Use of water potential measurements for assessing water stress in *Vitis vinifera* L. cv. Tempranillo grown in Southern Oregon

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Dissertação para obtenção do Grau de Mestre em

Vinifera EuroMaster – European Master of Sciences of Viticulture and Oenology

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Lisboa, 2012

Abstract

A study was conducted to investigate the practical use of four methods for measuring plant water potential (Ψ) of *Vitis vinifera* L. cv. Tempranillo (syn. Tinta Roriz, Aragónez) under field conditions within the Southern Oregon American Viticultural Area. Predawn leaf water potential (Ψ_{pd}), midday leaf water potential (Ψ_{l-md}), midday stem water potential (Ψ_{s-md}) and early morning stem water potential measured between 07:00h and 08:00h solar time (Ψ_{s-em}) were each measured on vines before and after the initiation of irrigation. Measurements were conducted on the same vines and on the same dates both before and after veraison. Irrigation was applied based on an estimation of vineyard evapotranspiration (ET_c) and consisted of four treatments: 70% of ET_c continuously (70-70), 35% of ET_c continuously (35-35), 70% ET_c before veraison and 35% ET_c after veraison (70-35) and 35% before veraison and 75% ET_c after veraison (35-70). Irrigation was initiated based on Ψ_{l-md}. When assessing vineyard variability before the initiation of irrigation, with Ψ_{pd} ranging between -0.05 and -0.53, all four measurement types were able to distinguish between high vigor (HV) and low vigor (LV) zones and able to categorize vines under previously established thresholds. However, Ψ_{l-md} showed a tendency to underestimate vine water status at levels greater than -0.9 Mpa. When comparing Ψ_{s-em}, Ψ_{s-md} and Ψ_{l-md}, early morning measurements showed significant differences between irrigation treatments on 3 of 4 measurement dates while midday measurements were able to distinguish significant differences on only 1 of 4 dates. Ψ_{s-em} measurements were generally able to distinguish differences between vine water status even when morning cloud cover was present. Linear regression analysis of Ψ_{pd} versus Ψ_{s-em}, Ψ_{s-md} and Ψ_{l-md} at one site resulted in significant r^2 values of 0.62, 0.69 and 0.58, respectively. Linear regression analysis of Ψ_{s-md} versus Ψ_{l-md} using data from both sites resulted in r^2 values of 0.88. Overall, Ψ_{s-em} seemed to provide a better option for differentiating plant water status of Tempranillo grapevines in Southern Oregon compared to Ψ_{s-md} and Ψ_{l-md} within the range of water deficit levels studied here. Ψ_{s-em} was able to show differences between the water status of vines before the initiation of irrigation as well as differentiate between irrigation treatments later in the year. However, questions remain about the environmental and physiological factors that might impact the results of this method before water deficit threshold levels can be clearly defined.

Resumo

Realizou-se um estudo de investigação com o uso de quatro métodos para a medição do potencial hídrico de uma planta (Ψ) de *Vitis vinifera* L. cv. Tempranillo (syn. Tinta Roriz, Aragonez), em condições de campo na zona vitícola americana no Sul de Oregon. As medições foram: o Potencial foliar de base (Ψ_{pd}), do meio-dia, o potencial de água na folha (Ψ_{l-md}), do meio-dia, o potencial hídrico do caule (Ψ_{s-md}) e o potencial de agua do tronco medido entre as 7h00 e as 8h00, horas solares (Ψ_{s-em}) e medidos em cada videira, antes e após o inicio da irrigação. As medições foram realizadas nas mesmas videiras e nas mesmas datas antes e após pintor. A irrigação foi realizada com base na estimativa da evapotranspiração da vinha (ET_c) e constou de quatro tratamentos distintos: 70% da ET_c de forma continua (70-70), 35% da ET_c de forma continua (35-35), 70% ET_c antes do pintor e 35% ET_c depois do pintor (70-35) e 35% antes do pintor e 75% após o pintor (35-70). A irrigação foi iniciada baseado em $\Psi_{l-md.}$ Quando se avalia a variabilidade da vinha antes do inicio da irrigação, quando Ψ_{pd} variam entre -0,05 e -0,53, todas as medições foram capazes de distinguir zonas de alto vigor (HV) e baixo vigor (LV) e capazes na maioria dos casos, de categorizar videiras sob limites previamente estabelecidos. No entanto, Ψ_{l-md} mostrou tendência para subestimar o stress hídrico da videira para níveis superiores a -0,9 MPa. Ao comparar Ψ_{s-em}, Ψ_{s-md} e Ψ_{l-md} verificou-se que as medições da manha conseguiam mostrar diferenças significativas entre os tratamentos de irrigação em 3 das 4 datas de medição, enquanto que as medições a meio do dia conseguiam mostrar diferenças significativas apenas em 1 das 4 datas. As medições de Ψ_{s-em} eram capazes de apresentar diferenças entre o stress hídrico nas videiras mesmo na presença de nuvens matinais. A análise de regressão linear entre Ψ_{pd} versus Ψ_{s-em}, Ψ_{s-md} and Ψ_{l-md} num dos locais resultou em valores de 0,62, 0,69 e 0,58 respetivamente. A análise de regressão linear de Ψ_{s-md} contra Ψ_{l-md} resultou em valores de $r^2 = 0{,}88$. Em geral, Ψ_{s-em} parece ser a melhor opção para diferenciar o stress hídrico das videiras de Tempranilho em Oregon do sul comparativamente com Ψ_{s-md} e Ψ_{l-md}, dentro do intervalo de stress hídrico estudado aqui, providenciando. Ψ_{s-em} revelou diferenças entre o estado hídrico de videiras antes do inicio da irrigação bem como diferenciou tratamentos entre irrigações no final do ciclo. No entanto, permanecem dúvidas sobre os fatores ambientais e fisiológicos que podem ter influenciado os resultados deste método.

Acknowledgments:

I wish to express my sincere appreciation and gratitude to:

The Vinifera Euromaster program for providing me with the amazing opportunity to explore the world of viticulture and enology on a global scale and for the knowledge and perspective gained from this experience;

Dr. Gabriel Balint and the Southern Oregon Research and Extension Center for allowing me to conduct my research as part of their program and providing me with the resources and support to do so;

Earl Jones, owner of Abacela Winery, and Richard Ellis, owner of Ellis Vineyards, for allowing me to conduct my thesis project in their vineyards and for the continuous assistance from them and their staff that made this project possible;

Dr. Carlos Lopes of the Instituto Superior de Agronomia in Lisboa, for acting as my co-advisor and for his patience and understanding throughout the process;

Dr. Gregory Jones for his council and generous contribution of time spent procuring climactic data;

Stephanie DiBrezzo for her constant patience, understanding, love and support;

My parents, Elizabeth and Jonathan Cole, for providing me the opportunities that made it possible to pursue my chosen path and for their consistent support and counsel through my life.

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Abbreviations:

RDI	Regulated deficit irrigation
Ψ	Water potential
Ψ_{pd}	Predawn leaf water potential
$\Psi_{l\text{-}md}$	Midday leaf water potential
$\Psi_{s\text{-}md}$	Midday stem water potential
GDD	Growing degree days
AVA	American Viticultural Area
Ψ_l	Leaf water potential
FTSW	Fraction of transpirable soil water
Ψ_s	Stem water potential
$\Psi_{s\text{-}em}$	Early morning stem water potential
VSP	Vertical shoot positioned
SD	Standard deviation
ET_c	Crop evapotranspiration
ET_o	Reference evapotranspiration
K_c	Crop coefficient
SWC	Soil water content
PDT	Pacific Daylight Time
VPD	Vapor pressure deficit
LAI	Leaf area index
A	Net photosynthesis
g_s	Stomatal conductance
LV	Low vigor
HV	High vigor
SL	Silt loam
GSL	Gravelly silt loam

1.0 Introduction:

1.1 Irrigation and Irrigation monitoring:

Irrigation has become a well-established practice in non-European grape growing regions across the world and its usage is increasing in many parts of Europe (Intrigliolo *et al.* 2005; Sousa *et al.* 2006; Baeza *et al.* 2007). The use of irrigation can ameliorate reduced stomatal conductance and photosynthetic activity caused by excess water stress experienced in many grape-growing regions (Kliewer *et al.* 1983; Lopes, 1999; Rubio *et al.* 2004; Santesteban and Royo, 2006; Intrigliolo and Castel, 2011). It has also been well established that controlled quantities of irrigation water can increase yield while maintaining or improving fruit quality (Matthews and Anderson,1988; Chaves *et al.* 2007; Rubio *et al.* 2011). However, these positive effects may be dependent on seasonal conditions, varietal selection and crop load (Bartolomé *et al.* 1996; Salón *et al.* 2004; Chaves *et al.* 2007; Intrigliolo and Castel, 2008). Differences in vine water status have also resulted in wines with significantly different composition, appearance and flavor (Matthews *et al.* 1990, Intrigliolo *et al.* 2008) and may influence the rate of grape ripening (Matthews and Anderson, 1988; Van Leeuwen *et al.* 2009). However, the over-use of irrigation water can cause excessive vegetative growth, reduction in grape quality, and the waste of valuable water resources (Matthews and Anderson, 1988; Baeza *et al.* 2007; Chaves *et al.* 2007). In addition, the need for both effective and efficient irrigation practices will likely become increasingly important in the future with the threat of global climate change in many grape-growing regions of the world (Mote *et al.* 1999; Schultz, 2000).

Deficit irrigation involves the use of supplemental irrigation to impose mild plant water stress and has been shown to effect both vegetative and reproductive development depending on the timing of application (Matthews *et al.* 1987; Matthews and Anderson, 1989). As such, the practice of Regulated Deficit Irrigation (RDI) has become a popular method for managing the balance between canopy development and fruit quality by imposing specific water deficits during different periods of the vine's physiological cycle (Chalmers *et al.* 1986; McCarthy *et al.* 2002). These strategies can be used in vineyard management to control vegetative growth, maximize water use efficiency, influence yield and change grape quality characteristics (Matthews and Anderson 1988; McCarthy,1997; Ojeda *et al.* 2002; Chaves *et al.* 2007; Girona *et al.* 2009; Santesteban *et al.* 2011b).

In order to optimize such precise irrigation strategies, a quick and reliable method for quantifying plant water availability is needed. One strategy is the use of a soil-water balance calculation which estimates the available soil water based on water added through irrigation and precipitation subtracted from water lost through runoff, drainage and evapotranspiration (Lebon *et al.* 2003). However, this method may not produce adequate irrigation recommendations because many of the factors included are difficult to accurately estimate under field conditions (Lebon et al. 2003; Girona *et al.* 2006; Pellegrino *et al.* 2006) and such errors are cumulative over time if not regularly confirmed with direct soil or plant based measurements (Jones, 2004). The measurement of soil moisture can provide a direct method of estimating soil water availability, but physiological stress responses are dependent on environmental conditions as well as actual soil moisture (Winkel and Rambal, 1993; Gruber and Schultz, 2005; Williams and Baeza, 2007). Problems may also arise when relying on soil moisture measurements to manage irrigation if the method used does not cover the full range of vine-sensitive soil water content or if readings are taken in locations and depths that are not representative of main vine root activity (Intrigliolo and Castel, 2008; Van Leeuwen *et al.* 2009).

Direct measurements of plant water status may represent the most straightforward and accurate indicators for evaluating water availability, since such methods provide an estimate of actual plant water status under the given conditions. The use of a pressure chamber to measure the water potential (Ψ) of the leaf (Scholander *et al.* 1965) has become one of the most popular and well established methods for directly measuring plant water status (Williams and Araujo, 2002). This method has also been proven to be more effective at managing irrigation regimes under field conditions compared to the water-balance method (Girona *et al.* 2006). A number of variations on the measurement of Ψ have been proposed and validated, primarily predawn leaf water potential (Ψ_{pd}), midday leaf water potential ($\Psi_{l\text{-}md}$) and midday stem water potential ($\Psi_{s\text{-}md}$) (Williams and Trout, 2005). However, there remains disagreement within the literature as to which of these three methods best characterizes vine water status under field conditions (Choné *et al.* 2001; Gruber and Schultz, 2005; Williams and Trout, 2005). In addition, some studies have suggested that methods relying on morning-time measurements can also be reliable indicators of vine water status (Salón *et al.* 2004; Baeza *et al.* 2007; Santesteban *et al.* 2011a). Conflicting reports within the literature on the best measurement of Ψ are likely a result of the many abiotic and biotic factors influencing the vine's physiological responses to water deficits (Jones, 2004). As a consequence, growers may be confused about how to choose the best method for assessing water status. Moreover, the incorrect interpretation of field data could negatively effect water management decisions if methods are not validated for specific regions and varietals.

1.2 Southern Oregon American Viticultural Area:

The Southern Oregon American Viticultural Area (AVA) consists of two major sub-AVAs, Rogue Valley and Umpqua Valley. These regions are located within the mountain valleys of the north-western coast of the United States and are largely protected from the cold Pacific Ocean's influence by the Coastal Mountains (Jones *et al.* 2003), as seen in **Figure 1**.

Figure 1. Map of the American Viticultural Areas of Oregon. Provided by the Oregon Wine Board at www.oregonwine.org.

The Umpqua Valley AVA was established in 1984 (U.S. Code of Federal Regulations, 2000) and is made up of multiple smaller valleys, primarily situated below 300 m elevations and surrounded by three mountain ranges with distinct geological characteristics: the Klamath Mountains, the Coastal Range and the Cascades. The sub-valleys of Umpqua generally run east-west which allows some Pacific breeze to penetrate, creating a climate that is usually cooler and wetter than the Rogue Valley (Jones *et al.* 2003). The average frost-free period occurs between April 10th and October 31st and growing degree day (GDD) accumulation generally puts the grape-growing climate between Region I and Region II on the Winkler Index (Winkler and Cook, 1974; Jones *et al.* 2003; Western Regional Climate Center, 2012).

The Rogue Valley AVA was established in 1991 (U.S. Code of Federal Regulations, 2000). It is the southernmost grape-growing region of Oregon and is also bordered by three distinct mountain ranges: the Klamath Mountains, the Siskiyou Mountains and the Cascade Mountains. Although the official boundaries of the Rogue Valley AVA go much higher, existing vineyard sites generally range between 275m and 670m. The Rogue Valley is divided into three main growing areas: the Bear Creek Valley, the Applegate Valley, and the Illinois Valley. However, only the Applegate Valley has been officially recognized as its own sub-AVA (U.S. Federal Register, 2000). The greater Rogue Valley is generally considered to be warmer and drier than Umpqua Valley (Jones *et al.* 2003). However, large differences exist within the three sub-regions of Rogue Valley, with much wetter and cooler conditions in the Illinois Valley and southwest parts of the Applegate Valley because of increased proximity to the Pacific Ocean (Jones and Light, 2001). The average frost-free period is typically shorter than in Umpqua Valley, occurring between May 10th and October 10th, due to the higher elevation. Historical GDD accumulation generally puts the grape-growing climate of the Rogue Valley between Region I and Region II on the Winkler Index, but values equivalent to Region III occur in some areas in some years (Winkler and Cook, 1974; Jones *et al.* 2003; Western Regional Climate Center, 2012).

Annual precipitation in the grape-growing regions of Southern Oregon range widely, from approximately 480 mm to 1518 mm and 780 mm to 1366 mm for Rogue Valley and Umpqua Valley, respectively (Jones *et al.* 2003). However, generally only 15% or less of the precipitation occurs during the growing season of April through October (Jones and Light, 2001; Jones, 2003), making irrigation an important tool for growers in these areas to maintain yields and vine health (Jones and Pierce, 2007).

