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Abstract: In this study the estimation of reflectivity at 1730 MHz (L-band), measured with a microwave digital cordless telephony (DCT) patch antenna, is presented as an easy-to-handle and non-destructive new method to assess the relative water content (RWC) of poplar leaves and filter discs at different levels of dehydration. The accuracy of this new method has been contrasted with the R1300/R1450 index, determined by a portable near infrared (NIR) spectrometer. The close correlations found between RWC and reflectance at a frequency of 1730 MHz, both for filters and leaves, indicate that microwave determinations are rather independent of the physical properties of the material analysed. On the contrary, the differences found between poplar leaves and leaf filters in the relationships established between RWC and the R1300/R1450 index demonstrate a strong influence of the properties of the material in NIR reflectance measurements, specifically as they relate to changes in leaf thickness during dehydration. Subsequently, the absence of changes in the R1300/R1450 index for poplar leaves above turgor loss point prevented its use for the estimation of leaf RWC above this point. Moreover, R-square coefficients were higher for microwaves than for the R1300/R1450 index and data obtained using the microwave technique had not to be corrected in relation to leaf thickness, which is one of the main advantages of this technique versus NIR reflectance. The use of a technologically simple, low cost and portable device, based on a microwave DCT patch antenna, could yield a solid support for the development of a commercial apparatus enabling the determination of plant water status under field conditions.

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Dear Sir/Madam:

In this study we present a novel technique for estimating leaf relative water content. This technique is based on the measurement of the reflectivity at 1730 MHz (L-band), using a microwave digital cordless telephony (DCT) patch antenna. Our aim is to develop a commercial portable tool for the determination of plant water status under field conditions. For this reason, the patent of our device is currently in full process.

Thank you very much in advance.

Microwave L-band (1730 MHz) accurately estimates the relative water content in poplar leaves. A comparison with a near infrared water index (R_{1300}/R_{1450})

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Abstract

In this study the estimation of reflectivity at 1730 MHz (L-band), measured with a microwave digital cordless telephony (DCT) patch antenna, is presented as an easy-to-handle and non-destructive new method to assess the relative water content (RWC) of poplar leaves and filter discs at different levels of dehydration. The accuracy of this new method has been contrasted with the R_{1300}/R_{1450} index, determined by a portable near infrared (NIR) spectrometer. The close correlations found between RWC and reflectance at a frequency of 1730 MHz, both for filters and leaves, indicate that microwave determinations are rather independent of the physical properties of the material analysed. On the contrary, the differences found between poplar leaves and leaf filters in the relationships established between RWC and the R_{1300}/R_{1450} index demonstrate a strong influence of the properties of the material in NIR reflectance measurements, specifically as they relate to changes in leaf thickness during dehydration. Subsequently, the absence of changes in the R_{1300}/R_{1450} index for poplar leaves above turgor loss point prevented its use for the estimation of leaf RWC above this point. Moreover, R-square coefficients were higher for microwaves than for the R_{1300}/R_{1450} index and data obtained using the microwave technique had not to be corrected in relation to leaf thickness, which is one of the main advantages of this technique versus NIR reflectance. The use of a technologically simple, low cost and portable device, based on a microwave DCT patch antenna, could yield a solid support for the development of a commercial apparatus enabling the determination of plant water status under field conditions.

Key words: L-band, microwaves, near infrared reflectance, plant water status, poplar clones

Abbreviations: DCT, digital cordless telephony; NIR, near infrared; PLT, percentage of loss of thickness; R, reflectance; RWC, relative water content.

1. Introduction

Poplars can be considered one of the main agro-forestry resources for biomass production (Meiresonne et al., 1999) and carbon sequestration (McKenney et al., 2004). Specifically, these species have been widely planted and cultivated in southern Europe (Sixto et al., 2007). The existence of atmospheric drought prevailing during the vegetative period in many arid or semi-arid regions of the world, increase significantly the rate of water consumption by the plant (Tognetti et al., 2009). Therefore, many commercial tree plantations are frequently irrigated in order to maintain the physiological activity and achieve a good production (Migliavacca et al., 2009). To prevent excessive water consumption in a global scene of decreased water availability, several methods has been developed to maximize the water use efficiency in agricultural and horticultural crops, highlighting the need for new methods of accurate irrigation scheduling and control (Jones, 2004). It has been suggested that the use of plant “stress sensing“ including both water status and plant response measurements are the main approaches to implement adequate irrigation scheduling, rather than only estimating the soil moisture status directly (Jones, 1990a, 2004, 2007).

Assessment of plant water status can be achieved via measuring the energy status (e.g., water potential) or by monitoring the amount of water (i.e., relative water content) (Jones, 2007). On the other hand, direct estimation of plant stress sensing can be carried out by evaluating physiological parameters based on stomatal closure, such as by porometry (Vilagrosa et al., 2003), by thermal imaging (Grant et al., 2006; Suarez et al. 2009), or by assessing the development of energy dissipation mechanisms which induce spectral reflectance changes around the green part of the spectrum (Peguero-Pina et al., 2008; Suarez et al. 2009).

