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## Long-term effects of water stress on hyperspectral remote sensing indicators in young radiata pine

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### ABSTRACT

The development of methods to spatially describe long-term water stress in plantations is going to become increasingly important as drought events increase in frequency under climate change. Despite this we are unaware of any research that has used hyperspectral imagery to describe long-term water stress for the most widely established exotic pine species, *Pinus radiata* D. Don (radiata pine). Hyperspectral imagery and foliage water content were repeatedly sampled over a five month period in one-year old radiata pine that were allocated to well-watered and drought treatments. These data were used to (i) determine how rapidly equivalent water thickness (EWT) and leaf fresh weight (LWC<sub>F</sub>) change in response to drought and (ii) identify the key hyperspectral indicators associated with changes in EWT and LWC<sub>F</sub>.

Both EWT and LWC<sub>F</sub> exhibited little treatment variation until 91 days after treatment (DAT) but diverged strongly after this time. By 108 DAT, mean values in the control exceeded the droughted treatment by 41.3% for EWT (0.0175 *vs.* 0.0124 g cm<sup>-2</sup>, P < 0.001) and 17.2% for LWC<sub>F</sub> (67.7 *vs.* 57.8%, P < 0.001). Using hyperspectral data captured 108 DAT, highly significant treatment differences (P < 0.001) in reflectance were found in the SWIR region with the most significant differences occurring between 1139 and 2497 nm.

There were strong correlations between a wide range of water indices and both measures of water stress using data captured 108 DAT. The indices that were most strongly correlated with EWT were found to be GVMI, NDWI<sub>1640</sub> and SIWSI with  $R^2$  values ranging from 0.86 to 0.87. Indices that had the strongest correlations with LWC<sub>F</sub> were SRWI<sub>1</sub>, GVMI and MSI with  $R^2$  values ranging from 0.89 to 0.90. Water indices were more strongly related to both measures of water stress than the physiological index PRI which was moderately correlated to both EWT ( $R^2 = 0.47$ ) and LWC<sub>F</sub> ( $R^2 = 0.62$ ). A wide range of structural and chlorophyll indices were less well correlated with both measures of water content and long-term water stress than either PRI or the water indices.

### 1. Introduction

Water is a fundamental component of trees and is a key determinant of carbon gain and transpiration. Physiological processes and phylogenic traits that are strongly affected by water include photosynthetic efficiency, transpiration rates and leaf vigour, structure and shape (Kramer and Boyer, 1995). Over the short-term water stress results in stomatal closure and lower assimilation rate with needle water potential declining over the short-medium term (Mitchell et al., 2013; Mitchell et al., 2014). The impacts of water stress over the long-term are reduced leaf water content, with either carbon starvation or hydraulic failure, often leading to tree mortality (Kramer and Boyer, 1995; Stone et al., 2012; Mitchell et al., 2014).

The characterisation of water content and long-term water stress effects in trees is important for understanding their general physiological status and the extent of drought stress (Hill et al., 2019). The quantification of water and dry matter content components also enables better characterization of canopy fuel moisture, which is a critical input

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for fire risk analysis (Chuvieco et al., 2020). The retrieval of leaf water content using remotely sensed data would provide a useful means of identifying trees and forests that are experiencing long-term water stress effects.

Within the remote sensing and ecological literature water stress is often described by equivalent water thickness (EWT) and gravimetric water content (GWT). EWT is quantified as the mass of leaf water per unit leaf area (i.e. g cm<sup>-2</sup> or cm<sup>3</sup> cm<sup>-2</sup> which decomposes to cm) and is physically related to radiation adsorption processes as it measures the optical path and depth of the water layer (Baret and Fourty, 1997). GWT expresses the mass of leaf water as either the percentage of the leaf dry weight (LWC<sub>D</sub>, %) or leaf fresh weight (LWC<sub>F</sub>, %). The LWC<sub>D</sub> has been widely used in fire risk research where it is also known as the fuel moisture content (Chuvieco et al., 2020) while LWC<sub>F</sub> is a commonly used leaf trait in ecological studies (Garnier and Laurent, 1994).

Hyperspectral imagery has been widely used to predict foliage water content. Leaf water absorbs radiant energy in the near infra-red (NIR) and short wave infra-red (SWIR) parts of the spectrum. Previous research has found liquid water absorption peaks of increasing size at 970, 1200, 1450, 1950, and 2500 nm (Knipling, 1970; Thomas et al., 1971; Tucker, 1980; Fourty and Baret, 1997; Datt, 1999; Danson and Bowyer, 2004).

Numerous studies have exploited these absorption features to develop strong negative correlations between reflectance in individual wavelengths and leaf water content (Hunt et al., 1987; Jacquemoud and Baret, 1990; Carter, 1991; Danson et al., 1992; Aldakheel and Danson,

1997). Generally wavelengths between 1300 and 2500 nm have been found to have strong correlations with leaf water concentration (Carter, 1991) but the weaker absorption bands between 900 and 1300 nm are also effective predictors as they penetrate further into canopies than the rapidly absorbed higher wavelengths (Sims and Gamon, 2003). Many studies have also used spectral vegetation indices that combine two different wavelengths to precisely predict water content and a large number of useful indices have been developed (Hunt and Rock, 1989; Gao, 1996; Penuelas et al., 1997; Datt, 1999; Ceccato et al., 2001; Danson and Bowyer, 2004; Buddenbaum et al., 2012; Buddenbaum et al., 2015). These indices often use a SWIR or NIR band to detect water content and a NIR band to normalise the effect of leaf structural variability on reflectance (Danson and Bowyer, 2004; Colombo et al., 2008). In addition, radiative transfer simulations have been proposed to estimate leaf equivalent water and dry matter content using model inversion techniques, linking leaf and canopy physical models (Riano et al., 2005). These methods have been successfully applied at airborne scales using hyperspectral imagers (Cheng et al., 2006). Scaling up has also been undertaken at the satellite level, using index-based indicators such as the Normalized Difference Water Index (NDWI) (Gao, 1996) to map fuel moisture content in shrubs using MODIS time series imagery (Zarco-Tejada et al., 2003).

