Project Title

Estimation of water potential components and pressure-volume curve parameters on intact olive vegetative organs

Investigator

Giovanni Rallo

Post-Doctoral fellowship. Department of Agricultural & Forestry Science, Viale delle Scienze 13, ED. 4 -90128- Palermo, Italy. E-mail: giovanni.rallo@unipa.it.

In the competition 2014, the Committee of Alexander Goetz support program selected this research for the proposed innovative use of FieldSpec 4 spectro-radiometer, launched by ASD Corporation, to estimate the pressure-volume (P-V) curve parameters based on vegetation spectral reflectance signature in the VIS-NIR-SWIR domains.

The proposed research should be considered as a crucial and innovative step to estimate the plant water status and particularly the vegetation water potential and its components, representing important variables of the plant-water relations, whose knowledge is essential when investigations on precision irrigation scheduling and drought monitoring has to be carried out.

Recently, based on the spectral signature acquired by means a Field Spec Pro (ASD inc.), two different approaches to detect crop water status in terms of LWPs, were assessed by the investigator's team (Rallo et al., 2014). Specifically, existing families of Vegetation Indices (VIs) and Partial Least Squares Regression technique (PLSR) were optimized and tested. The results indicated that a satisfactory estimation of LWP at tree canopy and leaf levels can be obtained using vegetation indices based on the near and shortwave infrared domains (NIR e SWIR) requiring, however, a specific optimization of the corresponding "centre-bands". According to the encouraging results obtained in the first step of the investigation, the new research had the objective to improve the quality of the relationships between spectral indices and plant water status, as well as to detect the components of water potential (turgor and osmotic) of intact vegetative organs of olive trees. These objectives could be achieved because of the significant improvements of spectral resolution characterizing the new Fieldspec 4, compared to the Field Spec Pro used in the past.

More in detail, the main objective of the research was to analyze the reflectance spectral response in the VIS-NIR-SWIR domain and the mechanical cell well properties expressed by the leaf elastic module, measured at various stages of crop development and for different regimes of soil water deficit. Estimation of the elastic module was carried out by considering the P-V curve approach (Boyer, J.S. 1995), namely examining the empirical and non-linear relationship between the leaf water potential and the corresponding water content.

At the same time, it was possible to investigate on:

- the possibility of indirect estimation of the P-V curve parameters based on vegetation spectral signature in the VIS-NIR-SWIR domains;
- the development of a spectral analysis protocol allowing to measure the two principal components of the vegetation water potentials, i.e. turgor and osmotic;
- the effects of changing in mechanical properties of cell walls, expressed by their the elastic module.

Background

Osmotic and turgor water potentials are the principal components of a global potential term; they are usually measured by pressure methods in vegetative organs, like leaves or pieces of shoot. Considering that the components of plant water potential have opposite sign (negative for osmotic and positive for turgor water potential), the simple measurement of total water potential does not provide any information about its components. To this aim, is therefore necessary to plot, together with the Pressure–Volume (P-

V) curve, the components of leaf water potential versus leaf water contents. In the literature, this kind of plot is known as "Höfler diagram" (*Richter*, 1978), .

The knowledge of the P-V curve, allows to identify specific parameters (turgor loss point and osmotic potential at saturation) indicating the drought tolerance of a certain vegetation species. Moreover, volumetric bulk elastic modulus, reflecting the mechanical propertied of cell walls, can be mathematically derived.