In the last 25 years the number of wine grape hectares under production has increased dramatically in Southern Oregon, making it more and more important to educate growers on the most up-to-date practices and maximize water resources. According to the U.S. National Agricultural Statistics Service (1988 to 2012), the number of wine-grape hectares increased by 614% and 413% from 1987 to 2011 for the Rogue Valley and Umpqua Valley, respectively.

There now exists over 850 hectares in the Rogue Valley AVA and 546 hectares in the Umpqua Valley AVA (U.S. National Agricultural Statistics Service, 2012).

1.3 Vitis vinifera L. cv. Tempranillo:

Vitis vinifera L. cv. Tempranillo (syn. Aragónez and Tinta Roríz) is the most planted varietal in Spain (Baeza *et al.* 2011) and among the most popular varietals in Portugal (Johnson and Robinson, 2007). It has also been planted in significant amounts in parts of Argentina, Australia, and the United States (Martinez, 2003; Rowley, 2008; Jennings, 2012). The first mention of the varietal Tempranillo in the Oregon Vineyard and Winery Report was in the 2003 vintage report in which it refers to the varietal as one of the "unfilled wine grape needs reported by wineries" (Oregon Agricultural Statistics Service, 2004). By 2011 there was reported to be 76.9 hectares of Tempranillo planted in Oregon (U.S. National Agricultural Statistics Service, 2012).

Tempranillo is typically a vigorous wine-grape cultivar (Baeza *et al.* 2011) with high crop load potential and good sugar production (Shellie, 2007). It is known for its relatively late bud-break and early ripening (Shellie, 2007) with rapid early-season canopy development (Baeza *et al.* 2011). Tempranillo is widely considered to be sensitive to water stress and often exhibits early leaf senescence as a result, which can significantly reduce total leaf area (Gomez del Campo *et al.* 1996; Lopes *et al.* 2007; Baeza *et al.*, 2011). Irrigation has been shown to reduce or prevent this issue (Bartolomé *et al.* 1996; Esteban *et al.* 1999). However, there exists a range of conflicting reports on this varietal's typical physiological responses to drought (Chaves *et al.* 2010). The behavior of different cultivars under water stress is often described as either isohydric, in which water use is tightly regulated, or anisohydric, in which such regulation is limited (Schultz, 2003). In the case of Tempranillo, both behaviors have been observed depending on growing conditions (Lovisolo *et al.* 2010).

Despite the large pool of academic knowledge about Tempranillo, most existing research has been done in locations outside of Oregon. In particular, published studies on water status monitoring involving Tempranillo have been performed in regions of Spain or Portugal representing either different soil types or different weather conditions compared to the growing regions of Southern Oregon (Rubio *et al.* 2004; Intrigliolo and Castel, 2006; Santesteban and Royo, 2006; Sousa *et al.* 2006; Lopes *et al.* 2007). Comparisons between some basic climactic characteristics of Southern Oregon and several important Tempranillo growing regions in Europe are represented in **Figures 2 and 3.**

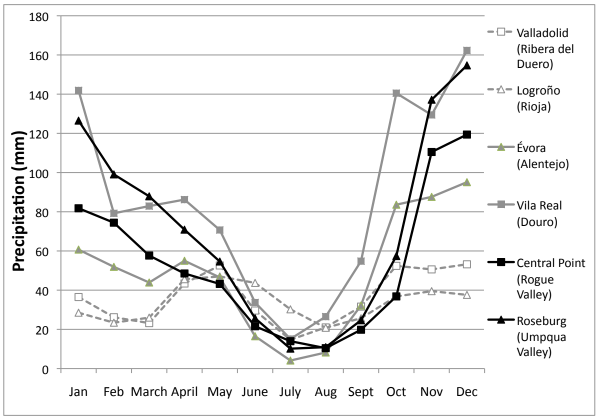

Figure 2. Comparison of monthly precipitation (mm) for regions of Southern Oregon (Rogue Valley and Umpqua Valley) and the Iberian Peninsula (Ribera del Duero, Rioja, Alentejo and Douro). Values represent 30-year averages (1981-2010). European data provided by Dr. Gregory Jones (Southern Oregon University) and U.S. data provided by the Western Regional Climate Center.

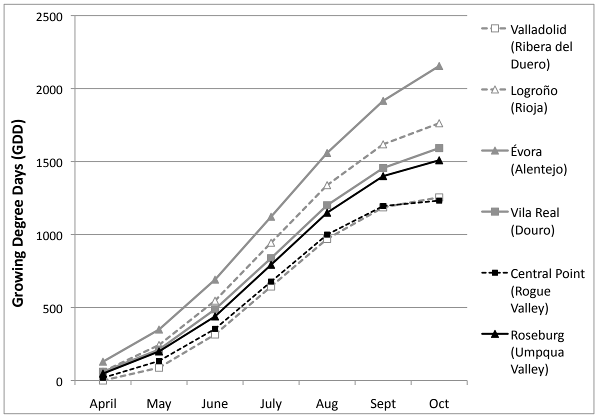

Figure 3. Comparison of monthly growing degree day (GDD) accumulation between April and October for regions of Southern Oregon (Rogue Valley and Umpqua Valley) and the Iberian Peninsula (Ribera del Duero, Rioja, Alentejo and Douro). Values represent 30-year averages (1981-2010). European data provided by Dr. Gregory Jones (Southern Oregon University) and U.S. data provided by the Western Regional Climate Center.

1.4 Objectives:

The goal of this project is to investigate the Ψ response of Tempranillo under different irrigation strategies and to evaluate methods for monitoring the water status of this varietal under Southern Oregon conditions. The results of this study are intended to provide local growers with a better knowledge of how these irrigation and monitoring methods function as well as offer practical recommendations on how best to monitor irrigation for the varietal Tempranillo.

2.0 Literature Review:

2.1 Introduction:

When irrigating grapevines, growers must decide on a method or methods for determining both the timing and quantity of water to be applied. Monitoring plant water status directly has become a common practice for grape-growers attempting to implement deficit irrigation strategies. While numerous methods are available, plant-based measurements have the advantage of taking into account more of the factors influencing water status, including soil moisture, evaporative demand and the plant's physiological responses to both (Jones, 2004). In his review of plant-based methods for irrigation monitoring, Jones (2004) described the use of a pressure chamber to measure Ψ as a "widely accepted reference technique". This method provides a direct measurement of the plant's water status, utilizing equipment and procedures that are fairly straightforward and practical to implement in both experimental and production situations.

2.2 Measuring Plant Water Potential:

The use of a pressure chamber for measuring Ψ has been included in the majority of studies describing the water relations of grape vines (Smart, 1974; Hardie and Considine, 1976; Bartolome *et al.* 1996, Schultz, 1996; Lopes, 1999; Sousa *et al.* 2006; Santesteban *et al.* 2009; Intrigliolo and Castel, 2010). A number of different procedures for using the pressure chamber to evaluate Ψ have been proposed. The three most common and well-established methods to date are $\Psi_{l\text{-}md}$, $\Psi_{s\text{-}md}$, and Ψ_{pd} (Gruber and Schultz, 2005; Williams and Trout, 2005). All three of these common methods can be significantly correlated with other indicators of water availability including irrigation amount, soil moisture, shoot growth and other physiological indicators of water stress such as stomatal conductance (g_s) and photosynthesis (A) (Choné *et al.* 2001; Williams and Araujo, 2002; Baeza *et al.* 2007; Santesteban *et al.* 2011a). However, each method has advantages and disadvantages and it is important for growers to understand how to interpret the information provided by the method they have chosen for managing irrigation. For example, in their study of multiple Tempranillo vineyards Santesteban *et al.* (2011a) concluded that neither Ψ_{pd} nor $\Psi_{s\text{-}md}$ could be said to be "statistically preferable" but that $\Psi_{s\text{-}md}$ may be a better estimate of the actual extent of vine water stress while Ψ_{pd} may be a better indicator of actual water availability because of the elimination of above ground influences.

Leaf water potential (Ψ_l) is a measurement of the water potential of an individual leaf. This procedure is often carried out close to "solar noon", a method known as midday leaf water potential ($\Psi_{l\text{-}md}$), which is generally intended to indicate the minimum leaf water potential experienced by the plant during the day (Van Leeuwen *et al.* 2009). This method may be the most common and practical plant tissue water potential measurement for growers to implement due to its simple, quick procedure and the fact that it can be done during normal work hours. Experiments have confirmed that $\Psi_{l\text{-}md}$ can show significant differences between water availability (Matthews et al. 1987; Williams and Trout, 2005) and is well correlated with other indicators of vine water stress (Baeza *et al.* 2007). However, because Ψ_l is regulated under high levels of water stress in some varietals (Schultz, 2003), this method may not always reflect the true water status of the plant. Sousa *et al.* (2006) indicated that, for Tempranillo, midday measurements showed little sensitivity to small differences in soil water content and attributed this to the regulation of stomata by the plant. Other studies in regions of Portugal and Spain have also reported that $\Psi_{l\text{-}md}$ measured on Tempranillo was less sensitive to differences in irrigation quantities compared to Ψ_{pd} and $\Psi_{s\text{-}md}$ (Lopes *et al.* 2007; Intrigliolo *el al.* 2005). Moreover, relationships between Ψ_l and variables representing water loss, photosynthesis, yield and quality parameters in Tempranillo were shown to be stronger for predawn measurements compared to midday measurements (Medrona *et al.* 2003).

Leaf water potential can also be measured just before daybreak, a method known as predawn leaf water potential (Ψ_{pd}). Taking measurements at this time is assumed to allow plant water potential to come under relative equilibrium with the soil and avoids climactic influences that exist during the day (Van Zyl, 1987; Gruber and Schultz, 2005). This seems to be confirmed by the fact that Ψ_{pd} can reliably estimate the fraction of transpirable soil water (FTSW) under the vine (Pellegrino *et al.* 2004; Gruber and Schultz, 2005). However under mild water deficits or when only a small portion of the rooting zone is humid, Ψ_{pd} may not reflect actual day-time plant water status if soil water is adequate to rehydrate the plant overnight but not adequate to meet day-time transpiration demand of the canopy (Ameglio *et al.* 1999; Van Leeuwen *et al.* 2009). Regardless, Ψ_{pd} has been shown to be more highly correlated with daytime A and g_s compared to midday measurements of Ψ in several varietals (Williams and Araujo, 2002; Medrano *et al.* 2003). In addition, relationships between Ψ and berry size were improved when measuring Ψ_{pd} compared to $\Psi_{s\text{-}md}$ (Baeza, *et al.* 2007). Thresholds for Ψ_{pd} have been recommended for estimating low, mild, and severe plant water deficits in Tempranillo specifically (Santesteban and Royo, 2006) and such thresholds have been used successfully for managing irrigation experiments on this varietal (Santesteban *et al*. 2011b).

Stem water potential (Ψ_s) involves covering an entire leaf with plastic and reflective foil before measuring the leaf's water potential. Isolating the leaf from ambient conditions in this way allows the leaf Ψ to come into equilibrium with the internal vasculature (xylem) of the stem (Begg and Turner, 1970). This procedure is intended to give a measurement of the entire plant's hydric-stress during daytime conditions while reducing the effects of leaf-microclimate conditions (McCutchan and Shackel, 1992; Choné *et al.* 2001). Strong correlations have been shown between midday measurements of stem water potential ($\Psi_{s\text{-}md}$) and $\Psi_{l\text{-}md}$ in Tempranillo (Intrigliolo and Castel, 2008; Girona *et al.* 2009) and other varietals (Williams and Araujo, 2002). However, when measured on *V. vinifera* cv. Cabernet Sauvignon, $\Psi_{s\text{-}md}$ produced more consistent results compared to $\Psi_{l\text{-}md}$ and was able to better distinguish significant differences between vines (Choné *et al.* 2001). Minimum $\Psi_{s\text{-}md}$ has also been well correlated with more "low-tech" water stress indicators such as shoot growth slackening and berry weight (Van Leeuwen *et al.* 2009). When tested in commercial vineyards in Navarre, Spain, $\Psi_{s\text{-}md}$ was proven to provide consistent estimations of water stress in Tempranillo vineyards under a variety of climactic conditions, soil depths, slopes aspects and water deficit levels (Santesteban *et al.* 2011a).

In order to use plant water potential for the scheduling of irrigation, thresholds must be set to determine how to interpret each of the above pressure chamber methods. Over the course of the last three decades, many studies of the behavior of grapes under water stress have provided clues about these threshold levels. For example stomatal conductance has shown a decline in *V. vinífera* L cv. Carignane as Ψ_l approaches values between -0.9 MPa and -1.0 MPa (Kliewer *et al.*1983); grape productivity was maintained in *V. vinífera* L cv. Thompson Seedless (syn. Sultana) at irrigation levels resulting in $\Psi_{l\text{-}md} > -0.9$ MPa (Grimes and Williams, 1990); evapotranspiration and available soil water began to be limited in Thompson Seedless at $\Psi_{l\text{-}md} < -1.0$ MPa (Williams and Trout, 2005); and shoot growth rate was shown to stop at $\Psi_{l\text{-}md}$ values less than -1.12 MPa in Cabernet Sauvignon (Baeza *et al.* 2007). In their review of vine water status assessments, Van Leeuwen *et al.* (2009) have proposed a set of relational thresholds for each of the three common measurement techniques discussed above, presented in **Table 1**. These threshold values are similar to those used successfully to schedule irrigation in field trials (Girona *et al.* 2006; Santesteban *et al.* 2011b).

Table 1. Threshold values for three pressure chamber methods (MPa) covering five categories of water deficit levels. Originally published by Van Leeuwen *et al.* (2009).

	Midday Stem Water Potential (MPa)	Midday Leaf Water Potential (MPa)	Predawn Leaf Water Potential (MPa)
No water deficit	> -0.6	> -0.9	> -0.2
Weak water deficit	-0.6 to -0.9	-0.9 to -1.1	-0.2 to -0.3
Moderate to weak water deficit	-0.9 to -1.1	-1.1 to -1.3	-0.3 to -0.5
Moderate to severe water deficit	-1.1 to -1.4	-1.3 to -1.4	-0.5 to -0.8
Severe water deficit	< -1.4	< -1.4	< -0.8

In addition to these three measurement types, a number of studies have demonstrated that Ψ_s measured in the early morning hours between 0700h and 0800h solar, called early morning stem water potential ($\Psi_{s\text{-}em}$), can also be a representative method for measuring the water status of grapevines (Salón *et al.* 2004; Salón *et al.* 2005; Intrigliolo *et al.* 2005; Intrigliolo and Castel, 2006; Intrigliolo and Castel, 2010). When measured on Tempranillo, $\Psi_{s\text{-}em}$ has been shown to be more sensitive for distinguishing differences between irrigation treatments over a range of seasonal conditions and water stress levels compared to midday measurements (Intrigliolo and Castel, 2010) and better correlated with g_s compared to other Ψ measurements (Intrigliolo and Castel, 2006). In addition, $\Psi_{s\text{-}em}$ was well correlated with berry weight, sugar content, yield and wine characteristics when measured on both Tempranillo and *Vitis vinífera* cv. Bobal (Salón *et al.* 2004). However all of these studies were performed in the same vineyard near Reguena, Valencia, Spain, which was grafted onto a single rootstock (161-49) growing in deep calcareous clay loam soils.

