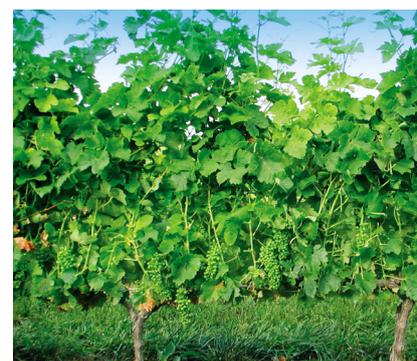




**Final International Symposium**  
Toulouse, South-West of France  
16 & 17 November 2016

# Book of abstracts



European collaborative project funded by the European Union

**FP7 Knowledge Based Bio-Economy (KBBE) program**

Grant Agreement n° 311775



# A few words about InnoVine and the 4 years of the project

The wine industry is a major economic sector through the European Union where wine production represented in 2010/2011 about 60% of the whole amount elaborated on the planet.

Yet, European wine producers must face several key issues and challenges such as :

- unwanted evolution of the wine quality due to climate change in a crop which is strongly driven by the product value,
- threats on the sustainability of the vineyards due to combination of biotic and abiotic stresses more and more in relation with the climate change,
- the necessity to reduce the use of pesticide on a crop for which very few qualitative resistant cultivars are available and for which the resistance of varieties has to be managed to be durable,
- a still growing competition on world markets that strengthens the importance to fit consumer's demands, which can be quite versatile while viticulture relies on long term plantings and investments.

The InnoVine project, funded through the European FP7 Knowledge Based Bio-Economy (KBBE) program, was launched in January 2013 with the aim to address some of these challenges. During 4 years, it has involved 27 different partners from 7 European countries (Bulgaria, France, Germany, Hungary, Italy, Portugal and Spain). During its four years of life, the 15 public and 12 private partners of the projects have de-

veloped a wide range of knowledge and tools to help viticulture to adapt to climate change and to the need of a drastic reduction of chemicals in the vineyards. The consortium has for instance experimented many practices aiming at manipulating the carbon balance of the plant at various stages of its development to test and understand their effects on the berry composition (quality) and its effect on diseases. Large panels of genetic resources have also been screened to provide the breeders with new sources of genetic resistance to foliar diseases and the producers with sets of grapevine varieties or of clones characterized for their modes of adaptation to drought stresses. DSS systems have been improved to take into account improved disease models, environment impacts, impacts on the vintage, new monitoring tools... A wide range of high throughput, non-destructive phenotyping and monitoring tools have been developed and tested.

Finally, brainstorming sessions aiming at proposing new viticulture systems (combination of several practices) based on the expertise of the participants but also on InnoVine results have been organized. The two days of conference will make a panorama of the results obtained during the project, the first day focussing on the knowledge developed and the second on results transferable to the users.

InnoVine was also a rich human adventure, mixing a wide range of expertise and of point of views in fruitful collaborations and we hope that it will be a seed for future successful projects.



Anne-Françoise Adam-Blondon  
Coordinator of the project

# Symposium schedule

**Wednesday, November 16th,  
8h30 - 18h00**

8h30 – 9h30. Registration

9h30 - 10h00. Welcome and introductions from the president of the Occitanie region, the president of the INRA regional center (Michèle Marin), the EU commission program officer (Massimo Burioni) and the coordinator of the project (A-F Adam-Blondon)

## Session 1 : Grape adaptation to diseases and climate change

Chair person A-F Adam-Blondon

10h00 - 10h40. Keynote lecture - Bruce Reisch : Grapevine Breeding in the United States: Perspectives from the VitisGen Project

10h40 – 11h05. François Delmotte : Durability assessment of resistance

11h05-11h30. Coffee break

11h30 – 11h55. Reinhard Töpfer : Needs for new disease resistance

11h55 – 12h20. Osvaldo Failla : Screening for new sources of powdery and downy mildew resistance

12h20-12h45. Ludger Hausmann : Developing tools and resources for breeding grapevines resistant to black rot or phylloxera.

12h45 – 13h05. José-Miguel Martínez-Zapater : Screening germplasm for adaptation to drought and temperature

13h05 – 14h30. Lunch and posters.

14h30 – 14h55. Manfred Stoll : Canopy management to support disease control : what do we learn from Innovine ?

14h55 – 15h20. F. Bove : Modeling downy mildew epidemics in susceptible and resistant genotypes

## Session 2 : Grape berry ripening: environmental drivers and spoilers

Chair person C. Lopes

15h20 – 16h00. Keynote lecture - Markus Keller : Grape berry ripening: Environmental drivers and spoilers

16h00-16h30. Coffee break

16h30 – 16h55. Serge Delrot: Phenotyping tools and usefulness for understanding biological traits growth and disease

16h55 – 17h20. Zhanwu Dai: Modeling the influence of environmental stress on berry composition

17h20 – 17h45. Stefano Poni: The uncoupling of berry ripening: what we learned from INNOVINE.

17h45 – 18h00. Round table with the advisory platform



## Thursday, November 17th, 9h00 – 17h00

9h00-9h20. Welcome speeches

### Session 3 : Resistant varieties/Sensors/ Decision Support Systems/Systems

Chair persons Eric Serrano, M. Torres/V. Rossi

9h20-9h40. Keynote lecture from the Gruppo Italiano Vini : Grower's perspective on innovation towards sustainability

9h40-10h15. Reducing the impact of viticulture through the development of new varieties

- Laurent Audeguin : Worldwide view of breeding for disease resistant grapevine varieties and what Innovine is bringing to the scope
- Elisa De Luca. What are the new findings obtained during the project?

10h15-10h50 Prototyping new viticulture systems

- Laurent Delière : Application of the approach system in viticulture
- David Lafond : Examples of systems conceived and tested in InnoVine

10h50-11h10. Coffee break

11h10-12h30 Assisting growers' decision

- Christophe Guizard : State of the art on sensors and monitoring tools in viticulture. New sensors and new applications developed during InnoVine by Force A, Noveltis, ICVV and AGRI-Ciência
- Vittorio Rossi : A review on the Decision Support Systems (DSSs) and models available in viticulture. New models and adaptation of existing models carried out in the frame of InnoVine by Horta, IFV

12h30 – 14h00. Lunch and posters session

14h30-17h00. Tasting, DSSs and sensors demonstration sessions

- Thematic wine tasting (resistant and new varieties, canopy management trial)
- Interactive demonstration of DSSs and sensors (Vite.net, AGRI-Ciência web solutions, Wineo, Multiplex, Smartgrape, Vitisflower, PTO).

# Talks

- p 9 ▶ Grapevine breeding in the United States: Perspectives from the VitisGen Project *Reisch et al.*
- p 10 ▶ A key to sustainable management of grapevine resistance to downy and powdery mildew *Delmotte*
- p 11 ▶ Needs for new disease resistance *Töpfer et al.*
- p 12 ▶ Screening for new sources of powdery and downy mildew resistance *Failla et al.*
- p 13 ▶ Developing tools and resources for breeding grapevines resistant to black rot or phylloxera *Hausmann et al.*
- p 14 ▶ Screening germplasm for adaptation to drought and temperature *Pinasseau et al.*
- p 16 ▶ Canopy management to support disease control: what do we learn from InnoVine? *Stoll et al.*
- p 17 ▶ Resistance components to *Plasmopara viticola* and *Erysiphe necator* in grapevine genotypes *Bove et al.*
- p 18 ▶ Grape berry ripening: environmental drivers and spoilers *Keller*
- p 19 ▶ Phenotyping tools and their usefulness for understanding biological traits related to growth, ripening and disease resistance *Goutouly et al.*
- p 21 ▶ Modelling the influence of environmental stresses on berry composition *Zhu et al.*
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- ▶ WINEO: Characterization of vineyard plots by airborne/drone imagery *Poustopis* p 30
- ▶ Vitisflower®, a new app to count the number of flowers per inflorescence under field conditions *Tardaguila et al.* p 31
- ▶ A review on models and Decision Support Systems available in viticulture *Rossi* p 32
- ▶ vite.net® a Decision Support System for sustainable management of the vineyard *Legler et al.* p 33
- ▶ Development of an a priori zoning of Physiological Behavior Units of vines, using precision viticulture techniques *Raynal et al.* p 34

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- p 37 ▶ Building the stakeholder platform to foster sustainability in the wine sector *Corbo et al.*
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- p 40 ▶ A new tool for the evaluation of the agronomic management of the vineyard *Lamastra et al.*
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- ▶ Placing Vitaceae proanthocyanidins in a phylometabolic perspective *Brillouet et al.* p 57
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- ▶ Evaluation d'un équipement de suivi maturité : Le Multiplex® en Aquitaine *Vinsonneau et al.* p 59
- ▶ Characterisation of muscadine resistance to phyloxera. *Lalanne Tisé et al.* p 60
- ▶ WINETWORK project: Identification and dissemination of innovative control strategies for Flavescence Dorée and GTD management *Prezman et al.* p 61
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# Grapevine breeding in the United States: Perspectives from the *VitisGen* Project

Bruce I. REISCH<sup>1</sup>, Elizabeth M. DEMMINGS<sup>1</sup>, Lance CADLE-DAVIDSON<sup>2</sup> and the *VitisGen* team<sup>3</sup>

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The *VitisGen* project ([www.vitisgen.org](http://www.vitisgen.org)) united public grapevine breeding programs in the United States expressly for the purpose of accelerating grapevine cultivar development via next generation sequencing technology, and both local and centralized trait evaluation. More than 20 Project Directors led efforts in breeding, genomics, computational biology, trait economics, and extension. To integrate unique genetic resources, cutting-edge genotypic data, and robust phenotypes, our research team 1) maintained breeding germplasm locally, 2) collected and analyzed high-resolution genotypic data centrally, and 3) collected and analyzed phenotypic data both locally and in three phenotyping centers specializing in fruit chemistry, low temperature responses, and powdery mildew resistance. Locally, our research teams phenotyped more than 100 unique traits ranging from disease resistance (to pathogens such as downy mildew, black rot, and

Phomopsis) to fruit quality attributes (such as berry skin color, berry diameter, and seedlessness). Here, we will share some of the advances derived from our experience maintaining 17 core mapping families, genotyping 28,000 individuals (mapping populations; seedlings for marker-assisted selection; and germplasm), and collecting over a million phenotypic data points. We will also highlight some of the individual projects within *VitisGen* that have specifically aided our goals to advance cultivar development for traits of importance to industry stakeholders.

The *VitisGen* project was funded by the USDA-NIFA Specialty Crop Research Initiative award number 2011-51181-30635.

# A key to sustainable management of grapevine resistance to downy and powdery mildew

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The use of grapevine disease resistant cultivars is certainly one of the most promising innovation to decrease grapevine dependency on fungicides. Varietal resistance introduces a new component into vineyards that is likely to modify in depth disease management strategies. Unfortunately, recent evidences suggest that fungal pathogens may be able to erodes partial resistance despite a limited deployment of these culti-

vars. The evolution of these new cropping systems is therefore facing a major challenge, which is to maintain the efficacy of resistance in the long term by slowing down the adaptation of pathogen populations. Several complementary approaches are currently explored to provide elements of knowledge on the durability of cropping systems based on these resistant varieties.

# Needs for new disease resistance

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Diseases like powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) are serious phytosanitary challenges in viticulture. They cause severe plant protection treatments which are applied in a preventive manner as the traditional wine grape cultivars are without exception susceptible to the mildews. One option to reduce the environmental impact of these treatments is the use of disease resistant cultivars. Resistances originate from introgressions from American or Asian wild *Vitis* species with a drawback for quality in early backcross generations.

In several countries resistance breeding has a long tradition and cultivars showing good field resistance and good wine quality became available since the middle of the 1990th. The origin of these currently available cultivars goes several decades back when their crosses were made. At that time selection was done exclusively phenotypically. Meanwhile breeders look back to a decade of intense genetic analyses in grapevine research resulting in genetic markers as an excellent tool to assist selection (MAS). Today breeders can make use of almost a dozen resistance loci and correlating genetic markers for both mildew diseases making selection easier. Genetic analysis of the currently available high quality new cultivars from resistance breeding programs revealed that they carry single resistances for either one of the mildews

(1&1). As consequence MAS is currently used to stack resistance loci generating plants with 2&2, 3&3 or intermediate combinations of resistances. Despite of this amazing progress new resistances are required in view of durability of resistance. In particular downy mildew resistance loci confer as single loci mostly medium strong resistance. On the other hand reduced plant protection treatments in vineyards with mildew resistant new cultivars resulted in the emergence of other fungal diseases such as black rot. As a consequence the breeding goals in resistance breeding were extended. Today important goals for grapevine breeders are resistance to powdery mildew, downy mildew, and black rot but also resilience against botrytis. The scenario for viticulture in future is the cultivation of new grapevine cultivars from resistance breeding programs which are managed with a minimal plant protection regime. These new cultivars shall be selected in the expectation of global warming, thus showing e.g. higher drought tolerance and/or later ripening.

In contrast to wine grape breeding for rootstock breeders phylloxera and nematode resistance are of primary importance. They also select genotypes for adaptation to abiotic stress factors.

# Screening for new sources of powdery and downy mildew resistance

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Powdery and downy mildew susceptibility of *Vitis vinifera* L. cultivars represent one of the main weakness of the traditional viticultural models. The sources of resistance from American species have been exploited since the end of the XIX century. More recently also sources from Asiatic species have been utilized for pyramiding resistance to mildews into *Vitis vinifera* genome by pseudo backcrossing breeding strategies. The recent easier access to East European and Caucasian germplasm, both wild and cultivated, has now opened new perspectives for the identification of novel sources of resistance to mildews within the *V. vinifera* cultigen.

In the framework of the INNOVINE WP3 Task 3.1, a large screening to search for resistance to downy (DM) and powdery (PM) mildews have been completed.

The plant material involved in the workplan was mainly from the Caucasian region (Georgia, Armenia and Azerbaijan). Other east European and oriental regions were represented: Balkans, North Black sea region, Central Asia and Near East. For wild grapevines (*Vitis vinifera silvestris*) also Mediterranean proveniences were selected.

DM resistance was screened by experimental inoculations of leaf disks with *Plasmopara viticola* natural populations collected in the field. PM by natural or handmade infections of *Erysiphe necator* in greenhouse.

The total number of tests carried out during the whole project was 1686 for DM and 1130 for PM.

Thirty-six accessions showed a degree of resistance to DM from medium-high to very high (score > 6 according the OIV scheme): 33 are from Caucasus (33 from Georgia). 22 accessions are *V. vinifera vinifera* and 14 are *V. vinifera silvestris*.

Two hundred and eighty accessions showed a leaf degree of resistance to PM from medium-high to very high (score > 6 always according the OIV scheme): 248 accessions are from Caucasus, and 199 of these are from Georgia. 251 are *V. vinifera vinifera* and 29 *V. vinifera silvestris*. 40 accessions did not show any PM symptoms in greenhouse (mainly from Caucasus, Georgia in particular).

