Measuring plant stress to design and steer agroecological systems: the grape vine example

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Abstract

Benefits of the measurement of the redox potential (Eh) and pH of leaves: Work and a recent bibliographic study have made it possible to correlate the development of pests and diseases with specific oxidation-reduction conditions (measured through the redox potential, Eh) and acid-base (pH) of plants, in relation to the conditions of soil, cultural practices, genotype and climatic conditions. It would thus become possible to diagnose or even quantify the effect of certain stresses undergone by cultivation via these measures. This opens up new perspectives on the management of practices for agro-ecological crop protection by regulation of Eh-pH conditions. Such a prophylactic approach would maintain the plant in good health, under conditions unfavourable to pests, rather than having to fight against these pests once they have developed, often by overoxidation.

Conventional electrochemical measurements are very delicate and time-consuming, which makes them impractical for farmers. Due to these measurement difficulties, the number of references on the conditions of plant balance and the impact of practices remains very limited. Portable near infrared spectrometry (NIRS) would make it possible to overcome these difficulties. This has been shown for wheat and rapeseed first with measurement campaigns combining classic electrochemistry (Eh, pH, electrical conductivity (EC)) and spectrometry with the spectrometer developed by the company SENSEEN, during 2020 and confirm now for rice and grape vine in 2021. The paper presents in detail the status of plan stress for grape vine with a large measurement campaign in 2021 and the ability to predict Eh and pH by low-cost portable spectrometer using innovative AI-based chemometrics.

In conclusion, the simple, reliable, fast and inexpensive measurement by portable near infrared spectrometry of these plant health indicators should make it possible to develop a unique expertise on the effects of agricultural practices and thus to have practical tools for redox control of the plant health.

Keywords: Agroecology, plant stress, potential redox, spectroscopy

INTRODUCTION

This paper presents an innovative approach with several associated multi-disciplinary researches in agronomy, photonics and Artificial Intelligence to provide unique tool to drive farms aiming to implement greener approach such as agroecology. Agroecology is a dynamic concept that has gained prominence in scientific, agricultural and political discourse in recent years. It is increasingly promoted as being able to contribute to transforming food systems by applying ecological principles to agriculture and ensuring are generative use of natural resources and ecosystem services while also addressing the need for socially equitable food systems .

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The strategy of agroecological management of weeds, pests and diseases is based on vegetation diversification and the promotion of soil health (Vega et al, 2020). The Food and Agriculture Organization of the United Nations (FAO) describes soil health as the "capacity of soil to function as a living system, with ecosystem and land use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health (Haney et al., 2018). The paper introduces the need for more measurements to have quality result indicators and compare them to new farming practices. Agronomical research may introduce innovative indicators as Eh and pH to understand health of the agriculture ecosystem. Researchers in photonics may present the state of the art in Near Infrared Spectrometry to acquire light spectra and finally research in Artificial Intelligence may provide the Eh and pH measurements in simple and cost-effective way that the agroecology community is demanding.

I. REDOX POTENTIAL: THE INNOVATIVE MEASURES TO STEER AGROECOLOGICAL SYSTEMS

Soil health or quality has been defined in many ways that usually include various aspects of physical and chemical soil properties and some biological indicators. Thus, various indicators proposed to assess soil health (Bünemann and all, 2018)] all reflect the importance of soil organic matter, nutrient cycle, biological activity and soil structure in soil health. Interestingly, these parameters both impact and are impacted by soil redox potential (Eh, assessing the availability of electrons) and pH (assessing the availability of protons), and it was proposed to assess soil health through Eh and pH (Husson et al., 2018a) Eh and pH signaling and homeostasis are also regarded as key processes on almost all aspects of plant biology (Rengel et al. , 2002), and as for soil health, the various methods developed to assess plant stress/health, as chlorophyll fluorescence, photo-oxidative stress markers (including photosynthetic pigments, PSII efficiency, ROS, reactive carbonyl species, antioxidant systems) are all related to Eh and pH. Thus, it was also proposed to use Eh-pH also as indicators of plant health (Husson et al. , 2018b). Figure 1 shows the difference areas where oxidation of plants leads to favourable conditions for pests and diseases. (Husson et al, 2018a) .Pests and pathogens thrive in specific Eh-pH niches. Plants become susceptible to pest and pathogen attacks if their compartments are subjected to imbalanced Eh-pH conditions with specific Eh-pH values for each pest or pathogen to thrive

