



**UNIVERSIDAD DE TALCA  
FACULTAD DE CIENCIAS AGRARIAS  
MAGÍSTER EN HORTOFRUTICULTURA**

**ESTIMATION OF VINE WATER STATUS USING CROP WATER STRESS  
INDEX BASED ON LEAF ENERGY BALANCE, AND NATURAL LEAF  
REFERENCES UNDER MEDITERRANEAN WEATHER CONDITIONS IN THE  
CENTRAL VALLEY OF CHILE**

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**TESIS PARA OPTAR AL GRADO DE MAGÍSTER**

**TALCA - CHILE  
2020**

## CONSTANCIA

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Talca, 2021

## Índice

RESUMEN .....	ii
ABSTRACT .....	iii
1. Introduction .....	1
2. Materials and Method.....	4
2.1 Study site description .....	4
2.2 Experimental design.....	5
2.3 Midday stem water potential ( $\Psi_{MSWP}$ ) measurement .....	5
2.4 Canopy temperature measurements.....	5
2.5 Environmental measurements .....	6
2.6 CWSI calculation .....	6
2.7 Statistical analysis .....	9
3. Results .....	10
3.1 Climatic conditions and general measurements .....	10
3.2 CWSI Calculation .....	12
3.3 $\Psi_{MSWP}$ estimation.....	14
4. Discussion.....	17
5. Conclusions .....	18
7. Anexos.....	24
7.1 .Artículo enviado y aceptado por la editorial del “IX International Symposium on Irrigation of Horticultural Crops for publication in Acta Horticulturae” .....	24
7.2 Confirmación de aceptación de trabajo de congreso.....	31

## **RESUMEN**

Un experimento en terreno se llevó a cabo con el objetivo de calcular el Índice de Estrés Hídrico del Cultivo (CWSI) de una parcela de viña ubicada en el Valle Pehuenhue, Región del Maule, Chile ( $35^{\circ} 20'33''$  S,  $71^{\circ} 46'41''$  W, 86 msnm). Para este estudio, se evaluó el CWSI calculado a través de la Metodología de Balance de Energía (EBM) y la Metodología de Hojas de Referencia (LRM) con mediciones del potencial hídrico de xilema ( $\psi_{MSWP}$ ) para obtener la mejor correlación y determinar la metodología más adecuada para estimar el estado hídrico del cultivo durante la temporada. Se utilizaron las temperaturas del dosel obtenidas por termometría infrarroja y datos meteorológicos de una estación meteorológica automática para calcular el CWSI. Los resultados muestran que el CWSI obtenido a través de la EBM fue capaz de estimar  $\psi_{MSWP}$  de una manera más precisa con un  $r^2$  de 0,69. Este resultado sugiere que el CWSI calculado a través de EBM podría ser una excelente herramienta para estimar el estado hídrico de la planta de una manera no invasiva.

## **ABSTRACT**

A field experiment was carried out to calculate the Crop Water Stress Index (CWSI) of a vine plot located in the Pehuenhue Valley, Maule Region, Chile (35°20'33"S, 71°46'41"W, 86 m.a.s.l.). For this study, CWSI calculated through the Energy Balance Methodology (EBM) and the Leaf Reference Methodology (LRM) were evaluated with midday stem water potential ( $\Psi_{MSWP}$ ) measurements in order to obtain the best correlation, and determine the more suitable methodology to estimate plant water status during the season. Canopy temperatures obtained by infrared thermometry and meteorological data from an automatic weather station were used to calculate the CWSI. Results show that CWSI obtained through the EBM was able to estimate  $\Psi_{MSWP}$  in a more accurate way with an  $r^2$  of 0.69. This result suggests that the CWSI calculated through EBM could be an excellent tool for estimating plant water status in a non-invasive way.

**Keywords:** Plant water status, *Vitis vinifera*, Crop Water Stress Index, Midday stem water potential

## 1. Introduction

Most of the cultivated vineyards in the world are located in Mediterranean regions where seasonal droughts are frequent (Chaves et al., 2010). Also, for these regions an even more severe decrease in available water for agriculture is forecasted (Chaves et al., 2010; Gerhards et al., 2019; Pou et al., 2014). This decrease is produced by various factors, among which climate change stands out as the main cause of the lack of water for agricultural activity (Rockström et al., 2010). In addition to this, there are other factors such as competition for resources between agriculture, industry, hydroelectric plants and urban consumption, and the unsustainable use of groundwater also negatively influences the availability of this resource (Ortega-Farias et al., 2009; Han et al., 2018). This issue becomes a great challenge, especially for grape growers in Mediterranean areas, such as central Chile. Therefore, to maintain a stable production of adequate quality, water must be managed in a sustainable way (Gutiérrez et al., 2018), which is why grape growers have implemented various management strategies that seek to mitigate water scarcity during the productive season (Wade et al., 2004).

Several researchers have proposed Regulated Deficit Irrigation (RDI) methodology as an irrigation strategy to adapt viticulture to water scarcity scenarios. RDI is a technique that allows reducing the water application to vineyards in phenological stages where the vines are less sensitive to water stress (Romero et al., 2010; Munitz et al., 2017), having as the main result the improvement in fruit quality, but at the expense of yield (McCarthy et al., 2002; Santesteban et al., 2011). However, the application of RDI strategy requires an adequate monitoring of vine water requirements and plant water potential to avoid irreversible damage to the fruit (Romero et al., 2016). According to the literature, monitoring the vine water status using the pressure chamber methodology is one of the most reliable tool to evaluate the effect of RDI at different levels of water stress (Choné et al., 2001). This tool better integrates the effect of soil, cultivar, and climate over vine water status (Greenspan et al., 1996; Choné et al., 2001; Girona et al., 2006). In this sense, predawn leaf ( $\Psi_{PD}$ ), midday leaf ( $\Psi_L$ ), and midday stem ( $\Psi_{MSWP}$ ) water potentials have been suggested to evaluate irrigation scheduling in vineyards (García-Tejero et al., 2016; Gutiérrez et al., 2018; Matese et al., 2018; Santesteban et al., 2011). Girona et al. (2006) in the cv. Pinot Noir and García-Tejero et al. (2016) in the cv. Tempranillo indicated that the  $\Psi_L$  provides reliable evaluation of vine water status because this measurement is less variable than the water balance, and provides site-specific information. However, Williams & Araujo (2002) in the cultivars Chardonnay and Cabernet Sauvignon concluded that although  $\Psi_L$  has been widely used to monitor plant water status, it is not representative enough, since it has a variety of factors affect its measurement, such as vapor pressure deficit and solar radiation (Choné et al.,

2001), which could vary from one leaf to another, which means it represents the measured leaf and not whole plant (Acevedo-Opazo et al., 2010).

On the other hand, midday stem water potential ( $\Psi_{MSWP}$ ) stands out as a more suitable indicator of plant water status (Choné et al., 2001; Valenzuela, 2011). It accurately represents the vine water status, even if the soil water content is not homogeneous (Shackel, 2007), being less variable than  $\Psi_L$  (Choné et al., 2001). Also,  $\Psi_{MSWP}$  can isolate micro-environmental factors because the leaf transpiration is interrupted in order to prepare the leaf for measurement (Shackel, 2007), accurately measuring vine water status and providing a representative value of the whole plant (Delrot, 2010), reducing the number of measurements necessary to characterize vine water status. However, this type of measurement is expensive, destructive and time-consuming, and it does not consider the spatial variability of the productive unit under study (Acevedo-Opazo et al., 2008; Bellvert et al., 2015). The foregoing raises the need to seek a different alternative to this measurement that allows indirectly and quickly estimating the vineyard water status, considering its spatial variability, being the use of the Crop Water Stress Index (CWSI) a promising option.

