



# Short vs. long-term drought stress effect on Grüner Veltliner: Water relations and berry composition

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## **Master Thesis**

Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Science

in the field of Crop Sciences

submitted January 17, 2020

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## **Acknowledgements**

I want to thank my supervisors Jose Carlos Herrera, for his proficient advice, expertise and support, and Astrid Forneck, for encouraging me to dare the step into the, indeed complex, research topic of viticulture, as well as the Institute of Viticulture and Pomology from the University of Natural Resources and Life Sciences Vienna for the great supply and friendly working environment. Finally, I want to thank my family and friends.

# 1 Abstract

The effect of water deficit on grapevine physiology and berry composition has received great attention of scientific community. However, the vast majority of studies were performed on arid to semi arid regions, with high focus on red cultivars and anthocyanin accumulation. Few research focused on white cultivars growing in temperate climates, mostly rainfed, with insignificant irrigation practices and subjected to transient water stress periods during the growing season.

Since water deficit in ripening grapes was often associated with smaller berries but beneficial solvent-solute interactions transferred to the must a similar effect was also expected in white cultivar 'Grüner Veltliner' and formed the fundamental assumption of this master thesis. The experiment was carried out in a warm temperate climate in 3-yr. old potplants, that comprised an irrigation trial where rain was excluded by a covered canopy. Physiological data of vine water relations and that of grape composition from one season were measured during ripening and at harvest in the expectation of lowest berry mass in sustained deficit irrigated (SDI) test plants compared to a well watered control. Additionally one group of these deficit irrigated vines, was reirrigated before harvest in order to research the differences of long-term vs. transient drought with a weaker expected decline in berry mass compared to SDI vines.

Berry mass was however not significantly modified so that inhibited sugar accumulation due to post veraison water deficit (35 %  $ET_0$ ) could emerge in lower measured Total soluble solids (°Brix) in the juice from harvested berries with no significant change in skin phenolics compared to a well watered control. This pattern did not change by reirrigating vines before harvest, except for a small shift towards lower pH compared to sustained water deficit during ripening.

Levels of moderate and severe post veraison water stress corresponded with stagnation of vegetative growth and partial leaf shedding in potted 'Grüner Veltliner' that exhibited a near anisohydric behavior as adaption to prolonging drought in a potted scenario. The results may be a hint for that berry dehydration is possibly prone to a softer outcome under mild climatic conditions.

**Keywords:** *Grüner Veltliner, white cultivar, drought sensitive, flavonols, total phenolics, temperate climate, grape composition, late deficit irrigation, post veraison water stress, berry size, water relations, anisohydric, leaf shedding, stem water potential, stomatal conductance, intrinsic water use efficiency.*

## Kurzfassung

Meist konnte Trockenstress in Weinreben während der Traubenreife mit einer relativen Abnahme der Beerenmasse durch Verlust an Beerenwasser, dadurch aber auch mit einem günstigeren Konzentrationsverhältnis im erzeugten Most in Verbindung gebracht werden. Da jedoch ein Großteil dieser Erkenntnisse zugrundeliegende Studien (semi)-ariden Regionen entstammt, wurde diese Annahme am Versuchsobjekt 'Grüner Veltliner' in einem gemäßigten Klima überprüft. Hierzu wurde ein Bewässerungsversuch im Feld an dreijährigen Topfpflanzen in einem Folientunnel durchgeführt. Physiologische Daten des Wasserhaushalts sowie jene der Traubenkomposition einer Saison wurden von Beginn der Reifung bis zur Ernte erhoben und statistisch ausgewertet um einen Einfluss von simulierter andauernder Trockenheit (35% ET<sub>0</sub>) mit denen einer kurz vor der Ernte wiederbewässerten Versuchsgruppe (simulierte vorübergehende Trockenheit) sowie einer kontinuierlich ausreichend bewässerten Kontrolle (120 % ET<sub>0</sub>; kein Trockenstress) zu vergleichen. Hierzu wurde ein entsprechender negativer Einfluss von Trockenheit je nach Dauer auf die durchschnittliche Beerenmasse erwartet. Dies konnte aber anhand der Daten nicht bestätigt werden, da die Beerenmasse in allen Versuchsgruppen durchgehend ähnlich war, ebenfalls zutreffend für die in den Beerenschalen analysierten phenolischen Parameter (Gesamt Phenole, Gesamt Flavonole) sowie für die Gewichtsanteile der untersuchten Beerenkomponenten (Schalen, Fruchtfleisch, Kerne). Lediglich der pH-Wert im Traubensaft von durchgehend Trockenstress ausgesetzten Mutterweinen war etwas erhöht. Beide Trockenstress-induzierten Gruppen wiesen somit einen deutlich geringeren Gehalt an gelösten Feststoffen °Brix im Traubensaft auf, da ein durch Assimilatmangel bedingt niedriger Zuckergehalt der Beeren nicht wie angenommen durch deren Dehydration kompensiert wurde. Als Reaktion auf fortschreitende Trockenheit waren bei den Versuchsreben mittelfrüher Stomatanschluss, erhöhte Wassernutzungseffizienz, fortlaufend sinkendes Pflanzenwasserpotenzial, Einstellung des vegetativen Wachstums sowie Laubwurf feststellbar. Die gewonnenen Ergebnisse sprechen tendenziell dafür, dass ein Trockenstress bedingter Einfluss auf die Beerenmasse unter milden klimatischen Bedingungen nicht begünstigt wird

**Schlagwörter:** *Grüner Veltliner, Weißbeerige Sorte, Trockenstress, gemäßigtes Klima, Traubenkomposition, Wasserhaushalt, Intrinsische Wassernutzungseffizienz, stomatäre Reguierung, Beerenmasse, Evapotranspiration, Flavonole, Phenole, Mittägliches Stammwasserpotential, Gesamtblattfläche, Defizitbewässerung.*

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## Abbreviations

Ø	average / mean
*	significant $p < 0.05$
**	significant $p < 0.01$
Δ	mean difference
...	defines a unit or a string
~	approximately / about
±	+ / - a value
↑	increase / higher
↓	decline / lower
5bb	refers to the rootstock 'Kober 5bb'
ANOVA	One Way Factor Variance Analysis (ANalysis Of VAriance)
A <sub>N</sub>	Assimilation rate/ Carbon Influx rate (Midday)
a.s.l.	above sea level
BF	refers to Bonferroni Post hoc test (applied per ANOVA)
cv	coefficient of variance as standard deviation to mean ratio
Ψ <sub>s</sub>	stem water potential measured in the petioles (Midday)
ET <sub>0</sub>	reference crop evapotranspiration (2m),
ET <sub>c</sub>	crop evapotranspiration
FC	Folin Ciocalteu
FI	full irrigation / fully irrigated = control
FT-IR	Fourier Transformation Infra Red - Spectroscopy
gs	stomatal conductance (Midday)
GV	<i>Vitis vinifera</i> . L. (cv. Grüner Veltliner)
i.e.	id est
IP	pot arrived irrigation (kg)
IRGA	infra red gas analysis
IT	technical administered irrigation (kg or L)
K <sub>c</sub>	Crop coefficient (ET <sub>c</sub> /ET <sub>0</sub> )
LA	leaf area
LL	leaf length
LSD	refers to Fischers Least Significant Difference Post hoc test (applied per ANOVA)
μ	if in a statistical manner -> classical mean value, otherwise 10 <sup>-6</sup>
n	sample size / number of individuals of a population
P	probability to refute or accept the H <sub>0</sub> Hypothesis (sig. two tailed)
ppm	parts per million; abbreviation for WUE <sub>i</sub> [μ mol CO <sub>2</sub> mol H <sub>2</sub> O <sup>-1</sup> ]
R <sup>2</sup>	coefficient of determination of a linear Regression analysis
RDI	regulated deficit irrigation
RMS	Residual mean square
RM	Residual mean
σ	standard deviation of the sample (n-1)
sig.	significance (two tailed) = p-value
SDI	sustained deficit irrigation / sustained deficit-irrigated
SE(M)	standard error of the mean
TSS	Total soluble solids measured in ° Brix
WUE <sub>i</sub>	intrinsic water use efficiency (A <sub>N</sub> /gs)



## 2 Introduction & Hypothesis

There is still the evergreen discussion about an indirect, detrimental berry 'dilution' effect due to inappropriate irrigation practices in ripening grapes (Zhang & Hansen, 2018; Cifre et al., 2005), and it is a commonly accepted practice to reduce or stop water supply at veraison for this reason (Keller, 2016). Berry water is an important solvent however also determines the concentration of juice solutes in the pulp as well as the concentration of secondary metabolites extracted from seeds and skins present in the must after crushing (Roby & Matthews, 2004; Kennedy et al., 2002; Coombe, 1987). This of course affects the intensity of taste characteristics in the must and later the wine and thus quality. However, another part of this discussion may be separated into a distinguishable skin dilution effect. Although the biosynthesis of secondary metabolites is generally promoted by water deficit (Castellarin et al., 2007; Chaves et al., 2007) what may lead to an enhanced production, a big part of the final secondary metabolite concentration is indirectly determined by berry size. This is because concentrations in the berry skin, where most of polyphenolic compounds are located, decrease with berry size due to skin extension facilitated by cell stretching and incorporation of water (Lang & Thorpe, 1989), while the total amount per berry is rather constant (Ojeda et al., 2002).

After the onset of ripening, known as veraison, the xylem water influx into the berries ceases due to changes in xylem pressure, but the xylem is still functionable and capable of depriving excessive berry water to a minor extent (Keller et al., 2006; Zhang & Keller, 2016; Greenspan et al., 1994). After veraison, the phloem becomes the main source for berry water (Greenspan et al. 1994; Poni et al. 2018) where sufficient supply maintains berry fresh mass while water deficit causes an irreversible decline in berry water by dehydration and subsequently higher concentration of solutes (Coombe & McCarthy, 2000). However, as a response to low plant water status and plant water in combination with decreased assimilation rates also a lower phloem stream may reduce berry size due to inhibited cell extension relatively to well watered conditions. Unlike the pattern of berry growth, its amplitude is generally affected by post veraison water deficits (Matthews et al., 1987; Roby & Matthews, 2004; Castellarin et al., 2007), what may result in lower yield, but this effect is of course weaker than size modification by water deficits imposed before veraison (Matthews et al., 1987; Matthews & Anderson, 1989; Poni et al., 2018; Ojeda et al., 2002) what even may alter the ripening pattern (Herrera & Castellarin, 2016; Castellarin et al., 2007). Since vine production is mostly defined by wine quality prior to the yield (Meidlinger, 2000; Fereres & Soriano, 2007; Van Leeuwen & Darriet, 2016), an enhanced concentration may be preferred over potential yield losses (Keller et al., 2006).

Keller et al. (2006) could show that changes in berry size due to water deficits prior to veraison are reversible by reirrigation, while post veraison water deficit steadily showed shrinkage phenomena where reirrigation could only stop further decline but failed to restore full berry size. It was hypothesized that berries from cv. 'Grüner Veltliner' should follow that same trend, where berries should show highest evidence of berry shrinkage by dehydration under sustained post veraison water deficit, while berries from reirrigated vines after post veraison water deficit should maintain higher berry fresh mass but lower than those of a well irrigated control accordingly.

At least, such results were already reproduced in the field by Ollé et al. (2011), Ojeda et al. (2002), Castellarin et al. (2007), Buchetti et al. (2011) or Matthews et al. (1987) just to denote a few, and are rather the rule than the exception.

Berry mass and its components skin, pulp and seeds as well as skin flavonols and skin total phenolics were measured around veraison, before reirrigation and at harvest in order to detect any differences. It was assumed that dehydrated berries should reveal a higher skin proportion

and a higher skin to pulp ratio under sustained water deficit than those of reirrigated vines due to differences in berry dehydration caused by a decline in pulp proportion.

Juice quality parameters as total soluble solids, total acidity and pH were determined in the harvested berries. For total soluble solids (° Brix) it was hypothesized that berries from deficit irrigated vines reach either equal, maybe higher concentration if the berry size was significantly decreased, otherwise lower concentrations relative to a well irrigated control were assumed, since water stress is generally assimilate limiting (Chaves et al., 2010; ). It was assumed that pH should not be affected by the treatment, according to most papers. The severity of water deficits -between moderate and severe water stress - were scheduled at a relative aim crop evapotranspiration of 35 % from that of the well irrigated control and additionally monitored via midday stem water potential which was approached and held at a maximal bottom value of -1.2 MPa. Leaf gas exchange parameters were frequently measured, and stomatal conductance was used to describe the different degrees of water stress after Lovisolo et al. (2010).

Since many recent studies have emphasized on describing cultivar-responses to water deficit in e.g. isohydric and anisohydric behaviors (Chaves et al., 2010), this was also worked out in this thesis for cv. Grüner Veltliner, where an anisohydric or near anisohydric behavior was assumed due to its known modest drought tolerance (Bauer et al. 2013; ÖWM, 2019) and the more general anisohydric behavior of grapevines (Soar et al. 2006; Tombesi et al., 2015).

Another often reported interaction with water stress is a shift in flavonoid pathway (Chaves et al., 2007; Castellarin et al., 2007) most consistently associated with higher content of anthocyanins [in case of red cultivars] in grape skins, or even increased skin- flavonols and flavan-3-ols (tannins) (Ojeda et al., 2002). In order to investigate the effects of late water deficit under the aspect of the three different water regimes skin total phenolics and skin flavonols were measured during ripening and at harvest. In theory, flavonols should increase with progressive season during ripening (Kennedy et al., 2002), while tannins (mainly flavan-3-ols (Adams, 2006)) should reach a peak at veraison, and decrease from then (Buchetti et al., 2011). Nevertheless, the majority of the papers could not show a clear promotive effect of late deficit irrigation on skin tannins (Poni et al., 2018). Also, the results in the literature regarding skin flavonols are controversial. For instance, Castellarin et al. (2007) could measure increased gene expression of flavonol precursors, which were not consistently associated with a higher flavonol content in berries from late DI vines. Even the trend of a decrease in skin flavonols due to post veraison water stress is possible. (Kennedy et al. 2002; Herrera et al., 2017). Although flavonol biosynthesis is strongly promoted by light (Adams, 2006), and it could be shown that leaf removal around the bunch zone - with no limitation of soil moisture - was associated with increased skin flavonols (Friedel et al., 2015), a similar modification in leaf area due to post veraison water deficit, which is a very typical effect (Poni et al., 2018), does not produce the same effect to the best understanding of the author. For this reasons a clear hypothesis for flavonoid biosynthesis regarding the experiment could not be scientifically justified due to too diverse results in the literature, and it was mainly focused on solvent-solute interactions hereto.

# Literature Overview

## 2.1 Background

Like all terrestrial plants, grapevines require water. However, many grapevine growing regions are located in arid and semi arid regions and demand therefore irrigation which is often in conflict with the regional water supply, sometimes not even available or expensive (Schultz, 2000; Mullins et al., 2004; Jones, 2004; Flexas et al., 2010; Chaves et al., 2010; Tortosa et al., 2016). However these regions always demand adequate irrigation for any agricultural practices even under best water supply because higher evapotranspiration punishes excessive irrigation with an increase in soil salinity (Schultz, 2000; Goodwin & Boland, 2002). Although the cultivation of particularly wine grapes is relatively water saving compared to other deciduous fruit or annual crop production (Mekonnen & Hoekstra 2010; waterfootprint.org), water deficits due to sustained droughts limit productivity (Bota et al., 2001). This promoted the development of more water saving irrigation practices better known as deficit irrigation (DI) and has been the topic of many published studies in the recent 30 years (Gruber, 2013; Wample & Smithyman, 2002). Of course, such 'conservative' irrigation below the evapotranspirative demand of a crop comes up with yield losses, which however are of less importance in grapevine growing. There are natural reasons for that like higher interest of maximizing fruit-(generative) and not biomass (vegetative) production or the weaker sensitivity to plant water supply regarding yield determining processes at fruit developmental stages (Johnson & Handley, 2000). Another aspect that makes grapevines suitable for lower soil water supply is the downregulation of transpiration under drought at high water productivity regarding carbon assimilation (Chaves et al., 2010). Further reasons were already mentioned in the introduction. Especially in Europe where irrigation use is still low and vineyards are mostly rainfed (Mekonnen & Hoekstra, 2010; McCarthy et al., 2002; Balint & Reynolds, 2014; Appendix 8.1) a persistent assumption that irrigation causes a detrimental dilution of the quality is still widely distributed for probably no scientific reason and under the assumption of maybe too simple yield to leaf area relationships as well (Zhang & Hansen, 2018; Poni et al., 2018). However, there are also governmental quantitative restrictions of the yield especially in the world greatest wine producing countries (EU: ITA, FRA, ESP) (Hemming, 2018; Keller, 2010; Poni et al. 2018) - that may be denoted as "defined quality" - which were originally established to control the wine price (Bauer et al., 2013). Nevertheless, global irrigation use in grapevine growing for wine production is already high (Mekonnen & Hoekstra, 2010), and very likely to rise if considering that the terrestrial average temperature has already increased to 2 ° C over the pre-industrial period which not only additionally aggravates with more frequent but also longer periods of drought amongst higher evapotranspiration (IPCC, 2018 & 2019; Schultz, 2000; Jones et al., 2005).

## 2.2 Deficit Irrigation Effect on Grapevine

Grapevines (sp. *vinifera*) are generally capable of overcoming periods of droughts relatively successfully (Balint & Reynolds, 2014), what can be led back to their large and deep root system on the one hand (Chaves et al., 2010) but also physiological response/compensation mechanisms like the stomatal downregulation of transpiration or osmotic adjustment (Patakas et al., 2002; Patsakas & Noitsakis, 1999) that avoid and delay dehydration and cavitation events on the other hand. Studies in grapevine could point out that there is a range of variation from deficit irrigation -effects being mostly cultivar independent (Van Leeuwen et al. 2004) until major differences (Jones et al., 2005; Schultz, 2000, de Souza et al. 2005; Tombesi et al., 2014; Bota et al., 2016) and it still seems that the true outcome of the interaction of deficit irrigation

in the triangle soil, plant & atmosphere is not that well understood as it seems (Van Leeuwen et al. 2004; Herrera et al. 2017). Even a short term interseason variability can interfere with the effects of water deficit regarding the impact on grape composition and metabolites, and affects the reproducibility of results, suggesting a more complex covariance (Herrera et al., 2017). From a physiological perspective short term effects of water deficits are stomatal closure and long-term effects a decline in plant water potential (in various tissues) however often under maintenance of minimum photosynthetic productivity (Souza et al. 2005; Chaves et al., 2010; Jones et al., 2005; Rogiers et al., 2011). The stomatal regulation in return is dominantly driven by hydraulic regulation compared to biochemical regulation by ABA [abscisic acid] (Rodrigues et al., 2008; Loveys, 1991; Morison et al. 2008), which rather sustains stomatal closure even under reirrigation after drought (Tombesi et al., 2015). Therefore, grapevines are generally considered as an anisohydric species (Soar et al., 2006), with some exceptions (Schultz, 2003, Chaves et al., 2010). Isohydric means per definition that plant water remains equal at least for short periods of drought and water scarcity and is more precisely defined in insignificant diurnal differences between midday leaf water potential and pre dawn leaf water potential in plants. In isohydric plants stomatal closure is often earlier induced by ABA. ABA is produced by drying roots and transported over the shoots to the guard cells where specific receptors induce stomatal closure after perception (Comstock, 2002). However, there are possibly two forms of ABA i.e. i) foliar produced ABA and xylem received ABA derived from long distance transport from drying roots, and plants may apparently differentiate between the two forms whereas the latter one is more potent in stomatal regulation processes (Davies & Zhang, 1991). Nevertheless, this regulation process is limited in many grapevine cultivars so that ABA in leaves peaks only after significant stomatal closure has already happened (Tombesi et al., 2015; Degu et al., 2019). While external dehydration is avoided by stomatal closure, internal dehydration is mostly maintained by incorporation of osmolytes that attract water. This regulation also allow plants to maintain transpiration to a limited degree under moderate drought, since a specific water content in the guard cells is necessary for stomatal aperture (Chaves et al., 2010). According to a study in cv. Victoria by Patakas & Noitsakis (1999) more than two thirds of diurnal changes in osmotic potential of grapevine leaves were regulated actively by net solute accumulation rather than passive dehydration under water stress.

Under drought, plant available water is only accessible through higher soaking force by the roots which not rarely results in embolism. Here higher pressure facilitates a change of the aggregate state of xylem water from the liquid to the gaseous phase (water vapour). This change is known as cavitation or embolism formation. Embolism widespread eventually leads to plant mortality for hydraulic failure, because it interrupts the water flow in the xylem which is most sensitive in the leaf-veins where these symptoms occur at first (Brodribb et al., 2015; Hochberg et al., 2016 b). Nevertheless, most plants that are faced to low soil water and dehydration respond with stomatal closure in order to restrict such embolism-events before they happen (Jones & Sutherland, 1991) or respond with leaf shedding in order to avoid the spread of air bubbles from the leaf veins into the shoot xylem, where they would cause the highest damage (Tombesi et al., 2015; Hochberg et al.; 2016b). Thus, a long-term effect as an adaption to developing water stress is the decrease in leaf area (Jones, 2004). It could be even shown that this regulation allows vines to restore vine water status under drought to some extent (Degu et al., 2019), where a decreased evaporative demand by lower total leaf area does also reduce the necessary amount of water required. The restriction of shoot development and leaf area can reduce trimming efforts (Chaves et al., 2010), can improve the light interception in the area around the bunches (Chaves et al., 2007; Poni et al., 2018) but may help to maintain vine balance (equilibrium between generative and vegetative growth and fruitfulness (Loveys, 1991; Chaves et al., 2010; Poni et al., 2018). Additionally, a less dense canopy may also be less susceptible to insects and pathogens (Wample & Smithyman, 2002). However, this also means

a loss of photosynthetic surface and may restrict fruit development and quality (Lebon et al., 2006; Wample & Smithyman, 2002). It may be mentioned that the manual regulation of the leaf area by pruning and physiological regulation of the leaf area by drought do not necessarily have similar effects on grape composition. This could be e.g. shown in a study in cv. Merlot by Herrera et al. (2015).

It is also worth to mention that although stomatal closure prevents further dehydration, it also abrogates the transpiration cooling process, what indirectly exacerbates heat stress pressure (Müller et al., 1999). Therefore, it is not always clear to separate water stress from heat stress correctly. Heat stress increases the fluidity of the phospholipids in the membranes, thus disturbs its functionality. Another associated factor may be the enhanced abundancy of reactive oxygen species (ROS) that depresses plant cells with free radicals. ROS inhibit enzymatic activity of essential catalysts which are involved in the biochemical metabolism and functionality of plant cells, and increased content in grapevines under drought e.g. in leaves was already reported by Degu et al. (2019). Other results as a response to water deficit concern an enhanced biosynthesis of secondary metabolites, most consistently anthocyanins in red cultivars that may be even independent of the berry size (Poni et al., 2018; Chaves et al, 2010; Castellarin et al., 2007). However, when it comes down to other important phenolic substances in berry skins, like flavonols, flavan-3-ols or phenolic acids, the role of water deficit becomes unfortunately rather controversial (Herrera et al., 2017; Castellarin et al, 2007; Buchetti et al. 2011).

## 2.3 Irrigation Types & Scheduling

The classical agricultural usus is to avoid plant water deficits because they limit crop production (Feres & Soriano, 2007; Gruber, 2013). So, if the precipitation level is undercut and the crops are affected by dehydration symptoms mostly loss in turgescence (hanging shoots and leaves) the missing plant water is compensated by irrigation water. Although the development of precise and efficient irrigation systems (especially trickle irrigation) has helped to reduce global water input significantly, this technological progress has probably reached saturation and further improvement was sought in a more efficient scheduling (Jones, 2004; Gruber, 2013). However, the increasing demand for irrigation water at sinking supply and rising costs in many arid areas have further led to the development of irrigation methods that minimize water use and even particularly include the approval of water deficits (Feres et al., 2003; Jones, 2004; Flexas et al., 2010; Chaves et al. , 2010; Tortosa et al., 2016).

Next to full irrigation (FI) - total avoidance of any assimilate limitation - types of deficit irrigation (DI) - have been established (Gruber, 2013). In a full irrigation regime, an optimal water supply is conventionally achieved by maintaining a soil water content close to field capacity thus avoid limitation of plant available water (Jones, 2004). However, there is no 'real' field capacity in pot plants, and hereto crop evapotranspiration is the better discriminante.

While in a FI-scheduling lysimeter balances, weather station data and soil moisture monitoring are often sufficient, a DI-scheduling requires a more plant-based monitoring (Jones, 2004). This is because many plant physiological responses to drought are stronger related to the plant water status than the soil-water status (Jones, 2004; Deb et al., 2012).

A plant-based monitoring typically includes frequent measurements of plant water status, stomatal conductance or sap flow (Jones, 2004). Different applies have been established to express the intensity of water stress. One common method is to relate the water status of plants to the evaporative demand that would be obtained by full irrigation of a reference plant given in % ET of such a reference plant. This method requires the use of water balances and a weather station. Crop evapotranspiration (ET<sub>c</sub>) per plant or per specific crop covered area can be obtained with a lysimeter in terms of daily negative measured massflows (weight loss), while

reference evapotranspiration ( $ET_0$ ) is commonly determined via weather station data including windspeed, radiation, relative humidity and temperature at a reference height of 2m above ground level and subsequent estimation via Penman-Monteith equation or derived equations (Allen et al., 1998). The evapotranspiration concept was established in order to standardize the different climatic conditions where agriculture is spread and irrigation volumes cannot be translated however  $ET_c$  can (Allen et al., 1998). Nevertheless, this approach is not free of difficulties neither, and may be prone to under or overestimation of actual required irrigation amounts (Hochberg et al., 2016a).

Another objective estimation equation is provided by measuring the crop water stress index (CWSI) (Jackson, 1982; Alderfasi & Nielsen, 2000; Mullins et al., 2004). However, water stress can also be fairly well interpreted through low plant water status in deciduous crops measured with mobile pressure chambers (Scholander et al., 1965). The plant water potential in any tissue depends on soil moisture, the rate of water flow through the plant as well as various hydraulic resistance forces between different tissues and the bulk soil (Jones, 2004). Nowadays plant water potential is fairly easy to measure via pressure bomb readings. A pressure bomb reading can result in three different water potentials - 1) pre-dawn water potential  $\Psi_{pd}$  - estimation of the water potential near the rootzone [measured short before sunrise solely], 2) leaf water potential  $\Psi_{leaf}$  - actual water potential in the leaf [measured almost instantaneously], 3) stem water potential  $\Psi_{stem}$  - water potential of the stem [leaves isolated and measured after acclimation time of > 10 min] (Tuccio et al., 2019; Deloire & Heyns, 2011; Amègilio et al., 1999; Jones, 2004; Santesteban & Royo, 2006). Most of them involve, that the leaves are covered in plastic bags short before reading, which should avoid transpiration what is crucial since a pressure bomb reading should represent the unbiased tension in the leaf, actually the leaf-petiole, as precise as possible. Alternatively, the plastic bags can be wrapped with foil (e.g. aluminum foil) that blocks UV-radiation, enhances the isolation of leaf from the atmosphere and provokes stomatal closure in this way as an additional conservation factor (non transpiring leaf), which is followed by an acclimatization time. The principle is to determine the pressure given in Bar or MPa that is necessary until first visible water flows out of the cutting surface of the petiole protruding from the chamber lid from a leaf enclosed in the chamber (each measurement loses a leaf). All water potentials show a good discrimination against different water regimes, however, when considering the leaf to leaf variability and positions leaf water potentials must require a higher sample size for reliability while pre dawn leaf water potential and stem water potential are more stable due to the equilibrium with the water potential near the roots and the stem respectively (Chonè et al., 2001). Predawn leaf water potential however hardly reveals the actual water deficit of a 'working' cultivar, because it is measured at a point of relaxation or even at the dewpoint and rather provides information about soil moisture. After different vertical positions within the soil plant atmosphere continuum it can be claimed that the leaf water potential is always lower than the stem water potential, and the stem water potential always lower than the predawn water potential. This could be well shown in 'Chardonnay' and 'Cabernet Sauvignon' by Williams & Araujo (2002) in the field, who additionally reported high correlation between all of these water potentials. In addition, obtained plant water potentials are sensitive to canopy height position and basal distance of the leaf that is used for pressure chamber reading and the corresponding daytime as well as the prior leaf isolation time (Begg & Turner, 1970). Leaf and stem water potential can be principally measured at all day hours, however midday pressure bomb readings have gained more popularity the last decades, and especially midday-stem water potential has proven some reliability in field grown and potted grapevines (Matthews & Anderson, 1988; Chonè et al., 2001; Williams & Araujo, 2002; Roby et al., 2004; Williams, 2012; Hochberg et al., 2016a; Tuccio et al., 2019).