# Developing tools and resources for breeding grapevines resistant to black rot or phylloxera

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In the middle of the 19th century four pathogens of severe grapevine diseases were introduced from North America to Europe: The fungi (i) powdery mildew (*Erysiphe necator*), (ii) downy mildew (*Plasmopara viticola*), (iii) black rot (*Guignardia bidwellii*) and (iv) the insect phylloxera (*Daktulosphaira vitifoliae*). In general there are two strategies to combat these diseases, either with pesticide treatments or by breeding highly tolerant new varieties based on resistant resources of mainly non-European *Vitis* accessions. Whereas tolerant rootstocks are used to control phylloxera, less environmental-friendly chemical treatments are still the common practice against the fungal diseases and only a small acreage is planted with mildew tolerant varieties. If the climate changes in an adverse direction and chemical plant protection is considerably reduced, new and serious threats like black rot could emerge in viticulture.

Meanwhile several different resistances against both mildew diseases have been genetically characterized. However, knowledge about black rot and phylloxera resistances is still very limited. Therefore we started to develop methods and protocols for the screening of different kind of grapevine materials regarding resistance against black rot and phylloxera. For black rot potted vines were artificially inoculated and maintained under controlled conditions in a climate chamber for three weeks. The degree of resistance was evaluated using a five class scale similar to the known OIV descriptor list. All the European traditional grapevine varieties tested proved to be highly susceptible. In

contrast, among the set of new bred varieties and breeding lines a few turned out to show good tolerance against black rot (e.g. 'Felicia', 'Calardis Blanc', 'Merzling'). Furthermore, a notable high number of resistant varieties were found in a set of so-called American-French hybrids. This means that there are many resistances in the grapevine gene pool available but they were mostly lost during the breeding process. To overcome this problem we started to genomically localize black rot resistance in biparental mapping populations and to develop molecular markers for marker-assisted selection useful in breeding programs. Current results indicate that there is a major resistance locus on chromosome 14 in different North American grapevine accessions.

The search for phylloxera resistance was similar as outlined for black rot. Here, the formation of nodosities, that are the disease symptoms at the root tips of the experimental plants, were evaluated to determine the degree of resistance. New resistance resources were detected in individual accessions of the North American *V. aestivalis* species. The chromosomal position of a highly resistant breeding line based on *Muscadinia rotundifolia* and of the variety 'Börner' were analyzed. Once we have molecular markers for different resistance loci new scion and rootstock varieties with combined and therefore durable tolerance towards black rot and phylloxera can be bred. These new cultivars would be more environmentally friendly and consider future negative impact of climate change.

# Screening germplasm for adaptation to drought and temperature

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Changing climatic conditions are imposing the need to search for adapted grapevine varieties and clones. The screening of genetic resources more efficient in the use of water and more tolerant to higher temperatures along berry ripening constitute the first step for cultivar innovation and for the selection of germplasm suitable for the breeding of new cultivars. With this goal, we have searched for grapevine genetic variation at species (inter-varietal) and at cultivar (intra-varietal) levels.

At the species level, a core collection of 279 varieties has been grown under two irrigation regimes and analyzed for morphological, agronomical and biochemical traits in pulp and skin extracts in two successive vintages (2014 and 2015). High throughput methods developed for quantification of phenolic compounds and aroma precursors have been used to phenotype this panel, highlighting differences in cultivar response to drought. At the intra-varietal level we used as a reference two large collections of cultivar Tempranillo/Aragonez accessions and selected those ones showing lower stress response as measured by a lower Surface Leaf Temperature (SLT) or those showing a longer

berry ripening period. All strategies have been successful in detecting putatively useful genetic variation as a starting point in the breeding of new more drought tolerant cultivars as well as for innovation of Tempranillo/Aragonez cultivar.

Further analyses were focused to understand the genetic and molecular basis of the identified variation. At the species level, genotyping of the core collection with large sets of SNP markers will allow scanning of the whole genome for significant genotype-phenotype associations (GWAS) not only for each trait separately but also jointly for multi-traits and with multi-year data. This genetic analysis is complemented by transcriptome analyses comparing the response of isohydric (Montepulciano) and anisohydric (Sangiovese) cultivars to water stress. The results of experiments performed on pre-veraison stressed-samples allowed the identification of molecular markers related to the distinct stomatal behavior of both cultivars and its impact on leaf and berry gene expression. Regarding the selected Tempranillo/Aragonez accessions, the expression

analyses of stress-responsive genes in low and high SLT accessions showed significantly high correlations with the predicted SLT genotypic values. Furthermore, RNA-seq experiments conducted in ripening berries of long and short ripening period accessions allowed the identification of genes and genomic regions putatively involved in the observed early or late ripening behavior. Altogether these results demonstrate the existence of grapevine genetic variation potentially useful to adapt to climate change conditions and open the way to its genetic and molecular characterization.

# Canopy management to support disease control: what do we learn from InnoVine?

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Grapevine is a crop particularly dependent on pesticide use requiring a treatment index markedly above ten. Nevertheless, fungal infection can cause extensive fruit quality and economic losses in almost any grape growing region around the world. Despite integrated pest management programs, fungicide application is the most common strategy to control fungal development. Most of the sprays usually applied in the vineyard are targeted at powdery (*Erysiphe necator*) and downy (*Plasmopara viticola*) mildew and bunch rot, caused by *Botrytis cinerea*. The aim within the InnoVine Work Package 2 (Designing optimized vineyard practices to reduce pesticides) was to assess practical solutions to reduce fungicide input in viticulture by applying phytosanitary and cultural methods – without detrimental losses in profitability or quality.

Field experiments at partners in France (INRA: Institut national de la recherche agronomique; ForceA, SME), Italy (UCSC, Università cattolica del Sacro Cuore; HORTA, SME; IGA, Università degli studi di Udine), Portugal (ITQB, Instituto de Tecnologia Química e Biológica; ISA, Instituto Superior de Agronomia) and Germany (GRC; former Geisenheim Research Centre – now Geisenheim University; JKI, Julius-Kühn Institut) were conducted to evaluate the impact of novel canopy management practices on the sanitary status of the plants and on the berry composition to evaluate their potential feasibility.

With all varieties tested the risk of bunch rot was mainly reduced by decreasing cluster compactness. Changes in leaf area to fruit weight ratio led to reduced fruit-set, loosening cluster compactness, reduced cluster weight, crop load and the susceptibility to bunch rot as well as impacting on the ripening process. Results also hint to the point that pre-flowering leaf removal, heavy shading and pruning systems with high bud load and afterward adjusted through bunch thinning are the best techniques to increase indirect tolerance to bunch rots. In normal seasons, a combination of these strategies seems to be an excellent tool to decrease bunch rot risk in the vineyard.

Whilst cluster compactness plays a key role on bunch rot development, there is no immediate relation between cultural practices and the severity of downy and powdery mildew. Under severe infection conditions an intensive fungicide application schedule with short intervals is strongly required for *Vitis vinifera* cultivars to overcome negative impacts in concern of quantity and quality. The canopy manipulation strategies trialed were not able to reduce *P. viticola* infection significantly. Instead, mildew resistant varieties have been shown to be the most promising strategy to produce healthy grapes with less fungicide input.

# Resistance components to *Plasmopara viticola* and *Erysiphe necator* in grapevine genotypes

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Resistance to downy mildew (DM) was evaluated in 15 grapevine genotypes (Felicia, Calandro, Calardis blanc, Villaris, Regent, Reberger, Bronner, Solaris, Johanniter, Palava, Rkatsiteli, Early Merlot, Merlot khorus, Cabernet volos and Fleurtaï) bringing one or more of the following resistance genes from *Muscadinia* and American *Vitis* spp.: **Rpv3**, **Rpv4**, **Rpv10**, **Rpv11**, and **Rpv12**. Resistance to powdery mildew (PM) was evaluated in 10 grapevine genotypes (Regent, Calardis blanc, Villaris, Reberger, Calandro, Fleurtaï, Cabernet volos, Bronner, Johanniter and Solaris) bringing the resistance gene **Ren3**. The following resistance components were assessed in monocyclic experiments with artificial inoculation on leaves (for DM and PM) and bunches (for DM only): i) infection efficiency; ii) AUDPC (area under the disease progress curve); iii) degree of resistance according to OIV standards; iv) incubation and latency periods (in degree-days); v) sporulation on lesions; and vi) infectivity of the inoculum produced

on lesions. Resistance components were expressed at different levels in resistant genotypes, with significant differences in comparison to the susceptible *Vitis vinifera* variety used as reference (Merlot for DM and Pinot noir for PM). Differences were also found for DM on leaves collected at different growth stages and between leaves and bunches. Data from these monocyclic experiments were inserted in mechanistic models for predicting disease development in susceptible and resistant genotypes. Model predictions were then compared with field collected data. Preliminary results showed satisfactory agreement between model prediction and reality.

Federica Bove carried out this work within the Doctoral School Agrisystem of the Università Cattolica del Sacro Cuore (Italy).

# Grape berry ripening: environmental drivers and spoilers

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Grape berries exist to enable the propagation of grapevine genes by means of seeds. When seeds are mature, the berries accumulate pigments and aroma volatiles to attract seed vectors and sugar to reward those vectors. Growers and winemakers exploit this natural propensity but have to cope with the large spatial and temporal variation in berry size and composition that arises due to differences in environmental conditions, viz. soil and climate variation.

Berry size is determined by the balance of water flow into and out of the berries. Inflow occurs in both the xylem and the phloem, while outflow may occur in the xylem and by berry transpiration. Our work has shown that phloem inflow increases strongly at veraison, which enables berries to ripen even under severe stress conditions, while xylem outflow facilitates ripening under humid conditions that hinder berry transpiration. Thus both excess soil moisture and high humidity may delay ripening. The sensitivity of berry size to changes in soil moisture declines at veraison, but the berries may still shrink if water outflow exceeds water inflow.

The major changes in fruit composition occur early during berry ripening. Production of tannin precursors (flavan-3-ols) ceases by veraison, though polymerization may continue during ripening. Rates of sugar and anthocyanin accumulation, and of acid (malate) degradation, are rapid while a berry softens and changes color, and slow down thereafter. The pH may continue to rise even as malate degradation slows, partly due to displacement of protons by potassium. After berries reach their physiological sugar maximum around 23-25 °Brix, sugar (and sometimes acid) concentration may continue to increase due to dehydration. Irrigation may slow such weight loss but does not dilute fruit quality, because the stiffening skin limits berry expansion in response to root water uptake. Nevertheless, ripening berries may split (crack) if water outflow cannot compensate for phloem inflow or water uptake across the skin, for example during rainfall or sprinkler irrigation. Even under favorable conditions, mesocarp membranes may begin to fail at advanced maturity.

Water status is an important driver of berry composition. By restricting shoot growth, water deficit limits canopy size and density. Smaller canopies are associated with greater sun exposure of the berries

that are heated by solar radiation and thus are often warmer than shaded berries. Our work suggests that decreases in acidity and increases in skin flavonols, flavan-3-ols, and tannins under water deficit are likely mediated by changes in canopy microclimate, especially light and temperature. Other changes, such as increases in sugar and anthocyanins, may result mostly from both decreases in berry size and accumulation of abscisic acid triggering changes in gene expression and enzyme activity. In contrast, components such as amino acids may decline under water deficit.

Another key driver of berry composition is temperature. Together with water status, temperature may be responsible for most of the site and vintage variation that is commonly observed in wine production. We have found that low temperature may slow or inhibit berry sugar accumulation by limiting both source and sink activity. High temperature, by contrast, favors berry transpiration, which affects both sugar accumulation and berry weight loss. High temperature also accelerates malate degradation leading to low acidity and high pH, alters anthocyanin accumulation, increases tannin accumulation, and may enhance volatilization of flavor and aroma components, which might spoil fruit quality. Because the optimum tissue (not ambient) temperature for many of these processes is in the range 25-35°C, warm ripening periods tend to be associated with high vintage quality in cool climates but with poor vintage quality in warm climates.

Plant nutrient status, which is often linked to soil moisture, may be considered a modifier and potential spoiler of berry ripening. Adequate nutrition is necessary for growth, photosynthesis, and berry development. Nitrogen deficiency stimulates phenolic metabolism but limits accumulation of sugar, amino acids (especially arginine), and some aroma-active compounds. Where soil moisture is not limiting, excess nitrogen, by contrast, is associated with continued shoot growth and high canopy density, both of which curtail berry ripening. Nitrogen surplus also enhances malate production and suppresses phenolic metabolism, and hence anthocyanin and tannin accumulation. Nitrogen (nitrate) uptake by the roots is often coupled with potassium uptake. Because potassium can substitute for protons, a high potassium status tends to increase the berry pH, which may be desirable in cool but not warm climates.

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

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Grape growers must maintain yield and berry quality in spite of environmental variations (vintage and terroir effects), climate change, and increasing constraints put on the use of pesticides. To this end, phenotyping is made frequently by the grape grower in order to take the most relevant decisions related to vineyard management. Phenotyping is also useful to assist the selection process for breeders, and for the scientists who model plant behaviour and need data to construct and to validate the models that will be later used to elaborate decision making tools. During the Innovine project, several non-destructive phenotyping tools were used to monitor plant vigor and physiological status, berry growth, yield and ripening, or disease resistance. These tools include fluorescence and reflectance measurements, hyperspectral imaging, and smartphone apps. Although destructive, other high throughput devices (Fourier Transformation Infra Red) analysis were also used to obtain a snapshot of berry composition. The presentation will summarize examples of the utilization of some of these tools, and pinpoint their interest and limits.

## Fluorescence Sensor

The Multiplex on-the-go system and the hand-held Multiplex were used to monitor leaf development and berry quality. The main trial were conducted since 2013 at INRA Bordeaux on the experimental plot VITA-DAPT, which encompasses 50 varieties all grafted on the same rootstock genotype. The Multiplex on-the-go system provides a fast way to monitor leaf development with a high density of data. The Multiplex foliar indices, biomass, chlorophyll, flavonols, vigor and nitrogen uptake, were monitored at the plot scale during the whole season. The performance of this phenotyping may be improved by high precision GPS acquisition (RTK).

The hand-held Multiplex provides field and lab indices based on a large sampling for the monitoring of the grape quality evolution from veraison to harvest. The use of the CUBA tool (<http://max2.ese.u-psud.fr/cuba/>) is in progress for the expression of the anthocyanin content. The establishment of a global database (2013-2015) with a maximum of foliar and fruit parameters for 3 selected varieties is underway. In 2015 and 2016, tools and knowledge from the experimental trial were applied on a commercial plot for selective harvest.

The hand-held Multiplex can furthermore be used to screen for disease resistances following the kinetics after inoculation. Downy mildew infections were investigated in the greenhouse in 2014. Infections could be detected six days after inoculation. The method has been transferred to the field in 2016 to test the detection of different levels of resistance directly in the field.

## Physiocap

The embedded Physiocap sensor measures automatically vine per vine vigor parameters of pruning woods in winter : diameter, number of woods, weight of pruning woods (biomass). This laser-based sensor may replace the tedious wood weighing and provides high-density sampling.

The measured parameters give useful information to better understand the grapevine physiological balance at the different scales of vine, plot and estate. This helps the decision making for pruning, nitrogen fertilization and grass-cover management, according to the production objectives of the grapegrowers. Physiocap mapping services are proposed by FORCE-A with interpreted maps visualization through its web platform in several vineyards.

