WHOLE-CANOPY PHOTOSYNTHESIS AND TRANSPIRATION UNDER REGULATED DEFICIT IRRIGATION IN *VITIS VINIFERA* L. CV. CABERNET SAUVIGNON

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of JORGE ESTEBAN PEREZ PEÑA find it satisfactory and recommend that it be accepted

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Abstract

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Influences of water deficits in grapevines have been studied worldwide in recent years because of their influence on wine quality and water savings. To approach these issues, grape growers have adopted regulated deficit irrigation (RDI) that consists of applying less water than full vine evapotranspiration (FVET) to limit water use to that amount just needed to ripen the crop, achieve the fruit characteristics demanded by winemakers, and end the season with a plant prepared for winter and the following budbreak. For a better understanding of RDI on vine physiology, whole-canopy gas exchange rates (CO_2 and H_2O_v) were measured by a six-chamber, mobile field laboratory designed, built, and tested in 2001. Measurements were taken in 2002 and 2003 at five times during the season (fruit set, pre- and post-veraison, and pre-and post-harvest) in a drip irrigated vineyard of cv. Cabernet Sauvignon under three regimens of RDI: 1) standard deficit (S; replacement of 70% of FVET); 2) early deficit (E; replacement of 35% of FVET between fruit set and veraison); and 3) late deficit (V; replacement of 35% of FVET between veraison and harvest). At the same time,

single-leaf photosynthesis and transpiration were measured. Effects of those regimens on carbohydrate dynamics were studied by sampling leaf tissue and dormant canes for determination of non-structural carbohydrates. Vine canopies under additional water fixed less CO_2 and transpired less water than those under standard deficit. Reductions were associated with lower canopy conductance. Vines under additional water deficits (E, V) had lower leaf starch concentrations in the afternoon, but no differences in soluble sugar concentrations. Pruning weights in S and V vines were up to 41% higher than those of E vines. No differences were found in water use efficiency nor intrinsic water use efficiency among irrigation regimens. Reductions in irrigation and water use occurred at the expense of reductions in carbon fixation. No consistent effect of additional water deficits were recorded on fruit yield or quality. A novel deficit irrigation index based on monitoring transpiration and vapor pressure deficit is proposed.

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LIST OF ABBREVIATIONS

θ	volumetric soil water content
µ	micro
ρ_a	density of air
A_{420}	absorbance at 420 nm wavelength
A_{520}	absorbance at 520 nm wavelength
A_{700}	absorbance at 700 nm wavelength
A_b	basal area of chamber
ABA	abscisic acid
ADP	adenosine diphosphate
A_n	net photosynthesis
ATP	adenosine triphosphate
A_w	wall area of chamber
$c_{deficit}$	irrigation deficit coefficient
c_p	specific heat of air
CRD	completely randomized design
d_e	distance between emitters
DCS	days with clear skies
DI	whole-canopy proposed deficit index for RDI
DM	dry matter
DOM	day of measurement

DOY	day of year, January 1= 1
d_r	distance between vine rows
DSD	daily sum of degrees base 10°C
DW	dry weight
DWC	days with partially cloudy skies
E	early deficit irrigation regimen
e_a	air vapor pressure
EC	enzyme commission number
e_s	saturated vapor pressure of air
$e_{s(leaf)}$	saturated vapor pressure at leaf temperature
ET	evapotranspiration
ET_o	reference evapotranspiration (grass reference)
ETR	electron transport rate
f	emitter flow rate
f_a	air flow rate
F6P	fructose-6-phosphate
FC	field capacity
FPR	fruit:pruning weight ratio
FVET	full vine evapotranspiration
FW	fresh weight
G6P	glucose-6-phosphate
G6PDH	glucose-6-phosphate dehydrogenase

GDD	growing degree days base 10°C
g_s	stomatal conductance
g_c	canopy conductance
H	heat absorbed
H_2O_v	water vapor
Kc	crop coefficient
LFR	leaf area:fruit ratio
LST	local standard time
M	molecular weight of air
NAD	nicotinamide dinucleotide
NCE	net CO_2 exchange
NCE_{SL}	single-leaf net CO_2 exchange
$NCE_{WL,d}$	whole-canopy net CO_2 exchange per unit leaf area per day
NCE_W	whole-canopy net CO_2 exchange rate
NCE_{Wd}	daily mean of NCER between times at which $NCE_{SL,d}$ was calculated
$NCE_{WV,d}$	whole-canopy net CO_2 exchange per vine per day
NPQ	non-photochemical quenching
NS	number of shoots per vine
P_a	atmospheric pressure
PAR	photosynthetically active radiation
PAW	plant available water
PAWS	Public Agriculture Weather System

PPFD	photosynthetic photon flux density
$PPFD_d$	daily photosynthetic photon flux density
PGI	phosphoglucoisomerase
PSII	photosystem II
PWP	permanent wilting point
RDI	regulated deficit irrigation
r_H	resistance to heat transfer
Rubisco	ribulose bisphosphate carboxylase oxygenase
RuBP	ribulose bisphosphate
ss	soluble sugars
S	standard deficit irrigation regimen
sd	soil depth
SL	shoot length
SLA	shoot leaf area
SW	portion of total soil area wetted
SR	ratio between slopes of predicted TR_{WL} and predicted VPD
t_{ir}	duration of irrigation (h)
TA	titratable acidity
T_{air}	air temperature
t_{DI}	time of proposed deficit index DI
T_{DSD}	mean temperature for the 12 min interval
Tr	transpiration rate

Tr_{WL}	whole vine transpiration rate on a leaf area basis
Tr_{WV}	whole vine transpiration rate on a per vine basis
$Tr_{WV,d}$	whole canopy transpiration per vine per day
$Tr_{WL,d}$	whole canopy transpiration per unit leaf area per day
$t_{sunrise}$	time of sunrise
t_{sunset}	time of sunset
V	late deficit irrigation regimen
VLA	vine leaf area
VPD	air vapor pressure deficit
VPD_{la}	leaf to air vapor pressure deficit
WUE	water use efficiency
WUE_i	intrinsic water use efficiency
WUE_{SL}	single-leaf water use efficiency
$WUE_{SL,d}$	single-leaf daily water use efficiency
$WUE_{i,SL}$	single-leaf intrinsic water use efficiency
WUE_{WL}	whole canopy water use efficiency per unit leaf area
$WUE_{WL,d}$	whole-canopy daily water use efficiency per unit leaf area
$WUE_{i,WL}$	whole-canopy intrinsic water use efficiency per unit leaf area
WUE_{WV}	whole-canopy water use efficiency per vine
$WUE_{WV,d}$	whole-canopy daily water use efficiency per vine

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Dedication

This dissertation is dedicated both to the memory of my father and to "The Disappeared" of Argentina.

“From the moment of their abduction, the victims lost all rights. Deprived of all communication with the outside world, held in unknown places, subjected to barbaric tortures, kept ignorant of their immediate or ultimate fate, they risked being either thrown into a river or the sea, weighted down with blocks of cement, or burned to ashes. They were not mere objects, however, and still possessed all the human attributes: they could feel pain, could remember a mother, child or spouse, could feel infinite shame at being raped in public...”

[Ernesto Sábato, 1984, prologue to *Nunca Más*]

CHAPTER 1

GENERAL INTRODUCTION

Washington is the second leading grape producing state in the USA with about 12,000 ha of wine grapes (*Vitis vinifera* L.) and 10,000 ha of juice grapes (*Vitis x labruscana* Bailey). Wine grapes are destined for premium, superpremium and ultra premium wine. Major red-wine cultivars planted are Cabernet Sauvignon (2448 ha), Merlot (2420 ha), Syrah (849 ha), and Cabernet Franc (750 ha), while the white-wine cultivars are Chardonnay (2687 ha), Riesling (890 ha), Sauvignon Blanc (287 ha), and Gewürztraminer (271 ha; Washington Agricultural Statistics Service, 2002). According to Winkler's classification (Winkler et al., 1974) based on heat summation as degree-days (GDD.; base 10°C), eastern Washington falls in Region II, averaging 1350 GDD for Prosser (Naor and Wample, 1994). The growing season is short (158 frost free days) and because of this there are some limits to the amount of photosynthate that can be produced by a vine; thus, the balance between yield and quality is very important. Average annual rainfall at Prosser is 198 mm with 75% of the rain events occurring from October through April. The average cumulative "Class A" unscreened pan evaporation is 1266 mm for April through October (Naor and Wample, 1994) with maximum daily reference evapotranspiration (grass) during the growing season of about 9 mm d^{-1} (Evans et al., 1993).

Early frost and cold hardiness are major challenges for Washington grape growers. The combination of a short growing season, low rainfall, hot summers, and cold

winters challenge Washington grape growers in several ways. Because of the large deficit between evapotranspiration and rainfall, winegrapes can only be commercially cultivated by applying irrigation. Furrow, sprinkler, and drip methods have been used, although the trend is towards drip irrigation, which gives grape growers greater flexibility in irrigation scheduling. However, a major challenge lies in determining the timing and amount of irrigation to balance canopy productivity with crop load so as to achieve the fruit characteristics desired by winemakers and reach the end of the growing season with a plant prepared for the cold winter (i.e., avoiding any delay in the lignification of canes and replenishment of reserves in the permanent structures of the vine). This challenge has been approached partly by regulated deficit irrigation (RDI), or deficit irrigation, a system of managing soil water to impose predetermined periods of plant or soil water deficit to elicit some desirable plant responses (Behboudian and Singh, 2001). Localized-pressurized irrigation systems installed in vineyards allow precise control of the amount of water delivered to the vines and offer the grape grower a tool to manage not only yield but also crop quality (Williams, 1996). Different methods are used to quantify the severity of water deficits, from instruments that measure soil moisture or soil water potential, to instruments that measure variables related to plant water status (Kramer and Boyer, 1995).

In contrast to other commercially-grown perennial fruit crops (e.g., stone fruits, apples, pears) where bloom precedes vegetative growth, grapevines produce much vegetation before bloom and fruit growth (Mullins et al., 1992). The vegetative stage in grapevines starts with budbreak and is followed by a period of rapid shoot growth

(Williams et al., 1994). During budbreak, the permanent structures of the grapevine are the primary sources of reserve carbohydrate (Williams, 1996). The time at which shoots stop growing is variable and dependent mainly on environmental conditions, although in commercial viticulture this usually happens sometime between fruit set (transformation of flowers into fruits) and harvest. Thus, flower clusters appear and bloom occurs while shoots are still growing. Reproductive development begins with inflorescence (flower cluster) initiation in the summer preceding its flowering and fruiting (Pratt, 1971). Within the compound buds cluster initiation for the next year's crop begins around anthesis and is complete prior to veraison (Srinivasan and Mullins, 1981; Williams et al., 1994). It has been suggested that flower differentiation begins prior to budbreak and continues until anthesis (flower cap fall). The time between budbreak and anthesis varies with the cultivar and environmental conditions.

The growth of grape berries follows a double sigmoid pattern with two stages of rapid growth, phases I and III, separated by a 'lag phase' of no or slow growth, phase II (Figure 1; Coombe, 1960; Harris et al., 1968; Ollat et al., 2002). Phase I starts after anthesis and is characterized by a short period of cell division accompanied and followed by a period of cell expansion (Hardie et al., 1996; Harris et al., 1968; Ojeda et al., 1999; Pratt, 1971). At the end of phase II veraison occurs, a term derived from the French viticultural language that denotes a change in color of the berry skin. During veraison the berry softens and its skin changes in red-fruited cultivars from green to red, while in white-fruited cultivars the berries acquire a more translucent appearance. Phase III is characterized by rapid cell expansion, sugar accumulation, reduction in

organic acids, and accumulation of phenolic compounds and flavors (Coombe and Hale, 1973; Hrazdina et al., 1984; Pirie and Mullins, 1980).

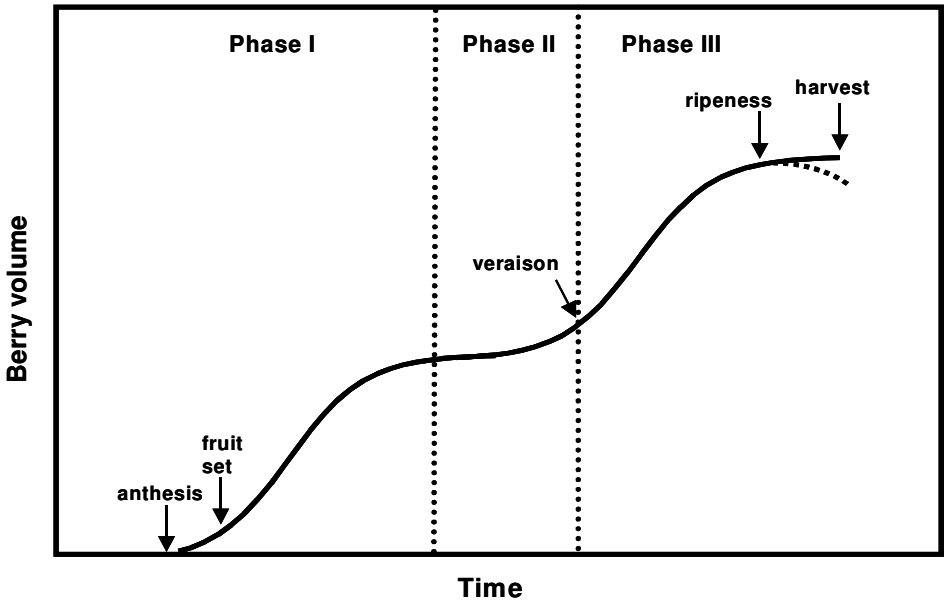

Figure 1: Diagram of a typical double-sigmoid pattern of growth by a grape berry, from anthesis to harvest. In some cultivars, berries may shrivel between ripeness and harvest, thus lose volume (dashed line; redrawn from Coombe and Hale, 1973)

The effects of water deficits imposed through RDI depend on the phenological stage of the grapevine and the severity of the deficit (Hardie and Considine, 1976; Williams and Matthews, 1990). Both vegetative and reproductive stages can be affected. Research suggests that a water deficit applied to winegrapes using RDI is a viable practice for controlling excessive vigor, reducing disease pressure, and improving wine quality (Bravdo et al., 1985; Esteban et al., 1999; Matthews and Anderson, 1988; Nadal and Arola, 1995). By contrast, water applied in excess can produce large canopies. Excessive shoot growth in grapevines can shade clusters, preventing proper development of flavor, color, and aroma (Price et al., 1995; Smart et al., 1988; Spayd et

al., 2002; Zoecklein et al., 1998). Furthermore large canopies favor development of diseases (English et al., 1989; Gubler et al., 1987). One of the goals of RDI is canopy control: reducing shoot growth and leaf area to optimize the balance between crop and canopy (Behboudian, 1997). However, an excessively limited canopy can result in insufficient carbohydrate sources for maturing the crop (Kaps and Cahoon, 1992; Kliewer and Dokoozlian, 2000; Kliewer and Ough, 1970; Koblet et al., 1994), insufficient reserves for the plant (Candolfi-Vasconcelos and Koblet, 1990; Petrie et al., 2000a), and can lead to excessive fruit exposure during berry growth (Bergqvist et al., 2001; Price et al., 1995; Spayd et al., 2002). Water deficits imposed early in the season generally reduce the rate of shoot growth, total leaf area, and subsequent pruning weights (Alexander, 1965; Kliewer et al., 1983; Neja et al., 1977; Poni et al., 1993; Reynolds and Naylor, 1994). Leaf expansion rate apparently is the most sensitive process to water deficit (Schultz, 2000; Schultz and Matthews, 1993; Winkel and Rambal, 1993), although the rate of shoot growth has been reported to be even more sensitive (Kliewer et al., 1983).