Diurnal patterns measured on many different varietals have typically shown grapevine water potential to be changing rapidly during the morning hours (Smart, 1974; Hardie and Considine, 1976, Van Zyl, 1987; Choné *et al.* 2001) which might make this appear to be an unreliable time-frame for measurements. However, diurnal Ψ curves for Tempranillo have also shown this timeframe to be more sensitive to differences in water status compared to measurements taken at midday or even predawn on some days (Intrigliolo and Castel, 2006). Choné *et al.* (2001) demonstrated the greatest differences in Ψ_s between Cabernet Sauvignon blocks grown on deep and shallow soils occurred in the morning (approximately 0900–1000) while showing no significant differences between $\Psi_{l\text{-}md}$ measurements for the same dates and sites. When compared side-by-side on Tempranillo, $\Psi_{s\text{-}em}$ showed statistically significant relationships with all three other methods described above but was chosen as the preferred method for tracking water status (Intrigliolo *et al.* 2005). Consequently, early morning measurements of stem water potentials may provide yet another option for accurately assessing plant water status. One practical advantage of this method is the timing of measurements during the first few working hours of the day, as compared to the inconvenient timing of the predawn method.

2.3 Influences on the measurement of Ψ:

There are a number of factors that affect plant water potential which should be taken into account when assessing the usefulness of a given method in a given situation including: temperature, evaporative demand, photon flux density (PFD), vapor pressure deficit (VPD), fruit load, rootstock, soil depth, slope orientation, total leaf area, dew point temperature and varietal (Smart and Barrs, 1973; Smart, 1974; Williams and Araujo, 2002; Medrano *et al.* 2003; Williams and Baeza, 2007; Olivo *et al.* 2009; Lovisolo *et al.* 2010; Perez *et al.,* 2010; Santesteban *et al.* 2011a). These factors may account for the disagreement between different studies carried out in different climates, sites, years and varietals and should be taken into account as best possible when interpreting Ψ values.

Climactic influences in particular play an important role in the results of Ψ measurements. In Tempranillo vineyards of Southern Navarre (Spain), variations in daytime measurements of Ψ_s were shown to depend primarily on temperature and evaporative demand while dew formation was shown to significantly influence Ψ_{pd} (Santesteban *et al.* 2011a). Light, temperature and VPD have been shown to have an especially large influence on both Ψ_l and Ψ_s measured during the day because of the influence of these factors on stomatal conductance (Smart and Barrs, 1973; Smart, 1974; Winkel and Rambal, 1993; Williams and Baeza, 2007). Changes in VPD have the greatest influence on Ψ_l when vines are under no water stress, with Ψ_l differences of as much as -0.24 MPa for well-watered vines as VPD increases from 2 to 5kPa (Williams and Baeza, 2007).

In addition, there is substantial variability in the behavior of different grape cultivars in their responses to water stress (Winkel and Rambal, 1993; Shultz, 1996; Gomez del Campo *et al.* 2004; Schultz, 2003; Santesteban *et al.* 2009). Grapevines are the first species to show both anisohydric and near-isohydric behaviors between cultivars (Schultz, 1996; Schultz; 2003). The mode of action for these differential responses is complex (Lovisolo *et al.* 2010), but both hydraulic signaling (Schultz and Matthews, 1988; Schultz, 2003; Chouzouri and Schultz, 2005) and chemical signaling (Stoll *et al.* 2000; Rodrigues *et al.* 2008) seem to play a role in the vine's ability to regulate stomata and thus influence transpiration and Ψ. Many varietals have now been classified as anisohydric, near-isohydric or isohydric but reports within the literature show disagreement as to how specific varieties should be classified (Chaves *et al.* 2010). Tempranillo is one example of this disagreement, having been classified by some authors as isohydric (Medrano *et al.* 2003; Sousa *et al.* 2006), by others as near-isohydric (Intrigliolo *et al*. 2005) and by others as anisohydric (Santesteban *et al.* 2009). Given these variations in water relations behavior between both varietals and conditions, assessments of water potential measurement techniques should be performed on both a regional and cultivar-by-cultivar basis.

3.0 Materials and Methods:

3.1 Sites:

All measurements were taken during the 2012 growing-season within an existing irrigation experiment being conducted in two commercial *Vitis vinifera* L. cv. Tempranillo vineyards grafted onto Millardet et Grasset 101-14 (*V. riparia x V. rupestris*), located within the Southern Oregon American Viticulture Area (AVA). Site 1 is located in the Bear Creek region of the Rogue Valley AVA at Ellis Vineyard (42°15'46.22"N, 122°49'32.89"W; approximately 490m above seas level). **Site 2** is located in the southern region of the Umpqua Valley AVA at Abacela Vineyard (43° 7'52.07"N, 123°26'47.36"W; approximately 210m above seas level). The two vineyards are characterized by differences in climate, soil, slope, aspect and vineyard design, which are outlined in **Table 2** and visually represented in **Figure 4** and **Figure 5**. Phenological timing for each site over the 2012 growing season is outlined in **Table 3**.

Table 2. Characteristics of two Tempranillo vineyard sites located in Southern Oregon. Soil descriptions provided by United States Department of Agriculture soil surveys (websoilsurvey.nrcs.usda.gov, 9/20/12). All 30-year averages of growing degree days (GDD) and precipitation provided by Western Regional Climate Center. Row orientations of North-South (N-S) and Northeast-Southwest (NE-SW) were confirmed using a compass in the field.

	Site 1	**Site 2**
American Viticulture Area	Rogue Valley (Bear Creek region)	Umpqua Valley (South Umpqua region)
Precipitation, 30-year average	517mm	858mm
Growing Degree Days, 30-year average (April 1 – October 31)	2861 GDD	2545 GDD
Soil description	Silt loam **or** gravelly silt loam (~18cm thick) over loam (>1.8m deep)	Silt clay (0.4m thick) over weathered bedrock
Slope	flat	~25%
Slope orientation	flat	West facing
Row Orientation	N-S	NE-SW
Vine Spacing (vine x row)	1.8m x 2.75m	2.4m x 3.0m
Plant Density per hectare	2020	1390
Year Planted	2000	2000

Figure 4. Aerial view of experimental Site 1. (Image: Google Earth, 6/12/2012).

Figure 5. Aerial view of experimental Site 2. (Image: Google Earth, 6/12/2012).

Vines at both sites were trained to a vertical shoot positioned (VSP) training system and hedged to maintain maximum canopy dimensions of approximately 1.4m tall and 0.4m thick. Vines were hedged 3 times at Site 1 and once at Site 2. Vines were pruned to a cordon system using two-bud spurs and shoot thinned in the spring at both sites using the grower's standard procedure. This resulted in average shoots per vine of 28.2 (SD = 3.1) and 23.1 (SD = 3.14) for Site 1 and Site 2, respectively, which corresponds to an average of 15.7 and 9.6 shoots per meter of cordon for Site 1 and Site 2, respectively. No significant differences in shoots per vine were shown between irrigation treatments at either site (data not shown). Leaves and laterals were removed from the fruiting zone before veraison on the east and north sides of the canopy for Site 1 and Site 2, respectively. A permanent cover crop was maintained between rows at both sites, consisting of a combination of native species and *Festuca sp.* grass, with a strip approximately 0.8 m wide under the vine row kept clear of vegetation by spring applications of herbicide. Cover crop was mowed 2 to 3 times over the growing season. A standard fungicide spray program was carried out by the grower at each site.

Table 3. Phenological events for experimental Tempranillo vineyards at two sites representing two sub-regions of the Southern Oregon American Viticulture Area. Dates determined by visually estimating 50% expression.

	Site 1 (Bear Creek Valley)	**Site 2** (S. Umpqua Valley)
Bud Break	May 2	April 23
Bloom	June 23	June 19
Verasion	Aug. 20	Aug. 28
Harvest	Oct. 18	Oct. 9

3.2 Irrigation Treatments:

As part of the existing trial, irrigation treatments in both sites were applied for two years prior to the measurement season (i.e. starting in 2010). At each site, experimental plots consisted of four treatments with three replicates per treatment and ten vines per replicate. At Site 1, four rows were randomly assigned with one treatment each and three uniform replicates were chosen along each row. At Site 2, irrigation treatments were randomly assigned to twelve individual rows, each representing a treatment replicate.

Irrigation was calculated as percentage crop evapotranspiration (ET_c) and applied through 1.9L/hour emitters placed on either side of each vine. Reference evapotranspitation (ET_o) was measured using an ETgage (Model E; ETgage Company, Loveland, CO, U.S.A.) placed within the vineyard and crop coefficients (K_c) were estimated by measuring the shaded area under the vine at solar noon (Williams and Ayars, 2005). At Site 2, irrigation was applied twice per week with each application representing half of the total application calculated for the given treatment. At Site 1, applications of 11.4 L per vine or less were applied and the number of applications per week was adjusted to meet total ET_c of the previous week, resulting in 2 and 4 applications per week. Irrigation was initiated when $\Psi_{l\text{-}md}$ on 3 or more plants within the experimental block reached -1.2 MPa or less. Treatments were applied as follows: 70% of ET_c continuously (70-70), 35% of ET_c continuously (35-35), 70% ET_c before veraison and 35% ET_c after veraison (70-35) and 35% before veraison and 75% ET_c after veraison (35-70). At Site 1, several minimal irrigation applications (<4L/vine) were applied evenly across the entire experimental plot prior to the initiation of experimental treatments in order to sustain some younger vines replanted in other parts of the block.

3.3 Soil Measurements:

At Site 2, soil water content (SWC) was measured weekly using an HH2 Moisture Meter and PR2/6 Profile Probe (Delta-T, Cambridge, UK). A single 27 mm teflon tube for each replicate was installed under the vine row on June 27th, approximately 0.2 m from an emitter on the down-hill side. Measurements were taken at 10 cm, 20 cm, 30 cm, 40 cm, 60 cm, and 100 cm depths.

3.4 Plant Measurements:

Ψ_{pd}, $\Psi_{l\text{-}md}$, $\Psi_{s\text{-}md}$ and $\Psi_{s\text{-}em}$ were measured by the pressure chamber technique (Scholander *et al.*, 1965) using a pressure chamber built and calibrated by PMS Instruments, Co. (Model 615D; Albany, OR, USA). Stem water potential bags used were designed for grape leaves and were manufactured by PMS Instruments, Co. (Albany, OR, USA). Predawn and midday measurements were conducted as described in Williams and Trout (2005), with a few modifications outlined below and taking into account recommendations by Williams and Araujo (2002). Specifically, Ψ_{pd} was measured during the last 2 hours of the night preceding sunrise. Midday measurements were taken during a 2-hour window centered on solar noon, which generally corresponded to approximately 1200h to 1400h Pacific Daylight Time (PDT). $\Psi_{s\text{-}em}$ was taken during a 1-hour window between approximately 0700h to 0800h (solar) as in Intrigliolo and Castel (2006). All leaves chosen for leaf water potential measurements were covered with a plastic bag and sealed 1-2 seconds prior to removal. Leaves chosen for stem water potential were located on the western side of N-S oriented rows and on the northeast side of NE-SW oriented rows, to minimize possible heating effects, and enclosed in plastic bags with reflective coating 60 to 90 minutes prior to removal (Choné *et al.* 2001). All leaves chosen for measurements were un-damaged, fully expanded and mature and were placed within the pressure chamber 15 seconds or less after removal from the plant. Midday measurements were always taken under full-sun conditions while $\Psi_{s\text{-}em}$ measurements were taken even on overcast mornings.

At both sites, $\Psi_{l\text{-}md}$ measurements were carried out on three vines from each treatment replicate (9 measurements per treatment) at bi-weekly intervals when weather allowed, and more frequently when possible, in order to track the seasonal irrigation treatment effects. Measurements began after the last recorded rainfall of the season at each site. Measurement dates were chosen so as to have at least 36 hours between the previous week's irrigation treatment and the first measurement of the day.

At Site 1, all four Ψ measurement-types listed above were carried out on one leaf per plant on the same three plants per replicate (9 measurements per treatment) on five dates (July 2nd, July 23rd, July 30th, August 13th and August 27th). At Site 2, $\Psi_{s\text{-}em}$, $\Psi_{l\text{-}md}$ and $\Psi_{s\text{-}md}$ were carried out on one leaf per plant on the same 3 plants per replicate (9 measurements per treatment) on four dates (August 7th, August 21st, September 11th and September 25th). At Site 2, vines were chosen to be centered around the soil moisture tubes as best possible. On July 23rd, significant dew was present on leaves for predawn and early-morning measurements. On August 7th and September 11th, skies were over-cast during $\Psi_{s\text{-}em}$ measurements. Skies were cloudless on all other $\Psi_{s\text{-}em}$ measurement days. Vapor Pressure Deficits (VPD) was calculated during measurement windows on each date using data from weather stations located at each site.

Leaf Area Index (LAI) was measured once just before irrigation and once after shoot-tip growth had completely stopped at both sites using a ceptometer (ACCUPAR LP-80, Decagon Devices, Pullman, WA). LAI measurements were taken on either side of the trunk at approximately solar noon on the same vines used for Ψ measurements described above. Canopy dimensions were also measured on either side of the trunk of the center vine and an average was used to calculate canopy surface area.

3.5 Vineyard variability:

On July 23rd and July 30th at Site 1 (prior to application of irrigation treatments), 16 locations ("spots") were chosen to represent the variability within the vineyard block where the experiment was located. Three neighboring vines, located within the same row, were used to represent each spot. The first 12 spots were located within the trial replicates described above and four additional spots were chosen to better represent the low vigor areas within treatment rows. These four additional spots were generally within the "gravelly silt loam" soil type shown on soil survey maps (**Figure 7**) and observed in the vineyard. Locations were chosen and categorized as low vigor (LV) or high vigor (HV) using an NDVI map provided by the grower (**Figure 6**) and confirmed using visual assessments of vigor and water stress (**Figures 8** and **Figure 9**). On each set of three vines, Ψ_{pd}, $\Psi_{s\text{-}em}$, $\Psi_{s\text{-}md}$ and $\Psi_{l\text{-}md}$ were measured on one leaf per vine. These measurements were also taken on all 16 spots during the irrigation period on August 13th (pre-veraison) and August 27th (post-veraison). On August 6th, only $\Psi_{l\text{-}md}$ was measured on all 16 spots.

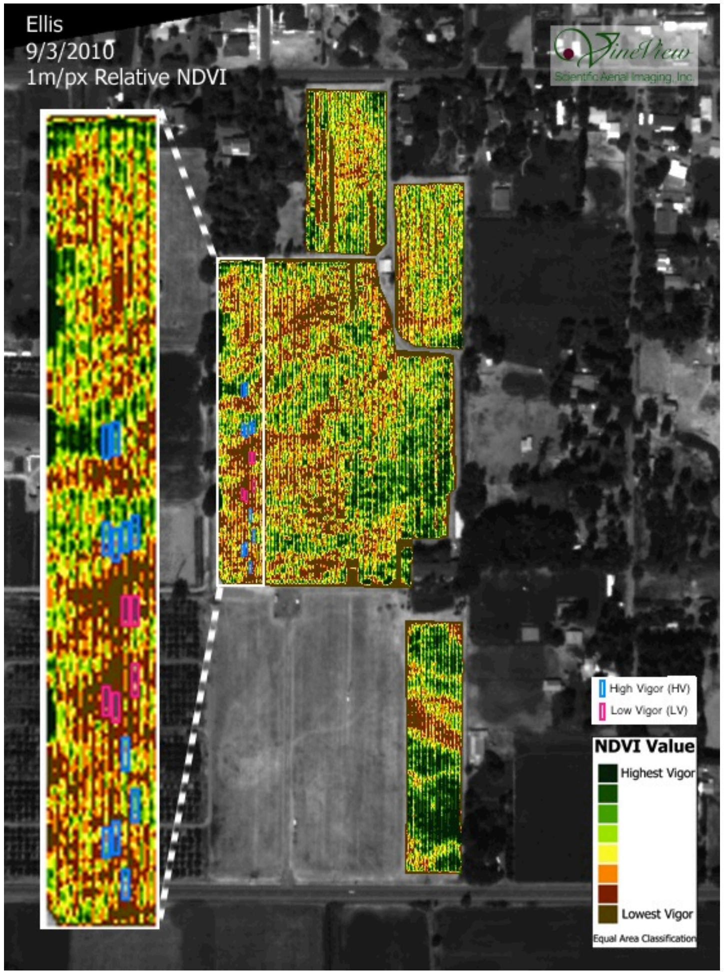

Figure 6. NDVI map of Site 1 ("Ellis" Vineyard), produced using aerial photography by Vine View Scientific Aerial Imaging Inc. (St. Helena, CA, USA). Rows of Tempranillo are outlined and expanded with 16 measurement locations marked and categorized by high and low vigor.