**Reflectance spectroscopy** was developed at the University of Milan (Italy) to achieve quantification of chlorophyll and carotenoid content in white grape (*Vitis vinifera* L.) skins and to study chlorophyll role in berry sunburn symptoms in different grape cultivars. This technique was also used to investigate the effects of water deficit on grapevine woody tissue. 10 different *Vitis* species have been studied in relation to their expected drought tolerance by reflectance spectroscopy. The spectral signature showed characteristic species features. Network analysis was able to predict the expected drought tolerance score. Differences between rough and spectra colored by SUDAN IV were used to develop a hydrophobicity index. This index correlated with the expected drought tolerance score.

### Smartphone based phenotyping

A Smartphone- based phenotyping tool using machine vision was developed to estimate the number of flowers and berries of a cluster under field conditions in a non-destructive way. This app, called vitisFlower was developed and tested by University de la Rioja (Spain). The app, freely available for Android devices at the Play Store, acquires an image of the inflorescence and has implemented a model based on image-analysis algorithm, which estimates the number of flowers in the inflorescence. The algorithm inside the app basically takes the following steps:

- Acquisition of the image
- Extraction of flower candidates
- Filtering the flower candidates results to remove false positives

The number of flowers per inflorescence was assessed at pre-flowering stage in a commercial nursery vineyard located in Navarra, Spain. Vitisflower successfully assesses the number of flowers per inflorescence on the 11 *V. vinifera* cultivars that were studied.

### Fourier Transformation Infrared Spectroscopy

INRA Bordeaux used a bar-coded system for sample tracking, and a system based on Fourier transformed infrared spectroscopy in order to monitor the chemical composition of the berries of the 50 varieties of the VITADAPT plot during ripening and at harvest. This technology is a well proven method for rapid and accurate analysis of liquid samples, and it is used to manage a large number of analyses on must. Major grape-quality and wine-component parameters are analysed quickly and efficiently. In just 30 seconds, one can analyse up to 20 main quality parameters in wine, such as ethanol, pH, sugars and organic acids. Up to 120 unattended tests can be completed in an hour. The reproducibility and precision are very good on berry juices. It was checked whether the calibration curves initially designed by FOSS with very few varieties could be applied on other varieties without changes. There was a very high correlation between the sugar concentrations estimated by the Foss and by Brix measurements on all the varieties studied. The same is true for comparison between Foss measurements and enzymatic determination of sugars, alcohol potential determined by Foss and Brix, total acidity determined by Foss and titration.

# Modelling the influence of environmental stresses on berry composition\*

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Grape quality is a complex trait that mainly refers to berry chemical composition, including sugars, organic acids, phenolics and other aroma compounds, which are dynamically changing with berry development and can be affected by genotypes (rootstock and scion), environment (light, temperature and water) and trophic factors (carbon and nitrogen). Moreover, the ongoing climate change affects the physiology of grapevine and ultimately wine quality and typicality. Therefore, a better understanding of the mechanisms controlling the accumulation of quality-related metabolites (both primary and secondary) in the grape berry is essential to adapting current viticultural practices to climate change. Process-based models can integrate various processes involved in defining fruit growth and composition, and reproduce the plant responses to weather and management practices, making them a promising tool to study the response of berry quality to growth factors. Within the Innovine project, we developed tools allowing to model the growth and quality of grape berry in changing environments. First, we developed models for simulating berry growth, sugar concentration, and anthocyanin composition over berry development under various growth conditions. Second, a functional-structural grapevine model that couples the

thermodynamics of water transport (adapted from Tardieu-Davies module), leaf gas exchange (an extended Farquhar-von-Caemmerer-Berry module), and a berry growth module was developed. The xylem water potential and phloem sugar concentration were simulated dynamically based on environmental conditions (e.g. CO<sub>2</sub>, light, temperature, humidity, soil water content etc.), and were fed into the berry growth model to simulate berry growth and sugar concentration. The model is able to make reasonable simulations under various virtual scenarios (e.g. different light and water stress conditions) and produce outputs which agree well with literature. Finally, a model giving a detailed description of the plant architecture and hydraulic structure under different training systems has also been built. Training system alters shoot and cane position within the canopy, and affects plant water use efficiency. The model can predict the effects of changing environmental factors on canopy's water use efficiency on a short time scale (e.g. 10 days). Overall, the presentation will provide an overview of recent progress made in Innovine on modelling of grapevine physiology, and open discussions about their use to better predict the effects of climate on grapevine.

# The uncoupling of berry ripening : what we have learned from InnoVine

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“Decoupling” berry ripening means piloting accumulation or degradation processes of biochemical components so that they can deviate from standard trends. For instance, it is well known that color accumulation starts in berries of red cultivars a few days later than sugar accumulation. Under such circumstances, being able to post-pone or delay sugar accumulation without hindering color formation is a quite suitable example of decoupling. Similarly, in white cultivars, allowing proper sugaring without compromising the maintenance of adequate acidity is likewise a fitting decoupling case. Decoupling can be achieved through either new genetic material or modified cultural practices in the vineyard. During the InnoVine project, CSIC evaluated the effects of two thermal regimes (24/14 °C and 28/18 °C day/night) on sugar and anthocyanins accumulation in 13 accessions of cv. Tempranillo differing in their cycle length. When total skin anthocyanins (mg g<sup>-1</sup>DM) was plotted vs. total soluble solids (TSS) the two thermal regimes nicely separated. While the onset of veraison was similar, at any given increase in TSS the 24/14 °C vines showed higher anthocyanins and such difference widened towards the end of maturity. This feature was essentially maintained in most of the accessions, although it was more consistent in the long ripening cycle variants.

The anthocyanins :sugar decoupling was addressed in another field study on the same cultivar by ITQB where grapes were sampled from East and West facing row sides of NS oriented rows subjected to either sustained deficit irrigation (30% ET<sub>p</sub>) or regulated deficit irrigation (10% ET<sub>p</sub>). The larger decoupling effect was observed for the two different row side exposure since at any TSS value East facing berries showed much greater anthocyanins concentration. Notably, for data pooled over 2013 and 2014 number of hours having T<sub>berry</sub> > 35 °C were 118 and 81 for East and West side, respectively. The anthocyanins : sugar decoupling was further investigated by an experiment conducted by INRA and UCSC, where sugar and anthocyanin profiles were

compared under two contrasting carbon supply levels in berries of cv. Sangiovese collected at 14 developmental stages. Results showed that carbon limitation led to a strong imbalance between sugars and anthocyanins. It seems that under carbon limitation, the grape berry can manage the metabolic fate of carbon in such a way that sugar accumulation is in priority for carbon use than for secondary metabolites.

Turning into vineyard cultural practices, UCSC showed that pre-flowering leaf removal applied on the white cv. Ortrugo in a warm climate where preservation of adequate acidity is becoming a serious concern was effective at accelerating sugar accumulation so that early picking could reach an optimal sugar acidity balance. UCSC also addressed in details vine response to the new apical- to-the cluster late leaf removal. Technique exploits the principle that removing part of the most efficient leaves located in the upper canopy should delay sugaring while berry pigmentation should be less affected since the microclimate around the fruiting area is left undisturbed. The preliminary pot study conducted on cv. Sangiovese subjected to either pre- and post-veraison leaf removal vs. non defoliated vines showed that despite a strong photosynthetic compensation was recorded (on a whole canopy basis) on the remaining leaves, at first picking date post-veraison leaf removal showed 2.4 Brix less than C vines while the color accumulation was very similar suggesting that the potential for decoupling is there. This outcome was then confirmed in a two-year field study on the same cv. that scored 1.2 Brix less at harvest in the late defoliation at similar anthocyanins accumulation. The anthocyanins : sugar decoupling was apparent since the first sampling date after leaf removal and carried through harvest.

The same technique was not as much effective in the white cultivar Ortrugo since while pre-veraison apical to the cluster leaf removal was quite effective at lowering the pace of sugar accumulation (1.4 Brix less at harvest), titratable acidity (TA) was already quite low

therefore rendering unlikely the approach of a retarded harvesting. In other words, TA in cv Ortrugo seems to be primarily driven by the local temperature rather than by its general (and reversed) relationship with sugar accumulation. Overall, trials conducted on Riesling and Muller Thurgau by GRC confirmed that within canopy manipulation treatments the intensity, timing and positioning of defoliation practices are the main clues to alter berry ripening

Another technique which UCSC proved to be useful for getting some degree of uncoupling in red grapes is the application of anti-transpirants (i.e. pinolene as commercial Vapor Gard). In the fairly dry 2013, Vapor

Gard applied either pre-veraison or both pre and post veraison was effective at slowing sugar accumulation (approximately 3 Brix less as compared to unsprayed) and, most importantly, sprayed vines showed an anticipated onset of coloring as compared to control vines.

Overall, information gathered throughout the InnoVine project has led to acquire valuable and new information about techniques which can suitably alter ripening dynamics in the field piloting selective accumulation of some components vs others. This represents, indeed, a body of knowledge useful for a more effective adaptation to climate change.

# Worldwide view of breeding for disease resistant grapevine varieties

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“Since 150 years, breeding has been a major tool to improve and adapt vine material while trying to answer questions such as : What are the identified targets and can we combine them in new varieties? What's going on in the world wine sector? Does this new material meet the expectations of the wine industry ? Are there any “Go – no Go” ?

### A bit of history...

The expansion of phylloxera in France in the middle of the 19's century and, meanwhile the development of fungi diseases such as powdery mildew (PM) and downy mildew (DM) led to the development of rootstocks that are still widely used today and of hybrids, which represented more than 400 000 ha in France until the middle of the 20's century. Most of this work was conducted by private breeders. In the 50's, public institutes started to investigate that field, working on rootstocks mainly for soil adaptation, and on scion varieties for production and quality (ie : Dornfelder, Marselan).

Fungal diseases started to be considered in the 70's giving birth to a new generation of varieties in Germany, Austria, Hungary, Switzerland and more recently in France: Bianca, Johanniter, Regent and others...

In France, A. Bouquet started a breeding program for disease resistance in the 80's based on introgressions of resistance genes (Run1, Rpv1) from *Muscadinia rotundifolia* with strong effects in *V. vinifera* through several generations of back-crosses. This program was re-oriented towards the development of varieties pyramiding resistances genes to ensure their durability 15 years ago. Finally, during the 90's, using cold hardiness of *V. Riparia*, University of Minnesota (USA) produced a series of new varieties for both for wine and table grape industry (Frontenac, La Crescent, Marquette...)

### What's going on today?

With the grapevine genome sequence release in 2007, the discovery of several loci of resistance to Powdery Mildew (5+) and Downy Mildew (10+), the routine use of screening with markers, the development of high throughput tools for genotyping and phenotyping, the

breeding strategies have evolved towards more efficiency.

The necessity to strongly reduced the use of pesticide in vineyards or more generally to control diseases is shared by most of the wine producing areas in the world and is one of the most widely shared breeding objective. Still, breeding objectives and priorities are highly variable across countries and some issues linked to the climate change are also getting more and more pregnant all across the world (e.g. adaptation to drought, winter frost). Finally, the adoption of the varieties will be highly driven by their ability to produce high quality wine in specific terroirs. All over the world, breeding programs are releasing or experimenting before release new generations of varieties:

- In US public institutes and the private sector: varieties resistant to Pierce Disease (PRD1) and rootstocks resistant to the root-knot and dagger nematodes (UC Davis), pyramiding resistance genes to diseases (Cornell University), resistance to fungi, yield and quality (mainly color; Gallo S.A.).
- In Australia, CSIRO is engaged in a breeding program using several sources of resistances derived from *Muscadinia*, *V. amurensis* or *V. cinerea* for root pest resistance. These genotypes are still at this stage under evaluation in the vineyards.
- In South Africa, ARC is pyramiding genes for improving the resistance of the table grape varieties.
- In Europe, many public institutes (e.g. Istituto de Genomica Applicata, JKI, WBI, Agroscope Changins, Geisenheim, INRA...) but also private or semi-private breeders (e.g. IFV) have increased their efforts during the last decade. For instance, in France, associations of winegrowers have signed long terms agreements with INRA and IFV to develop new varieties that would suit with the specificities of the wine of their area and that carry at least two sources of resistance to powdery and downy mildew.

### What's next ?

Due to the awareness of health concerns in relation with pesticide applications and to public policies addressing this problem, grapevine breeding programs have been dramatically growing over the last 10 years. Awareness on the problem of the durability of resistance gene is raising: e.g. OIV strongly recommended in 2013 that

new programs for resistance should take into consideration that resistance genes are precious common goods; INRA with IFV are building an observatory of the durability of resistance.

It is also leading to an evolution of legal rules associated to the registration of varieties and their use in some countries such as France, opening the possibilities for the wine industry to grow new varieties.

In parallel, new tools or approach such as gene editing or genomic selection will be investigated.

Even if developing a new variety at an industrial scale will take a while, it is obvious that the wine industry is opening a new page.

## Resistant varieties: new perspectives for a more sustainable viticulture

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Environmental sustainability and health protection of vineyard workers, consumers and other citizens are priorities in viticulture, which normally requires multiple treatments to protect the crop and secure grape quality. European standards have become more stringent in terms of pesticide use and the goal is to reduce chemical applications by 50% within 2025. In organic viticulture, the use of copper will be restricted to a higher extent, resulting in a more difficult pest management, higher costs of production and lower yield. As a consequence, there is an increasing interest in the use of new disease-resistant varieties in all grape growing regions.

We assessed characteristics and performances of six innovative varieties named Ud.30-080 (Sauvignon Maris), Ud.31-120, Ud.31-103, Ud.34-111 (Fleurtai), Ud.34-113 (Soreli) and Ud.76-026 (Sauvignon kretos), which carry resistances against downy and powdery mildews. These varieties were obtained through conventional breeding. Agronomical and oenological investigations have been carried out in high-fungal pressure sites of Fossalon di Grado (site 1) and Raus-

cedo (site 2), located in north-eastern Italy, and in a lower-fungal pressure site of Ripa teatina (site 3) in Central Italy. Resistant varieties (innovative practice) were compared with four traditional varieties (common practice), Merlot, Tocai Friulano, Sauvignon blanc (site 1 and 2) and Montepulciano (site 3), grown in the same vineyard. The potential for mildews infection was estimated using Horta's DSS (decision support system), that predicts number and timing of expected infections. Disease incidence was assessed by visual inspections of non-treated controls of sensitive varieties. A low-input pest management was applied to resistant varieties in sites 1 and 2; no treatments were applied to resistant varieties in site 3. The level of disease resistance has been evaluated by visual inspections and scored using OIV descriptors, according to a protocol shared with Innovine partners. Sensory evaluations of wines produced in the previous vintage (2015) have been carried out. Results of the four year investigations will be presented.

# Application of the approach system in viticulture

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Studies concerning the management of phytosanitary issues in viticulture are numerous, but mainly concern the individual assessment of isolated techniques. However, it appears clear that the stated objective of a significant reduction in the use of pesticides will not be achieved except by the addition of complementary techniques. Therefore, it is necessary to complement current research by more global approaches at the cropping system scale.

Developed for long a time in other agricultural sectors, studies on the design and assessment of cropping systems are more recent in viticulture, but are now developed since a few years.

A project, led in partnership by INRA, SupAgro Montpellier and IFV, has adapted this particular methodology to the case of viticulture and led to the design of the first low-input cropping system prototypes.

