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Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

- **Context**

Fluorescence

Physiocap

Reflectance

Smartphone

Infrared spectroscopy

Economical sustainability

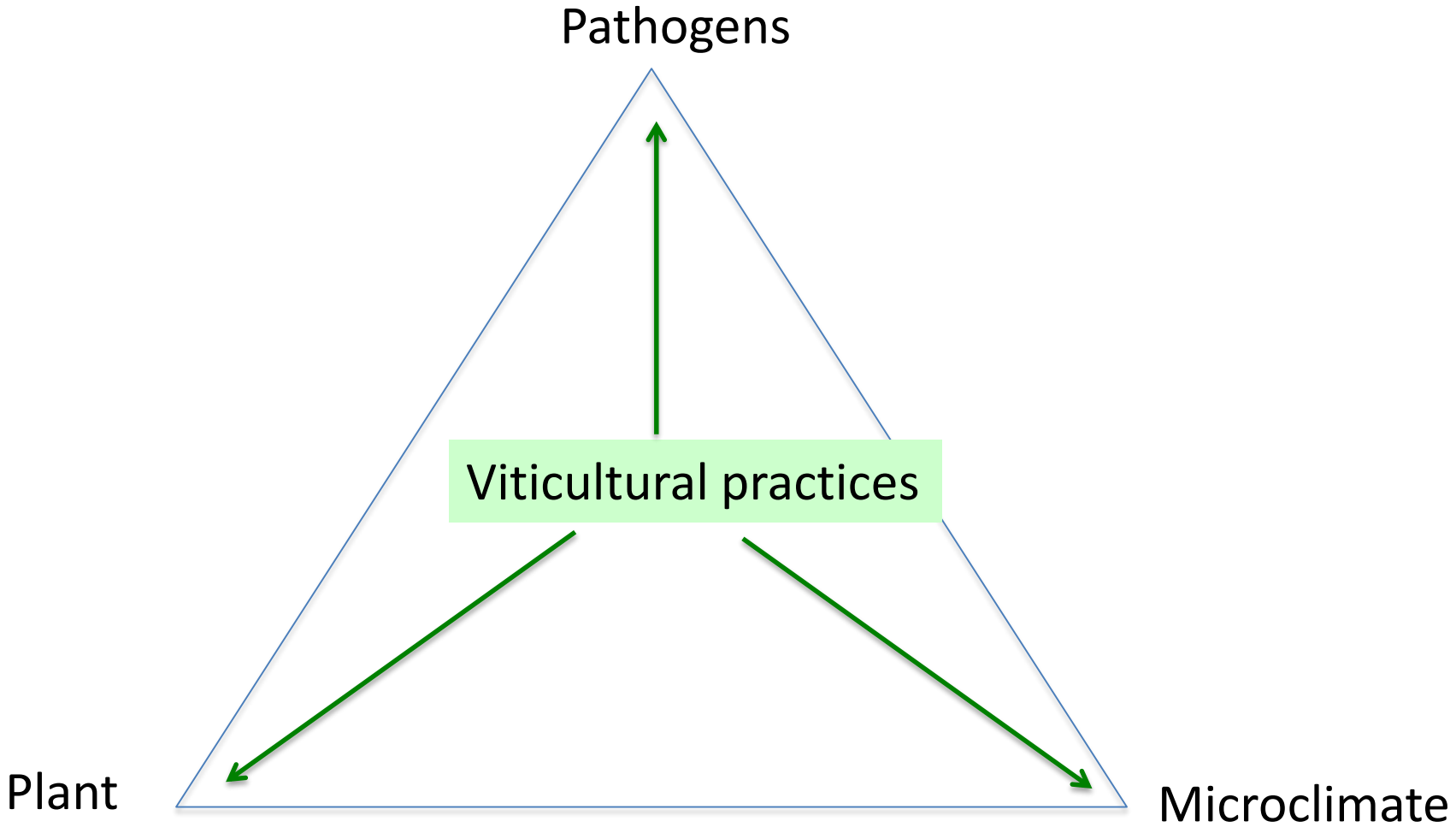
Vineyard management

Variety/rootstock

Water regime: ca. 200 mm

Yield :
50 to 60 hl/ha

Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance



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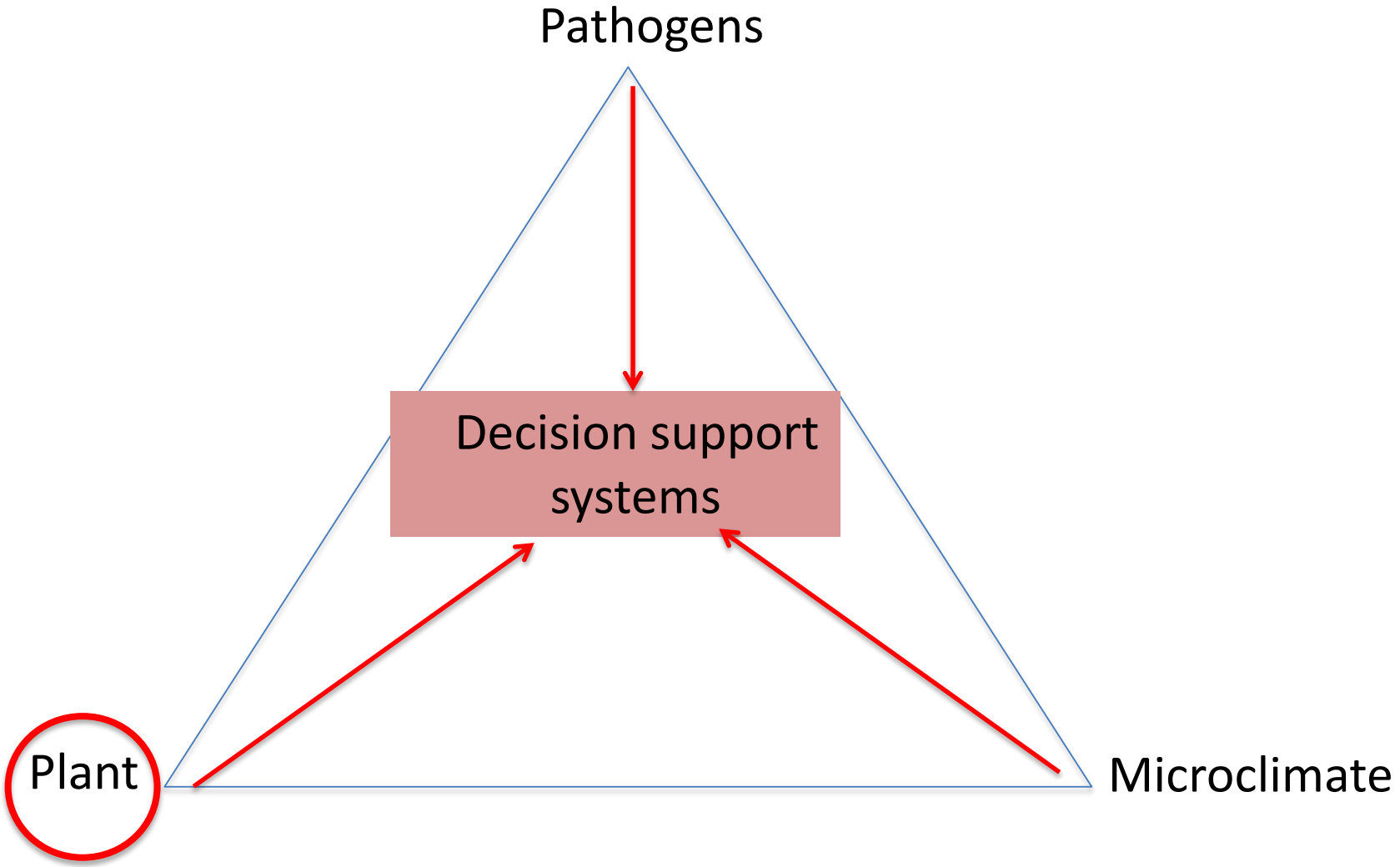

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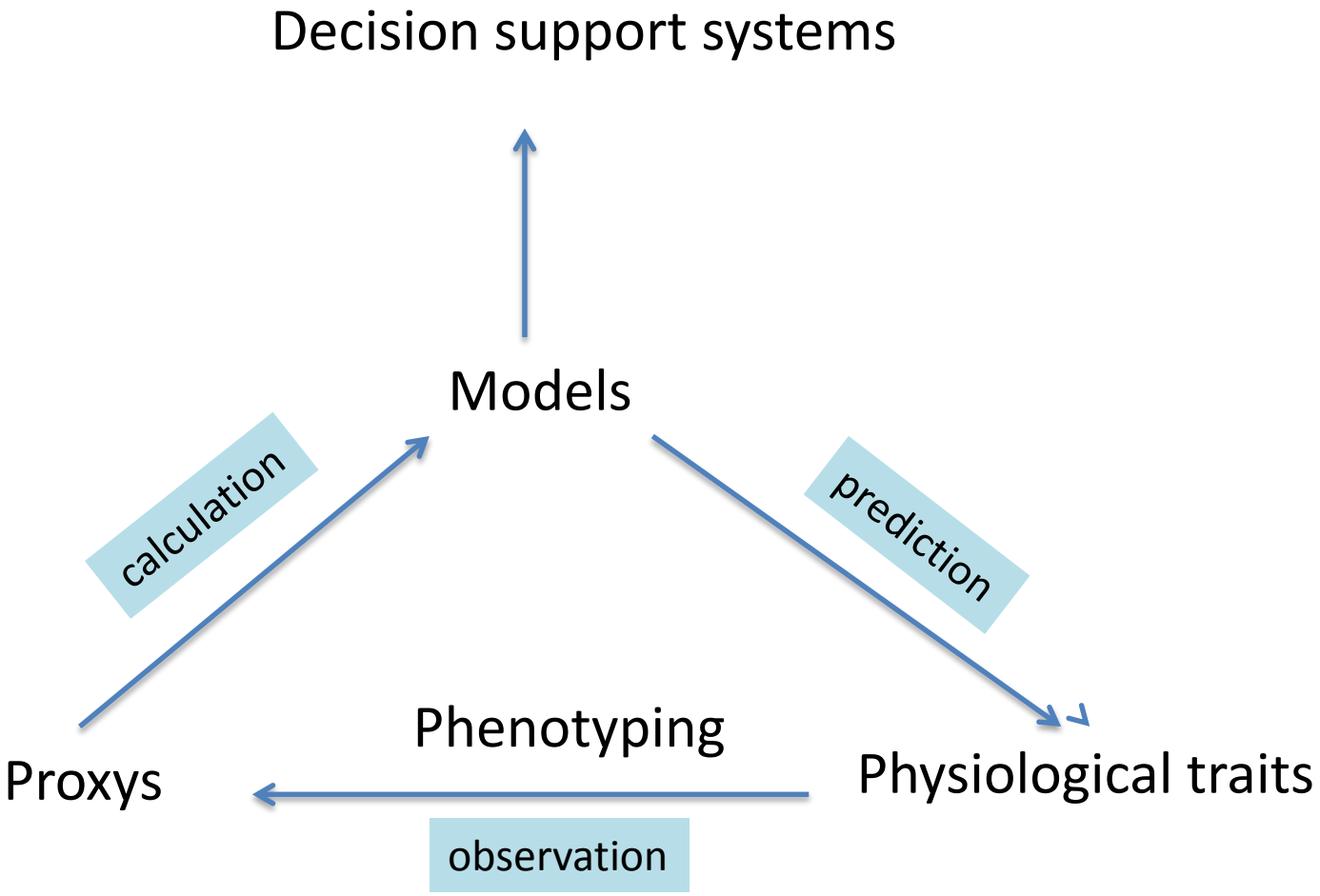
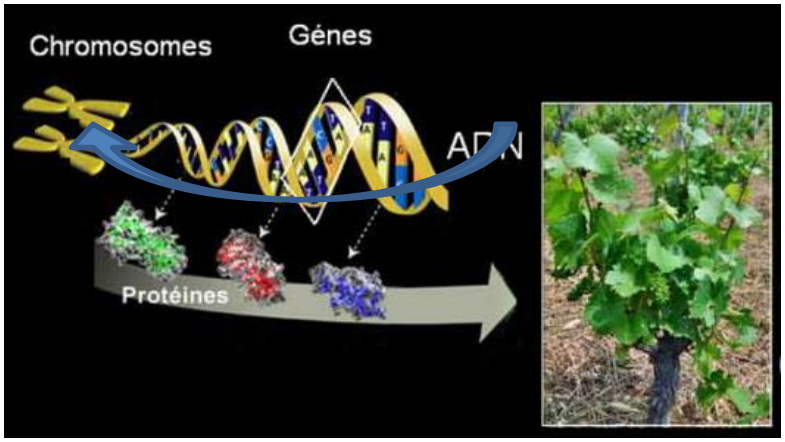
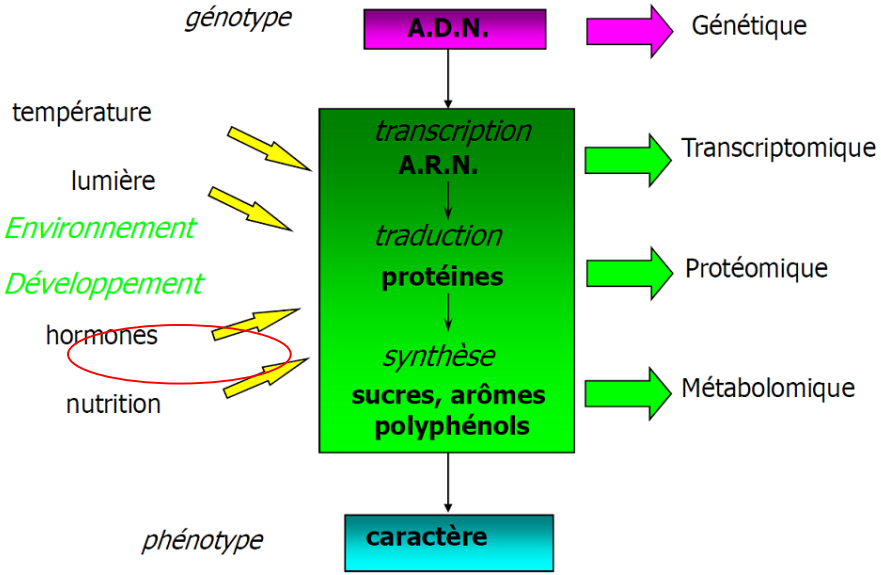


