



A non-invasive optical method for quality control of maturing and cured tobacco leaves

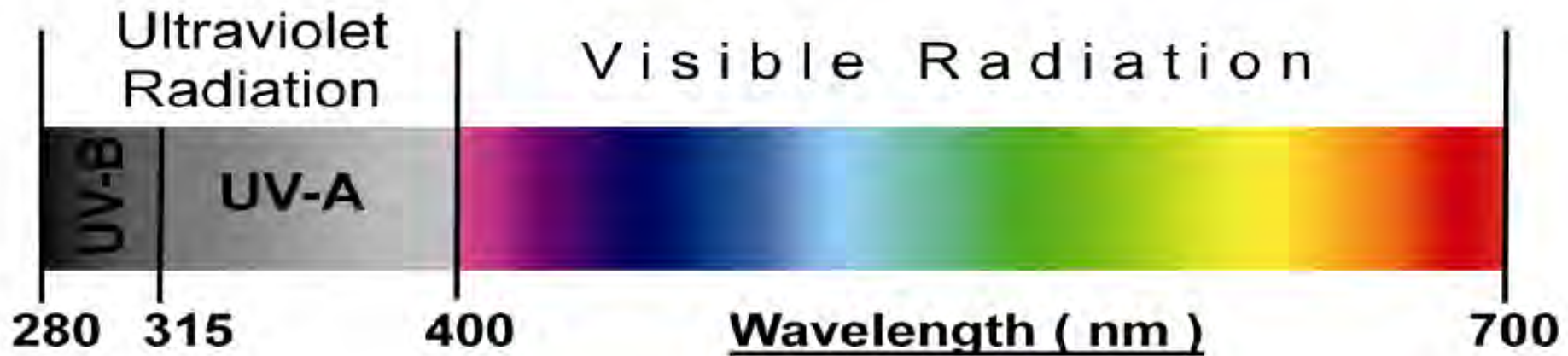
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NON-INVASIVE OPTICAL METHODS

THEY WORK ON LIGHT SPECTRAL RADIATION



AND TRY TO FIND CORRELATIONS BETWEEN SELECTED SPECTRAL EMISSIONS AND GIVEN PLANT COMPONENTS

IF ALL THE PROCESS RUNS SMOOTHLY YOU CAN DETERMINE HOW MUCH OF A GIVEN COMPONENT IS PRESENT IN THE PLANT

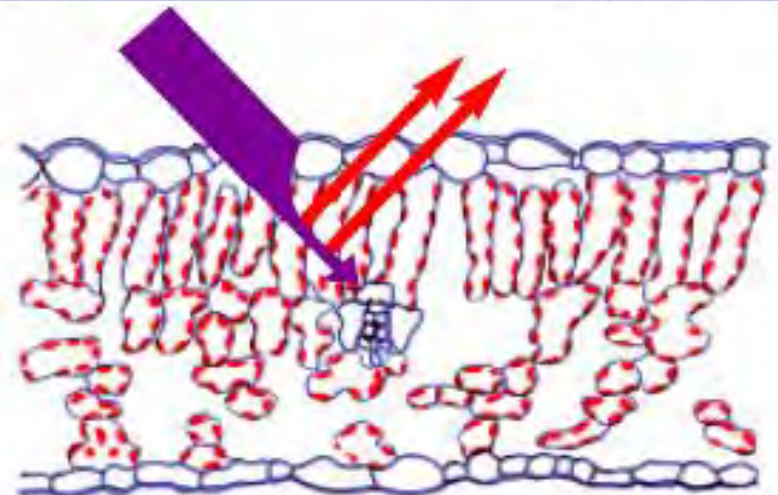
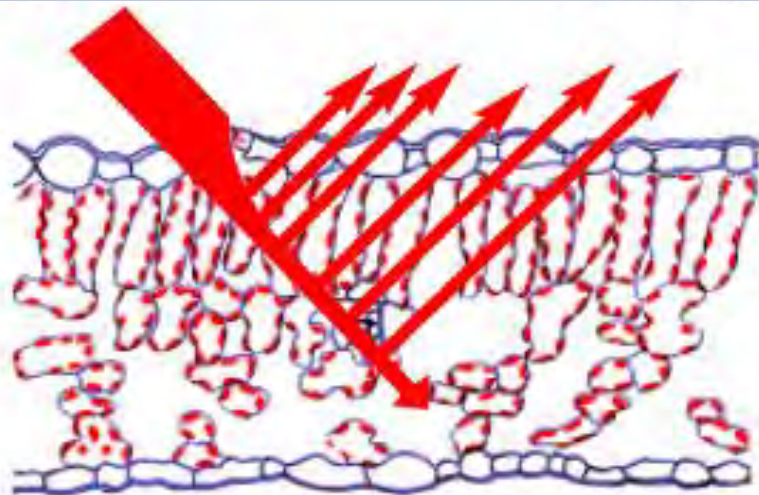
NON-INVASIVE OPTICAL METHODS