Another method that has gained some popularity recently are leaf-gas-exchange measurements via infra red gas analyzers (IRGA) that have proven its worth due to higher sample size at acceptable precision (Long et al., 1996; Gallé & Flexas, 2010; Williams et al., 2012; Hochberg et al., 2016a). Some important sizes that derive from such measurements are stomatal conductance  $g_s$  [ $\text{mmol or mol } \{H_2O\} \text{ m}^{-2} \text{ s}^{-1}$ ] - a relational size to plant transpiration -, the net assimilation rate  $A_N$  [ $\mu\text{mol } \{CO_2\} \text{ m}^{-2} \text{ s}^{-1}$ ] - a relational measure for carboxylation sometimes also denoted as net photosynthesis ( $P_N$ ) - and the ratio of them as a measure of intrinsic water use efficiency (Williams, 2012). Both sizes are strongly related and have proven a high connectivity that is very consistent among different cultivars as a response to soil water depletion respectively drought (Cifre et al., 2005; Lovisolo et al., 2010). Gas exchange measurements require sufficient replication due to large leaf to leaf-variation (Jones, 2004). It is commonly distinguished between three stages of water stress as a response to water deficit in grapevines based on the measured leaf stomatal conductance: mild [ $0.2\text{-}0.15 \text{ mol } \{H_2O\} \text{ m}^{-2} \text{ s}^{-1}$ ]; moderate [ $0.15\text{-}0.05$ ] and severe water stress [ $<0.05 \text{ mol } H_2O \text{ m}^{-2} \text{ s}^{-1}$ ] (Cifre et al., 2005, Lovisolo et al., 2010). Most papers aimed stress stages from mild to moderate while severe water stress is often not recommended since it highly reduces the yield and risks plant health by causing irreversible physiological damage, however may have the greatest potential of saving water if applied adequately in return and is most informative about a cultivar's adaption to severe drought (Keller et al., 2016; Wample & Smithyman, 2002).

Furthermore, the different cultivar-specific responses to drought need to be considered. After the relational progress of stomatal conductance vs. plant water potential plants are often partitioned in isohydric and anisohydric physiological behaviors (Limpus, 2009) and pessimistic and optimistic ecological behaviors (Schultz, 2003). Isohydric cultivars are supposed to maintain diurnal (leaf) plant water potential by immediate stomatal closure in order to not await more water to come (pessimistic) while optimists use all available resources in order to await further supply (Schultz, 2003). As a possible anisohydric consequence plant water status also declines faster over time than it would have in an isohydric response due to poor stomatal adaption adequate to evaporative demand and water status (Rogiers et al., 2009).

Dependent on the scheduled timing of water stress relative to the developmental stage regarding impact on berries, the difference between pre- and post veraison (before/after ripening) water stress can be made and effects were already sufficiently described in the introduction or see Zhang & Hansen (2018). Veraison is an important benchmark in evaluating maturity and prognose harvest dates. It is commonly approximated in advance with a model based on accumulated average growing degree days (GDD), and then determined directly as 50% of the berries are signaling ripening signs like berry softening in white cultivars or berry coloring in red grapes (Herrera & Castellarin, 2016). The impact on yield and berry quality but also shoot growth of pre veraison water stress is generally greater than those of post veraison water stress (Matthews et al., 1987; Goodwin & Boland, 2002; McCarthy et al., 2002) while the latter may come up with increased pH and lower malic acid content (Poni et al., 2018).

There is also the possibility of supplemental irrigation in case of drought which however can only be seen as deficit irrigation technique if it willingly accepts water deficits and does not balance them (Ferreles & Soriano, 2007). The simplest form is Sustained Deficit Irrigation, static, and vines can acclimatize to stress levels (Ferreles & Soriano, 2007). A hybrid form is Regulated Deficit Irrigation which seeks to take advantage of both worlds whereas the timing of reirrigation is crucial (Wample & Smithyman, 2002), however lacks a uniform meaning since all possible combinations (incl. pre and/or post veraison stress (Wample & Smithyman, 2002; Shellie, 2014; Poni et al., 2007; Balint & Reynolds, 2014; McCarthy et al., 2002; Goodwin & Boland, 2002)) are possible. A special form is Partial Root Drying (PRD), with the goal to avoid root adaption to water stress which should raise the stress level around the rootzone (increase in xylem ABA) with enhanced water use efficiency but without limited xylem sap flow (Dry et

al., 2000) and equal reduction in vegetative growth compared to other DI-techniques (Davies & Zhang, 1991). This is achieved by a more elaborate irrigation system where alternately only half of the rootzone per vine is drip-irrigated (McCarthy et al., 2002). It is believed that totally drying roots produce either more potent (root/xylem derived) ABA which gets long-distance transported to and recognized by the leaves, or different hydraulic signals in order to down regulate the transpiration and reduce merismatic growth (Davies & Zhang, 1991). In a PRD regime this assumption is exploited and the plant's perception of soil moisture is tricked out.

## 2.4 Pores, Channels & Vascular System

The primary long-distance water conducting vascular system is provided by the xylem, which is a 'vascular network' that goes through the roots, over the stem (trunk) to the shoots and finally over the petioles to the leaf veins or through peduncles over pedicels to the inflorescences which are later labeled as clusters. An elongation of the leaf veins to the leaf blade margin provides a channel which is called the hydathodes. Their function is believed to be stronger present around a dew point opposed with higher relative humidity and lower air but higher soil temperatures in order to get rid of excessive water (guttation). However, very little is known about their day hour activity, where it is assumed that their function is neglectable since both entities (active, passive) may evolutionary derive from aquatic plants which needed to maintain a bigger water flow through the plants in order to achieve adequate nutrient acquisition compared to soil-rooted plants (Mortlock, 1951). Nevertheless, the perimeter maximization of the grapevine leaf (lobbing), which also maximizes hydathode abundancy morphologically, still rises questions beyond the declaration as an atavism. Other forms of evapotranspiration are strongly limited through a 'waxy' cuticula that covers the epidermis of both sides of the leave as well as the shoots which are later additionally protected by lignification.

Among the leaf veins is the leaf blade or lamina. On the bottom side of the leaf lamina there are plenty of stomata. In dependency how many stomata are available per reference surface, the term stomatal density defines stomatal conductance and the rate of transpiration. The stomatal density can depend on climatic conditions but is rather genetically driven (Rogiers et al., 2009). It is self explaining that a higher stomatal density is harder to regulate and may be prone to anisohydric like responses. Here Rogiers et al. (2009) measured relatively high stomatal densities for white cultivars cv. Riesling and Chardonnay with 232 and 225 mm<sup>-2</sup> respectively, which which may be also comparable with cv. Grüner Veltliner to some extent. A stomatum primarily consists out of a pore that is surrounded by two guard cells. Besides light perception their activity is strongly coupled to the plant and soil water potential of ambient mesophyll and epidermis cells (passive/hydraulic regulation) as well as potassium concentration (active) of encompassing and the inner cells (Buckley & Mott, 2002). Stomatal activity is also sensitive to the abundancy of ABA (biochemical regulation). When the guard cells pump up with actively acquired potassium ions, water follows and the rising turgescence causes microfibrils in the guard cells to shape a bow, which results in stomatal aperture. If the water supply declines a higher guard cell pressure cannot be maintained and the stomata closes through collapse of the microfibril-tension. Today it is known that hydraulic regulation is the primary factor for stomatal closure in dehydrating grapevines, while ABA (abscisic acid) accumulation happens secondarily as a response to the hydraulic signal and mainly temporarily sustains the stomatal closure even after reirrigation in most grapevines (Tombesi et al., 2015). Daily stomatal aperture is required for leaf CO<sub>2</sub> import regarding photosynthesis. Besides water supply the capacity of photosynthetic active radiation (PAR) is the most limiting factor regarding photosynthesis. However, this factor can also be saturated and further increase comes with a high risk of oxidative- (Degu et al., 2019) and heat stress (Paciello et al., 2016) but a mostly stagnating (Baeza et al., 2010) if not declining assimilation rate due to light stress (Correia et



al., 1990). Indeed, in an experiment under controlled conditions Correia et al. (1990) could show midday depression in terms of declining stomatal conductance and net photosynthesis even in well watered grapevines where soil moisture was generally maintained [ leaf water potential  $\leq -0.3$  ] under high photon flux density [ $1450 \mu \text{mol m}^{-2} \text{s}^{-1}$ ], which effects were softened at lower light intensity [ $750 \mu \text{mol m}^{-2} \text{s}^{-1}$ ]. In their study the stomatal adaption to light until maximum stomatal aperture, after the onset of a constant radiation source was about an hour until it already steadily declined. Therefore, these results suggest a clear limiting effect of midday photosynthesis rates occurring independent of soil water depletion which can be another reason for midday depression (Tuzet et al., 2003) next to a temporally higher evaporative demand (Moutinho-Pereira et al., 2004).

In contrast hereto cloudy conditions may lessen the photosynthetic activity however only if such conditions correspond to very low photon flux densities, which are not homogenous among cultivars. Another essential factor is local atmospheric  $\text{CO}_2$  concentration. Kimball et al. (1995) could report partly enhanced  $A_N$  with free air  $\text{CO}_2$  enrichment in wheat, however with significant lower effect size than water supply and with reference to Correia et al. (1990) where high light was associated with saturated leaf-internal  $\text{CO}_2$  at declining photosynthetic performance,  $\text{CO}_2$  shortage is probably a neglectable factor in near future.

The last factor is the leaf area and its interaction with grapevine age or relative inhibition due to water stress or senescence. Higher leaf area and bigger vines have a higher evaporative transfer and demand, what affects the maximum of stomatal conductance that can be measured of an individual (Winkel & Rambal, 1993) which may not be true for vines under water deficit. Thus, it needs to be considered, that i) younger and ii) potted grapevines can not compete with vines that are older than 10 yr. and/or grown in the field even under excessive irrigation.

If all components fit together on a theoretical sunny day clear from clouds, the diurnal assimilation rate curve could theoretically fit a half sinus curve stretched to the light hours with maximum at midday and no activity in the dark hours. In nature however at least one resource is not saturated or even lacking at nearly all daily stages in leaf photosynthetic activity, and therefore this assumed model seems less reliable. A lot of published papers show variable daily curves with high variance throughout the day. Possible reasons are limited light access (clouds), midday depression (Tuzet et al., 2003), phase shifted daily temperature maximum (Costa et al., 2019), soil water status depletion etc.. It needs to be considered that plants are forced to consume available soil water almost immediately after light exposure, due to lacking storage mechanisms in non succulents, because soil water would otherwise irreversibly evaporate without any plant benefit. However, this also promotes plant water shortages in the afternoon. The phase-shifted daily temperature curve due to heat accumulation, where point of maximal radiation and temperature are asynchronous, is another important factor. Indeed, this means that in general the morning hours are more productive, while the afternoon hours are more challenging for plants leading to a scenario where conservative stomatal regulation contrasts the necessity of transpiration chill in order to prevent heat stress (Wehr, 2017).

All these described factors may result in a right-side flattening of the measured diurnal assimilation rate- and stomatal conductance curves (cf. Winkel & Rambal, 1993; Rogiers et al., 2009).

Only at night the stomata are 'probably' closed, associated with minimal neglectable transpirative activity, where the soil water depletion of plants is finally restricted. Technically, this means zero photosynthetic activity and stomatal conductance and even negative  $A_N$  values may be measured during this phase. However, this might not even be true as shown by Rogiers et al. (2009) where they could reveal significant stomatal aperture at night in some cultivars.

On cellular basis, various types of aquaporins, which are protein channels embedded in the plasma membrane, either facilitate or inhibit the cell-cell water movement dependent on intra-, and extracellular and adjacent cells' pH-, pCa, osmolite concentration at high speed (Vandeleur et al., 2009). They are considered to play a major role in the water use efficiency of cultivars

and are thus selection criteria in order to explore drought tolerant traits. Compared to plasmodesmata they are thus specific and regulative, thus can avoid water pass by signal stimulated closure.

## 2.5 Water Status & Plant Water Potential

The soil-plant-atmosphere continuum (SPAC) -model, which can be quickly explained as a sinking negative potential from the soil over the plant to the atmosphere with increasing height, is mainly based on the theory that water must be under tension in order to pass through the plant's xylem (Chone et al., 2001). Hereto the corresponding forces that are necessary for water to acquire the necessary potential energy to flow through the plant xylem against gravity is provided by a potential gradient between the soil and the atmosphere (Begg & Turner, 1970). In return these mass-flows from sites of high pressure (lower tension) to sites of low pressure (high tension), facilitated by osmosis and capillary forces (adhesion and cohesion) (Müller et al., 1999) can be reduced to the natural law of seeking the state of lowest energy/ resistance by approaching an equilibrium. Contrary to plants, in soils where no semi-permeable membranes are present the water flow is mainly driven by gravity and soil-moisture tension rather than solution concentration gradients (osmotic forces) (Richards & Weaver, 1944). While the roots have the job to induce overpressure through excessive water incorporations, the leaves produce negative pressure through transpiration which establishes and maintains this pressure gradient (tension). Since the atmospheric pressure is lower than the pressure in the leaves, when the air is not fully saturated ( $VPD > 0$ ), water vapor moves through plant stomata to the atmosphere and is taken up by the air. VPD is the difference of the actual air humidity vs. the relative humidity when the air is saturated at a specific temperature. The air can hold even more water at higher temperatures causing higher VPD, and wind removes saturated air and exchanges it by less saturated air, what promotes aridity in general and causes a higher evapotranspiration. In order to avoid lethal dehydration, plants are able to provide resistance forces (stomatal closure, osmotic forces) that inhibit water loss to the atmosphere under low turgescence. As a matter of fact, the more dehydrated the plants are, the higher is their capacity to retain water and the harder it is to remove free water from these cells (Deloire & Heyns, 2011). This provides a natural passive dehydration defense mechanism by the plants, that gets additionally supported by incorporation of osmolytes in cells compartmentalized by semi-permeable membranes, thus inhibits transpiration even if the stomata are adequately opened under these conditions. This can be the case when all plant available soil moisture is deprived around the permanent wilting point. Common parameters to describe the water status along the SPAC can be obtained by measuring moisture content or the matrix potential of soil layers near the rootzone, or plant water potentials per Scholander chamber in the field (Williams, 2012). Dependent on daytime all water potentials alternate due to temperature, radiation, evapotranspiration and mostly soil water content. The matrix potential among others depends on soil humidity, temperature and the distribution of coarse, middle and fine pores in the specific layers of the soil. It is generally known that fine pore water is hardly plant available since high soaking forces would be necessary in order to deprive this pore water, which is promoted by soil water depletion (e.g. drought) and defined as permanent-wilting-point in soil sciences. The opposite case is defined as field capacity, arrived by draining the soil after saturation for 2-3 days and determine the weight difference between fresh and dry weight from a soil sample or alternatively by tensiometer measurements. If translated to the potential concept in pressure, after an estimation by Richards & Weaver (1944), the field capacity of several soils should be around - 0.01 MPa. It may be mentioned that field capacity is a more useful parameter in field experiments or such experiments that use very big lysimeters ( $>0.5 \text{ m}^3$  soil).

## 2.6 Canopy Influence & Interactions

Even if all photosynthetic resources would be optimally allocated, it needs to be realized that not all leaves of the canopy are equally productive (Medrano et al., 2015; Müller et al., 1999). The ontogenetic constitution (leaf age), determines a big part of leaf productivity. A good leaf productivity goes hand in hand with net assimilate exports from the leaves. These "productive" leaves act therefore primary as sources, while less productive leaves with a net assimilate import act as primary sinks and may compete with the fruits of the vine. The maximal photosynthetic capacity of a leaf is reached with its final leaf size; a positive assimilate net export after half of the final leaf size (Müller et al., 1999); or after 50- 80 % of the final leaf size (Poni et al., 1992); followed by a steadily decline in productivity with growing leaf age (Wu et al., 2016). This was also confirmed for *Vitis vinifera* by Poni et al. (1992) where after about 40 days after unfolding the leaf carbon uptake declines. Hence it can be stated that older and younger leaves are generally less productive. However, the development of younger leaves needs to mobilize external assimilates until autarkic assimilate production which additionally requires preceded shoot elongation as carrier mainframe. This is why the development of younger parts of the canopy can be connected to an increased assimilate net import facilitated by the older leaves and is not rarely negatively assessed in viticulture (Müller et al., 1999), especially during the fruit bearing stages due to potential assimilate competition to the grapes (Ollat et al., 2002).

Furthermore, the demography of the canopy includes terms and factors like primary, secondary, tertiary leaves etc. and positional height and inclination, basal distance within the shoot and concurrency to each other for optimal light exposure just to denote a few.

While outer canopy layers are directly radiated, the remaining leaf area mainly receives diffuse shortwave radiation of lower density and thus photosynthesis (Mullins et al., 2004). Hereto Sánchez-de-Miguel et al. (2010) distinguishes between total- and external leaf area whereas the latter is assumed to carry out 90 % of all photosynthesis. This concept could be well shown by Kaps & Cahoon (1992) in 'Seyval blanc' grapevines grown in a container. By removing all leaves within a shoot except of one, a modification of the position from the remaining leaf with increasing distance to a basal cluster correlated with higher vegetative growth, sugar incorporation and fruit weight in their study. This shows that sun exposure is the most important factor for photosynthetic performance of a leaf, despite the fact that leaves that are more distant to the fruits need to establish and maintain a higher phloem concentration according to the proximity principle. However shaded leaves can also contribute as sources by the means of even a positive net export (Koblet, 1975).

Grapevines are in general perennial woody climbers (fixation per tendrils) and prerequisite static support either by a wired trellis system or are educated to small trees (e.g. gobelet-system) which is the oldest form (Mullins et al., 2004). The different trellis systems have huge impact on canopy formation and light acquisition and may aggravate comparisons between differently educated grapevines of the same cultivar.

Furthermore, in many cases pruning is necessary, since most cultivars are prone to high yield, which cannot be supplied with the right amount of assimilates (Mullins et al., 2004). A desired equilibrium can be either reached by trimming of excessive foliage that leads to increased light interception and improved microclimate within a row, or by removing excessive bunches or prior flowers.

It may be mentioned that a higher leaf area requires more water supply, with a higher evaporative demand but at better transpiration cooling performance if water is not lacking and modifications may bias temperature mediated impacts on grape composition like total acidity and pH (Wample & Smithyman, 2002; Paciello et al., 2016).

It is believed that calcium plays a crucial role in the osmotic adjustment of grapevine leaves to water deficit (Patakas et al., 2002; Degu et al., 2019).

## 2.7 Fruits

### *Phenology*

The production of fruits in grapevine extends over two years, whereas fruit primordia are formed in buds one year before they develop into an inflorescence the following year after bud burst (Keller, 2010). However, in between there is a dormancy necessary which requires cool temperatures before later bud burst in spring or summer of next year's season (Keller, 2010). These buds are also called latent buds since the primordium is not visible/ latent, and only after the full development after bud burst it becomes clear, what was developed out of the bud. The probability that an inflorescence derives from a latent bud is about a third, since a latent bud may also develop either into a tendril, a leaf or rarely also into a shoot (Mullins et al., 2004). However, mostly the first six to eight basal buds are actually producing an inflorescence primordia (Keller, 2010). This process, describing the development of a inflorescence or bunch primordium out of a bud, is called fruitfulness and is highly temperature, daylength, light-intensity dependent. Hereto Keller (2010) denotes necessary temperatures that can range from 20 up to 35 ° C, dependent on the preference of a cultivar and its origin. For instance, a trial performed by Buttrose (1970) denotes a fruitfulness of 0.6 per bud for Shiraz and Riesling at 20 °C, whereas higher temperatures up to 35 °C increased the fruitfulness. Broken down to seed maturing strains (most wine-grapes), since pathogenesis is a different story (more frequent in table grapes), after flowering (anthesis/ cap fall) where the flowers (petals, sepals) are opened, the gymno- and androecia are freed and wind pollination (self & cross-pollination) follows after few days (Mullins et al., 2004). After pollination, a pollen tube develops and grows down the style where it fuses with the egg cells and later form seeds (Keller, 2010). However only about 30% of all flowers can be converted to fruits, due to frost damage (<0°C) or incomplete pollination among other factors and will be discarded by the vine and are sometimes pruned by the cultivator in order to maintain an optimal amount that should promise better fertility and thus fruit set (Mullins et al., 2004). Finally, the fertilized receptacles slowly start to transform to the berries, whereas seeds are involved by Auxin production that triggers the Gibberellin-synthesis which promotes the cell division of the meso and exocarp (Keller, 2010).

### *Berry Development*

Three stages have established to describe the double sigmoid pattern of berry growth and enlargement (Mullins et al., 2004).

- I. Initial phase of rapid growth, mostly cell division and expansion, accompanied by a high accumulation of organic acids (40-60 days)
- II. Lag phase of slow or no growth, slow maturation of seeds, maximum of acidity. (7-40 days)
- III. Final phase of resumed growth and maturation starts with berry softening (vèraison) and is solely accompanied with cell expansion until final berry size while titratable acidity decreases and opposes a massive accumulation of hexose sugars (35-55 days); The osmotic gradient due to sugar accumulation enables higher possible water incorporations.

(Mullins et al., 2004; Bauer et al., 2013)

## *Pre & Post Veraison - Berry Hydraulic and Transpiration*

It is widely accepted that pre-veraison water deficit has a stronger impact on berry size mainly due to volumetric changes in the pericarp vacuoles, than post veraison water deficit (Ojeda et al., 2001; Poni et al., 2018; Chaves et al., 2010). The former one can be reversible if plant water status is timely restored before veraison and the latter one is rather driven by progressive transpiration of berry water (Greenspan et al., 1994; Keller et al., 2006, Zhang & Hansen, 2018). This is firstly because the net xylem flow rate into the berries is practically zero or even slightly negative then and since the phloem flow rate is one-way (into the berry) its stream may sooner or later be prone to be impeded since it can no longer overcome the osmotic resistance by high berry sugar (Greenspan et al., 1994, Poni et al., 2018; Lang & Thorpe, 1989). It is believed that this moment occurs at maximal berry size, and from then on higher concentrations of berry sugar are mainly due to progressive berry dehydration at practically zero water input and steadily declining osmotic potential in berries (Coombe & McCarthy, 2000; Bondada et al., 2017). Out of this it is often generalized that grapes are insensitive to soil water supply during the ripening phase (veraison - harvest).

While berry transpiration that corresponds with the stomatal & lenticellular activity is rather neglectable surplus berry water mostly gets discharged through cuticular transpiration where the main driving factors can be divided into the external factors VPD and the internal factors berry surface and cuticular conductance (Zhang & Keller, 2015). Hence it can be followed that high VPD and large turgescient berries tend to benefit berry transpiration processes in general. If berry transpiration is not compensated by further water influx through the phloem, the berries are faced to shrinking (shriveling) processes which are generally promoted by sustained water deficit in grapevines (Zhang & Hansen, 2018). It may be mentioned that berries contract during the day and expand over night, so if the expansion is higher than the contraction we can speak of berry growth and this day-night pattern is less expressed after the berries have reached the lag phase (Greenspan et al., 1994, Lang & Thorpe, 1989).

## **2.8 Grape Composition**

### *Components*

Grape berries consist out of the three components skin, seeds (1-4) and pulp which contains the major fraction of juice, sugar and acid, whereas most of phenolic substances which are important for the aromatic potential (odor, taste, astringency) of the must, are found in skins and seeds (Müller et al., 1999; Meidlinger, 2000). Although "quality" is a very subjective term and with emphasis on the research object in white cultivars, wine grape quality attributes often compromise a balanced sugar-to-acid ratio at moderate must pH with a clean varietal character (Poni et al., 2018).

### *Sugar*

The mobile form of sugar is sucrose, a disaccharide that consists out of the two monosaccharides glucose, which is an aldose based on a hexacycle ring, and fructose which is ketose based on a pentacyclic ring, also known as inverted sugar. The translation into the monosaccharides is accomplished enzymatic invertases and acids.

The long-term glucosereservoir is provided by a transformation from sucrose into starch, which allows plants to spare some of the photosynthetic produced sugar for times of shortages in the vacuoles (Keller, 2010).

Enhanced sugar content in berries is associated with a higher density and this fact is exploited in determination of berry sugar. Different methods have established like refractometry index, e.g. light source transmission through a dense liquid is weaker; or Grad Oechsle which reveals how much more weight relative to the reference of 1 L water is measured (Meidlinger et al., 2000). There are also FT-IR (Fourier Transformation - Infra Red) Spectroscopy methods, that exploit the absorbance of emitted infrared light and a Fourier transformation finally transcribes the quantity of a substance (Bruker, 2015).

### *Acid*

The acidity in grapes is one of the most crucial features may it be because of its contribution of sensory properties, or because of its responsibility for microbial and chemical stability of wines (Poni et al., 2018). The acid content consists mainly out of organic acids, actually mostly malic and tartaric acid, whereas tartaric acid is a better proton donor than malic acid and also slightly more abundant (Poni et al., 2018). These two acids are opposed by potassium concentration ( $K^+$ ) which is known to neutralize both and can have a huge impact on pH ( $\sim \pm 1$ ) what strongly depends on the used rootstock next to the used cultivar (Poni et al., 2018). Important indicators regarding the acid content of a must are pH value ( $-\log(c\{H^+\})$ ), total acid(ity) and titratable acidity both in g/L (Meidlinger et al., 2000). Total acid (acidity), measured via spectrometry or chromatography, and titratable acidity, measured with partial addition of a base, are often used synonymous but they are not the same (Boulton, 1980) and the latter one always lower since not all active content may dissociate. Nevertheless, both approach the dissociated fraction ( $H^+$  proton activity), not the actual acid content which remains latent.

### *Phenolics*

Polyphenolic (Phenylpropanoids) are various compounds and many of them are influencing the taste and the color but also the smell (odor) - since some of them are volatile - in grapes and have potential beneficial effects on human health like Quercetin (Ojeda et al., 2002; Andrade-Filho et al, 2009) either in an antioxidant or inflammatory way. After a coarse approximation by Singleton (1992) there are 4,000 mg phenolic substances per kg fresh grapes and only 5 % are located in the juice, one third in the skins and two thirds in the seeds. The juice contains mainly non flavonoids like hydroxycinnamates and caftaric acid, the skins anthocyanins in red grapes and other flavonoids including catechin and condensed tannins and in seeds mainly condensed tannins are present (Singleton, 1992; Mullins et al., 2004; Müller et al., 1999).

Non flavonoids refer to phenolic acids and their derivatives that are based on a C6-C4 hydroxycinnamic or a C6-C1 hydroxybenzoic acid skeleton (Nikfardjam, 2001) and stilbenes like resveratrol which provide defense mechanisms against biotic pathogens (Nikfardjam, 2001; Ojeda et al., 2002).

A fraction of the polyphenolics can be classified as tannins. Because of their properties they are able to inactivate and precipitate proteins (Ayabe et al., 2010), thus are responsible for astringency. One part of them are flavonoids (mainly Flavan-3-ols) and the other part of them gallic acid derivatives thus non flavonoids. Flavan-3-ols (mainly catechin and epicatechin derivatives) are major components of so-called condensed tannins or proanthocyanidins (Ayabe et al., 2010).