Alternative methods for plant stress sensing based on the response of the plant material to a certain stimulus have also been proposed, since the physical properties of plant tissues have been found to vary according to the degree of hydration. In this way, Gómez Álvarez-Arenas et al. (2009) and Sancho-Knapik et al. (2010) suggested that ultrasound resonances are sensitive to leaf microstructure and water content, and they provided evidence that changes in leaf relative water content and water potential can be accurately estimated by the corresponding changes in the acoustic properties of the leaf.

A more classical approach is based on the study of near infrared reflectance (NIR) and, specially, the so-called water bands. Carter (1991) and Carter and McCain (1993) found that a decrease in leaf water content was generally associated with an increase in reflectance throughout the 400 to 2500 nm wavelength range spectrum. Since then, several authors employed different techniques concerning infrared frequencies (e.g. Peñuelas et al., 1993; Seelig et al., 2008a, 2008b; Sims and Gamon, 2003). More recently, Wu et al. (2009) used normalized indices based on reflectance (R) at 1200, 1450 and 1950 nm and related them to the vegetation water content at the leaf scale. Following a similar procedure, Seelig et al. (2009) obtained a correlation for the ratio of reflectances between 1300 and 1450 nm (R_{1300}/R_{1450} index) and the relative water content (RWC) of cowpea and bean leaves. Although Seelig et al. (2009) concluded that the R_{1300}/R_{1450} index may be used as feedback-signal in precision irrigation control, they pointed out that IR reflectances are influenced by leaf thickness, which implies that this should be taken into account during the measurements. Recent advances in infrared technologies may lead to the widespread of this technique as a tool to measure leaf water status. Stemming from the complex and fairly expensive equipment mainly designed for laboratory use, nowadays there are available some portable and more affordable models that allow working at same range under field conditions (Zimmer et al., 2004).

Lower electromagnetic frequency ranges for measuring plant water status have also been tested (Jördens et al. 2009). A time domain reflectometry (TDR) method to estimate leaf disk water status was implemented by Martínez et al. (1995). Measurements were carried out on the X-band (7 to 12 GHz) using a complex and expensive laboratory equipment. The low dynamic margin of an oscilloscope may limit the application of the method, since lower magnitudes cannot be accurately determined. More recently, Menzel et al. (2009) described a non-invasive technique based on measuring dielectric properties changes in a microwave cavity resonator induced by the plant material inserted inside the system. Such method enabled measuring the water content of the whole plant, but it involved certain drawbacks associated with the complexity of the experimental set up and its low portability, which disable this technique to measure dynamic changes in single leaves and to be used under field conditions.

In spite of the good results obtained by Martínez et al. (1995) and Menzel et al. (2009) relating to the use of microwaves to estimate plant water status, the complexity of the experimental set up proposed prevents the applicability of such methods for the development of practical tools to characterize plant water status under field conditions. Therefore, the main objective of this study was to combine the potential effectiveness of the frequency range of microwaves in a technologically simple and portable device. For this purpose a microwave digital cordless telephony (DCT) patch antenna, commonly used in mobile phone technology has been employed to measure the reflectivity at a frequency of 1730 MHz (L-band) of poplar leaves at different RWC. As a second objective the accuracy of the new method was compared with the R_{1300}/R_{1450} index for the same materials, as measured by a portable NIR spectrometer.

2. Materials and methods

2.1. Plant material

Measurements were performed on mature *Populus x euramericana* (Dode.) Guinier leaves. In the early morning, branches were collected from the north side of the trees, placed in plastic bags and carried out to the laboratory. Once there, leaf petioles were re-cut under water to avoid embolism and kept immersed until full leaf rehydration during 24 hours at 4º C. Special care was taken to prevent leaf oversaturation, by detecting eventual water outflow from the sample when water potential (Ψ) was equal to zero (Kubiske and Abrams 1991). After rehydration, one set of ten leaves was destined for the measurement with the microwave technique and other set of ten leaves used for NIR measurements. Leaves were weighed and measured at constant time intervals at different levels of RWC, starting at full saturation (turgid weight, TW). Leaf dry weight (DW) was estimated after keeping the plant material in a stove (24h, 60ºC). The RWC was then calculated following the expression: RWC = (FW-DW)/(TW-DW), being FW the sample fresh weight at any moment.

In addition, all the measurements described in this study were also performed on filter papers (homogeneous cellulosic material; Whatman 3; diameter 125mm; thickness 0.39mm). Three millilitres of DI water were added to each filter in order to achieve a full water saturation state. Immediately after, filters were introduced in a water-saturated atmosphere at room temperature. Twenty four hours later, one set was measured by the microwave technique while the other was assessed by IR following exactly the same procedure as described above for plant leaves. The disc weight after saturation (SW), the disc weight at any moment (FW) and the overdried disc weight (after 24h, 60ºC) were used for the calculation of a RWC equivalent to that calculated for leaves.

