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# Changes in thermal infrared spectra of plants caused by temperature and water stress



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## ABSTRACT

Environmental stress causes changes in leaves and the structure of plants. Although physiological adaptations to stress by plants have been explored, the effect of stress on the spectral properties in the thermal part of the electromagnetic spectrum (3–16  $\mu\text{m}$ ) has not yet been investigated.

In this research two plant species (European beech, *Fagus sylvatica* and rhododendron, *Rhododendron cf. catawbiense*) that both grow naturally under temperature limited conditions were selected, representing deciduous and evergreen plants respectively. Besides TIR spectra, Leaf Water Content (LWC) and cuticle thickness were measured as possible variables that can explain the changes in TIR spectra.

The results demonstrated that both species, when exposed to either water or temperature stress, showed significant changes in their TIR spectra. The changes in TIR in response to stress were similar within a species, regardless of the stress imposed on them. However, changes in TIR spectra differed between species. For rhododendron emissivity in TIR increased under stress while for beech it decreased. Both species showed depletion of Leaf Water Content (LWC) under stress, ruling LWC out as a main cause for the change in the TIR spectra. Cuticle thickness remained constant for beech, but increased for rhododendron. This suggests that changes in emissivity may be linked to changes in the cuticle thickness and possibly the structure of cuticle. It is known that spectral changes in this region have a close connection with microstructure and biochemistry of leaves. We propose detailed measurements of these changes in the cuticle to analyze the effect of microstructure on TIR spectra.

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## 1. Introduction

### 1.1. Plant stress

Stress in plants can be generated from biotic and abiotic factors such as living organisms (bacteria, viruses and parasites), climatic conditions or natural disasters (Rhodes and Nadolska-Orczyk, 2001). Changing climatic conditions such as availability of water, irradiation or extreme temperatures, can create stressing conditions that change the normal growth of the plant and the canopy (Levitt, 1980). There are many known mechanisms to cope with stress, which can be grouped into two classes: (a) fast biochemical responses, followed by (b) microstructural re-arrangement of the

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cellular space and therefore the leaf structure, in the case of long duration stress or permanent stressors (Levitt, 1980; Rhodes and Nadolska-Orczyk, 2001). Under long duration stress, plants may change not just the microstructure but also the community physiognomy. Long duration and extreme stress conditions such as extreme temperature and water availability are well known evolutionary drivers of phytogeographical diversity and speciation, especially in alpine and desert environments (Charrier et al., 2014; Nevo, 2011).

### 1.2. Stress detection

Detecting stressors and their impact on vegetation are of importance for agriculture and for ecosystem conservation. Conventional methods of stress detection are based on the observation of physical changes, e.g. color or turgidity, with the risk of being detected only after a critical point of damage has been reached. Other methods focus on detecting early stress symptoms using biochemical and biophysical techniques such as leaf water potential and stomatal conductance (e.g. Hand et al., 1982; Hura et al., 2007; O'Toole

and Cruz, 1980; Zörb et al., 2004) or tracking changes in leaves and canopy temperature or Leaf Water Content (LWC) with thermal cameras and infrared sensors (e.g. Chaerle and Van Der Straeten, 2000; Costa et al., 2013; Mahlein et al., 2012; Oerke and Steiner, 2010). These techniques are time consuming, expensive and not applicable to the large areas observations.

### 1.3. Remote sensing

Remote sensing (RS) provides methods for early detection of plant stress based on changes in the reflection of different regions of the electromagnetic spectrum. The early availability of multi-spectral bands from air and spaceborne sensors promoted the development of spectral indices in the Visible (VIS), Near Infrared (NIR) and the Short Wave Infrared (SWIR) parts of the spectrum. More recently, the development of new technologies and remote hyperspectral sensors, have promoted the development of a new generation of more accurate hyperspectral indices which can detect a high variety of stressors. The electromagnetic spectrum can be divided in regions according to the interaction between energy and leaves, especially with the outer surface of leaves and their composition. The VIS–NIR is known for the strong interaction of light with pigment concentrations in the VIS and the strong reflectance and transmittance by the leaf in the NIR, which can be analyzed with different methods. The ultraviolet induced fluorescence in the VIS–NIR is used to detect the concentration of antioxidants and Reactive Oxygen Species (ROS), which are the initial responses to water and temperature stress (Chaerle et al., 2007; Frohnmeyer and Staiger, 2003). The VIS–NIR fluorescence can additionally be used to detect changes in pigments as a response to the reduction in the photosynthetic activity (Belasque et al., 2008; Lang et al., 1996; Lichtenthaler, 1996; Zarco-Tejada et al., 2009, 2002). Reflectance methods in the VIS–NIR allow the detection of leaf pigments, and their variations due to stress conditions (e.g. Carter, 1993; Cozzolino, 2014; Dobrowski et al., 2005; Eitel et al., 2006; Gao, 1996; Peñuelas and Filella, 1998; Peñuelas et al., 1997; Seelig et al., 2008). Changes in other biochemicals can also be detected in the VIS–NIR such as nitrogen, lignin and cellulose (Kokaly and Clark, 1999; Li et al., 2007; Martin and Aber, 1997; Serrano et al., 2002). The strong absorption features of water in the SWIR have been widely used for assessing water stress, by formulating water stress indices (e.g. Ceccato et al., 2002; Ceccato et al., 2001; Eitel et al., 2006; Feng et al., 2013; Fensholt and Sandholt, 2003; Seelig et al., 2008). While the Thermal Infrared (Karlson et al., 2004) range is conventionally used for detecting early signs of stress by observing canopy temperatures (e.g. Calderón et al., 2013; François et al., 1997; Grant et al., 2007; Jones et al., 2009; Leinonen and Jones, 2004).

