Real-Time Optical Solutions for Plant Sciences, Agri-food Industry and Agriculture

-

Fast and non destructive measurement of Polyphenols

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Summary

1. Force-A
2. Plant surfaces properties and polyphenols
3. The Log FER method
4. Dualex 3.3
5. Multiplex 3
6. Dualex 4
7. Applications
8. New Force-A technologies to come...

1. Force-A

Force A
VOIR POUR AGIR

***F**luorescence and*
***O**ptoelectronics*
***R**esearch for the*
***C**ommunication*
between
***E**cophysiology and*
-
***A**griculture*

CNRS spin off, University Paris 11.

Award-winner in 2002 and 2004 by the French government for its project of technology transfer

More than 15 years of R&D experience in the field of Photosynthesis and Optical Remote Sensing

- Located in Orsay (south of Paris)
- Today : 16 persons
- Born in 2004
- Completes € 2M Funding Round

- *Define and use new optical signatures of vegetation to monitor its physiological state*
- *Expertise : the **Fluorescence***

Leaf Clips

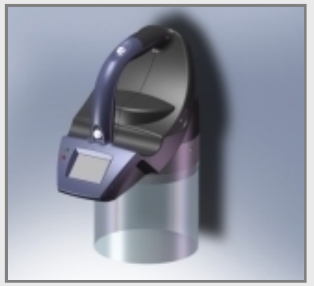
Dualex

Precision research

Nitrogen Management

Proximal Sensors

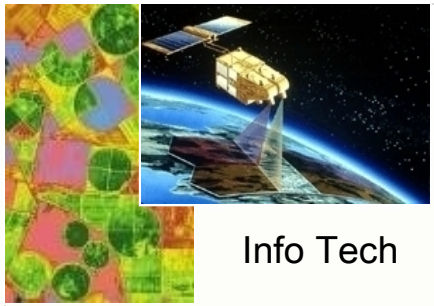
Multiplex



Hand-held

Multi-parametric

The New Agriculture



Info Tech

Sustainable Agriculture

Conservation

Integrated Crop Management

Force A
SEE TO ACT

Organic Agriculture

Crop Management - ROI

Precision Agriculture

Quality & Taste

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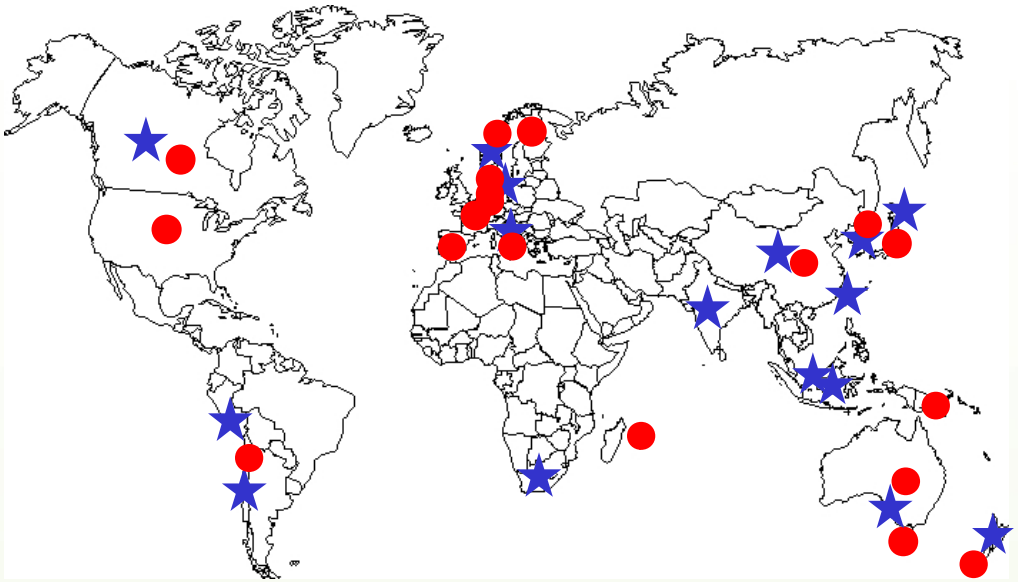
Distribution Channels and Sales to Date



International Distribution Network

Sales To Date

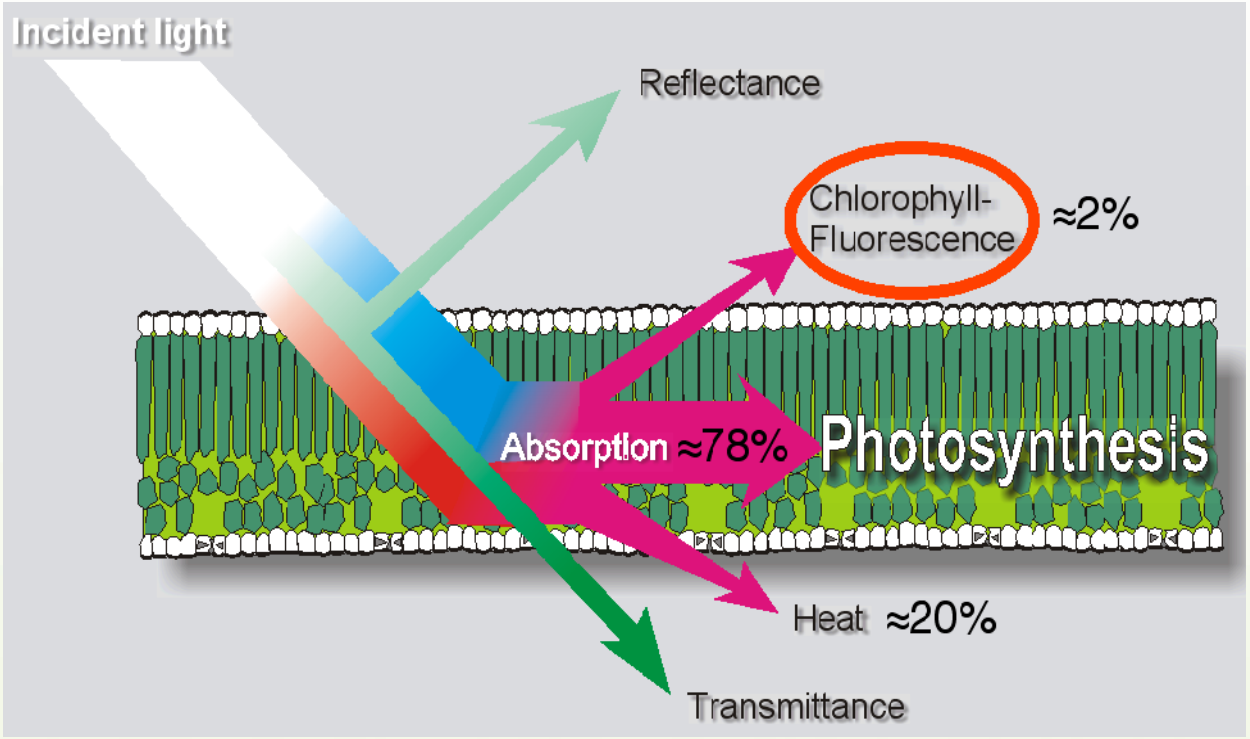
- **Plant Science customers**
- Direct Sales
- Distributor network

- **Agriculture customers**
- Direct sales (France & big players)
- Distributor network
- Foreign offices in selected countries (2010)