2.2 The microwave digital cordless telephony (DCT) patch antenna technique: Experimental set-up and procedure

The set up consisted of a microwave generator (oscillator) which injected its output in the antenna through a splitter device (3 dB hybrid coupler). The splitter allows to obtain at its output two identical signals in power (half of the input power), but with a phase difference of 90° (Fig. 1). The following basic properties of this splitter must be taken into consideration:

- The generator is always loaded with the same impedance what means it always delivers the same power to the splitter-antenna set.
- Power injected into the antenna through the two output branches of the splitter is ideally radiated (ideal situation in which the antenna is perfectly matched to the splitter) using a 50 Ω reference impedance.
- Possible power reflections of the antenna back to the splitter in ports 2 and 3 (Fig.1) (due to a defective design or, in our case, due to a dielectric material over the antenna) are fully routed to port 4, what allows to detect it in a quantitative manner, for instance with a rectifier diode.

In practice, elements inside the dashed box (Fig. 1) are an integral part of a typical microwave instrument: a vector network analyzer (VNA; E8364A 45MHz – 50GHz, PNA Series, Agilent Technologies Inc., Santa Clara, USA). This instrument injects microwave energy into port 1 and allows to simultaneously measure both the power reflected in port 1 (splitter matching) and the power flowing in port 4 (power reflected by the antenna). In a first calibration process, it was checked that the antenna was working properly at the desired frequency which implied that both powers must be low enough (i.e., the generator delivers its power and there is no reflection).

Water containing samples covered by a polystyrene layer were placed on top of the microwave patch antenna. When a strange media is introduced between the antenna and the surrounding air, it is expected to modify the matching conditions of the ideal antenna. The antenna is subsequently no longer well matched so part of the incident power is reflected back to the port 4 where it is measured.

2.3. Measured parameters

The R_{1300}/R_{1450} index (R_{1300}/R_{1450}), which was used as NIR parameter, measures the proportion of light that is reflected from leaves, i.e., leaf reflectance at the spectral region around 1450 nm with respect to the spectral region at approximately 1300 nm (Seelig et al., 2009). These reflectances were obtained by measuring the spectral reflectance region from 930 to 1690 nm using a Polychromix DTS NIR Spectrometer (Polychromix, USA). On the other hand, the parameter R_{1730}, which was determined by the microwave DCT patch antenna technique, is the reflectance coefficient at 1730 MHz, frequency commonly used in DCT antennae (see the experimental set-up described above for details).

The possible influence of the sample thickness on the R_{1300}/R_{1450} index (Seelig et al. 2009), both in leaves and filter discs, was estimated by measuring the thickness through the dessication process by using a digital contact sensor GT-H10L coupled to an amplifier GT-75AP (GT Series, Keyence Corporation, Japan). This ultra-low force sensor (having a measuring force of 0.2 N when installed facing up) applies a clamp pressure of 7 kPa, which is ca. 10 times lower than the one used by Zimmermann et al. (2008) for similar purposes. Thereby, it is ensured that leaf thickness measurements were not disturbed due to an excess of pressure over the leaf. Leaf thickness was standardized calculating the percentage loss of thickness (PLT) per sample, expressed as the ratio between the thickness measured for every particular RWC and the thickness determined at full turgor.

2.4. Statistical analysis

For leaves and filters, RWC was plotted both against R_{1300}/R_{1450} and R_{1730} values. Data were fitted to models and regression analyses were performed with the statistical programme SAS version 8.0 (SAS, Cary, NC, USA). The correction with the leaf thickness (Seelig et al., 2009) was made by multiplying the R_{1300}/R_{1450} of each value by the PLT obtained from the relation between RWC and PLT.

3. Results

In Figure 2 data concerning the relationships between RWC against NIR R_{1300}/R_{1450} (Fig. 2A) and against microwave R_{1730} (Fig. 2B) for the filter paper discs are presented. Data were fitted to an exponential model for the filters measured by NIR and to a linear model for the results derived from the microwave technique.

Values from poplar leaves measured by NIR were fitted to a linear segmented model because it explained the variability of the measured data better than a simple model (Fig. 3A). On the other hand, those values obtained by the microwave technique were subjected to a linear model (Fig. 3B). The segmented model in Fig. 3A is a non-linear model that fit a curve compound of two lineal models with different slopes. The point at which the switch between the two functions occurs is called a joint-point (Schabenberger and Pierce, 2002). In this case the RWC value of the joint-point calculated was 0.917 ± 0.022. From this value to a value of RWC = 1, the slope of the lineal model was approximately zero while the slope of the other lineal model (from RWC 0.917 to 0.250) was different to zero.

Figure 4 shows the relationship between RWC and the PLT of samples (either leaves or filter paper discs). Both for filter papers and leaves, it was observed that the PLT decreased as a result of a decrease in RWC. For filter papers, the PLT decreased linearly ranging from 1 to 0.9 for a decrease in RWC between 1 to approximately 0.25. However, such correlation was not linear for leaves, being adjusted to a polynomial square function which enabled the estimation of PLT values resulted from the RWC-R_{1300}/R_{1450} relationship. For the same RWC range indicated above, the decrease in PLT varied between 1 and 0.72.