Proximal spectral reflectance in the visible, near-infrared, and shortwave-infrared (VIS–NIR–SWIR) regions (350–2500 nm) have been successfully used for an accurate and rapid estimation of vegetation water status, as well as of biochemical and bio-agronomic properties involved into important physiological processes such as water exchange, photosynthesis, biochemistry of plant pigments, etc. Estimation of energy status of water in vegetative organs via proximal spectral reflectance is a convenient and rapid field-scale measurement approach, although it is applicable only to characterize the vegetation surface. Despite several field and laboratory experimental studies have been investigated the interactions between energy and water status, a poor scientific focus has been aimed to study the intrinsic interaction between the energy and the elementary components of the vegetation water potential, as well as the relationship between the energy and the mechanical properties of the cell walls. Only a research carried out by Peñuelas et al. (1993) presented some considerations about the effects of volumetric elastic module parameter on the reflectance response of gerbera plants. However, this study did not include the parametric approach to estimate the behavior of vegetative organs expressed in terms of Pressure-Volume curve.

Considering that the proximal spectral reflectance signatures contains information about vegetation water status and biomass structure, it can be assumed that the parameters describing the P-V patterns should be related to the vegetation spectral response.

Research Plan, methodology and first results

Research plan was developed in a period of about forty days during the mature phase of the first olive vegetative flux, in which an intensive field and laboratory experimental activities were carried out.

In each experimental day, P-V measurements and the related spectral signatures were detected on olive leaves collected on three plants maintained in absence of stress, during the soil drying processes following abundant watering (Treatment 1, T1). At the same time, investigation were also carried out on three plants maintained constantly under soil water deficit conditions (Treatment 2, T2). Operating in this way, it was possible to dispose of measurements acquired on a wide range of plant water status, and therefore to investigate on the dynamic of water potential components, so to examine different patterns of the pressure-volume curve.

Fig. 1 shows the experimental setup used during the research activity. Leaf spectral reflectance was monitored with an ASD FieldSpec 4 Standard-Res spectro-radiometer (Analytical Spectral Device, Inc.), whose features are indicated in table 1. The instrument covers a range of wavelengths from VIS to SWIR (350-2500 nm), is characterized by an internal field of view (IFOW) of 25° and samples with intervals of 1.4 nm and 2.0 nm, in the regions between 350 e 1000 nm and 1000 e 2500 nm respectively. Measurements required the use of the ASD Contact Probe and Leaf clip (Analytical Spectral Device, Inc.), specifically designed for plant leaves. Using these two accessories, leaf spectra were acquired on the adaxial surface of the same leaves used for the determination of P-V curve.

Fig. 1 - Experimental setup used during the research activity

Tab. 1 - Technical features of the FieldSpec 4 Standard-Res Spectroradiometer with annex a plant probe (exstracted from www.asdi.com)

Spectral Range	350-2500 nm
Spectral Resolution	3 nm @ 700 nm; 10 nm @ 1400/2100 nm
Sampling Interval	1.4 nm @ 350-1050 nm; 2 nm @ 1000-2500 nm
Scanning Time	100 milliseconds
Stray light specification	VNIR: 0.02%, SWIR 1 & 2: 0.01%
Wavelength reproducibility	0.1 nm
Wavelength accuracy	0.5 nm
Maximum radiance	VNIR 2X Solar; SWIR 10X Solar
Channels	2151
Detectors	VNIR detector (350-1000 nm): 512 element silicon array SWIR 1 detector (1000-1800 nm): Graded Index InGaAs Photodiode, TE Cooled SWIR 2 detector (1800-2500 nm): Graded Index InGaAs Photodiode, TE Cooled
Input	1.5 m fiber optic (25° field of view). Optional narrower field of view fiber optics available.
Noise Equivalent Radiance (NEdL)	VNIR: 1.0 X10-9 W/cm2/nm/sr @ 700 nm SWIR 1: 1.2 X10-9 W/cm2/nm/sr @ 1400 nm SWIR 2: 1.9 X10-9 W/cm2/nm/sr @ 2100 nm
Weight	5.44 kg (12 lbs)
Length	10" (25.4 cm)
Weight	1.5 lbs (.7 kg)
Power requirements	12-18 VDC, 6.5 W
Lightsource type/Life	Halogen bulb/1500 hours
Halogen bulb color Temp.	2901 +/- 10º% K
Spot size	10 mm