Several authors have pointed out that leaf temperature is directly related to the plant water status and its transpiratory rate (Baluja et al., 2012; Cohen et al., 2015; Costa et al., 2013; Fuchs, 1990; Jackson et al., 1981; Jones, 1999; Jones et al., 2009). This information is obtained using infrared radiometers or thermal cameras, and these devices work considering that bodies with temperatures above 0 °K emit infrared radiation (Morales et al., 2011; Diaz, 2012). Vadivambal & Jayas (2011) pointed out that infrared thermography can be used for various operations, such as estimating soil water status, crop water stress, stomatal conductance, biotic and abiotic stress detection, among others. In viticulture, Santesteban et al. (2017) and Fuentes et al. (2012) indicated that thermography is an excellent tool to estimate the water status of plants, since water stress reduces transpiration due to stomatal closure, increasing the plant canopy temperature (Matese et al., 2018; Stoll et al., 2008).

The information obtained from thermal sensors is integrated into indices that allow estimating the stress degree of the vineyard in a non-invasive way (Gutiérrez et al., 2018). However, a normalization of temperatures is required that considers the effect of environmental factors on the plant canopy temperature, such as shortwave incident radiation, relative humidity, and wind speed (Agam et al., 2013). Several reports indicate that the CWSI appears as a standardized index that would allow quantifying the degree of water stress of a crop, eliminating the effect of environmental variables (García-Tejero et al., 2016; Poblete-Echeverría et al., 2017). The CWSI ranges from 0 to 1, where 0

represents a well-irrigated crop that transpires at its potential rate, and 1 represents a crop with high water stress, that does not transpire (King & Shellie, 2016). The CWSI has been suggested in grapevines as an evaluation tool for irrigation scheduling with promising results (Baluja et al., 2012; Matese et al., 2018; Pou et al., 2014; Santesteban et al., 2017).

Three methodologies have been widely used to calculate this index, (i) the leaf reference methodology (LRM), proposed by Jones (1999), (ii) the empirical methodology (EM), developed by Idso et al. (1981) and (iii) the theoretical or leaf energy balance methodology (EBM) proposed by Jones (1992). For the purpose of this study, only LRM and EBM will be addressed. The LRM is based on the use of dry ( $T_{dry}$ ) and wet ( $T_{wet}$ ) reference leaves, in order to compare the canopy temperature with the temperature that the leaf would have under conditions of minimum and maximum transpiration, so the determination of temperature limits are more straightforward (Hamlyn G. Jones, 1999; Leinonen & Jones, 2004), but the choice of references, whether artificial or natural, could generate differences in the results, given the nature of the different type of used materials.

Studies such as that of Baluja et al. (2012) and Santesteban et al. (2017) used this methodology and they were able to determine that there is a significant correlation between the CWSI and  $\Psi_{MSWP}$  (with  $R^2$  of 0.5 and 0.6, respectively) on the vineyard.

Finally, the EBM estimates CWSI using the energy balance of the leaf. This methodology calculates the stress level of a crop considering the effect of environmental factors in addition to the crop, so factors such as net radiation, wind speed and environmental temperature are integrated. This methodology has been used in several studies. Alchanatis et al. (2010), for instance, tested energy balance equations to estimate crop water status in cotton ( $R^2 = 0.69$ ). Möller et al. (2007), who evaluated several CWSI calculation methodologies in grapevine, found promising results regarding the use of EBM ( $R^2$  ranging from 0.52 to 0.91), highlighting the issue of the variability of CWSI estimation between dates, explained by the authors due to the possible adjustments made by the plant in response to changes in water potential during summer. Osroosh et al. (2015), in apple trees, observed a relation between CWSI and  $\Psi_{MSWP}$  ( $R^2 = 0.91$ ), concluding that this method was suitable to characterize the water status of that crop.

Taking into account the above and the current situation of agriculture in Mediterranean areas in relation to the decrease in available water for irrigation, the use of this index is proposed as a viable tool to estimate plant water status, considering the spatial variability of the vineyard and incorporating



non-destructive measurements. Therefore, the main goal of this work is to estimate the  $\Psi_{MSWP}$  of an irrigated commercial vineyard, under different irrigation treatments, using the EBM together with the LRM to calculate the CWSI.

## **2. Materials and Method**

### 2.1 Study site description

The study was carried out during the 2017/2018 and 2018/2019 seasons on a commercial vineyard (*Vitis vinifera* L. cv. Cabernet Sauvignon) located in the Penciahue Valley, Maule, Chile (35°20'36.00"S, 71°46'42.19"O, 86 m.a.s.l.) (Figure 1). The vineyard was established in 2014 in an area of 4.5 ha, from which the actual study area covered 1.4 hectares, with a spacing between rows and over rows of 1 x 2 m, respectively (5,000 ha<sup>-1</sup> plants), with an east-west orientation, conducted in a simple vertical shoot positioned trellis system with a height of 2 m and drip-irrigated with a flow rate of 2 L h<sup>-1</sup>.

The climate is Mediterranean semi-arid, with an average temperature of 14.8° C, an average annual rainfall of 605 mm, mainly concentrated in the winter months (June – September) and a cumulative reference Evapotranspiration (ET<sub>0</sub>) of 1013 mm from September to May.

The soil of the study site belongs to Las Doscintas series (LDC) and is characterized by having a colluvial sedimentary origin. It has a sandy-loam texture, with an effective depth between 10 to 70 cm deep. The slope of the field varies between 2 and 10%, presenting a moderate to imperfect drainage.



**Figure 1.** Location of the study site, corresponding to the Penciahue Valley, Maule, Chile.

## 2.2 Experimental design

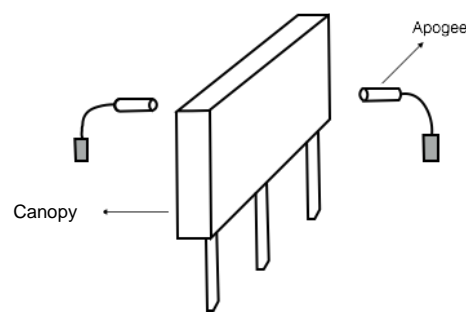
At the study site, two completely randomized treatments were established, which consisted of applying two irrigation thresholds based on values of plant water status (T0 = no stress, with  $\Psi_{MSWP}$  ranging from -0.8 to -1.0 MPa; T1= moderate-strong water stress, with  $\Psi_{MSWP}$  ranging from -1.0 to -1.4 MPa) established according to values proposed by van Leeuwen et al. (2008), between fruit set and veraison, with four repetitions each. Irrigation was suspended until the defined thresholds were reached, based on  $\Psi_{MSWP}$  measurements, to later reestablish irrigation.

## 2.3 Midday stem water potential ( $\Psi_{MSWP}$ ) measurement

An adult and healthy leaf was selected, from the middle third of the canopy that was completely exposed to the sun, which was covered with plastic film and aluminum foil to prevent the leaf from transpiring and performing photosynthesis. After one hour, and close to midday, the leaf was removed from the canopy and the measurement was carried out in a pressure chamber (model 600, PMS Instrument Company, Oregon, USA).

## 2.4 Canopy temperature measurements

For this measurement, a portable infrared radiometer (model MI-2H0, FOV 32°, Apogee Instruments, North Logan, UT, USA) was used to obtain temperatures from the north and south side of the canopy. The middle third of the canopy was measured, for which the infrared radiometer was placed at 30 cm from the canopy, and after 10-15 seconds, the temperature was recorded (Figure 2). To obtain a more accurate measurement, three repetitions were performed on each side of the canopy and then they were averaged.