Radiata pine (*Pinus radiata* D. Don) is the most widely planted exotic pine species in the world and large areas have been established in Spain, New Zealand, Australia, Chile, and South Africa (Lewis and Ferguson, 1993; Lavery and Mead, 1998). A significant proportion of these



Fig. 1. Set-up for reflectance measurement using the ASD FieldSpec Spectroradiometer showing (a) the instrument and computer set up (b) spectra obtained from two sample trees and (c) trees used in the experiment from the control (left of centre space) and droughted treatments (right of centre space). The photo shown in panel (c) was taken on the 12th April 2021 (73 days after treatment).

### Table 1

Indices that were included in the analysis.

	· · · · · · · · · · · · · · · · · · ·						
Index	Index name	Equation	Reference				
Chlorophyll an	d other photosynthetic pigment indices						
CI	Chlorophyll index	$\rho_{750}/\rho_{710}$	Zarco-Tejada et al. (2001)				
GM1	Gitelson and Merzlyak Index 1	$\rho_{750}/\rho_{550}$	Gitelson and Merzlyak (1997)				
GM2	Gitelson and Merzlyak Index 2	ρ <sub>750</sub> /ρ <sub>700</sub>	Gitelson and Merzlyak (1997)				
MCARI	Mod. Chl. Absorp. Rfl. Index	$[(\rho_{700}-\!\rho_{670})-0.2(\rho_{700}-\!\rho_{550})](\rho_{700}/\rho_{670})$	Daughtry et al. (2000)				
PSSRb	Pig. Spec. Simpl. Ratio Chl. b	$ ho_{800}/ ho_{635}$	Blackburn (1998)				
TCARI	Transf. Chl. Absorp. Rfl. Index	$3[( ho_{700}- ho_{670})-0.2( ho_{700}- ho_{550})( ho_{700}/ ho_{670})]$	Haboudane et al. (2002)				
TCA/OSA	TCARI/OSAVI	TCARI/OSAVI	Haboudane et al. (2002)				
VOG	Vogelmann Index	$\rho_{740}/\rho_{720}$	Vogelmann et al. (1993)				
Structural Indi	ces						
NDVI	Normalised Difference Vegetation Index	$(\rho_{800}-\rho_{670})/(\rho_{800}+\rho_{670})$	Rouse et al. (1974)				
OSAVI	Opt. Soil-Adjusted Veg. Index	$(1\!+\!0.16)(\rho_{800}-\rho_{670})/(\rho_{800}+\rho_{670}+0.16)$	Rondeaux et al. (1996)				
RDVI	Renormalized Diff. Veg. Index	$(\rho_{800}-\rho_{670})/(\rho_{800}+\rho_{670})^{0.5}$	Roujean and Breon (1995)				
Physiological /	Photochemical index						
PRI	Photochemical Reflectance Index	$(\rho_{531}-\rho_{570})/(\rho_{531}+\rho_{570})$	Gamon et al. (1992)				
Water indices	_						
Datt <sub>1</sub>	Datt <sub>1</sub>	$(\rho_{850} - \rho_{2218})/(\rho_{850} - \rho_{1928})$	Datt (1999)				
Datt <sub>2</sub>	Datt <sub>2</sub>	$(\rho_{850}-\rho_{1788})/(\rho_{850}-\rho_{1928})$	Datt (1999)				
DDI	Double Difference Index	$2\rho_{1530} - \rho_{1005} - \rho_{2005}$	Le Maire et al. (2004), Wang and Li (2012)				
fWBI	Floating position Water Band index	$\rho_{900}/min_{\rho_{930}-\rho_{960}}$ Strachan et al. (2002)					
GVMI	Global vegetation moisture index	$\frac{((\rho_{820} + 0.1) - (\rho_{1600} + 0.02))}{((\rho_{1600} + 0.02))}$	Ceccato et al. (2002)				
LWI	Leaf Water Index	$((\rho_{820} + 0.1) + (\rho_{1600} + 0.02))$ $\rho_{1300}/\rho_{1450}$	Seelig et al. (2008)				
MSI	Moisture Stress Index	$\rho_{1600}/\rho_{820}$	Hunt and Rock, 1989				
MSI1	Moisture Stress Index 1	$\rho_{1650}/\rho_{1230}$	Rock et al. (1986)				
MSI <sub>2</sub>	Moisture Stress Index 2	$\rho_{1660}/\rho_{930}$	Rock et al. (1986)				
NDII	Normalised Difference Infrared Index	$(\rho_{820} - \rho_{1600})/(\rho_{820} + \rho_{1600})$	Hardisky et al. (1983)				
NDWI <sub>1</sub>	Normalised Difference Water Index 1	$(\rho_{860} - \rho_{1240})/(\rho_{860} + \rho_{1240})$	Gao (1996)				
NDWI <sub>2</sub>	Normalised Difference Water Index 2	$(\rho_{870} - \rho_{1240})/(\rho_{870} + \rho_{1240})$	Rodríguez-Pérez et al. (2007)				
NDWI1640	Norm. Diff. Water Index 1640 nm	$(\rho_{860} - \rho_{1640})/(\rho_{860} + \rho_{1640})$	Chen et al. (2005)				
NDWI2130	Norm. Diff. Water Index 2130 nm	$(\rho_{\text{RER}} - \rho_{2120})/(\rho_{\text{RER}} + \rho_{2120})$	Chen et al. (2005)				
Ratio	Ratio <sub>975</sub>	$2mean(\rho_{0.00}, \rho_{0.00})$	Gao et al. (1993). Mutanga and Ismail (2010). Pu et al. (2003)				
D. (		$\frac{(mean(\rho_{920-940}) + mean(\rho_{1090-1110}))}{(mean(\rho_{1090-1110}))}$					
Ratio <sub>1200</sub>	Ratio <sub>1200</sub>	$\frac{2mean(\rho_{1180-1220})}{(mean(\rho_{1180-1220}) + mean(\rho_{1180-1220}))}$	Gao et al. (1993), Mutanga and Ismall (2010), Pu et al. (2003)				
SIWSI	Shortwave Infrared Water Stress Index	$\frac{(\mu_{1040}(\rho_{1090-1110}) + \mu_{1040}(\rho_{1265-1285}))}{(\rho_{1640} - \rho_{858})/(\rho_{1640} + \rho_{858})}$	Fensholt and Sandholt (2003)				
SRWI	Simple Ratio Water Index	$ ho_{860}/ ho_{1240}$	Zarco-Tejada and Ustin (2002)				
SRWI1	Simple Ratio Water Index 1	$ ho_{1350}/ ho_{870}$	Rodríguez-Pérez et al. (2007)				
SRWI <sub>2</sub>	Simple Ratio Water Index 2	$ ho_{880}/ ho_{1265}$	Rodríguez-Pérez et al. (2007)				
TM57	Ratio of Thematic Mapper B5 to B7	$\rho_{1650}/\rho_{2220}$	Elvidge and Lyon (1985)				
WBI	Water Band Index	$ ho_{970}/ ho_{900}$	Peñuelas et al. (1993)				
WI	Water Index	$ ho_{900}/ ho_{970}$	Penuelas et al. (1997)				