The system experimentation is the next step, consisting on the implementation of these prototypes on large plots and on the assessment of their running and performances.

Thus, long-term experimental platforms have been built since 2012 as part of the DEPHY network to test several prototypes. First results and experience feedback are now available.

## Examples of systems designed (and tested) in Innovine

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One goal of the Innovine project is to design new viticulture systems including technics tested in the various work packages of the project. The conception is based on a method developed on arable crops (Lançon et al., 2008) that has been adapted to vineyards at a National level in the French program EcoViti (Lafond et al., 2011). The vineyard systems will be designed as a function of the i) pedo-climatic environment and main environmental issues, ii) supposed new climate conditions iii) socio-economic demands iv) production objectives. These elements are summed up in Sets of Objectives and Constraints.

We did four design workshops during the project. The first one took place during the second annual meeting of the project in Geisenheim, two systems were designed, using two case studies:

- Continental vineyard, Rhine valley, Riesling production.
- Mediterranean vineyard, Languedoc, High hydric stress conditions.

The second system was refined during specific workshops organized with the Experimental Unit of INRA at Pech Rouge. During this second workshop, we discussed the opportunity of using minimal pruning (and some variations) in a system, and how this kind of training system could help to reduce pesticide use. Some ad-hoc experiments were designed to help to understand how this could be used, before going further in the system design.

The third workshop took place in Plovdiv during the third annual meeting of the project and we focused on two specificities of Innovine: use of new technologies and resistant varieties. Four parallel sessions were conducted (two on each topic), and the results confronted at the end of the workshop.

The last workshop took place in Sofia, and was oriented toward the design of systems adapted to the Bulgarian conditions. This workshop was however complicated by the fact that some attendants were not fluent in English. The system designed is to be refined with some local growers in the future before being tested.

# Grapevine optical sensors and decision support tools for precision viticulture

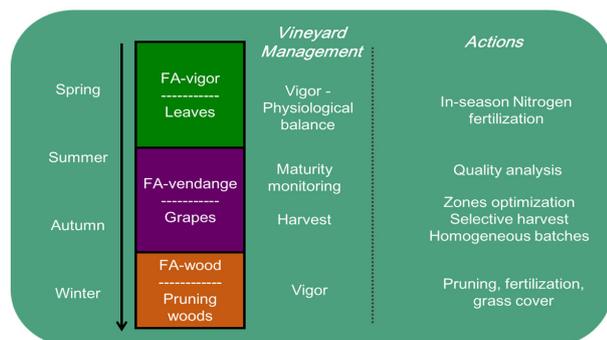
Naïma BEN GHOZLEN, Jean-Luc AYRAL

FORCE-A, Centre Universitaire Paris Sud, Bâtiment 503, 91893 Orsay Cedex - France

FORCE-A is a leading innovative company in precision viticulture providing diagnostic tools and solutions for grapevine monitoring and management. Pruning and fertilizing management, grape quality optimization and selective harvesting, high-throughput phenotyping are among the applications answered by FORCE-A's solutions.

These decision support tools are based on our hand-held and vehicle-mounted proximal optical sensors that assess and map the physiological balance and the nutritional, health and maturity status of the grapevine. The sensor data acquisition is associated to our FA-Server web platform for the processing and display of the interpreted plot maps, such as the zoned maps and the diagnostic maps.

Our grapevine services are organized into three product lines as described below. Each of them is operated at a different time of the year and grapevine phenological stage.



FA-vigor operates the Dualex leaf-clip and the On-the-Go Multiplex sensor for the assessment of chlorophyll and flavonols contents in leaves, Nitrogen status (NBI index), leaf density index and Nitrogen uptake (the 2 last indices with the On-the-Go Multiplex).

FA-vendange operates the hand-held Multiplex Anthocyanin sensor, which measures the surface-based content of berry anthocyanins at the cluster level. This unit can be converted into volume-based units by the CUBA tool (<http://max2.esse.u-psud.fr/cuba/>) developed during the Innovine project. FA-vendange is able to deliver both information of anthocyanin kinetics and anthocyanin maps for in-time and spatial decisions in the vineyard.

FA-wood operates the Physiocap laser-based sensor developed by the CIVC (Comité Interprofessionnel du Vin de Champagne) and the ERECA company, and provides a mapping service of pruning woods.

During the Innovine project, several tasks have been focused on the improvement of the in-the-field operability of the sensors and of the FA-server web platform in order to accelerate and simplify the process of data acquisition, processing and display. Several trials with academic and industrial partners have been defined and operated. These trials combined optical measurements with chemical analysis and wine tastings in order to improve the decision thresholds of the tools. The three grapevine services are commercialized in France and internationally.

Moreover, the Innovine project allowed the achievement of determinant progress in important thematics such as the demonstration of early detection of downy mildew in the field with the fluorescence-based Multiplex 330 sensor.

Our real-time and non-destructive tools are also well adapted for answering plant research needs. As such, FORCE-A is fully dedicated to efficient and sustainable viticulture.

# WINEO: Characterization of vineyard plots by airborne/drone imagery

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## Context

Knowing the characteristics and heterogeneity of a vineyard plot is crucial to improve the quality of its production. It enables the optimization of nutrient and phytosanitary product supply necessary for delimited areas and therefore allows the reduction of chemical treatments. Hence, producers make substantial economic gains, while protecting the environment. Using on-ground means for characterisation quickly turned out to be costly, time-consuming and of difficult management. NOVELTIS took another approach based on state-of-the-art technologies.

## Noveltis' solution

NOVELTIS implements high-resolution innovative mapping products for the characterization of vineyard plots. These products are based on airborne and/or drone high spatial resolution imagery and are provided in different formats, depending on user's preferences: online mapping, printed maps or GIS layers.

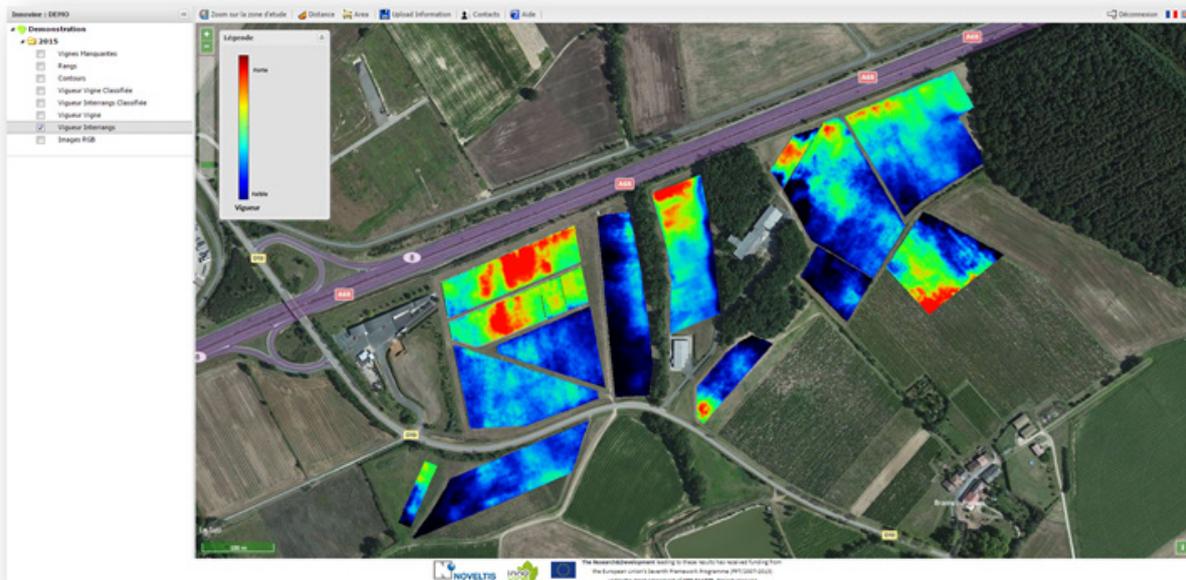
This new service provides users with a comprehensive and global view of the surface where vineyards are planted, together with a set of several key parameters, for each individual plot:

- Orientation and distance between ranks
- Missing plants: location and number/percentage
- Vine vigour and relative homogeneity
- Inter rank vigour and relative homogeneity

These products have been developed and validated in collaboration with the French Institute of Wine and the Vinovale wine cooperative.

## Noveltis' added value

NOVELTIS' top-level scientists are highly skilled in remote sensing, physics of measurement and in the analysis, exploitation, modelling and interpretation of environmental data. This expertise is completed by our IT engineers' who develop user-friendly and tailor-made interfaces in line with our customers' specific requirements.



# Vitisflower®, a new app to count the number of flowers per inflorescence under field conditions

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The number of flowers per inflorescence is an interesting parameter for plant phenotyping and early yield forecast. Unfortunately, a manual count of flowers is unviable as it is time and labor demanding. This work presents a new, easy-to-use, smartphone application for the automated actual flower estimation in grapevine inflorescences in the field, based on artificial vision techniques and mathematical modelling. The application, called VitisFlower®, offering a friendly interface, firstly guides the user to appropriately take an inflorescence photo (when inflorescences are at phenological stage BBCH 55) using the smartphone's camera. Then, by means of image analysis, the flowers in the image are detected and counted. Finally, the actual number of flowers in the inflorescence is inferred from the detected flower number using a mathematical model, and both, detected and actual values, are provided to the user. The process can be performed as many times

as needed. VitisFlower®, at first, is offered, free of charge, for devices powered by Android® OS, being the IOS® version under current development. VitisFlower® is an easy to use, reliable, non-invasive tool to automate flower counting of grapevine inflorescences in the vineyard, which can be a valuable information for yield forecast.

AQUINO, A., MILLAN, B., GASTON, D., DIAGO, M., TARDAGUILA, J., (2015) vitisFlower®: Development and Testing of a Novel Android-Smartphone Application for Assessing the Number of Grapevine Flowers per Inflorescence Using Artificial Vision Techniques. *Sensors* 15:21204 21218

# A review on models and Decision Support Systems available in viticulture

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Traditionally, disease control in vineyards is based on calendar applications of fungicides, in such a way to keep the plants constantly protected. Vine growers strongly rely on chemical pesticides, so that viticulture accounts for a relevant part of the pesticides used in Europe. Directive 128/2009/EC makes integrated pest management (IPM) mandatory across Europe in such a way to reduce the negative impacts of pesticides on human health and the environment. A key principle of IPM is to protect crops only when it is necessary, i.e., when there is the risk for pathogens to develop, infect plants and cause damage.

Weather is one of the main drivers for disease development. The study of the relationships between weather conditions and diseases is a long lasting story. First disease forecasters have been developed in the middle of '900 following an empiric approach, with simple tools showing relationships between particular stages of the pathogen and the concomitant weather conditions. Developments in weather monitoring and automatic data processing had a relevant role in increasing numbers of the forecasting models and their complexity. Nevertheless, empiricism predominated for long time. In recent years, new research approaches have increased our ability to investigate and understand these complex relationships. Relevant improvements were obtained with the mechanistic dynamic models which are able to model the entire life cycle of a pathogen with a great detail, its changes over time, and to account for the effect of all the influencing variables (weather conditions, host characteristics, control measures, etc.). Accuracy

and robustness of these models significantly increased compared to the empiric ones.

Advances in information and communication technologies (ICTs) made it possible to incorporate models in DSSs and to effectively deliver these systems to growers. In the past, there was a poor uptake of DSSs, because of different reasons: i) failure to support more than one or a few problems; ii) lack of a computer familiarity among the population; iii) system complexity; iii) use of inputs that the grower cannot easily provide; iv) difficult to show cost-benefits.

Modern DSSs are able to overcome these "implementation problems" and are useful tools for supporting informed decision-making in plant protection. They are characterised by: (i) a holistic vision of vineyard management problems with the focus on all the different individual operation issues (pests, diseases, fertilisation, irrigation, etc.) and on their interactions; (ii) incorporation of mathematical models to predict plant growth and development, disease development, fungicide efficacy, etc., (iv) provision of information on the focus of the decision in the form of easy-to-understand decision supports; (v) easy and fast access through the Internet; and (vi) two-way communication between users and providers, which make it possible to consider context-specific information, such as varieties, soil characteristics, etc., in addition to weather data. In some cases, information from DSSs is successfully supporting a great number of users.

# vite.net® a Decision Support System for sustainable management of the vineyard

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One important goal in modern agricultural crop production is to develop less intensive and integrated farming systems with reduced external inputs such as fertilizers and pesticides, as well as natural resources (water, soil, energy, etc.). The Directive on the Sustainable Use of Pesticides 128/2009/EC states that Member States shall establish or support the establishment of necessary conditions for the implementation of Integrated Pest Management (IPM). In particular, they shall ensure that professional users have at their disposal the information and tools required for pest scouting and decision-making, and that they also have access to IPM advisory services. Different tools have been developed for the support of decision-making in plant disease and pest control, and these tools can be grouped into three categories: i) warning services; ii) on-site devices; and iii) decision support systems (DSSs). Within Work Package 5 of the INNOVINE project, existing monitoring tools and DSSs were studied, further developed and integrated to better meet these new needs. In particular, Horta S.r.l., a spin off company of the “Università Cattolica del Sacro Cuore”, Piacenza (Italy), improved the DSS, called vite.net®, for the sustainable grapevine management. A properly designed DSS is an interactive software-based system that helps decision makers obtaining useful information from raw data, documents, personal knowledge, and/or models in order to identify and solve problems and make decisions.

vite.net® was initially developed as part of the EU-FP7 MoDeM\_IVM (GA n° 262 059) and consist of a web-based tool that analyzes weather and environmental data, and site-specific characteristics of the vineyard by using advanced modeling techniques for the key processes involved in the management of the vineyard, and provides up-to-date information for the management of the vineyard, in the form of alerts, and decision supports. vite.net® automatically collects, organizes and integrates the following types of information: (i) context information (e.g., the characteristics of the vineyard, such as geographical location, environmental conditions, presence of toxic elements on the ground);

(ii) crops information (e.g., vine, trellis system, and the line spacing of the plants, and the characteristics of canopy); (iii) status information (e.g., vine growth and crop development, pest and disease onset, and signs of water stress); and (iv) operational information (time, and the dose of pesticide application). Context and crop information are static, and are entered by the user on the web platform. The dynamic information is collected asynchronously; for example, the weather data are collected at regular intervals from meteorological stations. The end user is able to get information on the status of crops and the actions to be taken in real time through a web interface.

Within the EU-FP7-KBBE INNOVINE project this DSS was improved and enlarged with new functionalities, such as: i) a scouting module that allows the users to insert scouting observations for diseases, pests and abiotic stresses into the system, for both real-time calibration of the models and decision-making; ii) a model for estimation of the optimum dose of fungicides to be applied in the vineyard at any time; iii) indicators for the environmental impacts of the management actions undertaken into the vineyard; iv) a model for infections of grapevine buds by powdery mildew; v) a model for leaf development in single hire wire (SHW) trellis, having free vegetation and no foliage wires for supporting the growing shoots; and v) early detection of drought with improvements to connect this model with the model for leaf development in the different grapevine varieties and training systems.