The effects of water deficit on the reproductive stage, as with the vegetative stage, depend on the severity and the timing of the deficit (Alexander, 1965; Hardie and Considine, 1976; Matthews and Anderson, 1989; McCarthy, 1997; Poni et al., 1993; Van Zyl, 1984). Yield is reduced by water deficits imposed either before or after veraison, with greater effect when applied before veraison (Matthews and Anderson, 1989; Van Zyl, 1984). Reduced yields are explained mainly by fewer berries per cluster, fewer clusters per vine, and lower weight per berry. For example, severe water deficit applied

in young potted vines early in the reproductive stage (leaf water potential at dawn -0.8 MPa; 75% cap-fall) reduced fruit set and produced a loss of whole clusters (Hardie and Considine, 1976). In field-grown vines, similar responses also were reported (dos Santos et al., 2003; Matthews and Anderson, 1989). Berry shriveling and smaller berries at harvest have been observed when water deficits were applied to phases I, II or III. Generally, water deficits during phase I have been reported to have a greater effect on final berry size than deficits during phase III (Creasy and Lombard, 1993; Greenspan et al., 1994; Greenspan et al., 1992; Matthews and Anderson, 1988; McCarthy, 1997). The observed reduction in final berry size from water deficit during phase I can be explained mainly by a reduction of the mean size of pericarp cells (Ojeda et al., 2001).

Greater diurnal fluctuations in berry size were observed with water deficits during phase I than deficits during phase III. This phenomenon has been partly explained by the characteristics and dynamics of the xylem connection between the berry and the vine, which apparently functions until around the end of phase II, when xylem peripheral bundles start to stretch and break, gradually losing part of their function (Creasy et al., 1993; Düring et al., 1987; Findlay et al., 1987). However, xylem axial bundles apparently remain functional or at least partially functional for some time after veraison (Düring et al., 1987; Rogiers et al., 2001). Once the xylem loses its functionality, berries apparently become hydraulically isolated from shoot water relations (Greenspan et al., 1994). It has been shown that water enters the berry during phase III mainly through phloem tissue (Greenspan et al., 1996; Greenspan et al., 1992) but debate about the mechanism of water transport into the berry during phase III still exists (Coombe and McCarthy, 2000;

McCarthy and Coombe, 1999; Picaud et al., 2003; Rogiers et al., 2001). However, experiments with dye showed that after veraison xylem was intact but flow of water through the xylem stopped at the base of the berry (M. Keller, unpublished data). In other work, berry dermal plasticity but not elasticity was shown to differ between before, and after veraison (Matthews et al., 1987) which may have contributed to the more dramatically reduced diurnal fluctuations in berry size during phase III (Greenspan et al., 1996).

Water deficits have been reported to improve grape color and anthocyanin production in red-fruited varieties (Bravdo et al., 1985; Esteban et al., 2001; Freeman, 1983; Freeman and Kliewer, 1983; Hardie and Considine, 1976; Poni et al., 1993). Anthocyanin concentrations increase in two ways: directly, as content per unit skin surface area (Matthews and Anderson, 1988) or per unit skin weight (Ojeda et al., 2002), and indirectly by the reduction in berry size, resulting in higher skin:juice ratios. It has been suggested that post-veraison water deficits affect red wine flavonoid concentrations primarily by reducing berry size and secondarily by modifying flavonoid biosynthesis (Kennedy et al., 2002; Kennedy et al., 2000).

To better understand the effects of a water deficit imposed by RDI on vine productivity, its effects on photosynthesis need to be explored. Part of the organic phosphates that are produced by photosynthesis, photoassimilates, are used by the source organ while others are exported to sink organs. Sources are plant organs that are net exporters of a compound (e.g., a leaf is a source for carbohydrates and a root is a source for nutrients), while sinks are organs that are net importers of a compound

(e.g., a root is a sink for carbohydrates and a leaf is a sink for nutrients). Carbon partitioning is the distribution of photoassimilates within the plant, while carbon allocation refers to the regulation of the distribution of fixed carbon into the various metabolic pathways (Taiz and Zeiger, 1998).

Water deficits affect photosynthesis, carbon partitioning and carbon allocation, and can limit carbohydrates' availability to fill the fruit in source-limiting situations. But in contrast with other crops, especially many agronomic crops, where partitioning favors the harvested organs and harvest index (ratio of dry mass of harvested organs to total plant dry mass) is intended to be maximized (Gifford et al., 1984), in winegrapes this is not the case (Behboudian and Singh, 2001). On the contrary, in commercial viticulture it is the sink size, or number of grape berries per vine, that usually is limited deliberately by the grower because high yield is not the main objective, but rather a combination of fruit quality and quantity (Jackson and Lombard, 1993). This is important because the balance between sources and sinks of photoassimilates within the grapevine modulates the effects of imposed water deficits (Bravdo et al., 1985; Kliewer et al., 1983; Poni et al., 1993; Poni et al., 1994) and vice versa (Williams, 1996). The responses at the whole vine level appear to be based on reallocation of resources within the vine, driven by sink priority (Edson et al., 1993). Vineyard management practices must influence photosynthesis and dry matter partitioning in a way that enough carbohydrates are available for vegetative growth and reproductive development, but also assuring that enough reserves are retained in permanent tissues for subsequent seasons (Petrie et al., 2000a). Likewise, excessive vegetative growth that could be detrimental to grape

quality or vine cold hardiness must be avoided. Thus a balance between vegetative and reproductive growth and storage should be achieved. Winter pruning, along with flower and fruit cluster thinning are among management practices used to limit the size of the reproductive sink. Management practices used to control source size, mainly the green canopy, are shoot thinning, summer pruning, fertilization, and irrigation (Mullins et al., 1992; Williams, 1996).

Drawing conclusions or comparing results from experiments on the effects of water deficits on photosynthesis should consider deficit timing and severity, vine cultivar and age, and environmental conditions under which deficits were imposed. Effects of water deficits on photosynthesis have been studied sometimes in mature leaves and sometimes in young leaves. Likewise, water deficits have been imposed for long times and for short times, while some deficits have been severe and some mild (Farquhar et al., 1989). When water deficits are mild, leaf expansion can be affected without any inhibition of leaf photosynthesis rate (Hsiao, 1973) and a higher proportion of the carbon fixed may be directed to the roots. Roots generally grow at lower water potential than leaves (Boyer, 1988; Hsiao and Xu, 2000; Palliotti and Cartechini, 2000). A reduction in canopy growth rate caused by mild water deficit reduces the amount of photosynthetically active radiation (PAR) intercepted and photosynthesis per unit land area in crops like grapevines that have sparse canopies (Petrie et al., 2003; Poni et al., 2003). The reduction in growth rate could be interpreted as an adaptive response to limit transpiring surface area, but strategies of coping with the water deficit have been shown to vary according to the origin of varieties (Schultz, 2000; Winkel and Rambal, 1993).

More severe water deficits also affect total biomass production due to a reduction in the rate of photosynthesis (Delgado et al., 1995; Liu et al., 1978).

In general, photosynthesis in grapevines is reduced by water deficits (Escalona et al., 1999; Flexas et al., 1998; Kriedemann and Smart, 1971; Naor et al., 1994; Poni et al., 1993), which has been explained by stomatal and non-stomatal factors (Farquhar and Sharkey, 1982; Jones, 1985; Von Caemmerer and Farquhar, 1981), although the relative contribution of each factor to that limitation is still debated (Ball et al., 1987; Bota et al., 2004; Chaves et al., 2002; Downton et al., 1988; Flexas et al., 2002; Medrano et al., 2002a). Stomatal limitation of photosynthesis is due to stomatal aperture that changes the partial pressure of CO_2 (pCO_2) at the sites of carboxylation (Farquhar and Sharkey, 1982). Non-stomatal or metabolic limitation of photosynthesis is related to reduction in activity of the enzymes of carbon fixation, reduced electron transport rate, increased photoprotection mechanisms, increased photoinhibion, and reduced photophosphorylation. The relative contribution of stomatal and non-stomatal limitation apparently depends on the rate at which the water deficit is imposed or induced and its severity. When water deficits were mild and were gradually induced, the reduced rate of photosynthesis in grapevines primarily was related to reductions in stomatal conductance (de Souza et al., 2003; Delgado et al., 1995; Escalona et al., 1999; Flexas et al., 1998; Flexas et al., 1999; Osorio et al., 1995).

Stomata close in response to signals transmitted to the leaf from the roots sensing drying soil (Davies and Zhang, 1991; Wilkinson and Davies, 1997). Roots respond to water availability in soil that cause changes in root water status and mechanical

impedance by the soil. Partial dehydration of roots due to a water deficit causes a reduction in root water potential (Van Zyl, 1987; Williams and Matthews, 1990), an increase in xylem sap pH, and a stimulation of root abscisic acid (ABA) production (Davies et al., 2002; Wilkinson and Davies, 1997; Wilkinson and Davies, 2002). All of these signals are known to decrease stomatal conductance (Correia et al., 1995; Davies and Zhang, 1991; Hartung et al., 2002; Loveys and Düring, 1984; Sauter et al., 2001; Stoll et al., 2000; Tardieu and Davies, 1992). In field-grown grapevines under water deficit it was estimated that more than 80% of the variation in the rate of photosynthesis was explained by variations in stomatal conductance (Escalona et al., 1999). Gradual development of water deficit allows the plant to acclimate not only its photosynthetic machinery (Anderson and Anderson, 1988) but also its morphology (Palliotti and Cartechini, 2000). Under water deficit, grapevines have been shown to change the angle between the leaf blade and petiole (Smart, 1974), thus changing leaf orientation and reducing light interception. This could influence the rate of photosynthesis (Kriedemann and Smart, 1971), and possibly avoid photoinhibition, the inhibition of the light-dependent reactions of photosynthesis (Osmond, 1994). Greater numbers and more uniform development of stomata were observed in vines where water deficit was gradually induced than when the deficit was achieved quickly (Liu et al., 1978).

When water deficits are severe and imposed quickly, and below a certain leaf water potential or stomatal conductance, the contribution to the regulation of photosynthesis by non-stomatal effects apparently increases (Chaves, 1991). Under severe water deficit there is a reduction in quantum efficiency of photosystem II (PSII)

that could result in photoinhibition (Düring, 1998; Iacono and Sommer, 2000). Electron transport rate (ETR) was slightly reduced (Bota et al., 2004; de Souza et al., 2003) and non photochemical quenching (NPQ), the process that lowers the efficiency of PSII (Osmond, 1994), was increased (Flexas et al., 1998). Reductions in leaf ribulose bisphosphate (RuBP) regeneration and ribulose bisphosphate carboxylase oxygenase (Rubisco) activity also were reported to limit the rate of photosynthesis when severe water deficits were imposed and stomatal conductance was already reduced (Bota et al., 2004). The reduction in CO_2 uptake due to a reduction in stomatal conductance could lead to an excess of light to the phostosystems. Increased leaf temperature, due to a reduction in stomatal conductance, may increase photorespiration and also reduce rates of photosynthesis. However, the photosynthetic apparatus of grapevines apparently is very resistant to photoinhibition; the photochemical reactions are scarcely affected by drought (Bota et al., 2004; Chaumont et al., 1997; Flexas et al., 1998; Gamon and Pearcy, 1990). Moreover, other studies have indicated that even severely-stressed leaves had a low incidence of photoinhibition, with grapevines having a very effective safe dissipation of absorbed energy (Medrano et al., 2002b). Although stomatal limitation seems to be most important, photosynthesis and stomatal aperture have been reported to be tightly correlated (Correia et al., 1990; Escalona et al., 1999; Flexas et al., 1999; Flexas et al., 1999; Jacobs et al., 1996; Jarvis and Davies, 1998).

In considering the literature on grapevine irrigation and water deficit, it is worth noting that the technological and traditional context of the research location influence how irrigation may have been approached. For example, legal restrictions on irrigation in

some European countries have dictated that much of the research be done with potted vines, because irrigation may be used only to save field-grown vines in an emergency when drought is severe (Behboudian and Singh, 2001). In some of the countries where commercial and profitable viticulture is possible without irrigation, the purpose of irrigation is to increase profits, yield, and quality by offsetting crop water deficit during the growing season (Esteban et al., 2002; Medrano et al., 2003). On the other hand, in regions where commercial viticulture is not possible without irrigation, the purpose of irrigation is first to make the vineyard economically feasible, and then to influence grape quality and yield. In places like eastern Washington, where there are no restrictions on the use of irrigation in viticulture, results from many European studies may not be directly applicable (Behboudian and Singh, 2001; Reynolds, 2000).