plantations are located in areas that are subject to drought. Under climate change, the combined influence of lower rainfall and higher air temperatures are expected to increase the severity and duration of drought in many countries where radiata pine is grown (Clark et al., 2012; Head et al., 2014; Pachauri et al., 2014). Although radiata pine is a fast growing species, water stress significantly reduces carbon assimilation and growth rate (Mitchell et al., 2013; Mitchell et al., 2014) primarily through reducing stomatal conductance (Watt et al., 2003; De Diego et al., 2012; Mitchell et al., 2013). As drought conditions worsen, radiata pine becomes predisposed to attack from pests and diseases (McDowell et al., 2008; Verbesselt et al., 2009) and prolonged water stress commonly results in tree mortality (Stone et al., 2012). Although several spectral-based indices have been proposed to assess the leaf biochemistry in broad leaf species, the specific needle structure of coniferous species together with the canopy structural effects mean that not all hyperspectral indicators are suitable for monitoring long-term processes of decline (Zarco-Tejada et al., 2018). The identification of hyperspectral indices to quantify drought stress would provide a means of phenotyping radiata pine for this trait, potentially allowing drought resistant clones to be deployed to areas at risk of current or future drought.

Despite the potential of remotely sensed data for identifying longterm drought stress in radiata pine, we are unaware of any research for this species that has characterised relationships between indicators derived from hyperspectral imagery and the two measures EWT and GWT, specifically LWC<sub>F</sub>. Using data collected from a pot trial of young radiata pine, the objectives of this study were to (i) determine how rapidly EWT and LWC<sub>F</sub> change in response to drought and (ii) identify the key hyperspectral bands and indices associated with changes in EWT and LWC<sub>F</sub>.

### 2. Material and methods

### 2.1. Experimental set-up and measurement dates

The experiment was undertaken in the Scion nursery in Rotorua, New Zealand. Eighty radiata pine seedlings that were approximately one-year old were transplanted on the 5th of August 2020 into 20 L pots filled with potting mix. These trees were placed inside a glasshouse with a fluctuating ambient day temperature that was maintained above  $10 \,^{\circ}$ C and reached a maximum temperature of  $30.4 \,^{\circ}$ C during summer. The potted trees were well watered prior to the imposition of treatments in

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#### January 2021.

In January 2021, the 80 trees were randomly and equally allocated to droughted and control treatments. The droughted trees did not receive any water from the 29th January 2021 until the conclusion of the experiment on the 17th May 2021. Over this period the control trees were watered to excess twice per week. The full set of measurements, described below, were undertaken on 50 trees (25 per treatment) while additional measurements of only EWT and LWC<sub>F</sub> were made on 20 trees (10 per treatment).

The remaining ten trees (five per treatment) were set aside to continuously monitor root-zone volumetric water content ( $\theta$ ) throughout the duration of the experiment. Measurements were made using a pre-calibrated CS655 multiparameter smart sensor (Campbell Scientific Inc., Logan, Utah, US) that was installed in each pot to a depth of 12 cm and measured  $\theta$  across a soil volume of 3.6L. All sensors were connected to a CR1000X data logger and observations were recorded on an hourly basis.

The full set of measurements, made on the 50 trees, were taken during five campaigns that spanned five months. A set of pre-treatment measurements were undertaken on January 26th while four post-treatment measurements were undertaken on February 2nd, 7th, 19th, and May 17<sup>th</sup> 2021. These dates were selected to cover the range in  $\theta$  experienced by droughted trees with  $\theta$  averaging, respectively, 0.30, 0.15, 0.08, 0.03, and 0 m<sup>3</sup> m<sup>-3</sup>. Additional measurements of EWT and LWC<sub>F</sub> were taken prior to treatments being imposed on the 22nd January and following treatment on the 15th, 22nd, 29th March, 10th, 30th April and 11th May.

### 2.2. Hyperspectral reflectance measurements

Needle-level reflectance at wavelengths from 400 to 2500 nm was measured from needle mats using an ASD FieldSpec Spectroradiometer and a customised dark container setup. The fibre optic cable of the spectroradiometer with the 8° field-of-view (FOV) lens was fixed on a stand to point in a nadir direction at the needle mat sample (Fig. 1). A 100 W halogen lamp was used as the light source and placed at a 30° angle to the sensor and sample. This setup was fully enclosed during each data collection to minimise the effects of stray light.

For each tree, 4–5 fully extended fascicles were destructively taken from the upper third of the canopy. The fascicles were then laid out as



**Fig. 2.** Daily volumetric water content measured by the soil moisture sensors over the duration of the experiment. Shown are mean values (lines) and 95% confidence intervals (shading) for the control (green) and drought treatments (red). The dashed vertical lines denote the timing of the five full sets of measurements (n = 50). Shown at the top are the measurement set and days after the start of the treatment (DAT) on the 29th January 2021. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

needle mats against a non-reflective dark foam paper and held together using black tape. The mats were arranged such that gaps or overlaps between the needles were minimised and steps were taken to ensure that the mats covered the whole FOV of the sensor which was approximately 0.9–1.0 cm. For each tree, the spectroradiometer was first calibrated against a white reference and three reflectance spectra were recorded. Each reflectance spectrum was an average of 30 readings. The white reference reflectance spectra were also recorded before and after collection of the three sets of reflectance spectra. The data collection was undertaken using the ASD  $\mathrm{RS}^{3\,\mathrm{TM}}$  Spectral Acquisition software.