What is phenotyping ?

Collect on a large number of organisms a set of characters whose variability is analyzed with regard to the genetic and environmental context

(understanding effects of G x E interactions, breeding, modelling).



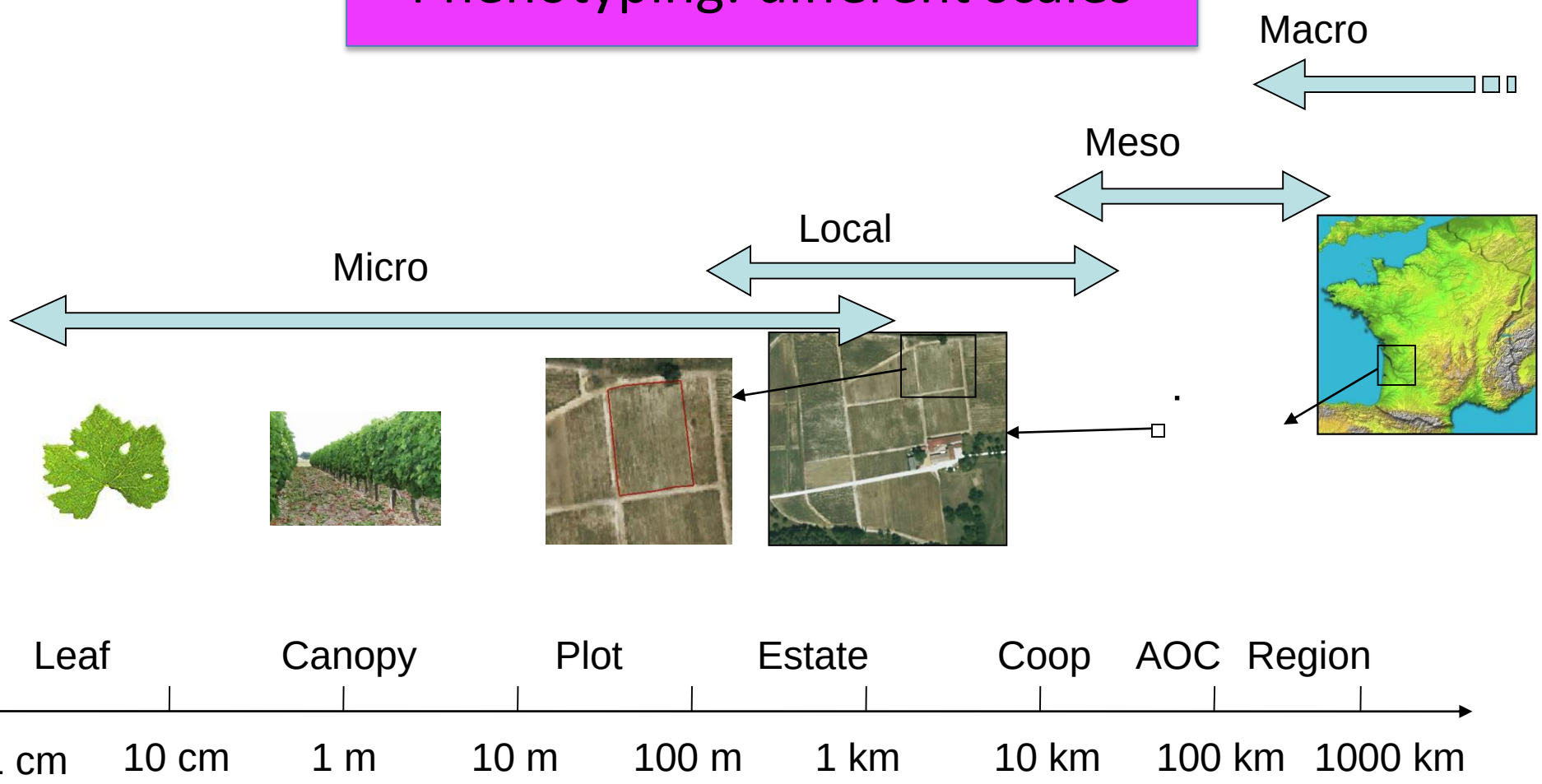


Need to report accurately all experimental conditions

Fieldomics (Vivier et al.,), Integrapomics (Pezzotti et al.)

Grape information system (Adam-Blondon et al., Horticultural Res., in press)

Phenotyping: different scales



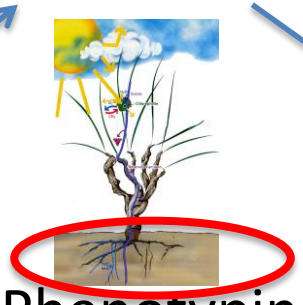
Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

Decision support systems

Models

calculation

prediction



Phenotyping

Proxys

observation

Physiological traits

Fluorescence
Laser
Reflectance
Image analysis
Infrared spectroscopy
Hyperspectral imaging
Thermal remote imaging
Trunk dendrometers
Sap flow sensors
Drones

Growth
Vigor
Phenology
Yield
Berry composition

Context

- **Fluorescence**

Physiocap

Reflectance

Image analysis

Infrared spectroscopy

Proximal sensing of vegetation

ACTIVE

artificial light source

Reflectance or **Fluorescence**

Type of functioning

PASSIVE

the Sun as light source

Reflectance or Fluorescence

Working distance

contact — small — medium — large

leaf clips — Multiplex — LIDAR — aircraft — MERIS satellite

CASI

(Fibre optics)

Spectral Domain

UV — visible — NIR — SWIR — thermal — radio

Phen Car Anth Chl LMA Prot H_2O

Mapping / Zoning / Block selection

Mounted Multiplex

Vine mapping
(N status, Chl)
Grape quality zoning

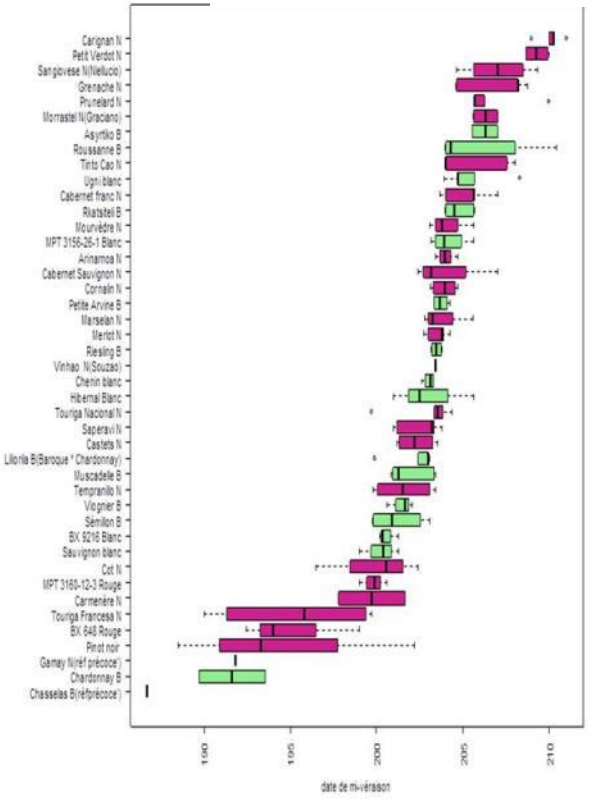
Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

late varieties, low sugar, resilient to high temperature, adapted to drought, resistant to diseases, suitable to elaborate specific types of wines

			31	25	44	27	11	2	22	10	20	36	41	17	21	24	4	43	51	45
			7	33	37	14	42	5	18	48	39	29	23	13	15	46	30	6	26	38
			3	19	50	32	9	8	1	47	35	28	40	34	12	49	52	16	29	7
			7	39	9	45	10	28	2	16	33	1	31	12	28	52	46	47	22	42
	43	44	46	35	25	50	43	39	11	40	4	41	21	46	50	32	11	9	33	21
26	50	49	22	27	38	35	25	14	47	33	19	45	17	34	8	17	49	51	43	13
11	5	12	30	34	15	27	19	24	22	31	25	9	36	27	18	28	25	39	2	41
33	13	32	42	41	14	3	6	41	37	15	13	24	30	42	7	48	38	10	30	14
1	20	37	29	16	23	13	4	7	51	38	10	2	32	6	23	1	37	45	19	27
8	28	47	24	18	36	26	52	42	21	34	39	48	3	11	40	31	4	18	6	15
31	4	19	40	48	44	49	29	20	5	30	49	51	52	20	26	8	20	34	16	23
6	45	3	36	2	17	1	46	32	48	44	35	47	43	38	37	50	35	3	26	24
10	51	17	52	21	12	23	8	9	18	29	14	15	22	16	5	36	12	44	40	5

1	Alvarinho B	14	Chenin B	27	MPT 3156-26-1 B	40	Saperavi N
2	Agiorgitiko N	15	Colombard B	28	MPT 3160-12-3 N	41	Sauvignon B
3	Arinarnoa N	16	Cornalin N	29	Muscadelle B	42	Semillon B
4	Asyrtiko B	17	Cot N	30	Nero d'Avola (Calabrese) N	43	Syrah N
5	BX 648 N	18	Gamay N	31	Petit Manseng B	44	Tannat N
6	BX 9216 B	19	Grenache N	32	Petit Verdot N	45	Tempranillo N
7	Cabernet franc N	20	Hibernal blanc B	33	Petite Arvine B	46	Tinto Cao N
8	Cabernet-Sauvignon N	21	Lilliorila B	34	Pinot noir N	47	Touriga Francesa N
9	Carignan N	22	Marselan N	35	Prunelard N	48	Touriga nacional N
10	Carmenère N	23	Mavrud N	36	Riesling B	49	Ugni blanc B
11	Castets N	24	Merlot N	37	Rkatsiteli B	50	Vinhao (Souzao) N
12	Chardonnay B	25	Morrastel N	38	Roussanne B	51	Viognier B
13	Chasselas B	26	Mourvèdre N	39	Sangiovese N	52	Xinomavro N