Studies of the effects of water deficits, as well as other environmental factors, cultural practices, and biotic stresses on photosynthesis in grapevines have been determined mostly from responses measured at the level of a single leaf. Factors studied include disease incidence (Orlandini and Giuntoli, 1998; Varadi et al., 1995), irrigation or water deficit (Delgado et al., 1995; Murillo de Alburquerque and Carbonneau, 1999; Nadal and Arola, 1995; Naor et al., 1993; Naor and Wample, 1994; Poni et al., 1993), solar radiation (Osorio et al., 1995; Schubert et al., 1996), leaf age (Petrie et al., 2000b; Schubert et al., 1996; Schultz et al., 1996), trellis systems or canopy management (Cavallo et al., 2001; Iacono et al., 1995; Katerji et al., 1994; Poni et al., 2000), and crop load (Hummel and Ferrere, 1998; Naor et al., 1997; Petrie et al., 2000a). Single-leaf measurements of photosynthesis are useful in comparing

experimental treatments and provide information that cannot be obtained by other biological indicators of vine productivity such as dry matter. Leaf-level measurements are instantaneous and non-destructive, and allow measurement of the total carbon gain by an individual photosynthetic organ (Long et al., 1996). However, leaf-level photosynthesis measurements can provide incomplete and potentially misleading information if extrapolated to quantify photosynthesis or infer differences in crop productivity at the level of the whole plant (Hsiao and Acevedo, 1974; Poni et al., 1997; Quereix et al., 2001). In general, single leaf photosynthesis measurements refer to a small area of the leaf, determined by the size of the leaf chamber. Thus measurements are not extrapolated from entire leaves but from 2 to 3 cm^2 to a whole canopy, which in commercial viticulture can have more than 6 m^2 of leaf area per vine (e.g., Dokoozlian and Kliewer, 1995; Perez Peña and Tarara, 2004). Scaling-up from single leaf to whole canopy photosynthesis is not straightforward because the latter includes leaves of different ages and degree of light exposure, and integrates organs like fruit, shoots, and trunks (Amthor, 1994; Bugbee, 1992; Buwalda, 1991; Buwalda et al., 1992; Grau, 1995; Intrieri et al., 1997; Long et al., 1996; Succi and Magnanini, 1994). Estimation of leaf area distribution and light extinction by the canopy combined with a directional treatment of incident light are needed to estimate whole canopy photosynthesis from single leaf measurements (De Pury and Farquhar, 1997). In addition, whole canopies may have higher apparent values for light compensation and light saturation than do single leaves (Corelli-Grappadelli and Magnanini, 1993; Francesconi et al., 1997).

To date, one common finding has been a lack of good correlation between single-

leaf and whole-vine measurements, with whole-vine photosynthesis expressed on a leaf area basis being lower than values from single-leaf measurements (Edson et al., 1993; Edson et al., 1995a; Edson et al., 1995b; Intrieri et al., 1997; Poni et al., 1997). Single leaf measurements overestimated whole-plant photosynthesis by as much as 40% in grapevines and fruit trees (Edson et al., 1993; Intrieri et al., 1997; Katerji et al., 1994). Differences in crop load affected rates of photosynthesis measured on single leaves, but not on whole vines (Edson et al., 1993). Trellis type (vertical shoot positioning and Lyre) affected rates of photosynthesis if they were expressed per unit leaf area (i.e., analogous to single-leaf measurements), but not when rates were expressed per vine (Katerji et al., 1994). One caveat is that whole canopy photosynthesis expressed per unit leaf area is not strictly a value of net leaf photosynthesis as the measurement also includes respiration by non-photosynthetic organs.

Contrary to single-leaf photosynthesis measurements, whole-canopy measurements provide an integrated value of net carbon fixed and water transpired, and overcome some of the limitations of the single-leaf gas exchange measurement by integrating the response of the entire canopy (Katerji et al., 1994; Knight, 1992; Ollat and Tandonnet, 1999; Petrie et al., 2003; Poni et al., 1997). Measurement of whole-canopy gas exchange facilitates instantaneous estimation of light conversion and water use efficiencies, and provides a tool for quantitative assessment of the impact of environmental changes upon biological processes like photosynthesis (Garcia et al., 1990). The two general approaches to whole-canopy gas exchange measurements are enclosure (i.e., whole-canopy chambers) and meteorological methods (i.e., flux-gradient

or eddy correlation; Field et al., 1989; Garcia et al., 1990; Long and Hällgren, 1989). Enclosure methods consist of enclosing a single canopy or groups of plants in chambers and measuring gas exchange. Meteorological methods estimate photosynthesis by concurrent measurements of CO_2 concentrations and air movements above the crop or canopies using rapidly responding instruments (Long and Hällgren, 1989). Enclosure methods offer more accurate estimates of whole-plant gas exchange, are well adapted to small plot sizes, and cost less than meteorological systems. They allow researchers to make replicated comparisons of treatment influences on whole-plant CO_2 assimilation throughout the growing season. Enclosure gas exchange systems are powerful tools for detecting differences related to leaf shape, leaf distribution, sun exposure, crop load, biotic and abiotic stresses, or cultural practices (Buwalda et al., 1992; Intrieri et al., 1997; Intrieri et al., 1998; Lakso et al., 1996; Leadley and Drake, 1993; Petrie et al., 2003; Wheeler, 1992). Although models for estimation of whole-canopy photosynthesis could be used (Amthor, 1994; De Pury and Farquhar, 1997; Poni et al., 2003; Quereix et al., 2001; Wermelinger and Baumgärtner, 1991), whole-canopy measurements are still needed to validate these models (Wünsche and Palmer, 1997). The most realistic solution to study gas exchange *in situ* of whole plants with several m^2 of leaf area is the gas exchange chamber technique (Daudet, 1987).

Much work has been done on whole-canopy gas exchange in perennial fruit crops. Some researchers have enclosed potted plants (Edson et al., 1993; Intrieri et al., 1997; Miller et al., 1996a; Poni et al., 1997), while others have enclosed field-grown plants (Daudet, 1987; Heinicke and Childers, 1937; Intrieri et al., 1998; Katerji et al.,

1994; Succi and Magnanini, 1994). Most studies on whole-canopy photosynthesis in grapevines have used potted vines (Edson et al., 1993; Edson et al., 1995a; Edson et al., 1995b; Intrieri et al., 1997; Intrieri et al., 1998; Miller et al., 1996a; Miller et al., 1996b; Poni et al., 1997) with a few exceptions (Intrieri et al., 1997; Intrieri et al., 1998; Katerji et al., 1994; Ollat and Tandonnet, 1999). Most experiments with potted vines have used soilless potting media (Flexas et al., 1999; Ojeda et al., 2002; Patakas and Noitsakis, 2001; Schultz and Matthews, 1993). It has been suggested that the results obtained on potted plants cannot be transferred directly to the field environment (Alleweldt, 1984; Flexas et al., 2002). What is important in studies of RDI is that the restriction in root growth and the greater diurnal fluctuations in root-zone temperatures in potted vines cause more extreme water deficits than in field grown vines (Chaumont et al., 1995; Intrieri et al., 1997; Intrieri et al., 1998). Drought conditions in potted plants can be achieved very quickly (Escalona et al., 1999) whereas under field conditions water deficits develop more slowly (Flexas et al., 1998; Loveys and Düring, 1984). For example, stomatal closure occurred around -1.3 MPa leaf water potential in potted vines and -1.6 MPa in field-grown vines; different hormonal behavior (e.g., abscisic acid levels) was observed according to the size of the pots (Liu et al., 1978). In potted vines under laboratory conditions, photosynthesis decreased with increasing water deficit, and a more rapid decrease in the rate of photosynthesis occurred when the water deficit was imposed before rather than after veraison (Poni et al., 1993). Elsewhere, no difference in photosynthesis was observed in potted vines when water deficit was not severe and when soil moisture was depleted slowly (Düring and Dry, 1995). Water deficit

experiments conducted in pots found in general that non-stomatal inhibition of photosynthesis is important (Düring, 1998; Liu et al., 1978; Murillo de Alburquerque and Carbonneau, 1999; Quick et al., 1992; Rodrigues et al., 1993), although stomatal limitation also was observed (Dry et al., 2000; Düring, 1987; Liu et al., 1978; Quereix et al., 2001; Rodrigues et al., 1993; Schultz, 2000). By contrast, experiments with field-grown vines found that a reduction in the rate of photosynthesis primarily was related to stomatal conductance (Delgado et al., 1995; Escalona et al., 1999; Flexas et al., 1998; Flexas et al., 1999; Osorio et al., 1995) and less severe reductions in electron transport rates were observed.

Another factor limiting the application of results from experiments with potted vines is that most experiments have been done with very young vines and with canopy arrangements very different from those in a typical vineyard (Buttrose, 1965; Dry et al., 2000; Düring, 1998; Edson et al., 1993; Edson et al., 1995a; Edson et al., 1995b; Hardie and Considine, 1976; Miller et al., 1996b; Miller et al., 1996c; Poni et al., 1993), altering the relation between sources and sinks that one would expect to be found in commercial vineyards (Dokoozlian and Kliewer, 1995; Esteban et al., 1999; Gladstone and Dokoozlian, 2003; Intrieri et al., 1997; Mabrouk and Sinoquet, 1998; Schultz, 2000). For example, the presence of larger sinks in field grown grapevines (roots and permanent structures) apparently could mitigate any depressing effect fruit removal may have on leaf CO_2 assimilation (Edson et al., 1995b; Petrie et al., 2000b; Williams, 1996). Although water deficit can reduce rates of photosynthesis on a leaf area basis, situations of large sources (big canopies) in relation to sinks (fruits) may mitigate the effect of

reductions of whole vine photosynthesis on yield or quality (Kliewer and Dokoozlian, 2000; Poni et al., 1993).

Commercial viticulture in eastern Washington requires irrigation, and the Washington wine industry uses RDI in vineyards as a standard irrigation practice. Knowledge of the integrated response of the vine to water deficit will provide valuable information on the balance between sources and sinks, and its relation to yield, quality, and vine water use. Whole-canopy photosynthesis rates under various RDI strategies will provide information that can be developed into guidelines for growers on the size of canopy required for an expected crop load under RDI. Direct measurements of vine water use by the canopy gas exchange technique also will suggest how RDI may be applied to maximize water savings, an economic and political issue in the semi-arid inland Northwest.

This project was conducted to study net gas exchange (CO_2 and water vapor) by whole canopies of field-grown grapevines, and the dynamics of leaf and cane non-structural carbohydrates under extra water deficits imposed through RDI during the berry growth period to modify grape composition and to save irrigation water. To measure gas exchange, a portable, whole-canopy gas exchange system for several mature, field-grown grapevines was designed and tested (Chapter 2). The system was deployed in a commercial vineyard block to investigate the effects on carbon sequestration (Chapter 3) and vine water use (Chapter 4) of three regimens of RDI that will be described in detail (Chapter 3). Single-leaf measurements of photosynthesis were recorded simultaneously with the collection of leaf tissues for correlation between carbon

fixation and non-structural carbohydrates (Chapter 3). Yield, yield components, and standard indicators of fruit quality were assessed (Chapter 5). These results have the potential to be used in the development of guidelines for growers on the appropriate timing and extent of imposed water deficits via RDI to achieve desirable canopy sizes and ripen crops of high quality fruit demanded by winemakers.

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CHAPTER 2

A PORTABLE WHOLE-CANOPY GAS EXCHANGE SYSTEM FOR SEVERAL MATURE FIELD-GROWN GRAPEVINES

2.1. Abstract

Six flow-through chambers (8 m^3 volume) were built to measure gas exchange (CO_2 and H_2O) of whole vines under deficit irrigation regimes that imposed water stress at different stages of berry development. Chamber design and materials were selected to minimize environmental effects, and to accommodate the trellis of a mature, field-grown vine. A framed design allowed the chambers to withstand sustained winds up to 13 m s^{-1}, overcoming one disadvantage of the balloon-type chambers. At mid-canopy height, 1.6 m, air temperature inside the chamber was no more than 2.5ºC higher than at the same height in the canopy of an unchambered vine. Over 24 h, solar radiation inside the chamber was 90% of ambient. For vines irrigated according to standard industry practice, maximum values of net CO_2 exchange approached 12 µmol m^{-2} s^{-1}, whereas in water-stressed vines the maxima approached only 6.5 µmol m^{-2} s^{-1}. Transpiration among water stressed plants was reduced, with maximum rates at 1 mmol m^{-2} s^{-1} while vines under standard irrigation were at 2.5 mmol m^{-2} s^{-1}. Apparent light saturation for canopy photosynthesis was approximately 1200 µmol m^{-2} s^{-1} PPFD (photosynthetic photon flux density) for vines under standard irrigation, and about 800 µmol m^{-2} s^{-1} PPFD for vines under water stress.

2.2. Introduction

Photosynthesis measurements indicate net primary productivity. Most photosynthesis measurements have been recorded on single leaves and there are several commercial instruments available. However, in large trellised canopies like grapevines and some fruit trees, single leaf measurements often provide incomplete and somewhat misleading data compared to whole plant net primary productivity (Corelli-Grappadelli and Magnanini, 1993; Long et al., 1996; Poni et al., 1997). The photosynthetic capacity among leaves in a grapevine canopy differs due to leaf age (Kriedemann et al., 1970; Schultz, 1993), light exposure (Iacono and Sommer, 1996; Zufferey et al., 2000) and position on the shoot (Hunter and Visser, 1989; Poni et al., 1994), crop load (Naor et al., 1997; Petrie et al., 2000), and leaf prehistory during lamina expansion (Gamon and Pearcy, 1989; Intrieri et al., 1992; Silvestroni et al., 1993; Schultz et al., 1996). Scaling-up from single leaf measurements to whole canopy photosynthesis measurements is not straightforward, as the latter is a measurement of leaves of different age and degree of light exposure, as well as other organs like fruit, shoots, and trunks (Bugbee, 1992). Estimation of leaf area distribution and light extinction by the canopy combined with a directional treatment of incident light is needed to estimate whole canopy photosynthesis from single leaf measurements (De Pury and Farquhar, 1997; Giaglaras et al., 1995). Transpiration is reduced by the boundary layer of the leaf and the entire canopy (Wullschleger et al., 1998). Single leaf measurements can overestimate true whole-plant photosynthesis by as much as 40% in grapevines and fruit trees (Edson et al., 1995; Katerji et al., 1994; Poni et al., 1997). Spatial and

temporal variation in gas exchange and complex interactions between the plant and the environment make extrapolations difficult (Buwalda, 1991; Buwalda et al., 1992; Intrieri et al., 1997; Long et al., 1996; Succi and Magnanini, 1994; Wünsche and Palmer, 1997). In addition, whole canopies may have higher apparent values for light compensation and light saturation points than single leaves (Corelli-Grappadelli and Magnanini, 1993; Francesconi et al., 1997).