A TOBACCO LEAF

How Chlorophyll fluorescence change w/

Red Excitation

UV Excitation



THE DIFFERENCE IS RELATED TO LEAF POLYPHENOL CONTENT

NON-INVASIVE OPTICAL METHODS

PRELIMINARY ACTIVITY

- ❁ A time-consuming and expensive calibration process (survey + sampling + optical measurements + destructive analyses)

BUT ... WHEN A CONSISTENT AND AFFORDABLE CALIBRATION HAS BEEN DONE

- ❁ Reliable, easy and quick detection of the given parameter (or the given parameters)
- ❁ Repeated measurements of the same plant part to investigate some critical phase in progress (e.g. leaf or fruit maturation)
- ❁ Remote sensing and GPS-driven treatments (=PRECISION AGRICULTURE) become an easier target

MULTIPLEX[®] 3 FLUORESCENCE SENSOR

(FORCE-A, FRANCE)

- ❁ It consists of a portable battery-powered fluorimeter with light-emitting diode (LED) matrices as light sources in the UV-A (370 nm), blue (460 nm), green (515 nm) and red (637 nm) spectral regions.
- ❁ The Chlorophyll fluorescence in the red (RF) band, at 680–690 nm, and in the far-red (FRF) band, at 730–780 nm, is acquired sequentially at all the excitation wavelengths.
- ❁ Different combinations of the RF and FRF fluorescence signals at the various excitation bands can be used as indices of different compounds, e.g. anthocyanosides (520 nm), flavonols (350 nm), etc..



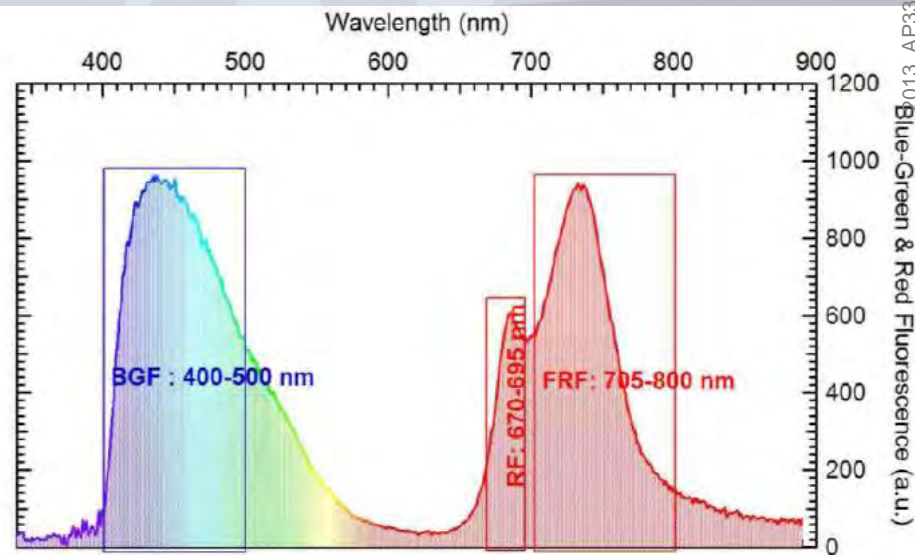
MULTIPLEX[®] 3 FLUORESCENCE SENSOR

(FORCE-A, FRANCE)