Mid veraison date

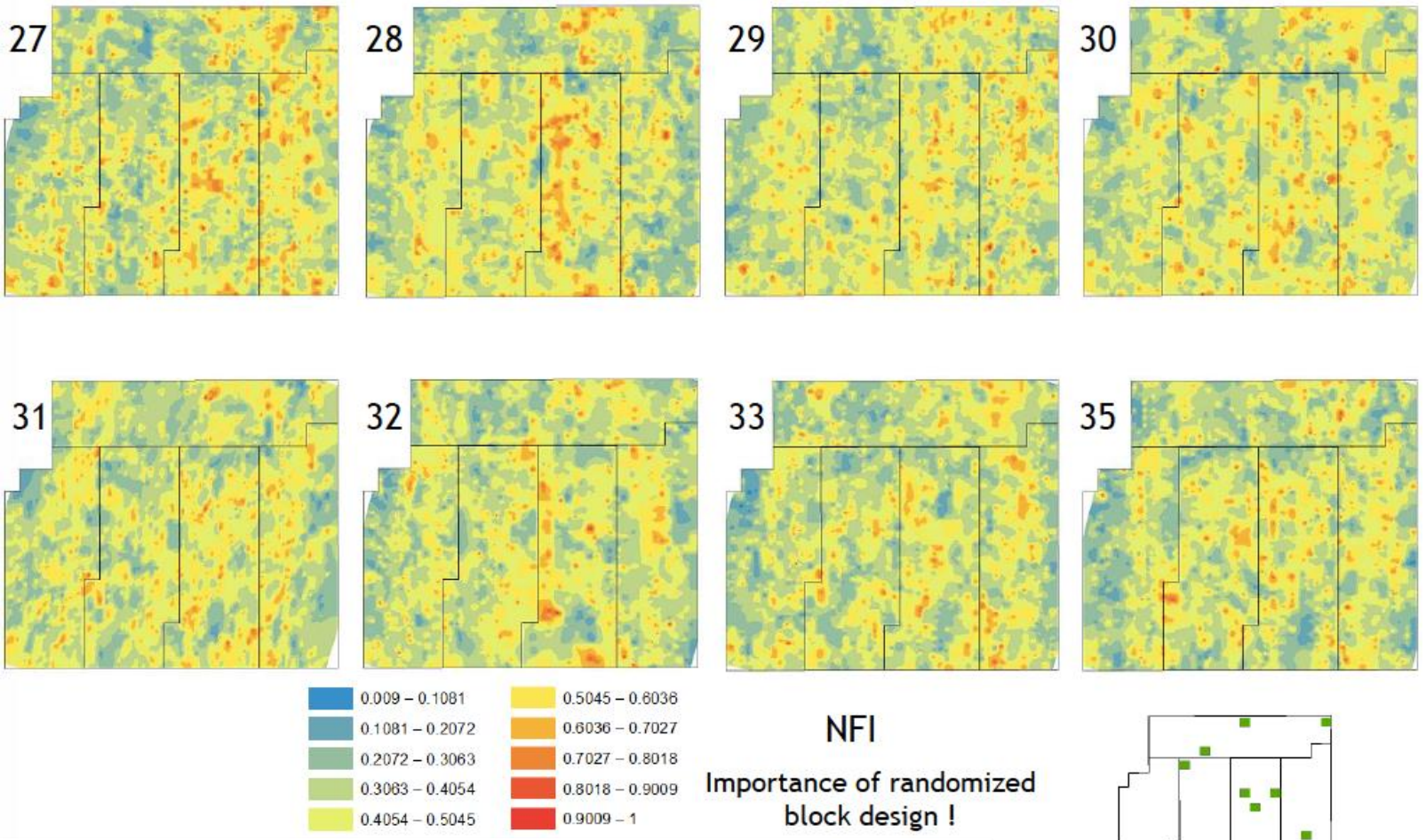


42 rows of 5 plants. Random distribution of 52 varieties in 5 blocks

Each number cooresponds to a mini-plot of 10 plants arranged in 2 rows (2*5 plants, face to face)

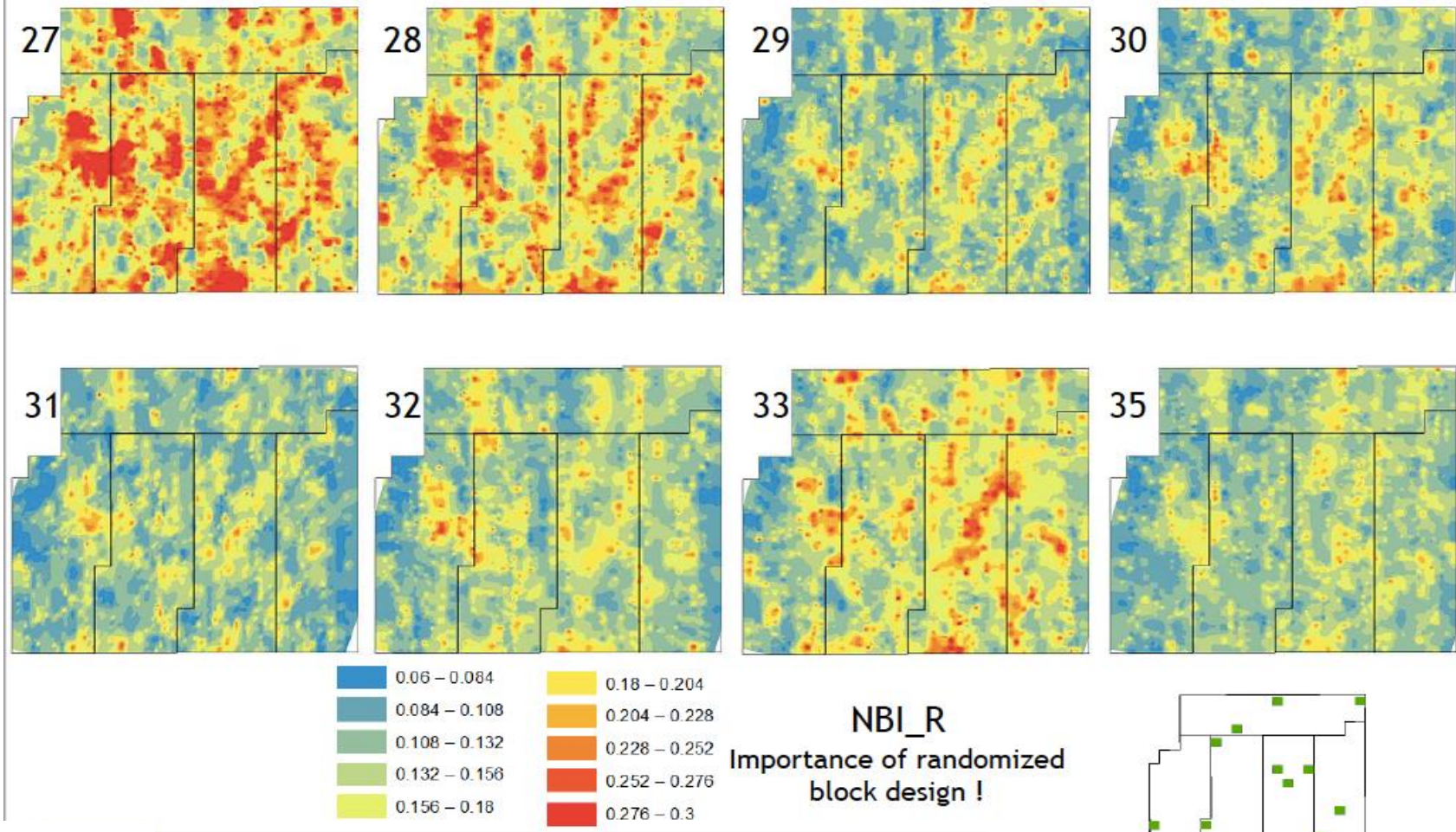
Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

Biomass Index



Bloom 2015	24
Veraison 2015	32

Nitrogen Balance Index (NBI)



Bloom 2015	24
Veraison 2015	32

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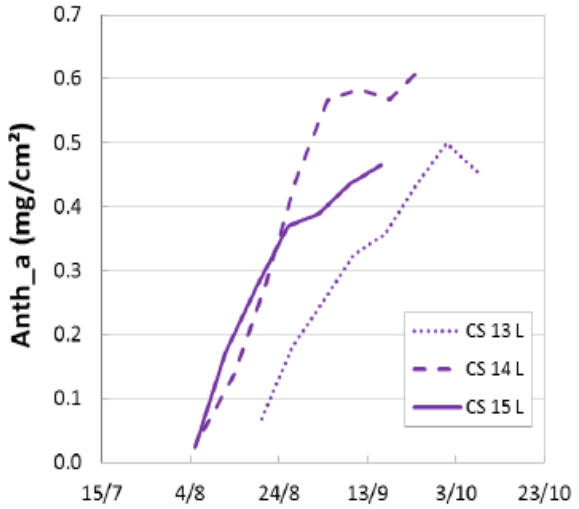
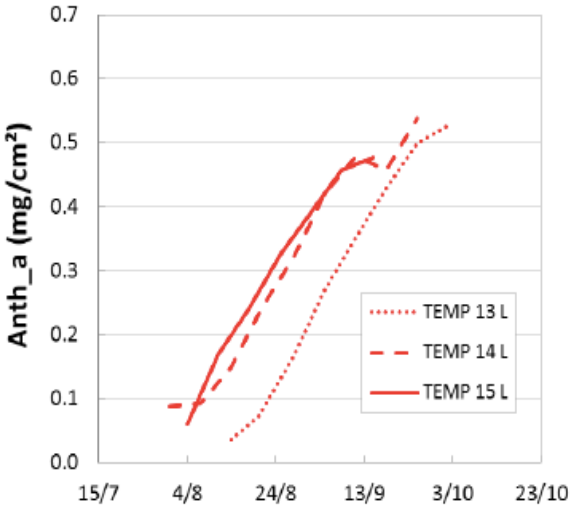
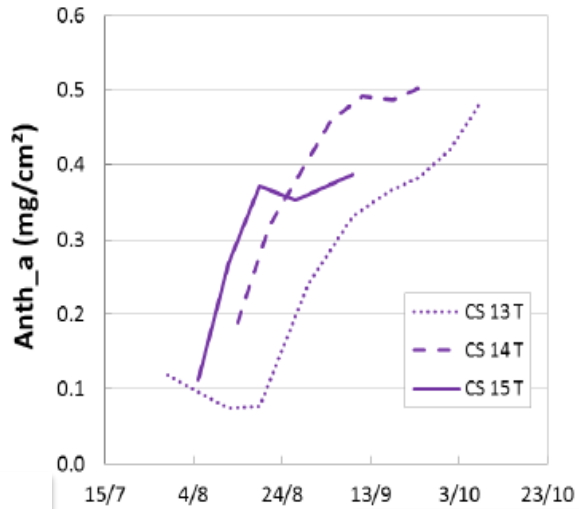


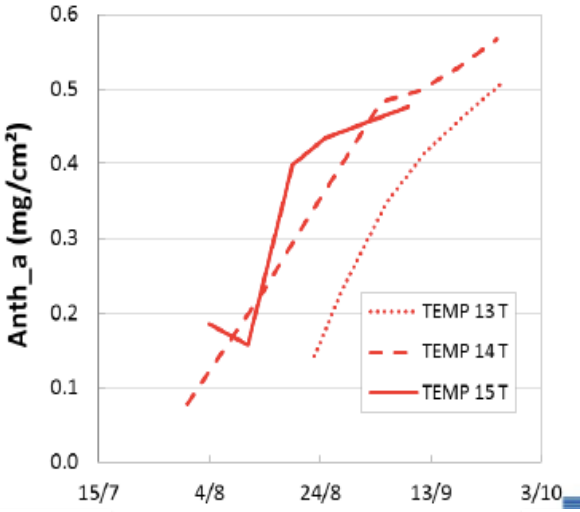
FIELD on GRAPES



Cabernet Sauvignon | **Tempranillo**



LAB on BERRIES



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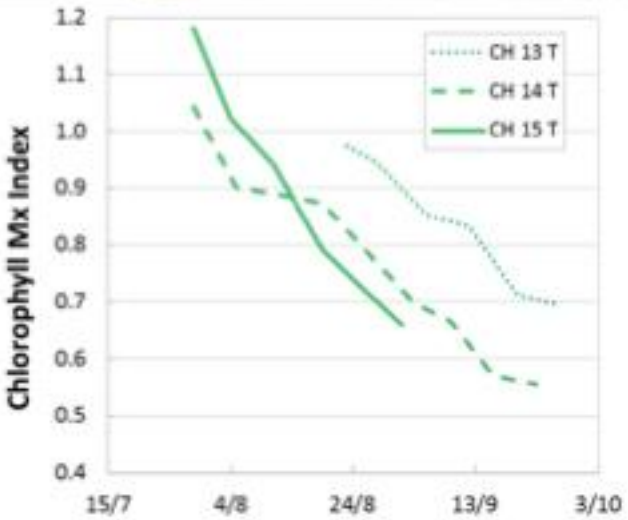