### 1.4. Thermal infrared

TIR is the region of the electromagnetic spectrum where the radiation emitted by objects due to their thermal state is more intense than the reflected solar radiation (Prakash, 2000). Sensors working in this region detect mainly the long-wave radiation of materials. The most explored regions in the TIR are the Mid-Wave Infrared (MWIR: 3–6  $\mu\text{m}$ ) and the Long-Wave Infrared (LWIR: 6–16  $\mu\text{m}$ ) which contain relevant information for the analysis of plant properties (Ribeiro da Luz and Crowley, 2007; Ullah et al., 2012b). Longer wavelengths, above 16  $\mu\text{m}$ , have not been used for plant studies.

Hyperspectral variations in the TIR region have so far been studied mainly for geological purposes, due to the strong spectral responses of minerals in this region (Van der Meer et al., 2012). Other scientific fields such as ecology and plant science have used this region less due to a lack of sufficiently accurate laboratory and

field instruments to detect the subtle spectral differences in the TIR that can be found in plants (Ribeiro da Luz and Crowley, 2007). Nevertheless, in the last decades the accessibility to new laboratory and field work equipment, as well as new airborne and satellite sensors with hyperspectral TIR detection capacity, is creating a need for further research in this field.

While the VIS, NIR and SWIR are dominated by the reflectance of the solar energy, longer wavelengths in the MWIR and especially the LWIR operate independently from reflected sunlight. The emissivity, i.e., the ratio of the energy radiated from a material's surface to that radiated from a blackbody (a perfect emitter) according to the Planck function at the same temperature and wavelength, is one of the most important factors in the variation of energy recorded in the TIR.

Thus emissivity is not the result of the interaction between solar energy and pigments like in shorter wavelengths, but is the effectiveness in emitting thermal radiation by the surface of the leaf. This means that the thermal radiation in the TIR can potentially contain information about the biochemistry and microstructure of single leaves and plants (Ribeiro da Luz, 2006; Ribeiro da Luz and Crowley, 2007, 2010). For instance, some researchers have found that single plant species can have a different 'spectral fingerprint' in the LWIR which could be used as a key for species identification (Ribeiro da Luz, 2006; Ribeiro da Luz and Crowley, 2010; Salisbury, 1986; Ullah et al., 2012a). Similarly some regions in the MWIR have a clear response to LWC (Ullah et al., 2012b), and the LWIR could be associated to biochemistry and microstructure of the leaves (Elvidge, 1988; Ribeiro da Luz and Crowley, 2007). This may be linked to physiological changes of plants and may be associated with responses to stressors.

Despite early efforts with exploring the TIR (e.g. Fensholt and Sandholt, 2003; Hunt and Rock, 1989; Ullah et al., 2013), the changes caused by water and temperature stress in the whole TIR has not yet been explored.

## 2. Materials and methods

### 2.1. Species

When selecting the species for this study, species which can grow up to the tree line in mountains (the line above which trees do not grow any more due to temperature limitations) were chosen, due to their differential plasticity and their capacity to cope with extreme conditions such as low temperatures and water depletion. These plants are, therefore, expected to have different mechanisms to cope with stressful conditions, such as structural changes in their leaves, when stress conditions continue for a long period (Prasad, 2001).

*Rhododendron spp.* are in general evergreen species whose leaves resist winter temperatures without senescence, and is found as a continuous tree line in Asia, Europe and America. *Fagus sylvatica* (beech) is a European deciduous species with a wide distribution in the mountains, where it forms tree lines in some localities. As a deciduous species, it loses its leaves seasonally when temperatures become colder. For this reason the low temperature treatment (see below) was not applied to beech, to avoid inducing premature senescence.

### 2.2. Experiment

A factorial experiment was established with two factors: temperature stress and water stress (Fig. 1). Temperature stress was defined as low or chilling temperatures between 1 and 10 °C, which can have a negative impact on the metabolism of plants, especially during the growing season (Bracale and Coragio, 2003;

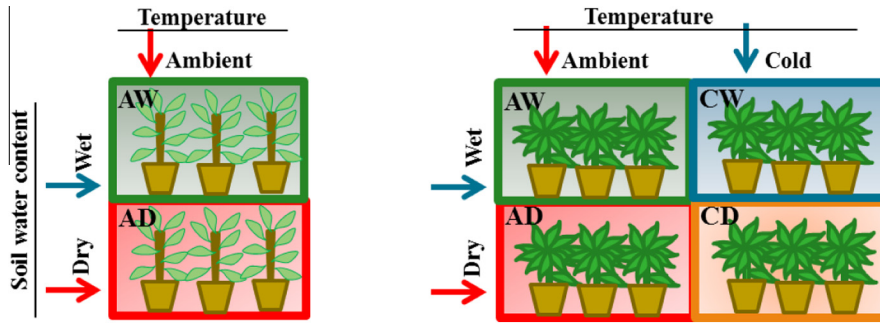


Fig. 1. Water stress experiment for beech (left) and 2 × 2 factorial design stress experiment for rhododendron (right).

Jouyban et al., 2013; Nilsen, 1987). This treatment had 2 groups: (a) ambient warm temperature (3 months outdoors in summer and 3 months warmed in the greenhouse in autumn; mean temperature: 19.1 °C), and (b) cold temperatures (3 months of inside a cooled greenhouse in summer, and 3 months of low temperatures outdoors in autumn; mean temperature: 9.9 °C). Table 1, shows the temperature variations for each period and location.