Leaf sampling was carried out at predawn (around 05:00 a.m. local time GMT+2), always choosing mature leaf localized on one-year-old shoot. Every sampling day and for each treatment, two leaves per tree were collected, with the aim to follow the two methodologies suggested in the literature and normally applied to investigate on the P-V curve. In the first method, it is suggested to put the leaf under water before cutting. The leaf is then re-hydrated for 24 h in a dark room at temperature of 4° C. In the second method, the cut leaf was not re-hydrated, but immediately sealed in polyethylene bags and brought to the laboratory for the measurements.
Figs. 2 and 3 show the different phases of the measurements related to both leaf spectral reflectance and leaf water potential, respectively.
Operatively, Field Spec 4 was used in white reference mode and selecting the foreoptic as "bare fiber". This was possible by means of the user-friendly interface that characterize RS^3 Spectral Acquisition Software. Before each spectral acquisition, an optimization adjustment was necessary to ensure that changing levels of drown welling irradiance did not saturate the detectors. After this optimization, the reflectance of a white standard panel mounted in the plant probe leaf clip was measured. The percentage of leaf reflectance was then obtained by dividing the spectrum of the sample by the white reference, as automatically provided in output by the instrument. For each leaf, reflectance spectral signature was acquired after placing the leaf in the plant probe, between the light source and the black target. In order to acquire a suitable signal to noise ratio, a number of 10 scans per spectrum was established. Immediately after each spectral acquisition, leaf water potential was measured on the same leaf with a Scholander pressure chamber (Skye, Powys, UK) according to the standard procedure usually adopted to detect the

P-V curve. In particular, the protocol suggests to follow a free-transpiration to dry the leaf (Lo Gullo et al. 1986, Vilagrosa et al. 2003). In order to improve the quality of leaf potential measurements, a stereomicroscope (40x magnification) was adopted.