Goals of vite.net® are to: i) maintain the natural resources of the vineyard for future grape production; ii) improve the economic viability of the crop through better management of resources and reduction of certain inputs (e.g., chemicals, water, etc.); iii) demonstrate good environmental performance to customers, neighbors and the general community; v) maintain/gain access to certain markets, particularly those with high environmental standards.

# Development of an *a priori* zoning of Physiological Behavior Units of vines, using precision viticulture techniques

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The systemic analysis of the performance of a vineyard at the scale of a wine-making exploitation is made possible by the use of sensors stemming from so called precision techniques, which allow precise and exhaustive geo-located measures.

The aim of our study is to exploit this kind of data and evaluate their information using geographical information systems (GIS) and crossing different layers representing characteristic and independent variables of the production system. The goal is then to elaborate an *a priori* zoning, likely to explain variations of the physiological development of vines and possible differences of the plants susceptibility to fungus diseases.

The study is based on the combination of two maps established on the property of Château Léoville Las Cases in the Medoc area of the Bordeaux vineyard (France). These maps represent the behavior of the two compartments, soil and plant, respectively determined by means of electric resistivity (R) and biomass index (B) measures. Three levels - low, medium, high- are defined for each type of data. The combination of these indicators allows the elaboration of 9 classes of islets, named Physiological Behavior Units (PBU), whose distribution is bounded by the GIS on the whole vineyard.

Six of these nine PBU were selected by exclusion of the medium class of the biomass index. Each PBU is replicated twice, thus establishing an observation device of 12 PBU likely to identify differences in terms of physiological development and disease susceptibility. For this purpose, treated and non-treated zones were delimited for each PBU, and a weekly monitoring of these areas has been performed during the 2014 to 2016 crop years. The first years's results of the study show that the PBU concept proposed seem to correlate with some of the significant variations observed for physiological and sanitary criterions.

Furthermore, the explanatory power of the set of other variables stemming from the Chateau's database (age of plantation, rootstocks, clones, exposure...) regarding the observed variability. It appears of our first approach that they have variable roles in the studied processes. Our next goal is then to combine all these factors to the existent PBUs, hierarchize their respective impact in order to define a zoning of plot sensitivity and incurred risks. This information could then be a basis to the reasoning of fungicide applications: a Decision Support System that can calculate the sufficient and necessary doses to stem epidemic cycles according to meteorological forecasts.

# Pests monitoring and warning system of Navarra (Spain): a collaborative approach for precise monitoring

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The objective of the pests monitoring and warning system of Navarra (ES), implemented by the Institute for Agrifood Technology and Infrastructures of Navarra (INTIA) in the framework of a comprehensive IPM strategy developed within the LIFE AGROIntegra project ([www.agrointegra.eu](http://www.agrointegra.eu)), is to generate accurate information concerning the status of pests, diseases and weeds (biotic agents), contributing to the overall goal of reducing the use of pesticides.

The participation of technical advisors and farmers on the system allows it to get better and more precise information on pests, diseases and weeds development in the region.

The new software application provides the possibility of modeling pests and diseases based on meteorological data and a 7 day weather forecast. The design of the system enables increasing the number of pests and diseases being monitored in the future, as well as the variables that assesses them.

The pests monitoring and warning system includes:

1. Modeling, monitoring, observation and tracking the health of crops and the evolution of pests, in more than 300 crops-biotic agents.
2. Historical risk maps and homogeneous behavior area maps of five biotic agents have been developed so far: Late blight of potato and tomato (*Phytophthora*

*infestans*), Mildew in grapevine (*Plasmopara viticola*), Apple Scab (*Venturia inaequalis*), Brown Spot of Pear (*Stemphylium vesicarium*), Rosaceae Fire Blight (*Erwinia amylovora*) and Great brome in cereal (*Bromus diandrus*).

3. Alerts and warnings for farmers to inform them of the risks related to the monitored biotic agents.

The Pests monitoring and warning system generates warnings and alerts information that can be directly applied by farmers in Navarra. Notices issued by the station are also built on the results of LIFE AGROIntegra field trials and will provide the following services:

- Risk cartography of biotic agents.
- Specific alerts on the more relevant risks of biotic agents.
- Recommended control methods of biotic agents according to their effectiveness in relation to the crop and the pest cycle, demonstrating the value of the innovations tested.

With the development of the tool, the communication of warnings and alerts in the region is expected to increase and improve. Stakeholders involved will be able to make use of these results to achieve the objectives of minimizing the environmental risk in crop protection under a sustainable use of pesticides, supporting the implementation of Directive 2009/128/EC.

# Screening genotypes of Aragonez for tolerance to abiotic stress in relation to berry composition

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High temperature is a major constraint of grapevine productivity, worldwide wine production and quality are severely limited by heat and climate change scenarios foresee an increase in this limitation. Berry quality decreases with extreme temperatures, due to deleterious effects on berry ripening and so does economic revenue. To overcome the effects of global warming in viticulture it is necessary to make a novel and non-conventional approach, using the rich genetic diversity within grapevine varieties.

To screen genotypes for sensitivity to abiotic stress surface leaf temperature (SLT), a novel indicator of abiotic stress tolerance, was used. Clones of Aragonez (syn. Tempranillo) were thus screened for adaptation to abiotic stresses in relation to berry composition: SLT was measured in 255 clones as a non-invasive indicator of abiotic stress tolerance and berry samples were analysed for quality traits.

The genotypic variability within the variety concerning SLT was quantified; the correlations among traits (SLT and must quality traits) were studied and a tolerant group of genotypes with good performance for berry characteristics was selected.

For SLT evaluation, an incomplete block design was used: each of five complete blocks comprised the effect of the original complete block of the experimental design of the field trial and the effect of the day. Within each complete block, each incomplete block comprised the effect of the time of day. In each plot, three

measurements were performed in three different leaves with ten technical replicates. Measurements were taken on peak heat hours on leaves exposed to the sun using a non-contact IR thermometer (Scan Temp 440). For the quality traits of the must, soluble solids (°Brix), acidity, pH of the must and anthocyanins were quantified in all genotypes in three complete blocks. Results concerning genetic variance component and broad sense heritability were analysed and the best linear unbiased predictors of the genotypic effects were ranked and a group of more tolerant genotypes was identified.

Significant genetic variability for SLT within the variety was found (for any usual significance level). The values obtained for the broad sense heritability were very interesting, 0.44 in 2014 and 0.51 in 2015, falling well within the values obtained for the quality traits. However, there was a genetic correlation for SLT between two of the seasons (2014 and 2015) of 0.15, a low value that indicates the existence of genotype-environment interactions. This result was probably due to the low levels of environmental stress in 2014 in contrast with the high temperatures and lack of water in the soil in 2015.

A group of the 40 more tolerant genotypes was selected in all the seasons, according to the values of PGV (predicted genotypic value) and 12 genotypes were common between the seasons studied.

# Building the stakeholder platform to foster sustainability in the wine sector

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Consultation, coordination and collaboration are essential to achieve sustainability. Sharing existing knowledge and disseminating new technical solutions, indeed, are considered essential to foster synergies and cooperation between the various actors in the wine value chain, in order to ensure that all the players can have the tools to improve the environmental, economic and social sustainability of wine production.

The WOS - Wine Observatory on Sustainability ([www.wineobservatorysustainability.eu](http://www.wineobservatorysustainability.eu)) is an international non-profit platform aiming to foster sustainability in the wine sector through cooperation and coordination between the various actors in the wine value chain. Launched in 2015, WOS aims:

- to create an international network and to build a base for useful synergies, involving all people and main organizations working on the issue of sustainable development of the wine sector;
- to share and combine experiences, programs, initiatives from different countries and actors about sustainability of the wine value chain;
- to sensitise to the topic the players in the wine chain (including the consumers), sharing events, documents, training and initiatives on the topic of sustainability in the wine sector;

In order to achieve these objectives, the main tool developed by the Observatory is the web platform [www.wineobservatorysustainability.eu](http://www.wineobservatorysustainability.eu). The platform includes and connects the most relevant practical tools, recognized authorities and best practices in the field of sustainable vitiviniculture. Built in order to promote a dynamic environment, where users are actively involved in its development, the platform addresses all the players in the wine value chain, working and promoting sustainability through specific projects (at whatever step of the chain, including consumers). WOS activity is constantly supervised by committees of experts.

In the poster presentation, we offer a view on the functioning of the WOS' platform, together with the presentation of some activities recently developed: i) a global map of sustainability initiatives ii) the ongoing initiatives of "Sustainability Guidelines" specifically designed for Small Trade Associations, to help companies in the implementation of sustainability programs and initiatives.

# Thermal patterns in the vineyard to support crop and soil management

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Irrigated viticulture expanded fast in Southern European countries such as Portugal to optimize berry yield and quality and to promote vine longevity. However, intensive irrigation increases pressure over the water resources in the region. In addition, row crops such as grapevine, are more vulnerable to heat stress due to the effects of soil heat fluxes, which can negatively influence canopy and berry thermal condition. Therefore, a better understanding of grapevine responses (diurnal and seasonal) to environmental factors (air temperature, soil water) and agronomic practices (deficit irrigation, soil management) are on demand by the industry.

Ground based thermography was used to monitor canopy and soil temperature patterns along the day and season as a tool to assess vine water status and predict risks of heat stress damage. In the frame of the EU-INNOVINE project, field trials were carried in 2014 and 2015 in South Portugal. We examined the diurnal and seasonal response of two *V. vinifera* varieties Aragonéz (syn. Tempranillo) and Touriga Nacional subjected to sustained deficit irrigation (SDI), and regulated deficit irrigation (RDI, about 50% of the SDI). Diurnal

canopy (TC), and soil surface (Tsoil) temperatures were assessed by thermography. Punctual measurements of leaf temperature with thermal couples, leaf water potential and leaf gas exchange were also done.

TC values were above the optimal temperature for leaf photosynthesis during the day light period (11-14h to 17h), especially under stressful atmospheric conditions (high VPD, high Tair) and under regulated deficit irrigation. Tsoil was on average about 10-15°C higher than TC. We also found in both years a strong correlation between TC derived from thermography and major eco-physiological traits (leaf water potential and leaf gas exchange). In addition, the good correlation between TC and thermal couple temperature data supported robustness of the thermal imaging approach. Our results suggest that canopy temperature can be explored as a simple but robust thermal indicator of crop performance and eventually as a parameter to feed growth models for the grapevine crop.

# First effects of global warming in the wine-growing area of Castelli Romani

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Research in viticulture and oenology is oriented to improve the quality of wine taking into account environmental sustainability: vineyard management and mutability of ecological conditions, characterized by warmer temperature and less frequent rainfalls. The aim of this work was to verify the presence of any changes in the vine-growing area of Castelli Romani as a result of global warming in the last two decades. We considered the maturation curves (sugar, total acidity and pH) of cultivars of great spreading on a local scale, particularly Cesanese, Malvasia puntinata and Trebbiano giallo. The Cesanese is fundamental for red wines typical of the Castelli Romani; the Malvasia puntinata and the Trebbiano giallo are the basis of the designation of origin Frascati DOCG, Velletri DOC, Marino DOC etc. The grape varieties observed are detected in the experimental vineyard of CREA-ENC, Velletri (RM); ripening curves were recorded since 1994 to present; we examined harvesting time differences, focusing on the periods 1994/96, 2001/2003, and 2010/2012.

Meteorological data used were detected by the weather stations of Ciampino and Latina (air Force meteorological network). We considered average temperature (minimum, maximum and annual temperatures) and total rainfall of two sites. The data indicate quite clearly that in the last 30 years, the weather trend has gone to meet some changes never recorded over the last centuries. From 1961 to 2010 the average temperature of the three periods has increased by 0.5°C in Roma-Ciampino to 0.4°C in Latina-Scalo. The annual cumulative rainfall showed an almost linear decrease of the data.

Regarding to the wine world, many authors agree that climate change would have serious consequences both on the grapevine crop and on wine process. The grape is not always harvested when the sugar content, pH and total acidity are at the optimum; this is due to the variability of the yearly weather, in particular when it is unfavourable for example, a rain occurred just before the harvest (it can alter the content of sugar) or particularly cold and dampish weather that could generate an increase in the virulence of some diseases and could also alter the normal function of plant nutrition; so, it was decided to standardize the data. Basically, it was assumed that the harvest takes place when the content of sugar in must has reached 18° Brix in white grape varieties and 20° Brix in the red ones.

Regarding the effects of global warming, it was found that the observed cultivars reacted in a significantly different ways, even though environmental, vegetative and nutrition conditions were equivalent. It is shown the difference (in days, DOY) of ripening time, for each cultivar, during the three triennium; the average period of maturation, for the same cultivar, has a different length in the three periods. Except Cesanese, the general trend is a reduction of the maturation process.

The most significant differences concerned Trebbiano giallo (-24 days). Also Malvasia puntinata (-16 days) presented a substantial advance of the time of harvest. Cesanese however, did not present significant gap and unlike other varieties, would have delayed maturation. In general, it can be asserted that changing climatic conditions have led to different modality and ripening times of the grapes respect two decades back. However, the climate extreme limits are not yet reached and the normal wine practice is not significantly altered. Noted variations would lead to think that it will be necessary to change some cultural practices. It is early to define these changes for the area in question, but surely they must prepare for new management types. The major experts of influence of global warming on the vineyards highlighted some possibility to counter its effects and to achieve the following objectives: to delay the ripening of the grapes; to combat drought; to counter the increase of the pathological potential, included the increase of irradiation.

To delay the ripening of the grapes (which would allow a more acceptable phenolic content) could be adopted measures such as later pruning, new canopy management, increase leaf/fruit ratio and the use of appropriate genetic material and rootstocks most suitable. To achieve the objectives above mentioned, actions are eligible (genetic material and rootstocks resistant, appropriate forms of cultivation, supplemental irrigation). Finally, to counteract the excess of irradiation would be appropriate to adopt different forms of shielding. We must to think innovative winery practices (e.g. quality of yeasts, operating temperatures in cellars, new constructive models). Furthermore, it would be appropriate to create a networks of available data (climatological, phenological, phyto-pathological data) from which we can draw an updated scenario of phenomenology in act that can better lead us to a strategy to contrast the effects of global warming.

# A new tool for the evaluation of the agronomic management of the vineyard

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In recent years, a number of sustainability programs, specifically dedicated to the wine sector, have been launched. Most of these programs are focusing on the environmental aspects, mainly through the assessment of indicators as the carbon and the water footprint. These indicators do not take into account important aspects related to the agronomic management of the vineyard.

In the framework of “VIVA – La sostenibilità della Vitivinicoltura in Italia”, the project developed by the Italian Ministry for the Environment, Land and Sea in collaboration with OPERA Research Center, a specific indicator to fill this gap, called Vigneto (Vineyard) has been created. “Vigneto” is a multidimensional indicator to evaluate the sustainability of management options adopted at field scale. The indicator considers the main agronomic aspects which can have an impact on the environment, including:

- pest management
- soil management (erosion and compaction)
- fertility management (soil organic matter management and fertilizer application)
- biodiversity management.