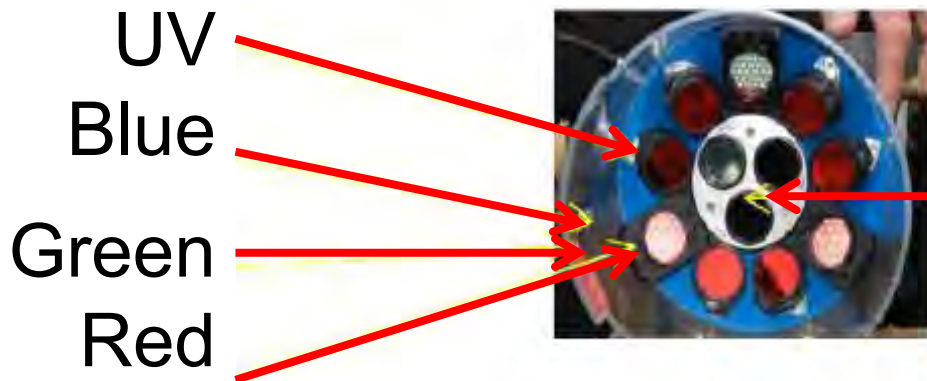
- ✿ Since the LED sources are pulsed and synchronized to the detection, the sensor is insensitive to ambient light and can be used directly in the field.
- ✿ The detection area of the sensor is circular, with a 8 cm diameter at a distance of 10 cm from the light sources (50 cm²)
- ✿ A single measurement consists in the sequence of 4 excitation flashes and detection of the respective fluorescence signals, repeated 500 times and averaged, for a total acquisition time of less than 1 s.



MULTIPLEX 3 FLUORESCENCE SENSOR (FORCE-A, FRANCE)