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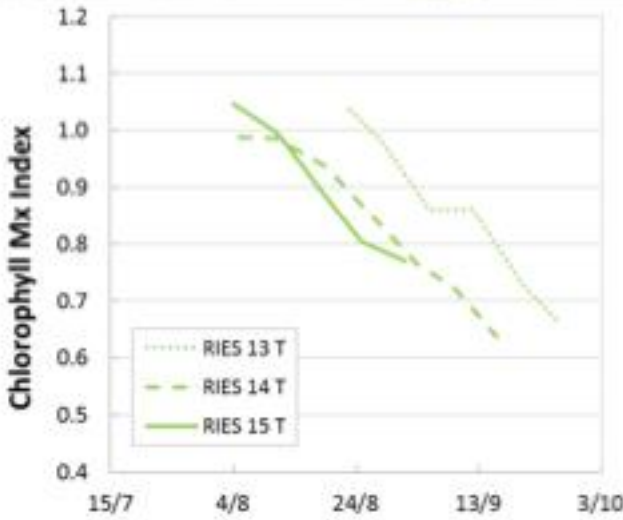
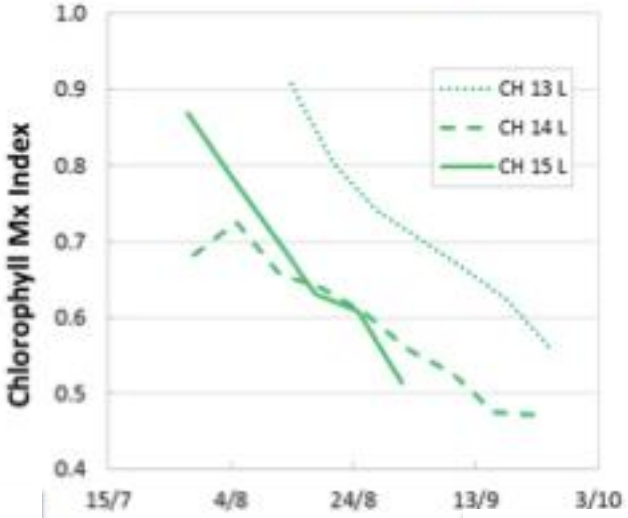
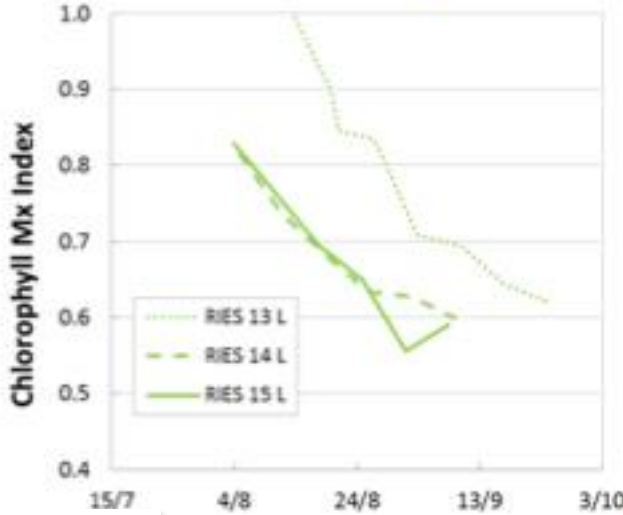


Chardonnay | **Riesling**



LAB on BERRIES



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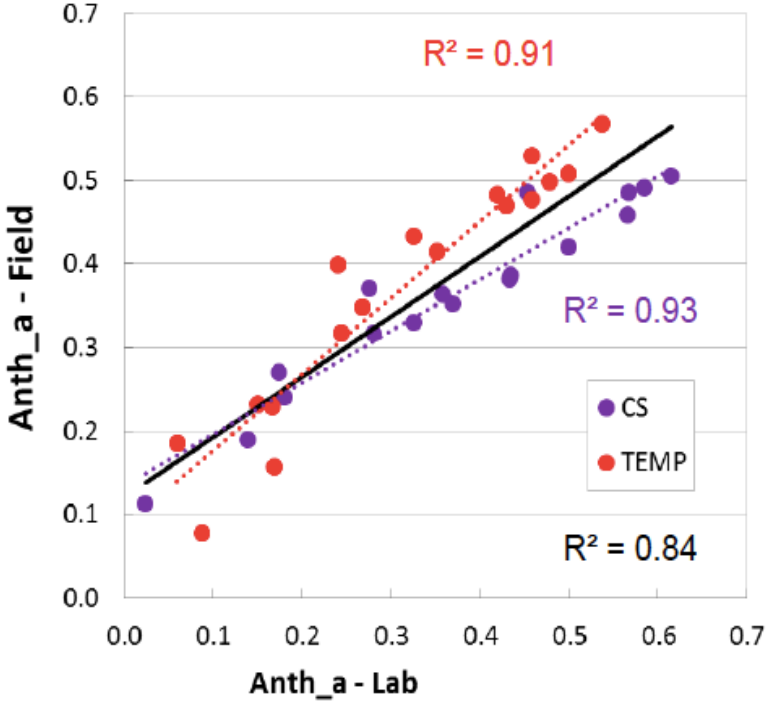
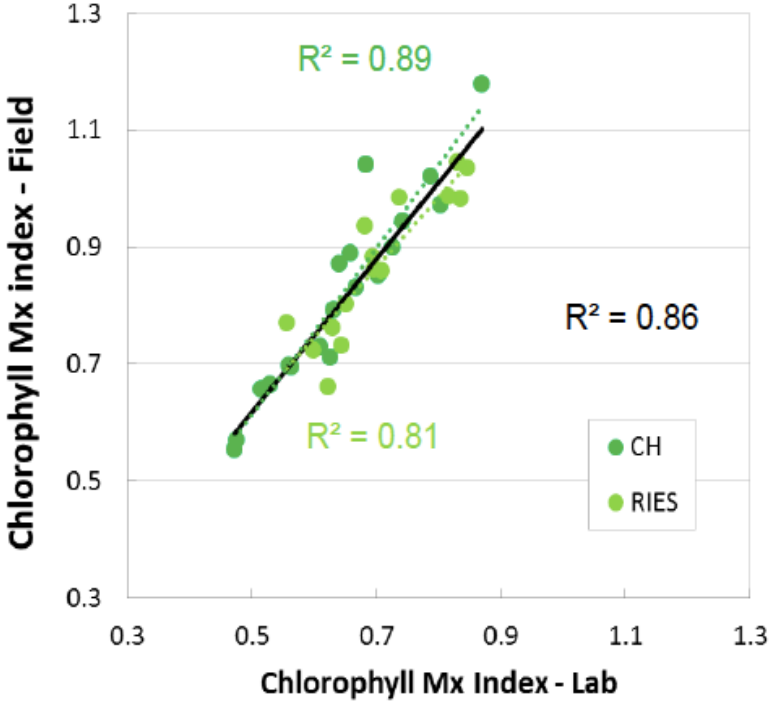


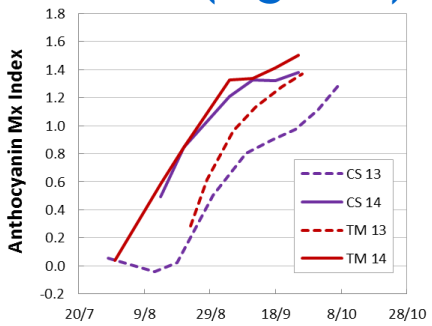


2013, 2014 & 2015 data, all blocks
For each point : LAB (around 30 meas.) vs FIELD (around 200 meas.)

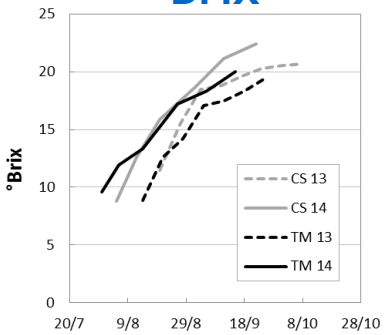
Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

MX optical indices

Anth a (mg/cm²)

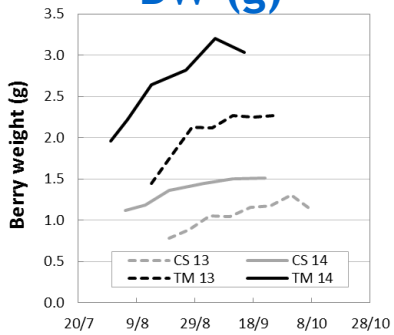


°Brix

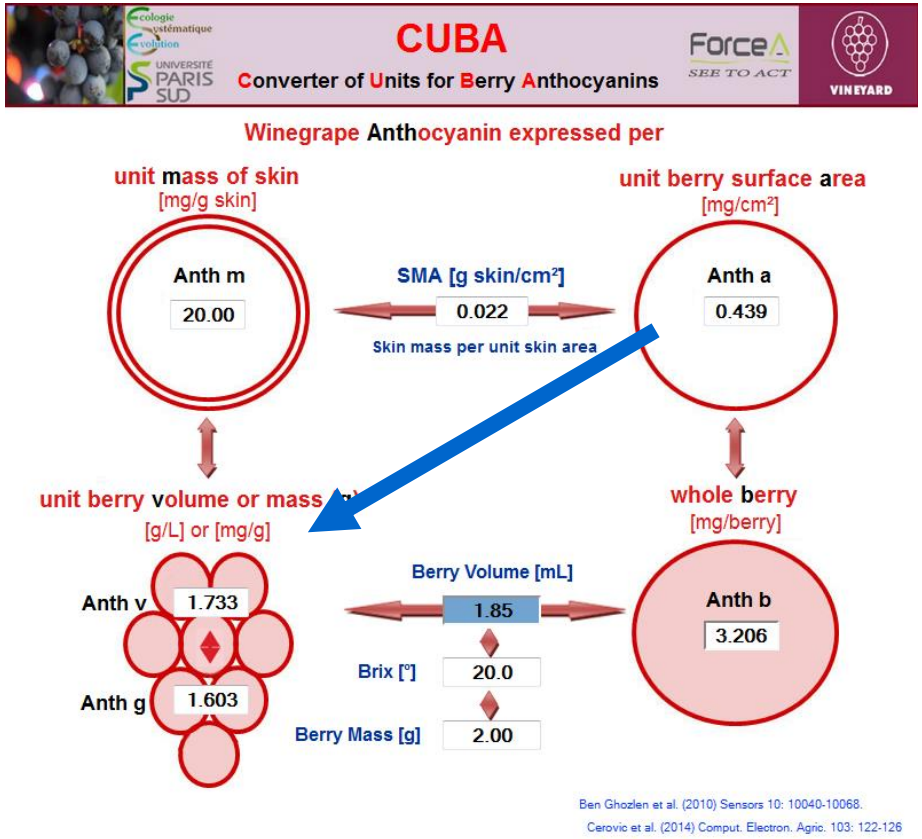


&

BW (g)



http://max2.ese.u-psud.fr/cuba/



Anth g & Anth v (common chemical analysis units)

Assessment of downy mildew (*Plasmopara viticola*) infection

Greenhouse 2014

- 10 potted plants per variety in greenhouse
- 5 inoculated with fungus
- 5 control plants (not inoculated)

Greenhouse 2014

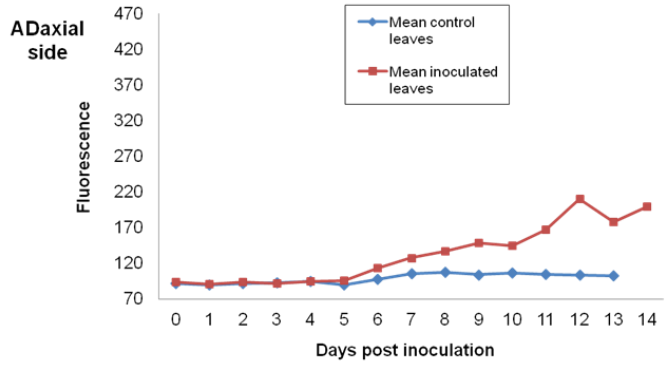
- Daily measurements (14 days)
- Adaxial (upper) and abaxial (lower) leaf side
- Same leaves scored over the entire time course !