Those aspects have been related by fuzzy logics and implemented in web GIS software. The application of

the model allows obtaining a general judgment of the agronomic sustainability of the vineyard management, in a range that varies from “A” (excellent) to “E” (completely unsustainable). The model output highlights that the tested wineries have different management strategies: indeed, producers manage vineyards in different ways, depending on the different geographical position. As an example, we report data from three wineries located in three different Italian regions: Piedmont, Friuli and Tuscany, assessed with the collaboration of the Centre of competence AGROINNOVA of the University of Turin. The results show that the main differences in the “sustainability performances” are related to the soil management.

The indicator “Vigneto”, as well as the related software, are tools useful to have a clear vision of the specific impact of the main activities related to the vineyard management. The developed model could be defined as an environmental decision support system, to be used to define the vineyard management practices according to a “sustainable vision”. Additionally, the use of an overall, single value obtained from the evaluation (A-E), makes the indicator an easy-to-understand and effective tool to communicate sustainability concepts to the final consumer and the stakeholder.

# The indicator “TERRITORY”: measuring the impact of wine production on the economy and the society

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It is generally accepted that the concept of sustainable development calls for a convergence between the three pillars of environmental protection, economic development and social equity. A long-lasting social and economic sustainable development could be achieved through an integrating approach which involves the protection and the management of natural resource. By extending the concept of sustainable development to wine production, it could be stated that sustainability is integrated into a food system whose features also entail “energy, environment, farmworker health and safety, consumer, distributional equity” and “economic cost” (Gómez, Barrett, Buck, & al, 2011). Therefore “sustainable” viticulture includes aspects such as Company’s contribution to the rural and local development, health and safety of workers, fair trade and traceability, economic viability and profitability of the measures taken. Furthermore sustainable viticulture has been defined by the Organisation Internationale de la Vigne et du Vin as a “global strategy on the scale of the grape production and processing systems, incorporating at the same time the economic sustainability of structures and territories, producing quality products, considering requirements of precision in sustainable viticulture, risks to the environment, product safety and consumer health, and valuing of heritage, historical, cultural, ecological, and aesthetic aspects”.

In order to include in the sustainability assessment also the social and economic dimensions of sustainability, a specific indicator has been developed in the framework of “VIVA – La sostenibilità della Vitivinicoltura in Italia”, the project launched by the Italian Ministry for the Environment, Land and Sea in collaboration with OPERA Research Center.

“TERRITORIO” (“TERRITORY”) is a kit of qualitative and quantitative indicators, built in order to evaluate the actions undertaken by the companies and their impact on their territory, intended as local environment, community (included consumers, local employees, local suppliers), landscape.

This indicator, take as methodological reference the “Sustainability Reporting Guidelines GRI G 3.1” and allows to evaluate many qualitative aspects of environmental sustainability (including landscape and biodiversity protections), employees’ rights, ethic towards consumers and suppliers, landscape and the economic and social end economic impact of wine production on the territory. The subjects of evaluation are i) landscape and biodiversity ii) society and culture iii) economy and ethic.

In order to accomplish the VIVA evaluation and the gain VIVA label, a winery have to successfully satisfy all the requirements included in the indicator TERRITORIO.

Thanks to this indicator, producers now have a tool to evaluate the consequences of their actions on the territory, gaining awareness on their capability to create shared value.

In the poster, we present how the indicator is build and disclosed to the final consumer through the label, together with some examples of positive actions implemented by VIVA’s wineries for the territorial promotion and the enhancement of the local communities.

# Modeling impacts of viticultural and environmental factors on rotundone in Duras wines

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A study was conducted to determine the key environmental and viticultural variables affecting the concentration of rotundone in Duras wines. In 2013 and 2014, berries were sampled 44 days after mid-veraison from 10 vineyards located in the South West of France and rotundone was quantified in wines prepared by microvinification techniques (1 L Erlenmeyer). For each plot more than 40 variables were monitored over the vine growing season and winter dormancy including fruit composition,  $\delta^{13}\text{C}$  and yield at harvest, pruning wood weights, leaf area, air temperature and rainfalls. The WaLIS water balance model was used to calculate stem water potentials at different time points. Rotundone concentrations were higher in the cooler and wetter 2013 growing season. Surprisingly, some high rotundone vineyards in 2013 showed low to moderate levels of concentrations in 2014. In the same way,

some blocks having high concentrations in 2014 had low rotundone in 2013 which led us to think that fixed variables such as year of plantation, altitude, training system, clone or rootstock don't have a large contribution to the rotundone model. For 2013, precocity of veraison, cumulative rainfalls over the veraison-harvest period and gluconic acid - a secondary metabolite of *Botrytis cinerea* - had negative regression coefficients to model rotundone. For the 2014 and 2013-2014 models, rotundone was best predicted by cumulative rainfalls over the 1st of April-30th of September and by stem water potentials 15 days before veraison in 2014. Important variables identified for modelling rotundone in wine were those associated with cumulative rainfalls and *Botrytis cinerea* damages. Our results show that mesoclimate is one of the key factors to explain the differences in rotundone observed among the sites.

# Certified clone and powdery mildew impact rotundone in Duras wines

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Few recent studies have been investigating the effect of clone on aroma compounds. The aim of this research work was to study the impact of certified clones from *Vitis vinifera* L. cv. Duras N on grape quality and rotundone, a sesquiterpene responsible for peppery aroma which has been reported recently in red wines made from this cultivar. The experimental site consisted of four consecutive rows, each row planted with one of the four certified clones of Duras N (554, 555, 627 and 654). For each clone, measurements were replicated on three experimental units per row. Each experimental unit consisted of twelve continuous vines. Rotundone concentration was measured in wines prepared by microvinification techniques (1-L Erlenmeyer flasks). For both vintages of study, rotundone concentrations were significantly higher in wines from clones 554 and 654 in comparison with clone 555, while clone 627

showed an intermediate level. In 2014, differences in powdery mildew (PM) severity on clusters were identified between the four clones and a positive logarithmic correlation ( $r^2 = 0.58$ ) was reported on the experimental site between rotundone in wines and PM severity on grapes. Our results found differences in rotundone concentrations in wines made from the four Duras N certified clones and suggest that grapevine defence response to PM could enhance rotundone production in berries. Clonal differences in susceptibility to biotic stress, such as PM, might explain the differences observed in rotundone concentrations between the four studied clones.

# Differential harvest: selecting within-vineyard areas to produce wines with enhanced rotundone concentration

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Recent research works highlighted large variability in rotundone within a vineyard. In order to further study and determine practical ways to map rotundone spatial variability, a study was carried in 2014 on a 0.41 ha Duras vineyard from the South West of France. The smart point method was used and in order to optimize vineyard sampling, variability in vine architecture of the plot was assessed through measurements of trunk circumference (TC). On the basis of the three classes of TC obtained, six smart points made of 50 vines were positioned and followed independently over the vine growing season and winter dormancy for shoot elongation, stem water potential ( $\Psi$  stem), mineral uptake (petiole analysis and Dualex® measurements), airborne multispectral video imagery (NDVI), fruit quality, pruning wood weights (Physiocap® and manual weighing) and yields at harvest. At each smart point, rotundone was

measured in wines obtained by microvinification techniques (1 L Erlenmeyer). Differences between smart points were very weak from an agronomic standpoint. However, a few plant physiological indicators (i.e.  $\Psi$  stem, Dualex® measurements) allowed discriminating vine performances. Despite this very low variability between plant and fruit measured variables, rotundone concentration was on average more than 50% higher in the wines from the high TC area. Thus, our data set suggests that a link exists between plant architecture (TC) and rotundone, and that TC can be used to approach rotundone spatial distribution. Our results also tend to strengthen the hypothesis that rotundone is very sensitive to fine variations of grapevine water status.

# The viva indicator “air”: carbon footprint and greenhouse gas inventory in the Italian wine sector

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Civil society actors are increasingly concerned about climate change and this issue will have, in the coming years, an increasing role in the political, economic and social choices. During the last United Nations Conference on Climate Change (COP 21), the 195 participating Countries have reached an agreement that aims to limit the rise in temperature to a maximum of 2 degrees Celsius by 2100. Both producers and consumers have an important role in the achievement of the COP 21 objective. Consumers are aware they can influence the logic of the market through a “green pressure” towards products with a low environmental impact. At the same time, many companies have understood that the production of lower emission goods combined with a transparent communication is a real opportunity to gain a competitive advantage.

In the framework of the Italian Ministry for the Environment, Land and Sea national project VIVA “Sustainability and Culture in the Italian wine sector” it has been developed a specific guideline for the application of the product carbon footprint.

This guideline allows Italian wine companies to adopt the ISO TS 14067 to account the greenhouse gas emissions directly and indirectly related to the life cycle of a bottle of wine (0.75 litres).

The project provide to companies, that have completed and certified the assessment, the VIVA label that can be used directly on the product, and that through

a QR code allows consumers to consult results and improvements achieved by the wine companies.

The life cycle of wine bottles includes five major phases: vineyard management, transformation of grapes into wine, bottling, distribution of bottles, refrigeration and disposal of glass (called respectively vineyard, winery, packaging, distribution and consumption). The greenhouse gas that should be included are: carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), hydrofluorocarbons (HFCs), sulphur hexafluoride (SF<sub>6</sub>) and perfluorocarbons (PFCs).

As example, we present the results and the communication instruments of two VIVA certified products, that show how the vineyard agronomic management, the production phase and some qualitative and environmental aspects of the life cycle can influence the final result.

This analysis allows producers and consumers to understand which are the processes within the product life cycle that have the greater impact on climate change, highlighting the proper action that can be implemented in order to rationalize the consumptions and to reduce the environmental burden of wine.

# Contribution à l'étude d'anciens cépages autochtones du Lot pour faire face au réchauffement climatique et à la réduction des intrants

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Face au réchauffement climatique et à la réduction des intrants phytosanitaires, de nombreux vignerons s'interrogent sur les cépages de demain, la façon d'entretenir le vignoble et de conduire les vinifications. En parallèle, les vignerons souhaitent l'expérimentation de cépages originaux, anciens ou nouveaux, permettant une diversification de leur production.

L'Association d'Expérimentation de la Ferme Départementale d'Anglars-Juillac, dans le département du Lot, étudie deux anciens cépages autochtones, un blanc : le Noual B et un noir : le Gibert N pour répondre à ces problématiques. Le Noual B et le Gibert N ne sont plus multipliés, ils ne sont ni classés, ni inscrits aux catalogues des variétés françaises ou étrangères. Le Noual (Oubal B) était cité dans le Lot en 1868 par Jules Guyot, confirmé par Pierre Galet en 1962. Le Gibert N était cité dans les années 1950 par Jean-Pierre Artozoul. Leur réintégration au vignoble est préalablement soumise à une expérimentation rigoureuse.

En 2010, deux parcelles expérimentales ont été mises en place, permettant de comparer le comportement agronomique, viticole et œnologique de chacun de ces anciens cépages autochtones avec celui de cépages référents : Chardonnay, Chenin, Viognier pour le Noual et le Cot pour le Gibert.

En 2010 également, une prospection ampélographique dans le département du Lot en collaboration avec l'IFV Sud-Ouest a permis d'identifier plusieurs souches de Noual B et de Gibert N. Après vérification sanitaire, ces souches ont été multipliées et mises en

conservatoire en 2012 sur le site de la Ferme Départementale d'Anglars-Juillac.

Chaque année des observations et notations sont réalisées sur les parcelles expérimentales et les conservatoires : ampélographie, stades phénologiques, comportement du végétal, aspect sanitaire du feuillage et des baies, sensibilité aux ravageurs et aux maladies cryptogamiques, sensibilité et à la coulure et au millerandage, estimation du rendement. Des mini-vinifications de 100L sont également réalisées sur chaque cépage (vinification en rosé et rouge pour le Gibert N). Les vins sont ensuite dégustés au printemps suivant la récolte (vin jeune).

L'intérêt d'une telle étude réside dans la valorisation de variétés anciennes, oubliées, pouvant répondre à des besoins techniques (longueur du cycle végétatif, tardiveté, moindre sensibilité aux maladies et ravageurs...) ou commerciaux (production, diversification) tout en apportant une originalité aux produits, ainsi qu'une communication liée à l'origine locale des cépages. Les premiers résultats révèlent une bonne adaptation du Noual B et du Gibert N aux conditions pédoclimatiques locales, de bonnes potentialités viticoles et œnologiques (cépages productifs) et une bonne appréciation par les dégustateurs en vins jeunes, souples et fruités, faciles à boire. Les prochains millésimes, ainsi que la mise en place de parcelles expérimentales chez des vignerons devront permettre de confirmer ces résultats, puis d'inscrire et de classer ces variétés avant de les diffuser plus largement.

# PATHOGEN : a European training Programme TO improve Grapevine virus knowledge and management

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PATHOGEN is a project co-funded by the Erasmus+ Programme of the European Union which aims to implement an innovative methodology for training in knowledge and management of viral diseases of the grapevine by mixing e-learning methods with field trainings.

Every year, the production of EU is threatened by different diseases attacking grapevines. In particular, virus and virus like diseases such as fanleaf and leafroll, present in most vineyards in all winegrowing countries in Europe, are among the most dangerous ones decreasing the production and leading to huge losses for the whole sector. Moreover, viruses and virus like diseases strongly reduce the quality of grape and wine production, leading to reduction in antioxidant compounds, in sugar contents, and in other desirable compounds of grape, juice and wine.

One major issue responsible for the spreading of the virus diseases is the lack of knowledge and concern on this issue of the main actors, meaning winegrowers, advisors, consultants, nurserymen and all the professionals of the sector in general.

The new training content on grapevine viruses will be adapted to the specific requirements of participating countries (France, Italy and Spain) and the special characteristics of their viticulture systems. The training content will be available through an e-learning platform in English, French, Italian and Spanish. Among its other features, the e-learning platform will also integrate assessment tools that allow us to track the progress of participants and a gallery of images and pictures to illustrate the symptoms of different vineyard viruses. The courses proposed are organized in 3 different levels: BASIC, ADVANCED and TEACHING.

BASIC and ADVANCED LEVEL will include introduction to the most relevant viruses, the main paths for

transmission of viruses, the most common detection methods and the legislation around the grapevine viruses. The difference in the two will be the level and depth of knowledge received by the trainees, according to their everyday needs. Moreover, the Advanced Module will include also two further parts, dealing with emerging virus diseases and research perspectives in virus management.

The TEACHING LEVEL will be focalized in training teachers, to enable them to teach the PATHOGEN course to practitioners of the wine sector in the future, after the end of the project. Indeed, during the project the educational platform will be prepared and the pilot courses will be launched. The project aims to spread the knowledge after the end of the project by replicating the courses in other areas and other countries. Several modules will be developed:

- Module 1. Introduction to main and regulated virus diseases, agronomic and economic impact:
- Module 2. Transmission of grapevine viruses
- Module 3. Detection methods
- Module 4. Control and management of virus diseases and their vectors
- Module 5. Emerging virus diseases (reserved to advanced and teaching level)
- Module 6. Research perspectives in management of grapevine viruses (reserved to advanced and teaching level)

From May 2017, the e-learning platform will be open. Field sessions are foreseen to start in September 2017 and May 2018. Finally, PATHOGEN's courses will be capitalized promoting its use among potential end users interested in the training, both within the participating countries and in other EU Member States.

