Irrigation decision support based on leaf relative water content determination in olive grove using near infrared spectroscopy

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ABSTRACT

The relative water content (RWC) provides a measurement of the water deficit of the leaf and may indicate a degree of stress endured under conditions of drought and high temperatures, its measurement therefore, being essential for the appropriate management of irrigation. This study sought to ascertain the viability of near infrared spectroscopy (NIRS), using a handheld portable NIR instrument for the non-destructive and *in situ* determination of RWC in olive tree leaves cultivated under higher temperatures than ambient. Different combinations of pre-treatments and first and second derivative were assayed to obtain information of spectral data and to develop calibration models. A calibration equation with enough prediction performance for supporting irrigation decision-making (standard error of cross-validation, SECV = 1.52%; r^2_{cv} = 0.61; residual predictive deviation for cross-validation, RPD_{cv} = 2.01) was obtained. The findings obtained from the external validation of the model (standard error of prediction, SEP = 1.63%; r^2_{p} = 0.64; residual predictive deviation for prediction, RPD_{p} = 2.17) suggest the viability of the on-tree use of NIRS technology for the instant measurement of RWC in olive groves, ensuring a major saving in time and avoiding the disadvantage of transporting samples to the lab, thereby favouring real-time decision-making in the field regarding the optimal amounts of irrigation to be applied; this is of enormous significance for the future, given that the availability of irrigation water for such vital crops to the Mediterranean region as the olive could be limited in years to come by a gradual increase in planetary temperatures.

Keywords: Olive grove; *In situ* RWC measurement; NIRS technology; Irrigation management; Climate change

1. Introduction

Olive (*Olea europaea* L.) is the most prevalent crop in the Mediterranean basin and has enormous ecological and economic importance to the region. It is well suited to the Mediterranean climate, which is characterised by hot and dry summers, mild winters, and relative lack of rainfall. However, the climatic conditions of this region are expected to change in the near future due to global warming. Climate experts have predicted an increase in average air temperature in the range of 2–5ºC (Giorgi, 2006; Gualdi et al., 2013; IPCC, 2014) together with more frequent occurrence of extreme events such as droughts and heat-waves (Giorgi & Lionello, 2008; Tanasijevic, Todorovic, Pereira, Pizzigally, & Lionello, 2014). In the climatic conditions being predicted for the region therefore – lower precipitation and higher temperatures – it is likely that this species will undergo frequent periods of water and heat stress, with concomitant effects on yields.

The leaf is the organ of the olive tree that is most responsive to environmental conditions (Nevo et al., 2000). The RWC of a leaf is an important indicator of a plant's water status. In this sense, RWC provides a measurement of the 'water deficit' of the leaf and may indicate a degree of stress expressed under unfavourable conditions such as drought or high temperature (Barrs & Weatherley, 1962; Barrs, 1968). This parameter has long been used as a reliable indicator of plant wellbeing and could be highly useful in ascertaining whether olive trees subjected to the climate conditions of the future are suffering from stress at any of the phenological stages of their reproductive cycle (Mullan & Pietragalla, 2012; Rallo & Cuevas, 2017). It can be useful for indicating plant water needs (Jones, 2004, 2007) aimed at reducing potential stressful situations for olive trees, especially in those phenological stages where the species is more vulnerable to extreme conditions.

The traditional method used to determine RWC is by measuring the differences in weight between the fresh, dry and turgid leaf (Stocker, 1929). This method is time-consuming– it requires more than 24 hours – and labour intensive in the laboratory. Moreover, although the procedure is straightforward, the taking of samples is prone to errors, because it can be accompanied by a modification in the water content prior to the start of the analysis. There is therefore a need for a fast and efficient method for the determination of the RWC in a way that is non-destructive and *in situ* (on-tree), allowing growers to make accurate irrigation decisions depending on the water deficit of the tree. In this context, near-infrared spectroscopy has significant potential as an appropriate method, since it is a non-invasive, rapid, economical and accurate alternative to traditional methods. The technology is simple, so fewer errors are introduced than in conventional analytical techniques (Osborne, Fearn, & Hindle, 1993). At the same time, NIR spectroscopy is a powerful tool for general process monitoring in real time (De la Roza et al., 2017; Zhang et al., 2017); this is of particular interest for many agricultural practices such as irrigation.

NIRS technology has been successfully used to determine various parameters in the leaves of a range of species, using both laboratory (Menesatti et al., 2010; Fernández-Martínez et al., 2017) and portable equipment (Itoh, Tomita, Uno, & Naomasa, 2011; Steidle-Neto, Lopes, Pinto, & Zolnier, 2017). In the case of olive leaves, the research that has been published makes reference to measuring nutrient content (Fernández-Cabanás, Garrido-Varo, Delgado-Pertiñez, & Gómez-Cabrera, 2008; Rotbart et al., 2013) and differentiation between juvenile and adult leaves (León & Downey, 2006), both carried out in laboratory conditions. However, there is no trace in the scientific literature of any research into the measurement of RWC in olive leaves using NIRS technology. Several authors have demonstrated the feasibility of NIRS technology in the non-destructive measurement of RWC in the fresh leaves of *Epipremnum aureum* and *Miscanthus (M. sinensis, M. sacchariflorus, M. lutarioriparia, M. floridulus and M. giganteus)* (Zhang, Li, & Zhang, 2012; Jin, Shi, Yu, Yamada, & Sacks, 2017) using lab-based monochromator instruments, and in seedling eucalyptus leaves using a portable MEMS-NIRS instrument (Warburton, Brawner, & Meder, 2014).