**Figure 2.** Diagram of canopy temperature measurements performed from the north and south side of the canopy, during 2017/18 and 2018/19 growing seasons, using an infrared radiometer (Apogee).

## 2.5 Environmental measurements

Environmental variables used to calculate CWSI were obtained from an automatic weather station located within the study site at a height of 2m from the soil, which is measured and recorded every 15 minutes. Relative humidity (RH) and air temperature ( $T_a$ ) were measured with a relative humidity sensor (HMP60, Vaisala, Logan, UT, USA). Wind speed ( $u$ ) was measured with an anemometer (03101-5, R. M. Young Co., Traverse City, MI, USA), and net radiation ( $R_n$ ) using a four-way net radiometer (NR Lite, Kipp & Zonen, Delft, The Netherlands).

## 2.6 CWSI calculation

CWSI was performed using the following two methodologies:

**2.6.1 Leaf Reference Methodology (LRM):** this methodology is based on the equation proposed by Jones, 1999, where  $T_{wet}$  and  $T_{dry}$  were obtained from an infrared radiometer, choosing the highest canopy temperature as  $T_{dry}$  and the lowest as  $T_{wet}$ , therefore CWSI is calculated as:

$$CWSI = \frac{T_c - T_{wet}}{T_{dry} - T_{wet}} \quad (1)$$

where,  $T_c$  = canopy temperature ( $^{\circ}C$ ),  $T_{wet}$  = temperature of a fully transpiring leaf ( $^{\circ}C$ ) and  $T_{dry}$  = a leaf with fully closed stomata ( $^{\circ}C$ ).

**2.6.2 Energy Balance Methodology (EBM),** or theoretical method: in this case,  $T_{wet}$  and  $T_{dry}$  were estimated through the leaf energy balance as follows:

$$T_{wet} = T_a + \frac{r_{HR} r_{aW} \gamma R_{ni}}{\rho_a C_p (\gamma r_{aW} + \Delta r_{HR})} - \frac{r_{HR} VPD}{\gamma r_{aW} + \Delta r_{HR}} \quad (2)$$

where,  $T_{wet}$  = reference of a leaf transpiring at its maximum rate ( $^{\circ}C$ );  $T_a$  is air temperature ( $^{\circ}C$ ),  $r_{HR}$  is the resistance to the radiative heat transference ( $s\ m^{-1}$ ),  $r_{aW}$  is the resistance of the boundary layer

to water vapor ( $s\ m^{-1}$ ),  $\gamma$  is the psychrometric constant ( $Pa\ K^{-1}$ ),  $R_{ni}$  is isothermal net radiation ( $W\ m^{-2}$ ),  $\rho_a$  is air density ( $1.2\ kg\ m^{-3}$ ),  $C_p$  is the specific heat of the air ( $1005\ J\ kg^{-1}\ K^{-1}$ ),  $\Delta$  is the slope of saturated water vapor pressure versus temperature curve ( $Pa\ K^{-1}$ ) and VPD is vapor pressure deficit (Pa).

$$T_{dry} = T_a + \frac{r_{HR} R_{ni}}{\rho_a C_p} \quad (3)$$

where;  $T_{dry}$  = reference of a leaf with fully closed stomata ( $^{\circ}C$ ).

$R_{ni}$  was estimated as:

$$R_{ni} = (R_n + g_R) * \rho_a C_p (T_c - T_a) \quad (4)$$

where;  $R_n$  = net radiation ( $W\ m^{-2}$ ),  $g_R$  = conductance of radiative heat transference ( $m\ s^{-1}$ ),  $T_c$  = canopy temperature ( $^{\circ}C$ ).

It is important to note that two types of  $R_n$  were used to calculate  $R_{ni}$ , therefore, from now on, EBM will be addressed in two ways: EBM Model 1 ( $M_1$ ) and EBM Model 2 ( $M_2$ ), where  $M_1$  refers to  $R_{ni}$  calculated using measured  $R_n$  and  $M_2$  refers to  $R_{ni}$  calculated, according to Ortega-Farías et al. (2016).

$R_n$  was estimated as:

$$R_n = R_s(1-\alpha) + R_{lin} - R_{loutc} - (1-\epsilon_c) * R_{lin} \quad (5)$$

Where;  $R_s$  = solar radiation ( $W\ m^{-2}$ ),  $\alpha$  = albedo (0.16),  $R_{lin}$  = longwave incoming radiation ( $W\ m^{-2}$ ),  $\epsilon_c$  = canopy emissivity (0.98) and  $R_{loutc}$  = longwave outgoing radiation from the canopy ( $W\ m^{-2}$ ).

$$R_{lin} = \epsilon_{atm} \sigma T_a^4 \quad (6)$$

Where;  $\epsilon_{atm}$  = emissivity of the atmosphere (dimensionless)

$$\varepsilon_{\text{atm}} = 1.02(-\ln(T_{\text{sw}}))^{0.236} \quad (7)$$

Where;  $T_{\text{sw}}$  = atmospheric transmissivity (dimensionless)

$$T_{\text{sw}} = \frac{R_s}{R_a} \quad (8)$$

Where;  $R_a$  = extraterrestrial radiation ( $\text{W m}^{-2}$ )

$$R_{\text{loutc}} = \varepsilon_c \sigma T_c^4 \quad (9)$$

$r_{\text{HR}}$  was estimated as follows:

$$r_{\text{HR}} = \frac{1}{g_{\text{aH}} + g_{\text{R}}} \quad (10)$$

$$g_{\text{aH}} = \frac{1}{100 \sqrt{\frac{d}{u}}} \quad (11)$$

$$g_{\text{R}} = \frac{4\varepsilon\sigma T_a^3}{\rho_a C_p} \quad (12)$$

where;  $g_{\text{aH}}$  = conductance of leaf boundary layer to the transference of heat ( $\text{m s}^{-1}$ ),  $g_{\text{R}}$  = conductance of radiative heat transference ( $\text{m s}^{-1}$ ),  $d$  = leaf dimension (m) and  $u$  = wind speed ( $\text{m s}^{-1}$ ),  $\sigma$  = Stefan-Boltzmann constant ( $5.6703 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$ ) and  $T_a$  = air temperature ( $^{\circ}\text{K}$ ).

$r_{\text{aW}}$  was calculated as:

$$r_{\text{aW}} = \frac{1}{g_{\text{aW}}} \quad (13)$$

$$g_{\text{aW}} = \frac{g_{\text{aH}}}{0.92} \quad (14)$$

where;  $g_{aW}$  = conductance of boundary layer to water vapor

It is important to note that two methods were used to obtain  $R_n$  and be able to calculate  $R_{ni}$ , therefore, from now on, EBM will be addressed in two ways: EBM Model 1 ( $M_1$ ) and EBM Model 2 ( $M_2$ ), where  $M_1$  refers to  $R_{ni}$  calculated using measured  $R_n$  and  $M_2$  refers to  $R_n$  calculated according to the methodology proposed by Ortega-Farías et al. (2016).

## 2.7 Statistical analysis

The evaluation between MSWP and CWSI was performed by linear regression analysis and to evaluate the degree of error of these models, a validation was carried out using the root-mean-square error (RMSE), and mean absolute error (MAE) (Willmott, 1982), calculated as:

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (E_i - O_i)^2}{N}} \quad (15)$$

$$MAE = N^{-1} \sum_{i=1}^N |E_i - O_i| \quad (16)$$

where;  $N$  = number of data,  $E_i$  = estimated value and  $O_i$  = observed value.

In addition to this, differences between treatments for variables such as yield,  $\Psi_{MSWP}$  and CWSI, were determined through an ANOVA using the R software (R Core Team, 2019).