The water stress treatment (dry) was set at 20% of the field capacity. The field capacity was calculated in relation to the weight of the pot and controlled regularly by weighing the pots (Fig. 2). Plants under the ‘dry treatment’ had their pots covered with plastic to avoid additional rainfall in the pots. The control group (wet) was watered weekly up to field capacity during the experiment.

Fig. 2 shows the factors in the experiments for both species: cold and dry, cold and wet (CW), ambient and dry (AD) and, ambient and wet (AW). The beech experiment consisted only of the last two factors (AD and AW) and lasted 3 months (July–September), while the rhododendron experiment consisted of all 4 factors (water and temperature stress) and lasted 6 months.

60 healthy rhododendron plants, ranging between 45 and 60 cm in height, were evenly divided over the four treatments, and 30 healthy beech plants between 80 and 120 cm of height were evenly divided over two treatments (15 plants in each treatment). For each plant, five healthy and representative leaves of the spring cohort were marked to be measured during the experiment

(Fig. 2). These leaves were mature but growing, therefore changes may be caused by the stress imposed on them. In total, 75 leaves were measured per treatment before applying the treatments, after three months (rhododendron and beech) and after six months (rhododendron only). The experiment for beech was terminated after 3 months to avoid interference with the natural senescence of the leaves after September.

2.3. Leaf spectral measurements

All plants were measured at the University of Twente with a Bruker Vertex 70 FTIR spectrometer, adapted with an external integrating sphere. This extension allows measuring absolute directional-hemispherical reflectance, which in turn can be recalculated to emissivity using Kirchoff’s law ( $emissivity + reflectance = 1$ ), assuming that leaves behave as opaque objects and therefore don’t produce transmittance (Fabre et al., 2011; Gerber et al., 2011). For consistency reasons emissivity was calculated for MWIR and LWIR, even though MWIR is in remote sensing practice influenced by reflected as well as emitted radiance. An Infragold plate with known spectral emissivity was used to calibrate each measurement. Details on the spectrometer design and calibration procedures can be found in Hecker et al. (2011).

Each leaf was placed under the external integrating sphere. Two additional leaves were placed below the marked leaf and taped to

Table 1  
Temperatures recorded continuously during the experiment.

Treatment	Period	Location	Mean (°C)	Standard deviation (°C)	Minimum absolute (°C)	Maximum absolute (°C)	% hours below 15 °C or above 25 °C	% hours below 5 °C or above 15 °C
Ambient	July–September	Outdoors	18.1	4.8	7.0	35.0	12.0	N/A
Ambient	September–December	Greenhouse	20	1.8	17.5	21.4	0.0	N/A
Cold	July–September	Greenhouse	11.2	1.5	9.4	15.6	N/A	0.0
Cold	September–December	Outdoors	8.7	4.2	0.0	20.0	N/A	10.0



Fig. 2. Experiment settings: low temperature controlled at greenhouse (left), water control (center), and leaves marked (right).

the 3 cm entrance of the integrating sphere, to prevent any possible transmittance in the region 3.5–5.7 μm (Gerber et al., 2011).

Spectra were measured for the range 4000–600 cm<sup>-1</sup> (2.5–16.7 μm) with a resolution of 4 cm<sup>-1</sup>. Per leaf, 520 scans per sample, and eight samples in total were taken. These measurements were averaged and the results were calculated per leaf. Five leaves per plant were measured in the same way for a total of 75 leaves per treatment at every stage of the experiment. Although the spectra were measured in the wavenumber domain, their wavelengths are displayed in micrometers as is customary in the remote sensing community.

2.4. Leaf Water Content (LWC) and cuticle thickness

LWC was destructively measured at each stage of the experiment using leaves from the same cohort of the marked leaves which were used for the spectral measurements. The relative gravimetric LWC was calculated using the equation:  $LWC = 100 * (W_w - W_d) / W_w$ , where  $W_w$  is the weight of the fresh leaf,  $W_d$  is the weight of the dried leaf. Leaves were dried in an oven at 65 °C. Cuticle thickness was measured from a thin transverse section of the marked leaves, using a Leitz Wetzlar microscope, with an amplification of 250×. This trait was measured at least 3 times in each leaf and the measurements averaged and expressed in μm.

2.5. Statistical analysis

To test whether all groups of plants belong to the same population before the treatment, an ANOVA test was run on the spectra of four groups of rhododendron and a *t*-test was performed for the two groups of beech to test for significant differences.

Differences in emissivity before and after the treatment for every spectral band were analyzed with a paired Students *t*-test (six months for rhododendron, three months for beech). The

resulting spectra of *P*-values were used to highlight areas that had spectrally changed in a significant way over the course of the treatments (for *P*-values lower than 0.05).

Overall changes in emissivity were expressed as a percentage of the spectral contrast. Spectral contrast is defined here as the difference between the minimum and the maximum emissivity values over the entire measured spectrum. The spectral contrast measured in our experiment was 0.038 in the MWIR and 0.018 in the LWIR for rhododendron and 0.043 in the MWIR and 0.036 in the LWIR for beech.

3. Results

After 3 months treatment for beech, and 6 months treatments for rhododendron, plants visually changed under all stress treatment regimes, compared to the control group (ambient and wet). Leaves from plants under stressed treatments changed from dark green to a light green. Loss of turgidity of the leaves was also observed especially for the dry treatments.