Figure 7. Soil survey map of Site 1. Soil types are indicated by the following codes: 157B) "Ruch silt loam, 2 to 7 percent slopes"; 158B) "Ruch gravelly silt loam, 2 to 7 percent slopes". Provided by the United States Department of Agriculture, NRCS (http://websoilsurvey.nrcs.usda.gov). *Exact soil borders may not be reliable to this scale.*

Figure 8. Photo of Tempranillo vines growing in a high vigor (HV) location with the block at Site 1.

Figure 9. Photo of Tempranillo vines growing in a low vigor (LV) location with the block at Site 1.

3.6 Repeatability and procedure testing for $\Psi_{s\text{-}em}$:

On August 19th at Site 1, three adjacent vines irrigated at 70% of ET_c and three adjacent vines irrigated at 35% ET_c were chosen and four leaves from each vine were bagged in the afternoon (approximately 18:00-18:30 PDT). On the next day (August 20th), an additional four leaves on each of the same shoots were bagged approximately 60 minutes before 0700h solar time. $\Psi_{s\text{-}em}$ was then measured on all leaves, with less than 90 seconds between measurements of leaves on the same shoot.

3.7 Diurnal Ψ patterns:

On August 27th at Site 1 and August 21st at Site 2, Ψ_l was measured every one to two hours on the same three vines from two different irrigation treatment rows (six vines total) starting just before dawn. Diurnal curves for Ψ_s starting at sunrise were produced in the same way on August 27th at Site 1 and August 7th, August 21st and September 11th at Site 2. Each set of three measurements was taken within a 10-minute time window. The average percentage of canopy illuminated by full-sun (%SUN) was estimated just before each set of Ψ_s measurements by measuring the canopy vertically above the trunk of each vine and averaging the three measurements.

3.8 Statistics:

Data was subject to analysis of variance (ANOVA). Means were compared using Duncan's multiple range test. Statistical analysis was done using Statistica® version 10 software (Statsoft, Tulsa, OK, USA).

4.0 Results and Discussion:

4.1 Vineyard Variability:

When spot measurements were taken prior to the initiation of full irrigation at Site 1, significant differences were shown between spots initially identified as low-vigor (LV) and high-vigor (HV) (**Table 4 and Table 5),** which were assumed to be growing on gravelly-silt loam (GSL) and silt-loam (SL) top soils, respectively. Differences between spots became clearer from the first measurement date to the second. On the day before irrigation treatments began (July 30th), locations could be classified as experiencing "no water deficit" (HV 1-11), "weak water deficit" (LV 4 and LV 5), "moderate to weak water deficit" (LV 2 and LV 3) and "moderate to severe water deficit" (LV 5) using classifications for Ψ_{pd} proposed by Van Leeuwen *et al*. (2009). The same significant differences between LV and HV vines were also seen when comparing LAI on July 30th and canopy surface area at the end of the season (**Table 6**).

In general $\Psi_{s\text{-}em}$ was the most sensitive measurement method, consistently distinguishing significant differences between categories of water deficit and even showing significant differences between spots under "no water deficit" on both dates. However, $\Psi_{s\text{-}em}$ was unable to show a significant difference between spots under "moderate to weak" and "moderate to severe" water deficits in one case (Table 5). Results for Ψ_{pd} showed similar sensitivity to differences in water status although this method was unable to show significant differences between "weak" and "moderate to weak" water deficits on July 23rd (Table 4). By comparison, data produced using $\Psi_{l\text{-}md}$ measurements showed no significant differences between means when values were higher than -0.8 MPa (Table 4 and Table 5) and did not always categorize spots under the same levels of water deficit as Ψ_{pd} and $\Psi_{s\text{-}md}$ (Table 5). Of the four methods assessed, $\Psi_{s\text{-}md}$ produced the most consistent significant differences between previously defined water deficit categories.

Other publications have also demonstrated the ability of both $\Psi_{s\text{-}md}$ and Ψ_{pd} to distinguish significant differences between non-irrigated vines within the same block or between different soil types (Choné *et al.* 2001; Van Leeuwen *et al.* 2006) while $\Psi_{l\text{-}md}$ was unable to distinguish these same significant differences in most cases (Choné *et al.* 2001). However, results from these studies were generally obtained on vines experiencing higher levels of water deficits or under conditions of higher VPD compared to those in the present study (**Table 7**).

Table 4. Table of predawn leaf (Ψ_{pd}), midday leaf ($\Psi_{l\text{-}md}$), midday stem ($\Psi_{s\text{-}md}$) and early morning stem ($\Psi_{s\text{-}em}$) water potentials values measured on July 23rd at 16 spots spread across a single vineyard block representing low vigor (LV 1-5) and high vigor (HV 1-11) areas. Values are shaded by water stress deficit thresholds according to those proposed by Van Leeuwen *et al.* (2009) as follows: no water deficit (▨), weak water deficit (▥) weak to moderate water deficit (▩) and moderate to severe water deficit (▤). Each spot was represented by 3 neighboring vines and values represent the mean of 1 measurement per vine (n=3).

	23-July															
	LV 1	LV 2	LV 3	HV 11	HV 8	LV 5	HV 5	HV 2	LV 4	HV 6	HV 3	HV 1	HV 10	HV 9	HV 7	HV 4
Ψ_{pd} (MPa)	-0.30^{a}	-0.27^{a}	-0.23^{a}	-0.10^{b}	-0.10^{b}	-0.09^{b}	-0.05^{b}	-0.07^{b}	-0.06^{b}	-0.13^{b}	-0.07^{b}	-0.07^{b}	-0.05^{b}	-0.08^{b}	-0.09^{b}	-0.07^{b}
$\Psi_{s\text{-}md}$ (MPa)	-1.17^{a}	-0.75^{b}	-0.80^{b}	-0.42^{c}	-0.47^{c}	-0.47^{c}	-0.37^{c}	-0.40^{c}	-0.42^{c}	-0.35^{c}	-0.35^{c}	-0.37^{c}	-0.30^{c}	-0.33^{c}	-0.38^{c}	-0.30^{c}
$\Psi_{l\text{-}md}$ (MPa)	-1.28^{a}	-0.98^{b}	-1.02^{b}	-0.62^{c}	-0.60^{c}	-0.68^{c}	-0.53^{c}	-0.57^{c}	-0.62^{c}	-0.52^{c}	-0.53^{c}	-0.67^{c}	-0.52^{c}	-0.48^{c}	-0.55^{c}	-0.52^{c}
$\Psi_{s\text{-}em}$ (MPa)	-0.55^{a}	-0.20^{c}	-0.33^{b}	-0.18^{cd}	-0.15^{cde}	-0.13^{cde}	-0.11^{cde}	-0.11^{cde}	-0.10^{cde}	-0.09^{cde}	-0.09^{cde}	-0.09^{cde}	-0.08^{cde}	-0.07^{de}	-0.07^{de}	-0.05^{e}

Table 5. Table of predawn leaf (Ψ_{pd}), midday leaf ($\Psi_{l\text{-}md}$), midday stem ($\Psi_{s\text{-}md}$) and early morning stem ($\Psi_{s\text{-}em}$) water potentials values measured on July 30th at 16 spots spread across a single vineyard block representing low vigor (LV 1-5) and high vigor (HV 1-11) areas. Values are shaded by water stress deficit thresholds according to those proposed by Van Leeuwen *et al.* (2009) as follows: no water deficit (▨), weak water deficit (▥) weak to moderate water deficit (▩) and moderate to severe water deficit (▤). Each spot was represented by 3 neighboring vines and values represent the mean of 1 measurement per vine (n=3).

	30-July															
	LV 1	LV 2	LV 3	LV 4	LV 5	HV 1	HV 2	HV 3	HV 4	HV 5	HV 6	HV 7	HV 8	HV 9	HV 10	HV 11
Ψ_{pd} (MPa)	-0.53^{a}	-0.37^{b}	-0.30^{bc}	-0.28^{bcd}	-0.26^{bcde}	-0.15^{cdef}	-0.14^{def}	-0.13^{def}	-0.13^{def}	-0.12^{def}	-0.11^{ef}	-0.10^{ef}	-0.08^{f}	-0.07^{f}	-0.07^{f}	-0.05^{f}
$\Psi_{s\text{-}md}$ (MPa)	-1.27^{a}	-0.96^{b}	-0.98^{b}	-0.60^{cd}	-0.60^{cd}	-0.70^{c}	-0.37^{d}	-0.44^{d}	-0.37^{d}	-0.53^{cd}	-0.43^{cd}	-0.42^{d}	-0.46^{d}	-0.5^{d}	-0.43^{d}	-0.43^{d}
$\Psi_{l\text{-}md}$ (MPa)	-1.38^{a}	-1.12^{b}	-1.07^{b}	-0.73^{c}	-0.83^{c}	-0.77^{c}	-0.65^{c}	-0.63^{c}	-0.73^{c}	-0.72^{c}	-0.72^{c}	-0.67^{c}	-0.72^{c}	-0.77^{c}	-0.70^{c}	-0.77^{c}
$\Psi_{s\text{-}em}$ (MPa)	-0.53^{a}	-0.45^{ab}	-0.38^{abc}	-0.25^{cde}	-0.34^{bcd}	-0.10^{e}	-0.08^{e}	-0.18^{de}	-0.18^{de}	-0.12^{e}	-0.12^{e}	-0.06^{e}	-0.13^{e}	-0.11^{e}	-0.06^{e}	-0.07^{e}

Climactic conditions can play an important role in the results of daytime Ψ measurements (Jones, 2004). When the influence of VPD on Ψ_l was studied using multiple wine grape varietals and locations in California, climactic influences were greatest on vines under no water stress (Williams and Baeza, 2007). Comparatively, Olivo *et al.* (2009) indicated that the influence of VPD deficits on Ψ_s for Tempranillo grown in Spain might be greatest later in the season or under higher levels of water stress (Ψ_s <-0.8 MPa). These reports could explain part of the reason why $\Psi_{s\text{-}md}$ performed better than $\Psi_{l\text{-}md}$ under lower water deficits on the date with a wider ranger of VPD during the midday measurement period (Table 5 and Table 7).

Water deficit thresholds for Ψ_{pd} and $\Psi_{s\text{-}md}$ proposed by Van Leeuwen *et al*. (2009) were generally consistent with each other in the present study (Table 4 and 5). In comparison, using the proposed $\Psi_{l\text{-}md}$ threshold for “weak” water deficit (<-0.9 MPa) seemed to underestimate plant water status when compared to the other measurement methods. Such inconsistencies in the interpretation of $\Psi_{l\text{-}md}$ thresholds can also be seen in data published for different varietals and different climates. In their study on Thompson Seedless in the San Joaquin Valley of California, Williams and Trout (2005) confirmed the $\Psi_{s\text{-}md}$ threshold for “weak” water deficit presented here in Table 1 (-0.6 MPa) but reported a conflicting $\Psi_{l\text{-}md}$ threshold of -1.0 MPa. Conversely, Girona *et al.* (2006) demonstrated that many water deficit thresholds for $\Psi_{l\text{-}md}$ presented in the literature underestimated water stress levels in *Vitis vinífera* L cv. Pinot Noir grown in Raïmat Lleida, Spain and proposed a $\Psi_{l\text{-}md}$ level of -0.6 MPa as a trigger for initiating irrigation in control treatments.

Table 6. Mean Leaf Area Index (LAI) and canopy surface area (m^2 m^{-1}) of vines measured at Site 1 and Site 2. Measurements at Site 1 were separated by replicate soil type based on vigor and vine water potential measurements before irrigation. LAI was measured on July 30th and July 31st at Site1 and Site 2, respectively. Canopy surface area was calculated using external canopy dimensions measured close to harvest. Mean values within the same column followed by the same letter do not differ significantly ($p \leq 0.05$).

	Leaf Area Index (LAI)	Canopy surface area (m^2 m^{-1})
Site 1 (Silt Loam)	4.08a	3.20a
Site 1 (Gravelly Silt Loam)	3.16c	2.64b
Site 2 (Silt clay)	3.62b	3.21a

Table 7. Vapor Pressure Deficits (VPD) and Temperature during approximate measurement windows for midday and early morning vine water potential on two dates at Site 1. Data was provided by property owner and was collected from an onsite weather station.

Date	Time Window (PDT)	Vapor Pressure Deficit (kPa)	Temperature (°C)
23-Jul	8:00 - 9:00	0.45 - 0.72	11.0 - 12.3
	12:00 - 14:00	0.92 - 1.07	15.3 - 16.6
30-Jul	8:00 - 9:00	0.43 - 0.70	14.0 - 16.7
	12:00 - 14:00	1.82 – 2.55	24.3 - 27.6

4.2 Effects of irrigation treatments:

Seasonal $\Psi_{l\text{-}md}$ patterns for Site 1 are presented in **Figure 10** for the original irrigation experiment. Mean $\Psi_{l\text{-}md}$ declined steadily from approximately -0.4 MPa starting June 6th (DOY 158) until the first minimal irrigation application on July 20th (DOY 213), which caused a slight increase in $\Psi_{l\text{-}md}$ for all treatments. Values continued to drop thereafter, until irrigation treatments were applied starting July 31th (DOY 213). Values of $\Psi_{l\text{-}md}$ gradually increased as irrigation treatments continued and returned to levels seen just prior to the start of irrigation, or higher, by the last measurement date. Measurements from the most stressed treatment replicate (replicate LV 2 from Tables 4 and 5) were used to initiate irrigation at Site 1, resulting in treatment $\Psi_{l\text{-}md}$ means ranging between -0.70 MPa and -0.85 MPa before irrigation was started (Figure 10). At Site 1, $\Psi_{l\text{-}md}$ never fell below -1.0Mpa (Figure 10). Differences in mean values did not generally correspond with irrigation amounts of the previous week at Site 1, although means of the treatment receiving the highest quantity of water (70-70) were always among the highest values following irrigation applications. Replicate LV 2 was located within the 70-35 treatment row, which is likely the reason for this treatment showing the lowest $\Psi_{l\text{-}md}$ means throughout the majority of the season (Figure 10).