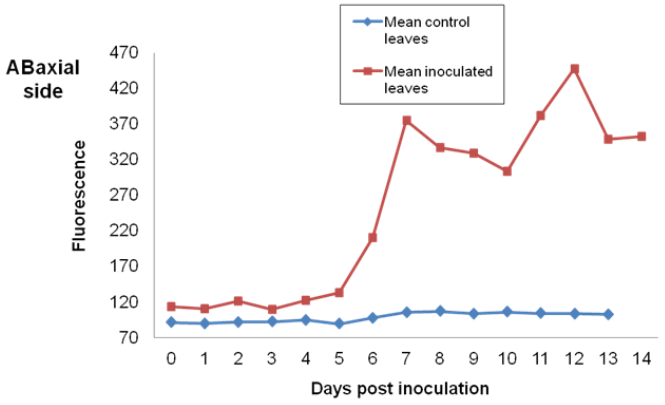
Greenhouse 2014

Results for downy mildew (example for sus. *vs.* res.)

Müller-Thurgau (susceptible)

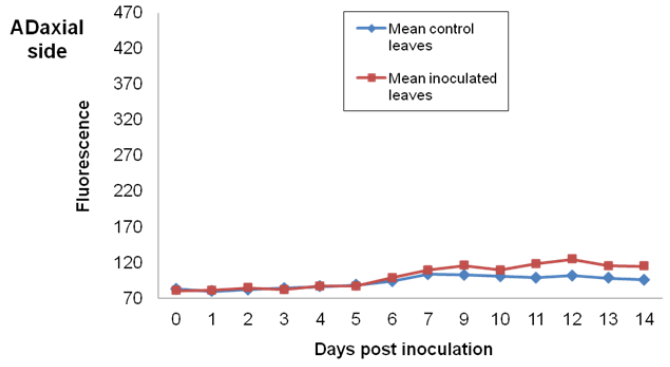
A

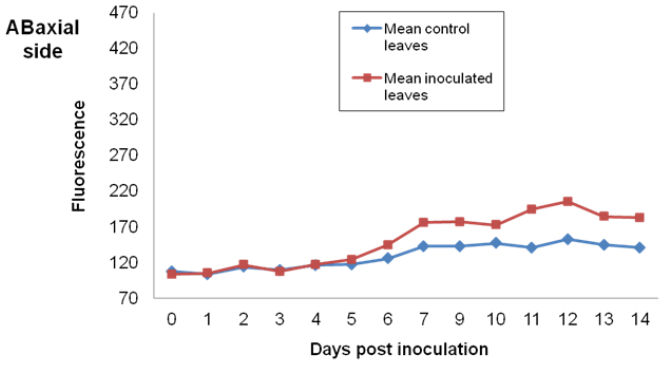




Regent (resistant)

B





Greenhouse 2014

Conclusion

- DM infection time course can be monitored *in situ* with the MX-330 sensor (change in BFG_UV level)
- Diseased vines can be clearly differentiated from healthy vines
- First significant signals detectable after 5-6 days after inoculation

Field 2016

- 6 different varieties:

Chardonnay → white grapevine variety

Cabernet Sauvignon → red grapevine variety

} Susceptible varieties

Solaris → white grapevine variety

Regent → red grapevine variety

} Resilient varieties

2011-003-0021

2011-007-0128

} Highly resilient varieties

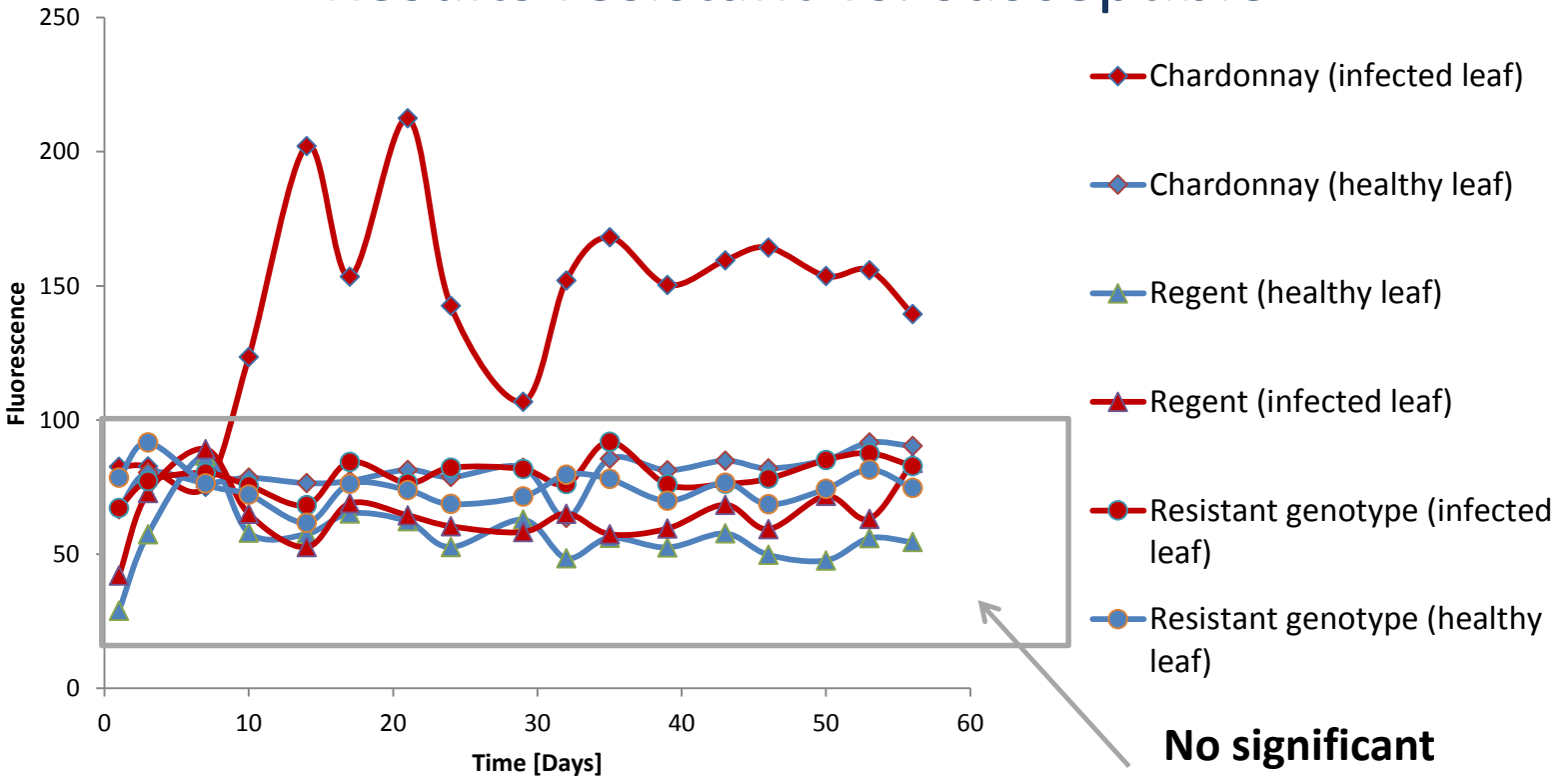
- 40 leaves per variety (2 heights: leaf on the height of first bunch and 8th – 10th leaf from cane); 10 leaves on each side of the canopy
- Measurement period started at BBCH 57 (beginning of June) until 100 % of infection

Field 2016

- 2 Measurements per week (3 – 4 days between) at the same time of day
- Adaxial (AD) and Abaxial (AB) leaf side
- Same leaves during the trail (marked at the beginning)
- Simultaneously to monitoring by Multiplex, visual screenings of measured area (6 cm diameter) → using OIV descriptors for downy mildew (DM) and powdery mildew (PM) (in 2016 no powdery mildew infections)
- Using BGF_UV index (BGF_UV = Blue Green Fluorescence under UV light excitation) → detecting stilbenes in leaf tissue

Field 2016

Results resistant vs. susceptible



No significant differences between healthy and infected leafs for different resistance levels

Field 2016

Conclusion

- Disease detection with Multiplex Mx 330 sensor is possible at abaxial side of leaves using BGF_UV index
- Healthy leaves of susceptible varieties can be clearly differentiated from infected leaves
- Downy mildew infections can be detected with Multiplex Mx 330 sensor from 5 – 6 % of infected leaf area onwards
- No differences between different levels of resistance were found using BGF_UV index

→Fluorescence signal of Regent and both highly resilient genotypes showed no significant deviation from each other and between infected and healthy leaves

Context

Fluorescence

- **Physiocap**

Reflectance

Image analysis

Infrared spectroscopy

Measurement of cane vigor provides information adding to leaf density and nitrogen content

Weight of wood prunings = number of canes * cane diameter

Number of canes

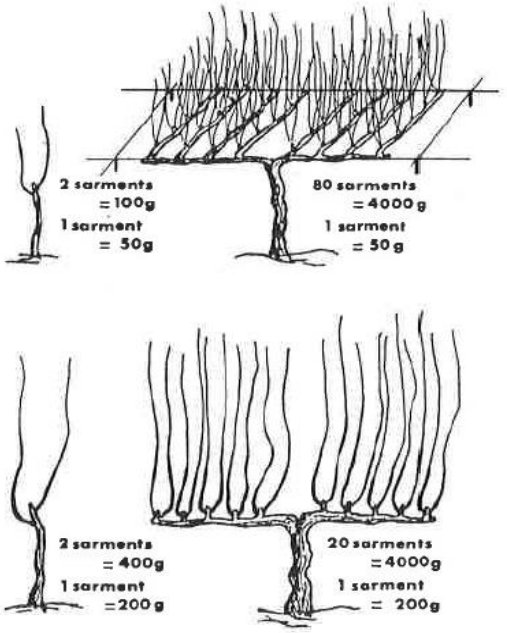
Low High

Small

Différent situations :

Diameter of canes

Large



FA-wood

Embarked Physiocap

Stage of measurement : winter phase before pruning

70 ha /week in a plot.