From the flavonoids flavan-3-ols are more abundant in the seeds than in the skin, while flavonols and anthocyanins are mostly present in the skin (Ojeda et al., 2002). White cultivars lack anthocyanins which are the main color pigments in the skins of red grapes due to a missing VvMybA1 gene transcription that was very likely caused by the former introduction of a retrotransposon in their ancestors (Kobayashi et al., 2004; Walker et al., 2007). In white cultivars therefore the co-pigments, the flavonols, are easier visible due to their yellowish color,

probably caused by chalcone, in the berry-skin (Ayabe et al., 2010). The biological function of the flavonols is similar to the carotenoids (terpenes), but without co-photosynthetic activity, they are contributing to bitterness in wines (Ojeda et al., 2002). Flavonols act furthermore as a sunscreen thus may absorb irradiance, and thus act as antioxidants in order to prevent oxidative stress by buffering free radicals via hydrogen bonds by a free hydroxyl- group (cf. Andrade-Filho et al, 2009). They are synthesized by the flavonoid pathway and form glycosides from which quercetin is most abundant (Ojeda et al., 2002), however also Kaempferol (Andrade-Filho et al., 2009). Both flavonols and flavan-3-ols are synthesized out of Malonyl CoA and Coumaroyl CoA which is enzymatically transformed to Chalcone followed by Naringenin and finally Dihydroflavonol (Ferreira et al., 2010; Nikfardjam, 2001). As previously reviewed by Poni et al. (2018) skin flavonol content is likely affine to light and even less dense canopies (Friedel et al., 2015) with clear discrimination against different latitudes. For instance, a supra-national study by Del-Castillo-Alonso et al. (2016) on cv. Pinot Noir showed a significant negative correlation with rising latitude (from Spain to Germany) regarding skin total flavonols. By carefully approaching denotable amounts in berry skins of that different fractions based on Friedel et al. (2015) with reference to cv. Riesling, a white cultivar comparable to Grüner Veltliner, from the measured total phenolics in their study about a third was total flavonols, two thirds Hydroxycinnamic acids and the rest flavan-3-ols. During ripening tannins (incl. flavan-3-ols) may decline, while anthocyanins and flavonols rather accumulate (Ojeda et al., 2002; Buchetti et al., 2011). Common analysis tools that are used in order to isolate and determine phenolic substances are either based on Gas chromatography-Mass spectrometry (GC-MS) concerning volatiles or Liquid chromatography-Mass spectrometry (LC-MS) methods for more stable solutes (Rusjan, 2010), like HPLC-DAD. All of these methods require a preceded extraction (polar and nonpolar solvents at different pH) and the use of a reference standard. Finally, physical and chemical properties of the target compound(s) are exploited and subsequently transferred into equivalents of the known reference standard amounts. Here the target maximum absorbance of light of a specific wavelength dependent on the reference phenolic compound is a decisive factor, which may be summarized in a range between 300-800 nm, however also depends on the used solvent to a minor degree. A more classical method is the Folin Ciocalteu method, which expresses total phenolics in equivalents of gallic acid after Singleton & Rossi (1965).

### 3 Materials and Methods

#### 3.1 Trial

Site of trial was Tulln an der Donau 3430, Lower Austria, Austria, with a warm temperate semi humid climate with warm summer (cfb after Köppen-Geiger climate classification), accumulated Ø yearly precipitation 625 mm, Ø yr. 9.7 ° C, 180 m a.s.l., central position: 48° 19' 11" N, 16 ° 4' 11" °E (Google maps; climate.org). Rows were NW-SE orientated, row distance ~2.4 m , plant distance ~ 1 m, plant density ~0.43 vines/m<sup>2</sup>. The trial was led as a repetition trial where test subjects received the same treatment as the year before, however only the results of the year 2018 were reviewed, and vintage effects could therefore not be tested. The trial was carried out as a randomized, imbalanced block design in rows (block size 13) with 3 replicates and factor steps were the different irrigation volumes that defined the treatment groups. Each row end was buffered with one marginal plant. Natural water impact through precipitation was excluded by an Ethylene-vinyl-acetate awning (round shape, highest point ~ 3m, lowest point ~ 2.50 m, installed at 5.06.18) . Factor steps respectively treatment groups were: FI... fully irrigated vines (~120 % ET<sub>0</sub>), SDI... sustained deficit irrigated vines (~35 %

ET<sub>c</sub> FI) & RDI... regulated deficit irrigated vines, whereas the latter were treated like the sustained deficit irrigated vines but additionally irrigated through the fully irrigated waterline one week before harvest. Until the day of reirrigation on 17 July (70 DAA), measured samples from sustained and regulated deficit irrigated vines were treated as one homogenous group. Deficit irrigation treatments were started one week before estimated veraison, estimated after cumulative growing degree days (GDD) respectively days after anthesis (BBCH (Lorenz et al., 1994)). Trial days were generally illustrated as days after anthesis (DAA) in this study, where anthesis was estimated as 50 % cap fall (BBCH 65), or days after veraison (DAV), where veraison was determined as 50% berry softening (BBCH 81-85) after Herrera & Castellarin (2016). For GDD calculation a base temperature of 10° C was used (cf. Parker et al., 2011) and for indoor GDD's a room temperature of 20° C was assumed (GDD=10°).

Two dripping irrigation main tube lines, fed by the local well, were time alternated programmed driven by two separate electric valves, to give water at 23:00 at a rate of 2 L/h through two secondary dripping tubes per pot plant. One pump fed the 'control' tubes with a programmed irrigation time of (~ 45 min). The other pump fed the 'deficit irrigation' tubes with alternated programmed irrigation times (11;6; 8 min). This alternation between 11, 8 & 6 min happened to accelerate the stress level as compensation to higher humidity and VPD caused by rainy respectively cloudy weather, which was reversed later in the trial due to hints of severe water stress (Appendix 8.6). The initial irrigation times for pump B (11min) was calculated after the daily ET<sub>c</sub> from a test cycle of the installed lysimeter plants between the 16. & 27.06.2018. Hereto the ratio of ET<sub>c</sub> and pot arrived irrigation known as irrigation factor was determined and finally the unknown irrigation volume calculated via known irrigation volume from the FI group and translated to a goal crop evapotranspiration of 35% similar to the method used by Hochberg et al. (2016a). The post adjustments were calculated adequately but with greater respect to already known water potentials and stomatal conductance values. The control water line was found disconnected on 2.07.18. The data analysis revealed that this suboptimal condition lasted for 4 days. The connection was restored and control plants were additionally irrigated for ~1h (~4 L) the same day.

The test subjects were 3-year-old cv. 'Grüner Veltliner'/Kober 5 bb cultivated in 20 L pots (holed) with 8:2 commercial potting media and perlite substrate. The potted grapevines overwintered in the greenhouse; the generative phase was therefore accelerated. Grüner Veltliner is known for medium strong vegetative growth, big dense berries with medium high sugar content, medium -late fruit maturity and high yield with a preference to medium heavy soils, however dislikes dry sites with bad water capacity as well as heavy soils and lime (Bauer et al., 2013; ÖWM, 2019). Kober 5bb, a common universal rootstock, supports intensive growth, with a good uptake in N, Mg, Ca and Mn but less in P, K and B and is little demanding to soil types with a good tolerance to lime, phyloxera and drought, but sensitive to heavy soils (Bauer et al., 2013). The pots were positioned on 2 stapled concrete slabs (50x50x4 cm x2) to exclude scenarios where the pot plants could acquire i) accumulated precipitation-water ii) drainage water from neighboring plants. The test subjects were positioned outdoor on 29.5.18 and vertically attached to the trellis system. Grapevines were more or less normalized to an equal amount of shoots (~4) and inflorescences (~5). Additional application of fertilizer NPK + Mg (40 g/ pot) equally to all groups once at the beginning of July.



## 3.2 Water Stress Monitoring & Irrigation Scheduling

### 3.2.1 Lysimeter Balances & Weather Station Data

2 PL-100 weighing platforms (METER Group, Inc. USA) were positioned under pots of one FI respectively one SDI pot plant and accounted for mass flows through irrigation and evapotranspiration. They were connected to a DT80M Data logger (Metergroup), an interface system and transmitter, that sent the measured data to a receiver computer every 15 min. The necessary system energy was fed via solar panel. The measure-cycle started on 16. June and ended at harvest on 24. July (77 DAA). The lysimeter balances surface was  $0.0707 \text{ m}^2$ , the upper inner pot diameter 30 cm with a surface area  $0.09 \text{ m}^2$  (not covered). Crop evapotranspiration ( $\text{ET}_c$ ) was calculated based on Hochberg et al. (2016a) as difference between the mean mass between 4:00- 5:00 (after irrigation/ drainage impact neglectable) and mean mass between 22:00-23:00 (before irrigation). Pot-arrived-irrigation was calculated as difference from the average mass between 22:00-23:00 (day before) and 00:00 - 1:00 (actual day). As a simplification the  $\text{ET}_c$  ranges were assumed to happen per  $1 \text{ m}^2$  ( $=1 \text{ mm m}^{-2} \text{ d}^{-1}$ ) according to the user manual. Daily  $\text{ET}_0$  was calculated from weather station data (below described parameters refer to 2m above ground level) via FAO Penman-Monteith equation (Soil heat flux was ignored) and daily VPD via difference from saturated vapor pressure and actual vapor pressure per average daily relative humidity, all by following the instructions described by Allen et al. (1998) (Appendix 8.3).  $\text{ET}_c$  from harvest day was corrected about the harvested yield of the lysimeter plants.

### 3.2.2 Leaf Gas Exchange Measurements

The interval for the measurements, around |11:00 -13:00 | , accounted for midday values because according to Williams et al. (2012) midday leaf-gas exchange measurements should gain the most reliable values. For 'representable' leaves the gas exchange parameters ( $A_N$ ,  $g_s$ ) were measured using an infrared gas analyzer LC-Pro (ADC Bioscientific Ltd.). The instrument settings included constant light intensity ( $1080 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), ambient  $\text{CO}_2$  and humidity. A measure cycle per leaf took approximately 2 min for adequate value stability. Selection criteria for a 'representable' leaf included: height in the canopy, basal distance, leaf position, -size, -age, -color, - order & sun exposure (outer canopy position). It was tried to fit this same selection criteria for all further leaves during a measure cycle.

### 3.2.3 Water Potential

Midday stem water potential was measured using a pressure chamber, at clear sky only, model: 3000 series Plant Water Status Console (Soilmoisture Equipment Corp, Santa Barbara, California). 15 min before measurement the target leaves were put into transparent plastic bags and wrapped with aluminum foil. Subsequently the leaves were cut with a razor blade at the petioles (remaining petiole 2-3cm) and immediately positioned into the instrument. Then the chamber was closed with the petiole protruding from the chamber lid, and the pressure was steadily increased with a valve at a slow increase rate until water dropped out of the cutting surface which was observed with a loupe. The valve was immediately closed as soon as any form of visible liquid dropped out of the petiole-cross section and the obtained value of the barometer taken as result.

### 3.3 Outcome Variables

#### 3.3.1 Total Leaf Area

Total leaf area was estimated according to the leaf lengths of the lamina and estimated on basis of a regression equation gained by the same trial plants from 2017 of the Institute of Viticulture and Pomology from the University of Natural Resources and Life Sciences Vienna, and evaluated once 1 day after veraison (58 DAA / 5.07.18) and 1 day after harvest (78 DAA / 25.07.18). Shoots were counted and all their major leaves, i.e. only primary leaves. From only one shoot the leaf lengths were measured per measure scale (0.5 cm accuracy). Subsequently, from all obtained leaf lengths the single leaf area was calculated and the mean value from that multiplied by the counted leaf number per vine in order to estimate the total leaf area.

$$LA = 1.177 \cdot LL^2 + 1.4871 \cdot LL$$

#### 3.3.2 Grape Composition

##### *Harvest Samples*

All clusters of an individual were harvested and weighed in order to determine the yield. Afterwards, 100 berries per sample were taken randomly and collected in conventional small freezing bags (tared) and the 100-berry-weight was weighed. The berries were manually squashed within the freezing bag and shaken several times. The released juice was transferred to falcon tubes (50mL) which were put into a centrifuge for 10 min at 1200 rpm. From the cleared juice a sample was put into a clean fresh syringe respectively and then injected into a Alpha I wine analyzer (Bruker Corporation) which was flushed with distilled water before each new sample injection. Brix content, soluble solids, density, pH, Total Sugar, Total Acid, Malic Acid content were measured. Additionally, refractometry index and pH was measured with an Atago PR301 - $\alpha$  Digital Brix Refractometer (Atago Co. LTD) and a HI 2211 basic pH-meter (Hanna instruments) in order to control the accuracy from the Alpha I measurements and the FT-IR method. Samples that could not been handled the same day were stored in plastic foam boxes in a cooling room with a target temperature at 4°C for approximately 12 hours and completed the next day.

##### *Temporal Samples*

At 58, 70 & 77 DAA randomly sampled berries were collected in falcon tubes (50 ml) and immediately snap-frozen in liquid nitrogen. The berries were detached per scissors with their pedicels (~ 0.5 cm) to prevent volatile losses and dehydration. Samples were led as a triplicate i.e. one sample per row and treatment, with variable number of berries. Here the limitation was the volume of the falcon tube. Therefore, more berries fitted in the falcon tube at 58 DAA because the berries were in average smaller at that time. The number can be denoted between 11 and 18 berries per falcon tube. Also, here SDI and RDI were treated as homogenous group until reirrigation at 17.07.18. (70 DAA) where they were divided. The frozen samples were kept stored in a -80 °C freezer until the later analysis, where the berries were counted, scaled and subsequently peeled. The berries and its components were always intermediary frozen in a bin containing liquid nitrogen after each working step. The aiding tools in order to remove the skins were a light defrosting step supported by hand rubbing/heating after which the berry skin was cut into four segments with a scalpel, which were finally removed via forceps, put into a new falcon tube and immediately blast-frozen again. Subsequently, the bulk weight of skins and tube was measured and tared with a ,at first, frozen, equal falcon tube. The seeds from the remaining samples were separated from the pulp, cleaned with paper towels from pulp residues,

and weighed in initially tared petri-dishes. The pulp mass was calculated from the total berry mass subtracted by the scaled weight of skins and seeds.

### *Skin Phenolics*

Skins were further processed to a powder, squashed with liquid nitrogen in a mortar with a pestle to increase the particle surface for a better extraction. From the skin powder, 0.20 g were scaled into a 2 ml microtube (tared) and filled up with 1.8 ml Methanol (50%). The extraction took two hours and was supported with a shaking device. At last the samples were centrifuged at max speed (15000 rpm) and room temperature for 10 min. The Folin Ciocalteu micro-protocol (Waterhouse, 2002) was then applied under dark conditions. Here, 20  $\mu$ l of each sample were treated with 100  $\mu$ l Folin Ciocalteu (FC) reagent and further diluted with 1.58 ml distilled water. After 6-7 min incubation time, 200  $\mu$ l of a before prepared sodium carbonate solution was added, and after further incubation of 2 h, 1 ml of the gained mixture was added into a 1 cm, 2 ml plastic cuvette compatible for the Genesys 10s UV-vis spectrophotometer (Thermo scientific) and analyzed at 765 nm absorbance. Later a gallic acid standard (gallic acid monohydrate) 5g/L was further diluted for a calibration curve for 10, 50, 100, 250 and 500 mg/L with 50% methanol respectively. From each one 20  $\mu$ l were used for the same just described FC protocol and analyzed with the spectrophotometer to construct a standard curve in order to transfer the gained absorbance values into gallic acid equivalents (GAE). Although the dilution factor for the processed samples was pretty low, which caused higher absorbance values, absorbance values for concentrations > 1000 mg/L showed still a good linearity and are therefore reliable (Appendix 8.2). Via linear regression gained gallic acid equivalents of the samples were finally corrected per dilution factor (1:9), exact weight and expressed as mg per g berry skin respectively per berry<sup>1</sup>. Flavonols were quantified with a 'Dionex UltiMate 3000 Basic Automated Systems' HPLC (Thermo Fisher Scientific) with a combined AAC 3000 Autosampler- column compartment and a UV-Vis DAD detector in 3D mode. Hereto the same centrifuged extraction-samples were used but first cleared from fine particle residues through a syringe nylon filter (0.45  $\mu$ m) and further 1:1 diluted with methanol (50%). The used mobile phase was: (A) 10% formic acid (in distilled water) and (B) 10% formic acid in acetonitrile. The separation was carried out using an Accucore C18 column, 2.6  $\mu$ m particle size, 100 mm  $\times$  4.6 mm (Thermo Fischer Scientific) kept at 25 °C. The gradient at 1 mL/min consisted in 0-8 min 95% A, 8-16 min 85% A, 16-22 min 70% A, 22-26 min 100% B. Peaks with maximum absorbance around 360 nm were considered flavonols. A quercetin-3-o-glucopyranoside standard (the most abundant flavonol in grape berries) was chosen for the calibration (Appendix 8.2), and the integrated areas under all obtained peaks from unknown compounds were transposed to equivalents for this compound and corrected about the dilution factor and exact weight and expressed as both i) skin concentration given in mg per g skin, and ii) per berry <sup>1</sup>by multiplying the obtained skin concentration with the mean skin mass in order to obtain a reference per berry based on Ojeda et al. (2002).

## **3.4 Data Analysis**

The data was processed via Excel 365 (Microsoft) & SPSS v.24. (IBM). All significance-values were calculated with  $\alpha = 0.05$ , two tailed expressed. An ANOVA was performed for normally distributed (Kolmogorov-Smirnoff test) and homogenous data (Levene). The chosen post hoc test was Bonferroni which was counterchecked via Fischer's LSD and if the homogeneity of variances was violated, a Games Howell Post hoc test was performed. If LSD and Bonferroni led to similar conclusions, only Bonferroni was reported, otherwise both. Due to partial refutation of assumed normal distribution and small but also imbalanced sample sizes leaf gas exchange and stem water potential data was handled via Mann-Whitney u-test. Correlation

testing was reduced to a Spearman-rho test for simplification. Scatterplots were illustrated as mean values with standard error bars and sample sizes denoted in captions. Levels of significance are shown as stars (\*) above data marks and refer either to all possible comparisons or are otherwise denoted. Only samples from repetition treated plants were included.

## **4 Results & Discussion**

### **4.1 Weather Station & Lysimeter Balance Data**

#### **4.1.1 Climate**

The climate during the trial in 2018 can be described as relatively stable and typical for the site however it was milder than the year before (climate-data.org). The daily mean temperatures can be summarized as 19.9 °C regarding the total time where the potplants were positioned outdoor and 19.5 °C speaking for the trial time regarding the different irrigation regimes. The sum of growing degree days (GDD) from beginning April until harvest (24.7.18) can be denoted as 1148.15 °C. There were hardly days with a temperature maximum  $\geq 30$  °C and only few  $\geq 25$  °C. 7 days of the trial time (27.06.18-24.07.18) including 59, 63-64, 74-76 DAA were affected by relevant precipitation and lowest VPD, temperatures and radiation. Although the experiment was protected from rain, at 74 DAA a precipitation incident occurred that was probably facilitated by higher wind speed. This was detected by the lysimeter balances but also marked by significant correlations with midday leaf gas exchange parameters as well as stem water potential with the daily sum of rain in FI vines, because water accumulated. Several cloudy days occurred in the trial and were approximately as abundant as sunny days. The harvest was carried out relatively early, but to the authors knowledge at technical maturity according to TSS in berries in FI vines and after cumulative GDD (cf. Friedel et al., 2015; Ojeda et al., 2002). Nevertheless, the pressure for berry dehydration was evaluated as relatively low compared to most available literature from more arid-climates, e.g. daily VPD was never above 2 kPa although this could also reach values of 5 kPa (cf. Herrera et al., 2017).

#### **4.1.2 Soil Water & Evapotranspiration**

Low irrigation volumes were accompanied by relevant total weight reduction in the first days in both lysimeter plants (FI due to the unauthorized disconnection of the irrigation line). While the FI-lysimeter plant relaxed quickly from the supply error between 50-55 DAA within 1 day (55-56 DAA), the weight decline regarding the SDI-lysimeter plant flattened out after approximately 10 days after the deficit irrigation treatment and had in general a weaker irrigation-evapotranspiration-amplitude. Compared to the response to the stomatal conductance to lower irrigation volumes, the weight curve declined a bit more delayed before stagnation, however reacted more accelerated than midday stem water potential to water deficits. It was checked if the midday soil water depletion in the SDI-lysimeter plant was relatively higher evident than in the FI-lysimeter plant,  $ET_{c_{mid}}$  hereto was calculated as difference of average mass between 4:00 and 5:00 and average mass of 11:00-13:00 and expressed as proportion of daily  $ET_c$ , however a t-test could not confirm a significant difference or imbalance. Both test plants have achieved ~35 % of the daily calculated crop evapotranspiration at midday in average. Thus, it can be summarized that lower soil water supply resulted in a lower base line and amplitude at a similar diurnal dynamic (Fig.2), and accordingly soil water depletion did probably not even happen in SDI plants otherwise the baseline in the SDI lysimeter plant would not have stabilized but further declined.

Crop evapotranspiration ( $ET_c$ ) correlated with  $ET_0$  and daily vapour pressure deficit (VPD) in both lysimeter plants significantly, however the correlation of daily  $ET_c$  and temperature was only significant in well irrigated vines according to the lysimeter balances. Therefore, evapotranspiration from free soil water was stronger in these pot plants accordingly, and insignificant in deficit irrigated vines.

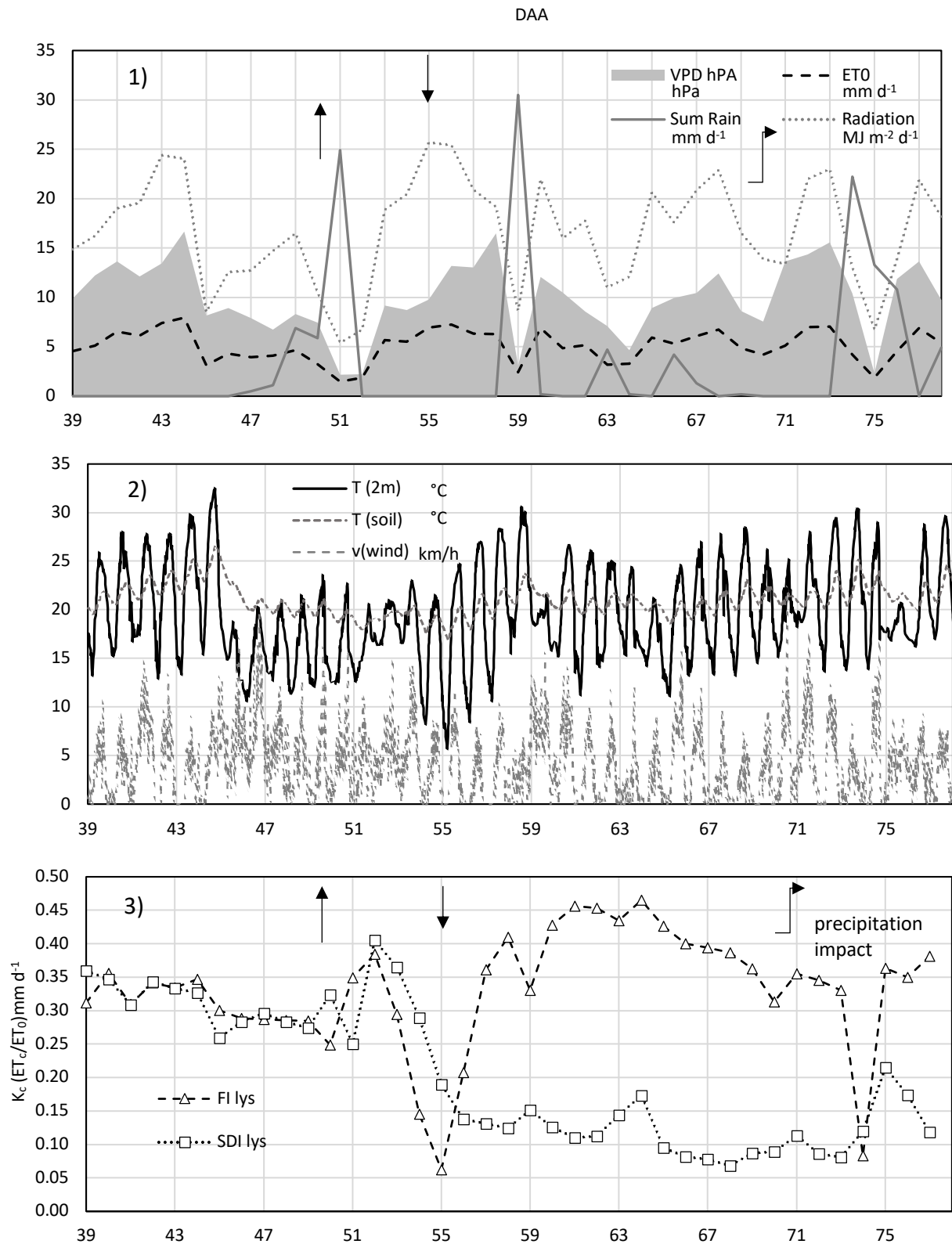
The crop coefficient  $K_c$  ( $ET_c / ET_0$ ) had a better connectivity with most measured water relational parameters than both of its forming parameters (measured in significance and effect size), including stomatal conductance  $g_s$  ( $R^2=0.82$ ; Fig. 4 a ) as already reported by Hochberg et al. (2016a) or Williams et al. (2012).  $K_c$  of the SDI lysimeter plant responded almost inversely than the FI lysimeter plant to rain events simply because, water gained through retention due to lower evapotranspirative pressure was actually consumed by the SDI plant while it merely caused a calculation error of  $ET_c$  in the FI plant by masking the actual evapotranspiration curve. Here water was not fully consumed by the FI plant until the next irrigation cycle and could therefore accumulate. Thus, the lower calculated  $ET_c$  respectively  $K_c$  values in FI vines at the end of the trial were because of water accumulation, which was clear since the base line of the balance curve actually raised. This could be linked to a precipitation event that was associated with relatively higher wind speed what could explain how rain could infiltrate the tunnel. According to the lysimeter plants a plus of 1.292 L in the FI plant and a plus of 0.908 L in the SDI plant could be detected hereto at 74 DAA.

$K_c$  and  $ET_c$  of the SDI lysimeter plant showed higher values towards the end of the trial firstly due to the slight change in irrigation time (+ 133 mL/day) and secondly due to the precipitation impact and third due to low reference  $ET_0$  at 75 DAA where both parameters peaked and then fell again to lower values.

#### **4.1.3 Irrigation Investment & Achieved % $ET_c$ factor steps**

The FI group theoretically received 4.12 and RDI vines 2.18 times more volumes of water than SDI vines in total during the trial time (50-77 DAA). The calculated total volumes hereto are in order for FI 73L , RDI 38.74 L and SDI 17.726 L. Expressed per average trial day for the FI vines 4.12 times more water was invested (2.703 L/day) and for the RDI vines 2.18 times more water invested (1.43 L/day) than for the SDI vines (0.657 L/day).

The SDI treatment was pretty much held at a range between 20-35 %  $ET_c$  - as referred to the FI lysimeter  $ET_c$  - after most of the soil moisture was exhausted, however the irrigation failure and the very likely precipitation impact at 74 DAA (see Fig.1 1,3) caused severe outliers. Nevertheless, the coarse estimation in reference to a well irrigated lysimeter plant was relatively accurate, though. After referencing the actual crop evapotranspiration (calculated after a full model approach including the estimated leaf area multiplied with 2 and also considering upper und lower surface area of the pot) with the reference evapotranspiration at 58 DAA the FI lysimeter plant achieved 108.17% the SDI lysimeter plant 34.03 % of  $ET_0$  and at harvest (77 DAA) the FI lysimeter plant achieved 149.12 % the SDI lysimeter plant 35.05 % of  $ET_0$ .