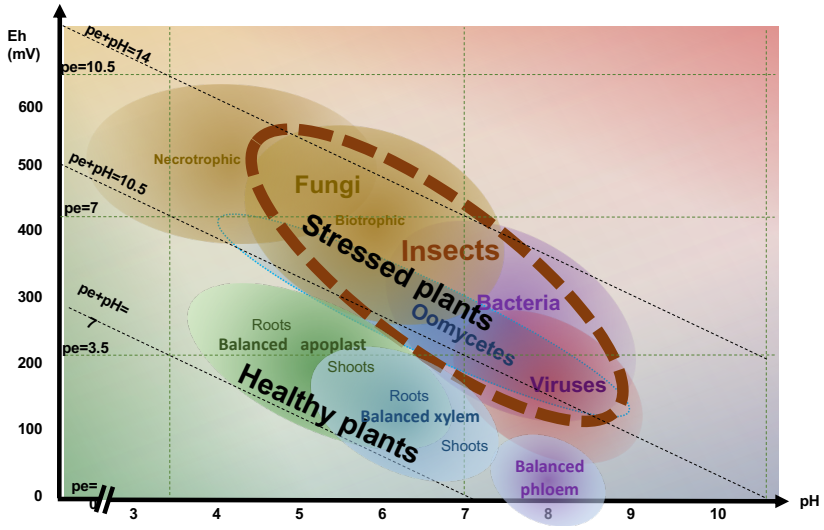

Figure 1 Characterization of the Eh-pH conditions for the development of different types of pests and the balance of different parts of plants

However, measurement of these parameters based on electrochemistry, face several difficulties especially the redox potential. Variations in the methodology and instrumentation for measuring the associated voltages often lead to imprecise and inaccurate estimates of redox potential (Rabenhorst et al., 2012) and electromagnetic fields can dramatically disturb Eh measurement (Husson et al., 2016) Furthermore, even when these constraints are overcome, electrochemical measurements are time consuming and fastidious, which strongly limits the possibility to use such measurement to drive the design and the implementation of agroecological practices. Hence, there is an urgent need to develop innovative measurement tools for in situ rapid and reliable measurement of Eh and pH in plants and soils

THE INTEREST OF MEASURING REDOX POTENTIAL FOR WINE MAKERS

The vineyards are suffering

A few economic figures show that viticulture is one of the jewels of the French economy. It represents the largest item of agri-food exports. In 2019, France was the world's second largest wine producer in terms of volume (17% of world wine) and the world's leading exporter of wine and brandy in terms of value (13 billion euros in turnover).

Despite the knowledge acquired concerning the development of the main pests which are partly responsible for the progressive decline of the vineyard and the progress already made in the means of control put in place to cope; it appears that the state of the French vineyard is in a critical situation and as such the various interprofessional bodies have decided to join forces since 2016. A national plan to combat vineyard dieback (PNDV) has been drawn up

Many pathogens are the cause of part of the decline of the vineyard and require the implementation of various means of control. Beyond pathogens, other causes of decline are linked to the genetics of the plant system and need to be better understood. In addition to crop dieback, the wine industry must deal with various seasonal pests likely to cause heavy losses. The means of control are costly for the sector and can represent up to 30% of the production costs per hectare for a winegrower for phytosanitary protection and up to 20% to replace the losses caused by dieback . Beyond the economic impact, the means implemented can have harmful effects on the soil microbiota. They also represent a major risk to human health; and therefore the challenges to which the project responds are public health, agronomic performance and economic impact.