# New classification of grape berry resistance responses to Black Rot

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*Guignardia bidwellii* (Viala and Ravaz) is the causal agent of Black rot of grapevine. The pathogen has become remarkable in Europe in the last decade causing necrotic spots on leaves and canes this way degrading the assimilation capacity of the stock. However, the most severe economic loss is caused by destroying the berries. In Hungarian plantations maintained without any chemical plant protection, severe berry symptoms occurred during the latest years. Beside quantitative loss of the crop, black rot damaged berries contaminate the wine by their bitter taste even if rotted berries are present in small quantities.

In earlier experiments accessions were classified for Black rot resistance on the basis of their leaf resistance, since only leaf resistance was investigated. The correlation of leaf and berry resistance however, was not established before. Therefore, in two successive years we have studied the berry resistance to Black rot of accessions proved to be resistant in artificial leaf resistance tests.

*Guignardia bidwellii* was propagated *in vitro* on 1/2 strength PDA medium on 25°C and under permanent light. Conidia were harvested after 15-20 days by flooding with distilled water and concentration was adjusted to 5-6 x 10<sup>6</sup> conidia per ml.

Potted grapevine cuttings were used for the artificial cluster infection experiment in between „post-bloom” to „berries before pea sized” phenological stages. Clusters were infected with the inoculum of the pathogen. Climate conditions were set to 99% relative humidity in the first 2 days, than mitigated to 70% with temperature around 22°C under artificial light.

Cultivars for this experiment were selected on the basis of leaf tests conducted in the former years, and only accessions showing leaf resistance were selected for the berry resistance tests.

22 cultivars were tested for berry resistance to *Guignardia bidwellii*. The symptoms were divided into three categories:

1 - Resistant without any symptom: Only one cultivar Csillám' showed this type of resistance. No visible symptoms occurred.

2 - Resistant with symptoms but no berry damage: In case of five cultivars the fungus was able to develop spots, but atypically to normal berry symptoms the pulp was not affected. Spots arose on the surface with some picnidia in the middle. Spots did not grow with the berry, so in some cases they peeled off, leaving intact skin tissue behind. The pathogen had no detrimental effect on the ripening process. The following cultivars belong to this group: 'Merzling', 'Seyve Villard 5276' ('Seyval blanc'), '5-10-6', '5-11-2', '5-11-6'.

3 - Sensitive: In this case disease cycle proceeded as described earlier for this pathogen. Berries turned brown and mummies developed. The majority of cultivars with moderate level leaf resistance had mummified berries in the clusters. Cultivars which were classified sensitive according to the leaf test, had also sensitive berries. The following cultivars were grouped into the sensitive section based on the berry test: 'Malverina', 'Teréz', 'Seibel 7053' ('Chancellor'), 'SV12375' ('Villard blanc'), 'GM318-57', 'GM7810', 'Bianca', 'SV18315' ('Villard noir'), 'Bolgár rezi' ('V25/20'), 'Moldova', 'Pannonia', 'Kismis vatkana', 'MM27', 'MM20', '20/3', 'ECS 44'.

In 2 cases leaf and berry resistance showed discrepancy. 'Seibel 7053' showed high level of leaf resistance in 6 tests implemented in 3 successive years. However its berry resistance was low, several mummies developed in the clusters in both berry infection tests accomplished.

Reversely the cultivar 'Seyve Villard 5276' had moderate leaf resistance with several spots on the leaf, but its berry resistance was grouped into the spotted category, without any damage.

# Phytomonitoring as a decision support tool for grapevine deficit irrigation management – a case study at a Mediterranean vineyard

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Deficit irrigation and related mild water stress are used to optimize grapevine source sink balance and avoid excessive vigor, optimize fruitfulness, berry size and composition in winegrape production for red wines. In deficit irrigated vineyards this mild water stress demands precise regulation and adjustment to the vine's phenology ("Regulated Deficit Irrigation") for which it is needed to know, in real time major indicators related to the environment (climate, soil water content) and plants (vegetation appearance and eco-physiological variables). Within the frame of the INNOVINE research project (FP7/2007-2013, n° 311775) the company Agri-Ciência, in collaboration with ISA and ITQBNova developed a monitoring system with dynamic integration and analysis of real-time data from multiple sources (climate, soil and plant). Several phyto-monitoring sensors were installed in a vineyard of Esporão in Reguengos de Monsaraz (South Portugal), to assess and evaluate the impact of two deficit irrigation strategies in two red varieties (Touriga Nacional and Aragonéz). A digital panel «dashboard» was developed for collection, processing, analysis and delivering of information concerning both the phyto-sensors and

weather station data, allowing viewing and data manipulation in real time. The obtained set of data was used to study the relationship between environmental variables (climate and soil) and other eco-physiological and biophysical parameters (leaf water potential and leaf and berry temperature) in order to generate indicators/models that can be integrated into a Decision Support System (DSS) to manage deficit irrigation in vineyards. This paper presents and discusses some of the relationships found namely the relationships between the fraction of transpirable soil water (FTSW) and predawn leaf water potential. Leaf temperature (Tl) is also integrated in the DSS as it can work as an alert indicator for heat waves when a defined threshold value is attained. Furthermore the berry temperature data is also incorporated as an alert for the thermal efficiency for anthocyanins biosynthesis.

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## Complex genome rearrangements cause loss of berry color in Tempranillo Blanco somatic variant

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Somatic variants appear spontaneously during the vegetative multiplication of woody crops. Tempranillo Blanco (TB) is a new grapevine white berry cultivar originated as a bud sport of the black berry Tempranillo Tinto (TT) cultivar. To understand the origin of this variation, TT and TB genomes were sequenced. Structural variation and genetic segregation analyses uncovered that complex chromosome rearrangements consistent with chromothripsis, a catastrophic phenomenon recently described in human cancer studies, generated the variant genome of TB and the deletion of the color locus functional allele. Loss of heterozygosity and decreased copy number delimited alternating monosomic and disomic fragments in the distal arms of TB's linkage groups 2 and 5. Hemizygous fragments collectively extended over 8.1 Mb and comprised 313 annotated genes. Clustered breakpoints for complex chromosome rearrangements disrupting linkage

groups 2 and 5 were identified and junctions involved unbalanced inter- and intra-chromosome translocations and one unbalanced inversion. Signatures of blunt fusions or microhomology-mediated end joining mechanisms were detected at breakpoint junction flanks. Segregation distortion in TB-derived selfed progeny indicated linkage of rearrangements in a single copy of the affected chromosomes that was barely transmitted. Additionally to the loss of black color in the berry, these dramatic changes have further viticultural consequences in TB associated to a decreased sexual transmission of derivative chromosomes. Our findings show that chromothripsis spontaneously arise during somatic growth of grapevine, showing that this phenomenon could contribute to clonal variation in woody crops and to the evolution of plant genomes.

# Early defoliation induced carry-over effects on vigor and node fruitfulness of Aragonez grapevines grown in a Mediterranean non-irrigated vineyard

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Early leaf removal at the fruit-zone has been recently tested in several high-yielding grapevine varieties and sites as a canopy management tool aiming at reducing cluster compactness and hence, regulating yield and reducing the susceptibility of berries to botrytis bunch rot. In the scope of the Innovine European Research project, a field trial was set up in a commercial vineyard located at the Lisbon winegrowing region (Portugal), trained to a vertical shoot positioning. An early defoliation treatment (ED) was compared with the conventional practice used by the grower (D&T: defoliation + bunch thinning) and with an untreated control (C) during three consecutive seasons (2013-2015). The ED consisted of the removal of 6 basal leaves and any laterals, if present, from all shoots one week before the beginning of flowering. The D&T comprised a defoliation of 3 basal leaves at the east side of the vine (North-South oriented rows) at bunch closure, and a bunch thinning of all second order clusters, at veraison. In all the three seasons, ED induced a strong source limitation at pre-bloom, which significantly reduced percent fruit-set, bunch weight and bunch compactness, as compared to the other two treatments. In the first season (2013) ED and D&T produced similar yield but significantly lower than C. In the following two seasons (2014-2015) ED presented the lowest berry weight, bunch weight and yield. During the second and third season D&T and ED treatments presented similar bunch number but significantly lower than C. While for D&T this is explained by bunch thinning, for ED it shows a negative carry-over effect on bud initiation, induced by the early source limitation. Regarding berry composition, ED showed similar juice soluble solids (°Brix) as the control, except for 2015, when it presented a significantly higher °Brix and a lower titra-

table acidity than the other two treatments. However, no significant differences were detected on total skin anthocyanin content. During 2013 and 2015 seasons, the pressure of botrytis bunch rot infection was very low, C treatment showing the higher incidence and severity of the fungus, while the other two treatments presented no (2013) or very low symptoms (2015). In 2014, due to heavy rains all along the month of September (51 mm), botrytis bunch rot infection attained a very high incidence and severity in all treatments. Our results show that pre-bloom defoliation is a canopy management practice with high potential for replacing cluster thinning as a tool for regulating grape yield with benefits for berry health. However, the carry-over effects on vigor and node fruitfulness recommend the use of this practice with care in Mediterranean non-irrigated vineyards, since the absence of lateral leaf area compensation might induce an excessive reduction in vine vigor and yield. Further research is needed in order to evaluate if a lower intensity of primary leaf removal and/or the retaining of the laterals would produce similar results on the reduction of bunch compactness, without any carry-over effect on bud fruitfulness.

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Les systèmes de production sont aujourd'hui très dépendants d'une couverture sanitaire reposant sur l'utilisation de pesticides. Ainsi, l'usage agricole mobilise à lui seul 90% de la consommation des produits phytopharmaceutiques en France (Butault et al., 2010). Face à l'évolution de la société en faveur d'une protection durable à la fois en termes d'environnement que de la santé, la question du développement de système de culture innovant devient un enjeu important. De nombreux programmes ont été mis en place pour tenter de réduire l'utilisation de ces intrants phytosanitaires.

Parmi les plus déterminants, le Grenelle de l'Environnement (2008) a initié un tournant dans les politiques en adoptant l'engagement n°129 qui prévoyait dans un délai de 10 ans (2018), une réduction de moitié des usages de pesticides. Cependant les contraintes liées aux impératifs de production mais également à la pression des maladies, rendent difficile la mise en place d'une lutte raisonnée sans accompagnement. Promété s'inscrit dans une démarche agro-écologique avec le développement d'Outils d'Aide à la Décision (OAD) pour la filière viticole. L'aide à la décision a pour but d'éclairer et de guider l'agriculteur, en lui apportant des réponses aux questions qu'il se pose durant son processus de décision : « Faut-il traiter ? Oui ou Non ». Ainsi, nous avons mis au point une approche reposant sur l'intégration à l'échelle parcellaire des données

climatiques, pathologique et phénologique. L'objectif est de mettre au point des indicateurs infectieux à l'échelle de la parcelle avec notamment dans le cas des maladies fongiques, les risques infectieux primaire et secondaire, cumulé à la campagne ou depuis le dernier traitement. Ces indicateurs sont mis en corrélation avec un seuil de risque afin d'optimiser la date du premier traitement mais également d'adapter la cadence de traitement à la pression de la maladie ainsi que la sensibilité du cépage cultivé. De la même façon, l'association des données climatiques observées avec des prévisions météorologiques, permet d'anticiper les risques de contamination durant les 7 jours à venir et dès lors, prévenir l'apparition de foyer infectieux. Les essais menés sur des exploitations viticoles au cours de la campagne 2016, montrent une réduction jusqu'à 46% de l'Indice de Fréquence de Traitement (IFT) par rapport à un itinéraire technique conventionnel ou bio. Ces résultats montrent l'intérêt d'une stratégie de modélisation à l'échelle de la parcelle pour la mise en place d'une utilisation raisonnée des produits de lutte. En outre le paramétrage spécifique des OAD pour une exploitation donnée, permet d'affiner la sensibilité de la modélisation en fonction des conditions réelles auxquelles va être soumise la culture. Cette approche constitue ainsi une solution déterminante dans la gestion des cultures en accord avec une agriculture durable.

## Reduced gamete viability associated to somatic genome rearrangements increases environmental sensitivity of fruit set in Tempranillo Blanco cultivar

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Tempranillo Blanco (TB) is a novel cultivar developed from a white-berried somatic variant of Tempranillo Tinto (TT). Chromosome rearrangements caused the loss of berry skin colour in TB, associated with a tendency to increased millerandage and reduced yield. The objective of this study was to identify, developmental, genetic and environmental factors conditioning fruit set and yield in TB. First, to understand the biological origin of this variation, production parameters were compared between TB and TT plants cultivated within the same experimental vineyard. TB showed lower yield than TT, which positively correlated with cluster weight but not with the number of clusters per vine. Diminished fruit set (ranging between 19-29% depending on the year) and increased millerandage (24-42%) appeared as the main drivers of cluster weight differences considering that seeded berries reached similar weight and size in both Tempranillo lines. Concurrently, pollen viability and seeds developed within seeded berries were also reduced in TB by 29-65% and 0.7-1.1 seeds per berry, respectively. The effect of environmental conditions on TB's fruit set and yield variation was also assessed by comparing a network of 9 TB

vineyards of the same age, spread along La Rioja D.O.Ca. region, and cultivated under similar management practices. The altitude of the vineyards ranged between 340-740 m asl and their environmental differences were monitored by meteorological stations from the Sistema de Información Agroclimática para el Regadío (SiAR) del Gobierno de La Rioja located close to each vineyard. The study of TB production in this network showed that fruit set and yield were also correlated among vineyards. Furthermore, high relative humidity and rainy conditions in the week before flowering time correlated with reduced seeded-fruit set and consequently, with increased millerandage rate and reduced yield. Pollen viability and number of seeds per berry remained more stable. Collectively these results suggest that reproductive dysfunction in TB gives rise to looser clusters and lower yield. This genotypically-driven dysfunction is more strongly expressed under environmental conditions that are unfavourable for pollination and fertilization.

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## Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy

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The impact of water deficit on berry quality has been extensively investigated during the last decades. Nonetheless, there is a scarcity of knowledge on the performance of different varieties exposed to a combination of high temperatures/water stress during the growing season and under vineyard conditions. The objective of this research was to investigate the effects of two irrigation regimes (Sustained deficit irrigation (SDI, 30% ETc) and regulated deficit irrigation (RDI, 15% ETc)) and of two cluster positions within the canopy (east- and west-exposed sides) on berry ripening in red Aragonese (syn. Tempranillo) grapevines. The study was undertaken for two successive years (2013-2014) in a commercial vineyard in South Portugal, monitoring the following parameters: predawn leaf water potential, berry temperature, sugars, polyphenols, abscisic acid (ABA) and related metabolites. Additionally, expression patterns for different transcripts encoding for enzymes responsible for anthocyanin and ABA biosynthesis (*VviUFGT*, *VvNCED1*, *VvβG1*, *VviHyd1*, *VviHyd2*) were analysed. In both years, anthocyanin concentration was lower in RDI at the west side (RDIW- the hottest one) from véraison onwards, suggesting that the most severe water stress conditions exacerbated the negative impact of high temperature on anthocyanin. The down-regulation of *VviUFGT* expression revealed a repression of the anthocyanin synthesis in berries of RDIW, at early stages of berry ripening. At full-maturation, anthocyanin degradation products were detected, being highest at RDIW. This suggests that the negative impact of water stress and high temperature on anthocyanins results from the repression of biosynthesis at the onset of ripening and from degradation at later stages. Irrigation and berry position had small effect on free-ABA concentration. However, ABA catabolism/conjugation process and ABA biosynthetic pathway were affected by water and heat stresses. This later

indicates the role of ABA-GE and catabolites in berry ABA homeostasis under abiotic stresses. Free-ABA and total anthocyanins were highly correlated during both years, however a weaker correlation was observed at the west side (east  $r=-0.84$ ,  $p<0.001$  versus west  $r=-0.67$ ,  $p<0.05$ ), indicating a likely decoupling on the ripening process parameters due to berry high temperature at the hottest side. PCA showed that the strongest influence in berry ripening is the deficit irrigation regime, while temperature is an important variable determining the success of deficit irrigation. In summary, this work shows the interaction between irrigation regime and high temperature on the control of berry ripening. Furthermore, berries grown under SDI displayed a higher content in phenolics than those under RDI, as a result of the attenuation of the negative temperature effects at the west side of the canopy.