3.1. Leaf water content and cuticle thickness

Through the course of the experiment period, LWC of beech decreased from 65.2% to 55.4% for the control treatment (AW) and from 64.3% to 40.7% for the dry treatment (AD) (Fig. 3). For rhododendron, LWC decreased from 67.2% to 61.8% for the control treatment (AW), and decreased in the stress treatments from 66.1% to 42.5% for ambient-dry (AD), from 65.2% to 48.3% for cold-wet (CW) and from 66.5% to 41.1% for cold-dry (Zhang et al., 2005). Cuticle thickness of each leaf measured after the treatment (Fig. 4), showed that beech leaves had similar cuticle thickness for the control (natural growth) and the stress treatment (7.2 and 7.6 μm respectively), while rhododendron developed thicker

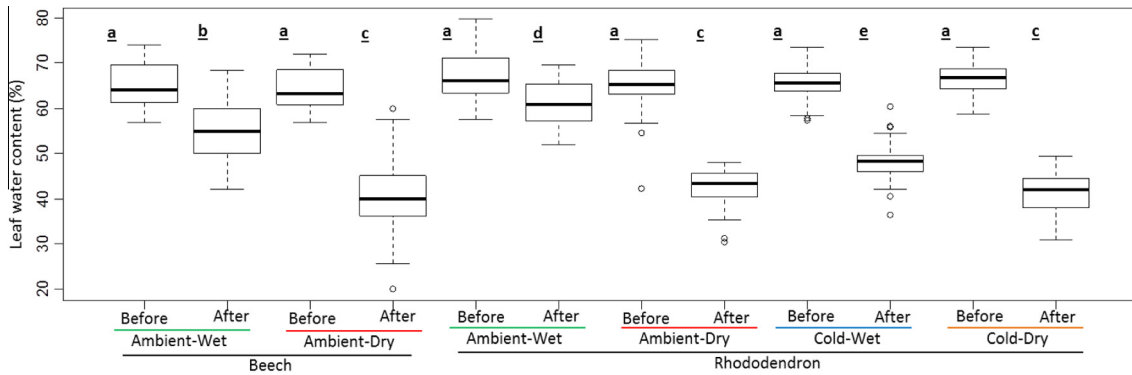


Fig. 3. Leaf water content for beech and rhododendron before and after each treatment. Same letters denote no significant differences according to an ANOVA test.

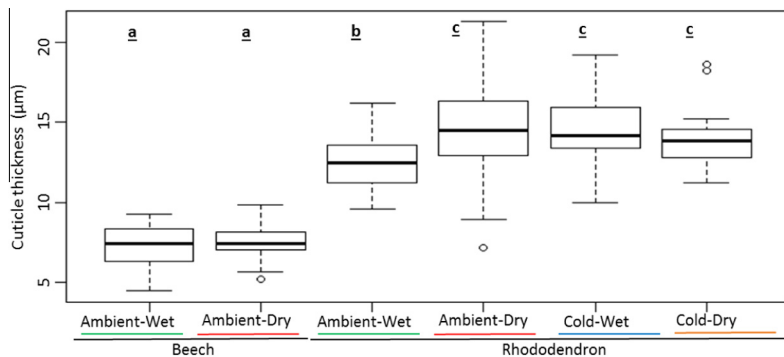


Fig. 4. Cuticle thickness for beech and rhododendron after the treatment. Same letters denote no significant differences according to an ANOVA test.

cuticles after the stress treatments compared with the control treatment (Control: 12.4  $\mu\text{m}$ , AD: 13.7  $\mu\text{m}$ , CW: 13.5  $\mu\text{m}$ , CD: 13.4  $\mu\text{m}$ ).

### 3.2. Emissivity spectra

There were no significant differences in emissivity between the plants of both species across the different treatments before the experiment started (Fig. 5). An ANOVA between these groups, composed of 75 leaves per group, confirms that for 98% of the spectral bands, there was no difference between the treatment groups of rhododendron before the beginning of the experiment. For beech a *t*-test showed that for 99% of the spectral bands there was no difference before the experiment between the two groups.

The mean spectra clearly differentiated both species, especially in the MWIR. The emissivity spectra of both species before and after the treatment showed significant differences (Figs. 6 and 7). As a general trend, after the treatments the emissivity along the spectra decreased for beech and increased for rhododendron. The atmospheric  $\text{CO}_2$  artifact that was not corrected for the measurements in the bands between 4.23 and 4.29  $\mu\text{m}$ , is still present in the results and will not be considered in the analysis.

For beech, after 3 months, the control plants (AW) showed changes in 60% of the spectral bands ( $P$ -value < 0.05) (Fig. 6). This change was more evident between 3.5 and 5.7  $\mu\text{m}$ . At wavelengths of 5.7  $\mu\text{m}$  and larger, the spectra changed less.

For the plants under dry treatment (AD) 84% of the spectral bands changed after 3 months of stress conditions. The MWIR changed similar to the control treatment, and the LWIR changed especially between 8.9  $\mu\text{m}$  and 13.7  $\mu\text{m}$ , with a significant reduction of the emissivity after the stress period. After 14  $\mu\text{m}$  the emissivity spectra were subject to more noise and the results are less reliable.

For the rhododendron control treatment (ambient temperature and wet; AW), less differences were observed between before (black line) and after 3 and 6 months (green lines) (Fig. 7a). A paired Students *T*-test showed that for 51% of the spectral bands

there were no significant differences between the time periods ( $P$ -value > 0.05, Fig. 7b).

In the ambient temperature and dry treatment (AD), the spectra for the rhododendron changed more than in the control with a general increase in emissivity, which was most pronounced between 7  $\mu\text{m}$  and 14  $\mu\text{m}$  where the emissivity also shows more variation (Fig. 7c). The  $P$ -value of the paired *T*-test showed that for 79% of the bands there is a difference between the beginning and the end of the treatment. The range 3.5–5.5  $\mu\text{m}$  changed except for the zone between 4.2 and 4.3  $\mu\text{m}$ , where  $\text{CO}_2$  artifacts can be found. The main changes in the spectra were found between 6.3 and 14.3  $\mu\text{m}$ , with an increase in emissivity (Fig. 7d).