Today, grape growers and nurserymen need practical and effective tools to help them with management strategies in compliance with environmental standards. An in-depth knowledge of redox in viticulture would provide grape growers and viticultural nurseries with an additional “decision support” tool and would have many economic, health and environmental benefits.

The need for new indicators

To give examples, the downy mildew control requires 2 to 8 treatments depending on climatic conditions and 10 to 12 applications in a catch-up situation. The plots present uneven health exposures that are difficult to qualify with current tools. Understanding them better is a performance issue for wine workshops and an interest in reducing inputs.

Rosé wine is a quality that is very sensitive to oxidation. While some wines keep well, others age prematurely. These behaviors are influenced in particular by the characteristics of the must and in particular by the polyphenols. An oxidative evolution of a vine during its vegetative phase reasons on the fruit by impacting its qualitative potential. The measurement of the redox potential provides the finesse of assessment necessary for the adjustment of the

transformation process. The development of these field measurements accessible by each producer is a guarantee of the necessary reactivity in these strategic moments when customer satisfaction is at stake.
Water stress is now an annual constraint that impacts the quality and profitability of workshops. Despite the development of irrigation projects, the development of farming practices that improve the storage of water in the soil and reduce its losses are more than ever a priority. The popularization of the measurement of the redox potential and conductivity by a tool accessible to the greatest number will allow each practitioner to evaluate the best practices on his territory by indicators sensitive to the evolutions of this stress.

MATERIAL AND METHODS

Measures on the field was made in partnership with the enterprise Ver de Terre Production which is recognized in France to lead the open-source diffusion of agroecology knowledge and pilot farmer portraits. This French agroecological farms ecosystem allowed to quickly find an high variability of farm practices from conventional agriculture until to no-till farming with soils rich in carbon. A campaign of measures has therefore been done on wheat and rapeseed, spanning several months, from December 2019 to June 2020. Around 1200 leaves of wheat and 650 leaves of rapeseed have been measured from several farming management systems. First NIR spectra were done, then, redox potential was measured directly by inserting a platinum electrode into the foil as described in [Husson et al., 2018a]. Once these measurements were made, a mortar and pestle then a syringe were used to extract the juice, on which the pH was measured with a Laquatwin pH 22 meter.
The collected data were directly sent with the app of the NIR sensor.
Although accurate, the method applied for the redox measurement has the following drawbacks:

- the equipment used for the measurement of the ORP being extremely sensitive to electromagnetic disturbances, care must be taken to analyse the samples collected in areas free from such disturbances.
- the electrodes used for the redox potential measurement being sensitive to temperature variations, it is necessary to accompany each measurement point with a temperature reading, which will subsequently make it possible to correct the measurement affected in the field.
- the redox potential measurement may vary slightly with the pressure exerted by the platinum electrode on the sheet.
- the redox potential measured on a sheet varies greatly with the day. We note a plateau from 11 a.m. to 4 p.m. which makes it possible to compare the different modalities among themselves (Husson et al. 2018)
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Figure 2: Measuring redox potential in the field with traditional electrochemical method

The NIR spectroscopy innovation

Portable near infrared spectrometry (NIRS) would make it possible to overcome these difficulties. This has been shown in wheat and rapeseed first with measurement campaigns combining classic electrochemistry (Eh, pH, CE) and spectrometry with the spectrometer developed by the company SENSEEN, during 2020 and confirm later for rice and wine leaf in 2021. SENSEEN which was created in May 2020 brings the state-of-the-art of miniaturized spectrometers combined with AI-based chemometrics. With several thousands of electrochemical measurements and corresponding spectrums, the company succeed to develop models using neuronal network and giving +87% accuracy of predictions