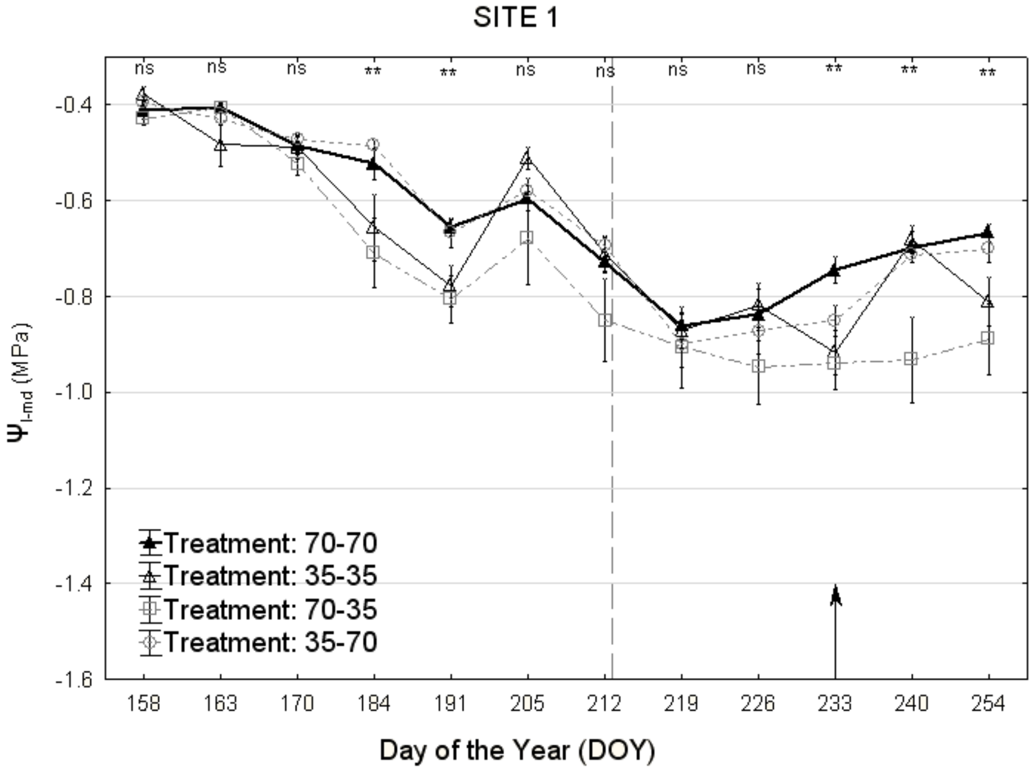

Figure 10. Pattern of $\Psi_{l\text{-}md}$ over the 2012 growing season for four irrigation treatments at Site 1. The vertical arrow indicates the approximate date of 50% veraison and the shift in irrigation applications for treatments 70-35 and 35-70. The vertical dotted line indicates the initiation of irrigation (DOY 213). Means consist of averages of nine measurements per treatment with standard error bars added. NS or ** indicates non-significant or significant differences between mean treatments values ($p \leq 0.05$).

Seasonal $\Psi_{l\text{-}md}$ patterns for Site 2 are presented in **Figure 11.** Mean $\Psi_{l\text{-}md}$ showed a steady decline from approximately -0.45 MPa on July 19th (DOY 171) until the initiation of irrigation on August 1st (DOY 214). On the day before irrigation was initiated, at least one $\Psi_{l\text{-}md}$ value less that -1.2 MPa was seen within each irrigation treatment (data not shown). However, $\Psi_{l\text{-}md}$ treatment means only ranged between -0.9 MPa and -1.0 MPa on the day before irrigation was initiated (Figure 11). Measurements of $\Psi_{l\text{-}md}$ before irrigation showed significant differences between treatments on one date. However, mean $\Psi_{l\text{-}md}$ values before the initiation of irrigation indicated that, on average, all treatment blocks were experiencing no water deficit during this period (Van Leeuwen *et al.* 2009). Once irrigation was initiated no significant differences between treatments were shown until after the second week of irrigation was applied (measurement DOY 227) and all expected differences between treatments were only seen after the fourth week of irrigation. The expected $\Psi_{l\text{-}md}$ differences between irrigation treatments were clearly shown just before veraison on August 28th (DOY 241) and after veraison on September 25 (DOY 269).

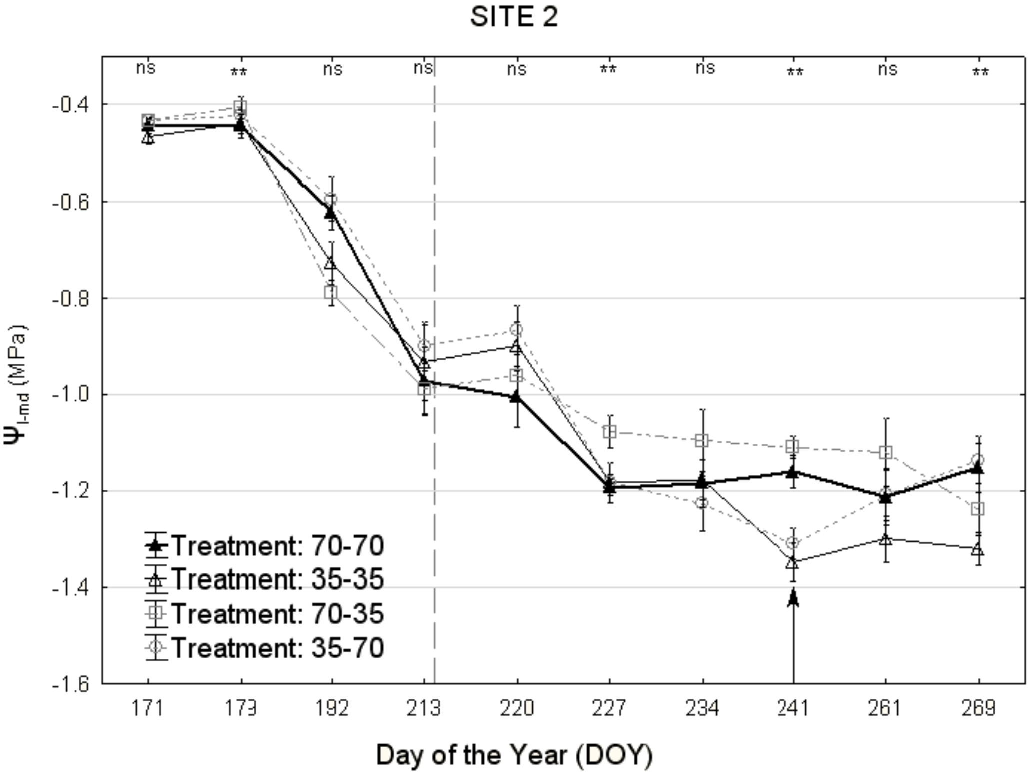

Figure 11. Pattern of $\Psi_{\text{l-md}}$ over the 2012 growing season for four irrigation treatments at Site 2. The vertical arrow indicates the approximate date of 50% veraison and the shift in irrigation applications for treatments 70-35 and 35-70. The vertical dotted line indicates the initiation of irrigation (DOY 214). Means consist of averages of nine measurements per treatment with standard error bars added. NS or ** indicates non-significant or significant differences between mean treatments values ($p \leq 0.05$).

Seasonal SWC values measured at Site 2 are presented in **Figure 12**. Several access tubes were placed slightly too high resulting in inaccurate data from the 10cm depth, so data from this depth was excluded. SWC at all depths and for all treatments declined slightly from July 10th (DOY 192) until veraison (August 28, DOY 241), demonstrating little to no effect from pre-veraison irrigation treatments. After veraison, all SWC levels increased dramatically at the 30 cm depth from <0.20 (m^3 m^{-3}) to ≥0.40 (m^3 m^{-3}) (Figure 12). SWC showed none of the expected patterns between irrigation treatments and no significant correlations with $\Psi_{\text{l-md}}$ means at any of the six depths measured (data not shown). This lack of irrigation response to SWC matches those obtained by Intrigliolo and Castel (2006 and 2008) when using a similar configuration of access tubes but contrasts with those obtained by Williams and Araujo (2002), who included more access tubes per vine and included some tubes between the vine rows.

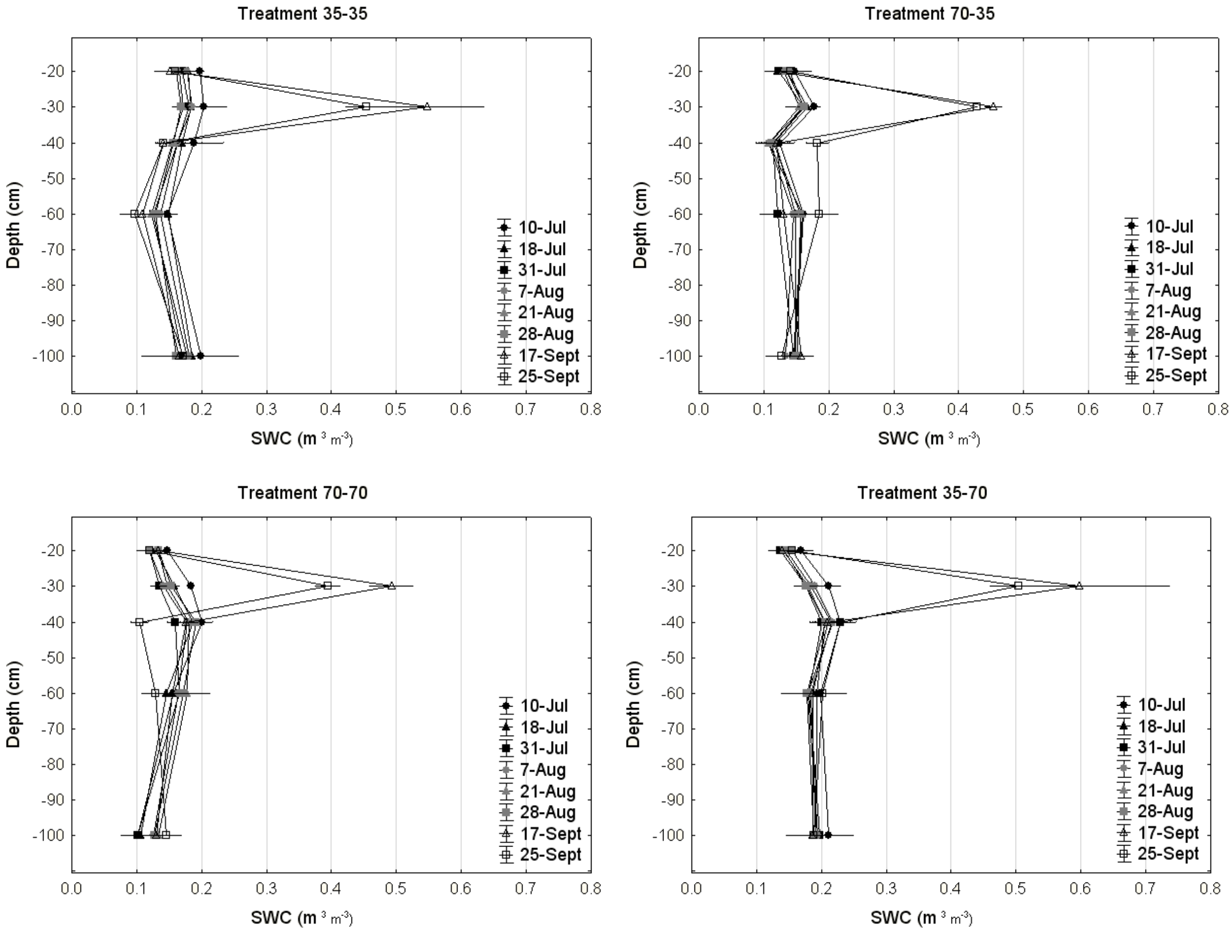

Figure 12. Soil water content at Site 2 measured under the vine row of four irrigation treatments (at depths of 20cm, 30cm, 40cm, 60cm, and 100cm) on eight dates over the course of the season. These dates represent periods prior to the initiation of irrigation (July 10, July 16, July 31), between the initiation of irrigation and veraison (Aug. 7, Aug. 21, Aug. 28) and post-veraison (Sept. 11 and Sept. 25). Values represent means of three measurements per treatment with standard error bars added.

Since $\Psi_{l\text{-}md}$ was able to reflect differences between irrigation treatments on several dates at Site 2 (Figure 11), data supports previous findings that SWC measurements alone do not reflect actual plant available water (Gruber and Schultz, 2005). High spatial variability within this thin soil type may have been a contributing factor to these results, making it difficult to represent the actual plant available water using a single access point and increasing the likelihood of calibration errors. In addition, the placement of the access tubes may have simply been too far from the wetted zone of the emitter to reflect the impact of irrigation. The apparent wetting of soil at the 30 cm depth without any increase in moisture content close to the soil surface suggests water was moving laterally in the soil at this depth. A sudden change in soil type may have created a “hard-pan” effect, causing irrigation water to spread out or run downhill (toward the access tube) only after soil directly under the emitter was fully saturated. The fact that this soil type is reported to have weathered bedrock subsoil starting at approximately 0.4 cm of depth supports this conclusion (Table 2).

The lack of expected differences in Ψ between treatments at Site 1 (Figure 10) was likely due to a combination of factors. The amount of irrigation applied could have been estimated incorrectly as the method used for estimating K_c was established under different climactic conditions and trellising systems (Williams and Ayars, 2005). In addition, the use of the ETgage has been shown to significantly underestimate ET_o when compared with the Penman-Monteith equation, especially under wind speeds greater than 1.6 kph (Chen and Robinson, 2009) which were seen frequently in the afternoons at this site (data not shown). However given that $\Psi_{l\text{-}md}$ generally decreased after irrigation was initiated, even in the treatment receiving only 35% of estimated $ET_{c.}$ (Figure 10), it appears that vines were in fact over-irrigated at Site 1.

Seasonal measurements at both sites (Figure 10 and 11) generally started at levels of $\Psi_{l\text{-}md}$ matching maximum field SWC capacity shown in other studies (Williams and Trout, 2005). Judging by $\Psi_{l\text{-}md}$ means just before the initiation of irrigation at Site 1 (Figure 10), irrigation might have been initiated before water from winter rainfall was depleted from the deeper soil profiles. Such deep ground water reserves would represent a serious interference with the effects of RDI irrigation treatments (McCarthy *et al.* 2002), as vines would not be dependent on irrigation water to maintain Ψ. In addition, the lack of buffer rows in this experimental design may have contributed to the lack of treatment effects if significant inter-row root activity was present after the initiation of irrigation.

When comparing Ψ_{pd}, $\Psi_{s\text{-}em}$, $\Psi_{s\text{-}md}$ and $\Psi_{l\text{-}md}$ treatment means at Site 1, all patterns were similar to those shown for $\Psi_{l\text{-}md}$ in Figure 10 with none of the expected effects of irrigation shown even when extra measurements from the GSL soil type were included (Annex Figure 1). However when data was pooled separately for all measurements within the different soil types established above (**Figure 13**), differences were clearly shown between soils for all measurement types and all measurement dates. These results are in agreement with others seen in dry-farmed vines growing on different soil types (Choné *et al.* 2001; Van Leeuwen *et al.* 2006), indicating that soil type may have influenced Ψ to a greater extent than did irrigation treatment in the present study. Minimum Ψ of vines growing in the GSL topsoil reached levels indicative of “moderate to weak” water deficits while the vines growing in SL topsoil experienced no water deficit for the duration of the irrigation season (Van Leeuwen *et al.* 2009). Since the majority of the vines growing in the GLS soil type were not included in the calculation of K_c for the irrigation trial, these vines were likely over-irrigated because of their smaller canopy size and therefore lower levels of water use (Williams and Ayars, 2005).