The spectra of plants under cold and wet treatment (CW) also diverged from the beginning to the end of the experiment (Fig. 7e). 90% of the spectral bands showed a significant difference after the experiment, which was distributed along almost the whole spectra except for some small regions that didn't change (3.5–3.6, 4.2–4.3  $\mu\text{m}$ , associated to remaining atmospheric artifacts, and 5.9, 12.4–13.0 and after 14.5  $\mu\text{m}$ , Fig. 7f).

Plants under low temperatures and dry treatment showed the most conspicuous changes in their general emissivity spectra (Fig. 7g). Almost the whole spectra from 2.5 to 14.5  $\mu\text{m}$  exhibited increased emissivity. For 92% of the bands significant differences in emissivity after 6 months were found. Some bands didn't change in this period such as the region between 3.4 and 3.5  $\mu\text{m}$  affected by a  $\text{CH}_2$  feature and the region 5.8–5.9  $\mu\text{m}$  (Fig. 7h).

## 4. Discussion

As it was suggested by and Ribeiro da Luz and Crowley (2010) and Ullah et al. (2012a) the TIR spectra of leaves show a clear separation that can be used for species identification. In this study the mean spectra is different for both species, especially in the MWIR. The mean spectrum of beech is similar to the spectra measured by Salisbury and Milton (1988) on another related beech species (*Fagus grandifolia*) with particular features at 4.0, 5.3, 5.7 and

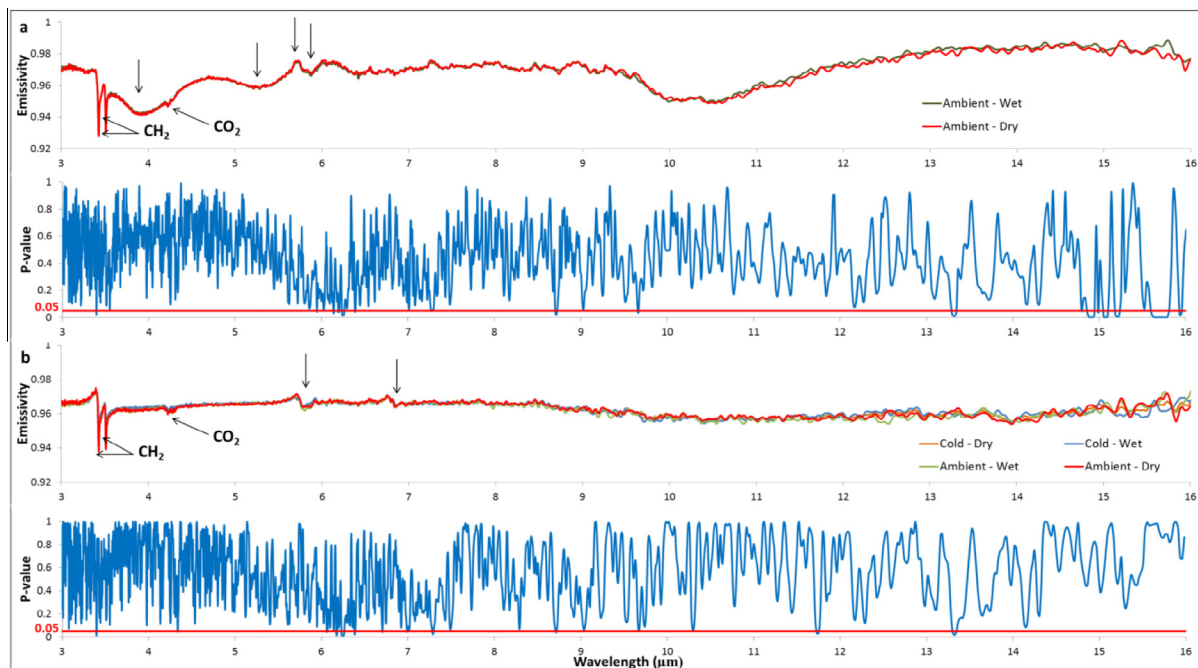
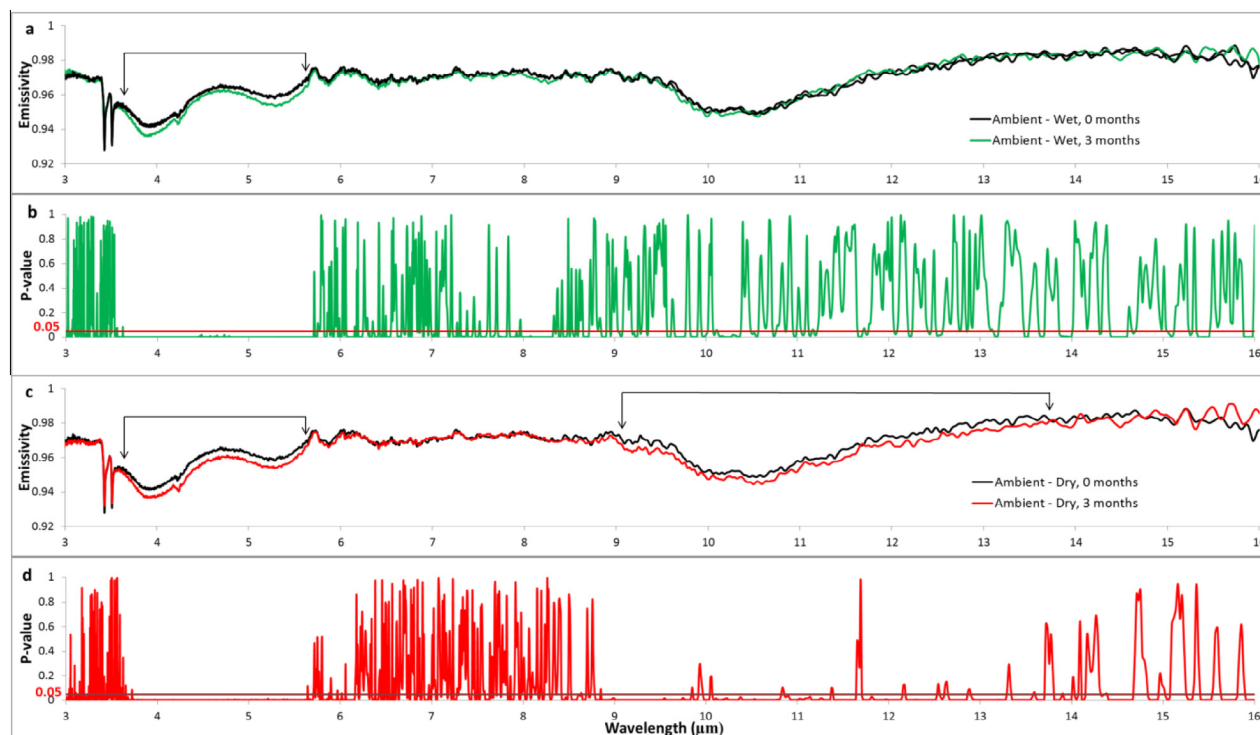