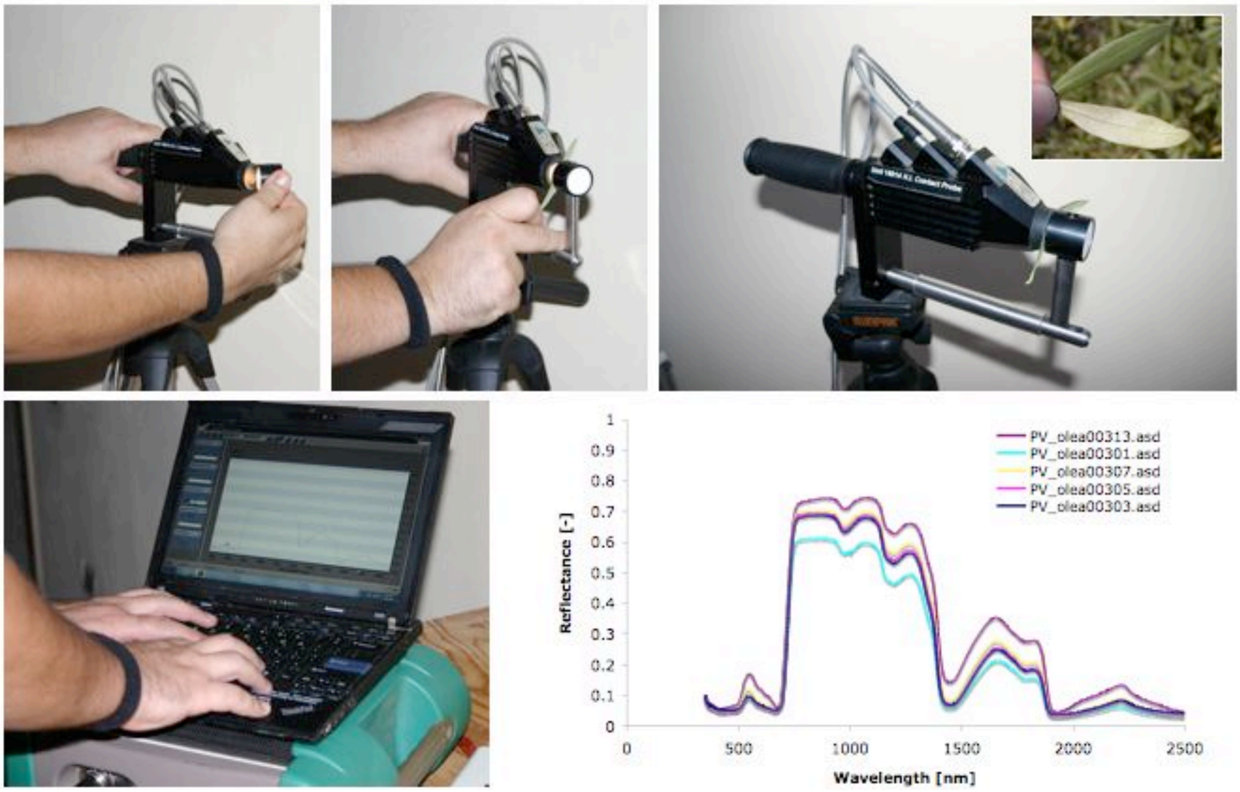
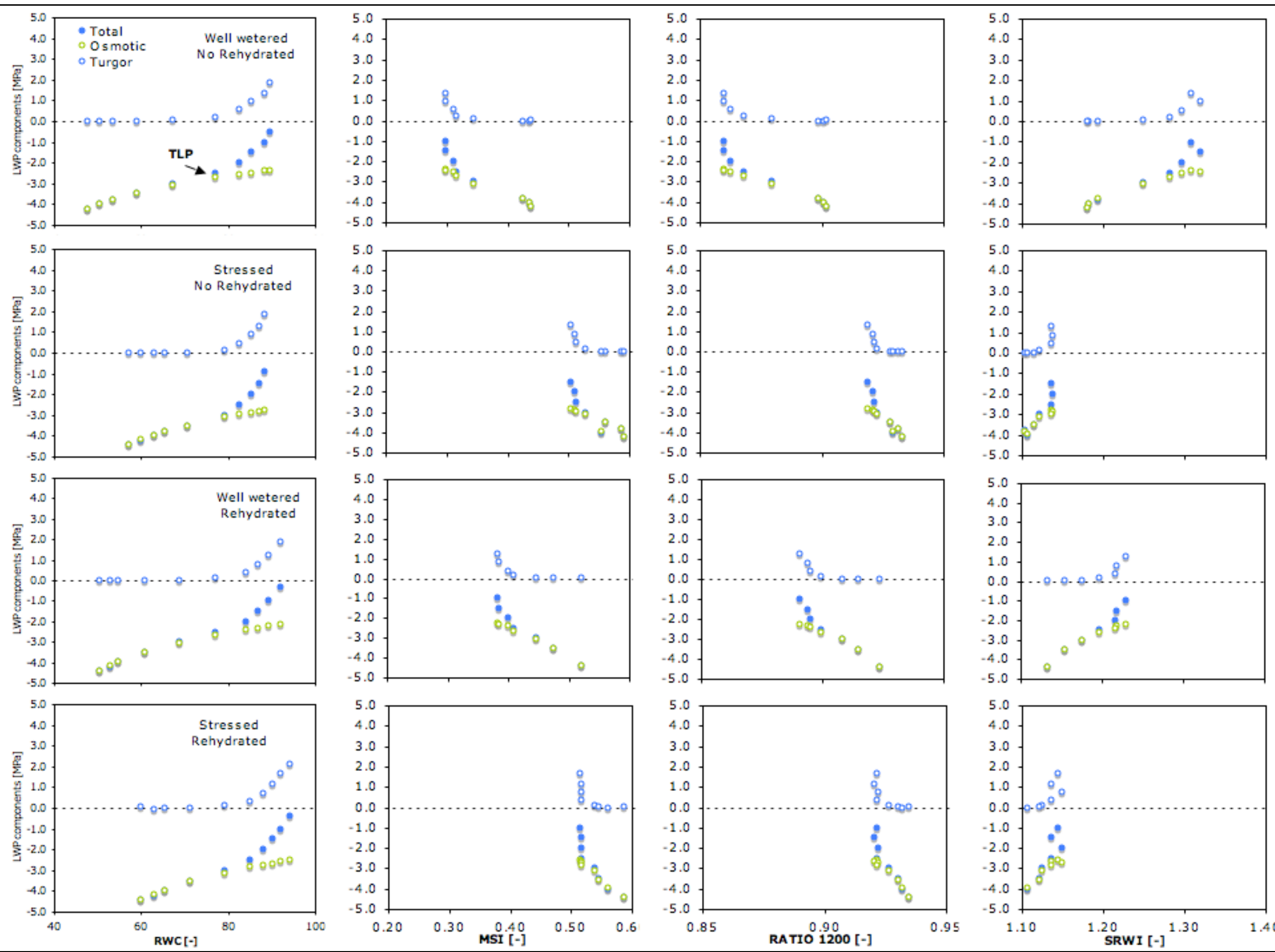