The aim of this study was to evaluate the feasibility of using NIRS technology for determining RWC in olive leaves growing *in situ*. The goal is to help growers to make irrigation decisions to mitigate negative effects of stress on crop performance under future weather conditions associated to climate change.

2. Material and methods

2.1. Plant material

Olive (*Olea europaea* L.) leaves from cultivars 'Picual' (N = 178 samples) and 'Arbequina' (N = 72 samples) were analysed. Each sample consisted of four fully-expanded leaves, which were located at the middle position of the canopy and exposed to sunlight. Samples were sequentially collected from March 2016 to July 2017 on 17 different days, covering the range of the distinct phenological phases of the olive tree (Table 1).

These olive trees were located in an experimental field at the Rabanales Campus of Córdoba University (Spain) and exposed to different temperature treatments (ambient temperature *versus* 4 ºC above ambient temperature), by the use of open top chambers equipped with heating and ventilation devices. These systems are able to maintain permanently a day/night temperature gradient between the tree and the surrounding environment of 4 °C throughout the complete reproductive cycle of this species (Benlloch-Gónzalez, Sánchez-Lucas, Benlloch, & Fernández-Escobar, 2018).

2.2. NIRS analysis

A handheld Micro-Electro-Mechanical System (MEMS) spectrometer (MicroPHAZIR™, Thermo Fisher Scientific, Wilmington, MA, USA) was used to collect the spectra of olive leaves *in-situ*. This instrument operates in reflectance mode (log 1/R) across the spectral range of 1600–2400 nm every 8 nm. Internal white reference was automatically collected every ten minutes.

Olive tree leaves are small and thick, so in order to avoid the loss of light during spectra collection and to ensure that the field analysis was carried out correctly, without detaching the leaf from the tree, a circular-(15 cm of diameter) black metal plate was used to hold the leaf.

At first, with the aim of establishing which side of the leaf was most appropriate for recording spectra, NIRS readings were carried out both on the adaxial and abaxial side of the leaf. Three spectral measurements were made per leaf (at the upper, middle and bottom parts) and per side (adaxial and abaxial). Since, four leaves were analysed per each olive tree, and a total of 12 spectra were obtained for each sample and for each leaf side. These 12 spectra per side were averaged to provide a mean spectrum for each olive tree, a mean for each sample and, initially, for each side.

2.3. Reference method

RWC was determined in accordance with the procedure set out by Stocker (1929). Briefly, leaves were collected at solar noon and quickly put inside a 10 ml-test tube, which was hermetically sealed with a lid and placed in a container filled with ice to avoid loss of leaf moisture. Once in the laboratory, the olive leaves were weighed (FW) and then rehydrated by adding 1 ml of deionised water to the test tube. After incubation at 4 ºC for 24 h, the leaves were re-weighed to determine the turgid weight (TW) and thereafter put into an oven at 70 ºC for 48 h to determine the dry weight (DW). The leaf RWC (%) was calculated as follows:

$$RWC\ (\%) = ((FW-DW)/(TW-DW)) \times 100$$

For the purposes of this research the Standard Error of Laboratory (SEL) was estimated by analysing 10 duplicated samples. In order to calculate the error, both the sampling error (selection of two consecutives leaves to analyse) and the error arising from the process of analysis in the laboratory (analysis was done by duplicated) were determined. Once these two errors had been calculated, the SEL value was obtained in accordance with Fearn (1986).

2.4. Spectral repeatability

The spectral repeatability was evaluated using the root mean squared (RMS) statistic, is defined as the averaged root mean square of differences between the different subsamples scanned at n wavelengths (Shenk & Westerhaus, 1995a, 1996). It indicates the similarity between different spectra of a single sample, in this case between the three spectra collected per sample. For this purpose, 10 leaves were selected from which three spectra were taken in the upper, middle and lower parts using the MEMS-NIR instrument.

An admissible limit for spectrum quality and repeatability was set following the procedure described by Martínez, Garrido, De Pedro and Sánchez, (1998) to calculate the standard deviation (STD) limit from the RMS statistic and obtain an RMS cut-off value.

2.5. Data processing

2.5.1. Principal component analysis

With the goal of studying the relationship between the RWC and the distinct phenological states in the olive tree's cycle, as well as conducting the possible identification of anomalous samples, Principal Component Analysis (PCA) was carried out. In this work, PCA was performed using the mean spectrum derived from each of the days being analysed. Matlab software (version 2015a, The Mathworks, Inc., Natick, Massachusetts, US) was used to conduct PCA, using mean centre, which subtracts the mean spectrum of the group from each spectrum, as a pre-treatment (Wise et al., 2006).