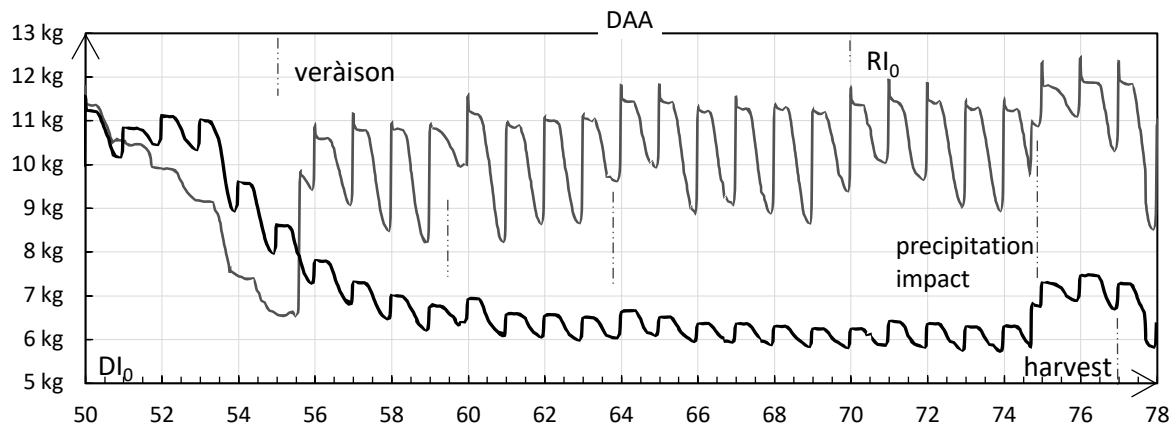
**Fig. 1** 1) Relevant Climate-Sizes for the 2018 trial: Daily- vapour pressure deficit VPD (hPa), -reference evapotranspiration ET<sub>0</sub> (mm day<sup>-1</sup>), -sum of rain (mm day<sup>-1</sup>) and radiation (MJ m<sup>-2</sup> day) from the start of the lysimeter setup until harvest of the trial site. upwards arrow...Irrigation regimes start (50 DAA), downwards arrow... veraison as 50% berry softening (55 DAA), slope arrow... reirrigation (70 DAA), harvest (77 DAA). DAA...days after anthesis. 2) Local temperature and wind speed (km/h) within 15min frequency . 3) Daily Crop coefficients K<sub>c</sub> as ratio of ET<sub>c</sub> / ET<sub>0</sub> of the two lysimeter plants FI...fully irrigated and SDI... sustained deficit irrigated in potted 'Grüner Veltliner' 2018. arrows in order: start stress imposition, veraison, reirrigation)

**Tab. 1** : Trial mean values from 50 DAA until 77 DAA for daily- climate data referred to 2m height above ground level: temperature (T), relative humidity (RH) and windspeed (W); as well as, irrigation: technical administered irrigation (IT), pot arrived irrigation (IP); and crop evapotranspiration respectively crop coefficient (Kc) and % ET<sub>c</sub> scheduling relative to the fully irrigated control, for fully irrigated (FI), sustained deficit irrigated (SDI) and regulated deficit irrigated (RDI) Grüner Veltliner potplants. lys... derived from a lysimeter plant, reg... estimated via multiple regression model

	ET <sub>0</sub>	VPD	T	RH	Radiation	W	Σ rain
Total	5.00	0.96	19.55	73.23%	16.67	3.58	4.23
Unit	mm d <sup>-1</sup>	kPa	° C		MJ m <sup>-2</sup> d <sup>-1</sup>	km h <sup>-1</sup>	mm d <sup>-1</sup>

	IT	IP	ETc lys	Kc lys	% ETc FI lys	ETc reg.	Kc reg.	% ETc reg.
FI	2.607	1.727	1.753	0.35	100.00%	1.64	0.32	100.00%
SDI	0.633	0.587	0.720	0.16	41.07%	0.68	0.15	41.77%
RDI	1.383					1.00	0.21	61.03%
Unit	L	L	mm d <sup>-1</sup>			mm d <sup>-1</sup>		



**Fig. 2:** Diurnal Lysimeter balance dynamic for a fully irrigated GV pot plant (upper line) and a deficit irrigated GV pot plant (lower line) during the irrigation trial time. DI<sub>0</sub>... start deficit irrigation regimes FI and SDI; RI<sub>0</sub>... start of reirrigation RDI.

## 4.2 Midday Leaf gas exchange & Stem water potential

### *Stomatal Conductance ( $g_s$ )*

In the initial days after treatment-application the measured midday stomatal conductance values declined very fast to values that reflected already levels of severe water stress after Lovisolo et al. (2010) [ $<0.05 \text{ mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$ ]. Here the FI group reflected lower values and a steeper decline (severe water stress already after 5 days) than the SDI group (severe water stress after 6 days) due to the irrigation-supply error. After that the irrigation was restored and the FI plants recovered high  $g_s$  values ( $> 0.15 \text{ mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$ ) within 2 days.

A similar decline within the first 4 days after deficit irrigation could also be shown by Degu et al. (2019) in potted Merlot where the grapevines remained at values in a range between 0.05 and  $0.015 \text{ mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$  after that.

SDI plants remained at the described low values with only little variability (around  $\pm 0.015$ ).

A deviating relaxation phase, where higher  $g_s$  was measured at 64 DAA in DI vines but also FI vines, was facilitated by mild climatic conditions [moderate radiation (possibly slightly clouded), low temperature at respectable  $ET_0$  and VPD and a slight precipitation event the night before], however returned to lower values again at 66 DAA.

Speaking for the total trial time both DI- vines (SDI & RDI) had significantly smaller  $g_s$  values than the fully irrigated control, as reported in many other studies (Patakas et al., 2002, Herrera et al., 2017, Hochberg et al., 2016; Ojeda et al, 2001, Buchetti et al., 2011; Castellarin et al., 2007). Although RDI vines recovered  $g_s$  values comparable to FI vines at harvest (both at moderate water stress though), a significant difference between SDI and RDI regarding the total trial time cannot be reported, since the timespan of reirrigation was too short for allowing such a difference.

The FI group displayed a suspicious declining pattern in measured gas exchange and stem water potential values in the last seven days that could not be properly explained with the lysimeter data. It was most plausible to interpret this decline as a response to higher VPD, since significant correlations with stem water potential and VPD were only found in FI vines not in DI vines. Such a response was at least already described by Rogiers et al. (2011). Thus, this pattern was mostly independent of the soil water supply and rather a response to higher evaporative demand, which was amplified when additionally considering a higher leaf area in FI vines. The decline in  $g_s$  was either a secondary effect due to lower plant water status or light stress according to a strong found relationship of  $g_s$  and daily radiation only significant in FI vines. This pattern was of course not evident in DI vines because i) high limitation of soil water (with greater effect size) did mask such effects and due to ii) ABA regulated stomatal inhibition (e.g. 73 DAA SDI Fig. 3. B, C vs. 3.A).

### *Assimilation Rate ( $A_N$ )*

Midday net photosynthesis behaved generally very similar to stomatal conductance inclusively speaking for the announced differences. Both responded pretty fast to lower irrigation volumes and stagnated at some bottom rate that was rather in equilibrium with the crop coefficient than with stem water potential. The lower  $g_s$  values were reflected with inhibited midday assimilation rates and thus photosynthesis in DI vines. In total both deficit irrigated groups undercut the FI control by far.  $A_N$  declined very fast within the first 5 days, however displayed higher fluctuation in contrast to  $g_s$ , what can be affirmed by higher variances of the assimilation rates in DI vines in total.

Both leaf gas exchange parameters achieved slightly higher values in DI vines at days that corresponded with precipitation, lower temperatures and radiation, and there was in general a positive correlation with relative humidity (daily & midday) stronger present in well irrigated



vines, though. A promotional effect on stomatal aperture to higher air humidity was already well reviewed by Schulze (1986). After site measurements that were performed at 55 DAA under sunny conditions at midday [pretty in between the trial average] the photon flux density in the tunnel was approximately  $1450 \mu \text{mol m}^{-2} \text{s}^{-1}$ . Although this value was already about  $300 \mu \text{mol m}^{-2} \text{s}^{-1}$  lower than outside the tunnel, this was apparently saturated enough to cause hints of midday depression in leaf gas exchange behavior due to light stress, as described by Correia et al. (1990). What might speak for this argumentation was a found negative correlation between midday leaf gas exchange parameters and radiation (daily & midday)  $\{p < 0.001, \text{Spearman's } \rho\}$ , however only significant in well irrigated vines and higher evident in the assimilation rate.

According to the fast recovery at 64 DAA of  $A_N$  and  $g_s$  proportional to  $K_c$  in SDI vines, or the fast recovery after restoration of the control waterline regarding FI plants, an ABA mediated role on stomatal regulation played a minor role in the early stages of water deficit. However, since stem water potential recovered faster than  $g_s$  later in the trial at 73 DAA in RDI vines, similar to to Herrera et al. (2017) in one year, it seems plausible that xylem ABA inhibited stomatal aperture after reirrigation (Tombesi et al., 2015), may correspond to the initial plant water status before reirrigation. Another evidence for this was that stem water potential in SDI vines was slightly higher at 73 DAA as well, although  $g_s$  did not change (Fig. 3 B). In potted GV (20 L) this "ABA effective" threshold was definitely below at a midday stem water potential of -0.8 MPa in the trail and may be first active below - 1 MPa.

#### *Intrinsic Water Use Efficiency ( $WUE_i$ )*

Intrinsic water use efficiency ( $WUE_i$ ) behaved inversely to  $g_s$  and  $A_N$  and was significantly higher in DI-treatments than in well irrigated FI vines in total, and correlated in general positive to water stress (Appendix 8.12.2). Speaking for the whole trial time the measured midday  $WUE_i$  was clearly over 90 ppm in both DI vines. After reirrigation the  $WUE_i$  from RDI vines recovered to values that were not significantly different from the FI control at harvest. There was no significant difference between both DI groups in total as well for the reirrigation phase, however.

#### *Midday Stem Water Potential ( $\Psi_s$ )*

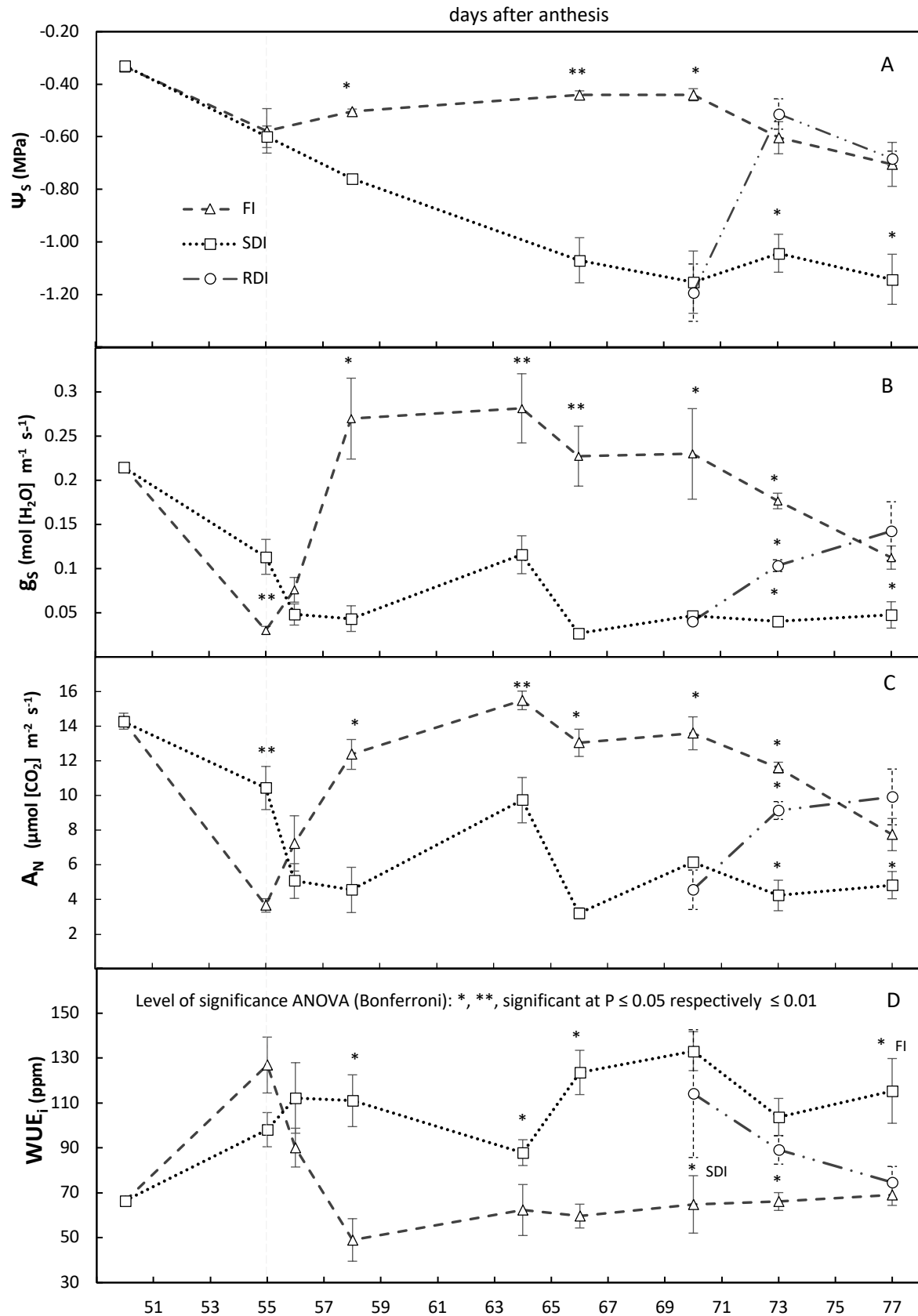
Midday stem water potential displayed a very slow, inert and lagging response compared to  $g_s$  or  $A_N$ , however recovered very fast after the vines have received higher irrigation volumes, similar as reported by Patakas et al. (2002), like observable in the RDI at 73 DAA (Fig. 3 A). The stem water potential was generally less sensitive to climatic changes which is however pretty clear e.g. due to the lagging response of soil temperature compared to air temperature (Allen et al., 1998), even in potplants. The negative responses of  $\Psi_s$  to changes in daily  $ET_0$  were most consistently proportional to radiation and were only significant in FI, maybe reflecting midday depression to some extent, but could also be mediated by the stronger relations of  $ET_c$  and daily temperature or VPD where higher amounts of free soil water ("luxury water") could evaporate on hot days.

It was kind of surprising that during 50-56 DAA, the  $\Psi_s$  of deficit irrigated vines (little supply) and the FI-control (no supply) declined in the same manner without discrimination between the factor steps (Fig 3 A). What could have played a role is that 51 DAA was accompanied by relevant precipitation what probably softened the effect of water stress in the not irrigated Control at this time, especially when considering the lagging adaption of the plant water status to low soil water content. Nevertheless, the crop coefficient was lower in FI vines either and according to the lysimeter plants a rainwater incident could have been excluded. Therefore, this approach is probably inappropriate, and a better explanation was probably found in stomatal

regulation, since recorded  $g_s$  of FI vines (no water) were significantly lower than in DI vines at that day, thus discriminated very well between no and little irrigation. This may be a hint for that GV vines react differently under root drying than under very low but frequent water supply (Davies & Zhang, 1991).

Speaking for the total trial records, significantly lower  $\Psi_s$  was measured in SDI vines compared to the FI control, with significant differences in 5 of 7 measuring days.  $\Psi_s$  of RDI vines already synchronized with those of the control at 73 DAA, already statistically distinguishable from SDI vines. However, also here the differences between the two deficit irrigated vines were too weak in total, even for the reirrigation timespan and therefore not significant. Contrary to stomatal conductance the measured midday  $\Psi_s$  from DI vines continuously declined in a nearly linear manner (-0.04 MPa per day) during the first 20 days of the trial and remained in a range between -1.1 and -1.2 MPa afterwards. Compared to potted Merlot in a two years study Herrera et al. (2017) the decline of  $\Psi_s$  was less steep, where it took less than one week to reach values below -1.1 MPa in a  $ET_c$  (35%) DI regime. Although there are plenty similarities to their experimental setup except different cultivar and slightly bigger pot volume (+20 L), the climatic scenario was probably more demanding in their study. A similar progressive decline of  $\Psi_s$  was also described in a two years trial on field Merlot grapevines by Herrera et al. (2015) where the steepest decline in DI-vines took place within the first 60 days and even reached values of -1.30 MPa in their trial while well irrigated vines remained at values around -0.5 MPa; or by Buchetti et al (2011) for the same cultivar in two years, whereas the latter study reported an actual bottom water potential of -1.4 MPa for stem water potential. Nevertheless, the field scenario in both mentioned studies needs to be considered, that should delay soil water depletion and thus prolong the adaption of lower water potentials in a relevant degree, whereas water potentials of field plants are lower by default.

Summarized, it can be followed that the differences between RDI and SDI vines were very weak in this experiment regarding the total trial time, while differences between FI and SDI were more relevant.



**Fig. 3 :** Mean values and vertical bars for standard error (n =3-5) for midday- stem water potential (A), stomatal conductance (B), net photosynthesis (C) and intrinsic water use efficiency (D) in potted Grüner Veltliner 2018 either fully (FI), sustained deficit (SDI) or regulated deficit irrigated (RDI). veraison...grey line.

### 4.3 Water Relations - Responses To Drought

*g<sub>S</sub>, A<sub>N</sub> and WUE<sub>i</sub>*

The high correlation between stomatal conductance and assimilation rate is a well reviewed topic following a saturated growth principle and it is known to be very homogenous among different cultivars (Lovisolo et al., 2010; Cifre et al., 2005). The curve response was very high, while the different factor steps of irrigation just moved the data points pretty much along the curve which was therefore also true for the relation between stomatal conductance and intrinsic water use efficiency. Regarding intrinsic water use efficiency stomatal conductance was the better predictor and showed a logarithmic relation, very similar to a illustration by Lovisolo et al. (2010), compared to net photosynthesis with a linear relationship. Thus, increase in drought were faced by downregulation of transpiration but at improved water use. However it may be mentioned that WUE<sub>i</sub> did not that clearly decrease under severe water stress contrary as described by Cifre et al. (2005), since A<sub>N</sub> was not severely reduced (Fig. 5: 1) & 2), while WUE<sub>i</sub> had a general positive correlation with falling midday stem water potential ( Fig. 6 c) because A<sub>N</sub> did rather stagnate but not decline under severe water stress. However, there was still a huge variation evident with high variance of A<sub>N</sub> and therefore also WUE<sub>i</sub> under severe water stress. Here A<sub>N</sub> followed a sinus-like swinging curve between values of 2 and 6  $\mu\text{mol} [\text{CO}_2] \text{ m}^{-2} \text{ s}^{-1}$  and lower values  $< 3 \mu\text{mol} [\text{CO}_2] \text{ m}^{-2} \text{ s}^{-1}$  could be actually linked to a linear correlation with the daily temperature ( $R^2=0.51$ ) suggesting non-stomatal limitations and thus not contradicting the review by Cifre et al. (2005). Negative correlations with the daily or midday temperature were generally only significant in SDI and RDI vines not in FI. The relationship of midday stomatal conductance with the daily VPD was negative as well and only significant in SDI vines. Both very in consensus with Rogiers et al. (2011) or Costa et al. (2019). This trait was already used as an isohydric indicator by Soar et al. (2006) where it was apparently mediated by ABA concentration in the xylem sap, higher evident in cv. Grenache than in cv. Shiraz but occurred in both cultivars.

*K<sub>c</sub>*

The crop coefficient had a high relation to stomatal conductance in the lysimeter plants, no matter which irrigation scenario, and an even stronger relationship to intrinsic water use efficiency. Interesting was that a prediction of ET<sub>c</sub> out of linear regression via WUE<sub>i</sub> allowed an estimation of negative values in DI vines (thus very poor) whereas a prediction out of g<sub>s</sub> did not, which however failed due to lacking linearity compared to a simple model that handled the more plausible ET<sub>0</sub> and applied irrigation volume as predictors (Appendix 8.4).

A maybe clearer discrimination was detected between crop coefficient and midday stem water potential, where only the SDI plant showed a significant correlation to some extent, as a response to the vine adapting to lower soil water supply.

Probably more relevant was, that SDI vines did more consistently use all of the water that arrived the pot, with less variance of ET<sub>c</sub>, thus used water more efficiently, which also matches with the increased intrinsic water use efficiency measured in these vines as well as with the ratio of ET<sub>c</sub> and pot arrived water. Thus, the stomata adapted very well to the apparent available soil water (Fig. 4 a) and the bottom rate of g<sub>s</sub> was very likely in equilibrium with the daily received water. Until this point a significant negative temporal correlation with little climatic variation occurred.

Although the stomata did react adequate to measured soil humidity in DI vines (regular amount of soil water supplied only at a lower volume), the measured stem water potential fell despite the described equilibrium. Plant water status declined further until stem water potential was between -1.1 and -1.2 MPa. From then on some extent of stagnation may have been interpretable, maybe not, since the irrigation time was slightly increased at 73 DAA, however also the evaporative demand was higher towards the end of the trial, aggravating a clear conclusion hereto. By clearly reserving some climatic variation in advance, an acclimatization of plant water status to a specific fraction of the full evaporative demand was generally achieved in some studies (Herrera et al., 2017), except those that withheld water until a measured water potential reached that were irrigated only once a week e.g. Buchetti et al. (2011), Herrera et al. (2015). Another possible pattern hereto, although in field plants, was described by Degu et al. (2019) where water potential ( $\Psi_I$ ) under a sustained ET 35% lys regime i) first declined to lowest values after which it ii) remained constant for a period of time and iii) finally recovered to some extent during the end of the season. Such a pattern was also exhibited by potplants in Herrera et al. (2017) following the same regime in one year. The recovery of water potential during iii) may be a result of water stressed vines acclimatizing to the altered conditions by decreasing the transpiration surface through leaf shedding after Degu et al. (2019), while shoot elongation stops after midday leaf water potential drops below -1.2 MPa according to a coarse estimation by Harb & Keller (2018) in ii), which was only present towards end of the trial. The just described process was pretty much in consensus with the performance of potted GV in the trial under the same deficit irrigation regime as well as with measured leaf area. Leaf shedding although present had a relatively weak impact and water stress induced effects on the canopy were mainly driven by nearly stagnation of shoot growth and leaf production according to the sampled data (6.4; Appendix 8.10). The outcome of this discussion is that the assumed stagnating pattern of the midday stem water potential is pretty plausible and water stressed vines were probably in the second phase ii) of the above described pattern.

A falling water potential as response to drought is the consequence in most studies that approved water deficits or withheld water, since stomatal closure can only prevent dehydration by transpiration while evaporation continues. The measured behavior of deficit irrigated GV might be partly interpreted that early stomatal closure nearly proportional to sensed soil water may have delayed the decline in plant water status, however, did not avoid it. Therefore at least one component either i) the adaption to the evaporative demand or ii) the adaption to actual soil water supply was not in consensus with the model of a pure isohydric behavior. A discrepancy of these aspects may be also a product of a 'drought incompatible' scion x rootstock interaction (Chaves et al., 2010).

For instance, medium drought-tolerant Kober 5bb (rootstock) may have sensed low soil moisture more 'optimistically' and subsequently underestimated the adequate transmission of either hydraulic or chemical signals in order to regulate the actual 'more pessimistically'-required stomatal conductance rate by less drought-tolerant scion 'Grüner Veltliner'. Nevertheless, this is just an in advance discussed aspect, that cannot be fully elucidated by the trial data, but may provide an approach following a simplified assumption of a control circuit inspired by Davies & Zhang (1991).

At least at 55 DAA it was evident that the used rootstock x scion system realized a different stomatal adaption dependent on low vs. no water supply. The neglected focus on self rooted cultivars under adaption to drought hereto was already criticized by Lovisolo et al. (2010). In order to return to the discussion and stick to the line of content, GV reacted partly pessimistic and partly optimistic in the trial but definitely not isohydric. To be more precise a relatively wide range in measured stem water potential between -1.4 and -0.8 MPa was practically

represented by the same range of stomatal conductance values ( $0.05 - 0.03 \text{ mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$ ) or crop coefficient values (0.12- 0.08) without any linearity (Fig. 6 a,d). Both relationships could be partly separated in a linear correlation until a threshold around  $\Psi_s = -0.8 \text{ MPa}$  and  $K_c = 0.15$  respectively  $g_s = 0.05 \text{ mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$  was reached. After this threshold any linear relation was gone which resulted then in a flat line that did no longer correspond to lower stem water potential (Fig.5, 6 a, d).

It may be mentioned that the standard error for the stem water potential increased with lower values, while the variance of the stomatal conductance behaved conversely. This may explain part of this weak response below the described threshold either i) in a statistically way e.g. higher measure error, or ii) under consideration of an increased likelihood of embolism events (Hochberg et al., 2016b), what may also bias the tension in pedicels in pressure bomb reading despite isolation of the leaf and acclimatization time. The latter should theoretically only concern the xylem water potential in the pedicels, not the measured stomatal conductance directly. This just discussed approach would at least not contradict the gathered intrinsic water use efficiency that correlated positively with declining stem water potential (Fig. 6 c).

On the other hand it may also be interpreted that the stomata were already closed at the described threshold since Rogiers et al. (2009) could show very well that expected zero values of stomatal conductance at "stomatal closure" are practically not present in real measurements and strongly depend on the stomatal density as well. For instance, comparable white cultivars 'Riesling' and 'Chardonnay' had a relatively high stomatal density in their study, what might be therefore also imaginable for 'Grüner Veltliner' and can exacerbate the measurement of actual zero values but also stomatal control on plant water status significantly. Another reference comes from a study by Tombesi et al., (2015) where stomatal conductance first froze at a comparable range as described and only reached actual zero values by suspending water until complete leaf abscission, which was by far not challenged in this experiment. Therefore, a zero rate of stomatal conductance is rather the exception than the rule especially in midday leaf gas exchange measurements. This is also plausible from a physiological perspective since total stomatal closure during midday and in the afternoon hours would be rather lethal for plants when considering the absolute abrogation of vital transpiration cooling as a consequence, and matches with the observed correlations of leaf gas exchange with the temperature only in DI vines.

Therefore, stomatal aperture was very likely also to some extent maintained by osmotic adjustment (Chaves et al., 2010) in DI GV in the trial, and because of the fact that the necessary guard cell pressure cannot be solely explained by passive regulation. Contrary, (xylem) ABA interaction had a inferior role since secondary symptoms hereto were only notable at the end of the trial at already very low measured midday stem water potential, thus did not significantly contribute to any impression of an isohydric behavior and it appeared that the adaption of GV vines to water deficit was primarily hydraulically regulated in consensus with Degu et al. (2019).

### *Classification of hydrodynamical behavior*

It is hard to generalize if potted GV reacted iso- or anisohydric in the trial, especially when considering the 'specific' pot scenario (20L), the temperate warm climate or that most of the accepted definitions work with responses of diurnal curves for stomatal conductance to nearly exclusively the leaf water potential (Tardieu & Simonnieau, 1998) or alternatively its response to soil moisture or by an ABA mediated regulation, under field conditions with much better soil moisture retention and often do not even include severe water stress. Another issue of this classification system is the heterogenous interpretation in the literature that lacks a clear

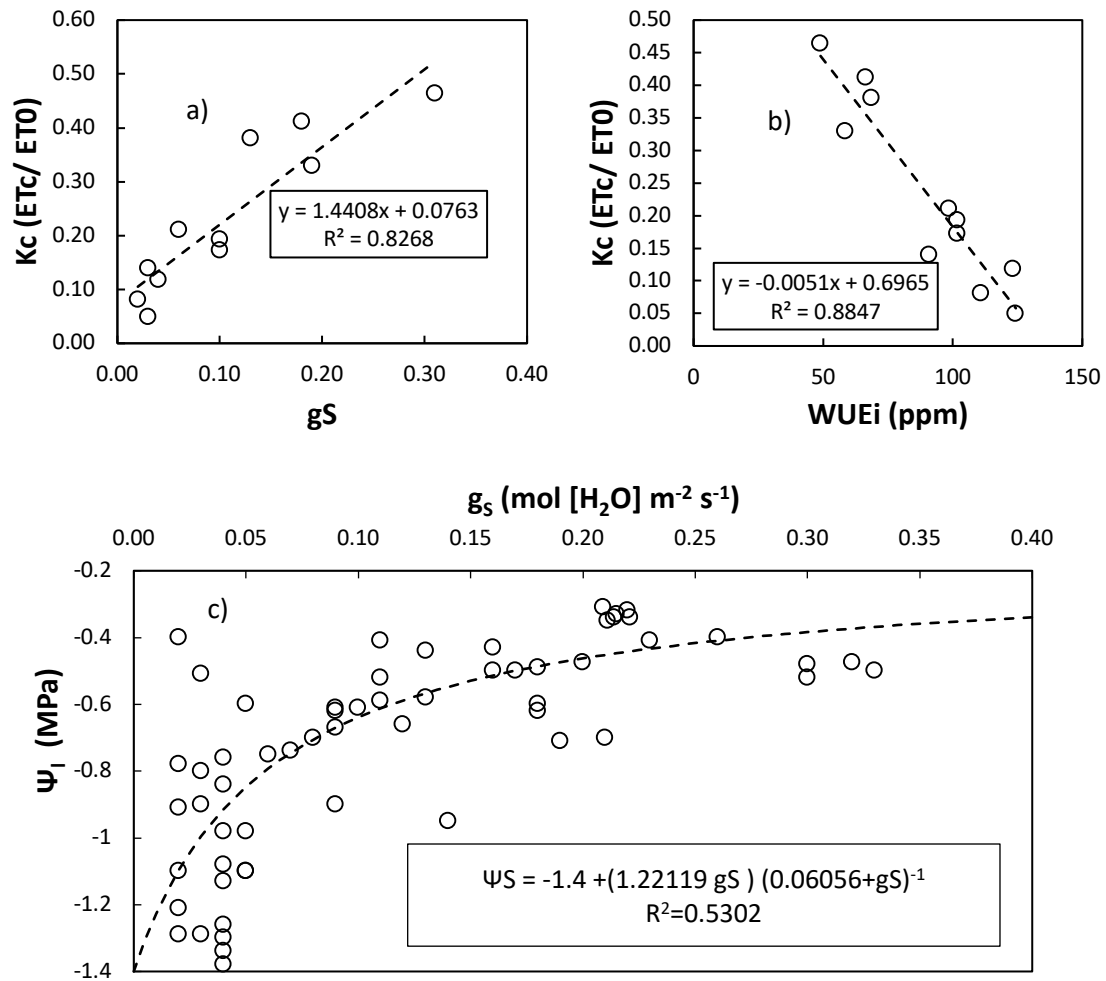
definition (Bota et al., 2016), while Klein (2014) declines such a dichotomous comprehension and suggested a continuum instead, whose sight is also shared by Chaves et al. (2010) or Harb & Keller (2018).