2. Plant surfaces properties and polyphenols

Leaf-light interactions

Wheat leaf under ambient light

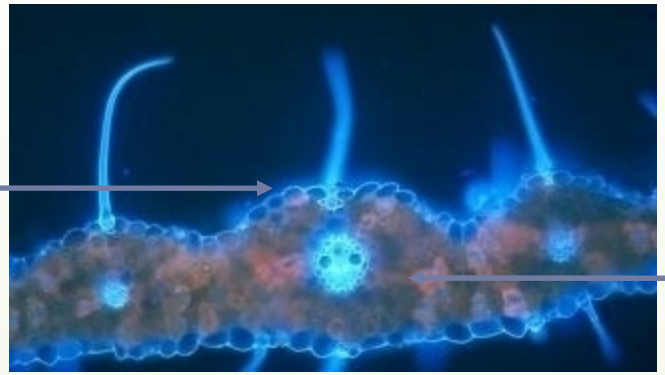


biomass = 3 %

Heat = 97 %

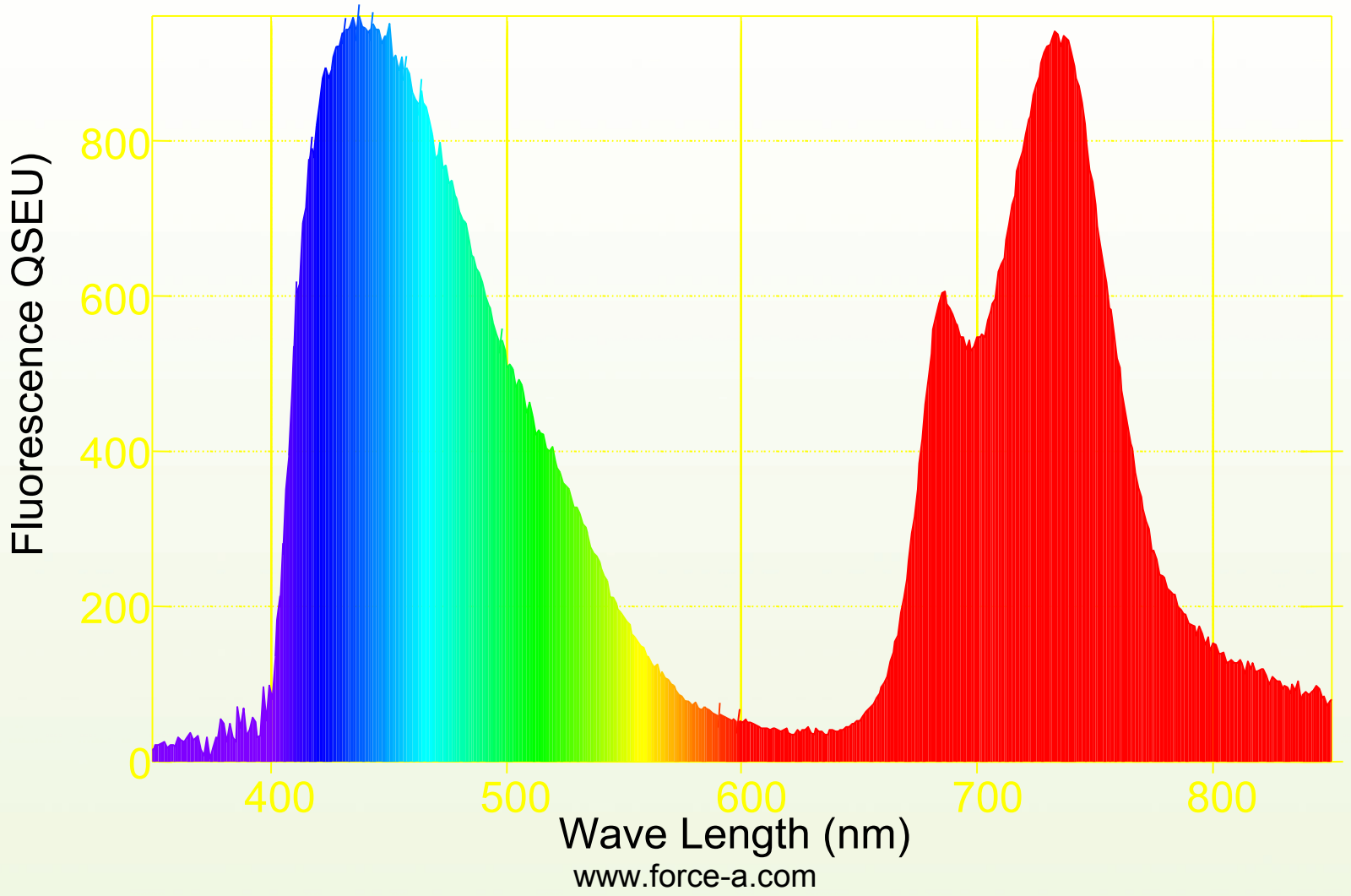
Fluorescence microscopy of a wheat leaf (337 nm)

Blue Green
Polyphenols
Fluorescence



Red
Chlorophyll
Fuorescence

Fluorescence emission spectrum of a wheat leaf



Polyphenolics Measurements Methods

Phen absorption properties

UV or visible absorption ranges

Use of the screening effect by epidermal phens of the excitation of chlorophyll fluorescence

Fluorescence excitation ratios of far red chlorophyll fluorescence

Phen fluorescence properties

UV or visible excitations

visible emissions

Fluorescence emission signals or ratios

Polyphenolics families

Flavonoids

Anthocyanins

Polyphenolics families

Hydroxycinnamic acids

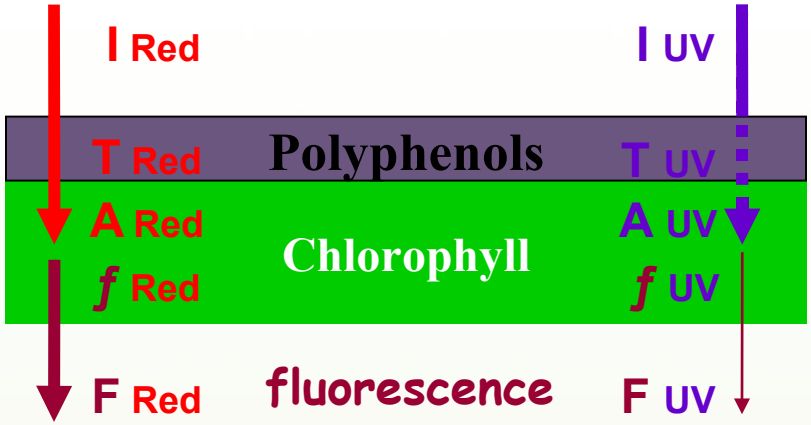
3. The Log FER Method

FER = Fluorescence Excitation Ratio

Calculation of the Optical Absorbance of Polyphenols.

- Screening effect of the polyphenols layer over the chlorophyll
- One beam screened by epidermis polyphenols
- One beam not screened by polyphenols
- Dual chlorophyll fluorescence emission detection

FER = Fluorescence Excitation Ratio



I Red = RED LIGHT EXCITATION

I UV = UV RADIATION EXCITATION

I = IRRADIANCE

T = EPIDERMAL TRANSMITTANCE

A = MESOPHYLL ABSORBANCE

f = FLUORESCENCE YIELD

F = CHLOROPHYLL FLUORESCENCE

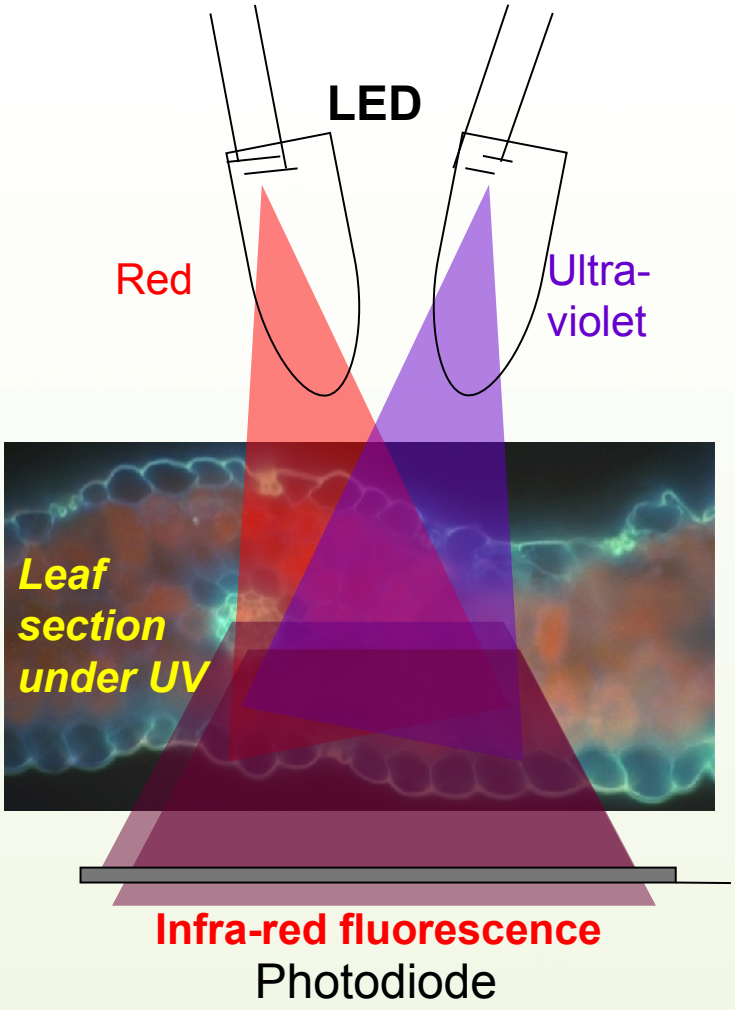
$$\frac{F_{Red} = I_{Red} * T_{Red} * A_{Red} * f_{Red}}{F_{UV} = I_{UV} * T_{UV} * A_{UV} * f_{UV}}$$

for $T_{Red} = 1$ and $\frac{I_{Red} * A_{Red} * f_{Red}}{I_{UV} * A_{UV} * f_{UV}}$ = constant

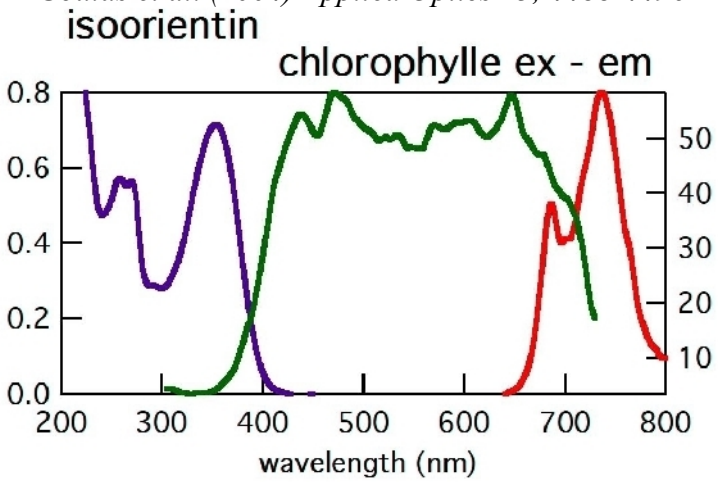
$$\log \frac{F_{Red}}{F_{UV}} = \log \frac{1}{T_{UV}} = A_{Flav}$$