Measuring device

✓ Conception : CIVC

+ Tractor / High clearance tractor / Quad

✓ Passage every 5 m

Shapefile of vineyards + **History of plots**

FA-wood

MAPPING OF VIGOR THROUGH CANE

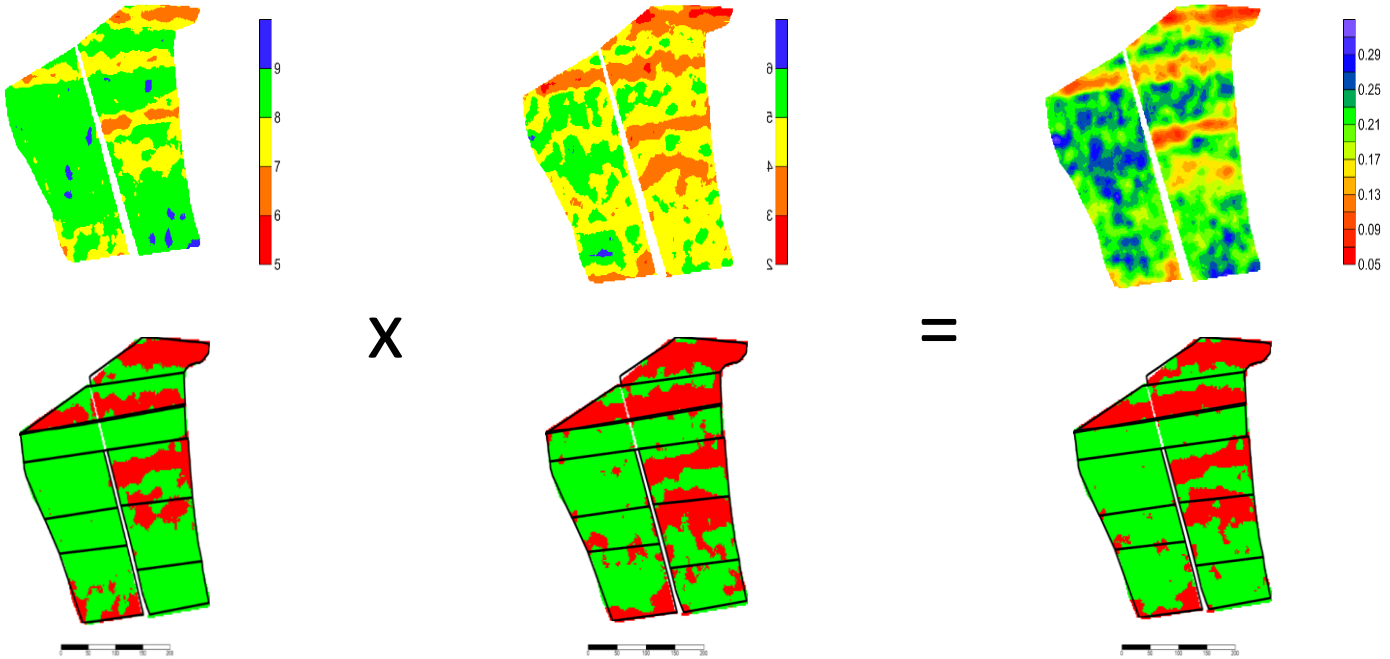
Indices measured :

- Cane diameter/ m^2 (or per plant)
- Cane number/m^2 (or per plant)

Deduced

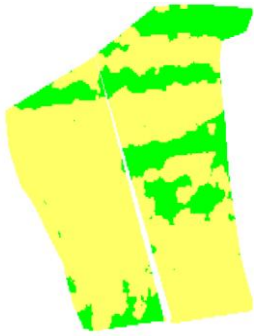
- Mean weight of pruned wood/ m^2 (or per plant)

Cane diameter mm /m^2 | **Cane number/m^2** | **Wood weight (Kg/m^2)**



+ Visual inspection (limited number of random points)

DIAGNOSIS MAP AND MATRIX USED FOR DECISION MAKING



	Low diameter <8 mm	Optimal diameter	High diameter >11 mmm
Low number of canes <5	Fertilize		Prune longer
Optimal number of canes			
High number of canes >7	Prune shorter		Cover crop

Unbalanced pruning

Unbalanced nitrogen

Thresholds are adjusted by calibrating with a reference plot

Context

Fluorescence

Physiocap

- Reflectance

Image analysis

Infrared spectroscopy

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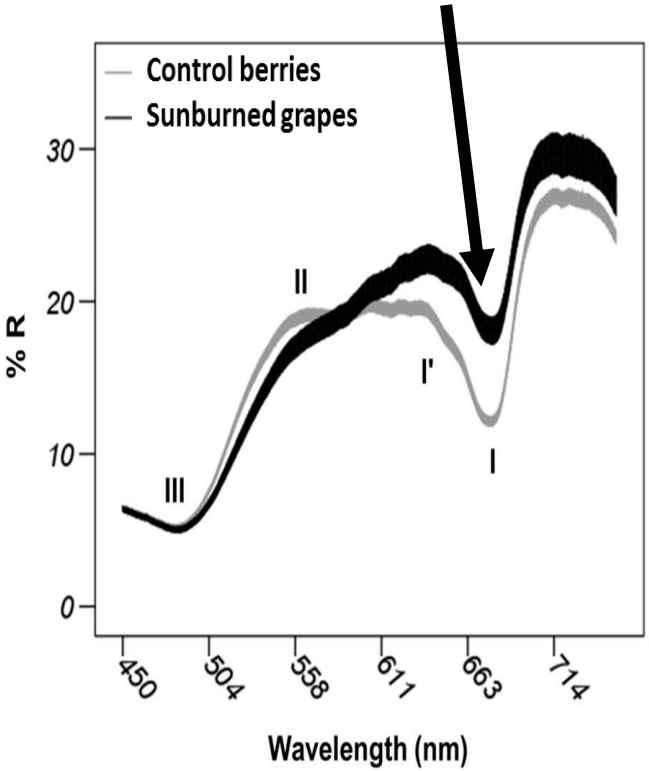
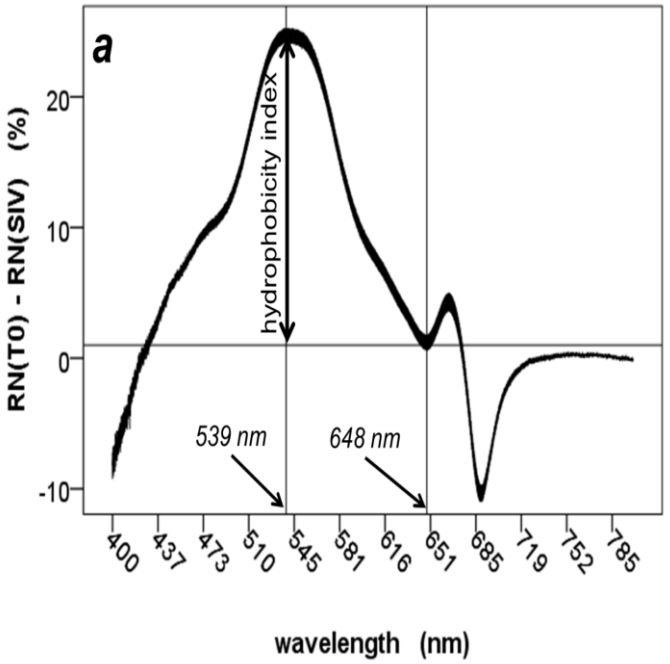


Example of white grape berry reflectance spectra (Rustioni et al., 2014)



Example of on-solid reaction for woody tissue hydrophobicity quantification (Rustioni et al., 2016)

RADIATIVE EXCESS AND SUNBURN SYMPTOMS

- A band related to the brown oxidized polymers has been identified.
- A method for the objective quantification of the sunburn symptoms has been developed.
- The central role of radiative excess have been demonstrated (temperature seems to play a secondary effect).
- Sunburn symptoms appeared related to the photosystems overexcitations.
- Berry susceptibility is increased by higher chlorophyll concentrations.

RIPENING

- Algorithms for the estimation of chlorophyll and carotenoid concentrations have been proposed.
- The yellow color of white grapes at ripening appeared to be mainly related to catabolic processes instead of accumulation of specific pigments

WATER STRESS

- Reflectance spectroscopy underlined modifications in the woody tissue pigmentations related to water deficit.
- Stem composition appears strongly related to the expected drought tolerance.
- Particular attention was paid to woody tissue hydrophobicity in relation to its physiological implications.

FUTURE APPLICATIONS

- Number of future applications.
- The Jaz System (Ocean Optics) used in these studies costed about 5000 Euros, and the rapid technological development continuously offer new solutions at lower prices.
- The analyses are very fast (few seconds/analysis), however an elaboration software for these applications is still missing.
- Working “on-solid”, the extraction procedures (and related limits and errors) are discarded.
- The methods are flexible and they can be adapted also to heterogeneous compounds (oxidative pigments, hydrophobic molecules…).
- This new knowledge could be applied for phenotyping screenings as well as for other physiological and characterization studies.

Context

Fluorescence

Physiocap

Reflectance

- Image analysis

Infrared spectroscopy

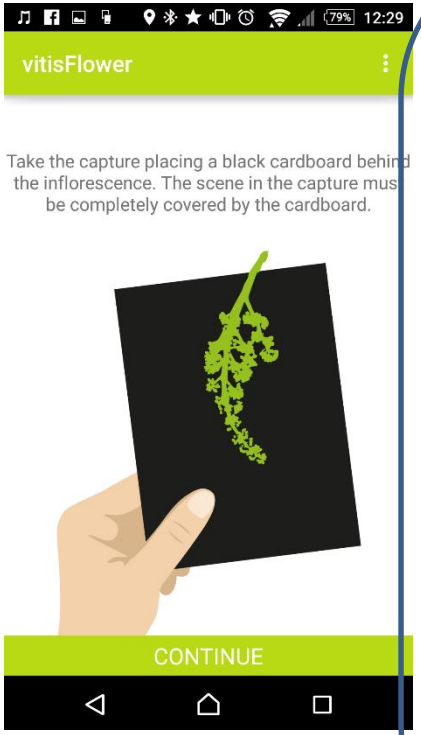
Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

1. To assess the number of flowers of an inflorescence using a machine vision model implemented in a smartphone app.

2. To develop a machine vision phenotyping tool to assess the number of berries in a cluster.

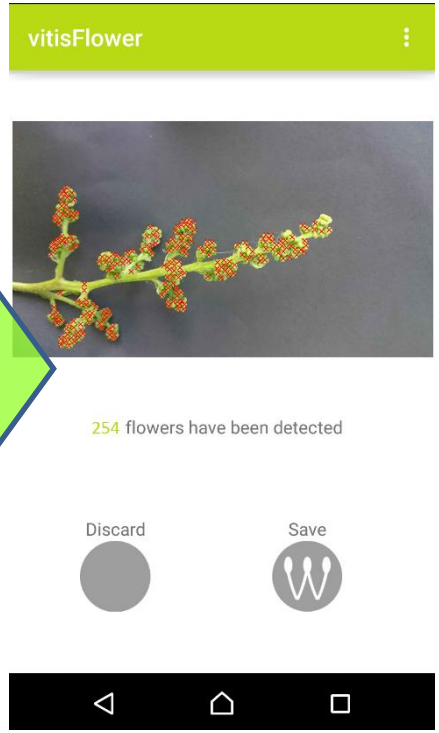
Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance

Home page



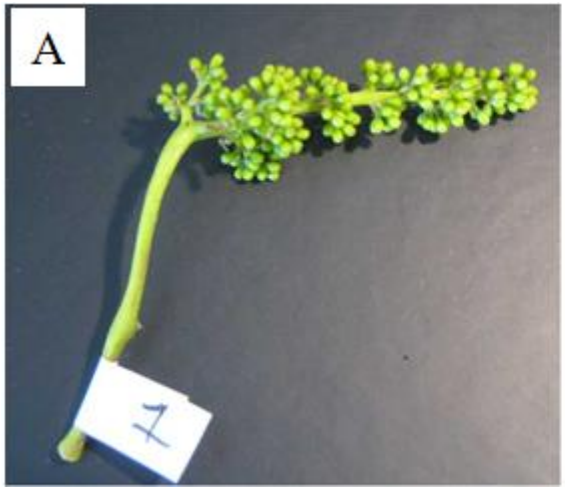
How to use the app

Image capture

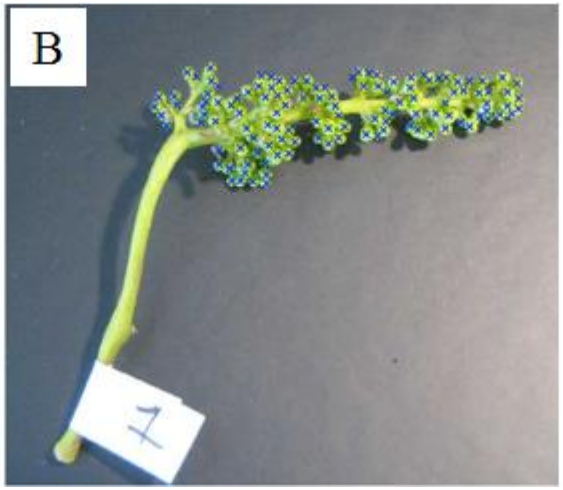


Results

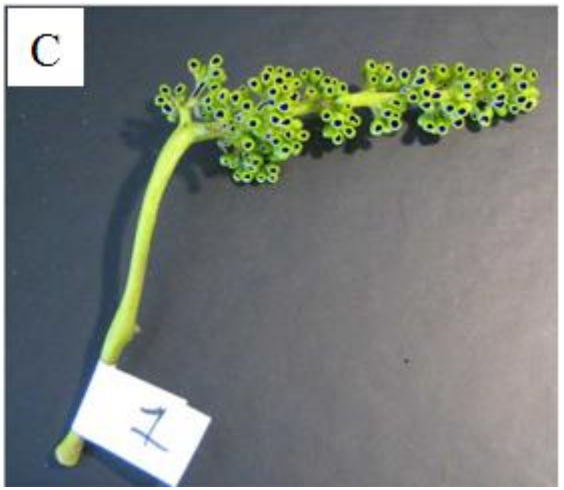
Detecting and counting flowers by means of image analysis:



Original image



Extraction of flower candidates



Final result after false positive filtering

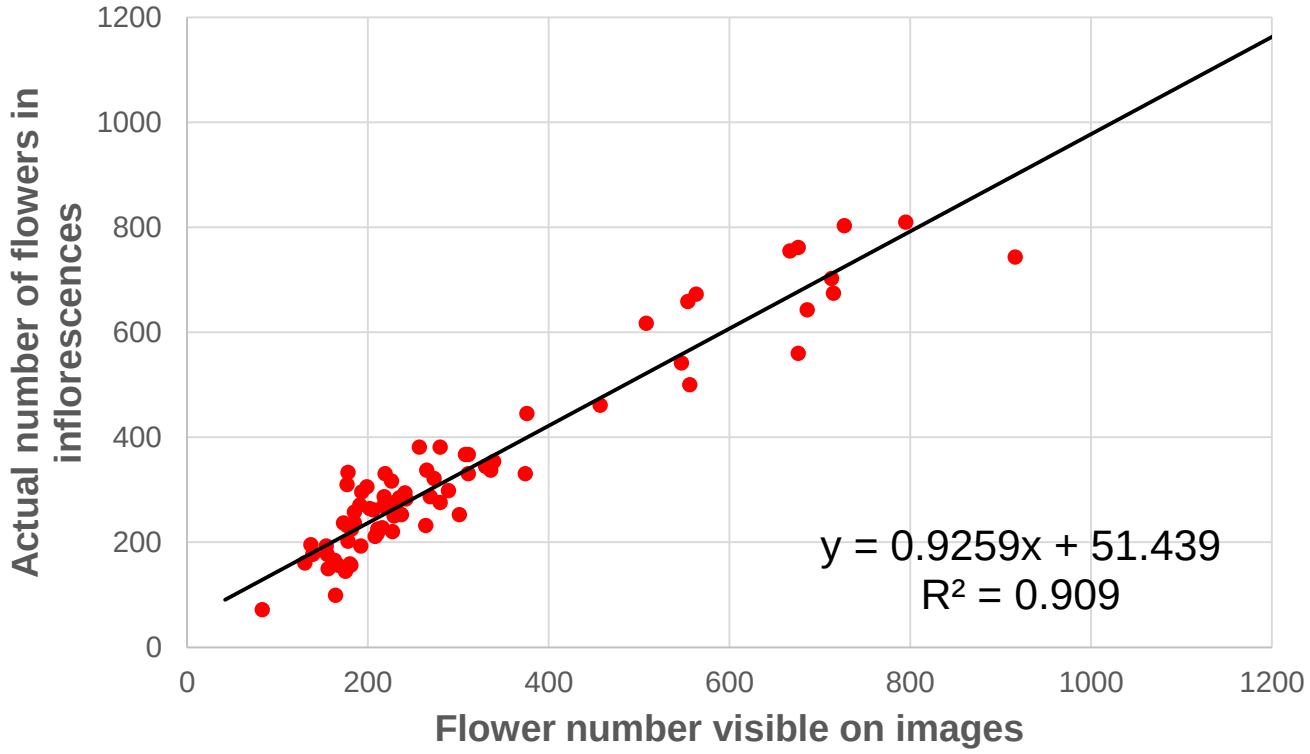
-The study was carried out at pre-flowering in a commercial nursery vineyard located in Falces (Navarra, Spain).

- Images of inflorescences from 11 Vitis vinifera L. varieties: Viognier, Verdejo, Touriga Nacional, Tempranillo, Syrah, Riesling, Pinot Noir, Grenache, Cabernet Sauvignon, Albariño and Airen were acquired. (12 inflorescences/variety). Total: 132 inflorescences.

#Berries assessment

- underway in the VitAdapt vineyard of the ISVV (Bordeaux, France).

Estimation of the actual flower number per inflorescence:



Context

Fluorescence

Physiocap

Reflectance

Image analysis

Infrared spectroscopy

Examples of results obtained with MULTIPLEX

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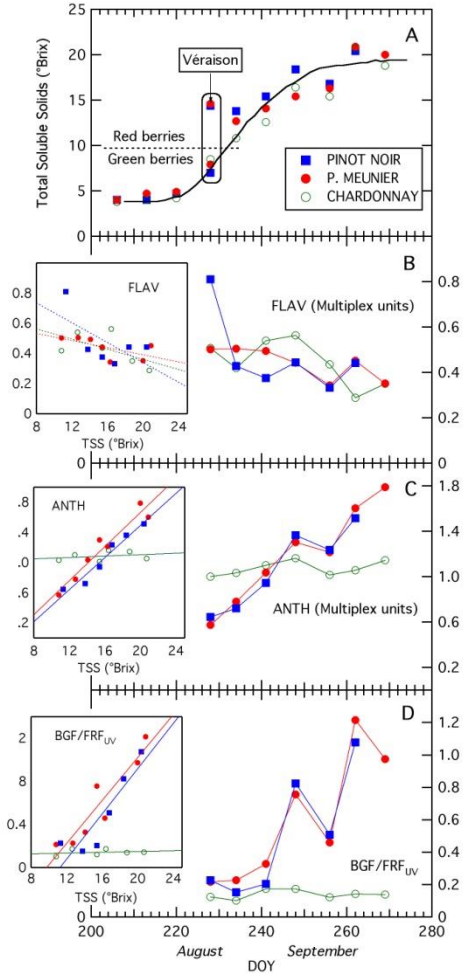
MULTIPLEX

labo



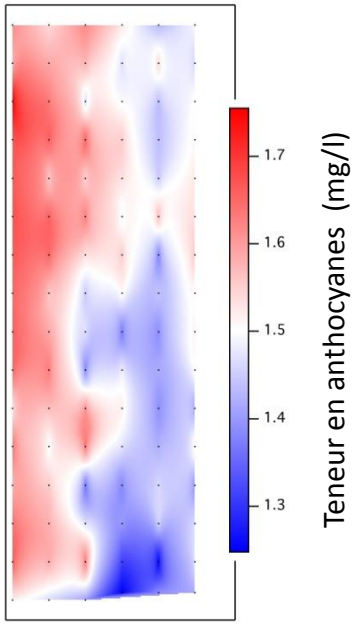
Seasonal monitoring

(Cerovic *et al.*, 2008)

Sugars

Flavonols

Anthocyanins



New indicator…under study
(depending on grape ripening)
Blue-Green fluorescence/Far-red fluorescence

Mapping

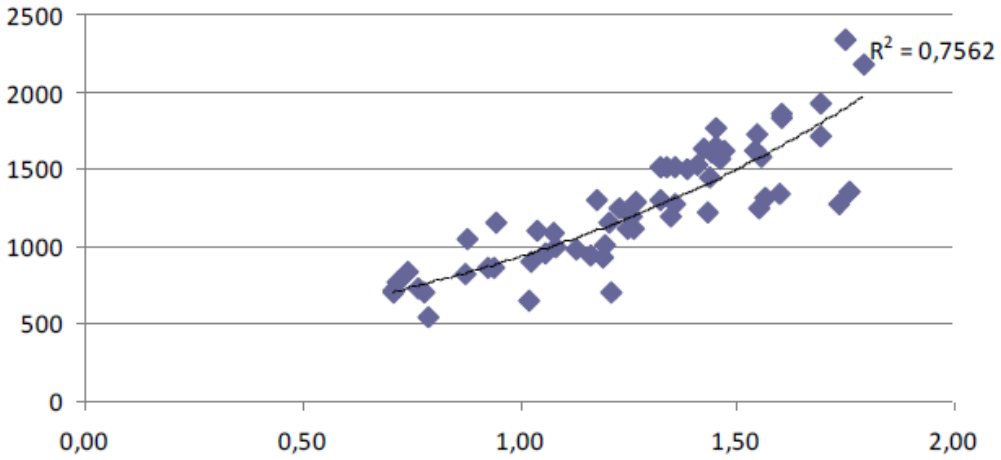
(Goutouly et Cerovic, 2008)

Main interest : possibility of establishing kinetics on the same berries or clusters with non destructive methods

« High-throughput » analysis of harvest

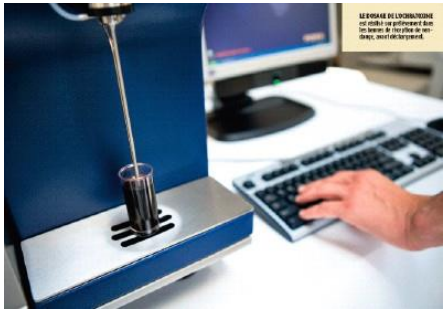
Multiplex

Anthocyanins (Glories)



Anthocyanin values (Multiplex units)

« High-throughput » analysis of the harvest in laboratory



OenoFoss – WineScan

(IRTF method: Infrared Spectroscopy with Fourier Transformation

MOUT

Mout de raisin

Brix
Densité
Acide malique
pH
Acide Tartrique
Acidité totale

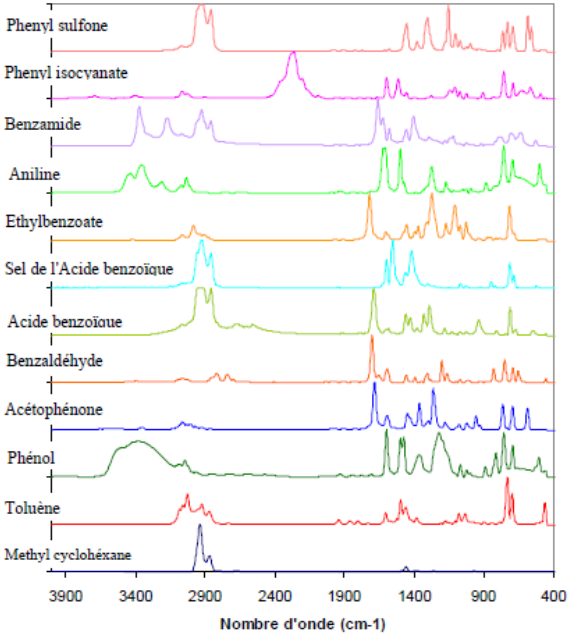
Pourriture Acide
Ethanol
Activité Fermentaire
Acide gluconique
Glycerol
Pourriture Grise
Activité Lactique
Acidité Volatile

Azote alpha Aminé
Azote Ammoniacal
Anthocyanes
Acide Citrique
Intensité Couleur Extrait
Extrait
IPT
Fructose
Acide gluconique
Glucose
Glycerol
Acide Lactique
Potassium
Sucres réducteurs
Acide Succinique
OD280
OD520

VIN FINI

Vin fini sec

A420***
A520***
A620***
Acide citrique
CO_2
Densité
Ethanol
Acétate d'Ethyle
Fructose
Acide gluconique
Glucose
Glucose+Fructose
Glycerol
Acide Lactique
Acide Malique
Methanol
pH
Sucres réducteurs
Acide Sorbique
Acide Tartrique
Acidité totale
IPT
Acidité Volatile



Berry

Parameter	Range	Repeatability (+/-) *	Precision (SE)**
Density	1.01 - 1.10	0.0002	0.001
Reducing sugars	40-350	0.5	2
Total acidity	2 - 20	0.05	0.10
pH	2.5 – 4.0	0.02	0.04
Volatile acidity	0 – 0.20	0.02	0.03
Malic acid	1 - 15	0.1	0.25
Tartaric acid	3- 10	0.1	0.4