Fig. 5. Mean spectra for each group of plants before stress treatments for (a) beech and (b) rhododendron. (a)  $P$ -value in a *T*-test for beech and (b)  $P$ -value in One-way ANOVA for rhododendron. Arrows mark spectral features.



**Fig. 6.** (a and c) mean spectra for beech leaves (0 and 3 months) and *P*-value for a paired *T*-test for (b) ambient wet treatment and (d) ambient dry treatment (d). Arrows mark spectral features.

5.8  $\mu\text{m}$ . The mean spectrum of rhododendron is similar to the spectra of *Rhododendron caucasicum* measured by Ullah et al. (2012a) which exhibits a narrower variability in emissivity between 0.95 and 0.97 in the whole TIR, and has lower TIR emissivity at 5.8 and 6.8  $\mu\text{m}$ . For both species the mean spectra of the leaves (Fig. 5) showed a region affected by  $\text{CO}_2$  between 4.23 and 4.29  $\mu\text{m}$  (Elvidge, 1988), and two strong C–H symmetric stretching features are visible at 3.4  $\mu\text{m}$ , and at 3.5  $\mu\text{m}$  associated with carbon–hydrogen fundamental stretching vibrations (Salisbury and Milton, 1988).

#### 4.1. Similarities between cold and water stress

We found that both long term stressors used in this study, (low temperatures and water stress), create a similar response in the two studied species. Visually, leaves for stressed treatments presented chlorosis, the LWC decreased, the cuticle thickness increased (rhododendron only) and the emissivity changed, decreasing for beech and increasing for rhododendron. In beech the LWC decreased by 15% for the control treatment and by 37% for the dry treatment. For rhododendron the LWC decreased by 33% on average for all stress treatments and just 8% for the control treatment. LWC loss in the control treatments can be expected due to natural aging of the leaves. This has been found also by Ackley (1954) and Kozłowski and Clausen (1965) in deciduous leaves where the drop in LWC is caused by an increase in dry matter compared with the leaf water content over the growing season. This change is less consistent in species with perennial leaves that exhibit slight declines, no change or even increments in LWC over the growing season (Gary, 1971; Jolly et al., 2014; Paganelli and Paganelli, 1971).

Our results showed that water and cold stress have a similar physiological responses, with both having depleted LWC and an increase in cuticle thickness (for rhododendron). These results agree with Stefanowska et al. (1999) who reported that plants of

*Brassica napus* L., var. *oleifera* L., growing in cold conditions showed similar modifications to plants under water stress (i.e. smaller leaf surface, thicker leaves).

Biochemically, under cold stress, plants biosynthesize Reactive Oxygen Species (ROS) such as  $\text{H}_2\text{O}_2$ , which can be damaging for cell walls (Prasad, 2001). Plants react to oxidative stress by favoring the concentration of osmoprotectors (antioxidants and enzymes) over water content in the leaves. This reaction is similar to that for water stress, where plants also react to oxidative stress by mobilizing osmoprotectors, creating a general depletion of water in the leaf (Prasad, 2001).