Figure 3: low cost handled NIR scanner used for wine leaf measurements campaign

THE MEASUREMENTS CAMPAIGNS IN VINEYARDS IN 2021

Estandon cooperative in Provence is a union of 9 cooperative cellars and 12 estates which produce nearly 20 million bottles of wine in AOC Coteaux Varois en Provence, Côtes de Provence, IGP Var and Mediterranean. Since 2018 Estandon has been committed to a "living soil" approach which aims to make the vines resilient to stress, whether climatic, health, nutritional or water related. The photosynthetic activity of a plant reveals the quality of its living space, its state of health and its capacity to produce. Maximizing the photosynthesis of a vine is therefore a quality issue in the broad sense. While a plant tends to oxidize due to its aging and external aggressions, photosynthesis acts as a regulator that buffers its biological balance. Making the measurement of Redox potential accessible through portable and easy-to-use tools is valuable for evaluating and optimizing cropping practices. Better production using fewer inputs, seeking soil self-fertility and stimulating the plant's natural self-defense systems are objectives that Estandon is pursuing.

In Summer 2021, about 1000 measurements have been made with the NIR scanner and reference measurements in Eh, pH, EC using electro-chemical methods (see figure 2, 3 and

Figure 4 pictures showing measurements on wine leaf with NIR scanner (left) and electro-chemical test set (right)

4)in order to help “calibrating” the scanner. Many observations have also been taken and an additional 1000 scans with observations but with no reference’s measurement have also been taken to get some feedbacks on potential redox and pH. About 12 different wine yards with different contexts and agronomic practices have been selected. Measurements at few win yards in the Bordeaux region have also been collected.

THE DATA ANALYSIS WITH AI-BASED CHEMOMETRIC

The measurements campaigns have provided a dataset made up of about 2000 scans. In details, this dataset is made up of:

- 912 scans associated to classic electrochemistry measurements (Eh, pH, EC),
- 845 scans not associated with any measurements, but with observations and annotations on the treatments(s) performed onto the plants.

Each scan is a 256-values array representing the absorbance values of the item being scanned, with the wavelengths ranging from 900nm to 1700 nm.

The goal was then to evaluate if Artificial Intelligence (AI) based models could provide reasonably accurate measurements predictions for (Eh, pH, EC). The advantage of the AI based models onto more standards models used in spectroscopy (e.g. PCR, PLSR) is that they could discover non-linear relationships between the explanatory variables (the spectral data) and the target variables. Indeed, the Universal Approximation Theorem, formulated in 1989 and proven by Kurt Hornik in 1991, states that “a neural network with one hidden layer containing a sufficient, but finite, number of neurons can approximate any continuous function to a reasonable accuracy” (Hornik, 1991).

For our use case we opted for an AI models based on Feed Forward Neural Network, a type of AI models which has been successfully used in a previous published paper (Cousin et al , 2020).

Here again, this model gave promising results for our three agroecological plant stress indicators (Eh, pH, EC) (see figure 5 and Table 1). In addition to absorbance values, the best models also include the ambient temperature as explanatory variable.

RESULTS

Results are summarized below:

Table 1: Performance of prediction models with training and validation datasets

Indicator	model	Training			Validation		
		R2	RMSE	RMSPE	R2	RMSE	RMSPE
Eh	Absorbance	0.97	2.9	0.9	0.9	5.6	1.7
	Absorbance, temperature	0.98	2.35	0.73	0.94	4.5	1.4
pH	Absorbance	0.98	0.02	0.85	0.96	0.05	1.5
	Absorbance, temperature	0.99	0.03	0.83	0.97	0.04	1.2
EC	Absorbance	0.97	0.28	6.2	0.95	0.36	7.1
	Absorbance, temperature	0.98	0.24	5.3	0.96	0.33	7

Eh Training (absorbance + T°)	Eh validation ((absorbance + T°)