If it comes down to define 'one component' of anisohydric behaviour according to Schultz (2003) or Harb & Keller (2018) by significant lower midday leaf water potential of stressed vines than the lowest potential of not stressed vines, under the assumption of a strong relationship between midday  $\Psi_s$  and midday  $\Psi_l$  (Williams & Araujo, 2002 [field plants], Poni et al., 2007 [potplants]), 'Grüner Veltliner' may have shown an anisohydric behaviour far beyond near isohydric behavior at most of the trial days. However, this is probably a very poor trait, and a falling stem- but also pre-dawn water potential was the consequence in many cultivars faced to water deficit independent from the isohydric classification. For instance, in a study by Tombesi et al. (2015) [8 year old potted] where apparently isohydric cv. Montepulciano and anisohydric cv. Sangiovese were compared, and soil content totally depleted until full leaf abscission, there was nearly no discrimination between those cultivars in midday stem water potential either although cv. Montepulciano initiated stomatal closure a bit earlier than cv. Sangiovese. Here probably  $\Psi_{pd}$  was the 'slightly' better discriminante, however did not change the overall picture. The curve response of  $g_s$  with  $\Psi_s$  in this thesis was optically more comparable to Tombesi et al.'s (2015) curve obtained by isohydric cv. Montepulciano, according to the decline in  $g_s$  after a  $\Psi_s$  of -0.8 MPa was measured.

This is just one example why the relationship of  $\Psi_l$  and  $g_s$  in elder field grown grapevines with a higher focus on mild rather than moderate water deficit is hardly comparable to any pot scenario.

An alternative and in the author's eyes more independent classification system was approached by Bota et al. (2016), discriminating grapevine cultivars after the four physiological criterions: i)  $WUE_i$  under non-stress conditions, ii)  $WUE_i$  under drought, iii) stomatal behavior under progressive drought ( $\Psi_s$ ) quantified via significance of linear regression and slope, and iii) long term WUE (leaf  $\delta^{13}C$ ); into three categories of "water productivity" following a grading scale (1-3, good- water productivity respectively). According to this system GV displayed i) 3: low  $WUE_i$  (<80 ppm) ii) 2: moderate  $WUE_i$  (100-120ppm); iii) 2: moderate water saver (regression slope  $g_s$  vs.  $\Psi_s$  < 0.25 and significant at  $P < 0.05$ ) and iii) was not measured.

After combining all these discussed concepts GV is probably a moderate until bad "water saver" respectively near anisohydric or "optimistic" if additionally considering the adjustment to water stress by the decreased total leaf area according to Winkel & Rambal (1993). It is well known that 'Grüner Veltliner' is not very drought tolerant after Bauer et al. (2013) so that this classification appears pretty plausible, especially if additionally considering its origin and adaption to rainfed temperate climate conditions which would justify this optimistic response. Also, the respectable average performance in net photosynthesis did not really reflect a pessimistic trait. Needless to repeat that *Vitis vinifera* is overall anisohydric (Soar et al., 2006).



**Fig.4:** a) Stomatal conductance and b) intrinsic water use efficiency from the lysimeter plants referred to their daily crop coefficient, and c) nonlinear relationship (inverted Michalis Menten model regression compatible for negative values) between stomatal conductance and midday stem water potential from all available trial data.

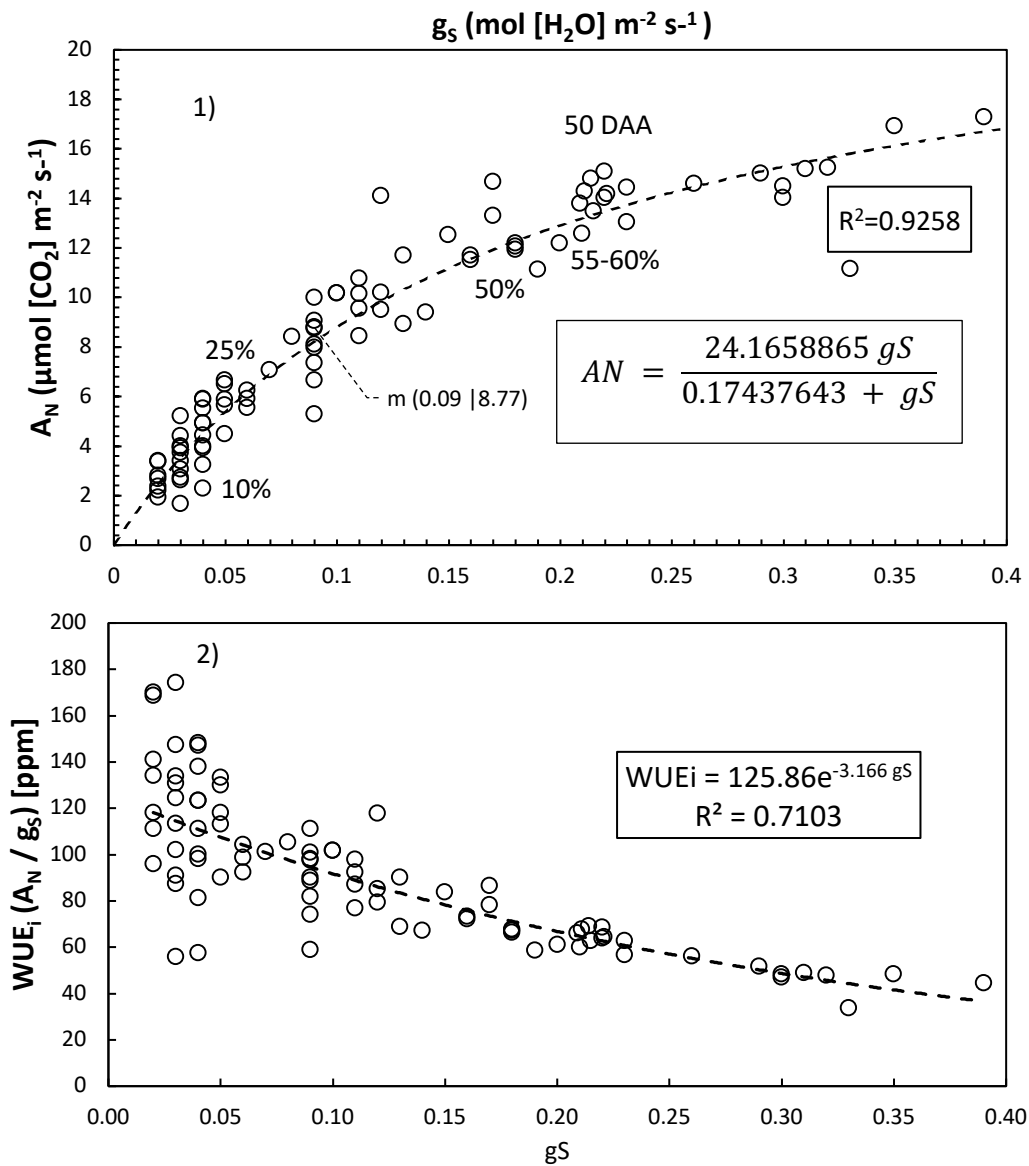
**Tab. 2:** Trial mean values of water related parameters. The relative crop evapotranspiration was calculated after the ET<sub>c</sub> gained by a regression function ( $R^2=0.86$ )

	$A_N$	$g_s$	WUE <sub>i</sub>	$\psi_s$	% ET <sub>c</sub> Lys
<b>FI</b>	11.54	0.194	70.37	-0.505	100
<b>SDI</b>	7.04	0.078	105.21	-0.876	41.77
<b>RDI</b>	7.99	0.094	98.38	-0.772	61.03
unit	$\mu\text{mol s}^{-1} \text{m}^{-2}$	$\text{mol s}^{-1} \text{m}^{-2}$	ppm	MPa	kg

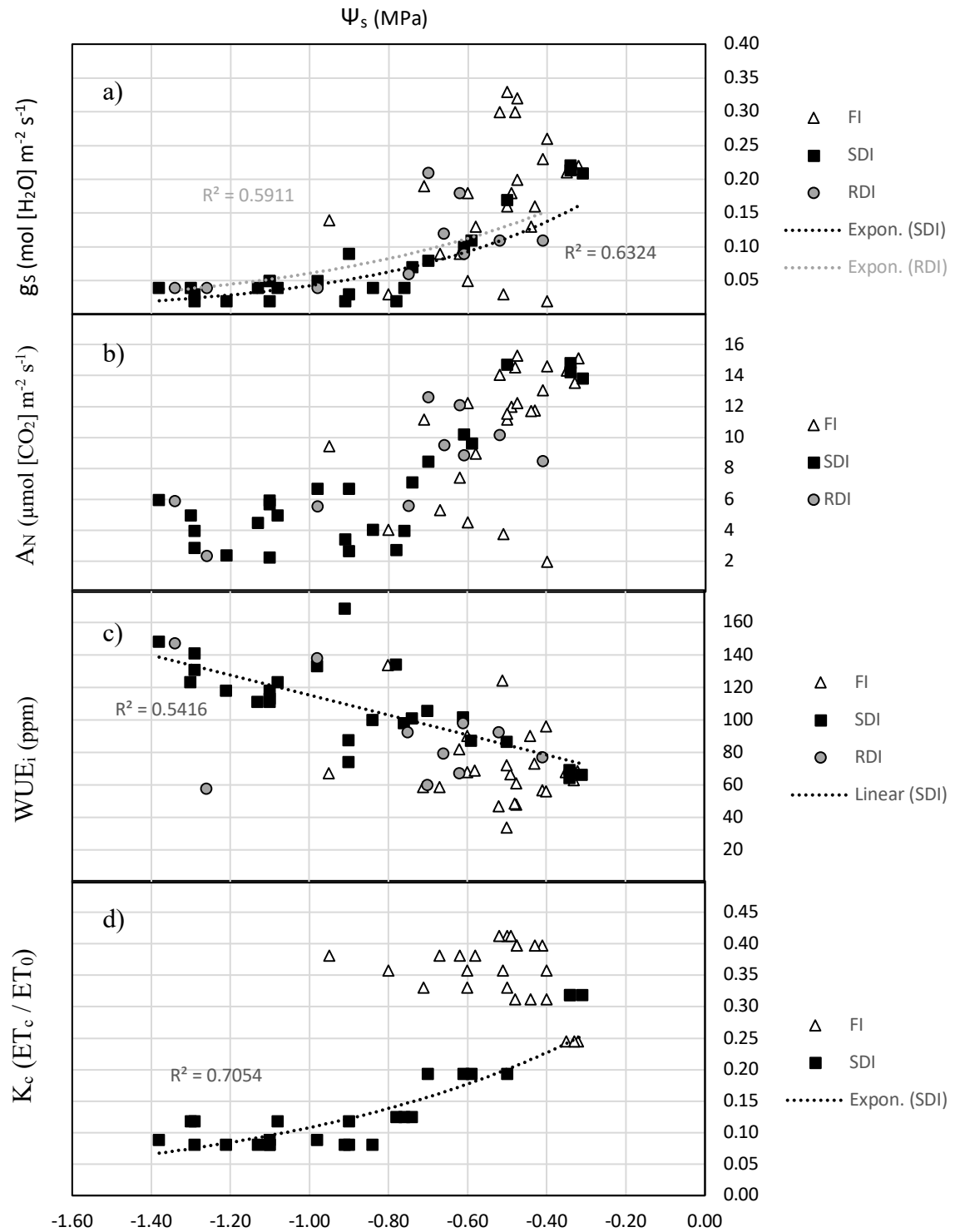


**Tab. 3:** days categorized after different stages of water stress for  $g_s$  in  $\text{mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$ : no  $> 0.2$ , mild  $0.15 < g_s < 0.2$ , moderate  $0.05 < g_s < 0.15$  and severe water stress  $g_s < 0.05$  based on Lovisolo et al. (2010).

Water Stress days	FI	SDI	RDI
No	15	1	1
Mild	5	3	3
Moderate	7	9	16
Severe	1	16	8



**Fig. 5:** Relation of midday stomatal conductance ( $g_s$ ) & 1) assimilation rate ( $A_N$ ) incl. Michaelis-Menten-model regression curve (dashed line), m... median; percentages in 1) refer to achieved assimilation rate compared to  $A_N \text{ max} = 24.16 \mu\text{mol [CO}_2\text{]} \text{ m}^{-2} \text{ s}^{-1}$ , 50% of stomatal aperture =  $0.17 \text{ mol [H}_2\text{O]} \text{ m}^{-2} \text{ s}^{-1}$  & 2) intrinsic water use efficiency, measured in differently irrigated (fully, sustained deficit- and regulated deficit irrigated) potted Grüner Veltliner 2018.



**Fig. 6 :** Relations of a) midday- stomatal conductance and b) -net assimilation rate, c) -intrinsic water use efficiency and d) daily crop coefficient with midday stem water potential in fully (FI), sustained deficit (SDI) and regulated deficit irrigated (RDI) potted Grüner Veltliner 2018. Crop coefficient values were assigned from only one lysimeter plant per denoted group and cannot reflect individual variability. True lysimeter data is shown in the Appendix 8.12.

#### 4.4 Total Leaf Area - Partitioning - Cluster Climate

As a typical result deficit irrigated vines were faced to lower leaf area (Loveys, 1991; Jones, 2004; Poni et al., 2018; Chaves et al., 2010; Wample & Smithyman, 2002). The total leaf area of FI vines was significantly higher than in deficit irrigated groups at harvest. Also, from a temporal view mainly in the FI group the total leaf area was higher at harvest than around veraison. Between RDI and SDI no difference could be reported at harvest nor a significant change between veraison and harvest, mostly because of the short reirrigation time span in RDI, where full recovery was additionally delayed what allowed therefore no continuation of shoot growth in such a tight timeframe.

In the canopy the different treatments had mostly two visible - opposing - effects: The well irrigated vines put emphasis on top shoot development, therefore showed preference on vertical light exploitation, while this effect was inhibited in deficit irrigated vines which showed higher tendency towards leaf abscission, especially the first and third (1<sup>st</sup> leaf after the cluster up the shoot) basal leaves were affected, while the inflorescence opposing leaves remained on the shoot in most of the cases. These effects as a response to droughts are probably rather typical, as reviewed by Poni et al. (2018) or Wample & Smithyman (2002). The field observations (leaf shedding) and estimated leaf area may display improved light interception and microclimate of the cluster zone in DI GV on the one hand but also as an adaptive effect in order to avoid cavitation events as described by Hochberg et al. (2016b). Why fruit-opposing leaves were not affected, beyond a priority, proximity- and supply- related explanation (Lang & Thorpe, 1989), may correspond with ABA interaction transduced by the roots, however, cannot be answered without sap flow- or biochemical data. It may be stressed that the impact of leaf shedding was generally low, and only visually observed, thus not found in the data, otherwise the leaf area in SDI plants at harvest would have been significantly lower at 58 DAA. Although the impact of few leaves on the estimation on total leaf area is generally doubtful, it may be objectively summarized that the data suggested merely a shorter canopy in DI vines compared to FI vines.

It is generally hard to separate the assimilate limiting influence of low regularly carbon fixation vs. low leaf area under water stress impact, however according to the literature the effect size of the leaf area hereto should be rather weak. As a reference, a study conducted by Keller et al. (2005) of several years in deficit irrigated 'Cabernet Sauvignon', 'Riesling' and 'Chenin blanc' could not confirm any significant variation in fruit composition although 30 - 39 % was removed by cluster thinning compared to a control, thus higher available leaf area for fewer fruits did not increase TSS accumulation here. Alternatively, by manipulating the leaf area, Herrera et al. (2015) reported that cv. Merlot of lower canopy under sufficient irrigation was associated with significantly lower TSS (°Brix) in one out of two years, while berry mass was not significantly affected in both years compared to a control. Friedel et al. (2015) reported no effect of leaf to fruit ratio on sugar accumulation in cv. Riesling by manipulating the cluster climate. When comparing the obtained trial values to Kaps & Cahoon (1992) where they denoted a yield per leaf area ratio of 8-10 cm<sup>2</sup>/g as most beneficial for berry weight and soluble solids in container grown 'Seyval blanc' grapevines, it appears that the trial values between 16 and 26 cm<sup>2</sup>/g are far beyond these measures [Tab. 5], suggesting no limitation. The achieved leaf area -yield ratio in DI vines was furthermore pretty much in a range far beyond suboptimal supply following a report by Kliewer & Dokoozlian (2005). Also, Herrera et al. (2017) observed even reduced TSS in berries from late deficit irrigated vines compared to a well irrigated control, although there were similar leaf area - yield ratios in one year.

Consequently, an under provision of assimilates just because of the lower leaf area is not really likely according to the literature, what makes the reduction of  $A_N$  due to water stress a better rationale in this experiment. To put emphasis on the aspect of discussion that both treatments

(leaf area manipulation or water deficit) may produce a similar modification in canopy, Herrera et al. (2015) reported hereto that both effects may occur independently from each other. It may also be discussed how far the sucrose supply of berries in both DI treatments was fed by the consumption of starch reserves. For instance, Patakas et al. (2002) observed a significant decrease of starch in leaves under drought stress, and Herrera et al. (2015) reported of significant higher sucrose in canes of Merlot in their study. Although the measured midday assimilation rate was generally not neglectable in DI vines in this trial, it cannot be excluded that berries from SDI and RDI GV were additionally supplied by starch consumption.

## 4.5 Berry Components & Growth & Yield

There were no significant differences in mean berry mass, berry skin, pulp and seed mass as well as its mass proportions and skin: pulp ratio due to the different irrigation regimes notable neither during ripening nor at harvest where also yield, cluster mass and 100 berry mass were not significantly different between the treatments against some subjective imbalanced impressions regarding some mean values. For instance: mean values for berry mass were higher both in the 100 randomly selected berries from a full harvest per individual ( 1.86 g) as well as in the randomly selected berries per row and treatment from harvest (1.95 g) of RDI vines with higher mean values for the yield, or berries from FI GV had even slightly higher mean values for skin-pulp ratio and skin-mass-proportion than those of DI GV at harvest. Nevertheless, both cases were not significant what makes a discussion obsolete.

Single seed mass in berries from RDI GV was significantly lower than in berries from FI GV, however the number of seeds per berry (2-3), that followed the inverted trend, not. According to Fischer's LSD test, a slightly different pattern appeared where the single seed mass in berries from RDI GV was significantly different from FI and SDI, and the number of seeds per berry only different from FI vines. If there should be a connection to the treatment it can only be a follow up effect of the previous year, since seed formation has already appeared before any treatment imposition in the trial. However this cannot be elucidated with data from only one year.

The mass of pulp represented about 80 % , that of skins 12 %) , and for seeds 7% of berry fresh mass in harvested berries of GV. Yield and mean cluster weight were not affected by the different irrigation treatments. The number of clusters per vine was pretty even between the groups (5-6).

Speaking for the berry development of all samples, growth and extension (berry-, pulp- & skin mass) was generally stronger in the first two weeks after veraison than afterwards. Surprisingly, berry, skin and pulp mass from only RDI GV samples significantly increased within the last week, which may indicate a potential effect (Appendix 8.9), that was however limited by possibly too early harvest. However, it also needs to be mentioned that here only an ANOVA was possible, with more power than used post-hoc tests in FI and SDI samples of course. Nevertheless, also the graphical impression may support this assumption given in Fig 11.

Temporal changes in seed mass followed an inverted trend as just described. The berry seed mass rather tended to decline, and this effect was much stronger between the 2<sup>nd</sup> and 3<sup>rd</sup> week after veraison. While all observed seed testa showed a greenish color in samples taken around veraison, most of them (80-90%) were already lignified two weeks later.

The Berry growth curve was overall homogenous between all groups and growth was generally not significantly affected by the treatment (including a relevant amount of days of severe water stress) in this study, leading to a similar report as described by Matthews & Anderson (1988) ,

Greenspan et al. (1994) or Roby & Matthews (2004) as response to water deficit during berry growth.

Therefore, berry fresh mass from GV appeared to be insensitive to variation in soil water supply during ripening and was not even affected by severe water stress compared to a well watered control in the trial similar to Casassa et al. (2015) in cv. Cabernet Sauvignon or as reviewed by Zhang & Hansen (2018) and in contrast to Ojeda et al. (2001 & 2002) in cv. Syrah and cv. Shiraz. They were further insensitive to higher irrigation as well regarding the RDI treatment. Similarly, Kennedy et al. (2002) could very well show in cv. Cabernet Sauvignon that a whole double irrigation treatment during ripening, had no impact on berry mass compared to a standard irrigation regime. The main driving factor to explain the observed outcome was possibly the mild climate. For instance, in a study by Herrera et al. (2017) berries were significantly smaller in DI vines in one year, but not in another.

Berries were maybe harvested too early, but to the best knowledge at least at technical maturity in FI vines (TSS 21-22), if referred to similar cv. Riesling in Friedel et al. (2015), and a decline in pulp proportion and increase in skin proportion of berry fresh mass between 70 DAA and 77 DAA was a general pattern visible in all treatments what possibly confirms that berries have already reached full size (Coombe & McCarthy, 2000), possibly already indicating impeded phloem sap flow into the berries where berry growth could no longer exceed or balance berry contraction (Greenspan et al., 1994).

Even after cumulative GDD (1148.15 °C) such hypothesized changes should already have occurred in the trial. For instance, Ojeda et al. (2001) displayed an already stagnating berry growth curve after 1100 °C in cv. Syrah und severe late water deficit while maximum berry diameter was already reached before 70 DAA in their study or see also Ojeda et al. (2002) where significant differences in berry mass were already detected 10 days after veraison as a consequence of late water deficit. Similar results were reported for the same cultivar by Ollé et al. (2011). Such effect was very likely not present in the trial, after the reviewed literature. Nevertheless, the mentioned studies derive from the Mediterranean Climate, thus another factor that needs to be considered, was the mild climate in the trial with relatively low VPD (0.5-1 kPa). Poni et al. (2018) denoted even possible necessary temperatures above 30 °C in correspondence to berry dehydration in their review, however conversely Herrera et al. (2017) reported no significant differences in berry mass in the "hotter" of two years.

The summarized relevant driving factors, why the hypothesized modifications in berry size or mass proportions of skin and pulp were not found in this trial may be the possible mild climate, different interaction of white cultivars adapted to such climatic regions, the relatively early harvest, while the role of leaf area was probably less important in this scenario. However, it also can be that cv. Grüner Veltliner is just a model plant for insensitivity of soil water supply in interaction with berry size after veraison as described by Zhang & Hansen (2018), and may be comparable to results produced by Casassa et al. (2015).

## 4.6 Grape Composition

### 4.6.1 Skin Phenolics

#### *Total Phenolics*

Skin total phenolic (TP) concentration [mg per g skin] of SDI GV berries was significantly higher than those of FI GV at only 1 sampling day (1 week before harvest). At harvest (77 DAA) this was no longer present. In FI vines skin total phenolics declined already significantly after 58 DAA and remained at that bottom low. In deficit irrigated vines the mean skin phenolic concentration slightly increased between 58 DAA and 70 DAA and then declined to values not significantly different from the fully irrigated control. This led to the slight impression of a weak delayed decline in DI vines. Nevertheless, temporal changes were not significant though. In total there was a likely negative time correlation in skin total phenolic concentration especially between 70 & 77 DAA.

A decreasing skin TP concentration in GV during ripening was already reported by Král et al. (2018), among other cultivars in their study ('Welschriesling' 'Chardonnay', 'Pinot Blanc', 'Noah'). Further studies that reported a similar decline including Obreque-Slier et al. (2013) in self rooted 'Carménère', 'Merlot', 'Cabernet Franc' and 'Cabernet Sauvignon'; Awad et al. (2017) in 'El Bayadi' table grapes. Other studies like Herrera et al. (2015) reported about a decline in the concentration of skin tannins (ed. note: a decisive fraction of total phenolics in white grapes ;mostly flavan-3-ols) with advanced season, which was stronger in irrigated than in deficit irrigated Merlot grapes in the late season of two years and may confirm the above described impression. However, the ripening time in their study was about 40 days longer and the highest mean differences between irrigated and deficit irrigated grapes were therefore observed with extended ripening, which would have probably not been the outcome with extended hangtime in this experiment. It also needs to be considered that their deficit irrigation treatment may have included pre-veraison water stress as well and was performed under field conditions, with significant differences in berry size. <sup>b</sup>Nevertheless, although the deficit irrigation regime in Herrera et al. (2015) was imposed at pea-size; BBCH 35, the stem water potential did first approach levels of moderate water stress around or after veraison, what makes it pretty legitimate as a 'late water deficit' reference in the author's eyes.

When this trial's skin phenolics were expressed per berry, there was no longer a correlation with berry skin mass and the amounts per berry were similar between FI, SDI and RDI GV even at a temporal view. This may speak for that skin phenolics concentration diluted with higher berry skin mass respectively berry mass and size in the trail, while the amounts per berry remained more or less equal, as described by Roby & Matthews (2004) or Roby et al. (2004). Both sizes correlated negatively with the midday stem water potential of the sampling days per treatment, however the correlation coefficient with the amount per berry was higher. This may be either a coincidence or may suggest that TP content in skins may depend on latest plant water status before berry sampling. However this cannot be clearly answered with these few data, since any natural variation can result in the same variation and because of the independent samples of course. A canopy effect was rather unlikely. For instance, although Friedel et al. (2015) could show a clear gradient of higher total phenolics from shaded leaves over a control to leaf removal in the bunch zone, this would have been insignificant if subtracting the total flavonols in their study, and the sum of flavan-3-ols and hydroxycinnamic acids were actually not significantly different between post veraison leaf removal and a control group. Thus, any variation in skin total phenolics due variation in total leaf area in this experiment are pretty unlikely. Although skin flavonols will be discussed separately in the next paragraph, this was mentioned because they comprise a respectable part in the analysed total phenolics in this thesis, however they are

less abundant (Adams, 2006) so that total phenolics in this experiment mainly represent flavan-3-ols.

The gained results in 2018 are pretty much in consensus with the discussion by Buchetti et al. (2011) and it may be summarized that skin tannin content may peak at veraison and decline afterwards very independent of imposed post veraison water stress in GV. Similar results with no significant difference between late water deficit and a control were also reported by Casassa et al. (2015) in Cabernet Sauvignon, where also no differences in yield and berry fresh mass were detected.