The relationship between RWC-R_{1300}/R_{1450} corrected by the PLT is shown in Figure 5. In this case and similar to the results obtained for non-corrected filter papers (Fig. 2A), the correlation obtained was better adjusted by an exponential model. Linear models for the near infrared RWC-R_{1300}/R_{1450} relationship indicated that the residuals were heterocedastic, while the exponential models presented homocedastic residuals (data not shown). In addition, the values corrected by the PLT of R_{1300}/R_{1450} for filter paper discs did not differ significantly from the raw data (data not shown).

To complement such information the regression coefficients of the relationships established for filter papers and leaves are presented in Table 1. For filters, the R-squared (R^2) obtained for NIR is 0.96 ($p<0.0001$) (Fig. 2A), while the resulting R^2 for the data recorded by the microwave technique (Fig. 2B) is 0.99 ($p<0.0001$). For leaves, the R^2 recorded by NIR once data was corrected by the thickness (Fig. 5), was 0.96 ($p<0.0001$) while the R^2 for the data recorded by the microwave method (Fig. 3B) was 0.98 ($p<0.0001$). In addition, results indicate that for the microwave technique higher F-ratios and lower standard errors (SE) of the estimation were recorded in contrast to the NIR method.

4. Discussion

In this investigation, an easy-to-handle and non-destructive microwave-based technique to estimate the water content of a material was developed, with special regard to plant leaves. An experimental set up chiefly composed by a standard DTC antenna and a network vector analyser was used to estimate the water content of an homogeneous (filter paper discs) and an heterogeneous (*Populus x euramericana* leaves) material, contrasting the results with those obtained after applying the R_{1300}/R_{1450} index.

The method assessed the dielectric behaviour of the two materials analysed and established very good correlations between RWC and reflectance at a frequency of 1730 MHz, both for filters and leaves. Although leaves are more heterogeneous than filters, the accuracy of microwaves for the estimation of RWC was almost the same for both materials (Table 1). The small differences found could be attributable to the irregular structure of plant leaf, in contrast to the structural homogeneity of the commercial filters used in this study. In spite of this fact, the technique may be considered quite independent of the physical properties of the material.

The frequency employed in this investigation is included in the L-band of the microwave spectrum, which has been previously used for remote sensing studies of the vegetation biomass and soil-moisture content (Ferrazzoli et al., 1992), and more recently for the study of vegetation water content at field level (Notarnicola and Posa, 2007). In contrast, this study proved an accurate determination of plant water status at leaf level, using a more simple methodology than the one described by Martínez et al. (1995) and Menzel et al. (2009), for plant leaves and shoots respectively. The use of this simple device has been possible due to recent advances in telecommunications technology, especially as it related to the development of the digital cordless telephony (DCT). Consequently, it is possible that a commercial portable tool for the determination of plant water status under field conditions based on our device could be developed and its patent is currently in full process.

On the other hand, the accuracy of this new method against the near infrared R_{1300}/R_{1450} index was compared in this investigation. It should be noted that the absence of changes in this index at leaf RWC values above the join point of the segmented model (Fig. 3A) prevents its use for the estimation of leaf RWC above this point. To solve this problem, Seelig et al. (2009), which stated that leaf reflectance is partly a function of the thickness and number of cell walls encountered by light on its path through leaves, proposed the correction of the R_{1300}/R_{1450} index taking into account the strong changes in thickness during leaf dehydration. The multiplication of this index by the PLT enabled to obtain a relationship between leaf RWC and the R_{1300}/R_{1450} index (Fig. 5) very similar to that found for filters (Fig. 2A).

Although both techniques can be used for the accurate estimation of leaf RWC, the microwave-based procedure show a certain number of advantages. First of all, R-square coefficients were slightly higher for microwaves than for the R_{1300}/R_{1450} index (Table 1). Furthermore, it is not necessary to correct the measured data by leaf thickness, which is one of the main advantages of this technique in relation to near infrared reflectance. For this reason, although near infrared reflectance might be used for plant physiological studies under field conditions, it can also be suitable to assess plant materials under an industrial point of view (e.g. quality control). On the other hand, the amount of energy received by the leaf is only 0.1 mW for the microwave technique, which is very much lower than that applied by the spectrometer used in this study for the measuring of the R_{1300}/R_{1450} index (2.5 W). Therefore, although both procedures are practically non-invasive, the microwave technique can be considered much less invasive than the near infrared reflectance.

5. Conclusions

In this study it has been shown that the changes in RWC of poplar leaves can be accurately determined by assessing changes in reflectivity at a frequency of 1730 MHz (L-band). The use of a technologically simple, low cost and portable device, based on a microwave digital cordless telephony (DCT) patch antenna, could yield a solid support for the development of a commercial portable tool for the determination of plant water status under field conditions in the future. This new technique, although tested on a specific poplar clone, can be also applied to other poplar clones and tree species with similar leaf size dimensions. The use of microwaves to estimate changes in leaf water status in species with smaller leaves would imply the application of other frequency ranges, which demands the development of different types of antennas.