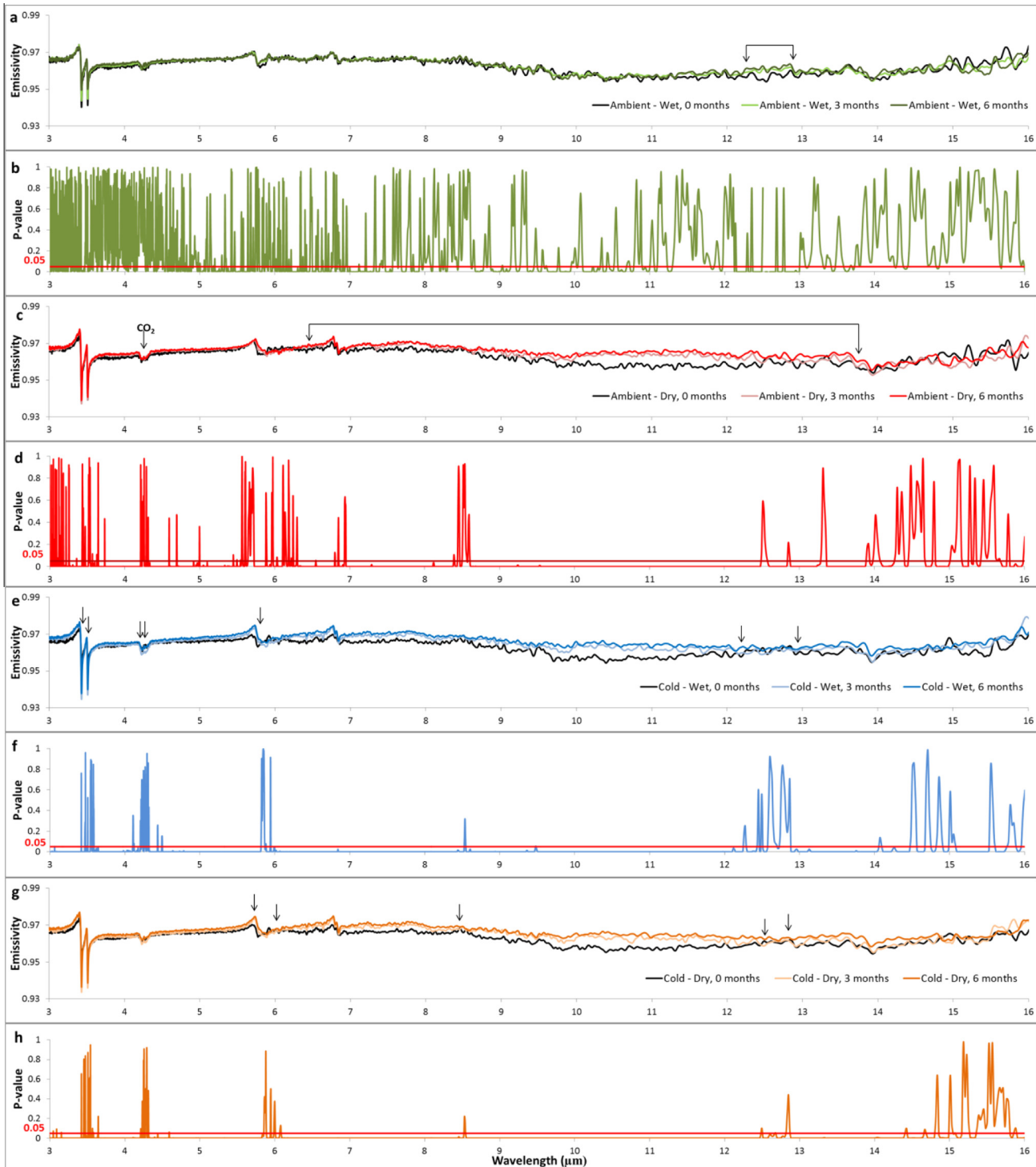
For the control treatments no water loss was expected. Nevertheless beech showed a reduction on LWC and a strong change in the MWIR spectra. These two changes are associated to the natural aging of the leaves especially when approaching senescent periods (Goss, 1973). The rhododendron control group showed no differences visually, in LWC, or cuticle thickness between the beginning and the end of the experiment.

#### 4.2. Changes in MWIR

There are two distinct regions with changes in the spectra as a result of the stress treatments for beech and rhododendron leaves, the MWIR (3–6  $\mu\text{m}$ ) and the LWIR (6–16  $\mu\text{m}$ ).

For the beech control treatment (AW), the emissivity decreased by 9.1% of the spectral contrast, and after the dry treatment, the emissivity decreased by 11.1% of the spectral contrast in the same region, with significant differences between both treatments.

A strong relation between water loss and decrease in emissivity in the MWIR has been widely reported for senescent leaves (French et al., 2000), and leaves under short term dehydration and different water contents (Fabre et al., 2011; Gerber et al., 2011; Salisbury and D'Aria, 1992; Salisbury and Milton, 1988; Ullah et al., 2012b). It has also been used for calculating the mid-wave infrared water index (MWI) (Ullah et al., 2013). More specifically, the same



**Fig. 7.** Mean spectra for rhododendron leaves in each treatment for 0, 3 and 6 months. *P*-value for a paired *T*-test before and after each treatment (0 and 6 months). Each graph shows the mean spectra before (black line) and after treatment (color line), and the *P*-value for AW (a and b), AD (c and d), CW (e and f) and CD (g and h). Arrows mark spectral features discussed in text.

strong positive correlation found for beech in this study between LWC and emissivity in the MWIR, has previously been reported by studies on LWC loss for deciduous species such as *Liquidambar styraciflua* (Ullah et al., 2012b), *Catalpa* sp., *Cercis canadensis*, *Sassafras albidum* (Gerber et al., 2011), *Prunus avium* (Fabre et al., 2011) and *Prunus serotina* (Salisbury and Milton, 1988).

For rhododendron, there were no significant changes between the start and the end of the treatment for either the control

treatment or the stress treatments (increase of spectral contrast by 2.7% and 4.0% respectively).

#### 4.3. Changes in LWIR

The LWIR between 6 and 16  $\mu\text{m}$  showed a strong difference before and after the stress treatments. For beech after the dry treatment, the emissivity in the LWIR decreased by 6.2% of the

spectral contrast, while LWC also decreased. The same correlation (decreasing emissivity with decreasing LWC) was found for other deciduous species like *S. albidum* (Gerber et al., 2011) and *L. styraciflua* (Ullah et al., 2012b). However, beech plants under the control treatment did not show significant differences in the LWIR compared to before the experiment, although the LWC was then also significantly reduced. These differences in response in the LWIR, while both treatments experienced a reduction in LWC, showed that the effect of leaf drying had a clearer connection to the MWIR than to the LWIR. These results showed that changes in LWIR must be showing stress responses other than changes in LWC.