Whole canopy enclosure methods are well adapted to small experimental plot sizes (Steduto et al., 2002) and overcome some limitations of single leaf gas exchange by integrating the response of the canopy (Buwalda et al., 1992; Daudet, 1987; Garcia et al., 1990). Good enclosure design minimizes disturbance of the plant's natural environment (Garcia et al., 1990). However, even with a highly transparent cover, an enclosure reduces solar radiation in the chamber as well as gas exchange between the plant and the atmosphere (Corelli-Grappadelli and Magnanini, 1993; Daudet, 1987; Mandl et al., 1973). This "chamber effect" must be minimized by sufficient rates of air exchange and air mixing within the chamber.

Our goal was to construct a gas exchange system that was unattended, moveable, wind resistant, and, using multiple chambers, capable of simultaneously recording measurements from several mature, trellised, field-grown grapevines. Each chamber enclosed a single vine. An open flow-through system was preferable because of the extended measurement period (>24 h) and the difficulty of establishing a perfect seal between plant and atmosphere with a large moveable chamber (Brown, 1988; Wheeler, 1992). Leakage of air from a well-mixed open chamber has little if any effect

on estimated gas exchange, as long as the flow rate of air entering the chamber is measured accurately (Garcia et al., 1990). The system was autonomous in that it was powered by a self-contained gas generator. This paper describes the design and management of a mobile system used to measure net gas exchange of whole field-grown grape vines (*Vitis vinifera* L. cv. Cabernet Sauvignon) under various deficit irrigation regimes in a semi-arid climate. The system can be applied in different areas where studies on whole canopy physiology are of interest.

2.3. Materials and methods

2.3.1. Chamber design

Six open, whole canopy gas exchange chambers were designed, built and tested during 2001 and 2002 for use with mature, field-grown grapevines supported by a multiple-wire trellis. Frequent strong winds (e.g., ≈12.5 m s^{-1}) at the experimental site required the chambers to be framed, which also simplified installation and repositioning in the field. Chamber dimensions were determined by the volume of canopy to be enclosed (maximum canopy height about 2 m, total leaf area about 8 m^2), the distance between rows (2.7 m), the distance between vines (1.8 m), and the trellis structures (3 horizontal wires at 0.80 m, 1.20 m, and 1.60 m). The modular design accommodated the trellis system without modification, allowing selection of any vine in the vineyard without disturbing vineyard management operations or the trellis wires (Weinstock et al., 1982).

Chambers were designed as standing cylinders (2.1 m diameter x 2.0 m height) topped by an open frustum (1.05 m high; Figure 2.1). An ideal shape for a chamber is spherical to maintain the angle of incidence of the radiation onto the chamber as

perpendicular as possible (Corelli-Grappadelli and Magnanini, 1997), while at the same time ensuring sufficient air mixing. A cylinder topped by a frustum improves air circulation by minimizing sharp angles at the outlet and reducing air incursion into the top of the chamber. The chambers were composed of two halves. Support legs (0.45 m high) raised the floor of the chamber above the drip irrigation lines and were retractable to facilitate chamber installation and leveling. Total chamber volume was approximately 8 m^3. Although frame members cast shadows on the canopy, the design was structured to minimize this effect. The ratio between frame area and chamber surface area (excluding floor) was 0.058. A small surface : volume ratio also reduces errors due to water adsorption (Knight, 1992), although the chamber cladding had stringent adsorption specifications. Framing members were lightweight aluminum (3.17 mm thick). A cylindrical chamber was attained with 4 horizontal rings of flat aluminum (25.4 mm wide; Figure 2.1) attached to the vertical parts. The basal ring was 50 mm wide and supported the chamber floor. Angle aluminum (25.4 mm x 25.4 mm) was used for the vertically oriented structures. To seal the chamber and avoid air leaks, closed-cell, adhesive-backed PVC foam strips (2.54 mm wide, 12.7 mm thick, 0.192 g cm^{-3} density) were attached to the vertical aluminum structure of one of the halves along the contact area with the other half. U-shaped aluminum clamps were used to join the halves, compressing the foam strip and sealing the chamber from incursion of outside air. Once installed in the vineyard, the chambers were secured to trellis posts in adjacent rows by 4 ropes. The chamber floor was 5 cm thick polystyrene foam (Styrofoam® ; Dow, Midland, MI). A piece of foam was wrapped and tied around the vine trunk to

approximate a circular section and eliminate irregularities that might cause air leaks at the base of the chamber. A semicircle (0.13 m radius) was cut at the edge of each half floor, so that when the chamber was closed, the flooring sealed the foam around the vine (Figure 2.2 A, B).

The chambers were clad in a biaxially oriented polypropylene (RX 140-Propafilm™ - UCB Films Inc, Smyrna, GA), having a nominal thickness of 0.035 mm, thermal transmission of 70% (2.5 µm – 20 µm), permeability to CO_2 of 4 x 10^{-9} µmol m^{-2} s^{-1} mm^{-1} (thickness) Pa^{-1} (gradient), and water absorption <0.005% over 24 h (Garcia et al., 1990). Propafilm™ has the highest long-wave transmissivity of suitable, commonly available claddings for gas exchange chambers (Hunt 2003), and is inexpensive. The absorbance of Propafilm™ between 200 nm and 1100 nm was measured with a spectrophotometer (DU® 640, Beckman, Fullerton, CA) at 50 nm intervals. Total radiative absorbance of the polypropylene film was about 0.06 to 0.08 in the visible range (400-700 nm) and about 0.05 to 0.06 in the near infrared (700-1100 nm). The cladding did not significantly modify the spectrum of incident solar radiation. Integrated over 24 h, the maximum reduction in irradiance was 10% for either cloudy or clear skies. Double sided tape for aluminum and plastic was used to adhere the film to the aluminum frame (9495 LE, 3M, Minneapolis, MN).

Ambient air was delivered to the chamber by a split capacitor blower (2.7 x 10^4 L min^{-1}; Dayton No. 7086-0201, Chicago, IL) with round inlet (0.16 m diameter) and rectangular outlet (0.14 m x 0.18 m). Galvanized sheet metal pipe was used for drawing ambient air into the blower and directing it to the chamber. Air intake

was 3.5 m above the ground to minimize fluctuations in ambient CO_2. The blower was connected to a transition duct that converted the rectangular exit to a circular section (0.20 m diameter) that then branched into two smaller (0.15 m diameter) delivery ducts beneath the chamber. A butterfly valve inside the 0.20 m diameter pipe was used to vary manually the flow rate (Figure 2.2 C). Air was distributed inside the chamber by two plenums (Figure 2.1), one in each half of the chamber, made of 0.15 m diameter, low density (100 μm thick) polyethylene tubes, with 132 uniformly distributed (19 mm diameter) perforations. The plenums were inclined approximately 35°. This inclination was determined after assessing visually the direction of the air streams during a test of the air circulation pattern inside the chamber by igniting smoke candles (Smoke No. 2B 60 second, Superior Signal Company, Spotswood, NJ) at the inlet pipe of the chamber. Smoke tests also indicated where in the chambers the air samples should be taken.

Air temperatures at the inlet and outlet of the chamber were measured by thermocouples (type T, 24 AWG). Inlet thermocouples were located inside the inlet pipe, after the blower and before the plenum. For the outlet air, shielded thermocouples were hung 0.5 m below the top of the frustum of the chamber. Shielded thermocouples also were hung in the canopy at 1.6 m (mean canopy height). Global irradiance was measured by a pyranometer (LI-200SA, LI-COR, Lincoln, NE) and incident photosynthetic photon flux density (PPFD) by a quantum sensor (LI-190S-1, LI-COR, Lincoln, NE), both located outside the chambers.

Air was sampled continuously from 0.75 m below the chamber frustum at a rate of 15 L min^{-1} with three, dual-head vacuum pumps (Mod. 400-2901, Barnant Company,

Barrington, IL) and was delivered to the infrared gas analyzer (IRGA, Ciras - DC, PP Systems, Haverhill, MA) by bi-layer tubing consisting of polyethylene liner and a shell of ethyl vinyl acetate (6.35 mm I.D., 7.93 mm O. D., Bev-A-Line®, Thermoplastic Processes Inc., Stirling, NY). Fittings were polypropylene (United States Plastic Corporation, Lima, OH).

Ambient [CO_2] and H_2O pressure were measured in air drawn continuously by a vacuum pump (UN815, KNF Neuberger INC, Trenton, NJ) from the middle of the vineyard at a height of 3.5 m and at a rate of 15 L min^{-1}. All sampled air was under positive pressure from the pumps to the IRGA. Concentrations of CO_2 and H_2O pressure at the chambers' outlets were measured by the IRGA. The measurement range was from 0 to 2000 µmol mol^{-1} with a precision of 0.2 µmol mol^{-1} at 300 µmol mol^{-1} for CO_2. For H_2O, its range was 0 to 75 mb with a precision of 0.02 mb at 10 mb. A gas multiplexer (GHU 161, ADC Bioscientific Ltd., Hoddesdon, England) switched the sample streams among the 6 chambers. All data were recorded by datalogger (Model CR7, Campbell Scientific, Logan, UT). The IRGA was zeroed every 30 min and calibration was checked after each field run using certified gas (359 and 305 ppm of CO_2, Air Liquid™, Houston, TX) and a humidity calibrator (PP Systems, Haverhill, MA).

2.3.2. System operation and testing

The 6 chambers (Figure 2.3) operated simultaneously, powered by a gasoline generator (5 kW; Onan Marquis 5000, Cummings, IN). A field trailer housed all instrumentation (e.g., IRGA, gas multiplexer, air sampling pumps, and datalogger). Air from one chamber at a time was directed to the IRGA by the gas multiplexer during a

2 min period. Air from the other 5 chambers was by-passed and vented outside. During the final 30 s of the 2 min interval, the datalogger recorded the IRGA output signal every 2.5 s, then computed a 30 s average. Every 2 min, the sample air stream from the next chamber in sequence was directed to the IRGA. Thus, data from each chamber were collected every 12 min. The time taken for the air sampled from the chamber to reach the gas multiplexer (60 m between the chamber and the gas multiplexer) was 7.6 s, and to the IRGA it was 20 s. Any vine within 60 m of the trailer housing the instruments could be measured.

On two days with clear skies (DOY 298, 2001, and DOY 192, 2002) temperatures inside and outside a chamber were measured at different heights above the ground (0.90 m, 1.40 m, 1.90 m, 2.40 m, and 2.90 m) with shielded thermocouples (type T, 24 AWG) mounted to a wooden frame. Temperatures were recorded every 5 s and averaged every 2 min. Irradiance was measured with two pyranometers (LI-200SA, LI-COR Inc, Lincoln, NE) installed inside and outside the chamber on DOY 299 (cloudy) and DOY 300 (clear) of 2001. Data were recorded every 5 s and averaged every 12 min by datalogger. Wind speed at the site was measured at 2 m height by a 3-cup anemometer (12102D, R. M. Young, Traverse City, MI). Both 12 min averages and daily maxima were recorded.

Because net gas exchange in an open system is proportional to the flow rate of air across the canopy it is critical to measure the flow rate accurately. Air flow through the chambers was monitored continuously by differential pressure sensors (Model PX170-07DV, Omega Eng. Inc., Stanford, CT) installed in each chamber and calibrated

against the flow as measured by a gas dilution technique (Garcia et al., 1990). Briefly, at the inlet pipe of the chamber before the blower, 98% pure CO_2 was injected with a highly accurate flowmeter (±5%, FM-1050 Series, Matheson, Montgomeryville, PA) specifically calibrated for CO_2. Before and after the injection point, air was sampled at 15 L min^{-1}. Post-injection sampling was beyond the blower but ahead of the plenums. This calibration procedure allowed us to calibrate the blower with the vine enclosed in the chamber, which is important because the plant can change a calibration curve performed on an empty chamber (Ham et al., 1993). The flow calibration was re-checked in all chambers at the end of every 7-day measurement run. Blower speed (rpm) was measured with a photo-tachometer and stroboscope (Model 4618258, Extech Instruments, Tampa, FL). With the butterfly valve completely open, flow through the chamber was about 16 m^3 min^{-1} (maximum flow), resulting in two chamber volumes being exchanged per minute. With the valve completely closed, flow was about 2.8 m^3 min^{-1} (minimum flow). The flow was kept at maximum during the day and at minimum during the night.

Net gas exchange rates were calculated from the differences in [CO_2] and H_2O pressure between the air exiting and entering the chamber, adjusted for the rate of air flow across the chamber. The decline in molar [CO_2] by photosynthesis is very small compared to that of H_2O addition, so the reduction in [CO_2] was not considered in transpiration calculations (Field et al., 1989). To express net gas exchange rate on the basis of canopy leaf area, total leaf area per vine was estimated by a 3 step process: (1) leaf width was regressed against leaf area for a sample of 200 leaves ($r^2 = 0.96$); (2) the

widths of all leaves on a sample of shoots (n = 8) were measured, and individual leaf areas computed from the relationship established in (1); and (3) leaf area per vine was calculated from the average leaf area per shoot in the sample and the recorded number of shoots per vine.

