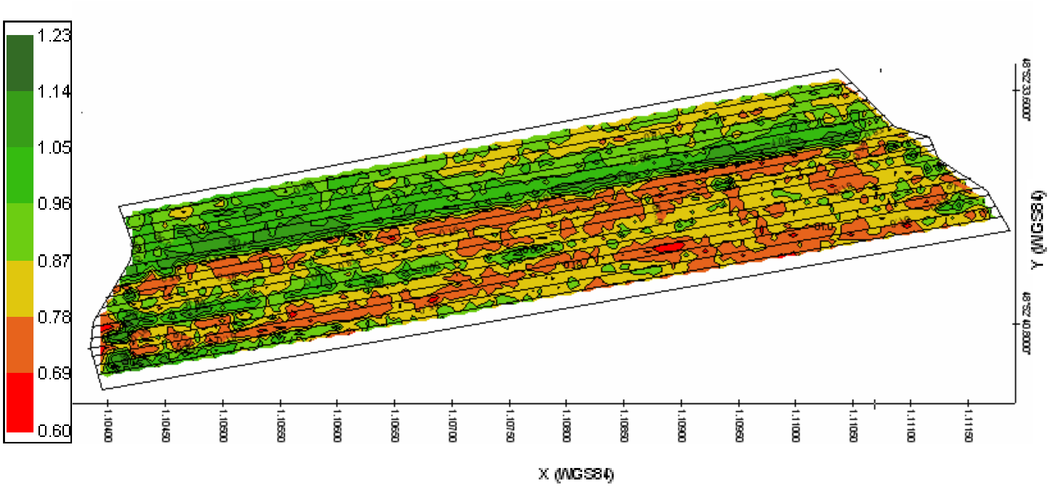

Fig. 6. NBI_R ratio on rapeseed experimental plot on December 20th, 2008. Inverse distance interpolation method. WGS 84.

Figure 5 shows that the NBI index is higher for Catalina seeded on September 20th, 2008. For small seeding densities, NBI is higher in light soil than in heavy and deep soil. So, rapeseed plants have a higher nitrogen status in these areas.

Table 3. Biomass destructive measurements and plant counting.

Soil	Cultivar	Seeding date	Seeding	Number of	Biomass	Biomass per

			density	plant		plant
			-seed/m^2-	-nb/m^2-	-g/m^2-	-g/plant-
Heavy and deep soil	Catalina	02/09/2008	20	12	600	50
			40	23	935	40.7
			60	33	1060	32.1
			80	37	915	24.7
			100	41	810	19.8
			120	47	1005	21.4
	Adriana	02/09/2008	20	19	950	50
			40	15	890	59.3
			60	32	1090	34.1
			80	41	990	24.1
			100	44	1025	23.3
			120	86	975	11.3
	Catalina	20/09/2008	20	19	185	9.7
			40	22	165	7.5
			60	28	360	12.9
			80	45	300	6.7
			100	63	695	11
			120	61	465	7.6
Light Soil	Catalina	02/09/2008	20	14	820	58.6
			40	10	655	65.5
			60	32	1130	35.3
			80	28	860	30.7
			100	51	740	14.5
			120	44	930	21.1
	Adriana	02/09/2008	20	14	460	31.9
			40	30	1140	38.3
			60	33	1100	33.5
			80	21	1175	55
			100	46	985	21.4
			120	42	985	23.5
	Catalina	20/09/2008	20	16	300	18.8
			40	16	160	10
			60	21	230	10.9
			80	30	305	10.2
			100	48	430	8.9
			120	30	245	8.2

In low seeding density areas, plants are large and not numerous; in high seeding density areas, plants are numerous and small. In low seeding density and light soil areas, plants benefit from available space and nitrogen. In other areas, nitrogen competition between crops takes place and limits crop growth.

Nitrogen status is higher, consequently NBI is higher in low crop density and light soils areas.

Table 4. Correlation matrix between crop weight (kg/ha) and single signals and Multiplex ratios on December 20th, 2008. (Pearson coefficients. In red if $p<0.05$).

Signal	Wheight
	-kg/ha-
FRF_R	0.65
FRF_UV	0.63
SFR	0.65
FLAV	0.64
NBI	-0.49

All the correlations are significant. Differences between crop weights and Multiplex signals can be explained because the sampling sites for biomass measurements and monitored surfaces with Multiplex are not exactly the same. The relationship between biomass and NBI is negative because of crop nitrogen competition (nitrogen dilution in whole crop canopy).

Thus Multiplex signals can be used to determine different areas of crop canopy density and different areas of crop nitrogen status in the field. Farmers can achieve their own maps for the management of treatments. Multiplex can be used to monitor crops and then manage fertilizing treatments.

Experimental fields #2

The following maps have been achieved with data monitored in the wheat fields near Saint-Illiers-la-Ville.