### **3. Results**

#### 3.1 Climatic conditions and general measurements

General atmospheric conditions for the two study seasons are shown in Table 1. Average temperature during the measurement period (between 12:00 and 14:00 pm) was 26.4 °C, being relatively stable throughout the entire period, with the exception of DOY 33 (2017/18), 10 and 17 (2018/19), which showed temperature values considerably lower than the rest of the day sampled, with values of 23.4, 21.3 and 22.8, respectively. These results coincide with the lowest values observed for the variables of RH and VPD. On the other hand, the average values for the variables of relative humidity (RH), vapor pressure deficit (VPD), net radiation (Rn), wind speed (u) and actual evapotranspiration (ETa), were 35.3%, 2.3 kPa, 670.6 W m<sup>-2</sup>, 1.2 m s<sup>-1</sup> and 4.74 mm day<sup>-1</sup>, respectively.

**Table 1.** Meteorological conditions at the time of measurements for each day of study, for the 2017/18 and 2018/19 growing seasons.

<b>Season</b>	<b>Date</b>	<b>T°</b>	<b>RH</b>	<b>VPD</b>	<b>Rn</b>	<b>u</b>	<b>ETa</b>
	<b>DOY</b>	<b>(°C)</b>	<b>(%)</b>	<b>(kPa)</b>	<b>(W m<sup>-2</sup>)</b>	<b>(m s<sup>-1</sup>)</b>	<b>(mm day<sup>-1</sup>)</b>
<b>17/18</b>	<b>363</b>	30,9	26,4	3,30	684,6	1,33	5,05
	<b>2</b>	26,2	27,2	2,49	665,9	1,25	4,40
	<b>28</b>	26,5	31,4	2,38	700,0	1,81	7,14
	<b>33</b>	23,4	50,6	1,4	677,4	1,9	3,87
	<b>38</b>	26,8	38,5	2,18	676,2	0,91	5,48
	<b>44</b>	30,5	28,0	3,15	676,9	1,63	6,02
	<b>52</b>	28,1	40,0	2,29	670,8	0,87	5,20
<b>18/19</b>	<b>362</b>	25,4	37,8	2,03	687,9	1,05	4,45
	<b>4</b>	25,5	38,6	2,03	676,0	0,66	2,06
	<b>10</b>	21,3	37,0	1,60	692,4	0,98	4,04
	<b>17</b>	22,8	37,7	1,75	649,9	0,63	4,21
	<b>24</b>	28,3	28,8	2,75	650,5	0,67	4,40
	<b>30</b>	27,2	35,9	2,33	652,6	1,07	4,68
	<b>45</b>	27,3	35,4	2,36	627,3	1,66	5,41

Where; T° = average temperature, RH = average Relative Humidity, VPD = average Vapor Pressure Deficit, Rn = average Net Radiation, u = wind speed and ETa = average Actual evapotranspiration.

Vine water status was determined by  $\Psi_{MSWP}$  measurements performed close solar noon, finding significant differences between treatments. Average data of  $\Psi_{MSWP}$ , CWSI and Yield for both seasons are shown in Table 2. In this regard, statistical differences were observed among treatments for all the study variables, which indicates that the irrigation treatments generated a significant effect on the plant variables during both study seasons.

For both study seasons, CWSI values were higher in the treatment with the greatest water restriction, as was the  $\Psi_{MSWP}$  measurement, which would indicate that the vineyard was sensitive to the different irrigation treatments applied. On the other hand, the lowest value of  $\Psi_{MSWP}$  was recorded in DOY 38 of the 2017/18 season, for T1, reaching values of -1.40 MPa, and the highest values for this measurement were recorded in DOY 362 and 24 of the 2018/19 season for T0, reaching values of -0.50 MPa.

In the case of the 2018/19 growing season, the level of water stress reached was lower than that of previous season, which would have been corroborated by the CWSI values estimated through EBM and LRM. In this regard, it is important to note that general CWSI-LRM values were higher than CWSI-EBM values during both study seasons, and CWSI values in general were lower in the south side of the vineyard canopy, which would be explained mainly because the south side of the canopy corresponds to the shaded side in this study site during the measurement period.

**Table 2.** Effect of irrigation treatments over seasonal values of Crop Water Stress Index, Midday Stem Water Potential and Yield.

		$\Psi_{MSWP}$ (MPa)	CWSI- LRM North	CWSI- LRM South	CWSI- EBM North	CWSI- EBM South	Yield (kg plant <sup>-1</sup> )
<b>A. Treatment</b>	<b>T0</b>	-0.75	0.35	0.30	0.23	0.19	6.5
	<b>T1</b>	-1.10	0.51	0.45	0.38	0.33	3.8
		*	*	*	*	*	*
<b>B. Season</b>	<b>2017/18</b>	-1.05	0.48	0.43	0.34	0.30	5.3
	<b>2018/19</b>	-0.80	0.38	0.33	0.27	0.22	5.0
		*	*	*	*	*	*
<b>A x B Significance</b>		n.s	n.s	n.s	n.s	n.s	n.s