### *Flavonols*

Only 3 days after veraison (58 DAA) a significant difference between berries from DI and FI vines was detected, where DI vines had higher values, which was however not significant in amounts per berry. Although there were no significant temporal differences or between the samples of differently irrigated groups, a small visible trend was notable where skin flavonols per berry had a weak preference towards higher irrigation supply (Fig. 12, C), but without significance. Therefore, the results are pretty much reflecting what Herrera et al. (2017) has reported in potted Merlot under post veraison water deficit. Contrary to Ojeda et al. (2002) where post- veraison water stress was associated with vastly higher skin flavonols compared to a control in cv. Shiraz, in Grüner Veltliner there was not such a detectable effect, whereas the results of this thesis may rather suggest the opposite as reported by Kennedy et al. (2002). Hereto an interference with specific glycosyltransferases or lacking substrate may be an imaginable factor (cf. Ayabe et al., 2010), since in all of the just cited studies, as well in this experiment, berry sugar was lower, as far there is a connection. A potential beneficial effect on skin flavonol accumulation due to improved cluster irradiance was in no way convincing in the trial, and may be impeded for the same reason as a secondary cause of lower photosynthesis and assimilates.

## **4.6.2 Berry Juice**

### *Sugar*

Significantly lower sugar content, TSS (°Brix) and density values were detected in the deficit irrigated groups SDI & RDI compared to the FI Control, however SDI and RDI were not significantly different from each other. The prior reported result may implicate that the awaited effect of equal or higher TSS at expense of berry size may only occur due to early deficit in berries from GV, comparable to Matthews et al. (1990) where only berries from late deficit irrigated cv. Cabernet franc were significantly lower in TSS compared to a control, early deficit but also a full deficit regime (early+late) that were all similar in juice TSS in two years. The latter reported insignificant result may be to some extent comparable to Shellie (2014) where there was not even a difference between a strong sustained deficit irrigation regime held at 35% (pre and post veraison) and a regulated deficit irrigation regime (35% pre- and 70 % post veraison) as referred to the  $ET_c$  of well watered conditions, in berry mass and TSS (° Brix). TSS measured with the handheld refractometer was about 0.5 ° Brix higher than the Alpha I results with lower standard error; however, both showed a good linearity (Appendix 8.8). The produced results might be for a big part well explainable by lower assimilation rates in the DI-treatments compared to fully irrigated vines, rather than the leaf area as discussed previously. Unlike studies like Herrera et al. (2015)<sup>h</sup>, Ojeda et al. (2002), Buchetti et al. (2011), Castellarin et al. (2007) no modification of berry size and component relations due to deficit irrigation can be confirmed by the data, and was thus not linked to enhanced TSS in the juice, nor similar concentrations like in Kennedy et al. (2002) or Wample & Smithyman (2002) can be reported

because of that same reason. Maybe such effects are not consistent and prone to a highly dependent interaction with climate respectively the year (Buchetti et al., 2011; Shellie & Bowen, 2014; Herrera et al., 2017). In the latter cited study that has also focused on post veraison water stress TSS of cv. Merlot was also significantly lower in two years in deficit irrigated vines, although many studies cited in the mainframe of this thesis did report the opposite for this cultivar. Water stress was mainly assimilate limiting in potted Grüner Veltliner in the 2018 trial, and reirrigation, although possibly, too late and too short, could not improve TSS in berry juice from RDI GV, under the assumption of  $A_N$  being a contributive factor. Irrigation did under best knowledge in no way dilute solvent-solute interactions.

### *Acid*

There was a strong discrepancy between the pH values obtained by the classical pH meter vs. the Alpha I wine analyzer by FT-IR with poor linearity (Appendix 8.8), whereas pH-meter values had a better accuracy according to lower standard error or Levene test. After the values obtained from the pH-meter SDI had significantly higher pH and lower  $H^+$  Concentration than other treatments FI, RDI according to HSD ( $P < 0.05$ ) which was not significant but close via more conservative Bonferroni with  $p = 0.067$  in comparison to FI and 0.109 in comparison to RDI. Contrary no significant difference between the treatments was notable in pH obtained by FT-IR method. This imbalanced linearity in pH or  $H^+$  between the two different instruments was also reflected in the found correlation of the total leaf area and pH respectively  $H^+$  concentration, where contradictorily FT-IR (Alpha I) had a higher coefficient of determination, though both were significant. In case of doubt, the classical pH-meter values are probably to prefer, since any potential measure error would have been equally distributed to all measured treatments and because of the fact that the Alpha I wine analyzer is generally better calibrated for wine samples than must. Another hint is the smaller mean total acidity in SDI, which was however not significant ( $P < 0.17$ ) also measured with the Alpha I.

A higher pH due to post veraison deficit irrigation was e.g. observed by Shellie & Bowen (2014) in cv. Cabernet Sauvignon and cv. Malbec consistently over four seasons, while differences in TSS were surprisingly weaker evident only in one year despite reduced berry mass in 3 years or by Shellie (2014). However, their study was conducted in a field scenario and included pre-veraison water deficit as well, amongst different cultivars. Alternatively, a higher pH due to late water deficit compared to sufficient supply during ripening in berries from cv. Cabernet Sauvignon was reported in two out of five years by Wample & Smithyman (2002) who reported a clear trend hereto as a consequence of late deficit irrigation compared to other treatments in their study.

Maybe a potential effect occurred in the population where reirrigation rapidly changed the pH of the berry juice to values comparable to the control, however the results and the statistic were not convincing enough to eliminate any doubt. Thus, the acid pattern in juice from harvested berries may be more comparable to produced results e.g. by Matthews & Anderson (1988) or Casassa et al. (2015) in Cabernet Sauvignon, or by Herrera et al. (2017) in Merlot.

### *Leaf area, Transpiration Cooling/ Temperature - impact (Acid)*

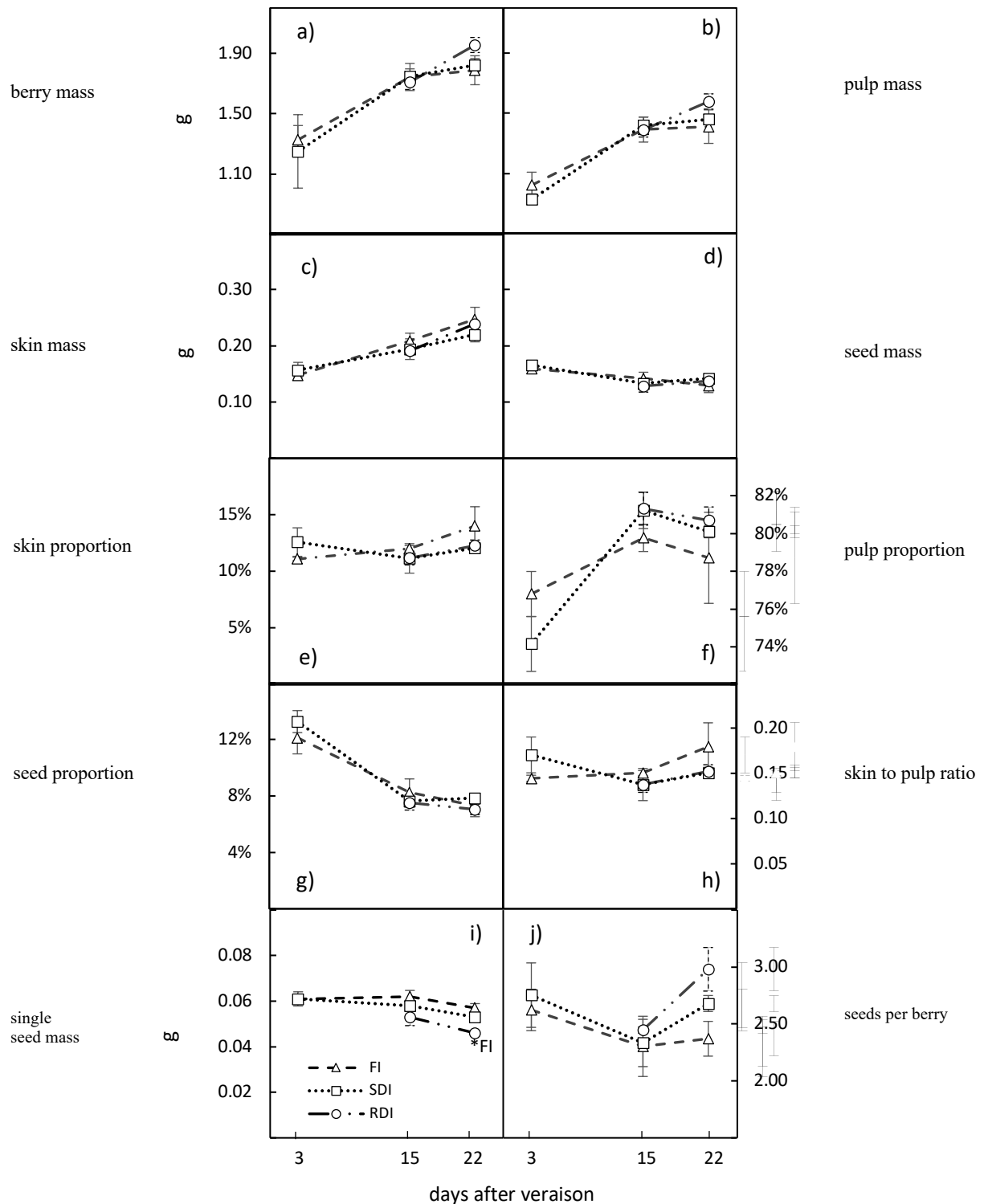
Although there were evident lower leaf area in DI GV and a correlation between the leaf area and juice pH respectively malic acid (Appendix 8.10), no discrimination against the different irrigation regimes was possible. However, hereto a study by Mabrouk & Sinoquet (1998), that included significant differences in leaf architecture with no limitation in plant water, also



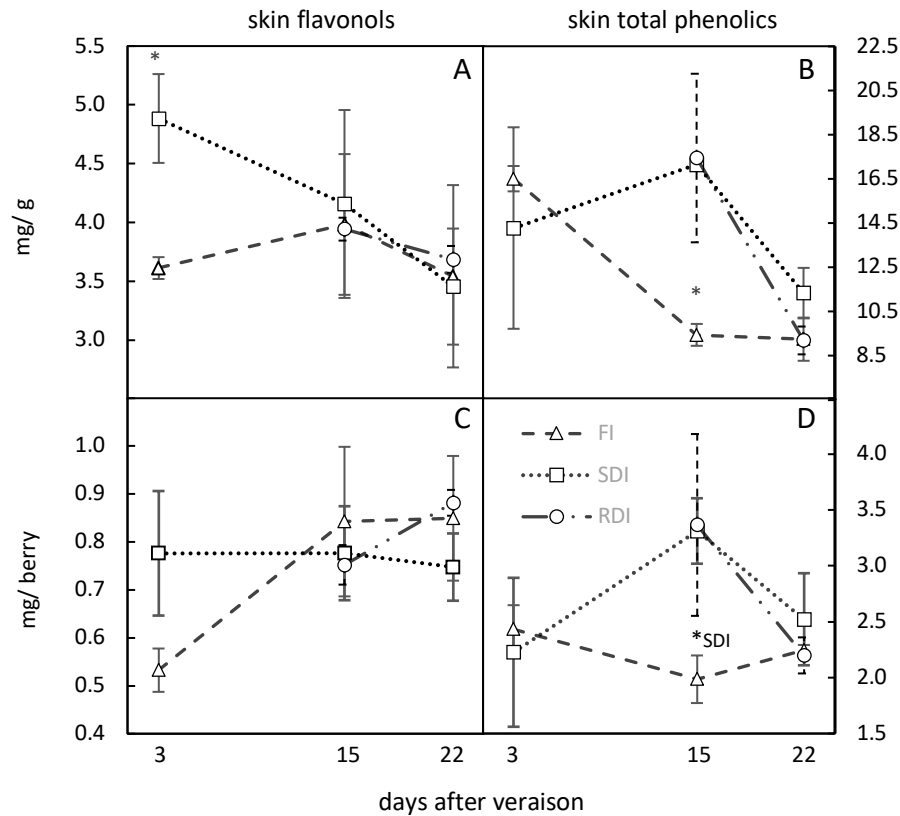
reported mostly no significant correlations with pH or titratable acidity as well, merely more vigorous plants facilitated by higher soil depth and thus soil moisture were prone to higher yield and pH in their study. Also, Friedel et al. (2015) could not report significant differences between post veraison leaf removal in the bunch zone and a control in cv. Riesling and mainly shaded leaves had significantly higher malate content in their trial. Therefore, a solely canopy effect on the acid metabolism should not have had a great effect size according to the literature. If SDI may support higher pH values in GV for some reason, it is more plausible that this was caused by the irrigation regime itself and not its secondary symptoms of ceased vegetative growth in the trial. One possible factor could have been higher heat stress exposure in SDI vines due to inferior transpiration cooling in the last week before harvest compared to the other treatments (cf. Paciello et al., 2017). This would be plausible this far, since temperature is principally known to have an impact on juice pH, whereas lower temperatures {far below 30° C} may come up with lower pH and higher temperatures conversely (Keller, 2010; Mullins et al., 2004; Poni et al., 2018, Casassa et al., 2015; Nicholas et al., 2011).

**Tab. 4 :** Grape composition and vine balance at harvest (24. July 2018, 77 DAA/ 20 DAV) from potted Grüner Veltliner 2018 either fully- (FI), sustained deficit- (SDI) or regulated deficit irrigated (RDI) from veraison until harvest with n=7, 8 and 8 respectively and n= 3, 3, 2 respectively regarding leaf area. Level of significance: \* significant at  $P \leq 0.05$  LSD (different from all comparable groups). Standard errors are shown in the Appendix 8. 8-10.

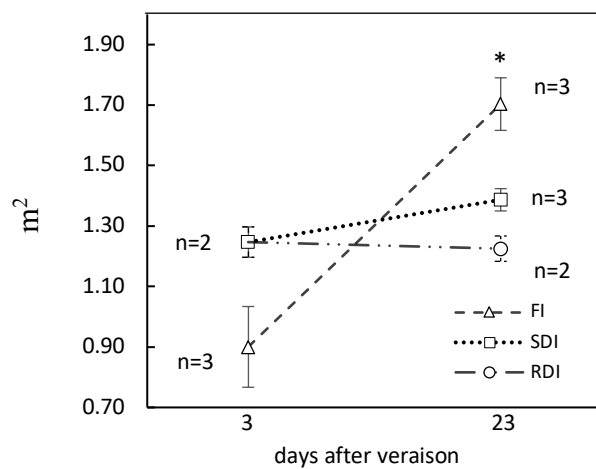
	FI	SDI	RDI	sign.
yield (g/vine)	629.06	661.33	764.4	n.s.
leaf area (m <sup>2</sup> /vine)	1.70*	1.39	1.23	s.
leaf area/yield (m <sup>2</sup> /kg)	2.71	2.10	1.60	n.a.
shoots	4	4	4	
clusters per vine	5.57	4.75	5.63	n.s.
cluster mass (g)	114.06	137.89	138.37	n.s.
berry mass (g)	1.83	1.81	1.86	n.s.
TSS (°Brix) [FT-IR]	20.36*	18.54	18.41	s.
TSS (°Brix) [refractometry]	20.81*	19.03	18.93	s.
Total Sugar (g/L) [FT-IR]	205.18*	187.66	187.00	s.
pH [pH-meter]	3.26	3.34*	3.26	s.
pH [FT-IR]	3.20	3.27	3.27	n.s.
total acidity (g/L) [FT-IR]	8.04	7.39	8.00	n.s.
malic acid (g/L) [FT-IR]	4.91	5.00	5.37	n.s.
skin mass (g)	0.25	0.22	0.24	n.s.
skin mass proportion	0.14	0.12	0.12	n.s.
skin to pulp ratio	0.18	0.15	0.15	n.s.
total phenolics (mg/g skin)	9.25	11.33	9.19	n.s.
total flavonols (mg/g skin)	3.54	3.46	3.69	n.s.
skin phenolics per berry (mg)	2.25	2.52	2.2	n.s.
skin flavonols per berry (mg)	0.85	0.75	0.88	n.s.



**Fig. 7:** Temporal Berry Growth and Change in Berry Components from 1 day after veraison (100% Berry Softening) until harvest of fully-, sustained- and regulated -deficit -irrigated 'Grüner Veltliner' potplants in 2018. Vertical bars are standard error (n=3). a) Berry Mass, b) Pulp Mass, c) Skin Mass, d) Berry Seed Mass, weight proportions of e) skin, f) pulp, g) seed, h) skin to pulp ratio, i) single seed mass and j) seeds per berry. Levels of significance: \* significant at  $P < 0.05$  (comparison denoted).



**Fig. 8:** Progression of measured skin polyphenolics in potted 'Grüner Veltliner' 2018 under three irrigation regimes (fully-, sustained, regulated deficit- irrigated respectively FI, SDI, RDI) from veraison to harvest. Vertical error bars are for standard error (n=3): skin flavonols and skin total phenolics given in skin concentration (A, B) and per berry (C, D). Level of significance: \* significant at  $P \leq 0.05$  (different from all available groups otherwise denoted (Games-Howell Post hoc for skin total phenolics (variances were not homogenous at 70 DAA), Bonferroni for skin flavonols).



**Fig. 9:** Total leaf area 3 days after veraison & 1 day after harvest (23 DAV) in fully-, sustained deficit- and regulated deficit - irrigated 'Grüner Veltliner'. Verticals bars are standard error. Levels of significance: \* significant at  $P < 0.05$  (all comparisons).

## 5 Conclusions

Grüner Veltliner (GV) faced to severe water stress exhibited a pattern of near-anisohydric behavior in the trial, with increased intrinsic water use efficiency at a low but respectable performance of the assimilation rate that was maintained decoupled from grapevine water status.

The impact of strong post veraison water deficit was mainly reflected by lower accumulated sugar in berries ( $-0.9^{\circ}$  Brix) and significantly decreased leaf area, separable in mainly inhibited shoot growth and to some visible extent in leaf shedding. In well irrigated vines where vegetative growth was not inhibited leaf area was higher by nearly 40 %. Typical reduction in berry size as a consequence of prolonged drought during ripening could not be reported, and DI GV revealed a high insensitivity in berry mass. It may also be considered that the warm temperate climate was probably less demanding regarding berry transpiration than those of e.g. a Mediterranean region.

Sustained Deficit Irrigation that simulated long-term drought in ripening berries of GV led to stomatal closure and carbon limitation that resulted in lower Total soluble solids in berries. The 'Regulated Deficit Irrigation' regime that simulated transient drought was not clearly associated with any advantage in grape composition over 'Sustained Deficit Irrigation' in any measured parameter except the restoration of vital plant physiology equal to the well watered control. Maybe this regime was applied too late since there were already hints of a decline in pulp proportion equally in all treatments that signalled that the berries were probably already isolated from the phloem stream. Thus the repaired assimilation rate had probably no beneficial impact on berry sugar accumulation anymore. Berries from SDI GV had a slightly higher juice pH, which was no longer present after a short period of reirrigation in RDI vines. Maybe an opportunity to control the acid content of wines more water efficient, however more trial replications and measurements of several organic acids and potassium cations in the berry juice as well as ambient temperature in the bunch zone may be helpful to elucidate such a potential effect more clearly.

Phenolics parameters analyzed here (i.e. total phenolics, total flavonols in the skin) were not significantly affected by the treatment, and skin flavonol content independent of differences in the leaf area between well irrigated and deficit irrigated vines. Merely the impression of weak tendencies where skin flavonol content rather correlated positively with irrigation supply, and the decline in skin total phenolics rather negatively. However, this remained just an impression and was not significant. Total phenolics decreased with berry size respectively higher skin surface independently of the variation in soil moisture.

A hypothesized late water deficit effect on berry mass was declined since it could not be supported by the gained data. According to the leaf area - yield ratio compared to the literature, the leaf area was probably not the limiting factor in berry sugar accumulation in DI vines but rather caused by the regularly lower assimilation rate and limitation of photosynthesis (Appendix 8.7).

Post veraison water stress related modifications in berry size may be prone to high climatic or annual variation, but this experiment is one example that berry size is an important discriminante in the success of a deficit irrigation regime, in this far, since if berry mass is not affected by water stress, the assimilate limiting role gets amplified at lacking beneficial modification in any solute-solvent, skin-pulp ratio pattern or that of the phenolic profile. Nevertheless, more trial replications are necessary to allow definite implications.

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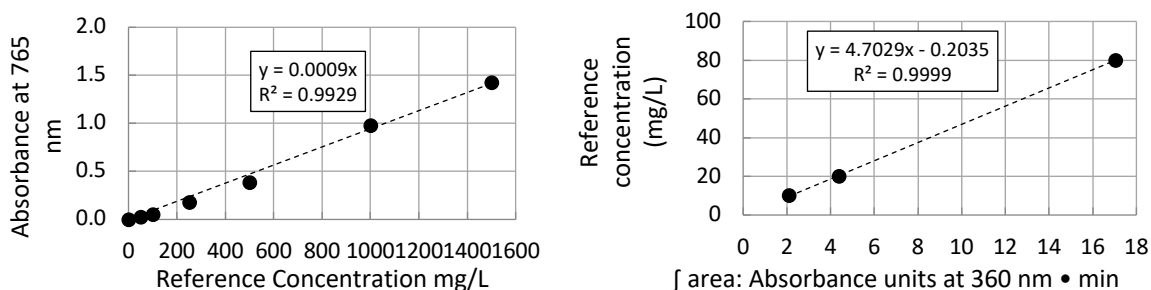
## 7 Appendix

### 7.1 Irrigation use in winegrowing

[m <sup>3</sup> /t]	Country Ø /							
	Province	Italy	France	Spain	USA	California	Australia	China
Water footprint	green	435	575	867	139	139	288	415
	blue	38	4	156	244	336	160	0
	grey	95	15	249	101	120	161	239
	Total	568	594	1272	484	595	609	654
	irrigated	8.03%	0.69%	15.25%	63.71%	70.74%	35.71%	0.00%?
	Rain Fed	91.97%	99.31%	84.75%	36.29%	29.26%	64.29%	100.00%?
[m <sup>3</sup> /t]	Country Ø /	South					Lower	
	Province	Africa	Chile	Argentina	Germany	Austria	Austria	Burgenland
Water footprint	green	253	377.68	198	333	674	679	684
	blue	182	6.2	287	0	9	11	3
	grey	56	163.67	46	21	35	35	35
	Total	491	547.55	531	354	718	725	722
	irrigated	41.84%	1.62%	59.18%	0.00%	1.32%	1.59%	0.44%
	Rain Fed	58.16%	98.38%	40.82%	100.00%	98.68%	98.41%	99.56%

Based on Mekkonen & Hoekstra 2010 (grapes: FAOSTAT 560; bottle wine ≤ 2L : HS 220421). The grey water footprint was neglected in the percentual irrigation calculations since it was the aim to display clean actual amounts free from environmental issues which are i) partly not avoidable and ii) hard to compare among the different regions. Collected water from precipitations (rainwater) however was included. Amounts refer to used m<sup>3</sup> water per produced t crop.

### 7.2 Calibration Curves for Phenolics



Calibration curve for the gallic acid standards: absorbance at 765 nm (y) vs. mg gallic acid per Litre (x) [left] & Calibration curve for the integrated area under the peaks for unknown flavonols into known quercetin - 3 - o - glucopyranoside reference standard concentrations [right]

### 7.3 Calculation of the reference evapotranspiration $ET_0$

$$ET_0 = \frac{0.408 \Delta (R_n - G) + \gamma \frac{900}{T + 273} u_2 VPD}{\Delta + \gamma (1 + 0.34 u_2)}$$

$$\Delta = 4098 \frac{0.6108 e^{\frac{17.27 T}{T+237.3}}}{(T+237.3)^2} \dots \text{slope}$$

$$VPD = e_s - e_a \dots \text{vapour pressure deficit}$$

$$\gamma = 0.665 \cdot 10^{-3} \cdot 101.3 \left[ \frac{293 - 0.0065 \cdot (\text{height over sealevel})}{293} \right]^{5.26}$$

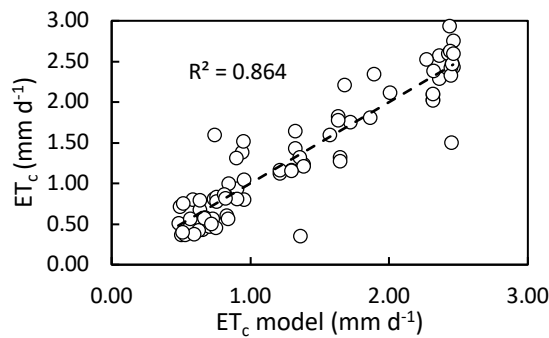
$$e_a = e^0(T_{min}) \frac{RH_{max}}{100} + e^0(T_{max}) \frac{RH_{min}}{100} \dots \text{actual vapour pressure}$$

$$e_s = \frac{e^0(T_{max}) + e^0(T_{min})}{2} \dots \text{saturated} \quad e^0(T) = 0.6108 \cdot e^{\frac{17.27 T}{T+237.3}} \dots \text{mean}$$

T... daily mean temperature °C (as mean of Tmax and Tmin),  $u_2$ ... daily Ø wind speed in  $m s^{-1}$ ,  $R_n$ ... daily net radiation  $MJ m^{-2} d^{-1}$ , G... soil heat flux  $MJ m^{-2} d^{-1}$  was neglected = 0 (Allen et al., 1998)

### 7.4 Estimated crop evapotranspiration model

Daily crop Evapotranspiration was pretty well predictable out of the two plausible effectors administered irrigation volumes and the daily reference evapotranspiration. This model was however only used to approximate crop evapotranspiration and derived sizes for RDI vines which were not equipped with a lysimeter balance.



$$ET_c \text{ model} = 0.358 + \frac{2.103}{[1 + 4.078 \cdot e^{(-7.175 \cdot [0.087 \cdot ET_0 + 0.075 \cdot IT \cdot ET_0] - 1.549)}]^{1/4.078}}$$

### 7.5 Growing Degree Days Formula

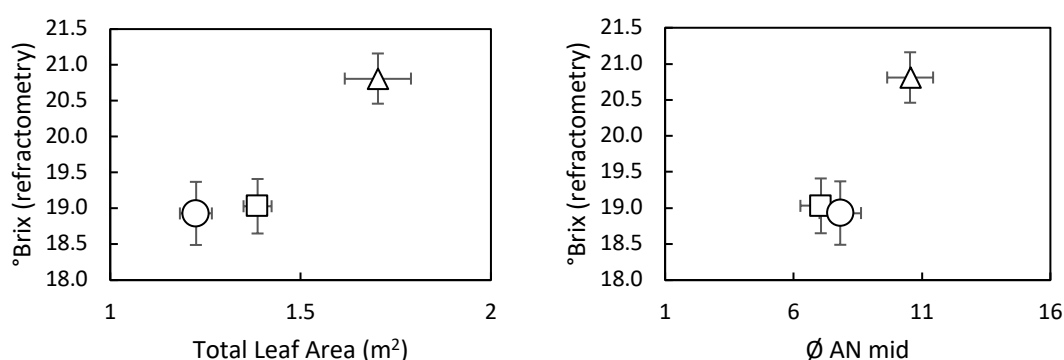
$$GDD = \frac{(T_{max} [^{\circ}C] + T_{min} [^{\circ}C])}{2} - 10^{\circ}C$$

## 7.6 Relevant Changes In Irrigation Times & Volumes

Relevant changes in the administered irrigation amounts in liter for the given days after anthesis (DAA). FI (full irrigation/ control), SDI (sustained deficit irrigation) & RDI (regulated deficit irrigation)

DAA	50	51	54	55	56	66	71	74	77
FI	3.00	0.00	0.00	4.00	3.00	3.00	3.00	3.00	3.00
SDI	3.00	0.73	0.73	0.73	0.53	0.40	0.40	0.53	0.53
RDI	3.00	0.73	0.73	0.73	0.53	0.40	3.40	3.53	3.53