2.5.2. Selection of the calibration and validation sets

Data pre-processing and chemometric treatments were performed using the WinISI software package ver. 1.50 (Infrasoft International LLC, Port Matilda, PA, USA). For the development of the model, the total set was divided into a calibration and a validation set. The selection of these sets was based on spectral information, using the CENTER algorithm (Shenk & Westerhaus, 1995a).

As spectral pre-treatments, Standard Normal Variate (SNV) and Detrending (DT) were used to remove scatter interferences (Barnes, Dhanoa, & Lister, 1989) together with the first derivative treatment '1,5,5,1', where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (Shenk & Westerhaus, 1995b).

Having ordered the population by spectral distances, samples that displayed GH values > 3 were removed. The validation set was selected by taking one sample out of every four in the initial set; the remainder constituted the calibration set.

2.5.3. Calibration development and validation procedure

Calibration models for the prediction of the RWC of the olive leaf were developed using Modified Partial Least Squares (MPLS) regression (Shenk & Westerhaus, 1995a) with six cross-validation groups to avoid overfitting. SNV and DT and Multiplicative Scatter Correction (MSC) were used as pre-processing for scatter correction (Barnes et al., 1989; Dhanoa, Lister, Sanderson, & Barnes, 1994). Additionally, four derivative mathematical treatments were tested: 1,5,5,1; 1,10,5,1; 2,5,5,1; and 2,10,5,1.

Best equations were selected according to the following statistics: coefficient of determination for calibration (r^2_c), standard error of calibration (SEC), coefficient of determination for cross-validation (r^2_{cv}) and standard error of cross-validation (SECV). However, in order to standardise the SECV value, another statistic, the residual predictive deviation (RPD), calculated as the ratio between the standard deviation (SD) of the calibration set to the SECV, was also calculated.

The best model obtained for the calibration set, as selected by statistical criteria, was subjected to external validation and evaluated in accordance with the protocol outlined by Windham, Mertens, and Barton (1989).

3. Results and discussions

3.1. Optimisation of in-situ olive tree analysis

After the spectra taken from both sides of the leaf at the beginning of the study, it was decided to take spectra only from the adaxial side, because the leaf of the olive has a highly-pronounced central vein on the abaxial side, causing greater dispersion of light during analysis. The procedure of taking spectra only from the adaxial side of the leaf is consistent with the practice of such authors as Zhang et al. (2012) in *Epipremnum aureum*, and Warburton et al. (2014) and Yang et al. (2017). in *Eucalyptus* leaves. Specifically, the study carried out by Warburton et al. (2014) on *Eucalyptus* seedlings, aimed at determining which side of the leaf was most appropriate for NIRS analysis, concluded that there were no significant differences enabling a particular part of the leaf to be established for recording spectra, although it is important to note that *Eucalyptus* leaves do not exhibit the very prominent central vein that is a feature of olive leaves.

After that and prior to the model development, it was necessary to optimise the NIRS analysis by means of the spectrum quality and repeatability measurement.

Firstly, the existence of noise in the spectrum was evaluated (spectral range 1600–2400 nm). To this end, the derivative treatment 1,1,1,1 was applied in order to determine the area of the spectral range affected by noise, given that it degrades the signal/noise relationship (Hruschka, 2001). After this process, the spectral range between 2312–2400 nm was eliminated (Fig. 1).

Secondly, spectral repeatability which is crucial to the construction of models that are both accurate and robust was evaluated. Statistical methods such as defined RMS cut-off limit can be useful for this purpose. The RMS cut-off was calculated as described in Section 2.4.

The STD_{limit} for the samples analysed using the handheld instrument was 42,663 μlog (1/R). Despite the importance of this parameter for fine-tuning new analytical methodologies and ensuring more robust models, no references have been found in the scientific literature that calculate STD_{limit} for the *in situ* analysis of olive leaves. In the present research, any sample whose triplicated screening scans yielded an RMS above this value was eliminated and repeated until values fell below that limit, thus ensuring a high degree of spectrum repeatability. It was found for example that the samples taken on 16 March 2017 exhibited values far higher than the established STD_{limit}, despite the analysis of the leaves being repeated on numerous occasions. A detailed study was carried out of the various factors that could have affected the analysis on that day, arriving at the conclusion that the variation arose from the fact that a few days prior to the analysis a copper-based treatment was been applied, with the consequence that the particles deposited on the leaves caused the analysis to be distorted. The samples taken on that particular day were therefore eliminated, leaving a set consisting of the 235 remaining samples.