2.4. Results and Discussion

2.4.1. Chamber design and operation

Due to frequent, strong winds at the experimental site, a balloon type chamber was impractical. The frame and the ropes that attached the chambers to the trellis made them stable enough to resist sustained winds over 13 m s^{-1} and higher gusts. A framed chamber also allowed us to maintain chamber shape and volume regardless of blower output or wind variation, thereby minimizing temporal variation in the vine boundary layer and reducing the incidence of air pockets, an advantage over balloon-style enclosures. Visual examination of smoke infiltration into the chamber showed thorough mixing in <40 s. The light weight frame allowed two people to move and secure the chamber from vine to vine in less than 30 min.

Flow rates to the chamber were selected according to three criteria: (1) the expected acceptable rise in air temperature in the chamber; (2) the acceptable reduction in [CO_2] between inlet and outlet; and (3) the available power for the blowers and instruments.

Expected ΔT_a according to flow rate and chamber design can be approximated by (Garcia et al., 1990):

$$\Delta T_a = H \times \left[\left(\frac{f_a \times M \times c_p}{A_b} \right) + \left(\frac{A_w \times \rho_a \times c_p}{2 \times r_H \times A_b} \right) \right]$$

where H is heat absorbed (W m^{-2}); ΔT_a is the temperature difference between inside and outside of the chamber (ºC); f_a is air flow (mol s^{-1}); M is the molecular weight of air (29 g mol^{-1}); c_p is the specific heat of air (1.01 J g^{-1} $ºC^{-1}$); A_b is basal area of chamber (m^2); A_w is wall area of chamber (m^2); ρ_a is the density of air (1.183 x 103 g m^{-3} at 25 ºC and 100 kPa); and r_H is resistance to heat transfer for wall surfaces under forced convection (s m^{-1}). Heat absorbed (H) was calculated following Campbell and Norman (1998). A rise in temperature of 3 ºC (Figure 2.4) was estimated for two chamber volumes per minute. A reduction in transpiration due to vine water stress would reduce the fraction of H dissipated by transpiration, consequently increasing the temperature of the chamber parts and the enclosed vine. For criterion (2), a depletion of 30 µmol mol^{-1} CO_2 between inlet and outlet was avoided, the practical level used in closed systems (Long and Hällgren, 1989). Criteria (1) and (2) are related as higher flow through the chamber (i.e., more chamber volumes exchanged per minute) will reduce the rise in temperature throughout the chamber, but at the same time also will reduce [CO_2] depletion. The depletion must be large enough to be detected by the IRGA, but not so large that [CO_2] is reduced significantly below ambient.

2.4.2. Chamber microclimate

On DOY 192, 2002 (Figure 2.5), maximum temperatures inside the chamber at the highest and lowest positions were 47.2ºC (2.9 m) and 42.5ºC (0.9 m). Outside

temperatures at the same positions were 42.5ºC and 40ºC, respectively. On DOY 298, 2001, also a day with clear skies (data not shown), maximum air temperatures (T_a) inside the chamber were 19ºC at the highest (2.9 m) thermocouple position and 15ºC at the lowest position (0.9 m above ground). Outside temperatures at the same heights were 15ºC and 13.8 ºC, respectively. The maximum ΔT_a consistently occurred at the two highest measurements positions (2.40 m and 2.90 m above ground) and were under 5ºC, regardless of absolute ambient temperatures. At measurement heights corresponding to the canopy (1.90 m and 1.40 m above ground) and at maximum flow rates (2 chamber volumes min^{-1}), air exchange was sufficient to keep T_a at canopy height within 2.5 ºC of that in an un-enclosed vine canopy during a warm day (40 ºC) with clear skies.

Higher T_a inside the chamber modifies gas exchange rates in at least two ways: (1) a direct effect of temperature on photosynthesis and (2) an indirect effect of temperature on vine physiology by its influence on leaf temperature and the vapor pressure deficit between leaf and air (VPD_{la}). Temperature effects on photosynthesis in grapevines have been better established at the single leaf level (Downton et al., 1987; Gamon and Pearcy, 1990a; Jacobs et al., 1996; Williams et al., 1994). At the whole-canopy level, the influence of temperature is not as clear as in single leaves, particularly in this case where the maximum ΔT_a at canopy height was 2.5-3.0ºC. In whole canopies, leaves are under different levels of irradiance, water status, and temperature, so the exact chamber effect on whole-canopy photosynthesis is not easy to predict. In single leaf measurements of *Vitis californica,* photosynthesis declined severely in high-light

leaves only if T_a exceeded 45°C (Gamon and Pearcy, 1990b). At the same high T_a, shaded leaves did not show the same degree of reduced photosynthesis.

Air temperature influences physiology indirectly via leaf temperature and stomatal behavior by way of VPD_{la} (El-Sharkawy et al., 1985; Farquhar and Sharkey, 1982; Lawson et al., 2002; Schulze, 1986). Assuming the water vapor pressure of the air (e_a) entering the chamber is ambient, and considering a relative humidity (RH) on a summer day of 0.25, with T_a of 40°C, the air VPD will be 5.53 kPa. An increase in T_a of 3°C due to the "chamber effect" would increase VPD_{la} to 6.87 kPa, 1.34 kPa higher than for a non-enclosed vine, if the increase in T_a is accompanied by the same increase in leaf temperature. Using the Penman-Monteith equation (Monteith and Unsworth, 1990) to estimate canopy transpiration, an increase in ΔT_a of 3°C would result in an approximately 2% increase in transpiration, which we considered a small environmental modification caused by the chamber.

In *Vitis* species an increase in VPD_{la} tends to decrease stomatal conductance (g_s; (Düring, 1987; Jacobs et al., 1996; Jarvis and Morison, 1981). In some single-leaf photosynthesis measurements on *Vitis* species (Chaves et al., 1987; Downton et al., 1987) both T_a and VPD_{la} varied simultaneously. This, together with the occurrence of stomata in patches on grapevine leaves, could make it difficult to interpret data and scale up from single leaf to whole canopy (Schultz et al., 1996). Jacobs *et al.* (1996) used a model that included net photosynthesis (A_n) and g_s to explain that VPD_{la} may have a larger effect on g_s than on A_n. With increasing VPD_{la}, the difference between ambient [CO_2] and leaf internal [CO_2] increases, and under these circumstances net CO_2

assimilation in grapes apparently is less affected than is transpiration (Chaves et al., 1987; Williams et al., 1994). The chamber effect, via an increase in the vine's boundary layer, results in leaves being less coupled to VPD_{la}, particularly if the ventilation rate through the chamber is less than that around an un-chambered vine (Jarvis and McNaughton, 1986; Wullschleger et al., 1998).

2.4.3. Net gas exchange

During mid-season under clear skies at an air exchange rate of two chamber volumes per minute (16 $m^3 min^{-1}$), the maximum differences in [CO_2] and H_2O between air entering and exiting the chamber were around 12 µmol mol^{-1} and 2.5 mb, respectively. Other values reported for open-system chambers are between 4.5 and 40 µmol mol^{-1} [CO_2] (Poni et al., 1997, less than 40 µmol mol^{-1}, Miller et al., 1996, between 15 and 35 µmol mol^{-1}, Corelli-Grappadelli and Magnanini, 1993, 30 µmol mol^{-1}, Wünsche and Palmer, 1997, 4.5 µmol mol^{-1} [CO_2] differential). In this experiment under standard irrigation, 70% of the calculated vineyard evapotranspiration (ET) was replaced weekly by drip irrigation. Stressed vines received irrigation equivalent to 35% of ET. Net CO_2 exchange was higher for vines under standard irrigation (maximum rate about 12 µmol $m^{-2} s^{-1}$) than under water stress (maximum rate about 6.5 µmol $m^{-2} s^{-1}$). Transpiration in both irrigation schemes responded to transient changes in solar radiation (Figure 2.6). Transpiration was higher in vines under standard irrigation (maximum about 3 mmol H_2O $m^{-2} s^{-1}$) than in stressed vines (about 1 mmol H_2O $m^{-2} s^{-1}$). Photosynthetic response to PPFD also differed between standard irrigated vines and stressed vines (Figure 2.7). Apparent light response curves were built combining the

data from the quantum sensor and net CO_2 exchange from predawn to noon. The apparent light response curve of a vine under water stress saturated at one-third lower PPFD (about 800 μmol m^{-2} s^{-1}) than did a vine under standard irrigation (about 1200 μmol m^{-2} s^{-1} PPFD). At PPFD levels > 800 μmol m^{-2} s^{-1}, photosynthesis may have been limited in the water stressed vines by water status rather than by light *per se*. Water use efficiency (WUE), the ratio of net CO_2 exchange (mmol m^{-2} s^{-1}) to transpiration (mol m^{-2} s^{-1}) averaged 4.0 for vines under standard irrigation and 6.4 for stressed vines. Other work in grapes, using single-leaf measurements, reported WUE between 1.2 to 3.2 (Poni et al., 1994) and between 1 and 8 (Schultz, 2000) depending on the leaf position along the shoot, water status, variety, time of day, and time of year. Differences in transpiration between irrigation levels may have caused the temperature of the air exiting the chamber around stressed vines to be 1 or 2ºC higher than the air exiting a chamber around a standard irrigated vine.

2.5. Conclusion

A whole-canopy gas exchange system was designed and used to measure photosynthesis and transpiration simultaneously on several mature field-grown, trellised grapevines. Modifications to light and temperature inside the chamber were minimal, with canopies no warmer than 2.5ºC above ambient and irradiance reduced by less than 10%. Net CO_2 and H_2O exchange rates between vines under different levels of imposed water stress were different. Maximum net CO_2 exchange rates were about 12 μmol m^{-2} s^{-1} for standard irrigated and about 6.5 μmol m^{-2} s^{-1} for water stressed vines. Maximum transpiration rates were under 3 mmol m^{-2} s^{-1} for vines irrigated by the industry

standard method, and about 1 mmol m^{-2} s^{-1} for vines irrigated at 50% of the standard amount. Differences in photosynthetic light responses were detected between irrigation levels. Because the chambers were portable and lightweight, the whole system (chambers and instrumentation) was quickly and easily assembled in the vineyard in under 20 man-hours, allowing for measurements during short periods. Moving a chamber from vine to vine along the same row required less than 1 man-hour. No modification to the original canopy of the grapevine or the trellis system was required (i.e., pruning, shoot removal, or cutting wires). The system can control at least 6 chambers depending on available power and labor. The cylindrical design of the chamber promoted air circulation and rapid mixing of the air inside the chamber, without formation of air pockets. High rates of air pumping (15 L min^{-1}) made it possible to choose any vine up to 60 m from the instrument trailer, thereby avoiding edge effects. Because the aluminum frame improved chamber resistance to wind, the chambers could be deployed during more days of the year, thus increasing the frequency of measurements and allowing for sufficient replication. The system designed here proved to be a suitable research tool for measuring whole-plant gas exchange and for understanding the effects of different environmental conditions and viticultural practices on whole vine physiology.

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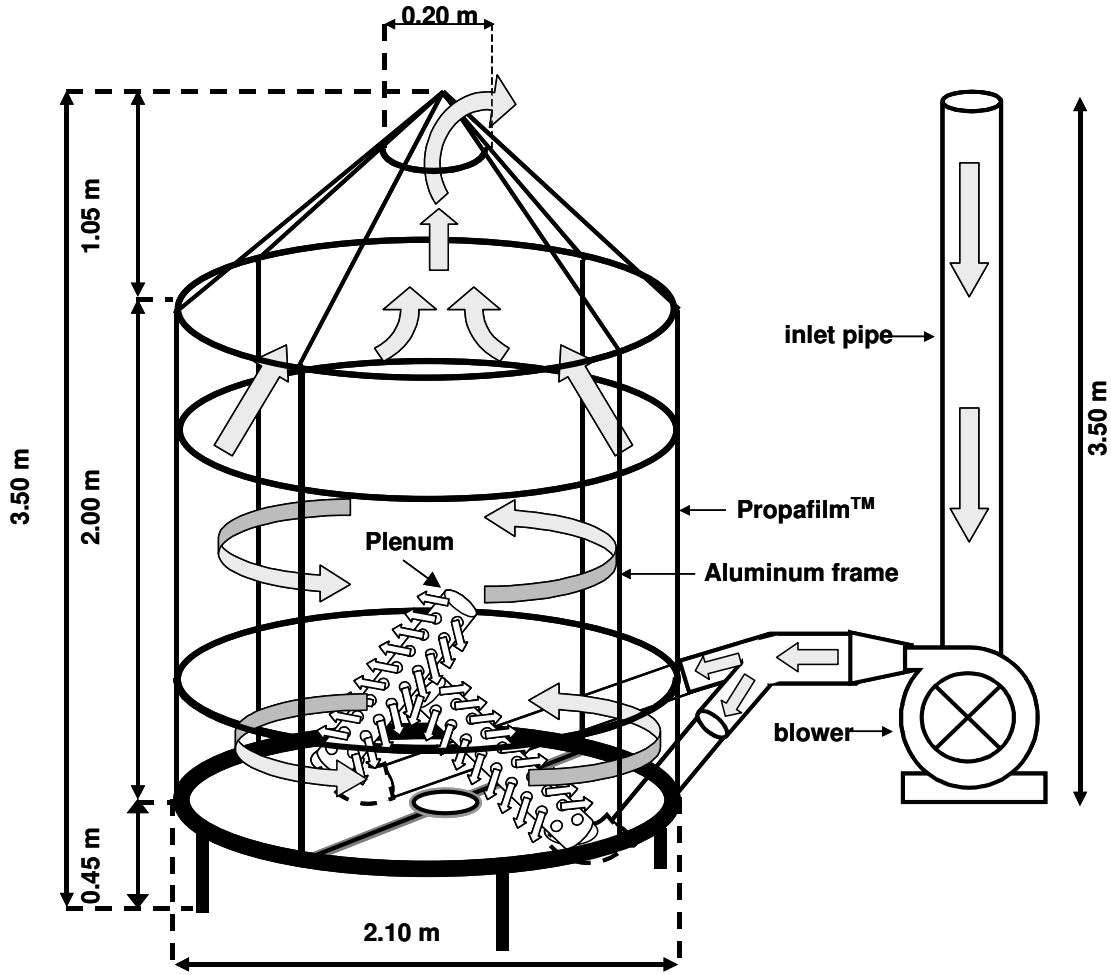

Figure 2.1: Schematic diagram of whole-canopy gas exchange chamber used on trellised, field-grown grapevines. Arrows denote air flow.