When comparing $\Psi_{s\text{-}em}$, $\Psi_{s\text{-}md}$ and $\Psi_{l\text{-}md}$ measurements at Site 2 (**Table 8**), no significant differences were shown between treatments for any of the measurement types the first week after irrigation on August 7^{th}. On all following measurement dates $\Psi_{s\text{-}em}$ was able to show significant differences between irrigation treatments, primarily indicating that the treatment receiving the least water (35-35) was significantly lower compared to the other three treatments. $\Psi_{l\text{-}md}$ was able to show these same effects, but only on the last measurement date. $\Psi_{s\text{-}md}$ was unable to show significant differences between irrigation treatments on any of the four dates. However, mean $\Psi_{s\text{-}md}$ values for treatments receiving 35% ET_c on the last measurement date were greater than -0.1 MPa compared to those receiving 75% ET_c (Table 8).

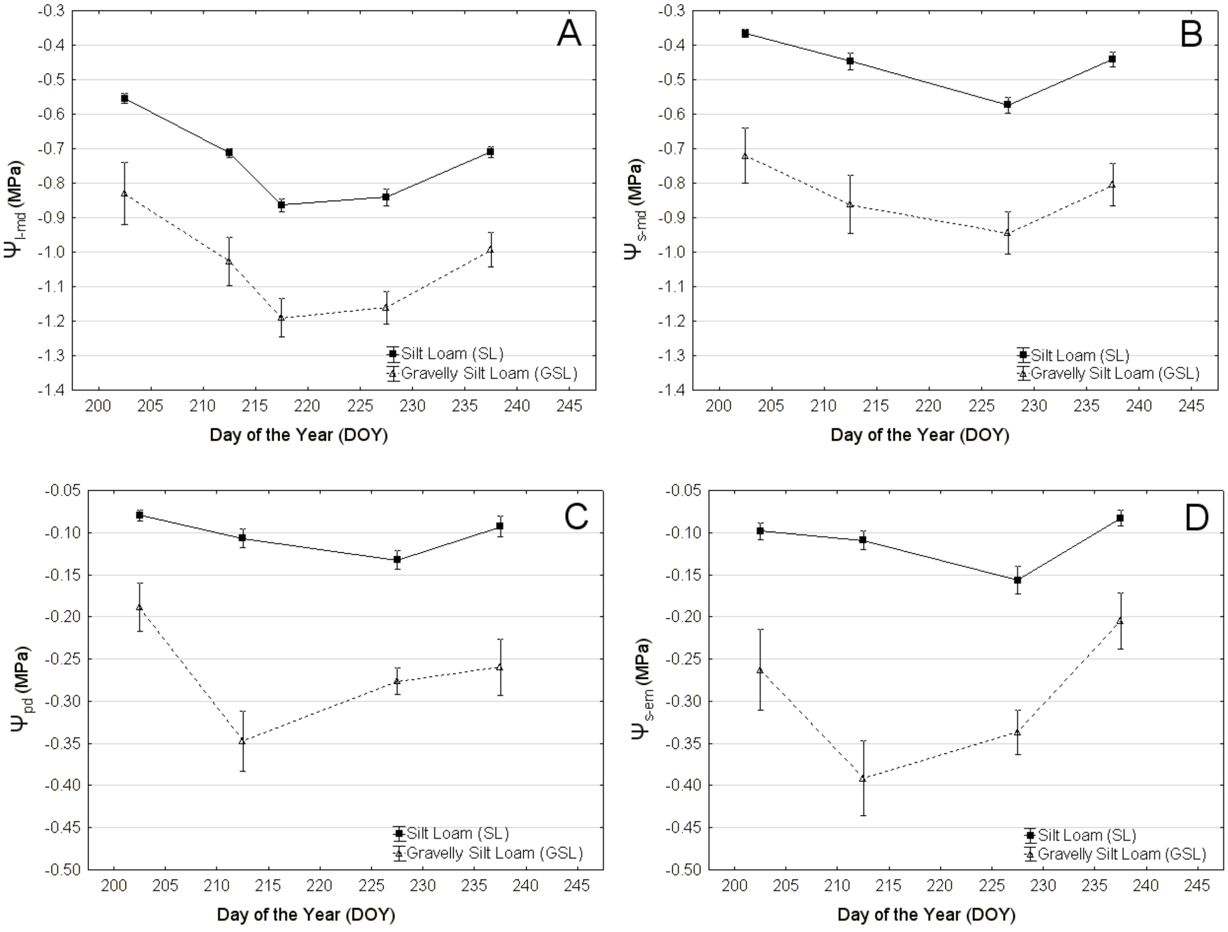

Figure 13. Seasonal patterns of $\Psi_{l\text{-}md}$ (A), $\Psi_{s\text{-}md}$ (B), Ψ_{pd} (C) and $\Psi_{s\text{-}em}$ (D) measured on vines growing in gravelly silt loam top soil (GSL) and silt loam top soil (SL) at Site 1. The first full irrigation was applied on July 31st (DOY 213). Values represent mean values with standard error bars added.

Results presented here confirm reports by Intrigliolo and Castel (2006) that $\Psi_{s\text{-}em}$ is better able to differentiate between irrigation treatments in Tempranillo compared to $\Psi_{l\text{-}md}$ and $\Psi_{s\text{-}md}$. This is despite VPD levels in the current study (**Table 9**) being lower than those reported by Intrigliolo and Castel (2006). Data presented here also indicates that $\Psi_{s\text{-}em}$ was able to differentiate between irrigation treatments even under cloudy conditions (Table 8). Similarly inconsistent $\Psi_{l\text{-}md}$ results (i.e. not always showing differences between irrigation treatments) have been attributed to stomatal regulation in Tempranillo vines under stress (Medrano *et al.* 2003; Sousa *et al*., 2006). Similar effects may be attributed to $\Psi_{s\text{-}md}$ since strong relationships have been shown between $\Psi_{s\text{-}md}$ and g_s in Tempranillo (Intrigliolo *et al.* 2005). However, results presented here may also support conclusions made by Williams and Baeza (2007) which suggest that the influence of VPD on Ψ_l decreases as vine water stress increases and that soil moisture availability becomes the primary factor affecting Ψ_l once levels begin to drop below -1.2 MPa. Conversely, greater influence of VPD on $\Psi_{s\text{-}md}$ later in the season and at higher levels of water deficit (Olivo *et al.* 2009) may have also played a role in the poor performance of this method during the irrigation season. Such environmental interferences would have been exacerbated by the use of a two-hour measurement period, compared to the one-hour period used in many other studies (Williams and Araujo, 2002).

Tables 8. Mean $\Psi_{s\text{-}em}$, $\Psi_{s\text{-}md}$ and $\Psi_{l\text{-}md}$ values on four measurement dates for four irrigation treatments at Site 2. Shaded values for $\Psi_{s\text{-}em}$ were taken on mornings with heavy cloud cover. The broken line represents veraison and the change in irrigation amount for RDI treatments. Mean values within the same date and measurement type followed by the same letter do not differ significantly ($p \leq 0.05$).

	Irrigation Treatment		**$\Psi_{s\text{-}md}$**	**$\Psi_{l\text{-}md}$**	**$\Psi_{s\text{-}em}$**
	%ET_c before veraison	%ET_c after veraison		(MPa)	
7-Aug	**35**	35	-0.71^{a}	-0.90^{a}	-0.17^{a}
	35	70	-0.65^{a}	-0.87^{a}	-0.13^{a}
	70	35	-0.69^{a}	-0.96^{a}	-0.13^{a}
	70	70	-0.76^{a}	-1.01^{a}	-0.19^{a}
21-Aug	**35**	35	-0.97^{a}	-1.18^{a}	-0.36^{a}
	35	70	-0.92^{a}	-1.23^{a}	-0.32^{ab}
	70	70	-0.91^{a}	-1.19^{a}	-0.13^{c}
	70	35	-0.86^{a}	-1.10^{a}	-0.18^{bc}
11-Sep	35	**35**	-1.06^{a}	-1.30^{a}	-0.60^{a}
	70	**35**	-0.91^{a}	-1.12^{a}	-0.44^{b}
	35	**70**	-0.93^{a}	-1.21^{a}	-0.29^{b}
	70	**70**	-1.00^{a}	-1.21^{a}	-0.39^{b}
25-Sep	35	**35**	-1.11^{a}	-1.32^{a}	-0.48^{a}
	70	**35**	-1.05^{a}	-1.24^{ab}	-0.25^{b}
	35	**70**	-0.92^{a}	-1.14^{b}	-0.21^{b}

70	**70**	-0.92^{a}	-1.15^{b}	-0.24^{b}

Table 9. Vapor Pressure Deficits (VPD) and Temperature during approximate measurement windows for midday and early morning vine water potential on four dates at Site 2. Data provided by property owner and was collected from an onsite weather station.

Date	Time Window (PDT)	Vapor Pressure Deficit (kPa)	Temperature (°C)
7-Aug	8:00 - 9:00	0.13 - 0.16	13.9 - 14.3
	12:00 - 14:00	0.67 - 1.43	20.4 - 25.8
21-Aug	8:00 - 9:00	0.27 - 0.60	15.1 - 18.1
	12:00 - 14:00	1.36 - 2.23	23.8 - 28.3
11-Sep	8:00 - 9:00	0.62 - 0.67	9.3 - 11.3
	12:00 - 14:00	0.76 - 0.79	17.4 - 21.1
25-Sep	8:00 - 9:00	0.09 - 0.13	11.4 - 12.2
	12:00 - 14:00	0.96 - 1.45	20.9 - 24.2

4.3 Diurnal Ψ patterns:

Diurnal patterns for Ψ_s and Ψ_l are presented in **Figure 14** and **Figure 15**, respectively. Measurements were taken on two dates before veraison and on two dates after veraison and include vines under "no water deficit" ($\Psi_{s-md} > -0.6$ MPa), "weak" water deficit (-0.9 MPa $> \Psi_{s-md} > -0.6$ MPa) and "moderate to weak" water deficit (-1.1Mpa $> \Psi_{s-md} > -0.9$ MPa). Diurnal Ψ patterns all showed a steady decrease throughout the morning hours with a plateau starting at approximately solar noon. These patterns generally agree with other diurnal curves measured for Syrah (Smart, 1974), Cabernet Sauvignon (Choné *et al.* 2001), Carignane (Kliewer *et al.* 1983) and Tempranillo (Bartolomé *et al.* 1996, Intrigliolo and Castel, 2006). Results also agree with those of other studies, which indicated that differences in water status are reflected in Ψ_s measured in the early morning (Choné *et al.* 2001) and sometimes show greater differences than Ψ_s measured at midday (Intrigliolo and Castel, 2006).

The vines measured at Site 1 showed Ψ_{pd} and Ψ_{s-em} values opposite of what might be expected, with lower values for vines receiving larger amounts of water (Figure 14, C and Figure 15, A). Both sets of vines measured at Site 1 were growing in the HV/SL locations described above (Section 4.1) and experienced no water deficit on this date (Van Leeuwen *et al.* 2009). It can be concluded that variation in soil between the two spots or between vines had greater influences on water status than did irrigation treatments in this case.

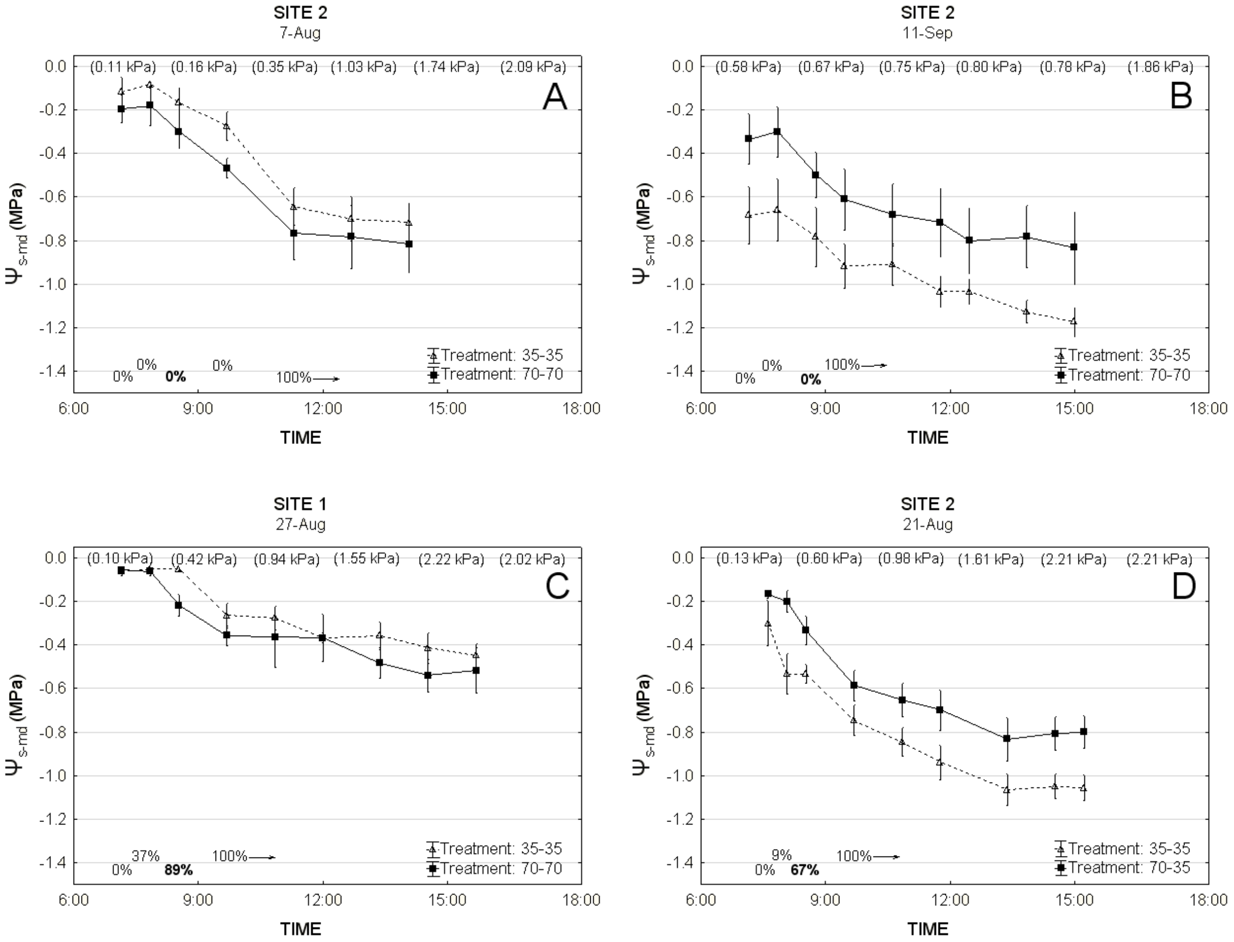

Figure 14. Patterns of diurnal stem water potential (Ψ_s) on cloudy days before veraison (A) and after veraison (B) and on sunny days at Sites 1 (C) and Site 2 (D). Approximate percentage of the canopy illuminated by full sun (%SUN) for morning measurement times is indicated at the bottom of each graph. Vapor Pressure Deficit (VPD) measured every two hours is presented at the top of each graph. Time represents Pacific Daylight Time (PDT). Values represent means of three vines measurements (n=3) from two different irrigation treatments with standard error bars added.