4. Dualex 3.3

Dualex Flavonols measurement principles



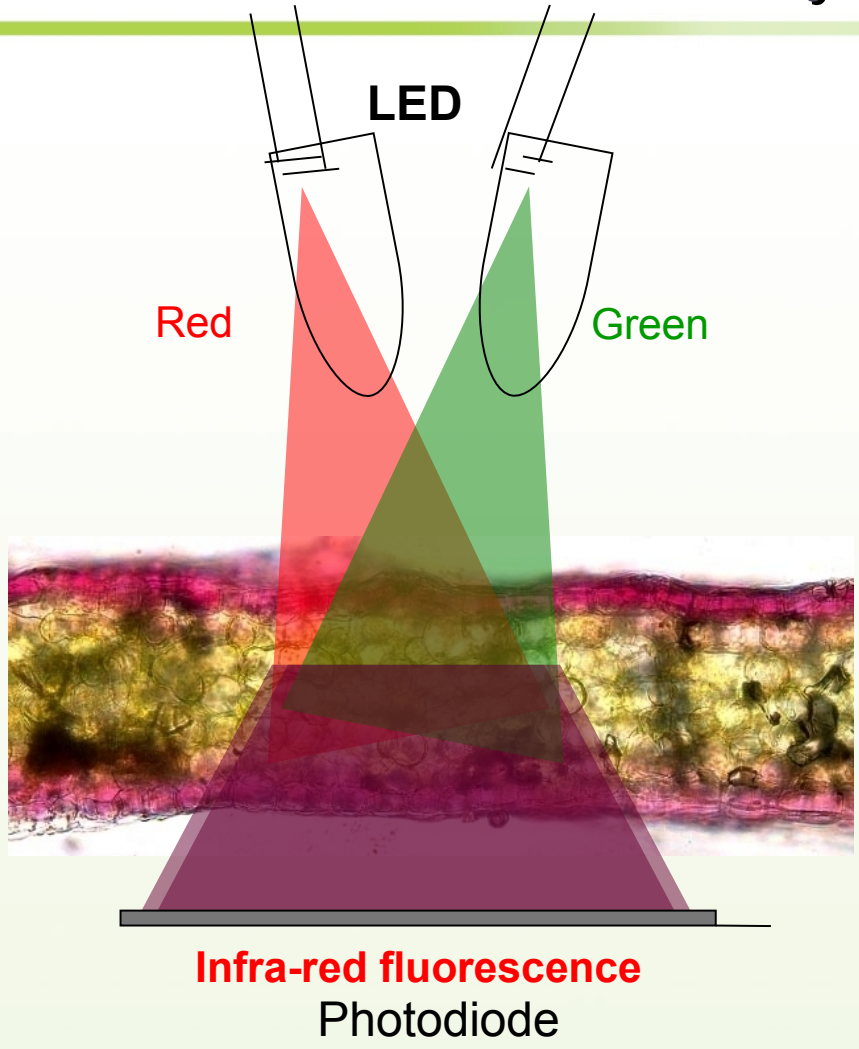
*Goulas et al. (2004) Applied Optics **43**, 4488-4496*

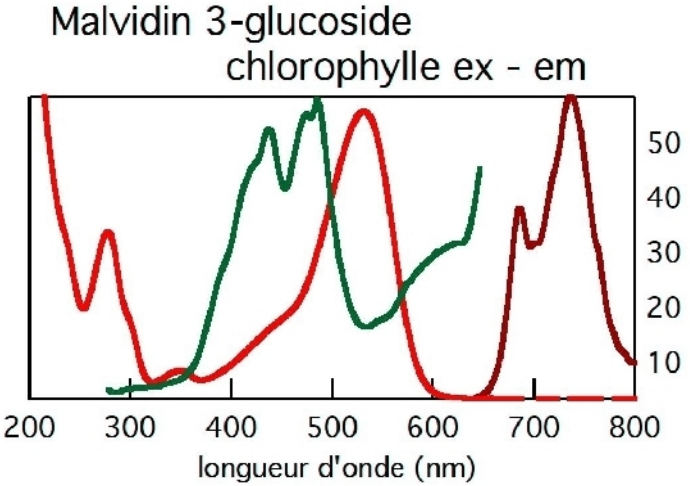


Flavonols are present in the epidermis. They absorb UV radiation and screen the mesophyll.

Chlorophyll from the mesophyll emits near-IR fluorescence measurable on both sides of the leaf.

Dualex Anthocyanins measurement principles





When anthocyanins are present in the epidermis they absorb green light and screen the mesophyll.

Chlorophyll from the mesophyll emits near-IR fluorescence measurable on both sides of the leaf.

3 versions for 3 types of polyphenols

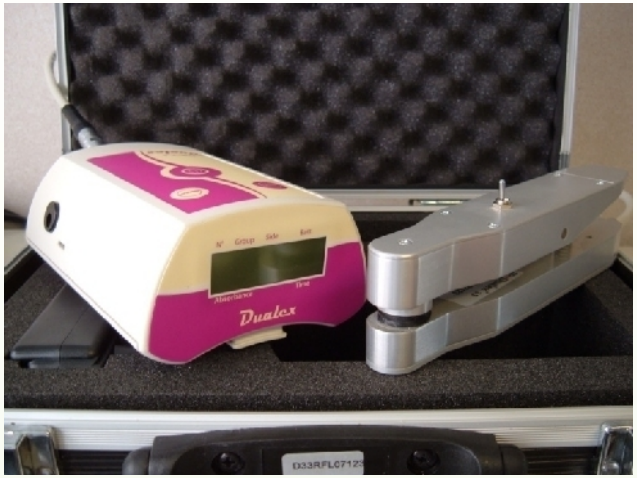
Three types of polyphenols:

- **DUALEX HCA:** In the UV-B at 315 nm
- **DUALEX FLAV:** In the UV-A at 375 nm
- **DUALEX ANTH:** In the VIS at 530 or 590 nm

Three types of polyphenols:

- Hydroxycinnamic acids
- Flavonoids
- Anthocyanins

The easy to use Dualex 3.3 series



Polyphenols measurement on leaves, berries, fruits and vegetables skins.

Measurement on non bearing chlorophyll samples with a specific optical filter

Dualex 3.3 Advantages

- Non-destructive, fast and easy-to-operate
- Field measurements
- No preparation of the plant
- Any ambient light conditions
- User-friendly leaf-clip design

5. Multiplex 3

Multiplex 3 Capacities

- 4 Excitations
- 3 Emission detections
- 12 parameters
- Log FER Method and other methods
- Remote sensing
- Geolocalized datas (GPS)
- SD car data logging and USB real time download

Multiplex 3 Calculations

- FLAV content

$$FLAV = \log \frac{FRF_R}{FRF_UV}$$

- Anthocyanins content

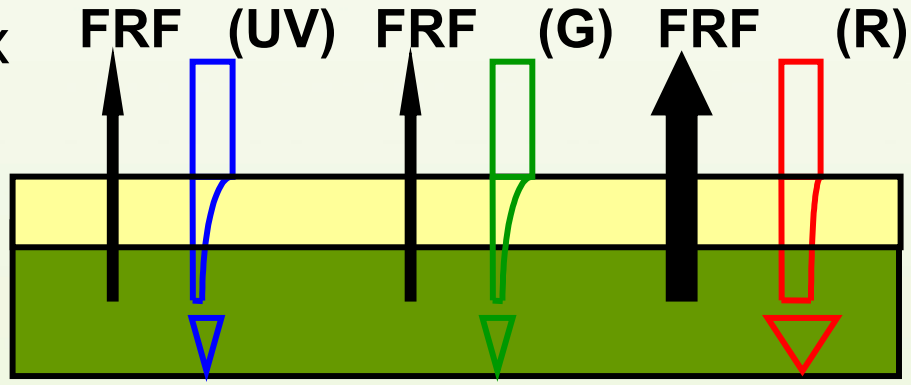
$$\begin{cases} ANTH = \log \dfrac{FRF_R}{FRF_G} \\ ANTH = \log \dfrac{5000}{FRF_R} \end{cases}$$

- Chlorophyll content

$$SFR = \frac{FRF_R}{RF_R}$$

- NBI: Nitrogen Balance Index

$$\begin{cases} NBI_R = \dfrac{SFR_R}{FLAV} \\ NBI_R = \dfrac{FRF_UV}{RF_R} \end{cases}$$



Multiplex 3 Advantages

- Real time, non contact sensing
- No preparation of the plant
- Active sensing. Measurements possible under any light conditions, day and night.
- Simultaneaous measurement of several optical signatures
- Hand held portable device
- Data Geolocalization