LED Excitation Flashes



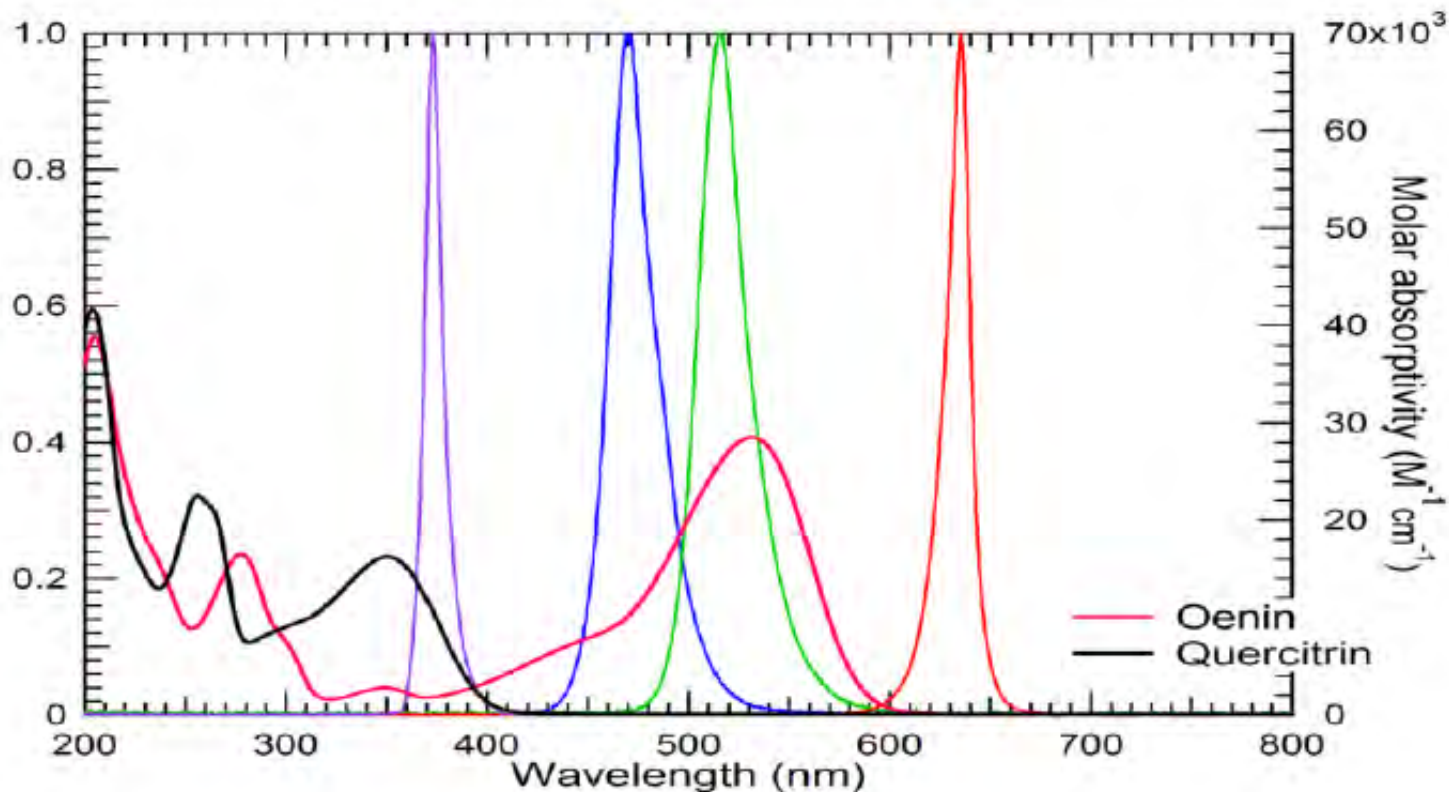
3 Light Diode detectors

- F Blue-Green
- F Red
- F InfraRed

MULTIPLEX 3 FLUORESCENCE SENSOR (FORCE-A, FRANCE)

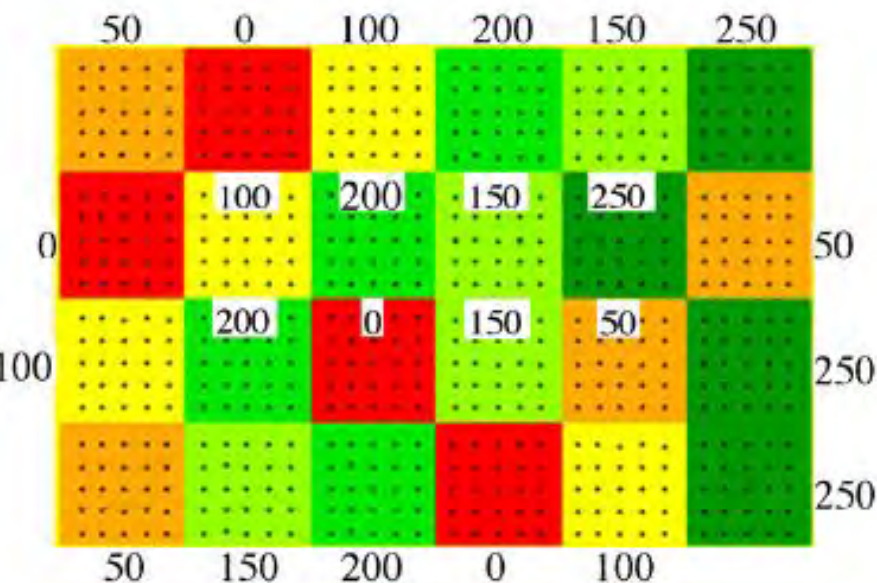


Multiplex emission bands and its use for Quercetin and Oenin detection & quantification in red grape skin