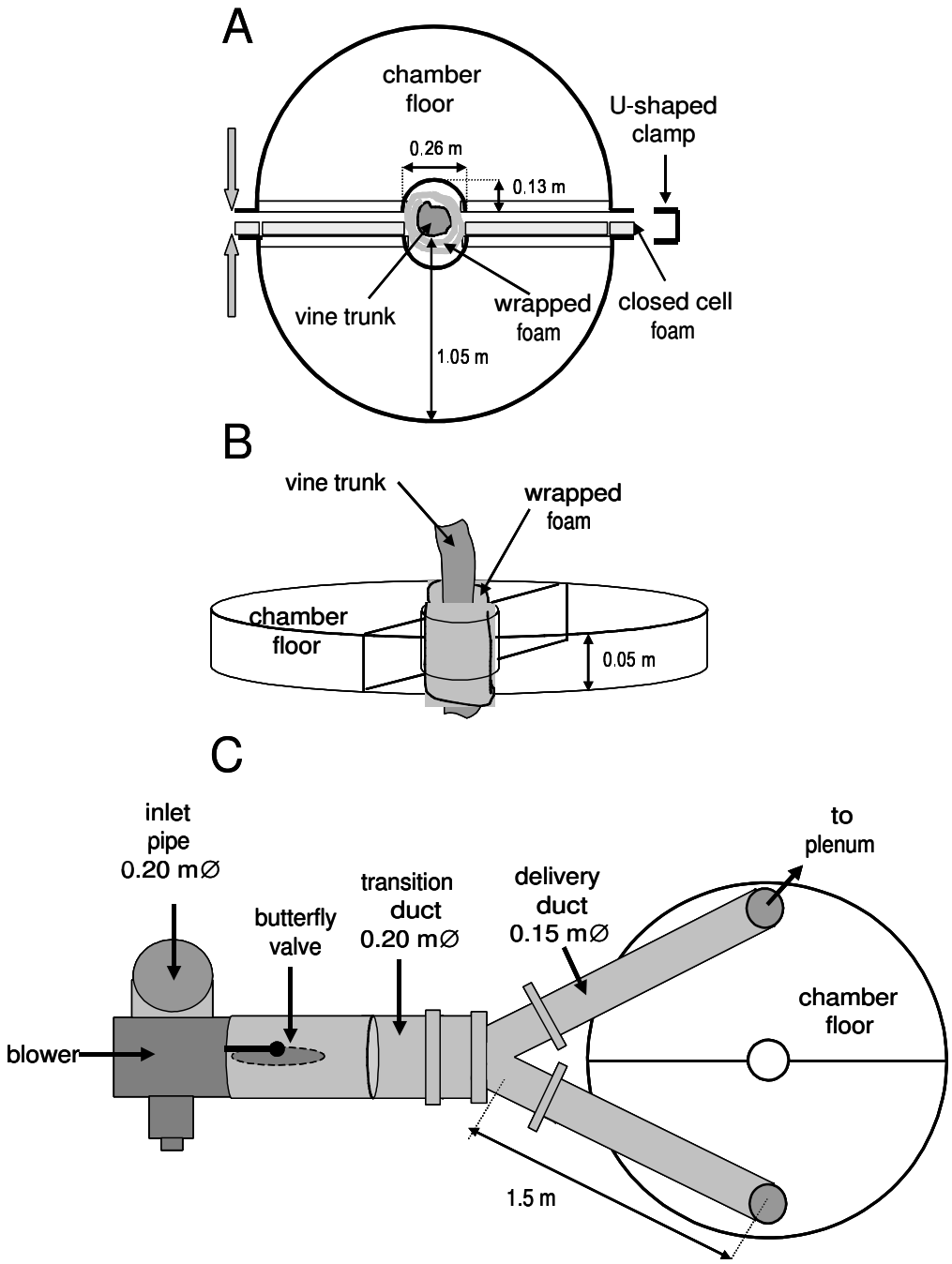

Figure 2.2: Schematic diagrams of the chamber floor (not drawn to scale). A: top view; B: side view; C: details of the air conducting system.

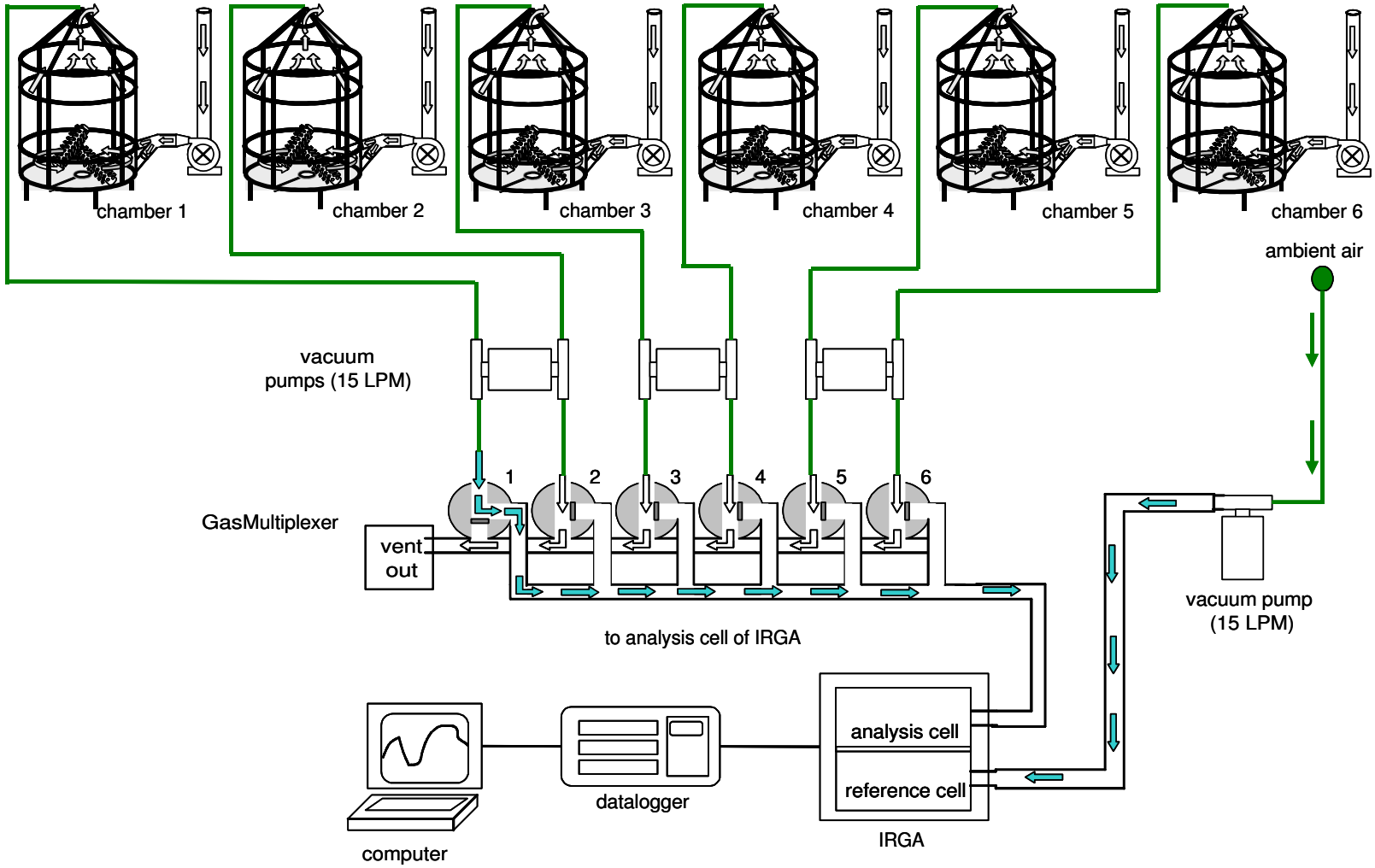

Figure 2.3: Schematic diagram of whole-canopy gas exchange system for several, field-grown vines.

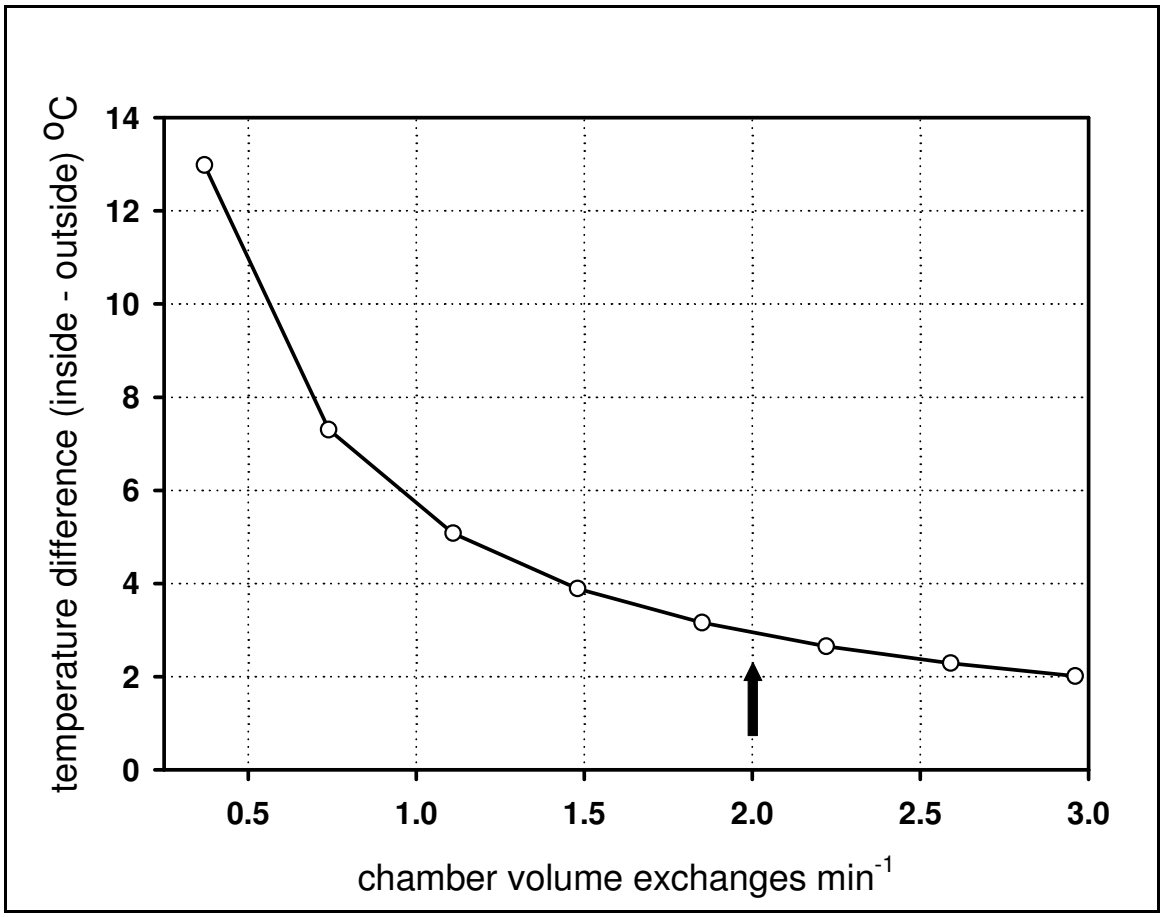

Figure 2.4: Estimation of the temperature difference between outside and inside the chambers based on the flow rate through the chamber. Arrow indicates the typical daytime ventilation rate. Calculations for DOY 157, under clear skies and 35°C ambient air temperature. Transpiration was assumed to dissipate half of the heat absorbed.

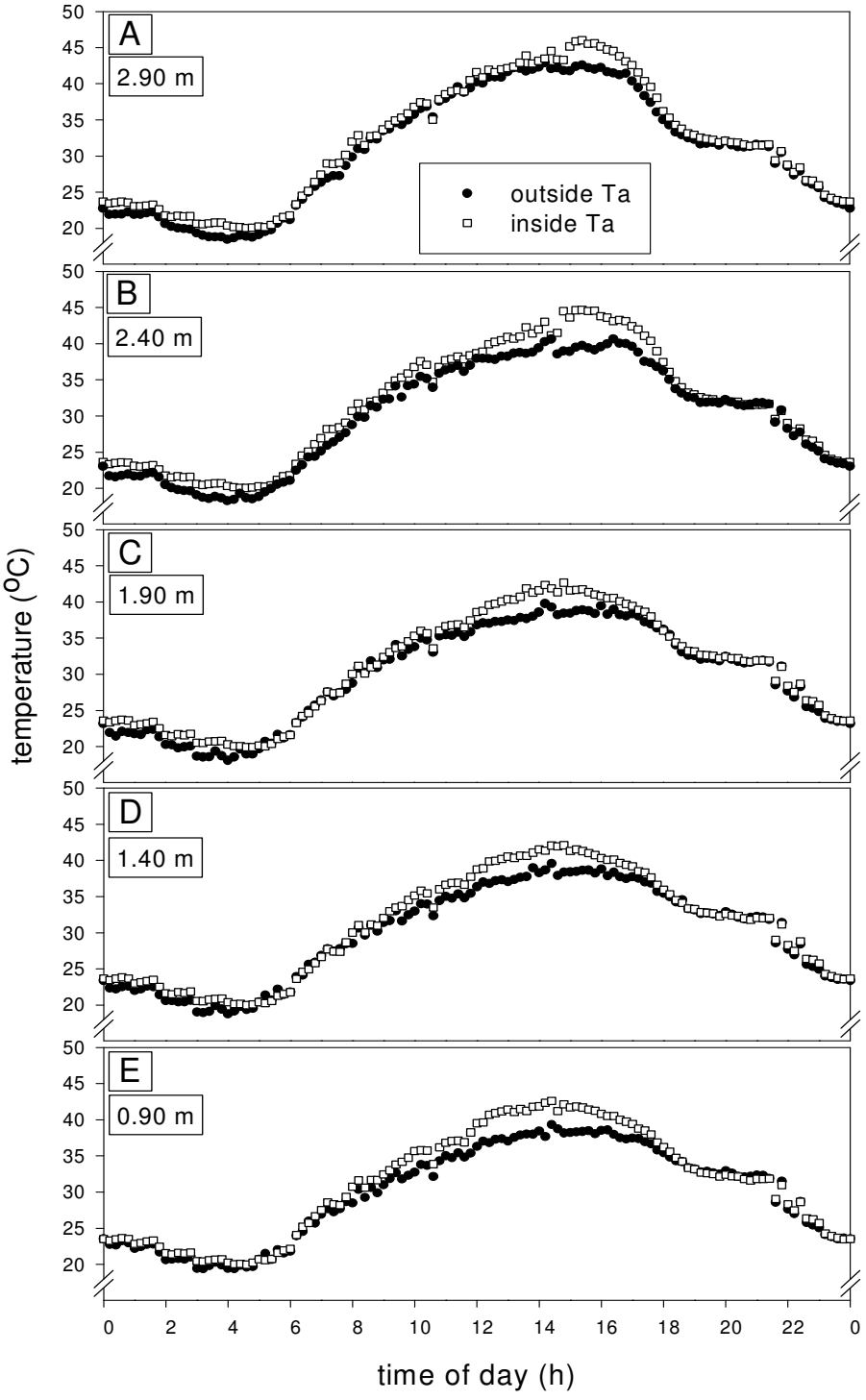

Figure 2.5: Diurnal pattern of air temperature inside and outside the chamber at 5 different heights above ground. Data were collected on DOY 192, 2002. Flow through the chamber was 16 m^3 min^{-1} (about two chamber volumes per minute).