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Tables

Table 1. Statistical parameters of the relationships between the relative water content and the Near Infrared (NIR) or Microwave, either for filter papers and leaves. *n* is the number of data points observed; *F-ratio* is the ratio of the variance explained by a factor to the unexplained variance ; R^2 is the R-squared; *S.E. of Est*. is the standard error of the estimation.

	Filter papers		Poplar leaves		
	NIR	Microwave	NIR	NIR corrected	Microwave
n	87	81	137	137	124
F-ratio	2289	14703	728	4195	7548
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
R^2	0.9642	0.9951	0.9426	0.9675	0.9849
S.E. of Est.	0.03253	0.00651	0.0219	0.05028	0.00394

Figure legends

Fig. 1. Schematic representation of the experimental set-up of the microwave patch antenna technique.

Fig. 2. Relationships between the relative water content (RWC) and (**A**) the R_{1300}/R_{1450} index for NIR and (**B**) R_{1730} for microwaves measured on filter paper discs.

Fig. 3. Relationships between the relative water content (RWC) and (**A**) the R_{1300}/R_{1450} index for NIR and (**B**) R_{1730} for microwaves measured on poplar leaves.

Fig. 4. Relationship between the relative water content (RWC) and the percentage loss of thickness (PLT) for filter papers and poplar leaves.

Fig. 5. Relationship between the relative water content (RWC) and R_{1300}/R_{1450} index corrected with the percentage loss of thickness (PLT) for poplar leaves.

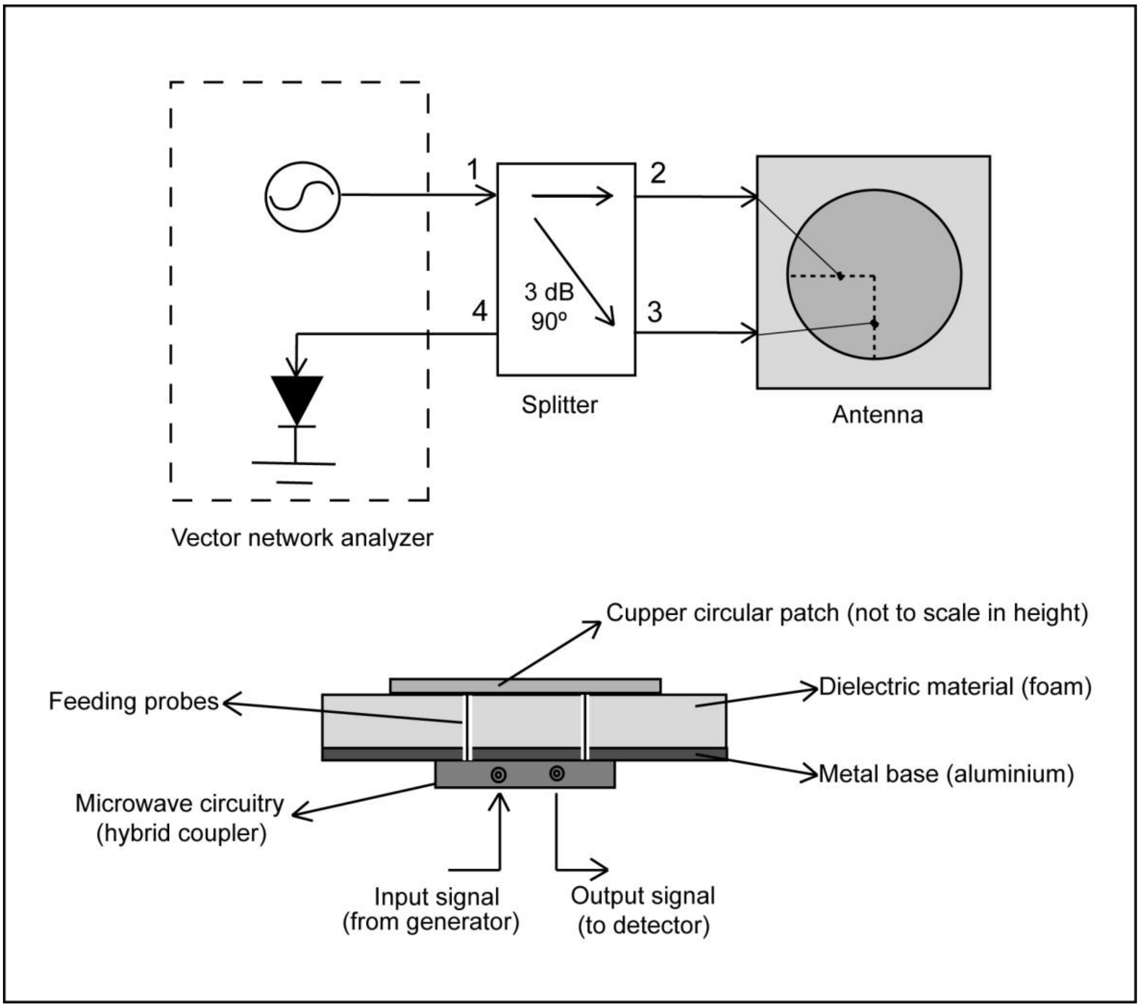
1
2
3 dB
90º
4
3
Splitter
Antenna
Vector network analyzer
Cupper circular patch (not to scale in height)
Feeding probes
Dielectric material (foam)
Metal base (aluminium)
Microwave circuitry
(hybrid coupler)
Input signal
(from generator)
Output signal
(to detector)