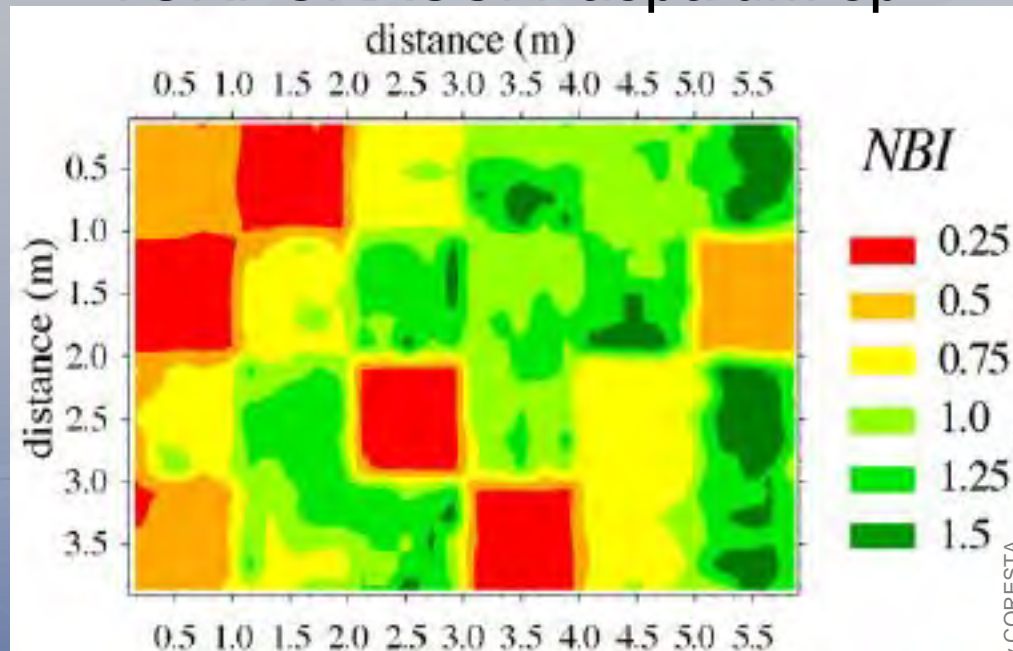


MULTIPLEX[®] 2 USE TO ASSESS LEAF NITROGEN CONTENT

G. Agati et al. / Europ. J. Agronomy 45 (2013) 39–51



TURFGRASS: Paspalum sp.

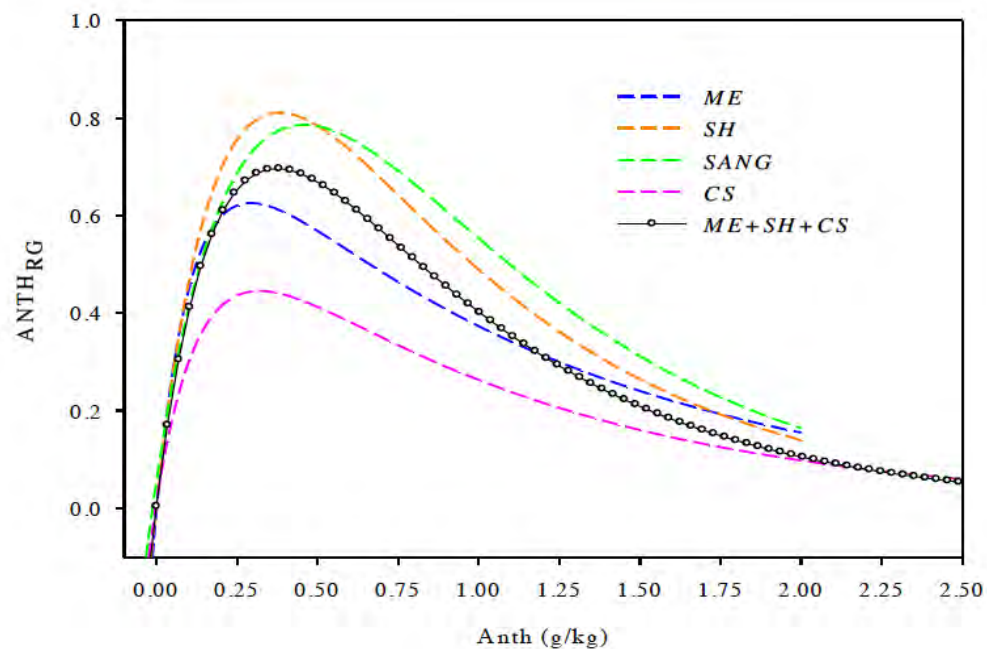
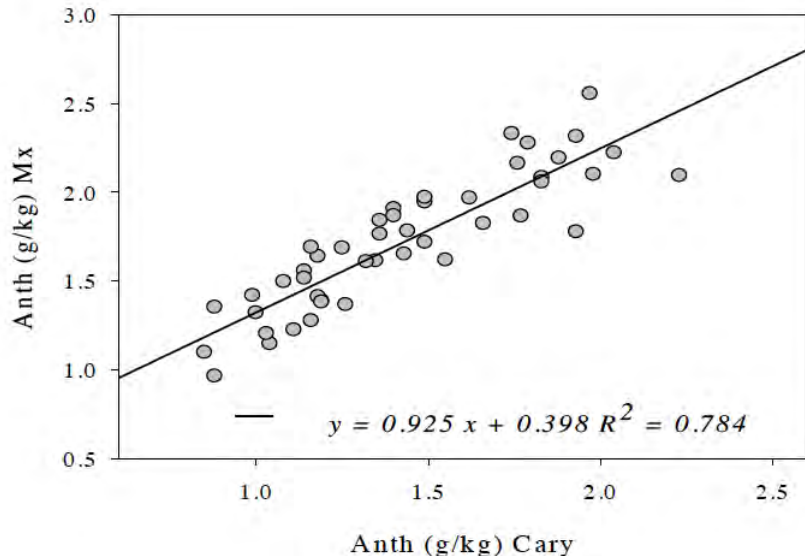