The resulting ASD files were loaded to ViewSpec  $Pro^{TM}$  and the reflectance datasets were exported as text files. The three reflectance spectra for each tree were averaged and divided by the average of the two white reference spectra to obtain corrected mean reflectance values. The indices summarised in Table 1 were derived from reflectance and grouped into four categories. Chlorophyll and other photosynthetic pigment-based indices were included in the first group. The second group was composed of structural indices while the third group included PRI which was classified as a physiological and photochemical index. The fourth category comprised water indices (Table 1).

### 2.3. Measurements of photosynthesis and stomatal conductance

Physiological measurements were made on the same days as the five hyperspectral dataset collections on all 50 plants (n = 25 per treatment). Net photosynthetic rates (A) and stomatal conductance ( $g_s$ ) were measured after a two-minute pre-illumination period, at 400 ppm CO<sub>2</sub> concentration. These measurements were taken by a GFS-3000 coupled with an Imaging-PAM chlorophyll fluorometer (M—series, Walz, Effeltrich, Germany) that was equipped with a CO<sub>2</sub> cartridge (to maintain CO<sub>2</sub> at a constant level).

### 2.4. Measurements of leaf area, leaf weight, EWT and $LWC_F$

Measurements of leaf weight and area were made on the same day as each hyperspectral capture and samples were placed on ice until these measurements were undertaken. Additional measurements of leaf area and weight were also taken on the dates listed in Section 2.1. Following Bown et al. (2009), needle area per fascicle was determined from [*nld*(1 +  $\pi/n$ ]/2, where *d* is fascicle diameter, *l* is fascicle length and *n* is the number of needles per fascicle. Total needle area (*A*) per sample was calculated as the sum of the individual areas for all 4–5 fascicles. The fresh weight (*FW*) was recorded and samples were then dried in a 70 °C oven until constant weight and the dry weight (*DW*) was measured.

Using these measurements EWT (g  $\rm cm^{-2})$  and  $\rm LWC_F$  (%) were determined from the following equations for all samples.

$$EWT = \frac{FW - DW}{A}$$
(1)

$$LWC_{\rm F} = \frac{FW - DW}{FW}$$
(2)

### 2.5. Tree dimensions

Tree height and diameter were measured four times over the duration of the experiment. Electronic calipers were used to measure rootcollar diameter and height was measured using a tape.

### 2.6. Data analysis

The data merging, post-processing calculations, statistical analysis, and visualisations were undertaken in Python 3.7.10 using pandas 1.1.5, numpy 1.19.5, scipy 1.4.1, matplotlib 3.2.2, and sklearn packages. Volumetric water content data was averaged to the daily level and plotted over time for both treatments. Temporal variation in water stress (EWT, LWC<sub>F</sub>), tree dimensions and physiological measurements were

3. Results

time



**Fig. 3.** Variation in (a) EWT and (b) LWC<sub>F</sub> over the duration of the experiment for the control (green bars) and drought (red bars) treatments. Shown are the mean (centre dots) and the 95% confidence interval (bars) for all measurements. The number of days after the start of the drought treatment (DAT) on the 29th January 2021 is shown at the top for all measurements. The dates that included a full set of measurements (n = 50) are denoted by dashed lines with all other displayed measurements were made on a smaller number (n = 20) of trees. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plotted and treatment differences were detected using a *t*-test.

Treatment variation in hyperspectral reflectance was plotted for all five captures and a *t*-test was used to detect significant differences in reflectance. The correlation coefficient between band reflectance and both measures of water stress was determined and plotted, for all five captures, to identify spectral regions that were most strongly related to EWT and LWC<sub>F</sub>. Bivariate relationships were constructed between both measures of water stress and all indices listed in Table 1. Comparisons of predictive precision for these relationships were made between the four index groupings (chlorophyll, structural, photochemical and water indices) and within each grouping the strongest relationships were plotted.

# high over the duration of the experiment. Values of mean daily $\theta$ in the control fluctuated between 0.27–0.38 m<sup>3</sup> m<sup>-3</sup> and averaged 0.33 m<sup>3</sup> m<sup>-3</sup> across the study period (Fig. 2). In contrast, values of $\theta$ in the droughted treatment declined exponentially from 0.30 m<sup>3</sup> m<sup>-3</sup> for the pre-treatment measurements to 0.15, 0.08, 0.03, and 0 m<sup>3</sup> m<sup>-3</sup> (Fig. 2) on measurement dates that respectively occurred 4, 9, 21 and 108 days after treatment (DAT).

Compared to changes in  $\theta$ , reductions in foliage EWT took longer to be expressed in the drought treatment (Fig. 3a). The EWT of both treatments fluctuated similarly through time and control values of EWT were not significantly higher than those of the drought treatment until 59 DAT (0.0199 *vs.* 0.0178 g cm<sup>-2</sup>; P = 0.034). These treatment differences converged over the next measurement and values in the control only exceeded those in the drought treatment by 3.09% at 91 DAT (0.0163 *vs* 0.0158 g cm<sup>-2</sup>; P = 0.524). Treatments did start diverging strongly and significantly after this point (Fig. 3a) and values of EWT in the control treatment exceeded those in the drought treatment by 41.3% at 108 DAT (0.0175 *vs.* 0.0124 g cm<sup>-2</sup>, P < 0.001).

Volumetric water content within the control treatment remained

3.1. Variation in root-zone volumetric and foliar water content through

Temporal and treatment related changes in LWC<sub>F</sub> were similar to



**Fig. 4.** Variation in reflectance showing the average (line) and standard deviation (shading) by treatment for the five sets of measurements (a–e) where the mean values for the control and drought treatments are shown as solid green and dashed red lines, respectively. Also shown is variation in treatment significance, as indicated by the P-value, for the five captures (f). The grey region shown at the top of panel (f) outlines the area of insignificance where P > 0.05 while the dashed line is drawn at P = 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 5.** Variation in the coefficient of determination ( $R^2$ ) between reflectance and (a) EWT and (b) LWC<sub>F</sub> within the 400–2500 nm range for the five full sets of measurements (n = 50). Horizontal dashed lines have been drawn to denote the relationship significance at P = 0.01 and 0.05.