3.2. Principal Component Analysis (PCA)

PCA was performed on the set comprising the spectra recorded per day (N = 16), after eliminating those mentioned in section 3.1. Figure 2a shows the PCA loadings for intact olive leaves in the spectral range 1600–2312 nm, while Fig. 2b displays scores of the second and third components of the PCA model. These two components were chosen because although the first two principal components (PC1 and PC2) represented a high proportion of the explained variance (82.23% and 16.57%, respectively), they did not facilitate the grouping of the samples in accordance with the phenological state; this grouping does however seem to become evident when the latent variables PC2 and PC3 are used.

The graphic representation of the loadings for PC2 and PC3 shows that the main absorption peaks for differentiating between the various phenological states of the olive tree are those related to water and carbohydrates respectively. Whereas the PC2 weighting coefficient exhibits a peak of water around 1900 nm, PC3 exhibits a band that is characteristic of carbohydrates (~1780 nm) (Shenk et al., 2008). The accumulation of carbohydrates in the plant differs in accordance with the phenological state that the plant is in at that time; thus, during the period of fruit formation and ripening; nutrients and carbohydrates will migrate from the leaf towards the fruit, accumulating in the latter (Fernández-Escobar, Moreno, & García-Creus, 1999). It therefore follows that the carbohydrate content in the leaf, represented by the third principal component, aids discrimination between the states the plant happens to be in.

Score plotting revealed apparent grouping by phenological stages (Fig. 2b), as shown in Table 1. Six groups emerge, which range from the period of winter dormancy to the maturation of the fruit, encompassing the intermediate phases of flowering, setting and growth of the fruit (Rallo & Cuevas, 2017).

In light of the PCA scores and bearing in mind the data set out in Table 1, it may be said that the phases of winter dormancy and flowering, which fundamentally occurs during the spring, when evapotranspiration is low (a rainy season), are related to PC2. The negative PC2 scores are associated with times of restricted water, which place the plants in situations of more acute hydrological stress. As it has already been mentioned, PC3 may be linked to carbohydrate content. This becomes particularly evident when analysing the group pertaining to the swelling of the fruit, which exhibits a positive PC3 score (Fig. 2b), setting it apart from the other samples and highlighting that in this phase there is a movement of carbohydrates from the plant's various organs towards the fruit, where it is subsequently assimilated (Fernández-Escobar et al., 1999).

León and Downey (2006) used PCA to differentiate between young and adult leaves in olive trees. They proposed that water content and various chemical compounds, particularly pigments, were responsible for this separation between the various ages of the leaf. In accordance with these authors, the distinction between the various phenological states could be due to the water and carbohydrate content of the leaf, although a depth study of the spectral characteristics of each state of the plant should be considered in future research.

3.3. Population characterisation

After applying the CENTER algorithm to the overall set (N = 235), two samples were identified as anomalous spectra. Once spectral outliers were removed, a set consisting of 233 samples was used to develop calibration models. As described in section 2.5, the set was divided into a training set (N = 174) and a test set (N = 59).

The distribution and statistics of the calibration and validation sets (mean, SD and CV) for the RWC are shown in Fig. 3. The structured selection based only on the spectral information treatments, such as CENTER algorithm, proved to be useful because the statistics for both sets were similar and the range in the calibration set encompassed the validation set.

Although *a priori* it may seem that the RWC parameter exhibits a wide range, both for the calibration (77.23–96.24 %) and for the validation set (78.22–95.61 %), this parameter actually exhibits severely restricted variability, as is evident from the low coefficients of variation obtained (Fig. 3). For the calibration set, 93% of the samples recorded an RWC of between 85% and 95%, while in the validation set 88% of the samples fell within this range, with very few samples (9 out of 174 and 5 out of 59 for the calibration and validation sets, respectively) recording RWC scores below 85%.

The low variability (CV_c = 3.37% and CV_v = 3.95%) is due to the RWC in olive-tree leaves not subjected to controlled water stress being around 90-95%, so this variation only derives from periods in which olives are suffering from water stress. Olive trees are drought tolerant, and leaves can reach extremely low relative water contents (75-80%) before losing turgor (Lo Gullo & Salleo, 1988). Therefore, values below 80% may correspond to extreme temperature events, which generally occur during the long dry season of the Mediterranean areas, where symptoms of dehydration are frequently observed and are generally associated with a low-potassium nutritional status (Fernández-Escobar, García, & Benlloch, 1994), something that was not applicable in the case of the current trial.

3.4. Calibration and validation for the prediction of the relative water content

Statistics for the best models obtained using the various pre-treatments to determine RWC in olive leaves measured on-tree are shown in Table 2.

According to Shenk and Westerhaus (1996) and Williams (2001), all models obtained enable classification of the RWC parameter between high, medium and low values ($0.50 < r^2_{cv} < 0.69$), being the best of them the one obtained using MSC and the first derivative of the spectrum (SECV = 1.52%; r^2_{cv}= 0.61; RPD_{cv} = 2.01).

In the present study, the estimated SEL was 0.87%. According to Fearn (1986), the SECV is determined not only by the SEL but also reflects the error of the NIRS method and the chemometric method. If the value of SECV is less than two times the SEL of the reference method, the NIRS equation is fit for use (Windham et al., 1989), meaning that this would be considered as appropriate for use in the field.