6. Dualex 4

Available in March 2009

- Flavonols
- Chlorophyll
- NBI : Nitrogen Balance Index

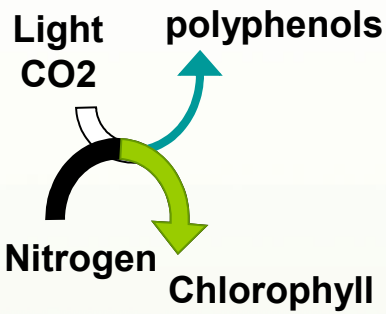
7. Applications

7. Applications

Nitrogen Management

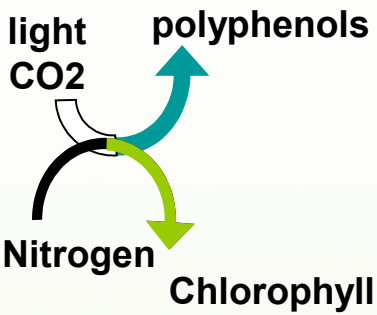
The Carbon Nutrient Balance

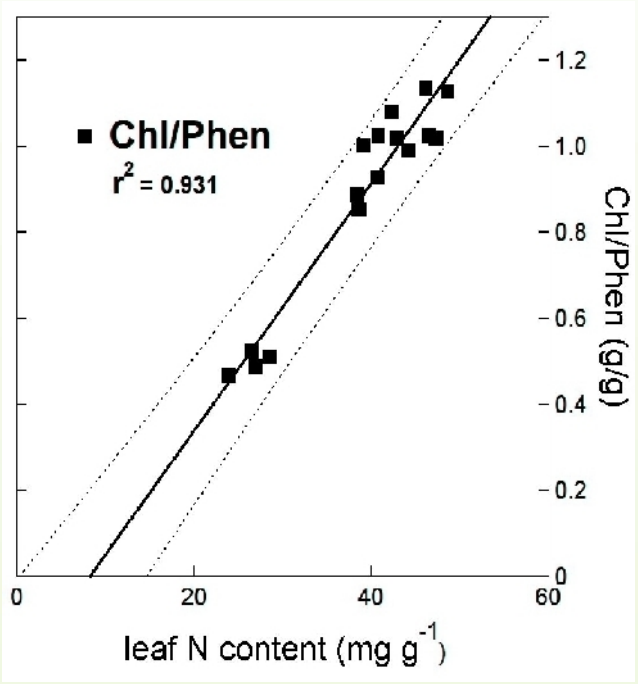
no N Deficiency



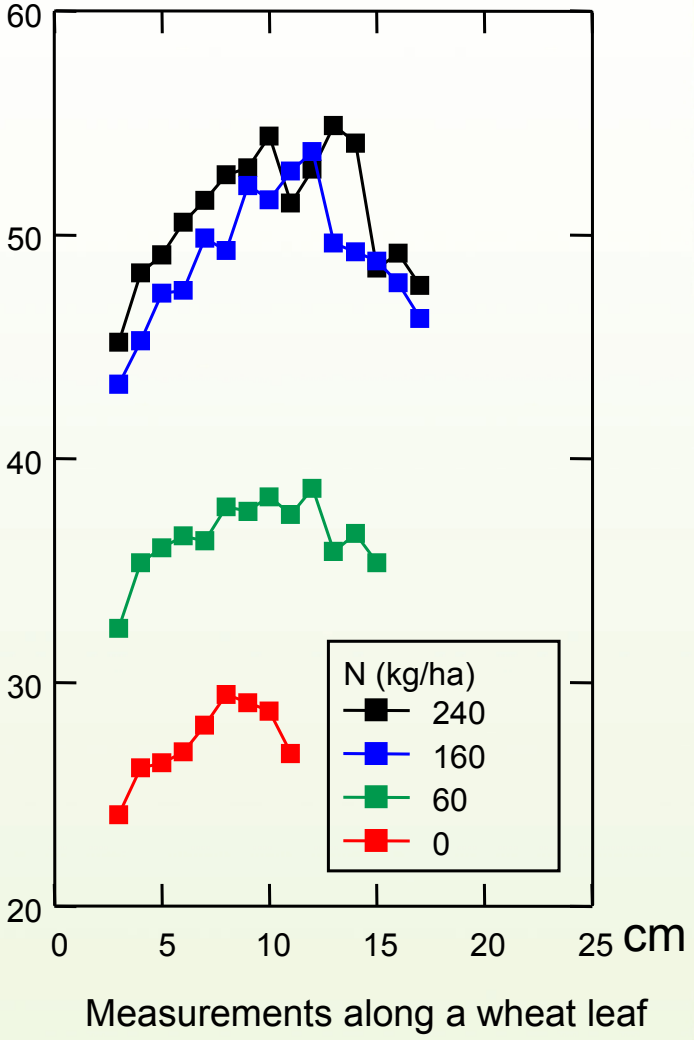
(Bryant et al. 1983)
(Coley et al. 1985)
(Herms & Mattson 1992)

N deficiency





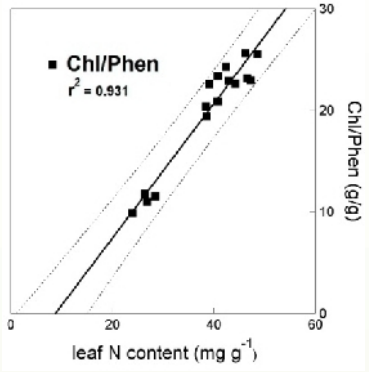
Chl/Phen = Index NBI



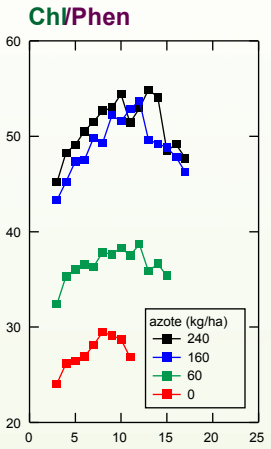
The Chl / Phen Ratio

Beneficial effects of **simultaneous Chl** and **Phen** assessment

1. The **opposite dependence** on N increases the dynamic range



2. The **parallel dependence** on leaf age decreases leaf position influence

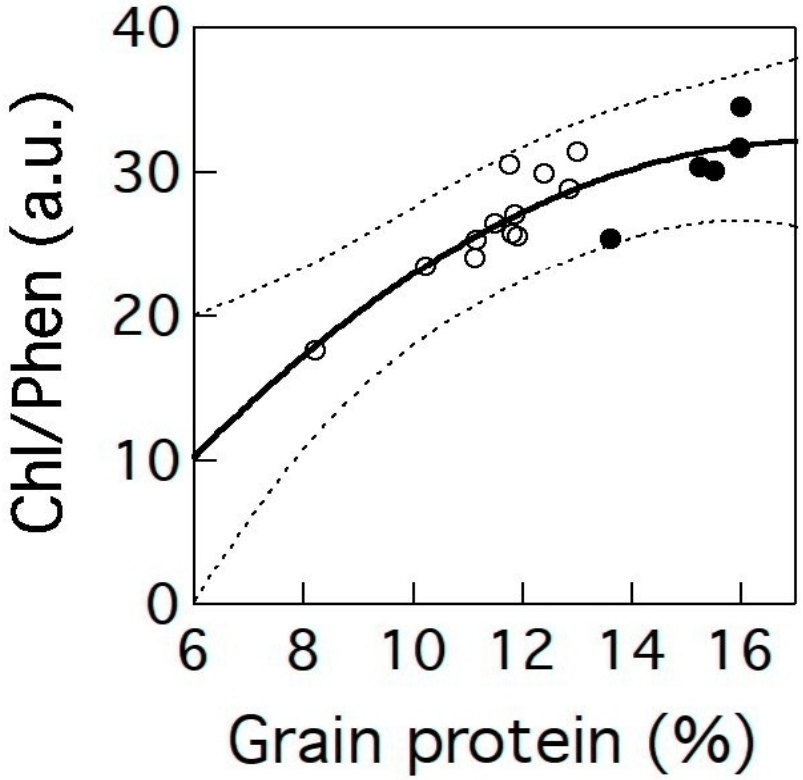


3. The ratio of **two surface-based measurements** avoids the influence of LMA

A new step towards real-time site-specific fertilisation

7. Applications

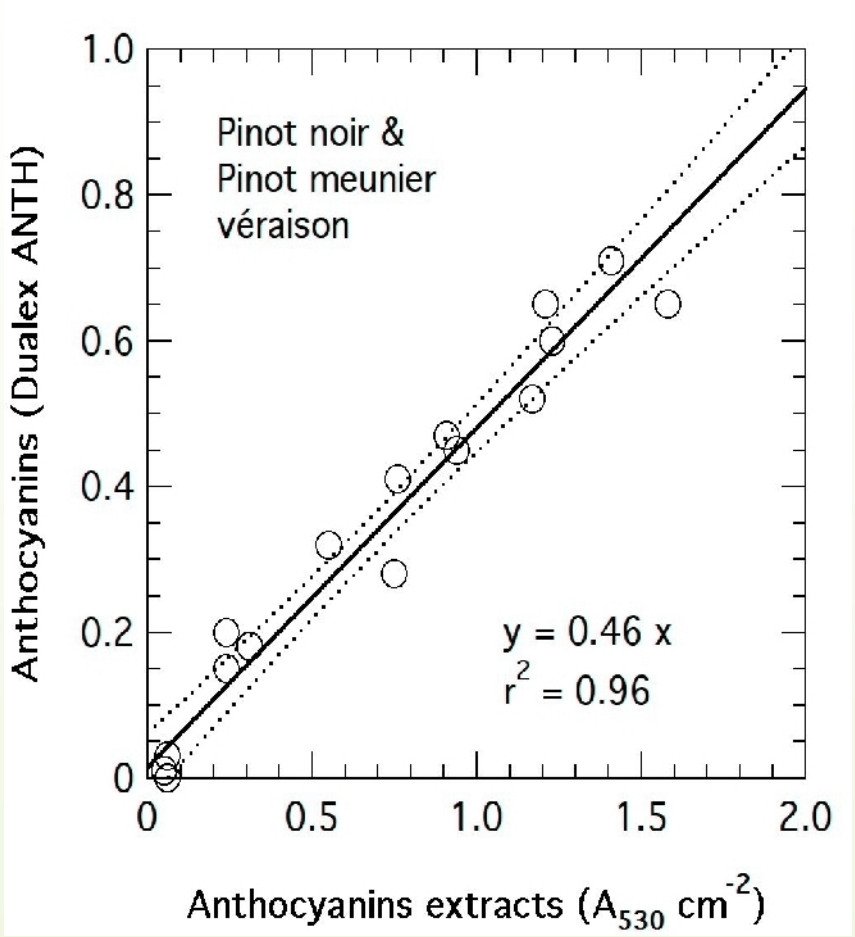
Protein Content Forecast

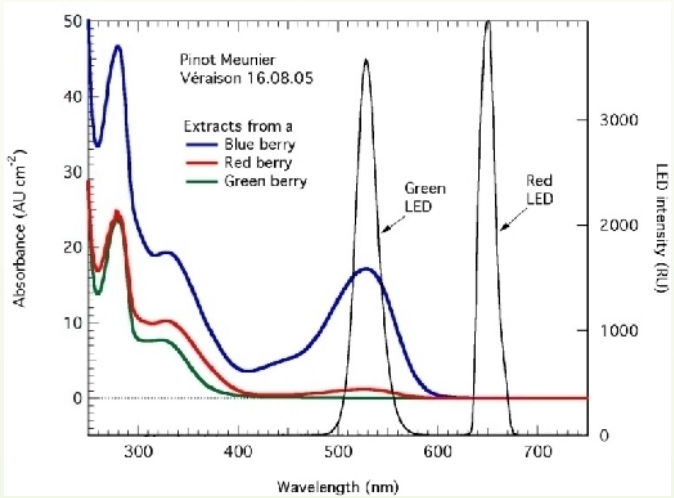

Chl/Phen (a.u.)
40
30
20
10
0
6
8
10
12
14
16
Grain protein (%)