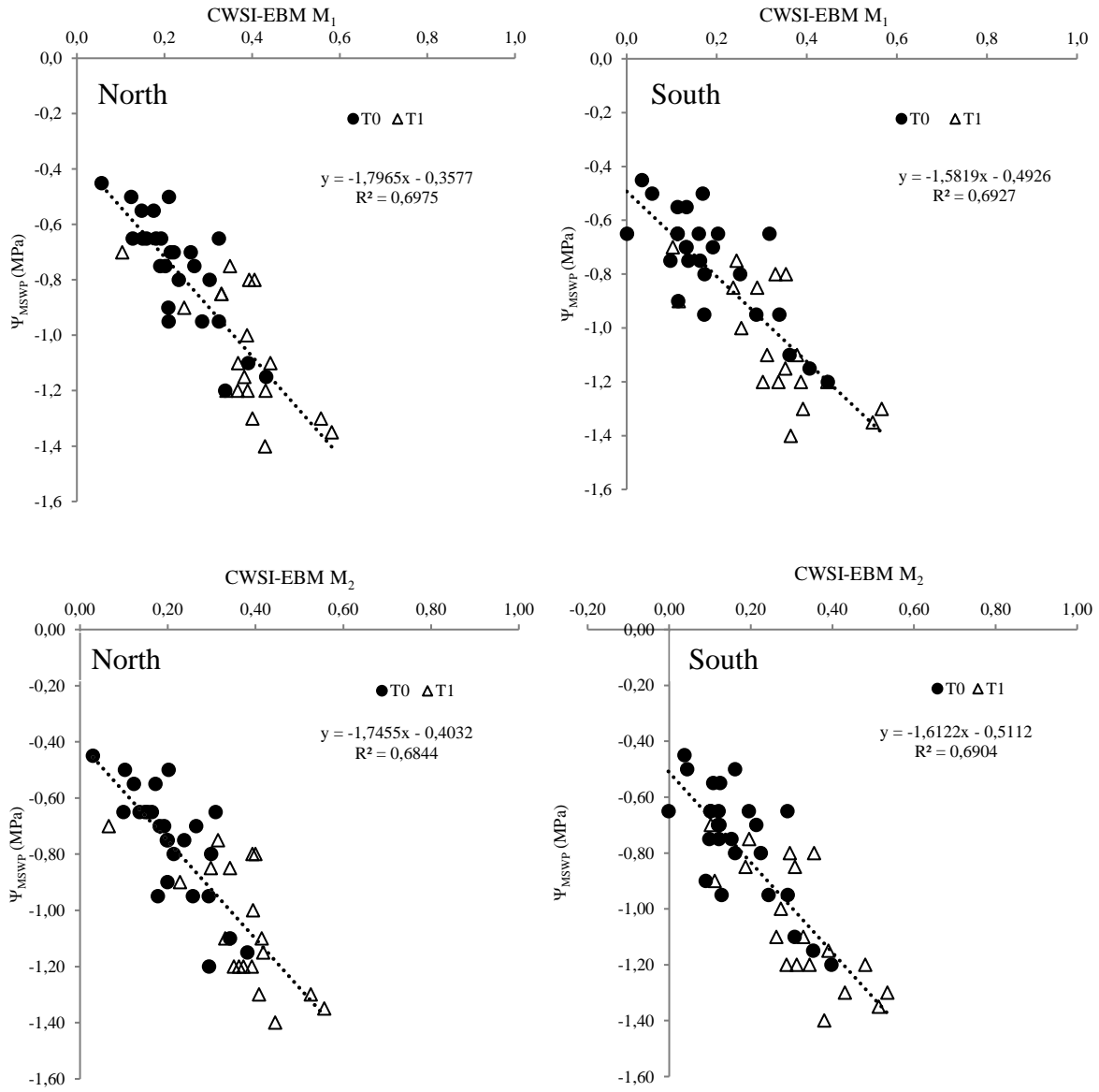


T0 = plants under no water stress; T1 = plants under moderate-severe water stress  
CWSI-LRM = CWSI calculated using the leaf reference methodology  
CWSI-EBM = CWSI calculated using the energy balance methodology  
\*p-value<0.05, which indicates significant differences.  
n.s = not significant.

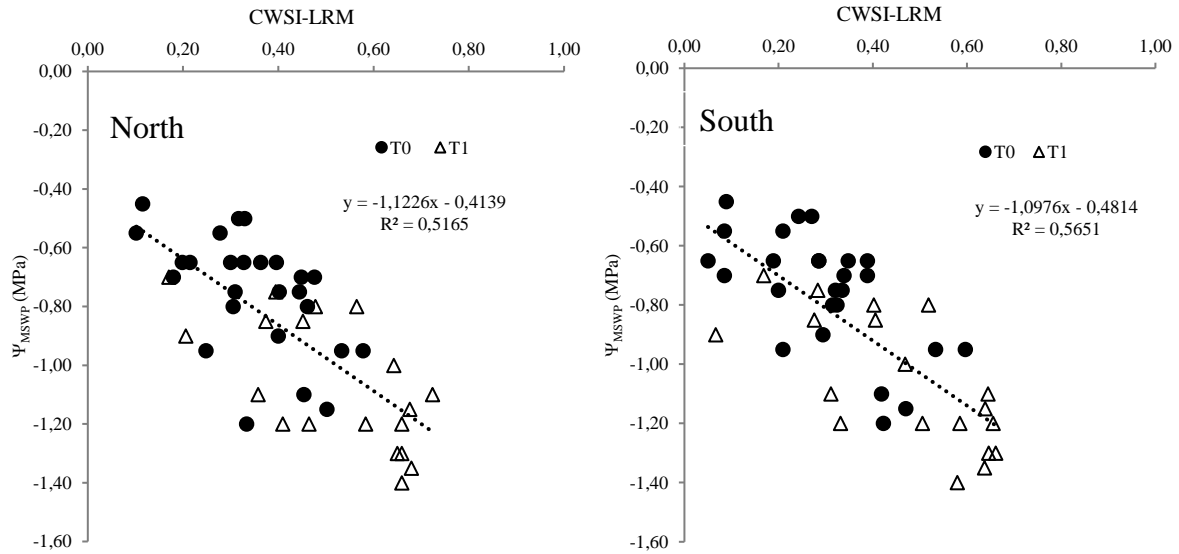
### 3.2 CWSI Calculation

After the CWSI calculation, linear regressions between  $\Psi_{MSWP}$  and CWSI-EBM ( $M_1$  and  $M_2$ ) and CWSI-LRM were carried out, showing high correlations in both cases (Figures 1 and 2), with the highest correlations for EBM ( $M_1$  and  $M_2$ ). These results indicate that EBM can be accurately correlate with  $\Psi_{MSWP}$  regardless of the side of the canopy where the measurement is made. Additionally, it is important to note that there are no significant differences between the methodologies used to calculate  $R_{ni}$  ( $M_1$  y  $M_2$ ) in terms of the correlation results observed between CWSI-EBM and  $\Psi_{MSWP}$ , which indicates that for the use of the leaf energy balance,  $R_n$  can be successfully estimated empirically, obtaining similar results to those obtained using measured  $R_n$  in the field.

For the case of LRM,  $R^2$  values obtained for the north and south side of the canopy were of 0.51 and 0.56, respectively. These results would indicate that this methodology (CWSI-LRM) is less accurate than the two previously presented in this document. On the other hand, the results would indicate that CWSI-LRM of the south side of the canopy can estimate  $\Psi_{MSWP}$  in a slightly more accurate way than the measurement performed on the north side of the plant.



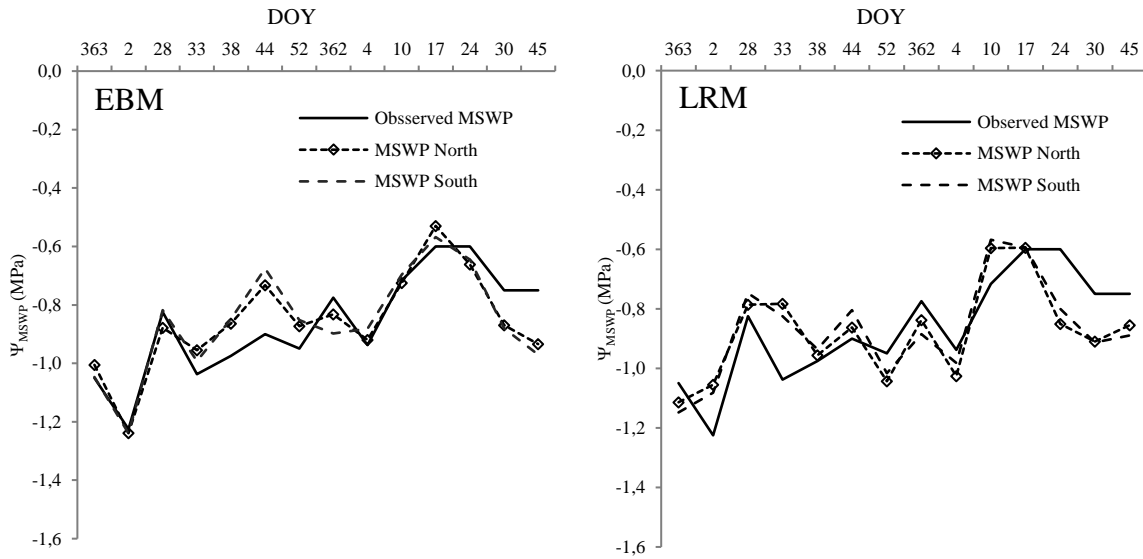
**Figure 2.** Relationship between global Crop Water Stress Index calculated through the leaf energy balance methodology (CWSI-EBM) for both seasons, for the north and south side of the canopy, calculated using  $M_1$  and  $M_2$ .



**Figure 3.** Relationship between global Crop Water Stress Index calculated through the leaf reference methodology (CWSI-LRM) for both seasons, for the north and south side of the canopy.