In order to compare the results obtained here to those obtained by other authors in leaves, the RPD_{cv} statistic was used to standardise the SECV value.

No other results have been found for determining RWC in olive leaves. However, various authors have used the technique to determine this parameter in a range of crops, initially using monochromator instruments in the laboratory. Zhang et al. (2012) reported good predictive capability (RPD_{cv} = 2.73) in determining the RWC in *Epipremnum Aureum* subjected to various water stress treatments, using a monochromator instrument with a spectral range of 200–1100 nm and a resolution of 1 nm. Jin et al. (2017) reported superior results to those obtained here (RPD_{cv} = 2.75) for *Miscanthus* leaves, using a monochromator instrument for the NIRS analysis with a wide spectral range (400–2500 nm, every 2 nm). These authors also had a calibration set for the parameter being studied that exhibited greater variability (CV = 6.53%), compared to the present case (CV = 3.37%), something that enables more robust models to be obtained (Shenk, Westerhaus, & Berzaghi, 1997). It is important to point out that both studies mentioned above carried out their RWC determinations with NIRS in the laboratory, whereas in the present study the analysis was conducted directly on the tree, with the MEMS-NIR instrument previously described. Moreover, the difference in predictive capacity between the first two spectrophotometers and the handheld instrument may reflect differences in spectral ranges, spectral resolution and in measuring area; the MEMS device measures an area of only around 4 mm^2, whereas both monochromators scan the whole sample.

While there are no reports of the use of portable instruments to measure RWC in olive leaves, various authors have used this type of instrument to measure RWC in the leaves of *Eucalyptus* seedlings. Thus, Warburton et al. (2014) measured RWC using a MEMS-NIR (MicroPhazir™ NIR spectrometer) instrument in the 1600–2400 nm spectral range; the results were better (r^2_{cv} = 0.88 and RER = 10.45) than those obtained here (r^2_{cv} = 0.61 RER = 12.51), possibly owing to the fact that they had a calibration set with a greater range (15.40–99.30%) than the one in the present study (77.23–96.24%). According to Fearn (2014), although r^2_{cv} can be useful for studying the predictive capability of the model, this is closely linked to the range of reference values, and this may provide a reason why the aforementioned authors reported a higher determination coefficient than that obtained here. In a similar trial and using a NIRS instrument that worked in the same spectral range (1600–2400 nm), Yang et al. (2017) obtained, for the *in situ* measurement of RWC in *Eucalyptus* seedlings, a predictive capability model (RPD_c = 2.59) that was slightly higher to the one obtained here (RPD_c = 2.09). This may be due to the fact that the authors in question had a calibration set with greater variability (SD = 6.33% and CV = 7.9%) than that in the present study (SD = 3.05% and CV = 3.37%), as well as the difficulties implicit in olive leaves in terms of thickness, sheen, enervation, etc., compared to *Eucalyptus* leaves, something that may have effect on NIRS analysis.

It should be noted that all these authors have conducted their experiments under controlled environmental conditions (temperature, humidity, irrigation, etc.), with situations involving induced water stress, thereby ensuring a set with a good and even coverage of the range. As Pérez-Marín, Garrido-Varo, and Guerrero (2005) point out, the distribution of samples within the calibration set is of great importance, because a uniform distribution throughout the range of the parameter being studied helps to obtain robust models.

Finally, Fig. 4 shows the regression coefficients for the best predictive model for the RWC parameter. The figure illustrates that the areas of the spectrum with greater weight in the model are located around 1720 nm, related to the C-H stretch first overtone and around 1936 nm, which corresponds to O-H bend second overtone (Osborne et al., 1993). This makes sense, because the RWC in olive leaves is very high, at around 90-95%. Furthermore, the area at around 2200 nm could be attributed to the C=O second overtone (Shenk, Workman & Westerhaus, 2008).

3.5. External validation procedure

After the development and analysis of the calibration models, the best model was subjected to external validation. For this purpose, a sample set not included in the calibration was used. Validation was performed using a set initially comprising 59 samples. Prior to the validation procedure, four samples were excluded from the validation set because they displayed values of RWC (78.22, 79.08, 95.60 and 95.61%) beyond the range obtained after the development of the equation (83.34–95.42%) for the parameter analysed. A graphic representation of the reference values *versus* the NIR predicted values for RWC in olive leaves is shown in Fig. 5.

The model developed for the prediction of the RWC complies with the limit established in terms of r^2_p for its implementation in routine ($r^2_p > 0.60$), as well as the confidence control limits for bias and SEP(c). The SEP value obtained shows a minor difference (0,09 %) compared to the SECV, and around 0.12% compared to the mean of the parameter, thereby confirming that the SECV provides a good estimate of the SEP

(Shenk et al., 2008). In addition, the slope (slope = 1.09) also falls within the established slope values (0.90–1.1) (Windham et al., 1989).