## 7.7 Discrimination of TSS (°Brix) in juice from harvested berries



## 7.8 Harvest Samples

Statistics for Juice parameters incl. instrument of the processed berry juice from harvested berry samples of either fully- (FI; n=7), sustained deficit- (SDI; n=8) and regulated deficit- irrigated (RDI; n=8) and in total including: Refractometry index in °Brix (Brix-meter & Alpha I), Total Sugar [g/L] (Alpha I), Density [kg/L] (Alpha I), pH and its delogarithmized c {H<sup>+</sup>} [mol] (pH-meter & Alpha I) {ANOVA respectively Bonferroni}

### TSS [°Brix] (Brixmeter)

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	20.81	19.03	18.93	19.54	p	*0.015	*0.010	1.000
SE	0.35	0.38	0.44	0.28	Δ	*1.789	*1.889	0.66
σ	0.92	1.06	1.25	1.36				

### TSS [°Brix] (Alpha I)

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	20.36	18.54	18.41	19.05	p	*0.017	*0.011	1.000
SE	0.37	0.38	0.47	0.29	Δ	*1.82	*1.94	0.13
σ	0.97	1.07	1.32	1.40				

### Total Sugar [g/L] (Alpha I)

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	205.18	187.66	187.00	192.76	p	*0.019	*0.015	1.000
SE	3.58	3.68	4.58	2.82	Δ	*17.52	*18.18	0.66
σ	9.48	10.40	12.95	13.52				



**Density [g/L] (Alpha I)**

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	1.0829	1.0755	1.0755	1.0777	p	**0.008	**0.008	1.000
SE	0.0014	0.0014	0.0017	0.0011	Δ	**0.0074	**0.0074	0.0000
σ	0.0038	0.0039	0.0048	0.0053				

**pH (pH-meter) [Bonferroni / LSD]**

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	3.26	3.34	3.26	3.28	p	0.109/*0.036	1.000	0.067/*0.022
SE	0.157	0.027	0.024	0.015	Δ	0.075	0.005	0.080
σ	0.042	0.076	0.068	0.072				

**pH (Alpha I)**

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	3.20	3.27	3.27	3.25	p	0.866	0.949	1
SE	0.05	0.04	0.03	0.02	Δ	0.065	0.062	0.00
σ	0.14	0.12	0.09	0.12				

**c {H<sup>+</sup>} (pH-meter) [Bonferroni / LSD]**

	FI	SDI	RDI	Total	Pair.	FI-SDI	FI-RDI	SDI-RDI
μ	5.52•10 <sup>-4</sup>	4.68•10 <sup>-4</sup>	5.62•10 <sup>-4</sup>	5.26•10 <sup>-4</sup>	p	0.138/*0.046	1.000	0.068/*0.023
SE	1.98•10 <sup>-5</sup>	2.77•10 <sup>-5</sup>	3.13•10 <sup>-5</sup>	0.18•10 <sup>-4</sup>				
σ	5.23•10 <sup>-5</sup>	7.84•10 <sup>-5</sup>	8.83•10 <sup>-5</sup>	0.84•10 <sup>-4</sup>				

**c {H<sup>+</sup>} (Alpha I)**

	FI	SDI	RDI	Total	Pair.	FI-SDI	FI-RDI	SDI-RDI
μ	6.54•10 <sup>-4</sup>	5.57 •10 <sup>-4</sup>	5.54•10 <sup>-4</sup>	5.86 •10 <sup>-4</sup>	p	0.816	0.778	1
SE	7.88•10 <sup>-5</sup>	5.69•10 <sup>-5</sup>	4.30•10 <sup>-5</sup>	0.34 •10 <sup>-4</sup>				
σ	2.09•10 <sup>-5</sup>	1.61•10 <sup>-4</sup>	1.22•10 <sup>-4</sup>	1.6 •10 <sup>-4</sup>				

**Total Acidity [g/ L] (Alpha I)**

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	8.04	7.39	8.00	7.80	p	0.480	1.000	0.506
SE	0.23	0.26	0.40	0.18	Δ	0.658	0.036	0.613
σ	0.61	0.73	1.12	0.87				

**Malic Acid [g/L] (Alpha I)**

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	4.907	5.000	5.375	5.102	p	1.000	0.657	0.940
SE	0.163	0.285	0.283	0.148	Δ	0.093	0.468	0.238
σ	0.431	0.807	0.800	0.710				

**100 Berry Mass [g]**

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
μ	182.64	181.06	185.97	183.25	p	1.000	1.000	1.000
SE	2.90	6.11	6.20	3.05	Δ	1.58	3.33	4.91
σ	7.66	17.27	17.54	14.61				

### Yield [g]

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
$\mu$	629.06	661.33	764.40	687.36	p	1.000	0.507	0.822
SE	65.68	69.14	63.07	38.46	$\Delta$	-32.28	-135.34	-103.06
$\sigma$	173.76	195.55	178.38	184.42				

### Clusters

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
$\mu$	5.57	4.75	5.63	5.30	p	0.466	1.000	0.357
SE	0.37	0.31	0.46	0.23	$\Delta$	0.82	-0.05	-0.88
$\sigma$	0.98	0.89	1.30	1.11				

### Cluster Weight [g]

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
$\mu$	114.06	137.89	138.37	130.81	p	0.519	0.494	1.000
SE	11.10	11.79	12.15	6.90	$\Delta$	-23.83	-24.83	-0.48
$\sigma$	29.36	33.36	34.37	33.07				

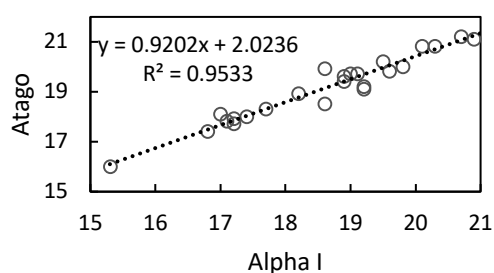
### Berries per Cluster

	FI	SDI	RDI	Total		FI-SDI	FI-RDI	SDI-RDI
$\mu$	61.88	75.17	73.96	70.71	p	0.451	0.567	1.000
SE	6.09	5.75	6.66	3.63	$\Delta$	-13.29	-3.33	-4.91
$\sigma$	16.11	16.26	18.83	17.43				

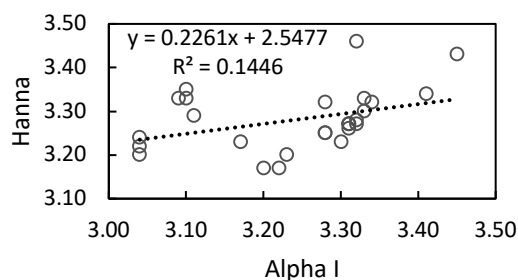
## Atago PR301- $\alpha$ Digital Brix Refractometer / Hanna HI 2211 basic pH-meter vs. Alpha I Wine Analyzer [FT-IR]

The linearity of both Brix quantities was pretty well, between the two pH metering methods rather poor.

Q-Q °Brix



Q-Q pH



## Row Effect

	Row	n	°BRIX	Density	Total Sugar	Malic Acid	Total Acidity	pH	c (H <sup>+</sup> )	Clusters	Mean Cluster Weight	Yield	100-Berry-weight	Mean Berries per Cluster
Spearman's rho	p	23	0.575	0.719	0.780	0.225	0.432	0.337	0.116	0.087	0.739	0.616	0.992	n.s.
ANOVA	p	23	0.693	0.598	0.552	0.222	0.506	0.293	0.294	0.121	0.113	0.297	0.741	0.151

No significant row effect detected

## 7.9 Temporal Samples

58 DAA n=6, n(FI/SDI) = 3; 70/77 DAA n=9, n (FI/SDI/RDI)= 3; various mass proportions are referred to berry fresh weight (berry mass). P values: Group and Date comparison (Anova, Bonferroni/ Games Howell)

	Treatment				DAA			
	DAA	FI-SDI	FI-RDI	SDI-RDI		58-70	70-77	58-77
berry mass (g)	58	0.470			FI	0.060	1.000	*0.039
	70	1.000	1.000	1.000	SDI	***0.000	0.734	***0.000
	77	1.000	0.392	0.636	RDI	-	*0.024	-
					Total	***0.000	0.103	***0.000
skin mass (g)	58	0.576			FI	0.086	0.387	*0.011
	70	1.000	1.000	1.000	SDI	0.417	0.784	^0.077
	77	0.701	1.000	1.000	RDI	-	**0.003	-
					Total	*0.003	*0.005	*0.000
pulp mass (g)	58	0.373			FI	0.098	1.000	^0.081
	70	1.000	1.000	1.000	SDI	**0.001	1.000	***0.000
	77	1.000	0.472	0.884	RDI	-	0.055	-
					Total	***0.000	0.442	***0.000
berry seed mass (g)	58	0.590			FI	0.902	1.000	0.294
	70	1.000	1.000	1.000	SDI	0.054	1.000	0.178
	77	1.000	1.000	1.000	RDI		0.541	
					Total	**0.006	1.000	*0.01
1 seed mass (g)	58	0.986			FI	1.000	0.151	0.264
	70	0.895	0.173	0.823	SDI	1.000	0.772	0.195
	77	1.000	0.025*	0.051	RDI	-	0.166	-
					Total	0.612	0.057	*0.005
seeds per berry	58	0.719			FI	0.937	1.000	1.000
	70	1.000	1.000	1.000	SDI	0.602	0.841	1.000
	77	0.553	0.077	0.590	RDI	-	0.051	-
					Total	0.236	0.178	1.000
skin mass proportion	58	0.303			FI	1.000	0.621	0.256
	70	1.000	1.000	1.000	SDI	1.000	1.000	1.000
	77	0.730	0.871	1.000	RDI		0.237	
					Total	1.000	0.272	0.833

	Treatment				DAA		
	DAA	FI-SDI	FI-RDI	SDI-RDI	58-70	70-77	58-77
pulp mass proportion	58	0.233			FI	0.717	1.000
	70	0.827	0.749	1.000	SDI	**0.008	*0.019
	77	1.000	1.000	1.000	RDI		0.611
					Total	***0.000	1.000
berry seed mass proportion	58	0.457			FI	0.091	1.000
	70	1.000	1.000	1.000	SDI	**0.001	**0.001
	77	1.000	1.000	0.945	RDI		0.505
					Total	***0.000	1.000
Skin: Pulp-ratio	58	0.267			FI	1.000	0.697
	70	1.000	1.000	1.000	SDI	0.574	1.000
	77	0.772	0.840	1.000	RDI		0.270
					Total	0.716	0.343
Flavonols skin concentration (mg/g)	58	*0.031			FI	1.000	1.000
	70	1.000	1.000	1.000	SDI	1.000	0.403
	77	1.000	1.000	1.000	RDI		0.162
					Total	1.000	0.749
skin flavonols per berry (mg)	58	0.151			FI	0.354	1.000
	70	1.000	1.000	1.000	SDI	1.000	1.000
	77	1.000	0.968	0.949	RDI		0.058
					Total	0.420	1.000
total phenolics skin concentration (mg/g)	58	0.652			FI	**0.001	1.000
	70 <sup>^</sup>	**0.002	0.291	0.997	SDI <sup>^</sup>	0.821	0.821
	77	0.505	1.000	0.476	RDI		0.100
					Total <sup>^</sup>	0.963	0.062
skin total phenolics per berry (mg)	58	0.783			FI	0.373	1.000
	70	0.353	0.319	1.000	SDI	0.489	0.874
	77	1.000	1.000	1.000	RDI		0.232
					Total	0.568	0.418

<sup>^</sup> Games Howell variances not homogenous, otherwise Bonferroni, significance however not detected via Bonferroni regarding skin total phenolic concentration at 70 DAA

No significant correlation with the row in any parameter from above detected (Spearman-rho) however on significant difference between the 2<sup>nd</sup> and 3<sup>rd</sup> row in skin flavonols per berry [mg] (p=\*0,03 Games-Howell, p=\*0.033 Bonferroni, p=\*0.011 LSD) as well as skin concentration [mg/g skin] from flavonols (p=\*0.035 LSD however p=0.104 Bonferroni). This was most likely because the 3<sup>rd</sup> row was more south exposed and a marginal row.

## Descriptive Statistic

		Mean				SE				$\sigma$				
		DAA	FI	SDI	RDI	Total	FI	SDI	RDI	Total	FI	SDI	RDI	Total
berry mass (g)	58	1.328	1.251			1.290	0.094	0.243		0.468	0.163	0.421		0.115
	70	1.743	1.746	1.709		1.733	0.090	0.051	0.049	0.033	0.155	0.088	0.084	0.100
	77	1.788	1.822	1.955		1.855	0.096	0.044	0.050	0.042	0.166	0.076	0.086	0.127
skin mass (g)	58	0.148	0.157			0.152	0.009	0.014		0.008	0.016	0.024		0.190
	70	0.209	0.194	0.191		0.198	0.013	0.018	0.006	0.007	0.023	0.032	0.010	0.022
	77	0.247	0.220	0.239		0.236	0.021	0.013	0.004	0.008	0.037	0.023	0.008	0.025
pulp mass (g)	58	1.022	0.928			0.975	0.086	0.036		0.469	0.150	0.063		0.115
	70	1.391	1.419	1.390		1.400	0.083	0.056	0.048	0.032	0.144	0.098	0.083	0.097
	77	1.411	1.459	1.578		1.482	0.111	0.032	0.051	0.044	0.193	0.056	0.088	0.132
berry seed mass (g)	58	0.159	0.166			0.162	0.008	0.008		0.005	0.013	0.014		0.013
	70	0.142	0.134	0.129		0.135	0.011	0.009	0.011	0.006	0.019	0.016	0.019	0.017
	77	0.130	0.143	0.138		0.137	0.013	0.001	0.008	0.005	0.022	0.002	0.015	0.014
1 seed mass (g)	58	0.061	0.061			0.061	0.002	0.003		0.002	0.003	0.005		0.004
	70	0.062	0.058	0.053		0.057	0.003	0.002	0.004	0.002	0.005	0.003	0.006	0.006
	77	0.057	0.053	0.046		0.051	0.002	0.002	0.000	0.002	0.003	0.003	0.001	0.005
seeds per berry	58	2.623	2.753			2.686	0.182	0.283		0.153	0.315	0.490		0.374
	70	2.303	2.333	2.447		2.361	0.264	0.208	0.027	0.100	0.458	0.360	0.046	0.300
	77	2.370	2.680	2.980		2.676	0.153	0.070	0.191	0.115	0.265	0.121	0.332	0.346
skin mass proportion	58	0.111	0.126			0.118	0.002	0.013		0.007	0.003	0.022		0.016
	70	0.120	0.111	0.112		0.114	0.003	0.013	0.006	0.004	0.005	0.022	0.011	0.013
	77	0.140	0.121	0.123		0.128	0.017	0.004	0.005	0.006	0.029	0.008	0.009	0.018
pulp mass proportion	58	0.768	0.742			75.476	0.012	0.015		1.028	0.021	0.025		2.517
	70	0.798	0.812	0.813		0.808	0.007	0.010	0.009	0.005	0.013	0.017	0.015	0.015
	77	0.787	0.801	0.807		0.798	0.024	0.003	0.007	0.008	0.042	0.006	0.012	0.024

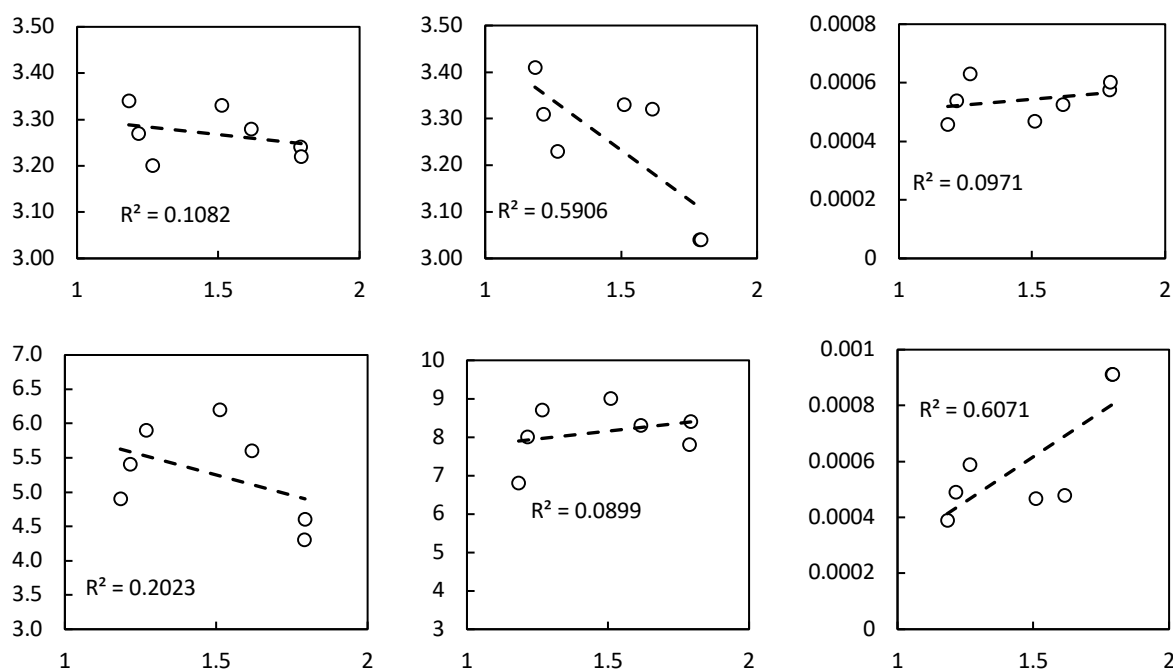
		Mean				SE				σ				
	DAA	FI	SDI	RDI	Total	FI	SDI	RDI	Total	FI	SDI	RDI	Total	
berry seed mass proportion	58	0.121	0.133		12.693	0.011	0.008		0.658	0.020	0.013		1.644	
	70	0.082	0.076	0.075	0.078	0.010	0.004	0.005	0.004	0.017	0.008	0.009	0.011	
	77	0.073	0.078	0.070	0.074	0.008	0.002	0.004	0.003	0.013	0.004	0.007	0.008	
Skin: Pulp-ratio	58	0.144	0.170		0.157	0.003	0.020		0.011	0.006	0.034	0.000	0.026	
	70	0.150	0.138	0.138	0.142	0.003	0.018	0.009	0.006	0.005	0.031	0.015	0.018	
	77	0.179	0.151	0.152	0.161	0.026	0.006	0.007	0.009	0.046	0.010	0.013	0.028	
Flavonols skin concentration (mg/g)	58	3.613	4.885		4.249	0.093	0.377		0.333	0.161	0.654		0.816	
	70	3.984	4.158	3.944	4.029	0.598	0.799	0.097	0.291	1.036	1.383	0.168	0.874	
	77	3.543	3.456	3.687	3.562	0.775	0.493	0.114	0.269	1.342	0.855	0.198	0.808	
skin flavonols per berry (mg)	58	0.533	0.776		0.654	0.045	0.130		0.082	0.078	0.225		0.201	
	70	0.843	0.777	0.752	0.790	0.156	0.098	0.041	0.056	0.270	0.170	0.071	0.168	
	77	0.849	0.748	0.882	0.826	0.130	0.070	0.027	0.048	0.225	0.122	0.047	0.144	
total phenolics skin concentration (mg/g)	58	16.509	14.276		15.392	0.573	4.558		2.114	0.993	7.895		5.179	
	70	9.443	17.150	17.443	14.679	0.495	0.264	3.813	1.718	0.858	0.458	6.604	5.155	
	77	9.248	11.331	9.190	9.923	0.977	1.143	0.630	0.588	1.692	1.979	1.091	1.763	
skin total phenolics per berry (mg)	58	2.433	2.227		2.330	0.216	0.666		0.317	0.373	1.154		0.776	
	70	1.986	3.313	3.367	2.888	0.213	0.293	0.814	0.342	0.369	0.507	1.409	1.026	
	77	2.247	2.523	2.199	0.232	0.046	0.412	0.161	0.138	0.080	0.714	0.280	0.414	

## 7.10 Leaf area

One significant correlation (Spearman-rho) with the row and difference (ANOVA) regarding number of shoots and counted leaves per vine probably between 2nd and 3rd row detected. However, variances were not homogenous, and this was not significant via Games Howell. No significant correlations of the row (Spearman-rho) nor differences of the row in the leaf length single or total leaf area at 58 DAA, 78 DAA was detected.

ANOVA <small>df1=1/</small> Bonferroni <small>df1=2</small>	58 DAA	78 DAA	78 DAA	78 DAA	FI	SDI
Compared Groups	FI-SDI	FI-SDI	FI-RDI	SDI-RDI	58-78 DAA	58-78 DAA
	P					
Shoots	0.495	1	1	1	0.495	
MeanLeafLength (cm)	0.099	0.417	1	1	0.071	0.417
SumLeaves	0.312	0.186	0.220	1	0.027	0.948
MeanSingleLeafArea (cm <sup>2</sup> )	0.100	1	1	1	0.066	0.373
EstimatedTotalLeafArea (m <sup>2</sup> )	0.144	0.040	0.013	0.290	0.022	0.104
58 DAA	FI (n=3)		SDI (n=2)			
	μ	SEM	μ	SEM		
Shoots	3.67	±0.333	4.00	±0		
MeanLeafLength (cm)	8.90	±0.345	9.96	±0.063		
SumLeaves	79.67	±7.535	92.00	±4.00		
MeanSingleLeafArea (cm <sup>2</sup> )	112.1	±7.72	135.5	±0.422		
EstimatedTotalLeafArea (m <sup>2</sup> )	0.900	±0.013	1.247	±0.05		
78 DAA	FI (n=2)		SDI (n=3)		RDI (n=2)	
	μ	SEM	μ	SEM	μ	SEM
Shoots	4.00	±0.000	4.00	±0.000	4.00	±1.000
MeanLeafLength (cm)	10.23	±0.258	10.62	±0.547	10.02	±0.484
SumLeaves	119.00	±1.000	91.33	±6.960	90.50	±11.500
MeanSingleLeafArea (cm <sup>2</sup> )	143.08	±6.105	153.75	±13.497	137.00	±12.771
EstimatedTotalLeafArea (m <sup>2</sup> )	1.703	±0.08	1.387	±0.04	1.225	±0.04

### Leaf Area Impact



Scatterplots illustrate the impact of leaf area per individual in m<sup>2</sup> (n=7) from left to right and top to bottom in order of appearance: pH (pH-meter, Alpha I), H<sup>+</sup> concentration [mol] (pH-meter, Alpha I), Malic Acid [g/L] (Alpha I) and Total Acidity [g/L] (Alpha I) from processed juice of berries from those individuals.

## 7.11 Midday IRGA and Pressure Chamber Measurements

sample sizes (n) for all gas exchange measurements  $A_N$ ,  $g_s$  &  $WUE_i$

IRGA	DAA									Total
n	50	55	56	58	64	66	70	73	77	50-77
FI	3	6	5	3	6	4	3	3	4	37
SDI	3	6	5	3	7	6	3	3	4	40
RDI	0	0	0	0	0	0	3	3	4	10
Total	6	12	10	6	13	10	9	9	12	87

sample sizes for all field measured midday  $\Psi_s$  during the trial time

$\Psi_s$	DAA							
n	50	55	58	66	70	73	77	Total
FI	3	4	3	5	3	3	4	25
SDI	3	4	3	5	3	3	4	25
RDI	0	0	0	0	3	3	4	10
Total	6	8	6	10	9	9	12	60

No significant correlation of the row was detected in any parameter (Spearman).

### Descriptive Statistic - Days

$A_N$	DAA									Total
$\mu$	50	55	56	58	64	66	70	73	77	50-77
FI	14.30	3.66	7.24	12.38	15.50	13.05	13.60	11.62	7.75	10.54
SDI	14.27	10.44	5.07	4.56	9.74	3.21	6.16	4.24	4.84	7.06
RDI							4.57	9.14	9.93	7.82
Total	14.28	7.05	6.16	8.47	12.40	7.15	8.11	8.33	7.50	8.66

$A_N$	DAA									Total
$cv(\%)^i$	50	55	56	58	64	66	70	73	77	50-77
FI	5.59	25.91	49.28	12.05	8.47	12.05	12.10	4.66	23.99	42.41
SDI	3.53	29.22	44.21	49.67	35.55	23.68	7.03	36.00	32.50	56.14
RDI							43.17	9.74	32.45	51.66
Total	4.19	58.76	49.27	54.50	31.90	72.65	53.94	40.58	40.40	50.80

$g_s$	DAA									Total
$\mu$	50	55	56	58	64	66	70	73	77	50-77
FI	0.215	0.030	0.076	0.270	0.282	0.228	0.230	0.177	0.113	0.170
SDI	0.215	0.113	0.048	0.043	0.116	0.027	0.047	0.040	0.048	0.078
RDI							0.040	0.103	0.143	0.092
Total	0.215	0.072	0.062	0.157	0.192	0.107	0.106	0.107	0.101	0.120



gs	DAA									Total
cv(%)	50	55	56	58	64	66	70	73	77	50-77
FI	2.09	36.51	41.19	29.40	34.08	29.89	38.64	8.65	23.38	62.48
SDI	2.81	42.74	55.90	58.08	49.11	30.61	12.37	25.00	62.87	78.86
RDI							0.00	11.17	46.68	70.38
Total	2.09	76.62	50.32	86.08	58.99	103.80	98.00	56.44	57.44	78.19

WUE <sub>i</sub>	DAA									Total
μ	50	55	56	58	64	66	70	73	77	50-77
FI	66.40	126.89	90.12	48.99	62.33	59.63	64.83	66.12	69.15	76.76
SDI	66.48	98.11	112.23	111.00	87.89	123.58	133.07	103.78	115.38	105.28
RDI							114.17	89.06	74.63	98.68
Total	66.44	112.50	101.17	80.00	76.09	98.00	104.02	86.32	86.38	91.49

WUE <sub>i</sub>	DAA									Total
cv(%)	50	55	56	58	64	66	70	73	77	50-77
FI	4.75	24.02	21.42	33.50	44.63	17.77	34.16	10.38	13.78	40.20
SDI	3.74	19.01	31.28	18.00	17.29	19.55	11.27	13.76	24.98	25.07
RDI							43.17	12.25	19.06	28.02
Total	3.82	25.24	28.80	47.12	32.56	38.89	39.83	22.06	32.13	34.46

Ψ <sub>s</sub>	DAA								
(-) μ	50	55	58	66	70	73	77	50-77	
FI	0.333	0.578	0.503	0.440	0.440	0.604	0.705	0.519	
SDI	0.330	0.600	0.760	1.070	1.153	1.043	1.143	0.887	
RDI					1.193	0.513	0.683	0.755	
Total	0.332	0.589	0.632	0.755	0.929	0.720	0.843	0.717	

Ψ <sub>s</sub>	DAA								Total
cv (%)	50	55	58	66	70	73	77	50-77	
FI	4.58	29.33	3.03	7.49	9.09	17.48	23.75	28.60	
SDI	5.25	13.68	2.63	17.94	17.80	11.98	16.70	35.77	
RDI	-	-	-	-	15.84	19.51	8.15	39.90 <sup>a</sup>	
Total	4.44	21.03	22.40	47.22	42.33	36.61	30.77	42.84	

<sup>i</sup> ...cV refers to standard deviation relative to the mean value.