7. Applications

Phenolic Maturity Monitoring

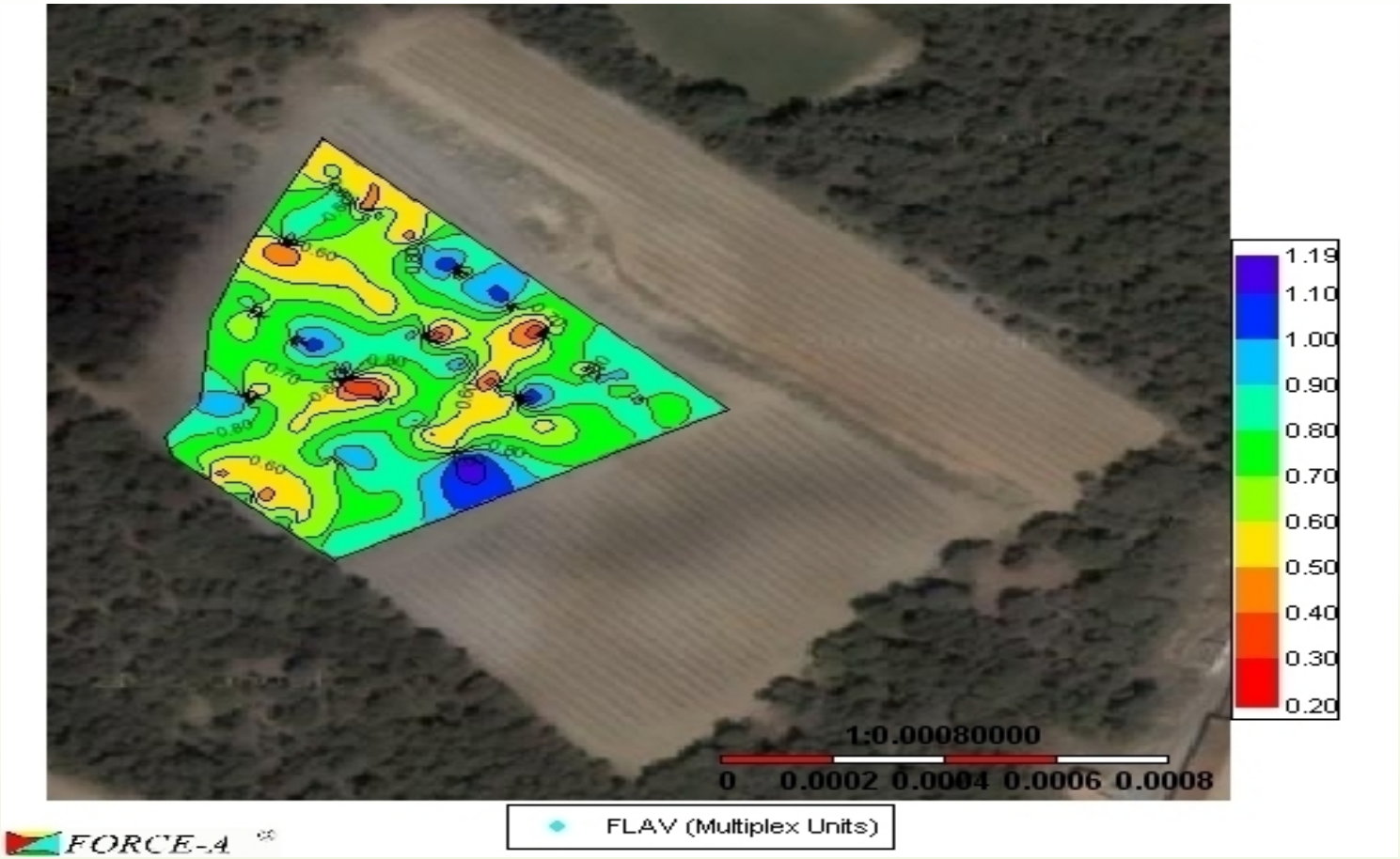
Optical Phenolic Monitoring on Berries





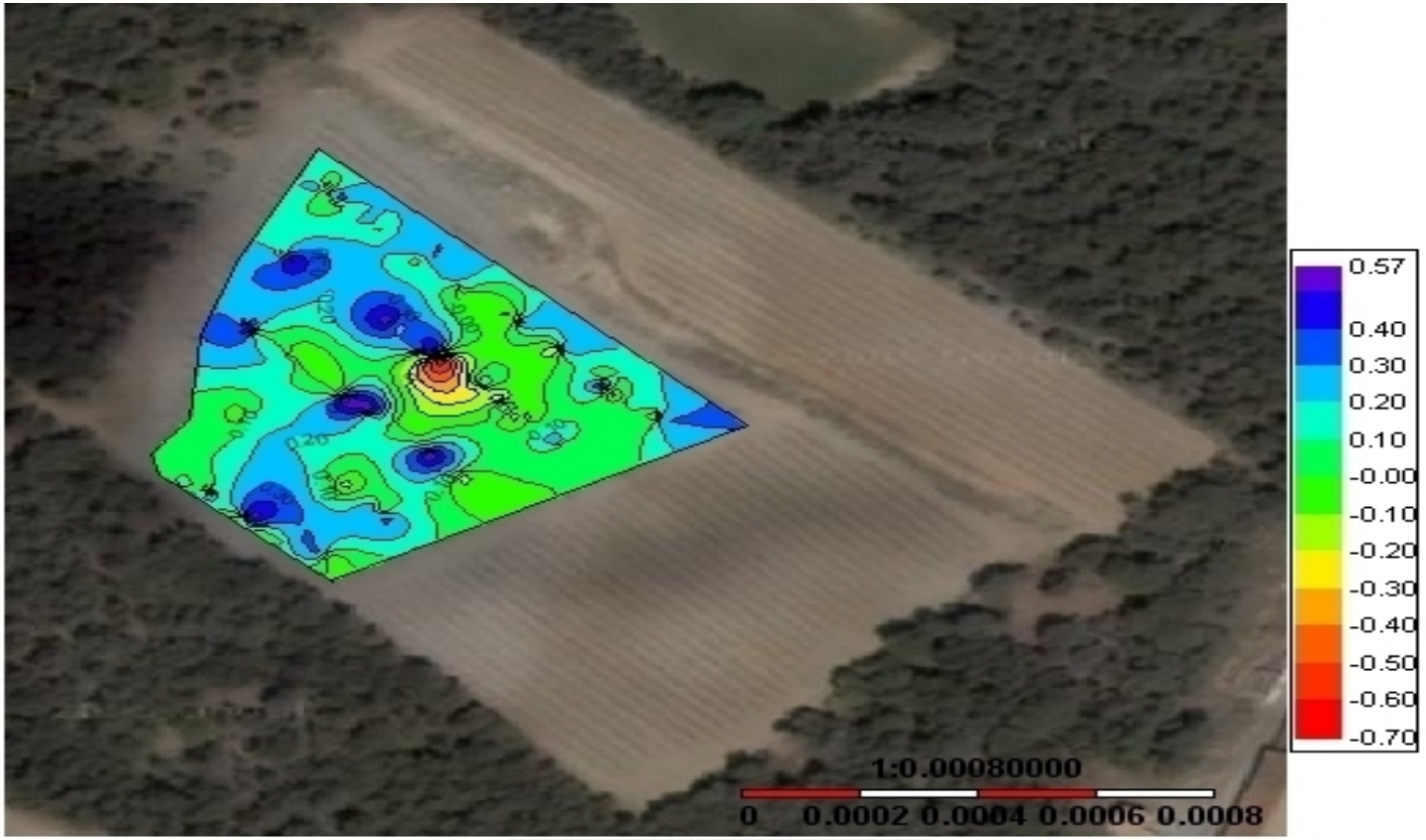
Vineyard Management – GPS Mapping

Mapping of the Flavonols content

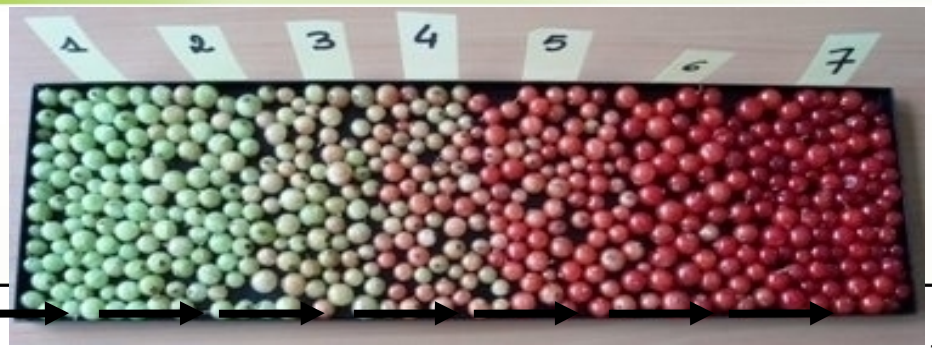


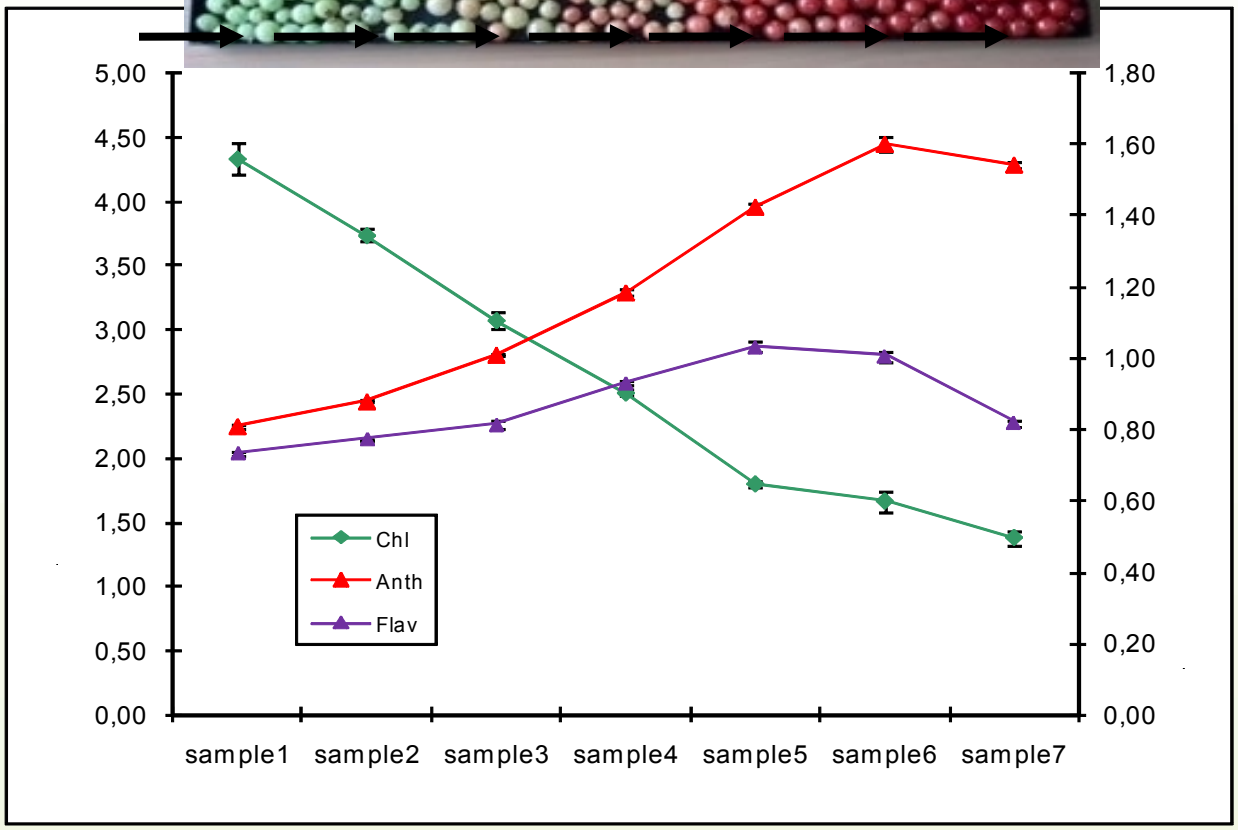
Vineyard Management – GPS Mapping

Mapping of the Anthocyanins content