Fig. 1

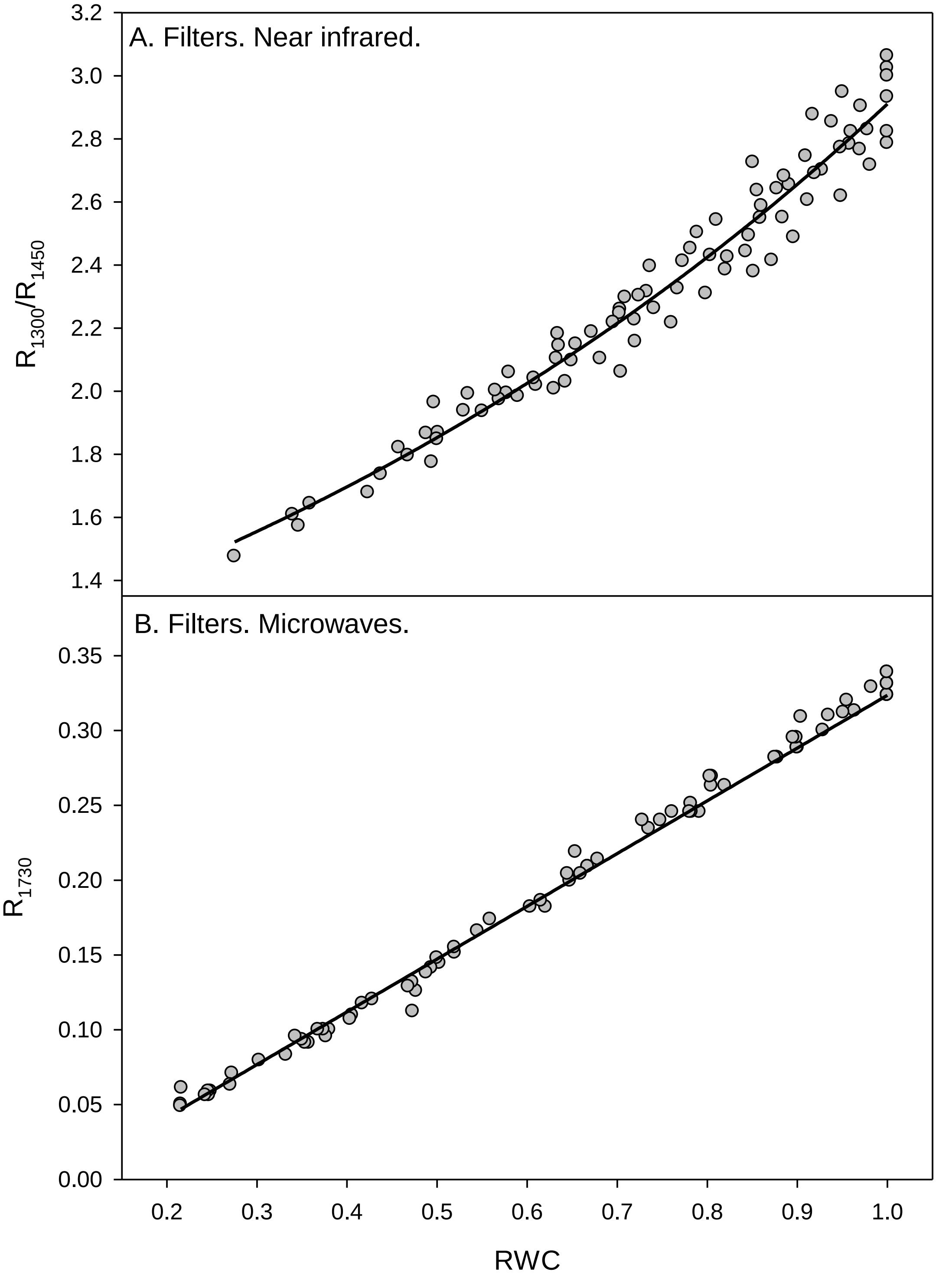
A. Filters. Near infrared.
B. Filters. Microwaves.
R1300/R1450
R1730
RWC
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Fig. 2