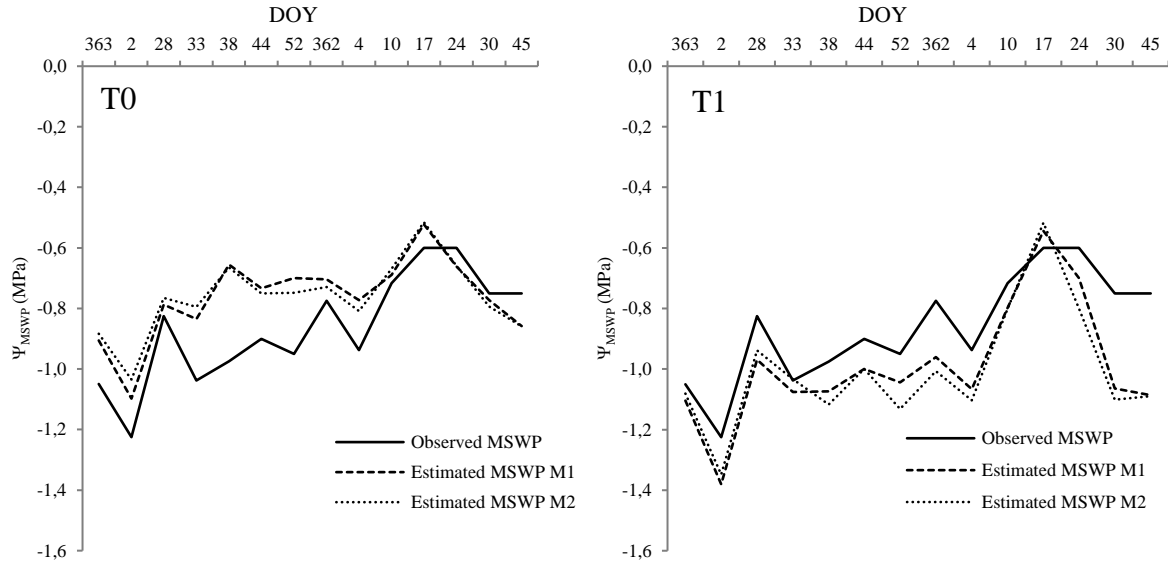
### 3.3 $\Psi_{MSWP}$ estimation

After the CWSI calculation, a comparison was made between the observed and estimated  $\Psi_{MSWP}$  values through CWSI-EBM and CWSI-LRM for the north and south side of the vineyard canopy (Figure 3). The results showed a better correlation between  $\Psi_{MSWP}$  and CWSI-EBM, due to this the estimation of vine water status was proposed using the  $\Psi_{MSWP}$  estimated by EBM for the north side of the canopy, and also a separation between irrigation treatments was proposed (Figure 4), incorporating  $M_1$  and  $M_2$ , which allowed to observe the effect that each treatment had on the estimation of  $\Psi_{MSWP}$  using the CWSI-EBM.



**Figure 4.** Observed versus estimated  $\Psi_{MSWP}$  for the north and south side of the canopy using the EBM and LRM for the 2017/18 and 2018/19 growing seasons.

After estimation  $\Psi_{MSWP}$ , error estimators were calculated to evaluate the degree of deviation of the models (Table 3). The RMSE and MAE values for EBM ranged from 0.09 to 0.11 MPa and 0.08 to 0.09 MPa, respectively, which would indicate that EBM can accurately estimate  $\Psi_{MSWP}$ . On the other hand, the LRM showed RMSE and MAE values of 0.13 and 0.10 MPa, respectively. This result would indicate a lower precision in the estimation of  $\Psi_{MSWP}$  compared to the EBM.



**Figure 5.** Observed versus estimated  $\Psi_{MSWP}$  for the treatment under no water stress (T0) and the treatment with moderate-strong water stress (T1), using the EBM (M<sub>1</sub> and M<sub>2</sub>) for the 2017/18 and 2018/19 growing seasons.

**Table 3.** Error estimators for  $\Psi_{MSWP}$  estimation using the Energy Balance Methodology (EBM M<sub>1</sub> and M<sub>2</sub>) and the leaf reference methodology (LRM), for the north and south of the canopy.

	EBM (M <sub>1</sub> )		EBM (M <sub>2</sub> )		LRM	
	N	S	N	S	N	S
<b>RMSE (MPa)</b>	0.09	0.11	0.10	0.11	0.13	0.13
<b>MAE (MPa)</b>	0.08	0.08	0.08	0.09	0.10	0.10

#### **4. Discussion**

The prevailing climatic conditions during the study period favored a water-demanding environment conditions, and as a result, a condition of moderate to severe water restriction was reached, specifically in the treatment with less irrigation input. This condition of lack of water generated differences in canopy temperature and  $\Psi_{MSWP}$  values, allowing to obtain significant differences between irrigation treatments during both study seasons. In addition, CWSI was highly influenced by environment conditions, acting as an accurate estimator of plant water status in the case of the treatment with the highest water restriction, which is in agreement with the literature, as indicated by Matese et al. (2018), who stated that, in Mediterranean areas, CWSI can be a reliable indicator of crop water status.

Regarding CWSI calculation, the use of energy balance equations demonstrated that this methodology is viable for an accurate estimation of vine water status. In this regard, results in this study showed a higher correlation between CWSI-EBM ( $M_1$  and  $M_2$ ) and  $\Psi_{MSWP}$  compared to LRM. The lower correlation between CWSI-LRM and  $\Psi_{MSWP}$  could be explained mainly by the influence of environmental variables such as wind speed, air temperature (Matese et al., 2018) and radiation (Pou et al., 2014), in addition of the involuntary inclusion of non-plant materials that could affect the temperature measurements (Jones, 2002).

In the case of CWSI-EBM and the use of two models proposed for the calculation of Rni, it is important to highlight the accuracy of the results obtained when using Rn calculated according to the methodology proposed by Ortega et al., (2016), indicating that the determination of this variable does not necessarily have to be done using a net radiometer, opening the possibility of reducing costs when calculating this index. This would allow the implementation of a more practical methodology for vineyard water management at the farmer level, through the introduction of low-cost spatialized sensors. In addition to this, the opportunity of taking this methodology and applying it through the use of Unmanned Aerial Vehicles (UAV) with high resolution thermal cameras arises as an interesting alternative to the use of ground-level sensors, as has been stated in literature (Baluja et al., 2012; Bellvert et al., 2014, 2015; Pagay & Kidman, 2019), having a great advantage in comparison with traditional water status sensors (Fernández, 2014), given that, in this way, it would be possible to overcome the main limitation of the currently used methodologies, which is the difficulty to characterize a whole plot in a rapid way.

## **5. Conclusions**

The current methodologies used to estimate plant water status are characterized by being time-consuming, labor-intensive, high-cost, and unable to account for the field spatial variability. That is why the use of thermal indices such as the CWSI, which can be estimated remotely, are presented as a possible tool to assess vine water status in a more accurate and faster way than the methodologies traditionally used. In this sense, results obtained in this study suggest that it is possible to accurately estimate  $\Psi_{\text{MSWP}}$  using CWSI calculated through the EBM. Estimated values of  $\Psi_{\text{MSWP}}$  using the EBM presented low errors compared to the observed  $\Psi_{\text{MSWP}}$  values measured in the field, with RMSE and MAE values of 0.09 to 0.11 and 0.08 to 0.09 MPa.

The results obtained are consistent with those observed in the literature. This would indicate that this methodology could be successfully implemented at the field level, with the possibility of improving this estimate by incorporating a network of low-cost spatialized thermal sensors that allow a better understanding of the natural spatial variability of the vineyard's water status.