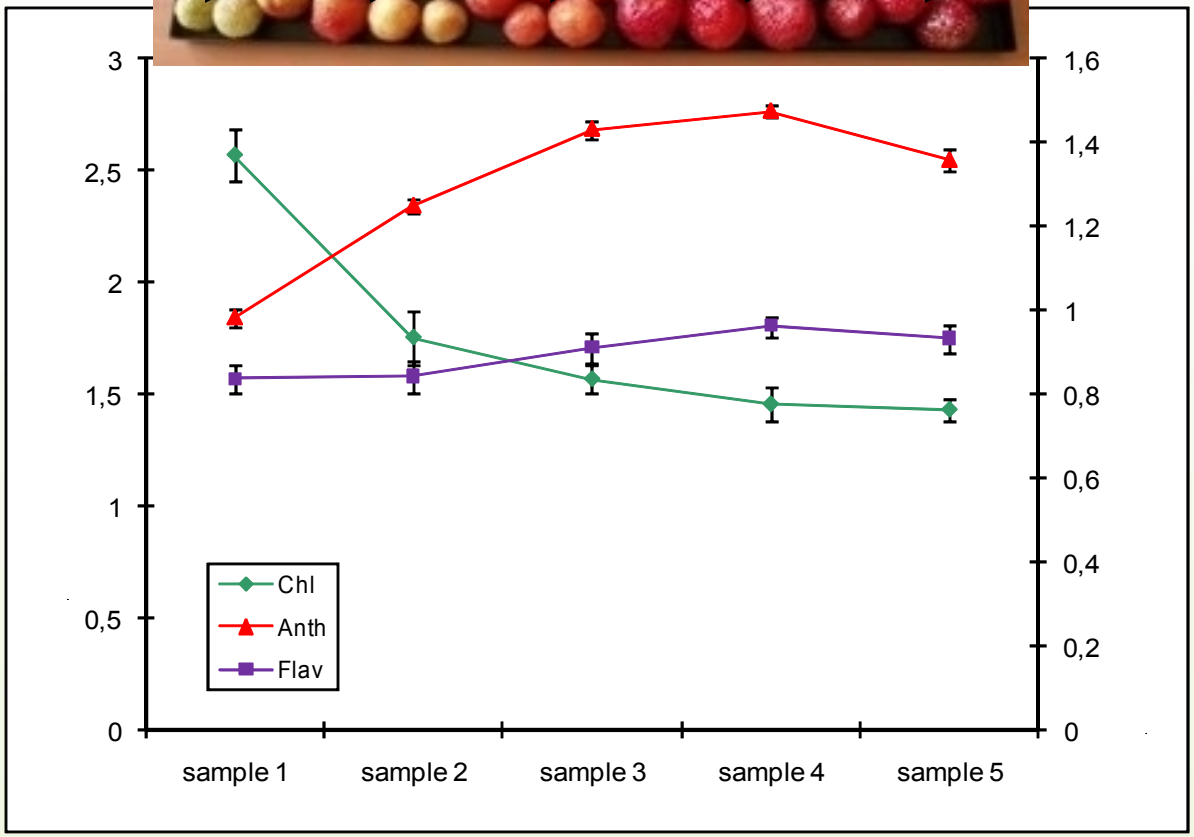


Chlorophyll, anthocyanins and flavonols, redcurrant berries





Chlorophyll, anthocyanins and flavonols measurements on strawberries



Outline on red fruits

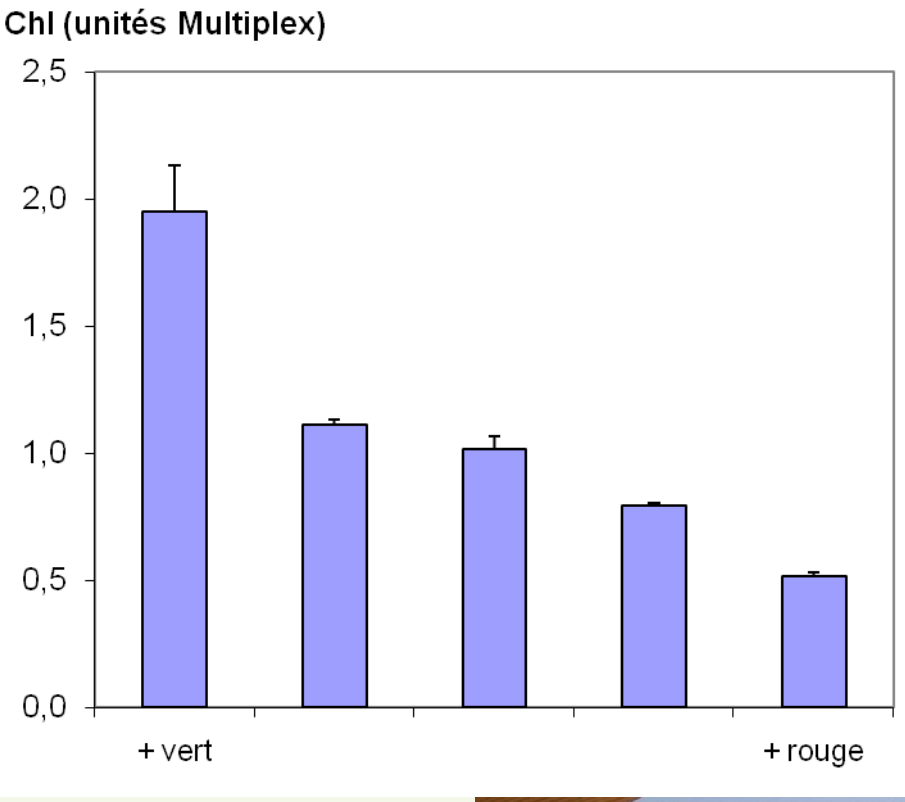
The maturity of red fruits is well assessed by Multiplex indexes

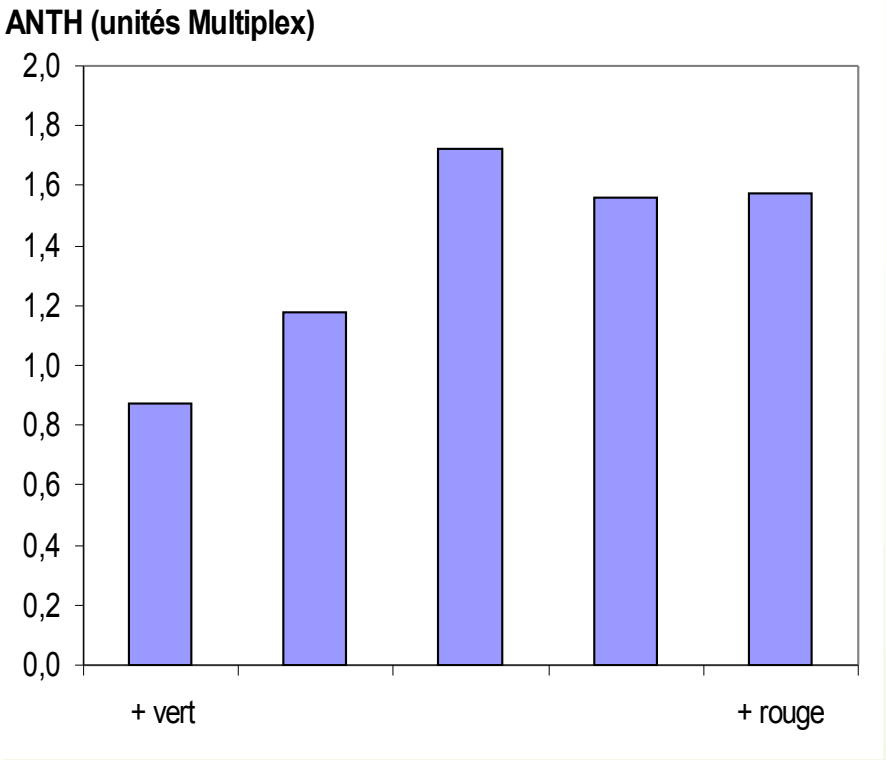
The Multiplex measurements show :