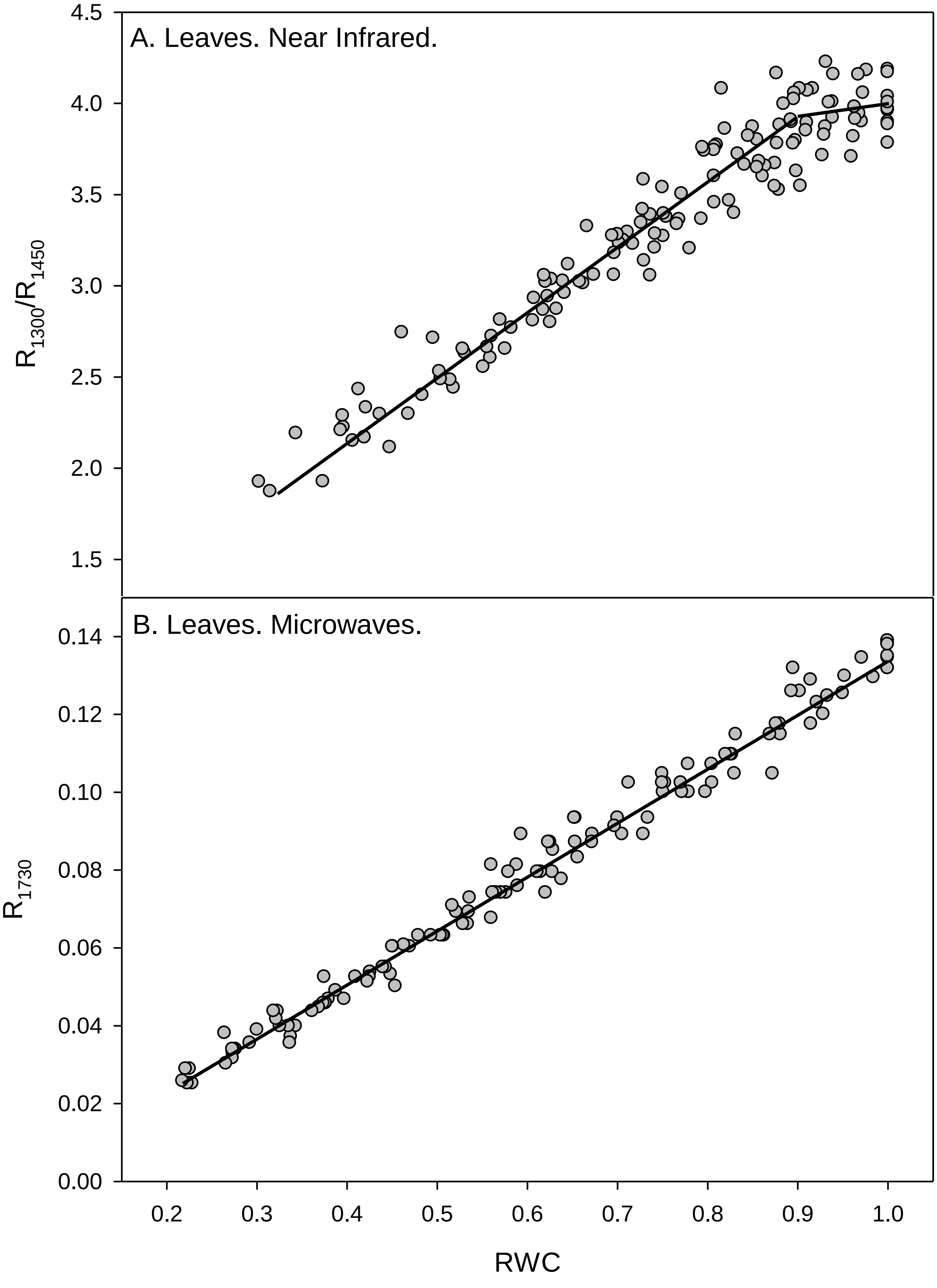
A. Leaves. Near Infrared.
B. Leaves. Microwaves.
R_{1300}/R_{1450}
R_{1730}
RWC
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Fig. 3