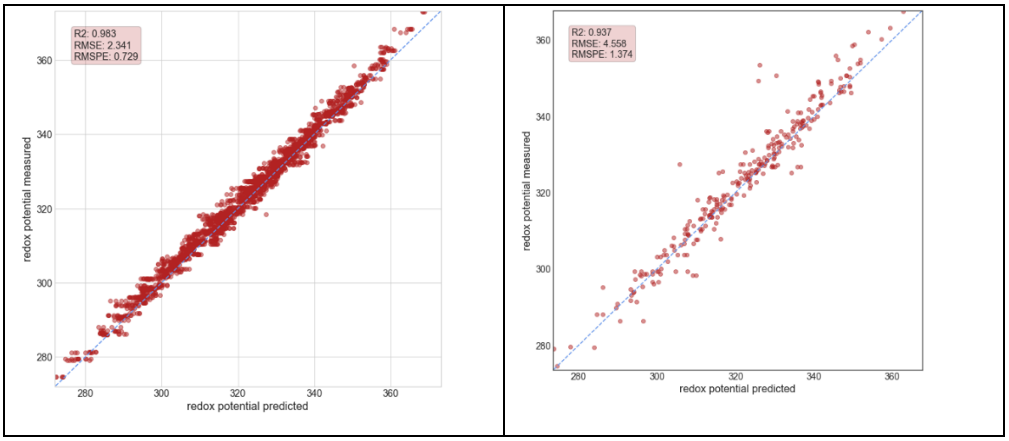

Figure 5 : Prediction models for Eh and Ph

INITIAL OBSERVATIONS

This first campaign of measurements provided a series of preliminary results showing the impact of agricultural practices on grapevine leaf Eh and pH conditions, as for below:

i) the changes with time, during the season and with aging vineyards. A 6 year-old Grenache variety showed marked changes in leaf pH at the beginning of the cropping season, and get oxidized latter in the season, while a 40 year-old Grenache was less oxidized at the beginning of the season and sustained better its pH, but at the cost of a progressive oxidation(figure 6)

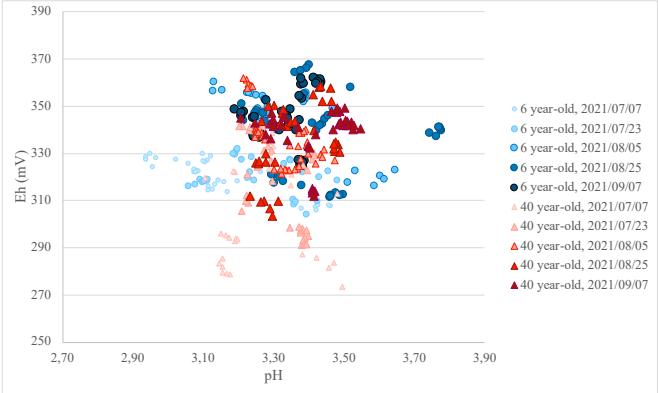

Figure 6 Changes in leaf Eh and pH across the season for a 6 year-old and a 40 year-old Grenache grapevine variety (Var, South Eastern France)

ii) the impact of maintaining a vegetal cover on the soil. Sustaining a vegetal cover on each row (one row over two with spontaneous vegetation, one row over two with seeded cover crops) resulted in more reduced grapevine leaves at the beginning of the cropping season and more alkaline leaves at the end of the season as compared to having one row over two with soil tillage, and one row over two with either spontaneous vegetation or seeded cover crops (figure 6).

CONCLUSIONS

The following conclusions can be drawn from the study:

- We succeed to calibrate a NIR scanner for Eh, pH, EC and achieved same prediction success as other cultures (e.g. Wheat, rapeseed) ;
- Wine makers can use an easy system for plan stress measurement and do as many as tests in routine

- Eh, pH, EC can be indicators to steer agroecological system and preliminary observations are encouraging
- more tests must be done and already planned in 2022.

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