- The decrease of chlorophyll content during maturation
- The increase of anthocyanins content during maturation
- Stable flavonols content during maturation

These results confirm that flavonols measurement do not depend on the chlorophyll content, whereas there are measured with the chlorophyll fluorescence signals

Maturity Assessment





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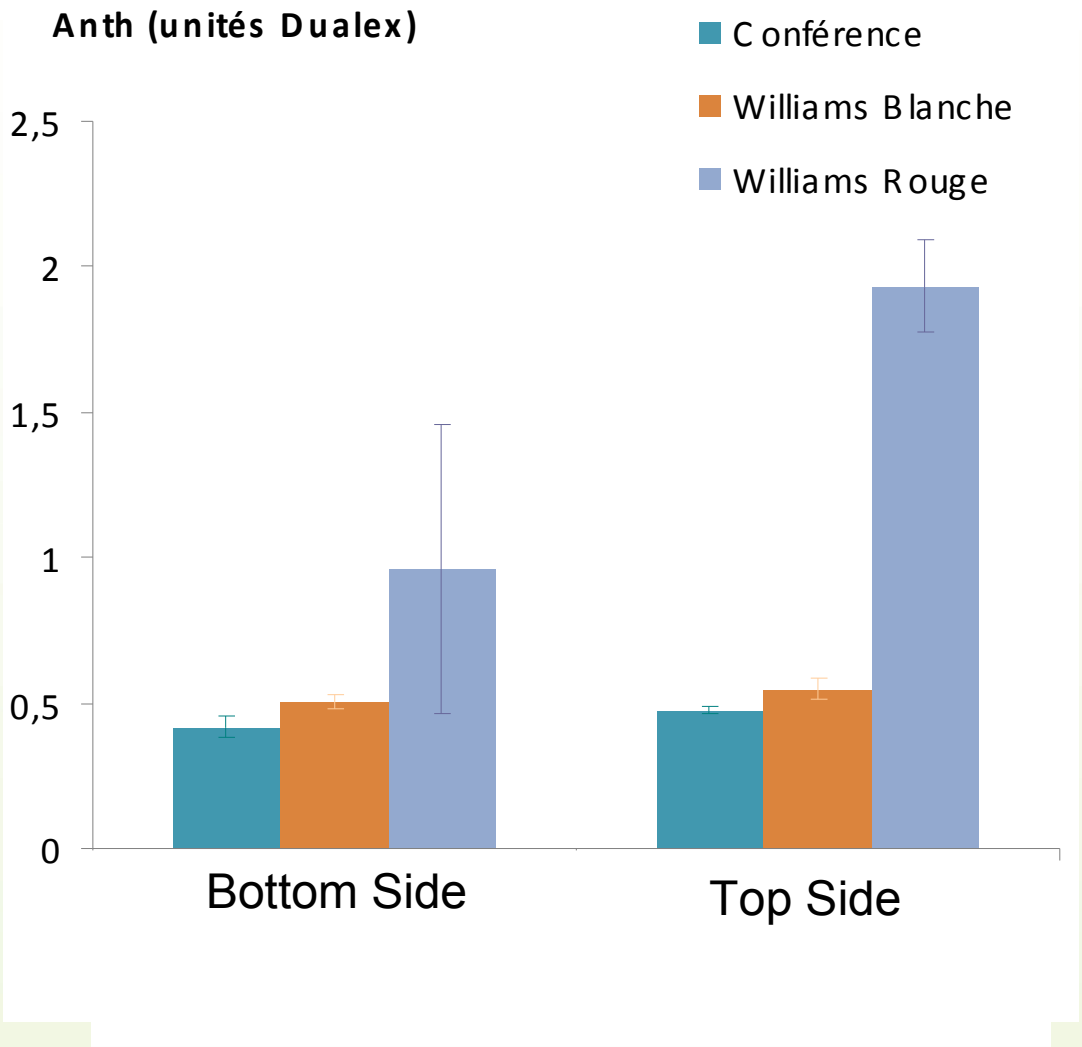
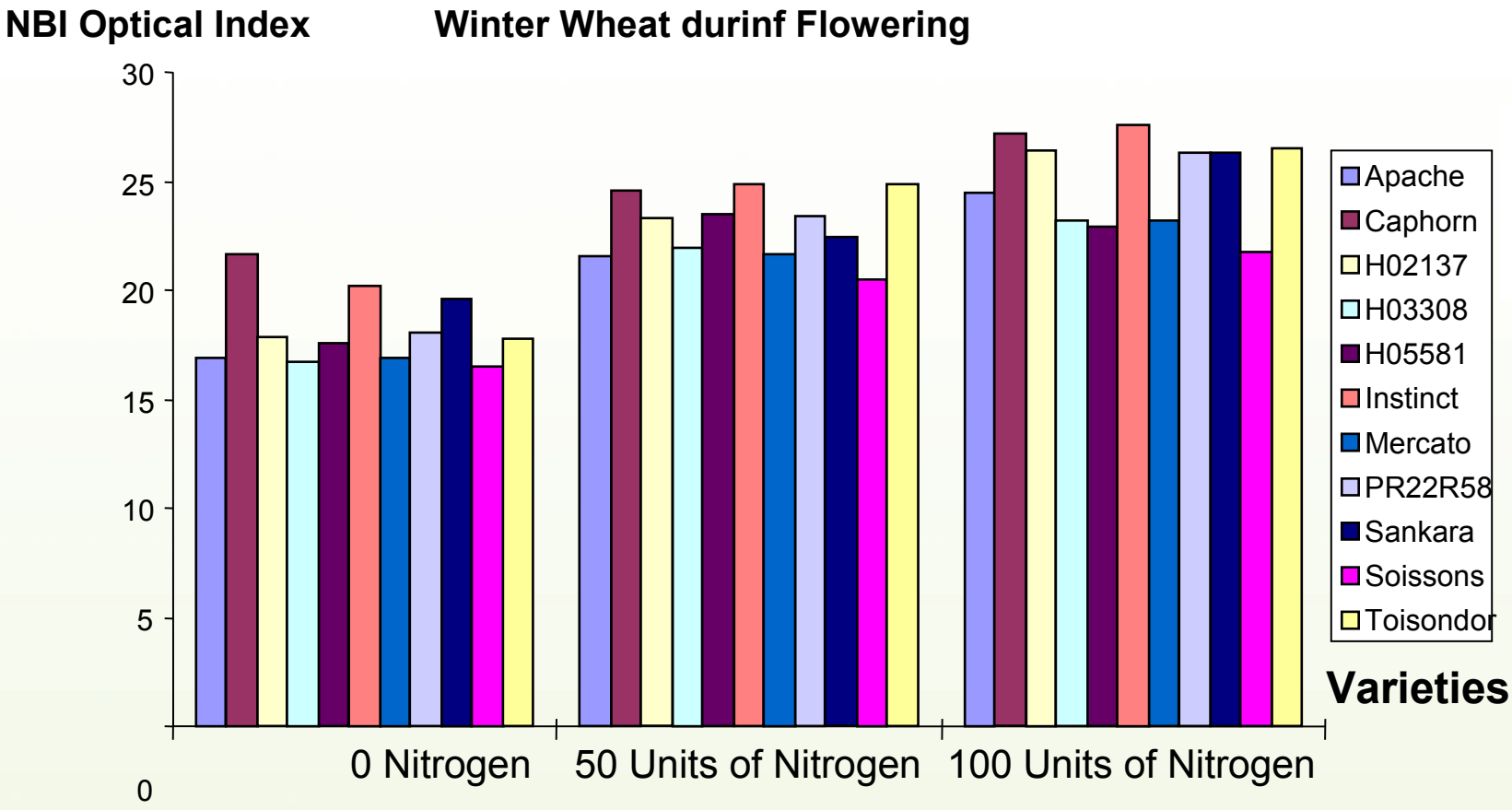
Consequences of different light expositions