On cloudless mornings canopies generally presented different levels of %SUN during the first part of the $\Psi_{s\text{-}em}$ measurement window at each site (**Figure 16)** due to both row direction and slope aspect. Since both light intensity and Ψ_s are related to transpiration in grapevines (Winkel and Rambal, 1993; Choné *et al.* 2001), it might be assumed that different levels of light interception at the moment of measurement would significantly impact resulting Ψ_s. However data presented here indicates that Ψ_s measured between 0700h and 0800h solar time (generally between 0815h and 0915h PDT) on cloudless days at both sites (Figure 14, C and D) showed clear differences between treatments despite different levels of %SUN. On days with cloudy mornings (Figure 14, A and B), $\Psi_{s\text{-}em}$ differences were less clear. However when comparing whole-treatment means representing a larger sample size (Table 8), significant differences in $\Psi_{s\text{-}em}$ were shown between treatments even on cloudy mornings while midday measurements showed no significant differences on these same dates.

Overall, the data presented here indicates that $\Psi_{s\text{-}em}$ varied between irrigation treatments despite cloud cover but may be less sensitive under such conditions. However, when Site 2 data was pooled separately for cloudy and clear conditions, relationships between $\Psi_{s\text{-}em}$ and $\Psi_{s\text{-}md}$ were shown to be significantly different from each other (**Figure 17).** These relationships indicate that at $\Psi_{s\text{-}em}$ tends to be lower on cloudy mornings compared to sunny mornings for the same $\Psi_{s\text{-}md}$ value.

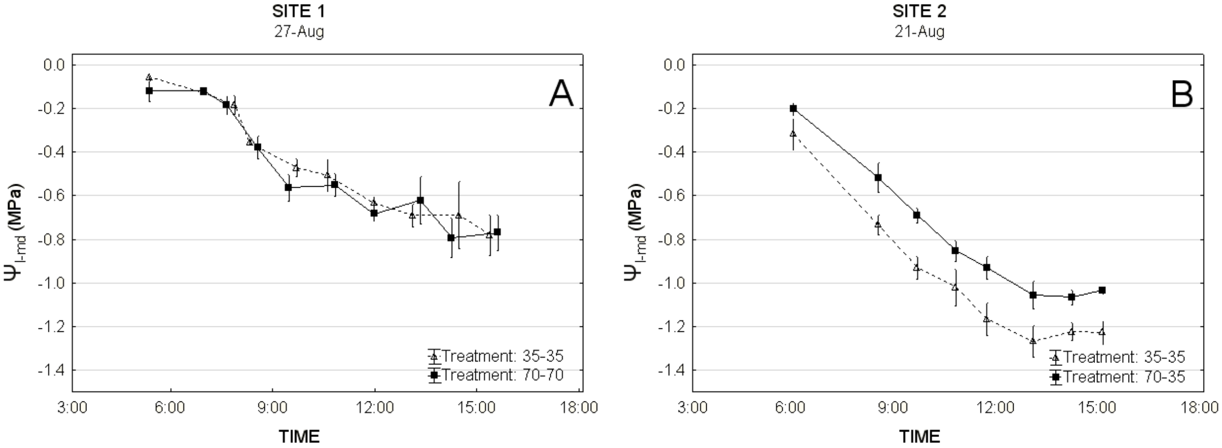

Figure 15. Diurnal leaf water potential (Ψ_l) on similar dates for Site 1 (A) and Site 2 (B). Time represents Pacific Daylight Time (PDT). Values represent means of three vines measurements (n=3) from two different irrigation treatments with standard error bars added.

Diurnal patterns of Ψ_l (Figure 15) showed differences before dawn were consistent with those seen for $\Psi_{s\text{-}em}$ on the same dates (Figure 14, C and D). In contrast, midday Ψ values were reversed compared to $\Psi_{s\text{-}md}$ on August 27th (Figure 14, C and Figure 15, A) when vines were under "no water deficit" (Van Leeuwen *et al.* 2009). As such, data presented here further supports the suggestion that midday Ψ measurements are subject to greater climactic interference when vines are not under water stress (Williams and Baeza, 2007).

Site 1
8:07 PDT - **6:50 solar** - 70 %SUN

Site 2
8:05 PDT - **6:48 solar** - 53 %SUN

Site 1
8:30 PDT - **7:13 solar** - 100 %SUN

Site 2
8:25 PDT - **7:08 solar** - 73%SUN

Figure 16. Photos of vineyard canopies taken just before the beginning of the $\Psi_{s\text{-}em}$ measurement time window at Site 1 (top left) and Site 2 (top right) and during the first part of the $\Psi_{s\text{-}em}$ measurement time window at Site 1 (bottom left) and Site 2 (bottom right). %SUN represents the percentage of full sun hitting the canopy. Photos taken on August 13th and August 21st at Site 1 and Site 2 respectively.

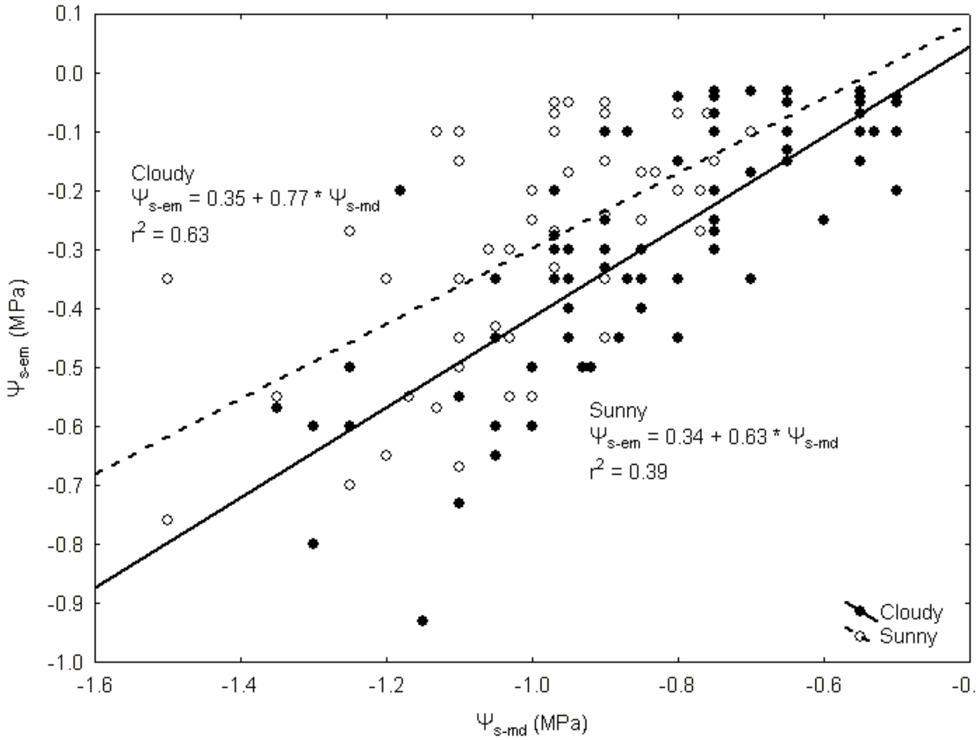

Figure 17. Relationship between midday stem water potential ($\Psi_{s\text{-}md}$) and early morning stem water potential ($\Psi_{s\text{-}em}$) on either cloudy mornings or sunny mornings. Points represent pairs of single measurements made on the same vine on the same date. R^2 values represent significances at $p \leq 0.05$.

4.4 Repeatability and procedure testing for $\Psi_{s\text{-}em}$:

A comparison between $\Psi_{s\text{-}em}$ measured on leaves bagged for 60 minutes or bagged the afternoon before are presented in **Table 10.** There were no significant differences seen between bagging methods, similar to data reported by Choné *et al.* (2001). This confirms that bagging methods should have no influence on the comparison of previous trials using $\Psi_{s\text{-}em}$ (Salón *et al.* 2004; Salón *et al.* 2005; Intrigliolo *et al.* 2005; Intrigliolo and Castel, 2006; Intrigliolo and Castel, 2010) and that growers can choose to attach bags at either time-frame for the measurement of $\Psi_{s\text{-}em}$. When values were pooled to produce mean $\Psi_{s\text{-}em}$ per vine, $\Psi_{s\text{-}em}$ was able to demonstrate significant differences between irrigation amounts and even between consecutive vines receiving the same irrigation amount despite mean differences of only 0.07 MPa. Although midday measurements were not performed on these vines on the same date, it may be interesting to compare these results with data presented by Choné *et al.* (2001) which showed significant differences between individual grapevines for $\Psi_{s\text{-}md}$ at differences of as little as 0.06 MPa while significant differences could only be detected for $\Psi_{l\text{-}md}$ with differences of 0.16 MPa.

Table 10. Mean $\Psi_{s\text{-}em}$ values measure on leaves bagged either 60 minutes before measurement or in the afternoon before measurement on two sets of three consecutive vines. Vines were either irrigated at 35% reference evapotranspirtation (ET_c) or 70% ET_c the week before. Measurements were taken before veraison on August 20th. Bagging trial means represent averages of four leaves measured on the same four shoots for each bagging method (n=4) and overall means represent an average of all leaves measured on the respective vine (n=8). Mean values within rows followed by the same letter do not differ significantly ($p \leq 0.05$).

	35% ET_c			70% ET_c		
	Vine 1 (MPa)	Vine 2 (MPa)	Vine 3 (MPa)	Vine 4 (MPa)	Vine 5 (MPa)	Vine 6 (MPa)
Leaves bagged ~1 hour before (n=4)	-0.28^a	-0.23^a	-0.22^a	-0.17^a	-0.15^a	-0.12^a
Leaves bagged ~14 hours before (n=4)	-0.28^a	-0.19^a	-0.20^a	-0.13^a	-0.12^a	-0.11^a
Mean (n=8)	-0.28^x	-0.21^y	-0.21^y	-0.15^z	-0.13^z	-0.12^z

4.5 Relationships between Ψ measurements:

Linear correlation models comparing Ψ_l and Ψ_s measurement methods are presented in **Figures 18 and 19.** Data was pooled across all irrigation treatments and all sunny days. Values generally represent vines ranging from "no water deficit" to "moderate to weak" water deficit, similar to data published by Intrigliolo and Castel (2006). In agreement with previous studies (Williams and Araujo, 2002; Williams and Trout, 2005; Intrigliolo and Castel, 2006; Santesteban 2011a), all methods showed statistically significant relationships with all other methods, though relationships between $\Psi_{s\text{-}md}$ and $\Psi_{l\text{-}md}$ were the strongest ($r^2 = 0.88$ in the present study). Relationships between $\Psi_{s\text{-}md}$ and $\Psi_{l\text{-}md}$ shown here are very similar to those published by Williams and Araujo (2002) for *V. vinifera* L cv. Chardonnay and Cabernet Sauvignon ($\Psi_{l\text{-}md} = -0.37 + 0.91\Psi_{s\text{-}md}$). However when relating midday measurements to either Ψ_{pd} (Figure 18, B and C) or $\Psi_{s\text{-}em}$ (Figure 19, B and C), correlations were always stronger for $\Psi_{s\text{-}md}$ compared to $\Psi_{l\text{-}md}$.

When data for relationships involving $\Psi_{s\text{-}em}$ was pooled separately by site, significant differences were seen between linear regression equations (Figure 19, B and C). For a given $\Psi_{s\text{-}em}$, midday measurements tended to be lower at Site 2 compared to Site 1. In these cases relationships at Site 1 had higher r^2 values compared to Site 2. However, this may have been due to the greater number of sunny days at Site 1 which resulted in a larger data set overall for this site.

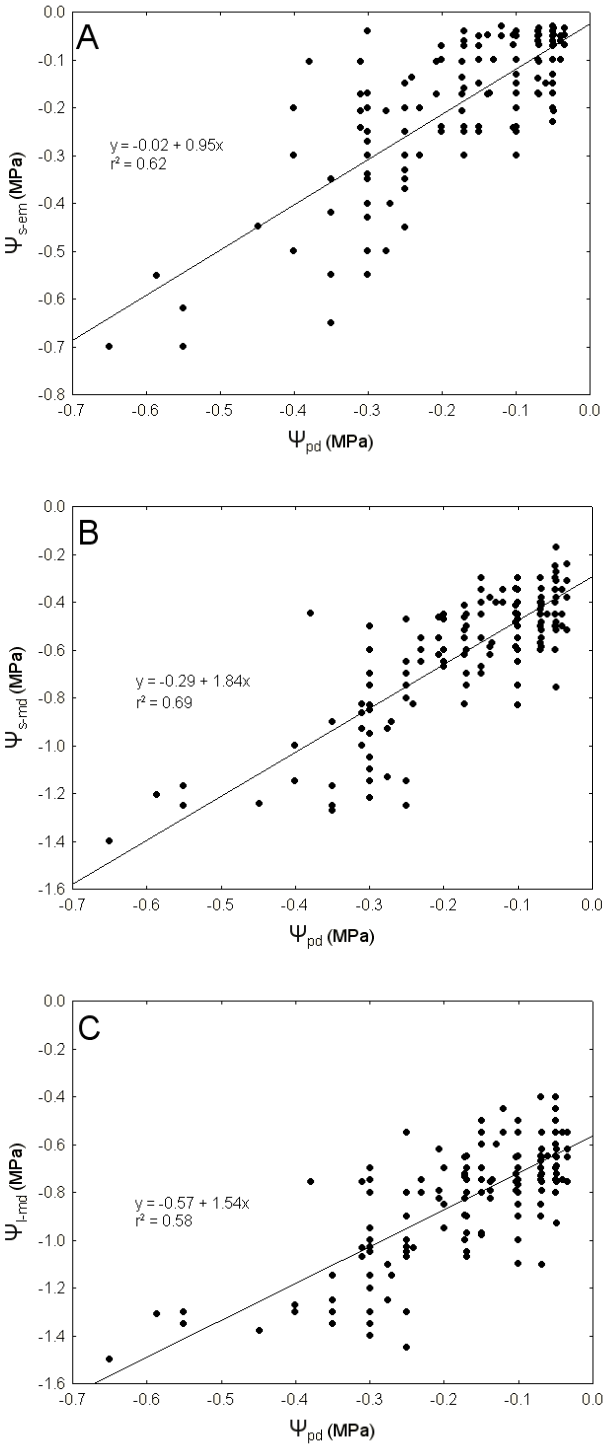

Figure 18. Relationships between predawn leaf water potential (Ψ_{pd}) and A) early morning stem water potential ($\Psi_{s\text{-}em}$), B) midday stem water potential ($\Psi_{s\text{-}md}$) and C) midday leaf water potential ($\Psi_{l\text{-}md}$) for Site 1. Values are single measurements made on the same vine on the same date. R^2 values represent significances at 5%.