These findings suggest that the NIRS equation obtained may be considered as a first step for the *in situ* measurement of RWC in olive leaves. This could eventually enable growers to ascertain the plant's degree of water stress in real time, and to take appropriate and informed decisions about the irrigation of the crop.

Under future scenarios, growers could use leaf RWC measures by NIRS technology to quickly determine *in situ* whether olive is suffering from water shortage, trying to prevent stressful conditions and supporting irrigation scheduling.

In a practical sense, the best strategy to follow is to make a protocol in which the value of RWC corresponding to each phenological stage and specie is established. Values of leaf RWC rapidly measured using NIRS technology which were below those indicate that irrigation treatments would be necessary. This would be an excellent complement to the different routine scanning usually made, such as soil water content and tree evotranspiration demand.

4. Conclusions

The results of this study, which used a handheld NIR spectrophotometer, confirmed the viability of NIRS technology for the measurement of RWC in olive leaves on the tree. Non-destructive and rapid determination of this parameter provides a quantitative measure of the hydration status of the olive tree in the field, enabling optimal and precise management of irrigation, something that will prove of great importance to olive cultivation in Mediterranean countries. Climate change forecasts are predicting major periods of drought and an increase in temperatures in the region, where water will become an increasingly scarce resource; this will make it imperative to be able to determine the RWC of olive trees with a view to maintaining the efficiency of photosynthesis and crop productivity.

Over the coming years, further studies will be needed in order to improve the calibration specificity, accuracy and robustness of this procedure.

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Table 1 - Olive phenological stages on date analysis.

Measurement date	Number of samples	Phenological stage	Mean temperature (ºC)	Mean relative humidity (%)	Leaf RWC (%)		
					Min	Max	Mean
1. 03/16/2016	10	Bud dormancy	14.40	74.10	89.81	93.31	92.40
2. 03/30/2016	15	Flower development	16.60	61.90	89.40	93.10	92.10
3. 04/06/2016	15	Flower development	17.60	54.80	90.40	95.10	93.20
4. 04/21/2016	5	Flower development	16.30	68.60	91.50	96.20	93.50
5. 04/28/2016	15	Flower development	19.20	64.80	91.70	95.80	93.50
6. 05/04/2016	8	Flower development	20.50	41.00	86.80	93.00	90.50
7. 05/23/2016	15	Fruit set	23.00	44.40	89.80	94.80	91.30
8. 06/09/2016	16	Fruit set	29.50	42.10	85.50	89.40	91.80
9. 06/15/2016	15	Fruit set	23.30	47.80	86.90	95.60	90.70
10. 06/20/2016	15	Fruit growth	27.70	37.30	89.00	95.40	91.70
11. 06/30/2016	15	Fruit growth	28.20	50.10	86.60	95.60	92.10
12. 07/26/2016	22	End of stone hardening	32.60	31.80	83.60	90.00	87.40
13. 10/06/2016	22	Fruit ripening	24.30	52.90	77.20	88.70	84.10
14. 11/02/2017	22	Fruit ripening	18.80	67.10	84.60	92.40	89.00
15. 03/02/2017	15	Flower development	13.70	72.30	90.40	92.60	91.60
16. 03/16/2017	15	Flower development	12.70	65.60	90.90	95.50	92.40
17. 04/05/2017	10	Flower development	17.10	49.40	90.40	92.90	91.70

Table 2 - MPLS regression statistics for NIR-based models for predicting RWC in olive leaves.

Scatter correction	Math treatment	N	Mean	SD	SEC	r^2_c	SECV	r^2_{cv}	RPD_{cv}
SNV + DT	1,5,5,1	167	90.82	2.55	1.68	0.57	1.78	0.52	1.71
	1,10,5,1	163	90.94	2.45	1.70	0.51	1.73	0.50	1.76
	2,5,5,1	163	90.71	2.50	1.51	0.64	1.58	0.61	1.93
	2,10,5,1	164	90.84	2.45	1.63	0.56	1.68	0.53	1.82
MSC	1,5,5,1	161	90.79	2.42	1.46	0.64	1.52	0.61	2.01*
	1,10,5,1	163	90.81	2.45	1.58	0.59	1.63	0.57	1.87
	2,5,5,1	162	90.75	2.46	1.49	0.64	1.54	0.61	1.98
	2,10,5,1	166	90.85	2.54	1.82	0.49	1.86	0.47	1.64

* Best model for RWC prediction.

Fig. 1 - First derivative spectra of olive leaves prior to removing the noise.