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## 7. Anexos

7.1. Artículo enviado y aceptado por la editorial del “IX International Symposium on Irrigation of Horticultural Crops for publication in Acta Horticulturae”

### Estimation of vineyard water status using infrared thermometry measured at two positions of the canopy

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#### Abstract

Thermal sensors have been widely used as a way to indirectly estimate plant water status. The information of these sensors can be integrated into the crop water stress index (CWSI), which is conceived as a normalized index able to estimate the midday stem water potential (MSWP). In this regard, several researchers have indicated that CWSI based on the leaf energy balance approach could be a suitable tool to evaluate vine water status. In this sense, a study was carried out during the 2017/2018 growing season to calculate CWSI through the leaf energy balance approach, over a drip irrigated vineyard located in the Pencahue Valley, Maule Region, Chile (35°20'33"S, 71°46'41"W, 86 m.a.s.l.). For this, two irrigation treatments were established after veraison, where temperatures from the north and south side of the canopy were obtained through infrared thermometry, along with environmental and MSWP measurements. CWSI was able to estimate MSWP from the north and south side of the canopy, with an R<sup>2</sup> of 0.62 and 0.45 respectively.

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**Keywords:** thermometry, crop water stress index (CWSI), midday stem water potential, leaf energy balance, grapevine

#### INTRODUCTION

Agriculture will face a reduction in the available water for irrigation, mainly because of the effect of climate change, which will reduce precipitations over important agricultural regions. In the Mediterranean regions, in particular, there is evidence that temperatures have risen and precipitations have decreased, along with a concentration of precipitations in a shorter period of time (del Pozo et al., 2019).

Therefore, irrigation strategies have been developed to reduce water applied during the growing season (Medrano et al., 2015), keeping in mind that the challenge is to reduce water use with non-negative effects in production (del Pozo et al., 2019). This is particularly important for grapevine, given that it is usually grown under Mediterranean regions (Permanhani et al., 2016). In this regard, a good alternative to optimize water use corresponds

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to the regulated deficit irrigation (RDI) technique, which consists in a reduction of applied water to induce a certain level of water stress, generally used to increase water productivity, manipulate vegetative development of grapes and to improve harvest quality (Romero et al., 2016; Santesteban et al., 2011).

A key element to consider when applying water stress is to have adequate measurements of plant stress conditions (Sepúlveda-Reyes et al., 2016). Several methodologies have been suggested based on physiological measurements, among which midday stem water potential (MSWP) has proven to be a reliable tool to quantify vine water stress (Choné et al., 2001). In this regard, the MSWP is more sensible plant water status estimations (Shackel et al., 1997), but has the disadvantage of being time consuming and destructive (Ihuoma y Madramootoo, 2017). In addition to this, it is not able to consider spatial variability of the field (Acevedo-Opazo et al., 2008), therefore, a new type of methodology is needed in order to overcome the aforementioned problems.

In this sense, the use of remote sensing arises as an alternative, specifically the use of thermal sensors (Khanal et al., 2017), which have been presented as an indirect way to measure vine water status through canopy temperature monitoring. These sensor can either be used for measurements at ground level, like infrared thermometers (Jones et al., 2009) or mounted on unmanned aerial vehicles (UAV) (Fuentes-Peñailillo et al., 2019). Their usefulness is based on the fact that leaf temperature depends on environmental factors (e.g air temperature, wind speed ( $u$ ), stomatal closure or opening, among others) (Jones et al., 2009). Therefore, canopy temperature, and more specifically, canopy temperature minus air temperature ( $T_c-T_a$ ), is proposed as an indicator of plant water status (Jackson et al., 1981).

However,  $T_c-T_a$  by itself is not able to properly estimate plant water status, given that other parameters such as net radiation and wind speed have a significant effect over this difference (Jackson et al., 1981), and must be considered to obtain a precise estimation of the plant water status.

Because of this, the use of the crop water stress index (CWSI), which is a normalized index developed to estimate plant stress (Bellvert et al., 2015), becomes a possible tool to assess plant water status more precisely. This index can be calculated through different methodologies, among which the leaf energy balance approach appears as a more stable methodology (Jones, 1999). This methodology uses the canopy temperature along with environmental measurements (net radiation, wind speed, and vapor pressure deficit) to simulate plant water status (Han et al., 2018). Thus, main objective of this study is to calculate the CWSI using thermal infrared temperature measured from two positions of the canopy. In addition, the leaf energy balance was used to estimate the CWSI.

## **MATERIALS AND METHODS**

### *Study site*

The study was carried out during the 2017/2018 growing season over a drip-irrigated vineyard (cv. Cabernet Sauvignon) with a north-south orientation located in the Penuhue Valley, Maule, Chile (35°20'33"S, 71°46'41"W, 86 m.a.s.l.). The climate is defined as Mediterranean-semiarid, with an average temperature of 14.8 °C and annual precipitation of 605 mm, which is mainly concentrated in winter (June – August). The soil has a sandy loam texture, with an effective depth between 10 and 70 cm and a slope ranging between 2 and 10%.

### *Experimental design*

Two completely randomized irrigation treatments (weak water deficit and moderate to severe water deficit) with three repetitions each were established after veraison. MSWP thresholds were defined according to van Leeuwen et al. (2008). These treatments were

applied by modifying the drip line, so when the defined thresholds were reached (-0.9 MPa for weak water deficit and -1.2 MPa for moderate to severe water stress), irrigation was reestablished.

#### *Physiological and remote sensing measurements*

Weekly MSWP measurements were made using a pressure chamber (model 1000, PMS Instrument Co., Corvallis, Oregon, USA) (Begg y Turner, 1970), and temperatures ( $T_c$ ) were obtained from the north (sunlit) and south (shaded) side of the canopy, pointing a portable infrared radiometer (model MI-2H0, FOV 32°, Apogee Instruments, Logan, UT, USA) horizontally to the middle third of the canopy (Figure 1), at a distance of 30 cm, recording the temperature after 10 – 15 seconds (with three repetitions per measurement point). Simultaneously, meteorological variables were obtained every 15 minutes from an automatic weather station located in the study site, which in combination with the variables mentioned above, allowed the calculation of the CWSI.

#### *CWSI calculation*

For this study, CWSI was calculated using the following equation (Jones, 1999):

$$CWSI = \frac{T_c - T_{wet}}{T_{dry} - T_{wet}} \quad (1)$$

where,  $T_c$  is surface canopy temperature (°C);  $T_{wet}$  is the temperature of a full-transpiring leaf (°C) and  $T_{dry}$  is the temperature of a stressed leaf (°C), with closed stomata.

Values of  $T_{wet}$  and  $T_{dry}$  were estimated through leaf energy balance (Jones, 1999) as:

$$T_{wet} = T_a + \frac{r_{HR}r_{aW}\gamma R_{ni}}{\rho_a C_p (\gamma r_{aW} + \Delta r_{HR})} - \frac{r_{HR}VPD}{\gamma r_{aW} + \Delta r_{HR}} \quad (2)$$

$$T_{dry} = T_a + \frac{r_{HR}R_{ni}}{\rho_a C_p} \quad (3)$$

where,  $T_a$  is air temperature (°C),  $r_{HR}$  is the resistance to radiative heat transfer ( $s\ m^{-1}$ ),  $r_{aW}$  is the resistance of the boundary layer to water vapor ( $s\ m^{-1}$ ),  $\gamma$  is the psychrometric constant ( $Pa\ K^{-1}$ ),  $R_{ni}$  is net isothermal radiation ( $W\ m^{-2}$ ),  $\rho_a$  is air density ( $kg\ m^{-3}$ ),  $C_p$  is the specific heat of dry air ( $J\ kg^{-1}\ K^{-1}$ ),  $\Delta$  is the slope of saturated water vapor pressure versus temperature curve ( $Pa\ K^{-1}$ ) and VPD is vapor pressure deficit (Pa).

#### *Statistical analysis*

A linear regression analysis was performed to determine the relationship between CWSI and MSWP. The difference between treatments was determined through an ANOVA using the R software (R Core Team, 2019).