EWT (Fig. 3b). Values of LWC<sub>F</sub> were very similar between treatments up to 21 DAT. For all measurements after this time LWC<sub>F</sub> in the control treatment significantly exceeded LWC<sub>F</sub> in the droughted treatment (Fig. 2b). However, these significant differences, from 45 to 91 DAT were relatively small (mean = 4.88%) and even at 91 DAT differed by only 4.08% (Fig. 3b). Strong treatment divergence occurred during the last two measurements when control values of LWC<sub>F</sub> were, respectively, 11.4 and 17.2% higher than those of the droughted treatment at 102 and 108 DAT (Fig. 3b).

### 3.2. Changes in tree physiology and dimensions

Although there were some fluctuations values of stomatal conductance ( $g_s$ ) and assimilation rate (A) in the control treatment remained relatively constant over the course of the experiment (Fig. A1). However, the imposition of drought had a strong and immediate effect on both  $g_s$  and A. Values of  $g_s$  in the drought treatment were reduced to 76, 56, 28 and 3% of the control values, respectively, at 4, 9, 21 and 108 DAT (Fig. A1). Similarly, A in the droughted treatment was reduced to, respectively, 87, 73, 52 and 0.5% of control values at 4, 9, 21 and 108 DAT (Fig. A1). These treatment differences in both  $g_s$  and A were significant (P < 0.05) by 4 DAT, and highly significant (P < 0.001) for measurements made 9, 21 and 108 DAT.

Mean tree dimensions in the control treatment rapidly increased over the duration of the experiment, from 62.1 to 77.4 cm for height and 9.4 to 11.9 mm for root-collar diameter (Fig. A2). These two tree dimensions for the droughted treatment did not significantly vary from the control treatment from the start of the experiment until 28 DAT in late February. However, by the end of the experiment (116 DAT) there was significant (P < 0.001) treatment divergence in these two dimensions which was attributable to the very small change in tree height and diameter within the droughted treatment from 28 to 116 DAT (Fig. A2).

### 3.3. Variation in needle-level reflectance through time

Treatment variation in mean needle-level reflectance spectra was consistent with the measures of foliar water content. The first four sets of spectra, captured from -3 to 21 DAT, did not show any visible differences between the two treatments (Fig. 4a–d). Within the 1st and 4th captures significant treatment differences (P < 0.05) were found in green to red (507–649 nm), red to red-edge (689–726 nm), and SWIR (1385–1469, 1831–1887, 2040–2496 nm) regions, but these differences were not strongly significant (Fig. 4f).

The last set of spectra, captured at 108 DAT showed a clear delineation between the control and drought means (Fig. 4e) that was particularly marked for wavelengths higher than 1000 nm. Highly significant treatment differences (P < 0.001) in reflectance values were found in the NIR and SWIR regions. With the exception of the

1912–1939 nm range, these differences were highly significant between 1139 and 2497 nm (Fig. 4f). In contrast, within the visible range there was little separation in mean reflectance between treatments and these differences were almost all insignificant (Fig. 4f).

Moderate to strong correlations between needle-level reflectance and foliar measures of water stress (i.e.  $R^2 \ge 0.6$ ) were found within the NIR/SWIR region of the spectra, but only for the last set of measurements captured 108 DAT (Fig. 5). For this capture significant correlations between reflectance and EWT were found within the SWIR wavelengths, and were most marked between the 1314–2474 nm range. The strongest correlation was found at 1537 nm ( $R^2 = 0.83$ ) while the strongest correlation in the visible region was at 690 nm ( $R^2 = 0.36$ ) (Fig. 5a). For this last capture LWC<sub>F</sub> exhibited moderate to strong correlations with reflectance between 1317 and 2479 nm, which peaked at 1510 nm ( $R^2 = 0.86$ ) and at 690 nm ( $R^2 = 0.37$ ) within the visible region (Fig. 5b). There were weak correlations between reflectance and both measures of water stress during the first four measurements (Fig. 5a, b). However, with the exception of the second capture, most of these correlations were insignificant at P = 0.05 (Fig. 5a, b).

### 3.4. Prediction of EWT and $LWC_F$ using the indices

All correlations between the indices and both measures of water stress were weak for the first four captures (Table 2). Of the four categories considered, the highest correlations were found using the water indices for all of the first four captures. Using the water indices, the correlations were highest for the first capture and predictions of EWT were stronger than predictions of LWC<sub>F</sub> across all four captures (Table 2). When averaged over the first four captures, the strongest predictor of EWT was Datt<sub>1</sub> (mean R = 0.54) while the strongest predictor of LWC<sub>F</sub> was SRWI<sub>1</sub> (mean R = -0.31).

The strength of correlations between water stress and structural, physiological and water indices markedly increased during the fifth capture at 108 DAT (Table 2). Correlations for these relationships were strongest using water indices, followed by PRI, then structural indices with chlorophyll indices showing the weakest correlations with EWT and LWC<sub>F</sub> (Table 2). Almost all water indices had *R* values that exceeded |0.8| (Table 2) with maxima absolute values of 0.93 for EWT and -0.95 for LWC<sub>F</sub>. The most strongly correlated indices ( $R \ge |0.9|$ ) were usually composed of a NIR and a SWIR band (GVMI, MSI, MSI<sub>1</sub>, MSI<sub>2</sub>, NDII, NDWI<sub>1640</sub>, and SIWSI), a NIR and two SWIR bands (Datt<sub>1</sub> and DDI), or two NIR bands (fWBI, SRWI<sub>1</sub>, WBI, and WI) (Table 2).