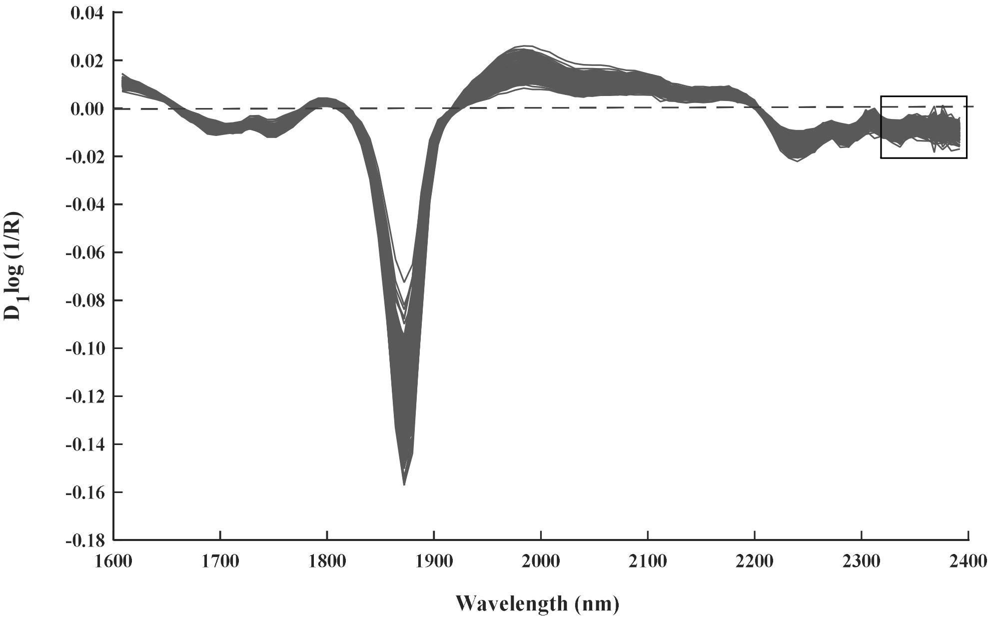
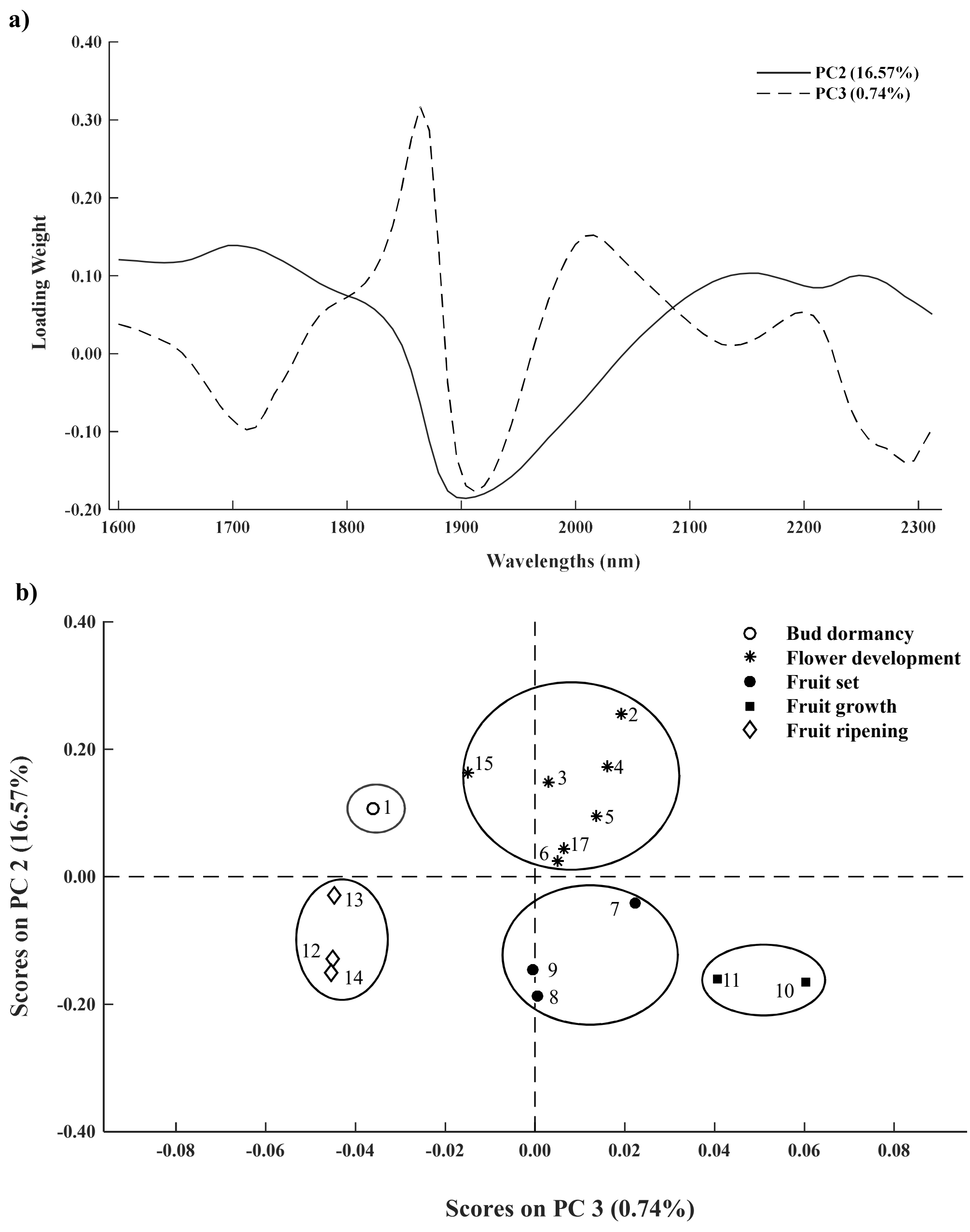


Fig. 2 - Loadings weight (a) and score plot (b) for the second (PC2) and third (PC3) principal components for olive leaf spectra.