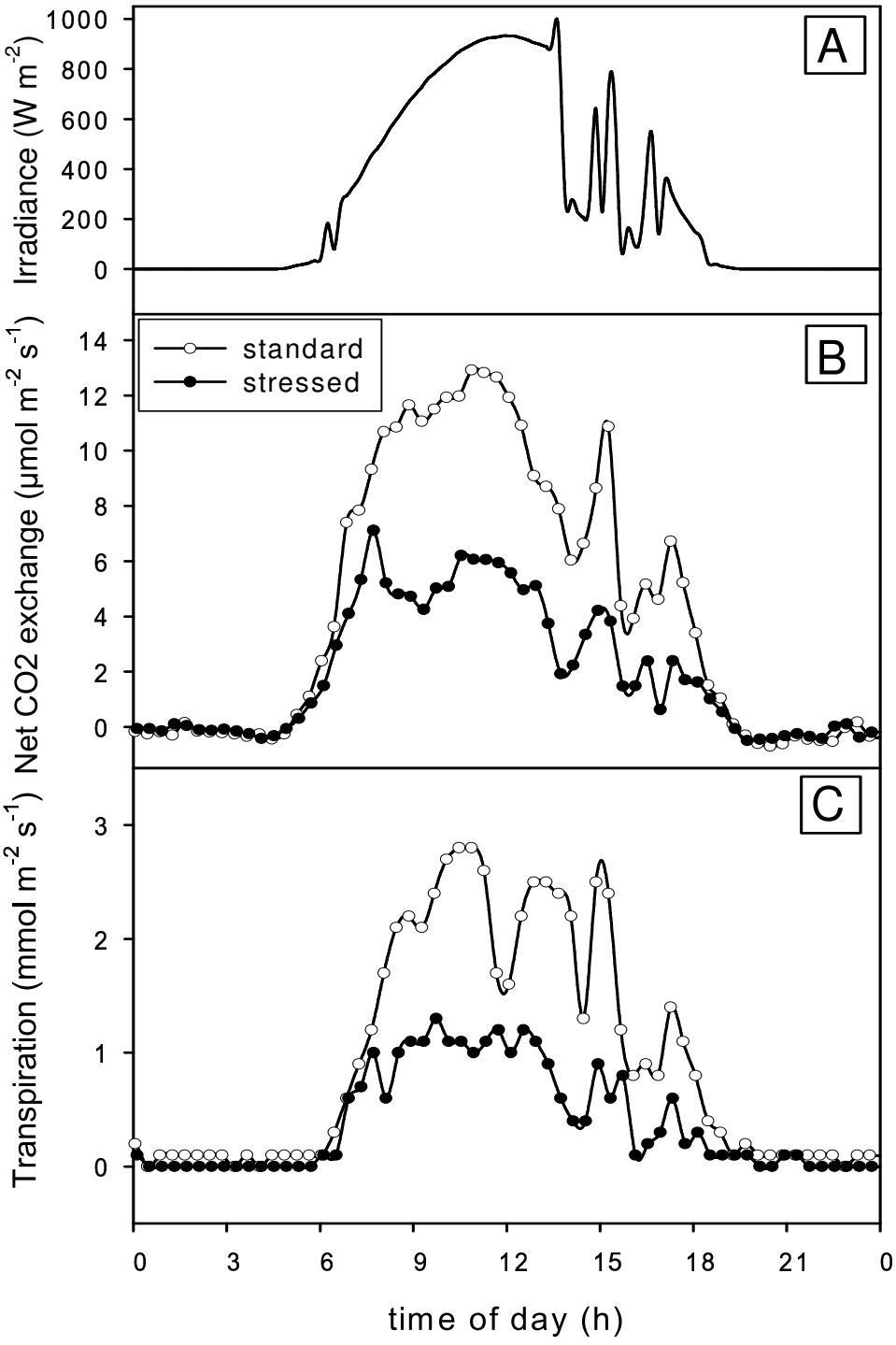

Figure 2.6: Diurnal curve of global irradiance (A), net CO_2 exchange rate (B), and transpiration (C) for an exemplary vine under standard irrigation and for a vine under imposed water stress. DOY 218, 2002.

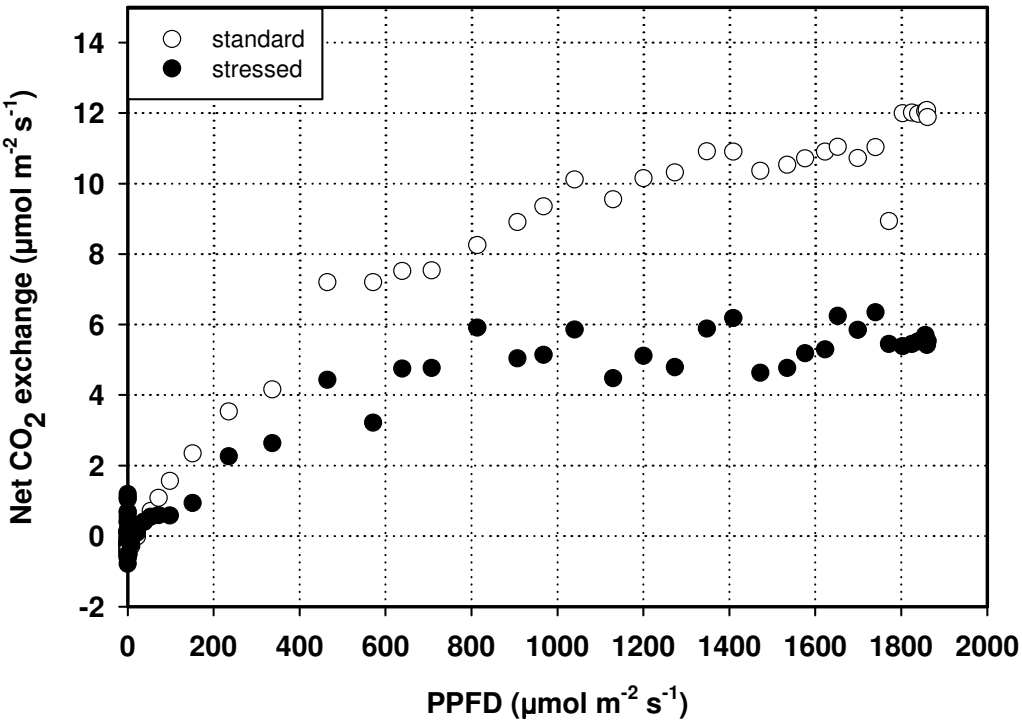

Figure 2.7: Apparent light response of photosynthesis for an exemplary vine under standard irrigation and for a vine under imposed water stress. DOY 218, 2002.

Figure 2.8: View of one gas exchange chamber installed in the vineyard.

CHAPTER 3

GRAPEVINE PHOTOSYNTHESIS AND NON-STRUCTURAL CARBOHYDRATES UNDER REGULATED DEFICIT IRRIGATION

3.1. Abstract

In order to improve grape fruit composition to satisfy winemakers and wine industry demands, and to save water, Washington wine growers have adopted regulated deficit irrigation (RDI) as a standard management practice in their vineyards. Reduction in irrigation affects key physiological processes like photosynthesis, and although water deficit effects on photosynthesis have been widely studied at the single-leaf level, to better understand these effects on vineyard productivity and fruit quality, whole-canopy research was conducted during growing seasons of 2002 and 2003. A six-chamber, mobile field laboratory was used to measure whole-canopy photosynthesis from field-grown, own-rooted, drip irrigated *Vitis vinifera* L. cv. Cabernet Sauvignon that were under three regimes of RDI: 1) standard RDI (70% of full vine evapotranspiration, FVET, was replaced weekly); 2) early deficit (35% of vine FVET was replaced weekly between fruit set and veraison); and 3) veraison deficit (35% of vine FVET was replaced weekly between veraison and harvest). When not under 35% deficit, vines in scenarios #2 and #3 were irrigated according to standard RDI practice. Whole-canopy measurements were conducted during 24 h at fruit set, pre- and post-veraison, and pre- and post-harvest. At the same time and on adjacent vines, single-leaf measurements of photosynthesis and leaf tissue for non-structural carbohydrate analysis were collected.

Large reductions were observed in net CO_2 fixation in vines under additional water deficit with respect to those under standard RDI. In the pre-veraison period, 'early deficit' vines fixed during 24 h up to ≈45% less CO_2 than did vines under standard RDI, while during the post-veraison period, vines under additional water defict fixed up to ≈33% less CO_2. A similar reduction was observed in sunlit, single leaves measured independently of the whole-canopy chambers, but poor correlation was obtained between the two types of measurements. Small differences were detected in daily whole-canopy net CO_2 fixation before and after harvest, when daytime temperatures were lower and day length was shorter. Leaf starch concentration in vines under additional water deficit was up to 32% lower in 2002 at pre-veraison during the morning compared to those under standard RDI, while in 2003 leaf starch concentration during the afternoon was up to 32% lower at pre-veraison and up to 20% lower at post-verasion in vines under additional water deficit compared to those under standard RDI. Average water savings were achieved of 40% in the early deficit regimen and of 23% in the late deficit regimen with respect to standard RDI.

3.2. Introduction

Vineyards in eastern Washington experience hot, dry summers and a ripening period characterized by warm days and cool nights. However, the growing season is short and extreme winter temperatures periodically cause significant cold injury to dormant buds and permanent vine tissues. Thus eastern Washington viticulture historically was classified as “cool climate,” but more recently the viticulture industry has recognized that Washington’s unique climatic challenges make it less meaningful to

adopt a strict warm- or cool-climate classification. In practical terms, the climate of eastern Washington demands that vineyard managers create a blend of “cool” and “warm” climate practices that is appropriate for their sites.

Although developed specifically for California, Winkler’s classification (Winkler et al., 1974) based on heat summation and expressed as degree-days (D.D., base temperature 10°C), locates Prosser, in the Yakima Valley, in Region II (1360 D.D.). The growing season is short (158 frost free days) and hot. August is the warmest month, with a mean temperature of 22.6°C, mean maximum temperature of 31.7°C, and daily maximum temperatures often reaching 43°C. January is the coldest month, with a mean temperature of 2.3°C, mean minimum temperature of -0.7°C, and some daily minimum temperatures falling below -21°C. Daily differences between mean maximum and mean minimum temperatures during the months of berry growth (July, August, September) are on average about 18°C. Average cumulative “Class A” unscreened pan evaporation is 1266 mm for April to October (Naor and Wample, 1994), while maximum daily reference evapotranspiration is 9 mm day^{-1} (Evans et al., 1993). Annual rainfall averages $\approx$200 mm, occurring mainly during winter months. There are 15.8 h of daylight on June 21 (DOY 172; U.S. Naval Observatory, www.usno.nav.mil) and total global irradiance averages 29 MJ m^{-2} on the same day (1998-2003 mean; Public Agriculture Weather System).

The combination of a short growing season, low rainfall, hot summers, and cold winters challenges grape growers in several ways. Due to the large deficit between evapotranspiration and rain, winegrapes can only be cultivated commercially by applying

irrigation. Furrow, sprinkler, and drip irrigation methods have been used, although the trend is towards drip irrigation. This last method gives grape growers much flexibility in irrigation scheduling. One of the challenges of the Washington wine industry is to determine the timing and amount of irrigation applied to balance canopy productivity with crop load, achieve the fruit characteristics desired by winemakers, and reach the end of the season with a plant prepared for the cold winter (i.e., avoiding any delay in the lignification of canes and replenishment of reserves in the permanent structures of the vine). This challenge has been approached partly by regulated deficit irrigation (RDI), a practice of managing soil water supply to impose predetermined periods of plant or soil water deficit to elicit some desirable responses in plants (Behboudian and Singh, 2001).

The effects of water deficits on grapevines have received considerable attention in the last few years, both in what are called “old” wine countries (e.g., de Souza et al., 2003; Escalona et al., 2003; Flexas et al., 2002; Flexas et al., 1999; Iacono et al., 1998; Maroco et al., 2002; Medrano et al., 2003; Palliotti and Cartechini, 2000; Rodrigues et al., 1993) and “new” wine countries (Dry et al., 2000; Liu et al., 1978; Murillo de Alburquerque and Carbonneau, 1997; Naor and Wample, 1994). One of the reasons for this interest is the tremendous influence of water deficits on all plant processes (e.g., photosynthesis), and particularly on berry yield and quality.