The indices that were most strongly correlated with EWT were found to be GVMI, NDWI<sub>1640</sub> and SIWSI (Fig. 6a – c) which were all band ratios consisting of a combination of NIR and SWIR (1600–1650 nm) bands. The relationships between these indices and EWT were best described by second order polynomial equations with  $R^2$  values ranging from 0.86 to 0.87. Indices that had the strongest correlations with LWC<sub>F</sub> were SRWI<sub>1</sub>,

### Table 2

Correlation between the indices and EWT and  $LWC_F$  for the five sets of measurements. The correlation is denoted by the correlation coefficient (*R*) which are coloured according to the strength of the linear relationships. Blue and red colours represent positive and negative correlations, respectively, with the darkness of the shade denoting the relationship strength (see key below). The asterisks \* denote the six strongest correlations for each variable.

Index	EWT					LWC <sub>F</sub>							
	1	2	3	4	5	-	1	2	3	4	5		
Chlorophyll indices													
CI	0.33	0.45	0.43	0.24	0.46		0.18	0.37	0.18	0.07	0.50		
GM1	0.30	0.48	0.43	0.28	0.10		0.16	0.40	0.17	0.11	0.10		
GM2	0.27	0.46	0.42	0.25	0.45		0.13	0.39	0.17	0.07	0.50		
MCARI	-0.24	-0.34	-0.37	-0.27	-0.31		-0.16	-0.36	-0.20	-0.08	-0.34		
PSSRb	0.22	0.47	0.40	0.24	0.44		0.10	0.41	0.17	0.09	0.46		
TCARI	-0.22	-0.38	-0.37	-0.26	-0.28		-0.16	-0.40	-0.19	-0.11	-0.29		
TCARI/OSAVI	-0.23	-0.41	-0.39	-0.26	-0.35		-0.16	-0.40	-0.19	-0.11	-0.38		
VOG	0.35	0.45	0.44	0.24	0.46		0.2	0.36	0.19	0.07	0.50		
Structural indices													
NDVI	0.18	0.45	0.37	0.22	0.56		0.08	0.38	0.17	0.06	0.60		
OSAVI	0.14	0.40	0.24	0.14	0.39		0.03	0.24	0.08	0.03	0.42		
RDVI	0.24	0.39	0.28	0.19	0.25		0.04	0.10	0.07	0.01	0.30		
Physiological / Pl	hotochemical	index											
PRI	0.24	0.33	0.22	0.21	0.67		0.10	0.35	0.12	0.19	0.77		
Water indices													
$Datt_1$	0.57	0.54	0.53	0.53	0.92		0.40	0.26	0.32	0.12	0.94*		
$Datt_2$	-0.51	-0.17	-0.28	-0.10	-0.69		-0.44	0.14	-0.10	-0.03	-0.71		
DDI	-0.61	-0.27	-0.36	-0.24	-0.89		-0.39	0.12	-0.13	0.02	-0.94*		
fWBI	0.53	0.54	0.48	0.49	0.91		0.38	0.29	0.22	0.19	0.94*		
GVMI	0.59	0.41	0.39	0.30	0.93*		0.43	0.12	0.19	0.10	0.94*		
LWI	0.33	0.41	0.30	0.32	0.89		0.31	0.30	0.20	0.17	0.93		
MSI	-0.60	-0.41	-0.39	-0.32	-0.92*		-0.44	-0.10	-0.18	-0.10	-0.94*		
$MSI_1$	-0.61	-0.39	-0.40	-0.28	-0.92		-0.42	-0.08	-0.15	0.01	-0.93		
$MSI_2$	-0.62	-0.42	-0.41	-0.32	-0.92*		-0.45	-0.09	-0.18	-0.09	-0.94		
NDII	0.62	0.42	0.41	0.32	0.92*		0.45	0.09	0.18	0.09	0.94		
$NDWI_1$	0.58	0.44	0.38	0.33	0.89		0.47	0.09	0.22	0.24	0.91		
NDWI <sub>2</sub>	0.57	0.43	0.38	0.33	0.89		0.45	0.08	0.22	0.24	0.91		
NDWI <sub>1640</sub>	0.62	0.41	0.41	0.32	0.92*		0.45	0.09	0.18	0.09	0.94		
NDWI2130	0.38	0.41	0.34	0.41	0.88		0.29	0.33	0.15	0.20	0.90		
Ratio975	-0.16	-0.30	-0.33	-0.16	-0.79		-0.05	-0.27	-0.07	0.11	-0.85		
Ratio <sub>1200</sub>	-0.58	-0.47	-0.50	-0.32	-0.88		-0.41	-0.17	-0.22	-0.16	-0.90		
SIWSI	-0.62	-0.41	-0.41	-0.32	-0.92*		-0.45	-0.09	-0.18	-0.09	-0.94		
SRWI	0.58	0.44	0.38	0.33	0.89		0.47	0.09	0.22	0.24	0.91		
SRWI1	-0.56	-0.52	-0.49	-0.49	-0.91		-0.45	-0.24	-0.26	-0.28	-0.95*		
SRWI <sub>2</sub>	0.56	0.43	0.39	0.33	0.89		0.46	0.08	0.23	0.24	0.91		
TM57	-0.05	0.36	0.23	0.39	0.76		-0.02	0.46	0.16	0.21	0.78		
WBI	-0.50	-0.56	-0.48	-0.48	-0.90		-0.33	-0.35	-0.22	-0.21	-0.94		
WI	0.50	0.56	0.48	0.48	0.90		0.33	0.35	0.22	0.21	0.93		
D 1	10.00	0.0 0.5	0 6 0 5	0.4.05	0.0.0		0.02	0.0.04	04.07	0 6 0 0	0.0 1.0		
K values	-1.00.8	-0.8– -0.6	-0.60.4	-0.40.2	-0.2-0		0-0.2	0.2–0.4	0.4-0.6	0.0-0.8	0.8–1.0		
Colour Key													

GVMI and MSI which were based on NIR and SWIR bands and these linear relationships had  $R^2$  values ranging from 0.89 to 0.90 (Fig. 7a – c).

Amongst the other categories PRI was the next strongest predictor of both EWT ( $R^2 = 0.47$ ) and LWC<sub>F</sub> ( $R^2 = 0.62$ ) using data from the fifth capture (Fig. 6d, 7d). Within the structural indices NDVI was the strongest predictor of both water stress indices (Fig. 6e, 7e). Among the chlorophyll indices VOG, CI and GM2 were the strongest predictors of both EWT (Fig. 6g – i) and LWC<sub>F</sub> (Fig. 7g – i). Although correlations were weaker overall for structural and chlorophyll indices the data does show a reasonable correlation between these indices and both measures of water stress for the droughted treatment (Figs. 6, 7).