*More information is displayed in Table 1.

Fig. 3 - Calibration and validation sets structure for the RWC.

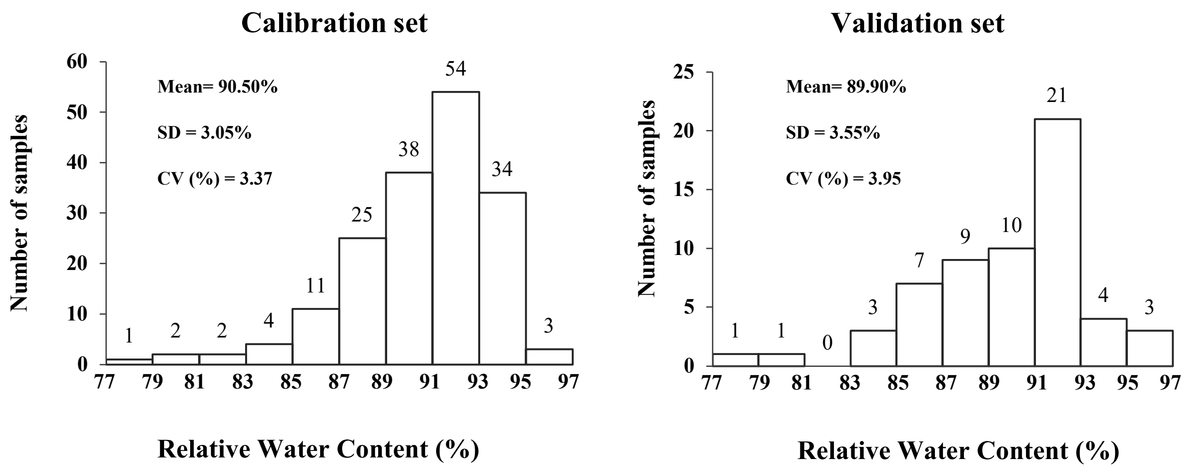


Fig. 4 – Regression coefficients for the RWC predictive model.

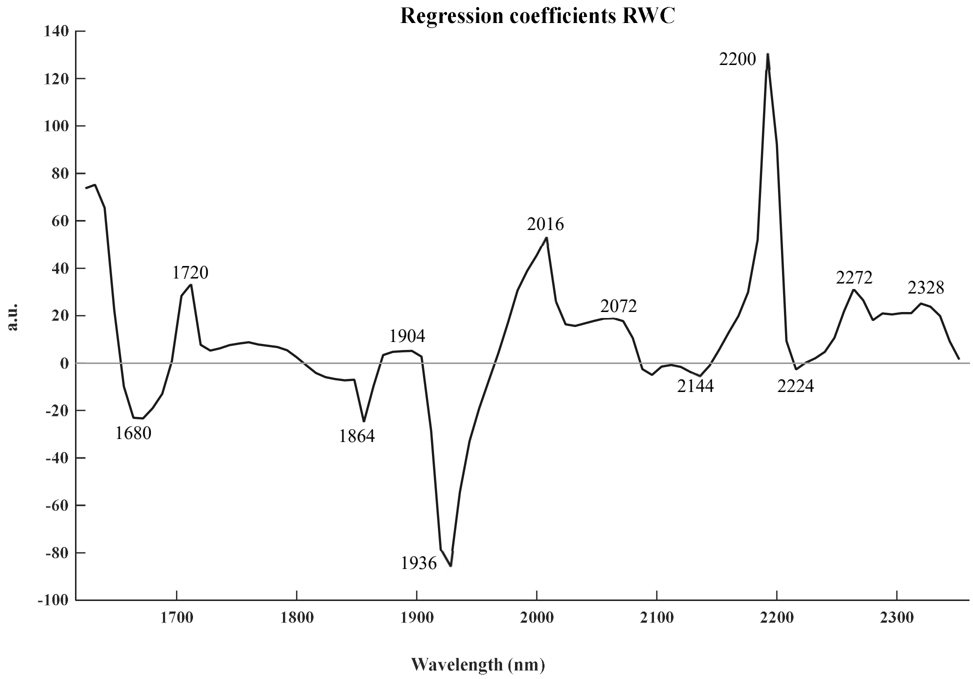


Fig. 5 - Reference vs. NIR predicted data for the validation set.