NBI = Index of Nitrogen Balance derived from Chlorophyll fluorescence measurements in the Far-Red and Red wavelengths and Leaf Flavonoids, measured in the Far-Red and UV bands

MULTIPLEX[®] 3 USE TO ASSESS GRAPE PHENOLIC MATURITY

Correlation between Cary's (spectrophotometric, destructive) and Multiplex (non-destructive) measurements

(Fierini et Al., 2012-2013)



MULTIPLEX[®] 3 USE & PRECISION AGRICULTURE

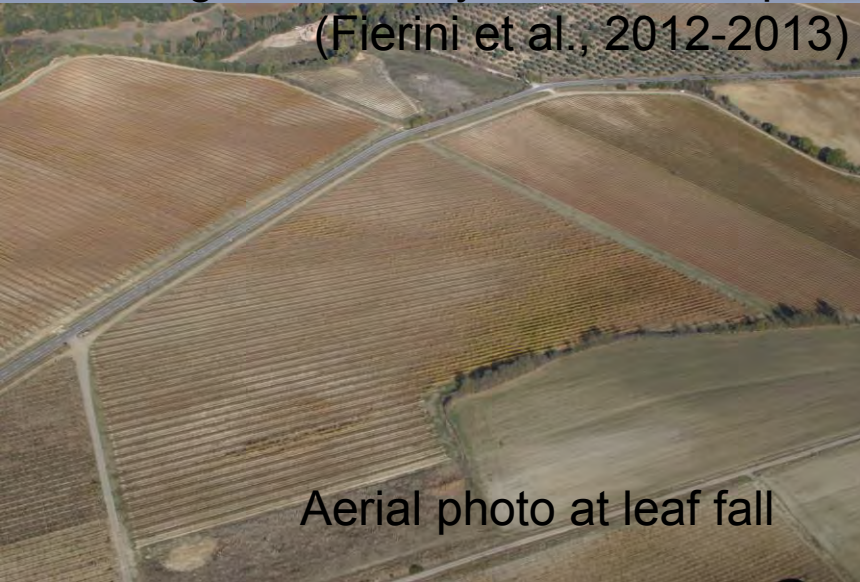


Vineyard 04.09 Castello Banfi
(42°58' N, 11°26' E)-Google Maps

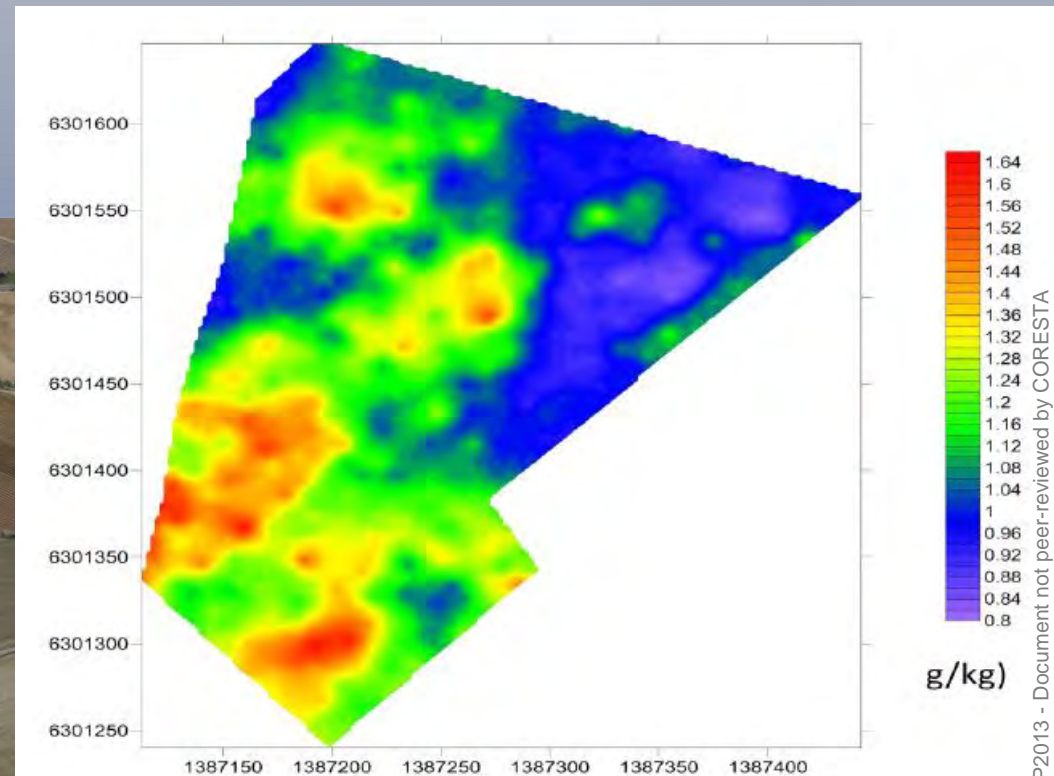


Courtesy Banfi,