Fig. 2 – Phases of Leaf spectral reflectance measurements

Fig. 3 - Phases of Leaf water potential measurements

On the same leaf, the measurements were repeated during a drying process, so to obtain a sufficient number of points of the P-V curve, in the part merely related to the osmotic component.
As an example, the left column of Fig. 4 shows the Höfler diagram obtained on four leaves, two of which collected in well watered trees (T1) and the other two on stressed trees (T2), in the cases they were not re-hydrated and re-hydrated. Particularly, leaf water potential, as well as its turgor and osmotic components are represented as a function of the relative water content (RWC).
On the other three columns of fig. 4, the same LWP components are plotted vs specific spectral reflectance indices, developed using formulations based on simple spectral mathematical operations (ratios or differences between the reflectance values at given wavelengths). The values of the indices considered in this preliminary analysis were determined based on the relationships indicated in the lower part of fig. 4. The choice of such indices considers the characteristics of water absorption in SWIR (sensitive to changes in leaf water content) and NIR (sensitive to changes in leaf internal structure) domains of the spectrum.
A first qualitative analysis of the figures shows how the considered indices reflect, in a certain way, the patterns recognizable on the Höfler diagram, for different examined leaves. Moreover, it has to be noticed that, whatever is the indices considered, it is possible to recognize characteristics points of the Höfler diagram, like those related to the turgor loss point.
Of course, the extension of the analysis to the entire dataset will allow to strength these preliminary results, as well as to identify and assess an easy procedure to parameterize the P-V curve based on simple spectral measurements.



$$RWC[\%] = \frac{W - DW}{TW - DW} 100$$
(Barr and Weatherley, 1962)

$$MSI = \frac{\rho_{SWIR}}{\rho_{NIR}}$$
(Hunt et al., 1989)

$$RATIO_{1200} = \frac{2\rho_{1180-1220}}{\rho_{1090-1110} + \rho_{1265-1285}}$$
(Gao and Goetz, 1994)

$$SWRI = \frac{\rho_{NIR}}{\rho_{SWIR}}$$
(Gao and Goetz, 1996)

Fig. 4 – Höfler diagrams and preliminary relationships between the components of leaf water potential with spectral indices. TLP: Turgor Loss Point

Future developments

The improved performance of FieldSpec 4, compared to the previous models, provide more detailed spectral features, allowing a better interpretation of the changes in the shape or in the position of peaks and therefore to reveal some information related to plant physical properties, hidden at a lower resolution. In particular, the active dynamic changes at the equilibrium between the plant water potential components, frequently recognized in olive trees, can be evaluated through the analysis and modeling of the water relationships existing in a single leaf or in more architecturally complex vegetative organs, like one-year old shoots.