## **RESULTS AND DISCUSSION**

Results of the regression showed a significant linear correlation between CWSI and MSWP for the north and south side of the canopy, with  $R^2$  values of 0.62 and 0.45 respectively (Table 1). These values agree with the results obtained by Möller et al. (2007) who observed a significant correlation between CWSI and MSWP with  $R^2$  values ranging from 0.52 to 0.91. In the same context, Fuentes et al. (2012) found an inverse correlation between these variables

( $R^2=0.75$ ) over different grapevine varieties, using leaf energy balance and reference surfaces, which corresponded to leaves coated with soapy water, for the wet reference, and with Vaseline, for the dry references.. Using a thermal camera placed on an unmanned aerial vehicle (UAV) and a hand-held thermal camera, Santesteban et al. (2017) obtained a significant correlation between CWSI and MSWP with  $R^2=0.69$ , highlighting the potential of CWSI to monitor vineyard water status.

Regarding the estimation of MSWP through the CWSI, given that the North (sunlit) side of the canopy presented a higher correlation with this index (Figure 2), the following analysis was carried out with the information from this sector of the canopy.

A significant difference was found between treatments, which can be seen in terms of MSWP,  $T_c$ , and CWSI. Higher values of CWSI were related to a lower yield, higher  $T_c$  and a lower MSWP, which indicates that irrigation treatments generated a physiological response over these variables (Table 2).

This estimation indicated that it is possible to predict MSWP values using CWSI calculated after veraison, for treatments under weak and moderate-severe water stress, as seen in Figure 3. This is similar to the results obtained by Poblete-Echeverría et al. (2017), who were also able to estimate MSWP using CWSI calculated from the sunlit side of a vineyard canopy.

Even though a significant correlation was found, improvements could be made by considering the possible source of errors attributed to the involuntary incorporation of non-plant material on the measurements made by infrared thermometers, given that ground-based temperature can contain other types of surfaces (Sepúlveda-Reyes et al., 2016) like soil or branches, which would induce measurement errors (Jones, 2002). In addition to this, several variables aside from  $T_c-T_a$  are needed to calculate CWSI through leaf energy balance, making it difficult to apply this method in practice (Matese et al., 2018).

## CONCLUSIONS

The irrigation treatments were able to generate a physiological change over the plants, in terms of MSWP, canopy temperature and yield. In relation to the CWSI, it can be said that it was able to estimate vine water status given that a significant linear relationship was found between MSWP and CWSI from the north side of the canopy ( $R^2 = 0.62$ ). This indicates that the use of CWSI from the north side of the canopy, calculated through the use of infrared thermometry and leaf energy balance, could be used as an aid in irrigation management, particularly for vines under weak water deficit.

## ACKNOWLEDGEMENTS

The research leading to this report was supported by the Chilean government through the projects CONICYT-PFCHA (No. 2018-21181790), FONDECYT (No. 1160997) and by the Universidad de Talca through the research program “Adaptation of Agriculture to Climate Change (A2C2)”.

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## **Tables**

Table 1. Determination coefficients ( $R^2$ ) of linear regressions between midday stem water potential (MSWP) and crop water stress index (CWSI) from different positions of the canopy.

<b>Part of the canopy</b>	<b><math>R^2</math></b>
North	0.62
South	0.45
Global	0.45

Table 2. Midday stem water potential (MSWP), crop water stress index (CWSI) from the north side of the canopy, canopy temperature ( $^{\circ}\text{C}$ ) and yield ( $\text{kg plant}^{-1}$ ) for every treatment for the 2017-2018 growing season.

<b>Treatment</b>	<b>MSWP (MPa)</b>	<b>CWSI (North)</b>	<b><math>T_c</math> (<math>^{\circ}\text{C}</math>)</b>	<b>Yield (<math>\text{kg plant}^{-1}</math>)</b>
Weak stress	-0.93	0.27	26.8	6.3
Moderate-severe stress	-1.24	0.40	29.8	4.2
<b>Significance</b>	*	*	*	*

\* p-value<0.05

## **Figures**



Figure 1. Measurement of canopy temperature

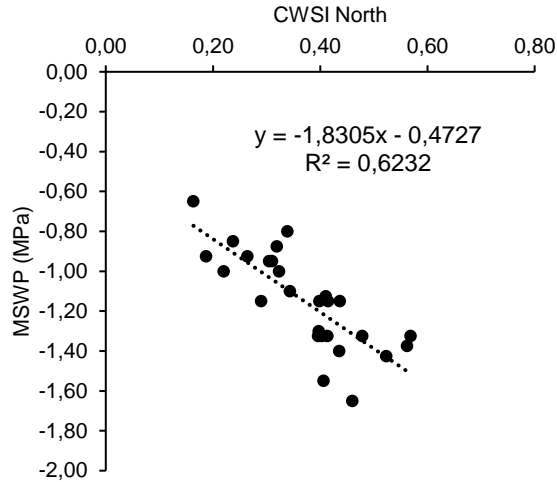


Figure 2. Linear regression between midday stem water potential (MSWP) and crop water stress index (CWSI) measured from the North side of the canopy.

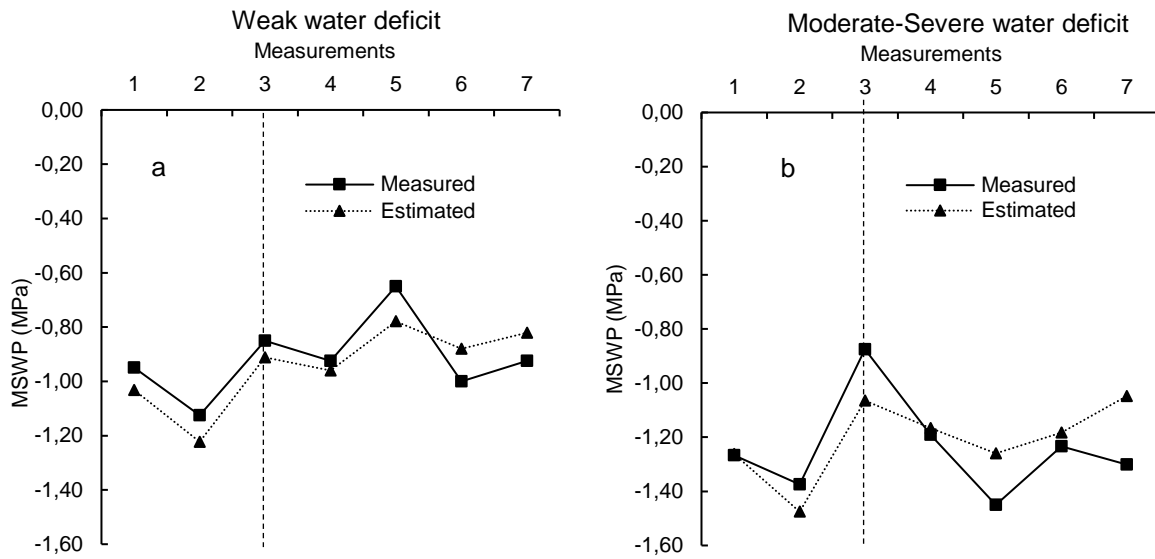


Figure 3. Measured v/s Estimated midday stem water potential (MSWP) using crop water stress index (CWSI) from the North side of the canopy, for plants under weak (a) and moderate-severe water deficit (b). The dotted vertical line indicates the start of veraison.

## 7.2 Confirmación de aceptación de trabajo de congreso.



Samuel Ortega Farias

mié 14/10/2020 8:18

Marcar como no leído

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Dear author,

This is to confirm that your article:

Estimation of vineyard water status using infrared thermometry measured at different positions of the canopy has been reviewed and is accepted by the editorial board of:

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