Mapping anthocyanosides in a Sangiovese vineyard with Multiplex (Fierini et al., 2012-2013)



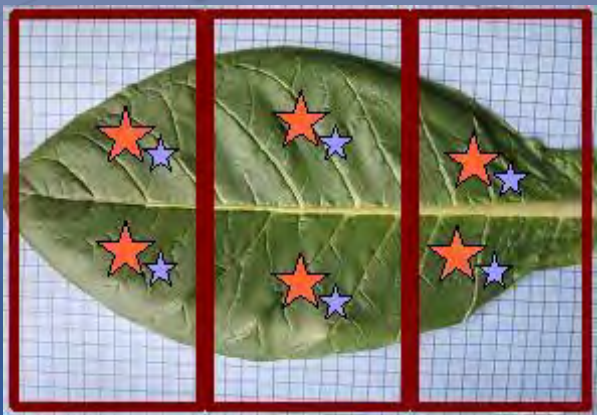
Aerial photo at leaf fall



MULTIPLEX[®] 3 AND TOBACCO

(FORCE-A, FRANCE)

- ❁ On our knowledge, this is the first calibration activity carried out on tobacco worldwide
- ❁ Calibration for leaf flavonols and nicotine derivatives has been in progress since 2011 (Phytolab & Consortium INSTM activity)
- ❁ An impressive No. of Multiplex (MU) measurements and parallel destructive analyses has been carried out, both on fresh and cured leaves of different varieties, to assess the feasibility of the technique