Future development will be addressed to the parameterization of the empirical nonlinear relationships between the inverse vegetation water potential (1/LWP) and the corresponding relative water content (RWC), based on the information extracted from high resolution hyper-spectral features, where cell wall/energy, water/energy and cell wall-water/energy interaction occur. This empirical relationship has been modeled by a continuous exponential model (Hellkvist et al., 1974) or by means of combination between a power curve, followed by a beeline, for relative water contents lower than the value corresponding to the turgor loss. The former is characterized by two parameters, whereas the latter by three parameters. In both the models, the parameters are related to:

-RWC associated to bound water in the cell walls, evaluated at the intersection between P-V curve and x-axis;

- Inverse of osmotic pressure at turgor-loss point and the corresponding RWC;
- bulk elastic module, identified as the slope of the linear relationship between imposed water stress and the corresponding strain value, in the range of RWC from saturation to the turgor-loss point.

Future investigations, on the acquired dataset will also allow:

- to select the wavelengths to which the different PV curve parameters affect the spectral features, in order to identify in which domains the spectral response of vegetative organs is sensible to modification of equilibrium in the water potential components;
- to characterize the geometry of spectral Reflectance features to which each investigated PV parameter is sensible;
- to identify simple and complex indices aimed to represent quantitatively the spectral features detected in spectral Reflectance signatures;
- to model the different PV curve parameters as functions of the selected indices;
- to assess the sensitivity of spectral water indices with the aim to verify how the equilibrium in water potential components influences plant water status.

Moreover, the contribute of active osmotic adjustment on plant water status will be also investigated, in order to improve the existing relationships between spectral indices and xylematic water potentials.

Finally, fractal analysis will be carried out in order to recognize if plant hyper-spectral signatures can be characterized by fractal dimensions, and if these dimensions are related with any of the parameters characterizing plant water status.

Bibliography

Barr, H.D. and Weatherley, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci. 15:413-428.

Boyer, J.S. 1995. Measuring the water status of plants and soils. Academic Press, San Diego. 178 p.

Gao, B-C. and Goetz, A. F. H., 1994, Extraction of dry leaf spectral features from reflectance spectra of green vegetation. Remote Sensing of Environment, 47, 369–374. Hunt, R.E.; Rock, R.N. Detection of changes in leaf water content using near- and middle-infrared reflectances. Remote Sens. Environ. 1989, 30, 43–54.

Gao, B.C. NDWI—A normalized difference water index for remote sensing of vegetation liquid water from space. Remote Sens. Environ. 1996, 58, 322–331.

Hellkvist, J., G.P. Richards and P.G. Jarvis. 1974. Vertical gradients of water potential and tissue water relations in sitka spruce trees measured with the pressure chamber. J. Appl. Ecol. 11:637--667.

Lo Gullo MA, Salleo S, Rosso R (1986) Drought avoidance strategy in Ceratonia siliqua L., a mesomorphic-leaved tree in the xeric Mediterranean area. Ann Bot 58:745–756.

Richter, H. Diagram for the Description of Water Relations in Plant Cells and Organs. J. Exp. Bot. (1978) 29 (5): 1197-1203. doi: 10.1093/jxb/29.5.1197A

Rallo G., Mario Minacapilli, Giuseppe Ciraolo, Giuseppe Provenzano (2014). Detection of crop water status in mature olive orchards using vegetation spectral measurements. Accepted on August, 21 2014, on Biosystems Engineering (DOI: 10.1016/j.biosystemseng.2014.08.012).

Vilagrosa A, Bellot J, Vallejo VR, Gil-Pelegrin E (2003) Cavitation, stomatal conductance, and leaf dieback in seedlings of two cooccurring Mediterranean shrubs during an intense drought. J Exp Bot 54:2015–2024.