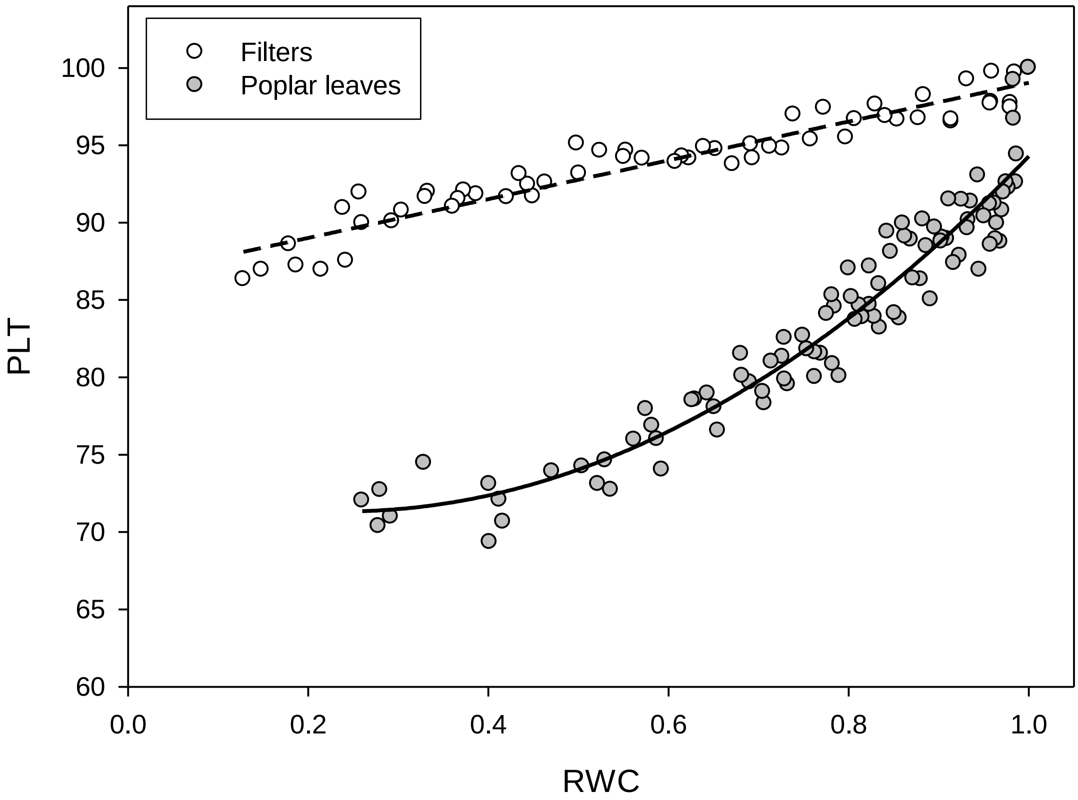
Filters
Poplar leaves
PLT
RWC
100
95
90
85
80
75
70
65
60
0.0
0.2
0.4
0.6
0.8
1.0

Fig 4

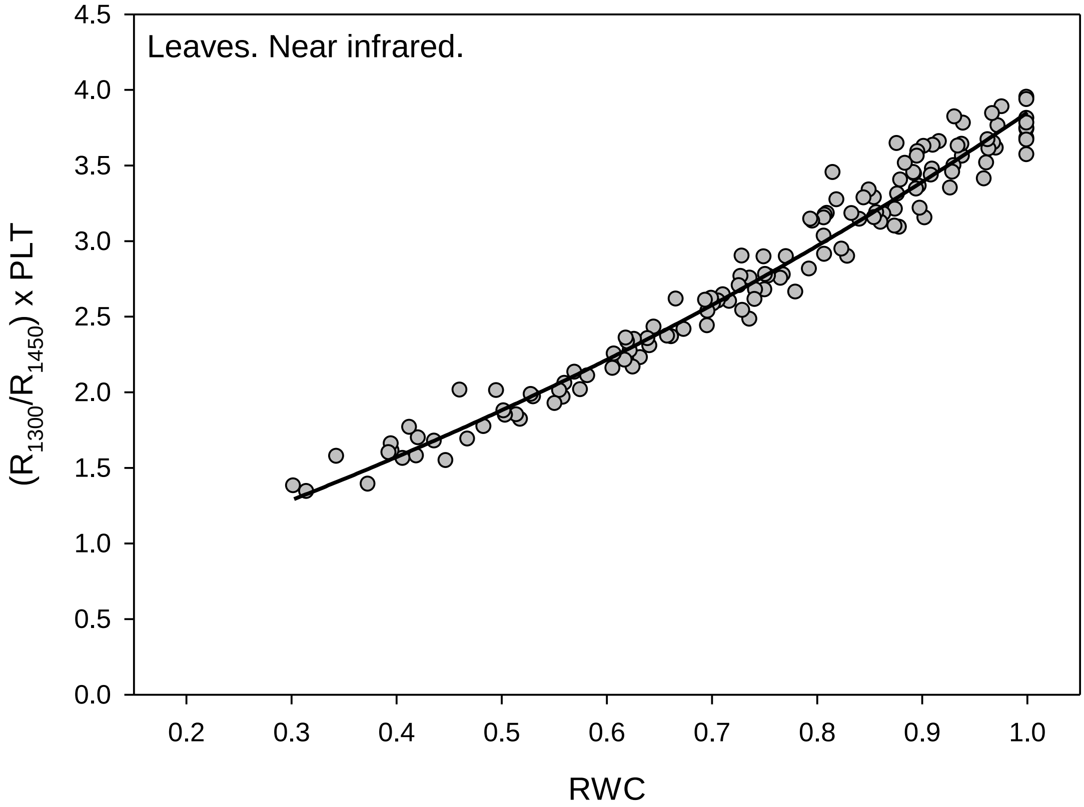
Leaves. Near infrared.
(R_{1300}/R_{1450}) x PLT
RWC
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Fig 5