MU LEAF UPPER SIDE



MU LEAF LOWER SIDE



HYDROALCOHOLIC
EXTRACT + HPLC

MULTIPLEX[®] 3 AND TOBACCO

(FORCE-A, FRANCE)

- ❁ Field calibration has begun at Fattoria Autonoma Tabacchi this 2013 season, testing different Virginia Bright and Kentucky varieties, and tobacco from experiments (nematode tests, fertilization tests, etc.) at various crop stages.
- ❁ These data are presently under investigation and will be presented in a dedicated paper



MULTIPLEX[®] 2 AND TOBACCO

(FORCE-A, FRANCE)



EXAMPLES OF 2011-2012 ACTIVITY

HPLC = $\mu\text{M/g FW}$

ITB 678

Part [?]	Under [?] test [?]				Multiplex [?]		HPLC [?]	
	FRF_R [?]	NBI_R [?]	FLAV [?]	FRF_R/FLAV [?]	flav [?] tot [?]	der [?] nic [?]		
DISTAL[?]	5429 [?]	3,3363 [?]	0,2204 [?]	-6527 [?]	1,631 [?]	10,648 [?]		
CENTRAL[?]	6696 [?]	3,6960 [?]	-0,0299 [?]	887 [?]	0,727 [?]	7,195 [?]		
PROXIMAL[?]	6398 [?]	6,3235 [?]	-0,3480 [?]	-37225 [?]	0,368 [?]	8,548 [?]		

[?]PVH 2310

Part [?]	Under [?] test [?]				Multiplex [?]		HPLC [?]	
	FRF_R [?]	NBI_R [?]	FLAV [?]	FRF_R/FLAV [?]	flav [?] tot [?]	der [?] nic [?]		
DISTAL[?]	3431 [?]	3,2841 [?]	0,5577 [?]	-3926 [?]	3,727 [?]	7,604 [?]		
CENTRAL[?]	3998 [?]	3,0482 [?]	0,0745 [?]	-1291 [?]	1,481 [?]	3,097 [?]		
PROXIMAL[?]	5293 [?]	4,9905 [?]	-0,4660 [?]	-24284 [?]	0,637 [?]	4,021 [?]		

MULTIPLEX[®] 2 AND TOBACCO

(FORCE-A, FRANCE)



EXAMPLES OF 2011-2012 ACTIVITY

ITB 678

mg/g Fresh Weight			
	DIST.	CENTR.	PROX.
acid monocaffeoyl quinic	0,277	0,148	0,182
acid monocaffeoyl quinic	1,436	1,071	1,489
acid monocaffeoyl quinic	0,491	0,335	0,424
quercetin rhamnosyl-glycoside	0,893	0,394	0,192
kaempferol rhamnosyl-glycoside	0,099	0,048	0,031
NICOTINE	1,725	1,166	1,385

MULTIPLEX[®] 2 AND TOBACCO

(FORCE-A, FRANCE)



EXAMPLES OF 2011-2012 ACTIVITY

PVH 2310

mg/g Fresh Weight			
	DIST	CENTR.	PROX
acid moncaffeoyl quinic	0,410	0,343	0,372
acid moncaffeoyl quinic	1,475	0,935	0,864
acid moncaffeoyl quinic	0,541	0,427	0,400
quercetin rhamnosyl-glycoside	2,088	0,833	0,350
kaempferol rhamnosyl-glycoside	0,181	0,069	0,038
NICOTINE	1,232	0,502	0,651

?

CONCLUSIONS

- ❁ Non-invasive optical methods have received so far great attention to evaluate grape and fruit maturation and quality, due to the previous, intensive calibration activity done
- ❁ This is the first application on tobacco, as far as we know
- ❁ Relationships between Multiplex indices and HPLC analyses for leaf nicotine and selected polyphenols are under developing due to an intensive lab calibration activity carried out by Phytolab & INSTM (2011-2012)
- ❁ 2013 activity at Fattoria Autonoma Tabacchi has extended this calibration activity to tobacco crops in the field, to test the “body of evidence” developed until now.



THANK YOU

QUESTIONS?