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## Towards genome-wide association studies under abiotic stress in *Vitis vinifera*

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Genome-wide association studies have proven their efficiency to decipher the genetic determinism of traits of agronomic interest in several plant species. In order to perform such studies in grapevine, we first designed an association panel of 279 *V. vinifera* cultivars from the French National Grapevine Germplasm Collection (Domaine de Vassal, INRA, France). It is composed of three subgroups of 93 cultivars representing the three main genetic pools, which differ in use and geographical origin: wine West, wine East, table East. This panel exhibited a good genetic and phenotypic representativeness of the whole collection. Linkage disequilibrium extent was quite small, 43 Kb for a corrected  $r^2$  predicted value of 0.2, emphasizing the need for several dozen thousands markers to achieve powerful genome wide association studies. The second step consisted in dense genotyping of this panel using a 18K SNP Infinium chip and genotyping by sequencing (GBS). While the first method provided useful genotypes at 12K SNPs, about 100K SNPs are expected using GBS with ApeKI. Data processing is in progress and this resource will be made available to the scientific community. The third step consists in phenotyping this panel for traits of interest. Vines were planted in

a randomized block design at Le Chapitre Research Vineyard (Montpellier SupAgro, France). Traits related to yield (cluster and berry weights) and phenology (maturity date) were measured in three years within the DLVitis program. Then within the Innovine program, grass was used as a cover crop between rows in the whole trial and part of the design was irrigated to obtain two levels of water availability. In addition to yield and phenology traits, berry composition (sugars, acids, anthocyanins, tannins and aromas), pruning weight and adaptation to drought ( $\delta C13$ ) have been measured during two growing seasons under both water availability conditions. This association panel, together with the accumulated data, represent highly valuable resources for genetic studies in grapevine. We are calling for an international collaborative effort to use such a panel in field and greenhouse trials to address questions on adaptation to changing environments.

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# Impacts of elevated CO<sub>2</sub> on interactions between *Vitis vinifera* L. and *Plasmopara viticola*, the causal agent of downy mildew on grapes

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The atmospheric level of carbon dioxide, one of the most important greenhouse gases, is increasing continuously. This increase is raising concerns also regarding plant pathogens as many reports predicted simultaneous impacts on the severity of plant diseases. However, these impacts differ between pathosystems; in some systems predicted impacts are rather positive (decrease of disease severity) while in others negative (increase in disease severity). In cool climate viticulture regions grapevine downy mildew (*Plasmopara viticola*) control demands a high fungicide input and is therefore of economic relevance for wine industry. Here, we report on the effects of elevated CO<sub>2</sub> (eCO<sub>2</sub>) concentration on the grapevine-*P. viticola* pathosystem. To this end, a grapevine free-air carbon dioxide enrichment (FACE) system (480 ppm; investigations in vineyard) as well as climate chambers equipped with CO<sub>2</sub> (750 ppm; investigations on potted vines) were used to study the effects of eCO<sub>2</sub> concentration on this pathosystem. On the fungal side, parameters

such as disease severity, length of incubation period, and vitality of zoospores were investigated. Alterations of pathogen-relevant morphological characteristics on grapevine leaves collected from plants grown in the FACE facility (ambient <sup>18</sup>CO<sub>2</sub> and eCO<sub>2</sub>) such as stomatal density were investigated by microscopic (anatomical) studies. In addition, transcriptome analyses of grapevine leaves infected and not infected by *P. viticola* and grown under aCO<sub>2</sub> and eCO<sub>2</sub> concentrations, respectively, were performed to analyze expression levels of relevant defense genes in grapevines. The data obtained will help us understand the grapevine defense mechanisms against *P. viticola* under future eCO<sub>2</sub>.

# Placing Vitaceae proanthocyanidins in a phylochemical perspective

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Genetically distant species within Vitaceae family, like *Mucadinia rotundifolia* and American or Asiatic *Vitis* species, enable to breed new *Vitis vinifera* cultivars more tolerant to diseases and pests and coping the massive use of phytochemicals in viticulture (1-7). In the future, Vitaceae may also provide tools to challenge abiotic stresses as consequences of climate change (8), which becomes problematic for berry yield and quality, if not detrimental to grapevine survival. Whatever, complex back crosses are needed in order to maintain the specific qualitative attributes of *V. vinifera*, and eliminate unfavorable traits for wine quality. In this respect, proanthocyanidins, or condensed tannins, are regarded as key-players in wine redox equilibrium, color stability and astringency as major sensory traits. The segregation of tannin structure in *V. vinifera* was documented in a Syrah x Grenache progeny and in a core collection representing the whole genetic diversity inside domesticated *V. vinifera* (9). It was recently reported that *V. vinifera* accumulates on average 30 fold higher amounts of condensed tannins than American *Vitis* species, with changes in galloylation and polymerization degree (10). In the present work, we have characterized the proanthocyanidin structure on a large set of Vitaceae, spanning different genera (*Nekemia*, *Ampelopsis*, *Parthenocissus*, and *Vitis*). The approach was focused on the accumulation of tannins in pericarp before ripening, excluding seeds. The 22 domesticated *V. vinifera* accessions formed a tight cluster, which was puzzlingly separated from the wild *V. vinifera* accessions richest in epigallocatechin. Tannins of Asiatic species were devoid of this component, excepted for *V. piasezkii*, which couldn't be distinguished from the domesticated *V. vinifera* genotypes.

The nine *Ampelopsis* species displayed a four times higher degree of galloylation, and spread over a large range of epigallocatechin/epicatechin ratio. Noticeably, the American *Vitis* species displayed all previous combinations of di and tri-hydroxylated compounds, and their galloylated forms. These results provide original insights in the phylogenomics of Vitaceae condensed tannins and open new horizons regarding the speciation and domestication of grapevine.

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## Grapevine adaptation to climate warning: new ways to breed varieties better adapted to elevated temperature

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The vine performance, including productivity and wine quality, is highly dependent on climate. In the long term, genetic improvement is the most pertinent strategy that will support sustainable wine production systems facing the announced climate changes. Unfortunately, the lack of knowledge about the mechanisms of adaptation of the grapevine to T° and of their genetic determinism limit the development of genetic improvement. In the last 5 years, we performed an interdisciplinary program to: i) characterize the impact of the increase in T°, at whole plant or fruit level, on the development and functioning of the plant (organogenesis, biomass variation, metabolism and transcriptomics of the berry), ii) identify the developmental (C balance) and molecular (transcription) mechanisms regulating the response of vegetative and reproductive systems to heat stress and ii) develop microvine progenies to map QTLs of development and study their stability against thermal variation.

Performing high-throughput genomic tools combined with the use of innovative experimental models (fruiting cuttings, microvines), this work was critical to decipher the ecophysiological and molecular mechanisms involved in the response of the vine to high temperatures and to develop tools and resources to research genetically determined tolerance traits. Using these better knowledge and original tools, we are performing a new program to select cultivars with berries growing at a lower osmotic pressure during ripening, in order to reduce the competition for photoassimilates between the vacuolar accumulation of sugar, and the synthesis of qualitative compounds from either primary (organic acids) or secondary metabolisms (pigments, aromas) in the cytoplasm.

# Evaluation d'un équipement de suivi maturité : Le Multiplex® en Aquitaine

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L'équipement Multiplex® est proposé par la société Force A, pour réaliser un suivi de la maturité phénolique de manière non destructive. Ce capteur piéton, dont le principe est basé sur les propriétés de fluorescence intrinsèque du végétal, permet, via des mesures dans l'Ultraviolet et le visible, de réaliser une mesure directement sur grappes et de donner des indices liés à la teneur en anthocyanes totaux (nommés ANTH\_RG et FERARI). Ce capteur peut également être utilisé en laboratoire sur un poste fixe ou non.

Sur les 9 parcelles étudiées dans le Bordelais, (merlot et cabernet sauvignon) sur les millésimes 2010, 2011 et 2012, les travaux suivants ont été initiés

- Une comparaison des mesures réalisées à la parcelle, entre les indices donnés par le Multiplex® et les valeurs de suivi maturité

- Une analyse statistique par régression linéaire pour concevoir des modèles de régression à partir des mesures effectuées au laboratoire.
- Une analyse métrologique (répétabilité, répétitivité, influence de l'eau et de l'obscurité sur la mesure) est également réalisée.

Par ailleurs, l'indice ANTH\_RG donné par l'équipement est plus spécifiquement étudié pour le suivi maturité. L'outil Multiplex® donne des résultats encourageants pour l'évaluation des anthocyanes totaux en particulier sur grappes pour le suivi à la parcelle. Cet équipement est répétable, répétitif et, dans sa forme actuelle (modification du diamètre de mesure, poids plus faible, intégration des tris sur les mesures réalisées à la parcelle), semble approprié pour déterminer un potentiel en anthocyanes totaux.

## Characterization of muscadine resistance to Phylloxera

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The objective of this study was to develop a reliable and repeatable assay under controlled conditions to characterize the level of resistance to phylloxera in an offspring resulting from a cross between the hybrid VHR 8771 [*M. rotundifolia* x *V. vinifera*] and Cabernet Sauvignon (*V. vinifera* L.) and to identify resistance QTLs to phylloxera on a genetic map produced from the offspring.

One-bud hardwood cuttings were planted individually in confined pots to avoid cross-contamination and grown in a greenhouse equipped with a cooling system. Two months after plantation (late spring) they were inoculated with 100 eggs of Pcf7 strain of phylloxera. In 2014, 19 individuals (5 replicates per individual, controls, progeny parents and 15 offsprings) were analyzed 3 and 6 months after inoculation. In 2015, the contamination was not successful, most probably because the inoculation was performed at high temperature preventing egg hatching. In 2016, 95 individuals (3 replicates, controls, both parents and 93 offsprings of which 77 had been genotyped to build the genetic map) were analyzed three months after inoculation. In parallel, the development over time of phylloxera larvae on isolated roots maintained in petri dishes was monitored on the parents, controls, and some offsprings. This assay was performed to study the relationship between the developmental capacities of phylloxera larvae on roots and the sensitivity of the whole plant. In addition, a genetic map was constructed with 188 SSR markers from 90 individuals of the 8771xCS descendants.

In 2014, records of nodosity numbers, made three months after inoculation allowed to differentiate sensitive from resistant individuals. Six months after inoculation, it was no more possible to observe differences between genotypes. Phylloxera was in its hibernation phase and old galls were not replaced by new ones. In 2016 the gall formation was generally lower in comparison to 2014. However the comparison of the response for controls and individuals tested in 2014 and 2016 showed consistent results, validating the repeatability of the assay. However it remains highly dependent of environmental factors (temperature and humidity). A QTL analysis was performed for gall numbers on the 77 individuals used to build the genetic map. No QTL were identified. This result can be explained by the low number of individuals studied, or by an insufficient density of SSR markers. Assays performed on roots displayed a correlation between the sensitivity at whole plant level and the development of phylloxera on roots.

The VHR 8771xCS genetic map is currently being densified with SNPs. New individuals obtained in 2015 and 2016 will be genotyped. A final phenotyping campaign will be carried out in 2017 on new individuals and controls with the objective to identify QTLs for phylloxera resistance in the genetic background of Muscadine.

# WINETWORK project: Identification and dissemination of innovative control strategies for Flavescence Dorée and GTD management

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Flavescence dorée (FD) is a grapevine disease that spreads epidemically if it is not managed properly. Control strategies based on vector monitoring, insecticide application and removal of infected vines and secondary hosts are mandatory in most European winegrowing regions. Grapevine trunk diseases (GTD) involve a large number of xylem-inhabiting fungal species predominantly associated to *Botryosphaeria* dieback, *Esca* and *Eutypa* dieback. Control strategies for GTD management are based on preventive techniques which minimize new infections. Both diseases cause significant economic losses in European vineyards. Innovative and good management practices developed by scientists and winegrowers need to be disseminated and adapted to routinely employed control strategies to maintain vineyard sustainability. The WINETWORK project funded through the European Union's Horizon 2020 research and innovation programme is developing an innovation-driven methodology, which promotes the exchange of knowledge between science and practice. This network is implemented in ten winegrowing regions, within seven European countries. The methodology is built on a network of winegrowers and regional experts gathered in regional Technical Working Groups, Scientific Working Groups (one for each disease),

and regional Facilitator Agents that stimulate their interaction. Bottom-up approach includes surveys, conducted and synthesised by Facilitator Agents, to identify winegrower's innovative practices, while scientists revise scientific data and adapt it to dissemination and practical use. Dissemination is adapted to different stakeholders and includes a summary of relevant practical and scientific knowledge available through a knowledge reservoir, newsletters, workshops, video clips, technical presentations, multimedia tools.

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## New tools and applications based on computer vision and spectroscopy developed within the InnoVine project framework at the Univer-

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New, non-invasive sensing technologies, including computer vision and spectroscopy, could be applied in precision viticulture with the aim of yielding useful, reliable and objective diagnosis of the vineyard status. This work presents some applications and tools based on computer vision techniques and spectroscopy developed within the InnoVine project framework at the University of La Rioja. NIR spectroscopy was used for grape composition assessment. Our results showed that total soluble solids can be determined in a vineyard using a non-invasive portable NIR spectrometer under field conditions. Moreover, computer vision systems were used to assess several features of the plant, such as the grapevine yield components. Hence, a new smartphone-based phenotyping tool has been developed that successfully assesses the number of flowers per inflorescence from a low-cost RGB image analysis. Similarly, the number of berries per cluster was also assessed in the field by means of non-invasive computer vision techniques. One step

forward was given and a computer vision set up was installed on a moving vehicle and used to assess the main features of the canopy within a vineyard using on-the-go. Using this methodology, our results indicate that good estimations of the percentage of exposed clusters, the canopy porosity and total wood pruning weight can be obtained from information extracted from images acquired under field conditions on-the-go. All these viticultural parameters were georeferenced, so they can be mapped, providing a very useful and friendly information to grapegrowers aiming at working following the principles of precision viticulture. Moreover, additional several non-invasive sensors could be embedded and mounted on a vehicle or in a robot for in field high-throughput plant phenotyping, enabling the assessment of multiple viticultural features simultaneously.

# Notes

# innovinePARTNERS

European collaborative project funded by the European Union

14 academic partners



12 private partners or SMEs



1 technical institute



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