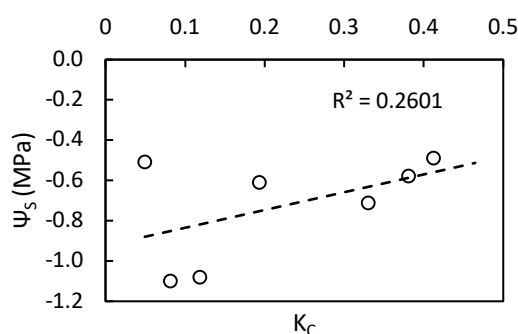
## Descriptive Statistics - Mean Performance for Sampling Dates and Reirrigation

WUE <sub>i</sub>						A <sub>N</sub>				
	$\mu$					$\mu$				
	50-58	58-70	70-77	58-77	50-70	50-58	58-70	70-77	58-77	50-70
FI	91.652	59.623	66.944	62.127	78.836	8.131	13.946	10.666	12.565	10.808
SDI	98.957	109.942	117.203	110.067	104.187	8.499	6.295	5.053	5.833	7.588
RDI			90.819	99.919				8.083	6.998	
total	95.305	89.089	91.655	88.116	93.117	8.315	9.380	7.934	8.839	8.914
	$\sigma$					$\sigma$				
	50-58	58-70	70-77	58-77	50-70	50-58	58-70	70-77	58-77	50-70
FI	36.680	20.315	12.393	17.713	33.838	4.699	1.850	2.961	2.925	4.814
SDI	27.346	25.475	22.753	24.342	27.274	4.368	3.611	1.426	3.240	4.138
RDI			30.542	27.617				3.240	3.730	
total	32.072	35.554	30.557	31.690	33.594	4.471	4.871	3.472	4.365	4.723
g <sub>s</sub>						$\Psi_s$				
	$\mu$					$\mu$				
	50-58	58-70	70-77	58-77	50-70	50-58	58-70	70-77	58-77	50-70
FI	0.119	0.256	0.167	0.221	0.17687	-0.482	-0.457	-0.595	-0.537	-0.463
SDI	0.100	0.065	0.045	0.060	0.08497	-0.567	-1.008	-1.116	-1.044	-0.804
RDI			0.100	0.081				-0.785	-0.860	
total	0.109	0.144	0.104	0.129	0.1247	-0.524	-0.788	-0.832	-0.789	-0.677
	$\sigma$					$\sigma$				
	50-58	58-70	70-77	58-77	50-70	50-58	58-70	70-77	58-77	50-70
FI	0.103	0.081	0.069	0.089	0.11571	0.146	0.041	0.160	0.137	0.110
SDI	0.071	0.053	0.018	0.047	0.06482	0.184	0.224	0.166	0.202	0.327
RDI			0.059	0.059				0.310	0.279	
total	0.088	0.116	0.072	0.101	0.10213	0.168	0.343	0.307	0.305	0.325

## 7.12 Water (Cor) Relations

Various parameter correlations are shown in tables below. Abbreviations: IT (technical administered irrigation), IP (pot arrived irrigation) and temperature (T), Radiation (Rad), RH (relative humidity), wind...windspeed (km/h) per average of day or at midday (mid). Other abbreviations were already mentioned or see Abbreviations. The German semicolon was used, thus a ',' = ' '. The tables refer to Spearman correlation and sometimes the Correlation coefficient is either abbreviated as Corr. Coeff. or C.C., significance as sig. is two-tailed expressed.

Relationship crop coefficient  $K_c$  ( $ET_c/ET_0$ ) with the midday stem water potential  $\Psi_s$  (lysimeter data)



### 7.12.1 Lysimeter plants x Climate Data

<b>Total</b>		<b>IT</b>	<b>IP</b>	<b>ET<sub>c</sub></b>	<b>ET<sub>0</sub></b>	<b>K<sub>c</sub></b>	<b>VPD</b>	<b>T<sub>day</sub></b>
<b>IP</b>	<b>Corr.Coeff.</b>	,872	1,000	,671	,084	,640	,150	,143
	<b>Sig.</b>	,000	.	,000	,471	,000	,197	,218
	<b>N</b>	76	76	76	76	76	76	76
<b>ET<sub>c</sub></b>	<b>Corr.Coeff.</b>	,663	,671	1,000	,526	,759	,461	,355
	<b>Sig.</b>	,000	,000	.	,000	,000	,000	,001
	<b>N</b>	78	76	78	78	78	78	78
<b>K<sub>c</sub></b>	<b>Corr.Coeff.</b>	,605	,640	,759	-,026	1,000	-,036	,070
	<b>Sig.</b>	,000	,000	,000	,823	.	,756	,541
	<b>N</b>	78	76	78	78	78	78	78
<b>ET<sub>c</sub>:IP</b>	<b>Corr.Coeff.</b>	-,266	-,329	,354	,720	,016	,622	,489
	<b>Sig.</b>	,024	,005	,002	,000	,893	,000	,000
	<b>N</b>	72	72	72	72	72	72	72
<b>FI (lys)</b>		<b>IT</b>	<b>IP</b>	<b>ET<sub>c</sub></b>	<b>ET<sub>0</sub></b>	<b>K<sub>c</sub></b>	<b>VPD</b>	<b>T<sub>day</sub></b>
<b>IP</b>	<b>Corr.Coeff.</b>	,547	1,000	,345	,145	,332	,174	,266
	<b>Sig.</b>	,000	.	,034	,386	,041	,296	,107
	<b>N</b>	38	38	38	38	38	38	38
<b>ET<sub>c</sub></b>	<b>Corr.Coeff.</b>	,350	,345	1,000	,833	,562	,735	,697
	<b>Sig.</b>	,029	,034	.	,000	,000	,000	,000
	<b>N</b>	39	38	39	39	39	39	39
<b>K<sub>c</sub></b>	<b>Corr.Coeff.</b>	,206	,332	,562	,118	1,000	,080	,311
	<b>Sig.</b>	,209	,041	,000	,475	.	,628	,054
	<b>N</b>	39	38	39	39	39	39	39
<b>ET<sub>c</sub>:IP</b>	<b>Corr.Coeff.</b>	-,167	-,429	,730	,751	,240	,730	,615
	<b>Sig.</b>	,344	,011	,000	,000	,172	,000	,000
	<b>N</b>	34	34	34	34	34	34	34
<b>SDI (lys)</b>		<b>IT</b>	<b>IP</b>	<b>ET<sub>c</sub></b>	<b>ET<sub>0</sub></b>	<b>K<sub>c</sub></b>	<b>VPD</b>	<b>T<sub>day</sub></b>
<b>IP</b>	<b>Corr.Coeff.</b>	,933	1,000	,720	-,104	,844	-,008	-,111
	<b>Sig.</b>	,000	.	,000	,536	,000	,962	,506
	<b>N</b>	38	38	38	38	38	38	38
<b>ET<sub>c</sub></b>	<b>Corr.Coeff.</b>	,737	,720	1,000	,405	,718	,362	,119
	<b>Sig.</b>	,000	,000	.	,011	,000	,024	,469
	<b>N</b>	39	38	39	39	39	39	39
<b>K<sub>c</sub></b>	<b>Corr.Coeff.</b>	,831	,844	,718	-,238	1,000	-,225	-,245
	<b>Sig.</b>	,000	,000	,000	,144	.	,168	,133
	<b>N</b>	39	38	39	39	39	39	39
<b>%ET<sub>c</sub>(FI)</b>	<b>Corr.Coeff.</b>	,833	,830	,635	-,276	,886	-,221	-,311
	<b>Sig.</b>	,000	,000	,000	,089	,000	,177	,054
	<b>N</b>	39	38	39	39	39	39	39
<b>ET<sub>c</sub>:IP</b>	<b>Corr.Coeff.</b>	-,139	-,119	,489	,746	,046	,579	,408
	<b>Sig.</b>	,406	,478	,002	,000	,785	,000	,011
	<b>N</b>	38	38	38	38	38	38	38

<b>Total</b>			<b>RH_day</b>	<b>Rad_day</b>	<b>wind_day</b>	<b>Σ_rain_day</b>	<b>%ETc(FI)</b>	<b>ETc:IP</b>
<b>IP</b>	<b>Corr.Coeff.</b>		-,229	,059	,004	-,072	,530	-,329
	<b>Sig.</b>		,047	,613	,970	,535	,000	,005
	<b>N</b>		76	76	76	76	76	72
<b>ET<sub>c</sub></b>	<b>Corr.Coeff.</b>		-,462	,501	-,072	-,417	,524	,354
	<b>Sig.</b>		,000	,000	,529	,000	,000	,002
	<b>N</b>		78	78	78	78	78	72
<b>K<sub>c</sub></b>	<b>Corr.Coeff.</b>		,001	-,035	,030	-,121	,700	,016
	<b>Sig.</b>		,993	,761	,792	,289	,000	,893
	<b>N</b>		78	78	78	78	78	72
<b>%ET<sub>c</sub> (FI)</b>	<b>Corr.Coeff.</b>		,006	-,160	,160	,014	1,000	-,152
	<b>Sig.</b>		,955	,162	,162	,906	.	,203
	<b>N</b>		78	78	78	78	78	72
<b>ET<sub>c</sub>:IP</b>	<b>Corr.Coeff.</b>		-,434	,694	-,137	-,504	-,152	1,000
	<b>Sig.</b>		,000	,000	,250	,000	,203	.
	<b>N</b>		72	72	72	72	72	72
<b>FI (lys)</b>			<b>RH_day</b>	<b>Rad_day</b>	<b>wind_day</b>	<b>Σ_rain_day</b>	<b>%ETc(FI)</b>	<b>ETc:IP</b>
<b>IP</b>	<b>Corr.Coeff.</b>		-,155	,172	-,305	-,098	.	-,429
	<b>Sig.</b>		,354	,302	,063	,558	.	,011
	<b>N</b>		38	38	38	38	38	34
<b>ET<sub>c</sub></b>	<b>Corr.Coeff.</b>		-,479	,805	-,291	-,490	.	,730
	<b>Sig.</b>		,002	,000	,072	,002	.	,000
	<b>N</b>		39	39	39	39	39	34
<b>K<sub>c</sub></b>	<b>Corr.Coeff.</b>		,127	,122	-,301	-,094	.	,240
	<b>Sig.</b>		,440	,460	,062	,570	.	,172
	<b>N</b>		39	39	39	39	39	34
<b>ET<sub>c</sub>:IP</b>	<b>Corr.Coeff.</b>		-,475	,679	-,063	-,461	.	1,000
	<b>Sig.</b>		,005	,000	,721	,006	.	.
	<b>N</b>		34	34	34	34	34	34
<b>SDI (lys)</b>			<b>RH_day</b>	<b>Rad_day</b>	<b>wind_day</b>	<b>Σ_rain_day</b>	<b>%ETc(FI)</b>	<b>ETc:IP</b>
<b>IP</b>	<b>Corr.Coeff.</b>		-,262	-,172	,387	-,032	,830	-,119
	<b>Sig.</b>		,112	,302	,016	,848	,000	,478
	<b>N</b>		38	38	38	38	38	38
<b>ET<sub>c</sub></b>	<b>Corr.Coeff.</b>		-,577	,375	,126	-,447	,635	,489
	<b>Sig.</b>		,000	,019	,443	,004	,000	,002
	<b>N</b>		39	39	39	39	39	38
<b>%ET<sub>c</sub>(FI)</b>	<b>Corr.Coeff.</b>		-,051	-,294	,333	,047	1,000	-,066
	<b>Sig.</b>		,759	,069	,038	,778	.	,694
	<b>N</b>		39	39	39	39	39	38
<b>ET<sub>c</sub>:IP</b>	<b>Corr.Coeff.</b>		-,457	,756	-,210	-,578	-,066	1,000
	<b>Sig.</b>		,004	,000	,205	,000	,694	.
	<b>N</b>		38	38	38	38	38	38

## 7.12.2 Leaf Gas Exchange and Water Potential x Lysimeter and Climate Data

### *Measured Values*

Total									
N				gS	An	WUE	ΨS	IT	IP
87	gS	Corr.Coeff.	1,000	,959	-,814	,739	,416	,509	
		Sig.	.	,000	,000	,000	,000	,000	
87	An	Corr.Coeff.	,959	1,000	-,654	,730	,380	,458	
		Sig.	,000	.	,000	,000	,000	,000	
87	WUE	Corr.Coeff.	-,814	-,654	1,00	-,654	-,415	-,489	
		Sig.	,000	,000	.	,000	,000	,000	
59	ΨS	Corr.	,739	,730	-,654	1,000	,666	,651	
		Sig.	,000	,000	,000	.	,000	,000	
FI									
N				gS	An	WUE	ΨS	IT	IP
37	gS	Corr.Coeff.	1,000	,913	-,845	,413	-,622	-,083	
		Sig.	.	,000	,000	,045	,000	,627	
37	An	Corr.Coeff.	,913	1,000	-,647	,591	-,597	-,152	
		Sig.	,000	.	,000	,002	,000	,368	
37	WUE	Corr.Coeff.	-,845	-,647	1,00	-,121	,570	,083	
		Sig.	,000	,000	.	,573	,000	,626	
24	ΨS	Corr.	,413	,591	-,121	1,000	-,178	-,140	
		Sig.	,045	,002	,573	.	,406	,513	
SDI									
N				gS	An	WUE	ΨS	IT	IP
40	gS	Corr.Coeff.	1,000	,965	-,722	,713	,653	,758	
		Sig.	.	,000	,000	,000	,000	,000	
40	An	Corr.Coeff.	,965	1,000	-,561	,647	,621	,729	
		Sig.	,000	.	,000	,000	,000	,000	
40	WUE	Corr.Coeff.	-,722	-,561	1,00	-,765	-,552	-,581	
		Sig.	,000	,000	.	,000	,000	,000	
25	ΨS	Corr.	,713	,647	-,765	1,000	,748	,678	
		Sig.	,000	,000	,000	.	,000	,000	
RDI									
N				gS	An	WUE	ΨS	IT	IP
10	gS	Corr.Coeff.	1,000	,923	-,523	,615	,810	,810	
		Sig.	.	,000	,121	,058	,004	,004	
	An	Corr.Coeff.	,923	1,000	-,297	,564	,722	,722	
		Sig.	,000	.	,405	,090	,018	,018	
10	WUE	Corr.Coeff.	-,523	-,297	1,00	-,200	-,266	-,266	
		Sig.	,121	,405	.	,580	,458	,458	
10	ΨS	Corr.	,615	,564	-,200	1,000	,798	,798	
		Sig.	,058	,090	,580	.	,006	,006	

*Mean values*

Total								
N			gS	AN	WUE	PSI	IT	IP
21	gS	Corr.Coeff.	1,000	,965	-,931	,745	,493	,604
		Sig.	.	,000	,000	,001	,023	,004
21	AN	Corr.Coeff.	,965	1,000	-,865	,786	,471	,531
		Sig.	,000	.	,000	,000	,031	,013
21	WUE	Corr.Coeff.	-,931	-,865	1,000	-,737	-,532	-,658
		Sig.	,000	,000	.	,001	,013	,001
17	PSI	Corr.	,745	,786	-,737	1,000	,703	,668
		Sig.	,001	,000	,001	.	,002	,003
FI								
N			gS	AN	WUE	PSI	IT	IP
9	gS	Corr.Coeff.	1,000	,850	-,900	,559	-,548	,067
		Sig.	.	,004	,001	,192	,127	,865
9	AN	Corr.Coeff.	,850	1,000	-,650	,883	-,548	-,183
		Sig.	,004	.	,058	,008	,127	,637
9	WUE	Corr.Coeff.	-,900	-,650	1,000	-,378	,548	-,300
		Sig.	,001	,058	.	,403	,127	,433
7	PSI	Corr.	,559	,883	-,378	1,000	-,206	-,162
		Sig.	,192	,008	,403	.	,658	,728
SDI								
N			gS	AN	WUE	PSI	IT	IP
9	gS	Corr.Coeff.	1,000	,933	-,683	,464	,725	,800
		Sig.	.	,000	,042	,294	,027	,010
9	AN	Corr.Coeff.	,933	1,000	-,583	,429	,651	,733
		Sig.	,000	.	,099	,337	,057	,025
9	WUE	Corr.Coeff.	-,683	-,583	1,000	-,929	-,853	-,850
		Sig.	,042	,099	.	,003	,003	,004
7	PSI	Corr.	,464	,429	-,929	1,000	,861	,714
		Sig.	,294	,337	,003	.	,013	,071
RDI								
N			gS	AN	WUE	PSI	IT	IP
3	gS	Corr.Coeff.	1,000	1,000	-1,000	,500	,866	,866
		Sig.	.	.	.	,667	,333	,333
3	AN	Corr.Coeff.	1,000	1,000	-1,000	,500	,866	,866
		Sig.	.	.	.	,667	,333	,333
3	WUE	Corr.Coeff.	-1,000	-1,000	1,000	-,500	-,866	-,866
		Sig.	.	.	.	,667	,333	,333
3	PSI	Corr.	,500	,500	-,500	1,000	,866	,866
		Sig.	,667	,667	,667	.	,333	,333

*Measured values*

Total								
N			ETc	ET0	KC	VPD	T_day	T_mid
87	gS	Corr.Coeff.	,390	-,347	,654	-,227	-,098	-,189
		Sig.	,000	,001	,000	,034	,367	,080
87	An	Corr.Coeff.	,324	-,400	,615	-,340	-,224	-,311
		Sig.	,003	,000	,000	,001	,037	,003
87	WUE	Corr.Coeff.	-,440	,201	-,612	,062	-,034	,026
		Sig.	,000	,062	,000	,570	,754	,808
59	ΨS	Corr.	,540	-,244	,684	-,238	-,336	-,320
		Sig.	,000	,062	,000	,070	,009	,013
FI								
N			ETc	ET0	KC	VPD	T_day	T_mid
37	gS	Corr.Coeff.	-,262	-,651	,464	-,222	,217	-,056
		Sig.	,118	,000	,004	,188	,197	,741
37	An	Corr.Coeff.	-,450	-,762	,411	-,454	,006	-,294
		Sig.	,005	,000	,012	,005	,970	,077
37	WUE	Corr.Coeff.	,081	,430	-,414	,014	-,356	-,147
		Sig.	,635	,008	,011	,934	,031	,385
24	ΨS	Corr.	-,772	-,823	-,319	-,607	-,546	-,545
		Sig.	,000	,000	,129	,002	,006	,006
SDI								
N			ETc	ET0	KC	VPD	T_day	T_mid
40	gS	Corr.Coeff.	,406	-,366	,744	-,571	-,700	-,703
		Sig.	,009	,020	,000	,000	,000	,000
40	An	Corr.Coeff.	,393	-,371	,746	-,593	-,710	-,706
		Sig.	,012	,018	,000	,000	,000	,000
40	WUE	Corr.Coeff.	-,356	,258	-,561	,340	,505	,507
		Sig.	,024	,109	,000	,032	,001	,001
25	ΨS	Corr.	,669	-,248	,746	-,319	-,469	-,458
		Sig.	,000	,231	,000	,120	,018	,021
RDI								
N			ETc	ET0	KC	VPD	T_day	T_mid
10	gS	Corr.Coeff.	.	,548	.	,622	,822	,889
		Sig.	.	,101	.	,055	,004	,001
	An	Corr.Coeff.	.	,539	.	,638	,681	,775
		Sig.	.	,108	.	,047	,030	,008
10	WUE	Corr.Coeff.	.	-,090	.	-,175	-,360	-,438
		Sig.	.	,805	.	,629	,307	,206
10	ΨS	Corr.	.	,944	.	,944	,405	,419
		Sig.	.	,000	.	,000	,246	,228

*Mean Values*

Total								
N			ETc	ET0	KC	VPD	T_day	T_mid
21	gS	Corr.Coeff.	,509	-,344	,746	-,218	-,116	-,177
		Sig.	,026	,127	,000	,342	,616	,443
21	AN	Corr.Coeff.	,401	-,419	,685	-,362	-,291	-,354
		Sig.	,089	,058	,001	,107	,201	,115
21	WUE	Corr.Coeff.	-,581	,227	-,748	,026	-,045	,008
		Sig.	,009	,322	,000	,910	,846	,973
17	PSI	Corr.	,572	-,261	,683	-,219	-,348	-,347
		Sig.	,026	,312	,005	,399	,171	,172
FI								
N			ETc	ET0	KC	VPD	T_day	T_mid
9	gS	Corr.Coeff.	-,200	-,733	,567	-,233	,200	-,017
		Sig.	,606	,025	,112	,546	,606	,966
9	AN	Corr.Coeff.	-,550	-,883	,267	-,583	-,200	-,417
		Sig.	,125	,002	,488	,099	,606	,265
9	WUE	Corr.Coeff.	-,017	,500	-,617	-,100	-,467	-,300
		Sig.	,966	,170	,077	,798	,205	,433
7	PSI	Corr.	-,847	-,955	-,360	-,649	-,595	-,595
		Sig.	,016	,001	,427	,115	,159	,159
SDI								
N			ETc	ET0	KC	VPD	T_day	T_mid
9	gS	Corr.Coeff.	,583	-,350	,917	-,667	-,783	-,767
		Sig.	,099	,356	,001	,050	,013	,016
9	AN	Corr.Coeff.	,500	-,433	,867	-,733	-,833	-,833
		Sig.	,170	,244	,002	,025	,005	,005
9	WUE	Corr.Coeff.	-,567	,267	-,700	,333	,567	,533
		Sig.	,112	,488	,036	,381	,112	,139
7	PSI	Corr.	,750	-,143	,679	-,143	-,393	-,393
		Sig.	,052	,760	,094	,760	,383	,383
RDI								
N			ETc	ET0	KC	VPD	T_day	T_mid
3	gS	Corr.Coeff.	.	,500	.	,500	1,000	1,000
		Sig.	.	,667	.	,667	.	.
3	AN	Corr.Coeff.	.	,500	.	,500	1,000	1,000
		Sig.	.	,667	.	,667	.	.
3	WUE	Corr.Coeff.	.	-,500	.	-,500	-1,000	-1,000
		Sig.	.	,667	.	,667	.	.
3	PSI	Corr.	.	1,000	.	1,000	,500	,500
		Sig.	.	.	.	.	,667	,667



*Measured Values*

Total									
N			RH_day	RH_mid	Rad_day	Rad_mid	wind_day	wind_mid	Σ_rain_day
87	gS	C.C.	,298	,348	-,420	-,422	,033	-,324	,297
		Sig.	,005	,001	,000	,000	,762	,002	,005
87	An	C.C.	,360	,355	-,427	-,443	,040	-,278	,341
		Sig.	,001	,001	,000	,000	,713	,009	,001
87	WUE	C.C.	-,178	-,233	,322	,303	,007	,318	-,232
		Sig.	,100	,030	,002	,004	,947	,003	,031
59	ΨS	C.C.	,119	-,085	-,135	-,090	,017	-,181	,372
		Sig.	,368	,520	,307	,496	,899	,170	,004
FI									
N			RH_day	RH_mid	Rad_day	Rad_mid	wind_day	wind_mid	Σ_rain_day
37	gS	C.C.	,424	,634	-,795	-,712	,157	-,493	,535
		Sig.	,009	,000	,000	,000	,352	,002	,001
37	An	C.C.	,618	,726	-,883	-,816	,170	-,442	,669
		Sig.	,000	,000	,000	,000	,315	,006	,000
37	WUE	C.C.	-,238	-,476	,589	,506	-,114	,413	-,317
		Sig.	,156	,003	,000	,001	,501	,011	,056
24	ΨS	C.C.	,631	,283	-,720	-,650	,618	,158	,686
		Sig.	,001	,180	,000	,001	,001	,460	,000
SDI									
N			RH_day	RH_mid	Rad_day	Rad_mid	wind_day	wind_mid	Σ_rain_day
40	gS	C.C.	,367	,245	-,199	-,286	-,033	-,121	,127
		Sig.	,020	,127	,218	,073	,842	,458	,436
40	An	C.C.	,379	,232	-,187	-,281	,027	-,038	,124
		Sig.	,016	,149	,248	,079	,868	,814	,444
40	WUE	C.C.	-,239	-,093	,206	,223	,187	,347	-,266
		Sig.	,137	,568	,201	,166	,248	,028	,098
25	ΨS	C.C.	-,030	-,310	,023	,114	-,097	-,171	,242
		Sig.	,887	,131	,915	,588	,646	,415	,244
RDI									
N			RH_day	RH_mid	Rad_day	Rad_mid	wind_day	wind_mid	Σ_rain_day
10	gS	C.C.	-,548	-,622	,548	,548	-,548	-,548	.
		Sig.	,101	,055	,101	,101	,101	,101	.
10	An	C.C.	-,539	-,638	,539	,539	-,539	-,539	.
		Sig.	,108	,047	,108	,108	,108	,108	.
10	WUE	C.C.	,090	,175	-,090	-,090	,090	,090	.
		Sig.	,805	,629	,805	,805	,805	,805	.
10	ΨS	C.C.	-,944	-,944	,944	,944	-,944	-,944	.
		Sig.	,000	,000	,000	,000	,000	,000	.

*Mean Values*

**All**

<b>N</b>			<b>RH_day</b>	<b>RH_mid</b>	<b>Rad_day</b>	<b>Rad_mid</b>	<b>wind_day</b>	<b>wind_mid</b>	<b>Σ_rain_day</b>
21	<b>gS</b>	<b>C.C.</b>	,277	,250	-,397	-,375	,019	-,299	,339
		<b>Sig.</b>	,224	,275	,075	,094	,935	,188	,132
21	<b>An</b>	<b>C.C.</b>	,393	,331	-,461	-,459	,117	-,217	,430
		<b>Sig.</b>	,078	,143	,035	,037	,612	,345	,051
21	<b>WUE</b>	<b>C.C.</b>	-,114	-,149	,329	,287	,061	,406	-,325
		<b>Sig.</b>	,624	,519	,145	,207	,793	,068	,150
17	<b>ΨS</b>	<b>C.C.</b>	,139	-,113	-,167	-,115	-,007	-,227	,483
		<b>Sig.</b>	,594	,665	,523	,662	,979	,380	,050

**FI**

<b>N</b>			<b>RH_day</b>	<b>RH_mid</b>	<b>Rad_day</b>	<b>Rad_mid</b>	<b>wind_day</b>	<b>wind_mid</b>	<b>Σ_rain_day</b>
9	<b>gS</b>	<b>C.C.</b>	,367	,583	-,767	-,667	,159	-,433	,366
		<b>Sig.</b>	,332	,099	,016	,050	,683	,244	,332
9	<b>An</b>	<b>C.C.</b>	,717	,783	-,967	-,917	,360	-,283	,693
		<b>Sig.</b>	,030	,013	,000	,001	,342	,460	,038
9	<b>WUE</b>	<b>C.C.</b>	-,067	-,317	,567	,450	-,134	,417	-,297
		<b>Sig.</b>	,865	,406	,112	,224	,731	,265	,438
7	<b>ΨS</b>	<b>C.C.</b>	,667	,324	-,847	-,739	,685	,180	,742
		<b>Sig.</b>	,102	,478	,016	,058	,090	,699	,056

**SDI**

<b>N</b>			<b>RH_day</b>	<b>RH_mid</b>	<b>Rad_day</b>	<b>Rad_mid</b>	<b>wind_day</b>	<b>wind_mid</b>	<b>Σ_rain_day</b>
9	<b>gS</b>	<b>C.C.</b>	,517	,183	-,233	-,283	-,033	-,083	,287
		<b>Sig.</b>	,154	,637	,546	,460	,932	,831	,454
9	<b>An</b>	<b>C.C.</b>	,533	,267	-,250	-,317	,192	,133	,218
		<b>Sig.</b>	,139	,488	,516	,406	,620	,732	,573
9	<b>WUE</b>	<b>C.C.</b>	-,167	,000	,150	,167	,301	,550	-,376
		<b>Sig.</b>	,668	1,000	,700	,668	,431	,125	,318
7	<b>ΨS</b>	<b>C.C.</b>	-,107	-,429	,071	,179	-,286	-,429	,401
		<b>Sig.</b>	,819	,337	,879	,702	,535	,337	,373

**RDI**

<b>N</b>			<b>RH_day</b>	<b>RH_mid</b>	<b>Rad_day</b>	<b>Rad_mid</b>	<b>wind_day</b>	<b>wind_mid</b>	<b>Σ_rain_day</b>
3	<b>gS</b>	<b>C.C.</b>	-,500	-,500	,500	,500	-,500	-,500	.
		<b>Sig.</b>	,667	,667	,667	,667	,667	,667	.
3	<b>An</b>	<b>C.C.</b>	-,500	-,500	,500	,500	-,500	-,500	.
		<b>Sig.</b>	,667	,667	,667	,667	,667	,667	.
3	<b>WUE</b>	<b>C.C.</b>	,500	,500	-,500	-,500	,500	,500	.
		<b>Sig.</b>	,667	,667	,667	,667	,667	,667	.
3	<b>ΨS</b>	<b>C.C.</b>	-1,000	-1,000	1,000	1,000	-1,000	-1,000	.
		<b>Sig.</b>	.	.	.	.	.	.	.