### 4. Discussion

The conservative growth and water use strategy of radiata pine accounts for the low initial rate of foliage water loss observed in this study. The response to drought by different species is determined by the extent to which plant water stress is hydraulically regulated (Mitchell et al.,



**Fig. 6.** Correlation between EWT and the strongest predictors from the four categories of indices for data extracted from the 5th measurement set (n = 50) taken 108 days after treatment. Shown are the strongest predictors from (a - c) water indices, (d) the photochemical index PRI, (e - f) structural indices and (g - i) chlorophyll indices. The observations are categorised by control (solid green circles) and drought (solid red circles) treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2013). Many species such as Eucalypt have high growth and water use during drought which results in a rapid depletion of soil water and expedites complete loss of hydraulic function (Mitchell et al., 2014). In contrast, radiata pine reduces water loss early under drought conditions through stomatal closure which results in a lower assimilation rate, but prolongs leaf turgor (Watt et al., 2003; McDowell et al., 2008; Stone et al., 2012). However, as drought conditions intensify needle water potential reduces over the short-medium term (1–3 months) and over the long-term foliage water content declines in radiata pine (Mitchell et al., 2013; Mitchell et al., 2014). Consistent with our findings turgor loss in radiata pine is reached 93 days after the imposition of drought which greatly exceeds, for example, the 44 days required to reach this point for *Eucalyptus globulus* (Mitchell et al., 2013).

Consistent with these slow changes in needle water loss, reflectance showed little treatment variation for the first three months following treatment. However, during the last set of measurements, reflectance of the drought treatment was significantly elevated over the control across the entire NIR and SWIR range. The strength of the correlations between needle water content and reflectance were highest for wavelengths that showed the largest treatment differences and the strongest correlations were found at wavelengths of, respectively, 1510 and 1537 nm for EWT and LWC<sub>F</sub>. These changes in reflectance in response to leaf desiccation are consistent with previously reported observations for other conifers including *Picea pungens* (Hunt and Rock, 1989), *Pinus patula* (Mutanga and Ismail, 2010) and *Pinus edulis* (Stimson et al., 2005). There was little treatment variation in reflectance within the visible range during the experiment which was consistent with visual observations that showed, with a few exceptions, little overall treatment difference in foliage colour (Fig. 1c).

Indices that accounted for variation in water content in the SWIR region were most strongly related to EWT and LWC<sub>F</sub>. Consistent with correlations using individual wavelengths, described above, the six strongest predictors of EWT used indices that included reflectance from wavelengths at either 1600 nm (GVMI, MSI, NDII) or 1640 and 1650 nm (NDWI<sub>1640</sub>, SIWSI, MSI<sub>2</sub>). The strongest predictors of LWC<sub>F</sub> used indices within this wavelength region (GVMI, MSI) but additionally included three indices with wavelengths from elsewhere in the SWIR/NIR region (Datt<sub>1</sub>, DDI, SRWI<sub>1</sub>). Floating band water index (fWBI) which uses reflectance from the NIR range (930–980 nm) was also a strong predictor of LWC<sub>F</sub>. These results are generally consistent with previous research that has shown foliage water content to be more strongly correlated to reflectance from wavelengths located in the SWIR than the NIR region (Tucker, 1980; Ceccato et al., 2002; Mutanga and Ismail, 2010). These results also agree with Danson and Bowyer (2004) who



**Fig. 7.** Correlation between LWC<sub>F</sub> and the strongest predictors from the four categories of indices for data extracted from the 5th measurement set (n = 50) taken 108 days after treatment. Shown are the strongest predictors from (a - c) water indices, (d) the photochemical index PRI, (e - f) structural indices and (g - i) chlorophyll indices. The observations are categorised by control (solid green circles) and drought (solid red circles) treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

found SWIR indices to be the best predictors of EWT while Water Index was a more precise predictor of the weight based index LWC<sub>D</sub> which is closely related to LWC<sub>F</sub>. Compared to SWIR based indices, Water Index is more sensitive to leaf dry weight (Danson and Bowyer, 2004) which may account for the slightly stronger relationships between fWBI and the weight-based metric LWC<sub>F</sub>.

Photochemical Reflectance Index, which is a widely used index of plant photosynthetic activity, was moderately correlated to foliage water content during the last capture. Research has widely demonstrated the utility of PRI for predicting light use efficiency (Garbulsky et al., 2011; Peñuelas et al., 2011) and key photosynthetic parameters under a range of stresses including limiting nutrition (Watt et al., 2020a), cold winter temperatures (Wong and Gamon, 2015a,b; Gamon et al., 2016) and herbicide damage (Scholten et al., 2019). The relationship between PRI and photosynthetic capacity has a strong theoretical basis as PRI can track plant photosynthetic activity through its intimate link with the dissipation of excess energy by nonphotochemical quenching (NPQ) via the xanthophyll cycle (Gamon et al., 1997). As water availability is a key regulator of photosynthesis, moderate to strong negative correlations have been found between PRI and mid-day needle water potential for a range of forest species (Ripullone et al., 2011). Our results are consistent with these findings and show that PRI is a useful determinant of long-term changes in foliage water content in radiata pine. Nevertheless, large effects due to photosynthetic pigments and leaf and vegetation structure on PRI require modelling methods to understand the direct links between stress and the photochemical index (Hernández-Clemente et al., 2019).

Predictors that were proxies for chlorophyll or structure were less strongly related to both measurements of water stress than PRI or water stress indices based on NIR and SWIR bands. Variations in chlorophyll may be attributable to water stress but are also more broadly affected by nutrition, phenology, disease and radiation stress (Larcher, 2003). As a consequence, there is no direct link between foliage water content and chlorophyll (Ceccato et al., 2001). This has been corroborated by case studies describing seasonal changes in leaf water content and chlorophyll that show little correlation between the two variables for five different forest species, including Pinus sylvestris (Gond et al., 1999). Similarly, although the structural index NDVI was moderately correlated with the two water stress indices, this index is an indirect proxy for water stress, typical of long-term sustained stress conditions. NDVI can be used to effectively discriminate between healthy and stressed foliage but the increases in reflectance within the red band that are characteristic of stress and needle dieback, are mainly attributable to reductions in chlorophyll (Asner, 1998; Rullan-Silva et al., 2013).