Wine

Parameter	Range	Repeatability (+/-) *	Precision (SE)**
Density	0.99-1.10	0.0002	0.001
Alcohol	0 – 15	0.02	0.08
Reducing sugars	0 – 220	0.7	2.5
Total acidity	2 – 7	0.06	0.14
pH	3.0 – 4.0	0.02	0.045
Volatile acidity	0 – 0.6	0.02	0.040
Malic acid	0 – 4	0.05	0.20

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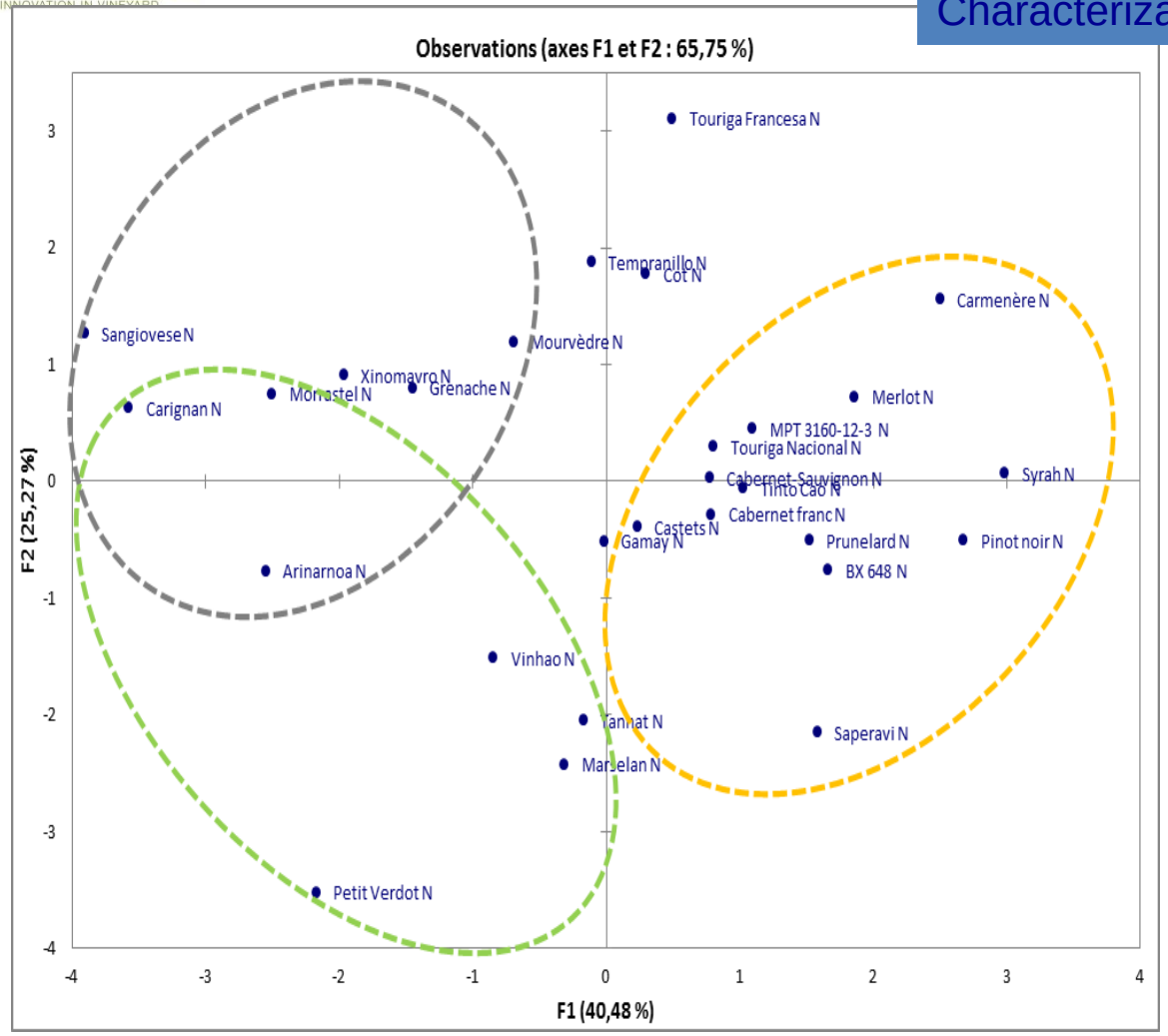
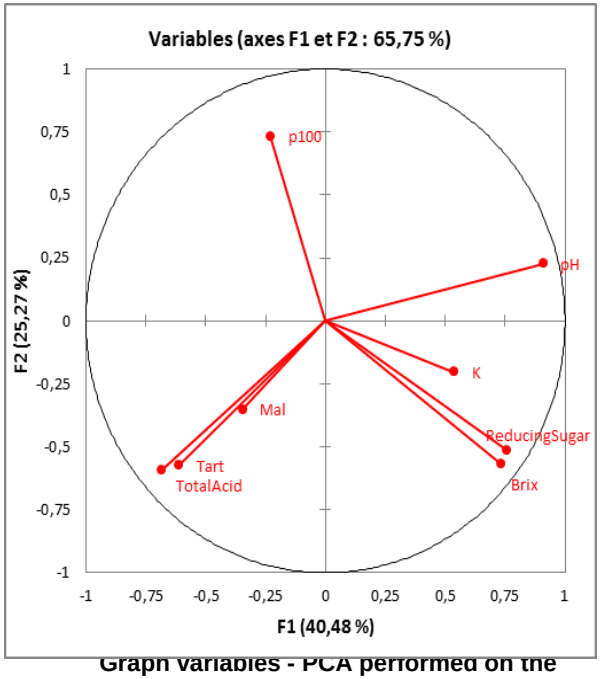
Characterization of groups of varieties



Graph individuals - PCA performed on the variables "size and composition of the berry" for all red varieties studied in 2013



Graph variables - PCA performed on the variables "size and composition of the berry" for all red varieties studied in 2013

High berry weight cultivars

High sugar content cultivars

High acid content cultivars

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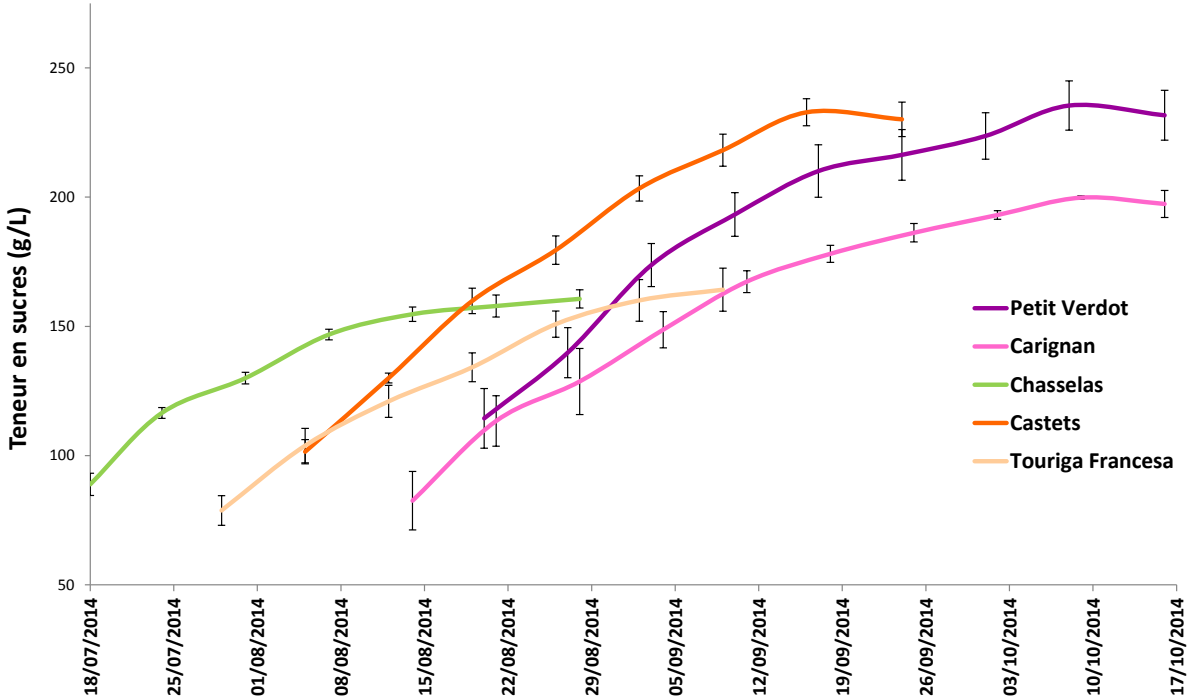
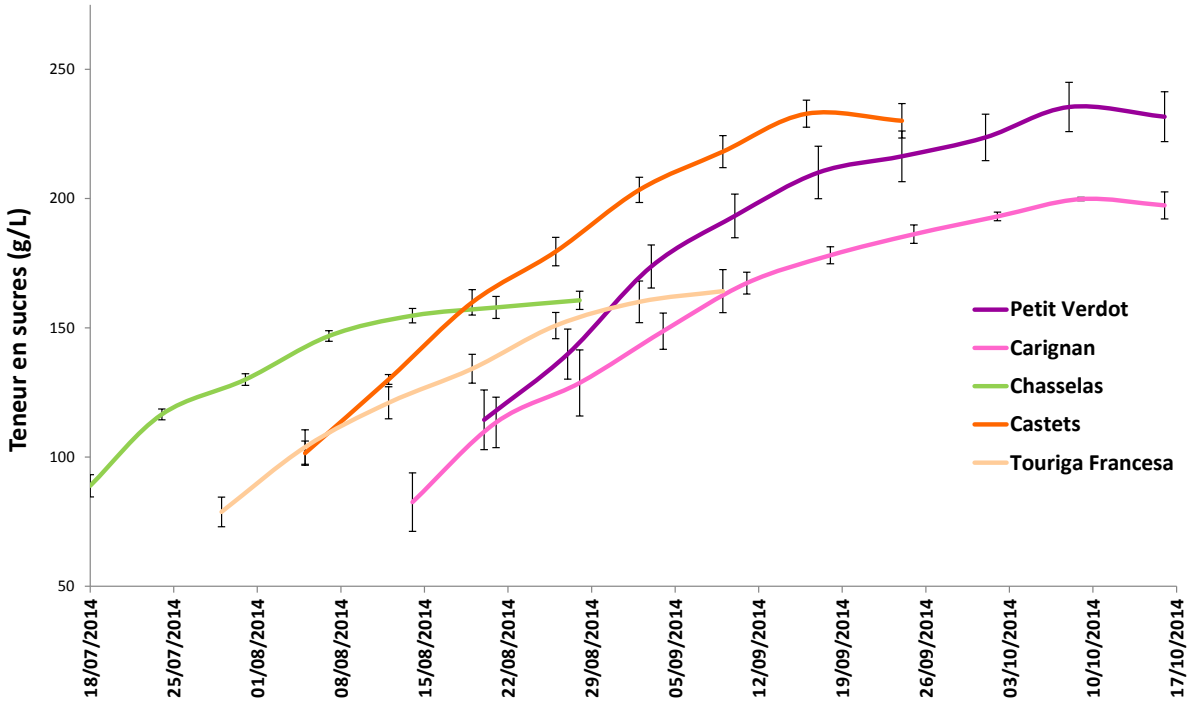


Main results and achievements: INRA Bordeaux



CONCLUSIONS

Many phenotyping approaches may be used and are in constant progress
Monitoring of grape development, ripening, and impact of disease

Modelling, Decision support systems

Further developments

Geopositioning
Hyperspectral imaging
Robots
Drones
Data bases

Thanks to

Jean-Pascal Goutouly[1], Naima Ben Ghozlen[2], Jean-Luc Ayral[2], ZhanWu Dai[1], Agnès Destrac[1], Susann Titmann[3], Manfred Stoll[3], Laura Rustioni[4], Osvaldo Failla[4], Javier Tardaguila[5], Anna Kicherer[6], Reinhard Töpfer[6], Cornelis Van Leeuwen[1], Serge Delrot[1]*

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[4] University of Milan, Italy ;
[5] University of la Rioja, Spain ;
[6] Julius Kühn Institut Siebeldingen, Germany.

Thanks for your attention