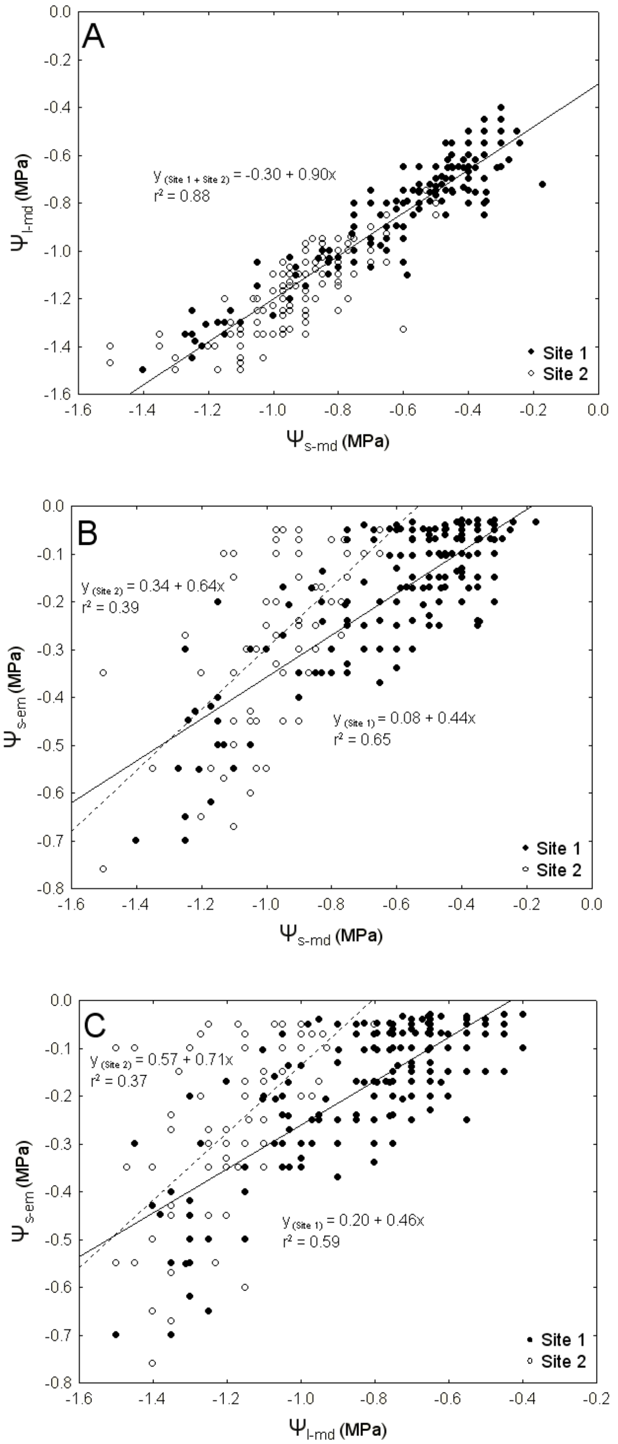

Figure 19. Relationships between A) midday stem water potential ($\Psi_{s\text{-}md}$) and midday leaf water potential ($\Psi_{l\text{-}md}$) and B) $\Psi_{s\text{-}md}$ and early morning stem water potential ($\Psi_{s\text{-}em}$) and C) $\Psi_{l\text{-}md}$ and $\Psi_{s\text{-}em}$ for sunny days at both Sites 1 and 2. Values are single measurements made on the same vine on the same date. R^2 values represent significances at 5%.

Differences between relationships at each site were possibly related to the west-facing slope at Site 2 (Santesteban, 2011a), which caused sunlight to hit the vineyard slightly later in the morning (Figure 16). However, Intrigliolo and Castel (2006) attributed similar differences between $\Psi_{s\text{-}em}$ relationships, seen between years in their case, to differences in total leaf area. Canopy height was also shown to effect $\Psi_{s\text{-}md}$ in the same Tempranillo vineyard located in Reguena, Valencia, Spain (Perez *et al*. 2011). While total leaf area was not estimated directly in the present study, values from Table 6 indicate that Site 1 represented a range of canopy sizes while Site 2 represented more uniform, mid-range canopy sizes. Judging from this author's observations of many vineyards throughout the area, these canopy characteristics cover a range that generally represents the normal canopy sizes for VSP trellising in the Southern Oregon AVA for Tempranillo.

4.6 Water Relations in *V. vinifera* L cv. Tempranillo:

Data presented here support the findings of other publications that Tempranillo exhibits an isohydric habit (Intrigliolo and Castel, 2005; Sousa *et al.* 2006), as midday measurements of Ψ_s and Ψ_l were unable to distinguish between different amounts of applied irrigation in several situations in which predawn or early morning measurements were able to show differences (Table 8 and Figure 14). In addition, analysis of all individual vine measurements taken over the course of this study revealed that Ψ_l values never dropped below -1.5 MPa (Annex Figure 2a and 2b), which is the minimum Ψ_l level reported to be maintained by isohydric varietals (Schultz, 2003; Lovisolo *et al.* 2010). However, the lack of a non-irrigated control makes it impossible to confirm the conclusion that these results were a result of stomatal regulation and not the application of irrigation.

Further investigation into the behavior of this varietal under the field conditions of Southern Oregon is needed since Tempranillo has been reported to demonstrate varying water relations behavior depending on site characteristics (Chaves *et al.* 2007*),* seasonal characteristics (Lovisolo *et al.* 2010*)* or before and after recovering from periods of water stress (Santesteban *et al.* 2009). Under the conditions studied here, $\Psi_{s\text{-}em}$ or Ψ_{pd} may be preferable methods for assessing the water status of Tempranillo vineyards in Southern Oregon given the possible influence of both climactic fluctuations and physiological regulation on Ψ during the midday period.

5.0 Conclusion:

Expected effects of irrigation treatments applied in this study were clearly reflected in Ψ data only at the site with shallower soils. At this site, mean seasonal $\Psi_{l\text{-}md}$ minimums reached -1.35 MPa and -1.21 MPa for treatments receiving 35% ET_c and 70% ET_c respectively. Using measurements from only the driest plants in the experimental plot for the initiation of irrigation generally caused irrigation to be applied at lower levels of plant water stress than intended for the majority of vines in the plot. Therefore, care should be taken to choose vines that correctly represent the entire plot when using Ψ measurements to manage irrigation. This is especially true for industry situations in which time restraints only permit small numbers of vines or locations to be measured in a single day. In addition, large variation within irrigation blocks due to soil type may greatly influence the effect of irrigation and should be identified in order to correctly judge vineyard water requirements.

Pressure chamber methods assessed in this study were able to show a reasonable ability to classify vines within the water deficit thresholds proposed by Van Leeuwen *et al.* (2009). However, $\Psi_{l\text{-}md}$ thresholds for low levels of water deficit may require additional adjustment for varietal and climate. In particular, the influence of VPD on Ψ_l should be taken into account when establishing thresholds for the initiation of irrigation.

All four methods (Ψ_{pd}, $\Psi_{s\text{-}md}$, $\Psi_{l\text{-}md}$ and $\Psi_{s\text{-}em}$) were able to clearly distinguish between differences in soil type, but only $\Psi_{s\text{-}em}$ showed a consistent ability to distinguish differences between effective irrigation treatments when methods were compared side-by-side. Overall, data presented here indicates that $\Psi_{s\text{-}em}$ or Ψ_{pd} are preferable methods for assessing the water status of Tempranillo vineyards in Southern Oregon.

Early morning measurements of stem water potential show promise as a practical technique for growers to assess variability within their vineyards and as a tool for managing irrigation. However further study is needed to determine appropriate water deficit thresholds for $\Psi_{s\text{-}em}$, taking into account the effects of canopy size and environmental effects unique to the early morning time-window.

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Annex:

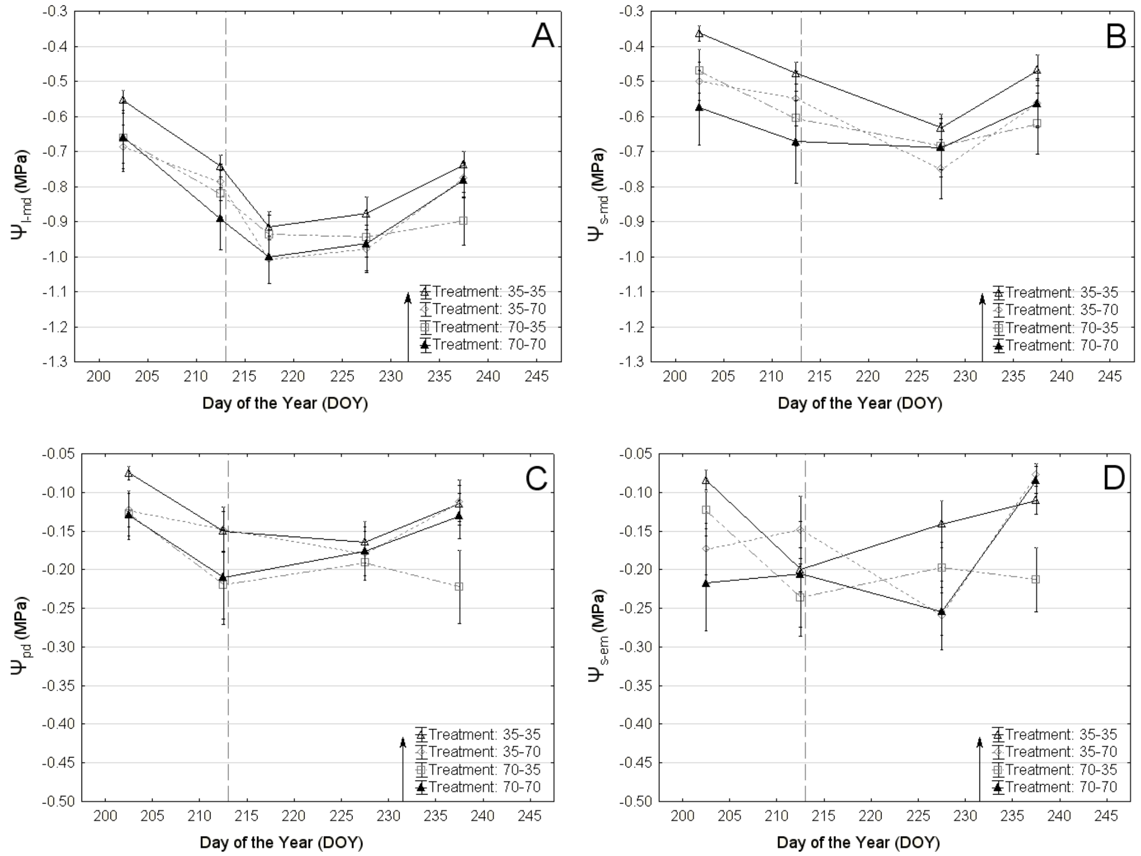

Figure 1. Seasonal pattern of $\Psi_{l\text{-}md}$ (A), $\Psi_{s\text{-}md}$ (B), Ψ_{pd} (C) and $\Psi_{s\text{-}em}$ (D) at Site 1 for four irrigation treatments. Vertical arrows indicate the approximate date of 50% veraison and the shift in irrigation applications for treatments 70-35 and 35-70. Vertical dotted lines indicate the initiation of irrigation (DOY 213). Means consist of averages of nine measurements per treatment with standard error bars added.

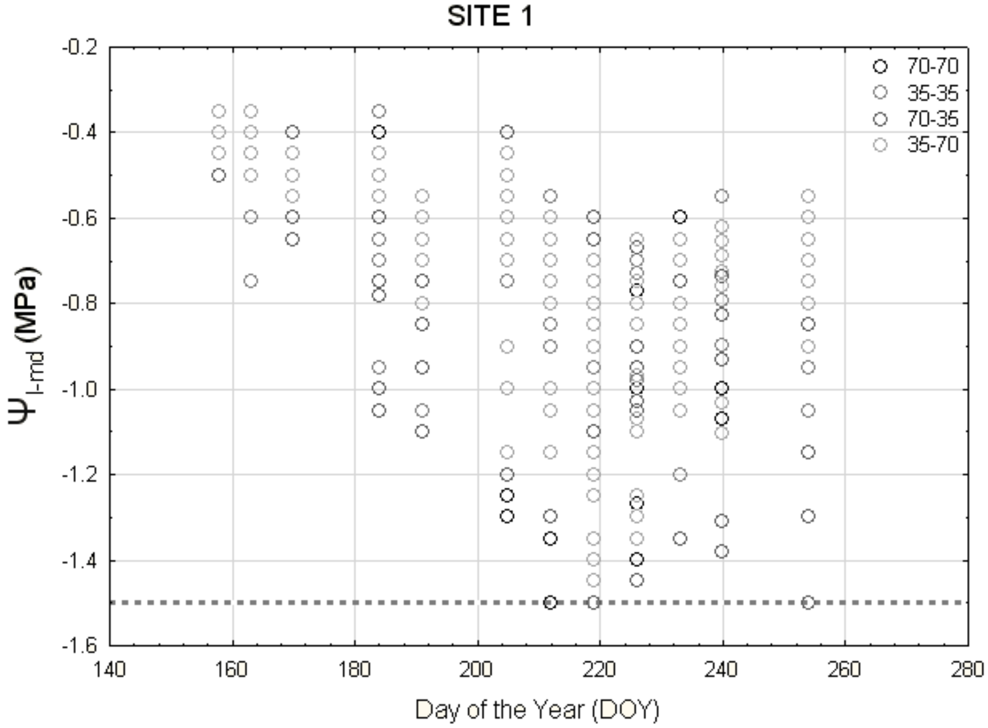

Figure 2a. All individual $\Psi_{l\text{-md}}$ measurements taken throughout the course of the season at Site 1 (n = 684), grouped by day of the year (DOY) and marked by irrigation treatment. Broken horizontal line indicates $\Psi_{l\text{-md}}$ = -1.5 MPa.

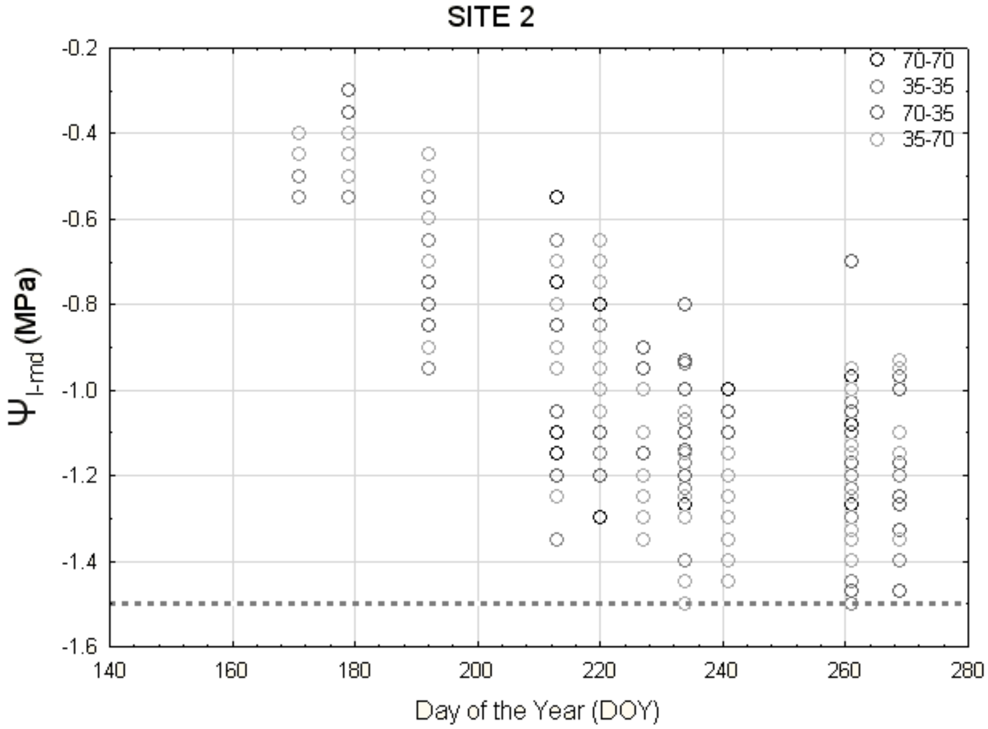

Figure 2b. All individual $\Psi_{l\text{-md}}$ measurements taken throughout the course of the season at Site 2 (n = 360), grouped by day of the year and marked by irrigation treatment. Broken horizontal line indicates $\Psi_{l\text{-md}}$ = -1.5 MPa.