Predictions of leaf water content made using the indices described here could be scaled up using hyperspectral imagery from unmanned aerial vehicles (UAV) and fixed wing aircraft if the effects of the canopy structure are accounted for using radiative transfer modelling methods. The recent development of many lightweight and affordable hyperspectral imagers that can be fitted to UAVs will facilitate data acquisition over smaller areas (Watt et al., 2019). Other studies have shown the use of compact multispectral images on board drones for forestry applications, including the detection of stress using photosynthetic pigments such as reductions in chlorophyll and carotenoids (Hernández-Clemente et al., 2012). Water stress across larger areas has been identified using hyperspectral cameras on board fixed wing aircraft (Colombo et al., 2008; Hernandez-Clemente et al., 2014; Dotzler et al., 2015) and most of these systems can acquire data at sub-metre spatial resolutions when flown at 1,000 m above ground level (Watt et al., 2019).

The use of satellite multispectral or hyperspectral imagery may also provide a useful means of scaling up predictions at a lower spatial resolution across broader spatial ranges. There are a number of operational and planned sensors on board satellites that can obtain imagery at spatial resolutions of ca. 30 m (Watt et al., 2019) with hyperspectral capabilities such as the PRISMA (Cogliati et al., 2021) and EnMAP (Guanter et al., 2015) satellite sensors. Water stress within forests has been robustly predicted at the landscape level using data derived from MODIS (Byer and Jin, 2017; Xulu et al., 2018) and Hyperion (White et al., 2007; Zhang et al., 2011).

The processes associated with long-term forest decline are characterised by reductions in photosynthetic and non-photosynthetic pigments along with structural changes which in combination affect the indicators derived from multispectral and hyperspectral images (Zarco-Tejada et al., 2018). Previous modelling work, has used Sentinel-2 data to accurately estimate chlorophyll content and physical models to account for the forest architecture (Hernández-Clemente et al., 2019; Zarco-Tejada et al., 2019). Similarly, the successful retrieval of water content using sensitive spectral indices will require appropriate modelling methods that can account for such coupled physiological and structural changes induced by stress.

One of the more promising indices identified in this study was Normalised Difference Infrared Index (NDII), which was a strong predictor of foliage water content, and can be extracted from the multispectral imager on board Sentinel 2 (S2) at a spatial resolution of 20 m. The high revisit frequency of S2 makes it a useful platform for detecting water stress. Severe drought within Italian forests has been successfully identified using S2 indices derived from the 10 m VNIR bands (Puletti et al., 2019). However, with this exception, multispectral imagery has been less widely used to detect water stress than other general stress conditions in forests (Coops et al., 2006; Meiforth et al., 2020) or water stress within orchard crops (Coops et al., 2006; Zarco-Tejada et al., 2009; Suárez et al., 2010; Stagakis et al., 2012; Meiforth et al., 2020). As multispectral imagery is relatively inexpensive to acquire and process greater use of this imagery to detect water stress should be further explored.

Further research should focus on detection of short-term water stress in radiata pine. In response to water stress, stomata partially or wholly close and this occurs relatively rapidly in radiata pine (Mitchell et al., 2013; Mitchell et al., 2014). As a result of this stomatal closure, assimilation rates are reduced (Mitchell et al., 2014) and the reduction in transpiration generates an increase in foliage temperature during the day due to reduced evaporative cooling (Raschke, 1960). Previous research has used thermal imagery to characterise drought induced increases in leaf temperature in a range of tree species (Scherrer et al., 2011; Zarco-Tejada et al., 2012) including *Pinus sylvestris* (Seidel et al., 2016). Solar-induced chlorophyll fluorescence and PRI are two plant based traits that have often been used to robustly predict changes in photosynthetic activity resulting from a range of stresses, including drought stress (Ripullone et al., 2011; Zarco-Tejada et al., 2012; Watt et al., 2020b). However, changes in foliage temperature have been shown to be the most sensitive indicator of short to moderate term water stress (Zarco-Tejada et al., 2012) which highlights the potential of thermal imagery for detection of short term drought effects.

### 5. Conclusion

Under the conditions of this experiment, this study shows that water loss from droughted radiata pine progresses relatively slowly over the first three months but progresses more rapidly after this point. These long-term changes in EWT and LWC<sub>F</sub> were found to be strongly correlated to a number of SWIR and NIR based indices. In contrast, routinely used indicators of photosynthetic activity, chlorophyll, foliage density and structure were less well correlated to the two water stress metrics. Further research should investigate the potential of hyperspectral and thermal imagery to detect short-term water stress indicators such as assimilation rate, stomatal conductance and needle temperature in radiata pine.

### CRediT authorship contribution statement

Michael S. Watt: Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration. Ellen Mae C. Leonardo: Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration. Honey Jane C. Estarija: Data curation, Methodology, Writing – review & editing, Project administration. Peter Massam: Data curation, Methodology. Dilshan Silva: Data curation, Methodology. Renelle O'Neill: Data curation, Methodology. David Lane: Data curation, Methodology. Rebecca McDougal: Methodology, Writing – review & editing, Project administration. Henning Buddenbaum: Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Pablo J. Zarco-Tejada: Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix

See Fig A1 and A2



Fig. A1. Variation in (a) stomatal conductance and (b) assimilation rate over the duration of the experiment for the control (green bars) and drought (red bars) treatments. Shown are the mean (centre dots) and the 95% confidence interval (bars) for all measurements. The number of days after the start of the drought treatment (DAT) on the 29th January 2021 is shown at the top for all measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. A2. Variation in (a) height and (b) root-collar diameter of trees over the duration of the experiment for the control (green bars) and drought (red bars) treatments. Shown are the mean (centre dots) and the 95% confidence interval (bars) for all measurements. The number of days after the start of the drought treatment (DAT) on the 29th January 2021 is shown at the top for all measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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