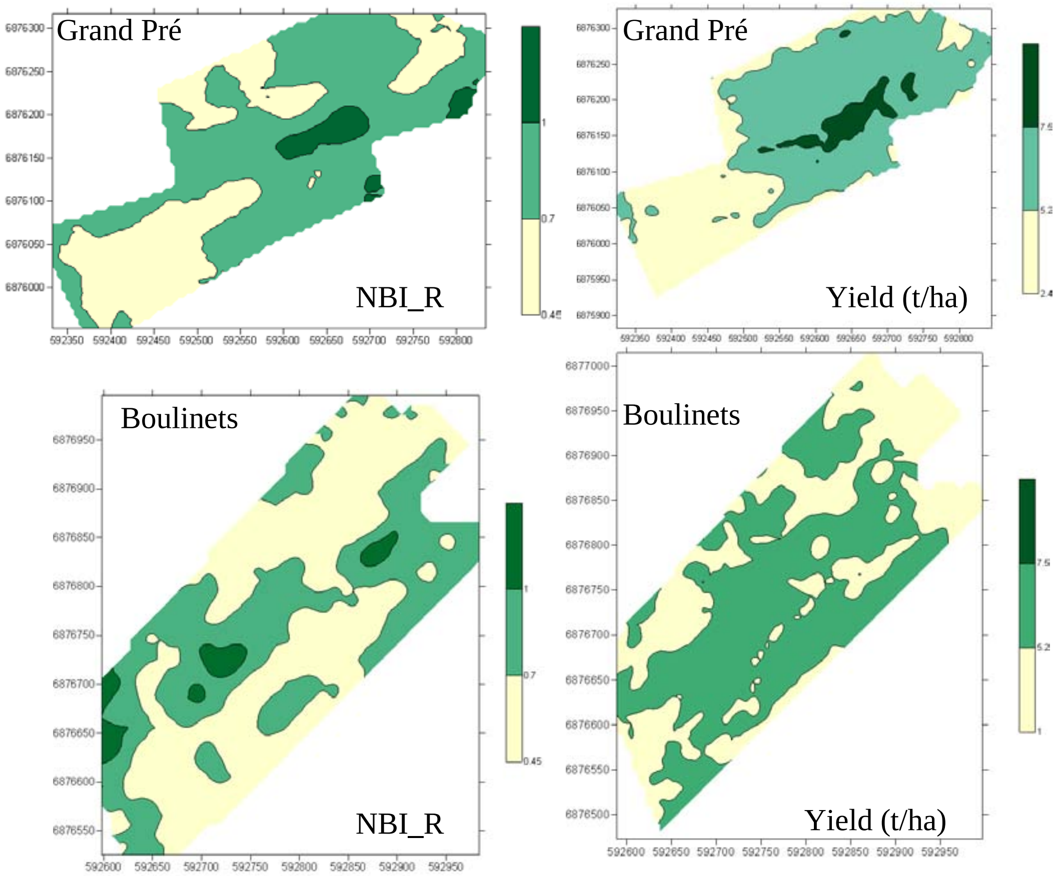

Fig. 7. NBI_R ratio on wheat experimental plots on April 27th, 2009, two nodes stage, and yield monitoring at harvest (t/ha). Wheat *cv.* Caphorn. Kriging interpolation method. Lambert 93.

NBI varies inside the field and between fields. NBI is lower in means in “Boulinets” than in “Grand Pré”.

In “Grand Pré” the large low NBI area in south-western corresponds to a low yield area and the high NBI area in the middle of the field corresponds to a high yield area.

We can observe that NBI map allows localizing some low NBI areas that are not noticed on yield map. It is due to the fact that the farmer performed its third nitrogen treatment after the achievement of the experiment, while modulating its nitrogen treatment according to the different areas he noticed through different previous mappings.

In “Boulinets”, we can observe a medium NBI and yield area in the middle of the field and two low NBI and yield areas in north and south of the field.

In this case also, little differences or similarities disappeared because of the management of the third treatment by the farmer.

NBI maps highlight same areas than yield's maps.

Dualex leaf-clip measurements realised in the different areas were used in the nitrogen management modelling system developed by Force-A in order to obtain nitrogen quantity to spread in the field. The decision rules adapted in the Dualex have shown through 2 years of trials in cooperative structures in France :
-good results for nitrogen management,
-good management of nitrogen treatment date,
resulting in yield and protein content increase in different conditions. It has also been compared to other nitrogen management tools.

Table 5. Dualex measurements of chlorophyll content (µg/cm²), flavonol content (Dualex units), NBI, and quantity of nitrogen to dress (uN = kgN/ha).

Field name	Fertilisation level	CHL		FLAV		NBI		NBI area/NBI X+	Nitrogen to spread
		Mean	Sd	Mean	Sd	Mean	Sd		
		-µg/cm²-		-Dualex units-					-uN (kgN/ha)-
Grand Pré	X+	121.2	10.2	2.1	0.2	57	7.4	1	0
	X	98.1	10.8	2.4	0.2	42.1	8.7	0.7	40
	X-	62.4	8	2.8	0.2	22	3.2	0.4	80
Boulinets	X+	101.8	11	2	0.3	51.8	8.7	1	0
	X	75.6	8	2.5	0.2	30.8	5.1	0.6	40
	X-	60.4	8.8	2.9	0.1	21.3	3.8	0.4	80

Sd: Standard deviation

We can observe that NBI and chlorophyll content increase while flavonol content decreases with nitrogen content.

Multiplex highlights crop areas with different nitrogen status. So nitrogen management maps can be achieved and then applied. The scale of nitrogen to dress can be adapted to the Multiplex maps. Indeed, Dualex measurements can be compared to Multiplex measurements and so Dualex nitrogen advices can be linked to areas assessed by Multiplex.

CONCLUSION

Dualex is a new leaf-clip that assesses crop nitrogen status in order to manage nitrogen treatments.

Multiplex is a new proximal optical sensor that measures crop canopy density and crop nitrogen status in the fields. It can be associated with Dualex in order to create nitrogen management maps.

Multiplex can be used in many data acquisition systems according to its wide range of functionalities and softwares aiming shortly at real-time management of crop treatments. Thanks to the use of fluorescence, Multiplex can be used to monitor a large range of physiological parameters on crops.

Force-A is continuously developing new accessories and softwares to automatize data processing for real-time zoning and crop management. In next few years, Force-A will develop Multiplex's own decision rules in order to get free of Dualex use.

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