7. Applications

Breeding – Cultivars Selection

Winter Wheat Varieties



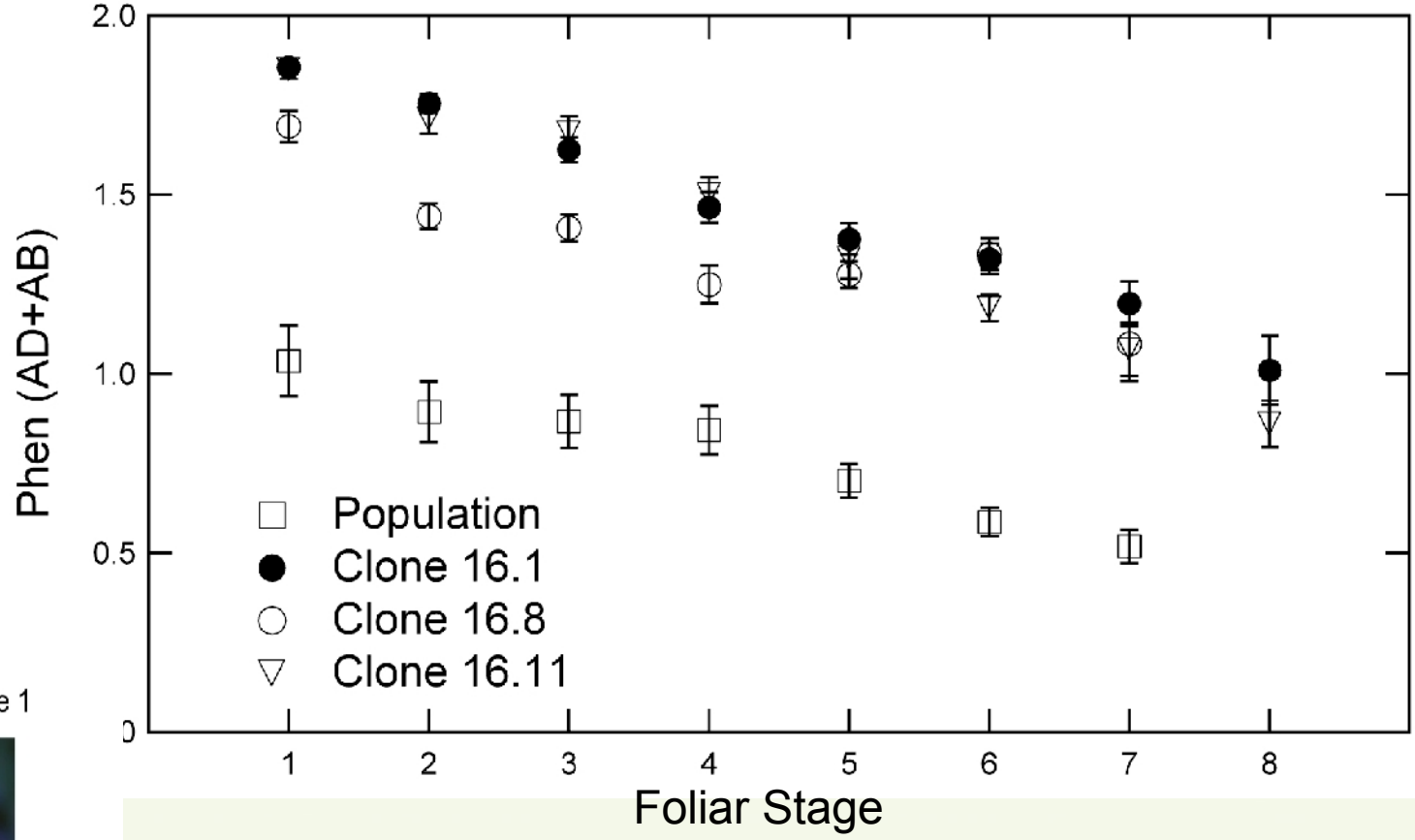
◎ Differences between varieties in temrs of Nitrogen Use Efficiency

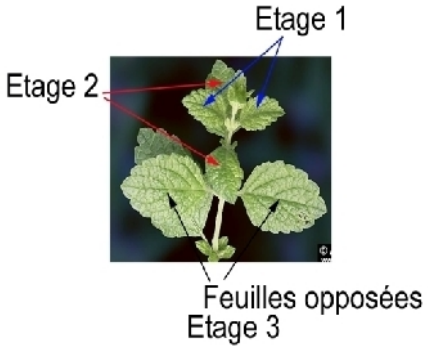
◎ Differences upon the fertiliser spreading → Nitrogen deficiency diagnosis compaored to a over fertilized band.

7. Applications

Herbal and Medicinals Herbs Quality Assessment

Mutants Screening - Melissa officinalis





Average of the polyphenols content for each foliar stage for the population and the cultivards 16.1, 16,8 and 16.11

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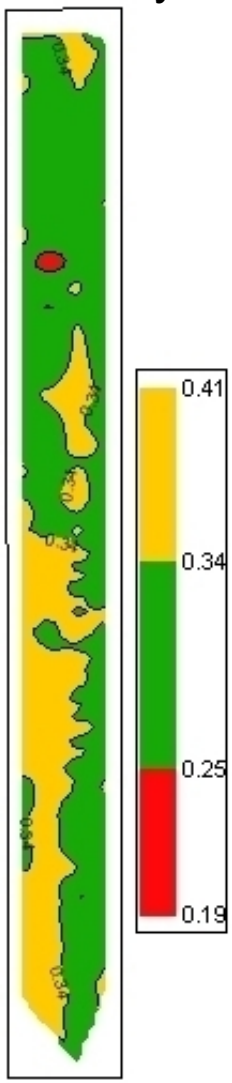
7. Applications

Precision Agriculture

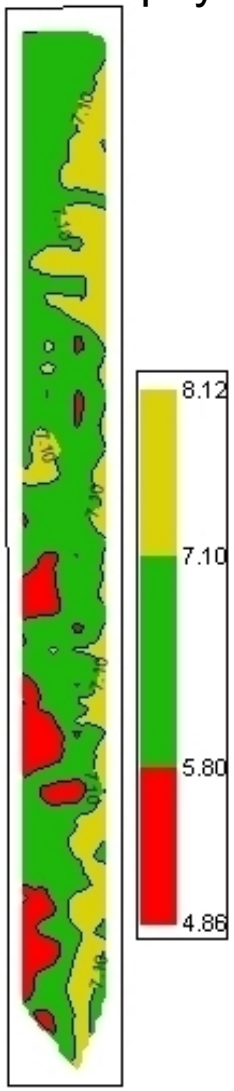
Salad field mapping with Multiplex

Salad field mapping with Multiplex

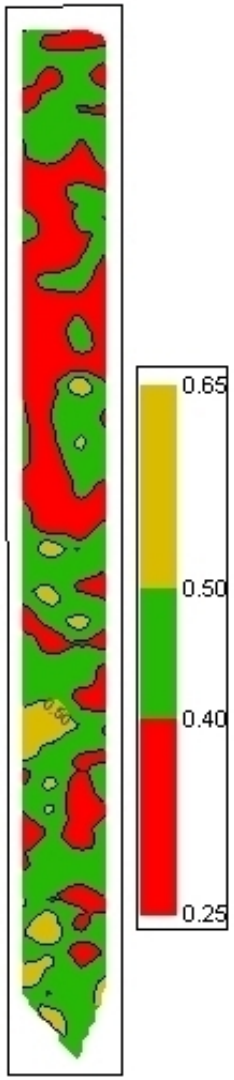
Anthocyanins



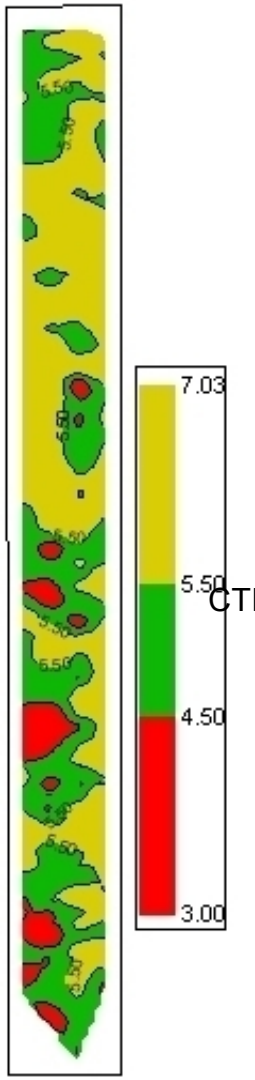
Chlorophyll



Flavonols



NBI Index



CTIFL, 2007

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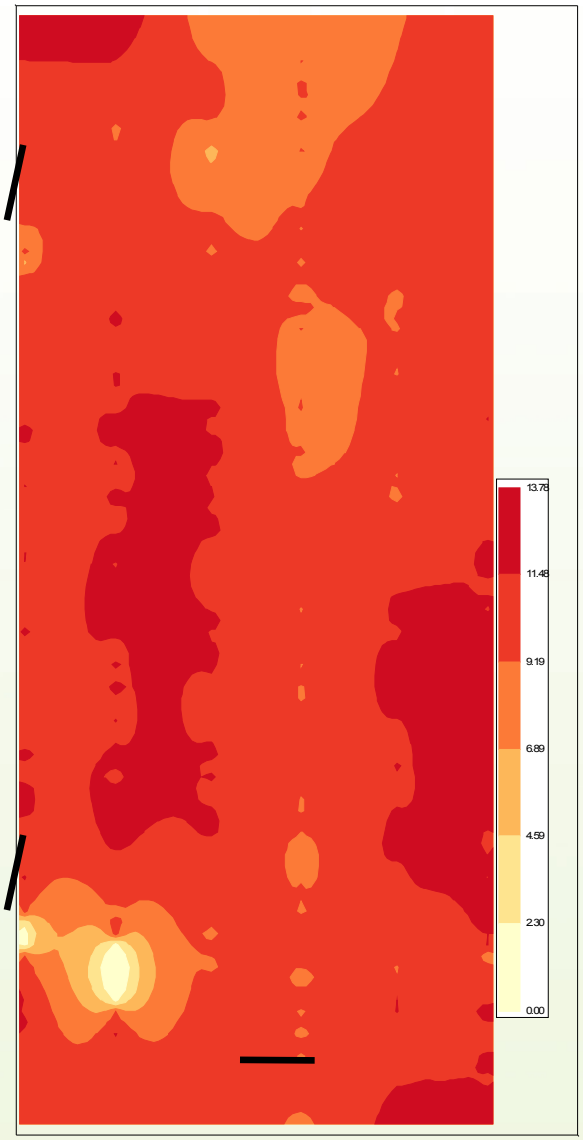
Turf grass N-Management

Chlorophyll



Representation of the heterogeneity of grass density on a soccer playground.

Mapping Interest :

- Identification of risk zone
- Assessment of the turf quality
- Manage chemicals and fertilizers

Leaf-light interactions

- Nitrogen Balance Index (NBI) increases when nitrogen is applied whereas control spot keeps constant

- NBI increases with the N-fertilization rate

- NBI indicator shows better N-rates discrimination than Chl or Flav indicators

→ NBI is a good indicator of N-nutrition level of turf grass
NBI is useful to manage the fertilization on turf grass.

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8. Force-A new technologies to come...

- Early fungal diseases detection
- Water stress detection
- Mycotoxines : Fast and non destructive measurement
- Early detection of phosphorus deficiency.
- Specific weed killing : distinction between monocotyledons and dicotyledons

Embedded Sensors

Multiplex

On-the-go Mapping

Site-specific Management

Thank You !

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