The spectral differences found in the LWIR are probably due to changes in the leaf microstructure, since it has been reported that in this region spectral features can be species dependent and rely especially on mesophyll and cuticular differences (Fabre et al., 2011; Gerber et al., 2011).

In the LWIR, rhododendron had a trend which was opposite to beech. The control treatment didn't show significant differences, while the stress treatments all showed an emissivity increase by 23.6% of the spectral contrast for this species. Gerber et al. (2011) analyzed the spectra of *Catalpa* sp. and *C. canadensis*, that had a decrease in the MWIR associated to fast LWC loss, but in the LWIR had a similar behavior as the rhododendrons with an increase in emissivity. *Catalpa* sp. had an emissivity increase between 6 and 11  $\mu\text{m}$ , and *C. canadensis* had an increase in emissivity after 10  $\mu\text{m}$ , while drying out the leaves.

#### 4.4. Importance of cuticle for TIR spectra

Thicker and more complex leaf cuticle can create a mask for the spectral features from underneath the cuticle. For instance Fabre et al. (2011) found spectral differences between adaxial and abaxial leaf surfaces of the same leaf, with the adaxial side having a thicker and more complex cuticle than the opposite side. Ribeiro da Luz (2006) also found that for thinner cuticles, the spectra is more strongly influenced by the materials from the inner tissue layers, such as cellulose and cutin.

This experiment includes two parallel species, a deciduous beech with a yearly foliage change and thinner leaves and cuticles, and an evergreen rhododendron with thicker cuticles and blades, which stay on the plant for multiple years. The complexity of both leaf surfaces differs: beech (*F. sylvatica*) has a smooth surface with sunken nervature and lack wax crystals (Barthlott and Neinhuis, 1997; Gülz et al., 1992; Hardin and Johnson, 1985; Neinhuis and Barthlott, 1998), while *Rhododendron* spp. has a thicker striated cuticle with scales and crystals which create a more complex texture (Balsdon et al., 1995; Hardin and Gensel, 1982; Wang et al., 2008). Moreover cuticle thickness tends to increase when plants experience stress such as low temperatures and drought (Kosma et al., 2009; Le Provost et al., 2013; Shepherd et al., 1995; Wang et al., 2008).

We expect that the more complex waxy cuticle in rhododendron and the increase in thickness experienced during the stress period is causing the increased emissivity and the loss of spectral contrast. This loss of spectral contrast related to an increase in emissivity is known as the cavity effect and is observed on complex and rougher surfaces (Ribeiro da Luz and Crowley, 2007; Kirkland et al., 2002, 2003). A complex cuticle could increase multiple surface reflections resulting in higher emissivity across the entire emissivity range spectra (Ribeiro da Luz and Crowley, 2007; Salisbury and Eastes, 1985; Salisbury and D'Aria, 1992; Kirkland et al., 2002, 2003) which was observed on the rhododendron spectra.

#### 4.5. Impact on energy budget

A better understanding of how stress generates variations in the LWIR spectra of different species is important, because the values

and variability in emissivity of plants are key parameters for atmospheric models. Often emissivity of vegetation is assumed to be either unity or at least constant across the entire LWIR spectrum. In this study we found that rhododendron and beech have an average emissivity of 0.9674 and 0.9683 respectively and that the emissivity can vary down to 0.9583 in the case of rhododendron.

By using the Stefan Boltzmann equation  $T_R = \varepsilon^{1/4} T_K$ , we can calculate that due to reduced emissivity ( $\varepsilon$ ) a rhododendron leaf with a kinetic temperature ( $T_K$ ) of 295.15 K (23 °C) is actually showing a radiant temperature ( $T_R$ ) of 293.52 K, causing a considerable temperature error of 0.8135 K comparing with the average emissivity of an unstressed individual, and an error of 2.4354 K when comparing with an emissivity of 1 for rhododendron. This quick calculation shows that even small changes can have a substantial effect on temperature retrievals, if left uncorrected.

## 5. Conclusions

The results of this study showed that long-term stress had significant effects on the thermal infrared spectra of plants. Both the MWIR (3–6  $\mu\text{m}$ ) and the LWIR (6–16  $\mu\text{m}$ ) sections of the TIR region showed significant changes after 3–6 months of water and cold temperature stress. We further found that both stress treatments created a similar spectral response for a species no matter the type of stress.

The two species investigated in this study showed opposing stress responses in the thermal infrared spectra: the emissivity decreased for beech while it increased for rhododendron under all stress treatments.

To explain these contrasting observations, we inspected leaf water content and cuticle thickness as the two main contributing candidates. A decrease in LWC should reduce the emissivity of leaves, as was observed in beech. Since LWC significantly decreased in both species during the course of the treatment while the TIR spectral response in beech and rhododendron were opposite, we rule out LWC as the main explanation for the opposing spectral behavior. Opposite to that, our results demonstrated that stress increased the cuticle thickness, especially in rhododendron, making most of the spectral information in stressed rhododendron coming from the cuticle itself, while masking constituents of the leaf's interior. Since rhododendron has thicker and more textured cuticle than beech, we conclude that the interaction between radiated energy and the textured surface (i.e., 'cavity effect') will tend to shift the emissivity spectra toward unity, increasing its emissivity in the process.

Future research should identify the cuticle properties that affect emissivity and quantify its impact on the energy balance of plants. This will help our understanding of plant response to stress.

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