Much of the vegetative growth in grapevines occurs between budbreak and anthesis. During this period in irrigated viticulture, if winter rains have filled the soil profile, vines may be allowed to draw from this reservoir with no supplemental irrigation. In places where there is too little winter rain, irrigation is applied at the beginning of the

growing season in such a way that controlled water deficits are imposed to limit shoot growth and achieve a canopy area that will ripen the crop. During the berry growth period (i.e., fruit set to harvest) different effects on berry composition have been observed whether water deficits were imposed between fruit set and veraison, or between veraison and harvest (Matthews and Anderson, 1988). Water deficits applied between fruit set and veraison have caused an irreversible reduction in final berry size (Matthews and Anderson, 1989; McCarthy, 1997), due mainly to a reduction in mean cell size (Ojeda et al., 2001), which has been shown to increase the skin:pulp ratio, changing must composition. Clusters are weak sinks until about fruit set (Hale and Weaver, 1962), with the fruit cluster becoming a much stronger sink after veraison (Candolfi-Vasconcelos et al., 1994). Water deficits applied between veraison and harvest have had less effect on berry size, but still some effect on berry composition, with the effects being explained primarily by the reduction in berry size, and secondarily by modifying flavonoid biosynthesis (Kennedy et al., 2002; Ojeda et al., 2002). If an imposed water deficit during the growth period of the berry reduces photosynthetic rates on a per vine basis, not only will fewer carbohydrates be available for the different vine sinks (e.g., berries), but also a different partitioning pattern of those carbohydrates could occur (e.g., proportionally more carbohydrates could be directed to the roots; Palliotti and Cartechini, 2000). The effects of a reduction in photosynthesis on berry size and composition depend on the magnitude of that photosynthetic reduction, the ratio between the sources (leaves) and sinks (fruit), and competition among sinks. Because of the different effects observed between water deficits imposed before and after veraison, and the

importance of the source:sink ratio in this response, it is necessary to study and measure photosynthesis of whole canopies of field-grown vines to better understand the physiological effects of RDI in vineyards.

Most research in grapevines on the effects of water deficit on photosynthesis has relied on measurements recorded from single leaves or small areas (2 to 3 cm^2) on a single leaf, a decision determined mainly by the type of instrument used (de Souza et al., 2003; Escalona et al., 2003). The lack of good correlation between single-leaf and whole-vine measurements (Edson et al., 1993; Miller et al., 1997; Poni et al., 1997) suggests that it is inadequate to infer whole-plant responses from measurements of photosynthesis on single leaves. Other studies of grapevines have measured whole-canopy photosynthesis, but mainly in relation to training systems and canopy manipulations (Intrieri et al., 1997; Intrieri et al., 1998; Katerji et al., 1994; Petrie et al., 2003). The only study of water deficit and photosynthesis on whole canopies in field-grown vines compared extreme conditions: irrigated vs. non-irrigated (Ollat and Tandonnet, 1999), which is a logical approach in a non-irrigated viticultural region. In irrigated viticulture as in eastern Washington, a non-irrigated option usually is not feasible. With this in mind, a system to measure photosynthesis of whole canopies in the vineyard (chapter 2, this volume) was used in a deficit-irrigation experiment in which RDI was applied at different times and intensities, between fruit set and harvest. In eastern Washington, RDI is a standard practice for growers of premium and ultrapremium winegrapes. It is applied with the primary goal of improving wine quality.

The objective of this experiment was to determine the effect of water deficits

imposed by RDI at different periods during berry growth, on whole-canopy photosynthesis of field-grown grapevines. To evaluate the suitability of estimating whole-canopy photosynthesis based on single-leaf measurements, single-leaf photosynthesis was also measured and compared with whole-canopy results. To evaluate whether different intensities of RDI affected the primary products of photosynthesis, leaf non-structural carbohydrates were measured during the berry growth period and after harvest. A secondary benefit of the study will be to better understand the consequences of RDI management on aspects of whole vine physiology related to carbon balance and to support more informed irrigation decisions by vineyard managers.

3.3. Materials and methods

3.3.1. Site and treatments

The study was conducted within an experiment that had been initiated in 1999 in a 4-ha block at the Canoe Ridge vineyard of Ste. Michelle Wine Estates (45.88° N, 119.75° W, 125 m above mean sea level, west of Paterson, WA). Vines, *Vitis vinifera* L. cv. Cabernet Sauvignon, drip-irrigated and own-rooted, were double-trunked, trained to a bilateral cordon at 1.06 m on the bottom wire of a two-wire sprawl trellising system, and spur pruned with 20 to 23 buds per m of row. The vineyard was planted in 1992 on a uniformly deep (≈1 m) Burbank loamy fine sand (sandy-skeletal, mixed mesic Xeric Torriorthents) at 1.8 m x 2.7 m (vine x row spacing) in a north-south row orientation with a 14% slope facing south. Soil field capacity was 14.6% by volume and permanent wilting point was estimated as 7.1% by volume. Soil water content was monitored up to

1.0 m depth weekly by neutron probe. The irrigation system had one drip line per row with three, 1.8-L h^{-1} pressure compensated emitters for every two vines.

The vineyard was irrigated early in the growing season (ca. April) if winter rains were insufficient to adequately fill the soil profile to field capacity. Full vine evapotranspiration (FVET) was calculated from reference evapotranspiration (ET_o) using data collected by the PAWS (Public Agriculture Weather System) weather station at Alderdale, WA (10 km west of the site), and a crop coefficient for Cabernet Sauvignon determined in eastern Washington (Evans et al., 1993; see appendix; Figure A3.1). Water consumption for vines under RDI was estimated as 70% of FVET in 2002 and 60% of FVET in 2003. Between budbreak and fruit set, irrigation was withheld until shoots were 0.9 to 1.2 m long and the rate of shoot growth was minimal. The target shoot length was chosen with an expectation that each shoot would ripen an average of 1.5 clusters of approximately 60 to 80 g each, given the suggestion that 7 to 12 cm^2 of leaf area are necessary to ripen 1 g of fruit (Alexander, 1965; Kliewer and Dokoozlian, 2000; Kliewer and Weaver, 1971; May et al., 1969). At fruit set, three irrigation regimens were imposed: standard RDI (S), early deficit (E) and late deficit (V). From fruit set to veraison, irrigation was applied within S and V to replenish 70% of FVET weekly (2002; 60% in 2003) with the goal of maintaining soil moisture in the top 1 m at 10% by volume. In the same period, irrigation in E replenished 35% of FVET weekly (2002; 30% in 2003) with the goal of maintaining soil moisture at 8.3%. From veraison to harvest, irrigation was applied in S and E plots to replenish weekly 70% (2002) and 60% (2003) of FVET with the goal of maintaining soil moisture at 10%, while V plots replenished 35% (2002)

and 30% (2003) of FVET weekly with the goal of maintaining soil moisture at 8.3%. After harvest, all vines were irrigated to replenish 100% of FVET. The managed soil depth was 1 m. Irrigation application was calculated from:

$$\textbf{Irrigation required} = c_{deficit} \times \text{FVET} + \frac{(\theta_{target} - \theta_{actual})}{100} \times sd \qquad (3.1)$$

where irrigation required is the amount of water to be applied per week (mm), $c_{deficit}$ is the deficit coefficient corresponding to the irrigation level, θ_{target} and θ_{actual} (%) are the desired and measured volumetric soil water contents, respectively, for a given irrigation level, and sd (mm) is the soil depth to be managed. The weekly irrigation was applied in three or four sets.

In 2002, the research department of Ste. Michelle Wine Estates provided data on total water applied by week for each irrigation level. During 2003, sensors installed in the drip irrigation lines detected pressure inside the drip line, corresponding to the irrigation lines being operated. The applied water (mm) was calculated:

$$\textbf{Irrigation applied} = \frac{f \times t_{ir}}{d_e \times d_r} \qquad (3.2)$$

where f is emitter flow rate (L h^{-1}), t_{ir} is the duration of irrigation (h), d_e is the distance between emitters (m), and d_r is the distance between rows (m).

3.3.2. Physiological Measurements

3.3.2.1. Whole-canopy net CO_2 exchange rate (NCE_{WV})

During 2002 and 2003, whole-canopy net CO_2 exchange rates (NCE_{WV}; μmol $vine^{-1}$ s^{-1}) were measured during five periods corresponding to different phenological stages (Figure 3.1): 1) fruit set, before treatment initiation (DOY 183 to 192, 2002, and DOY 177 to 184, 2003); 2) pre-veraison (DOY 217 to 224, 2002, and DOY 212 to 219, 2003); 3) post-veraison (DOY 234 to 240, 2002, and DOY 233 to 241, 2003); 4) pre-harvest (DOY 255 to 261, 2002, and DOY 254 to 262, 2003); and 5) post-harvest (DOY 278 to 284, 2002, and DOY 274 to 281, 2003). Cumulative net CO_2 exchange (24 h) was expressed both per vine ($NCE_{WV,d}$; g CO_2 $vine^{-1}$ d^{-1}) and per unit leaf area ($NCE_{WL,d}$; g CO_2 m^{-2} d^{-1}). Based on vine spacing, row spacing, and the anticipated canopy size, chambers of an approximate volume of 8 m^3 were used to fully enclose one vine each (Perez Peña and Tarara, 2004; chapter 2, this volume). Briefly, each chamber enclosed a vine without any modification of the canopy size, shape, or the trellis system. Six chambers operated simultaneously. At each sampling day, chambers were installed by 0800 h on 6 vines (two in each irrigation level), and were operated for 48 h. Only data recorded continuously during 24-h periods were used (0000 h to 2400 h). The six chambers were then moved to another six vines, run for 48 h and the process repeated. Total measurement duration was 7 to 9 days for 18 sample vines.

The experiment was arranged as a completely randomized design with a three-way treatment structure: irrigation (3 levels), year (2 levels), and day of

measurement (DOM; 3 levels). Repeated measurements were made five times during the season with two-vine replicates, with the vine as the experimental unit. The first analysis included data averaged across all measurement days: those with clear skies (DCS) and those with clouds (DWC). When only DCS data were used, analysis was for a completely randomized design with two-way treatment structure (irrigation and year).

3.3.2.2. Leaf area estimation

To calculate NCE_{WL} (whole-canopy net CO_2 exchange rate on a leaf area basis; μmol m^{-2} s^{-1}), total leaf area per vine on each of the 18 sample vines was estimated twice during 2002 (veraison and pre-harvest) and four times during 2003 (fruit set, veraison, pre-harvest and post-harvest). In 2002, total leaf area was estimated by a 3-step process: 1) leaf width was regressed against leaf area for a sample of 200 leaves from vines near the 18 sample vines; 2) the widths of all leaves on a sample of shoots (n=8) were measured, and individual leaf areas computed from the relationship established in #1; and 3) leaf area per vine was calculated from the average leaf area per shoot in the sample and the number of shoots per vine. In 2003, about fifty shoots were sampled from adjacent vines and their leaf area measured destructively (LI-3100, LI-COR, Lincoln, NE). A regression model between shoot length and shoot leaf area was fitted to the 50-shoot sample. On the 18 experimental vines, shoots were counted and the lengths of 50% of the shoots were measured. Vine leaf area was estimated using the length:area regression, the number of shoots per vine, and the length of the shoots. In 2002, the total canopy leaf area estimated at pre-harvest was used for calculating $NCE_{WL,d}$ and NCE_{WL} during the post-harvest measurements. In 2003, the total

canopy leaf area estimated at veraison was used for calculating $NCE_{WL,d}$ and NCE_{WL} during the pre- and post-veraison measurements.

3.3.2.3. Single leaf net CO_2 exchange (NCE_{SL})

During 2002 and 2003, net CO_2 exchange was measured on single leaves. Between bloom and fruit set 27 uniform vines near the 18 sample vines used for the whole-canopy measurements were randomly selected (9 vines per irrigation regimen). On each of these vines, 4 shoots $\approx$1 m long (2 on the north cordon and 2 on the south cordon of the vines) bearing one cluster were labeled. On each shoot, a fully expanded leaf (located about 6 to 8 leaves from the shoot tip) was selected, labeled, and used for NCE_{SL} measurements during the entire season. Single-leaf net CO_2 exchange was measured in 2002 at three of the phenological stages at which NCE_{WV} was measured: pre-veraison, post-veraison, and pre-harvest, and all five stages in 2003. Measurements were recorded four times during the day in 2002 and six times in 2003, from early morning until afternoon. A portable photosynthesis system (CIRAS-2, PP Systems, Haverhill, MA) was used. The leaf cuvette (PLC6(U) - PP Systems, Haverhill, MA) enclosed 2.5 cm^2 of leaf area. Air flow through the cuvette was 200 mL min^{-1}, and reference [CO_2] was set at 365 ppm. Zeroing and matching between reference and analysis cells of the instrument occurred every 30 min. Calibration was checked between phenological stages with certified gas (359 and 305 µmol mol^{-1} CO_2 with N_2 as balance gas, Air Liquid, Houston, TX) and a humidity calibrator (PP Systems, Haverhill, MA).

3.3.3. Leaf non-structural carbohydrates

At the same time that NCE_{SL} measurements were taken, a total of eight leaf discs (6.3-mm diam.) were collected from the two leaves above and the two leaves below the leaf used for NCE_{SL} measurements on the same shoot. Leaf discs were collected between veins with a modified commercial hole puncher, to which a 1.5 mL microtube was attached. When the leaf discs were cut by the puncher, they dropped into the microtube, which was immediately snap frozen in liquid nitrogen. The samples were then stored at -80°C until analysis.

3.3.3.1. Soluble sugars (ss) and starch extraction

Leaf discs were counted and weighed. An extraction method (Hendrix, 1993) was modified by adding a grinding step of the leaf discs. Two 2.5 mm diameter beads (zirconia/silica, Biospec Products, Bartlesville, OK) were added to the microtube and snap frozen in liquid nitrogen to facilitate grinding using a bead-beater type homogenizer (Mini-BeadBeater-8, Biospec Products, Bartlesville, OK) for 45 s. After homogenization, an aliquot of 1.25 mL of 80% v/v aqueous ethanol was pipetted into the microtubes and incubated for 15 min at 80°C in a water bath. After incubation, the ethanol was decanted into a 15 mL centrifuge tube and replaced with another 1.25 mL of 80% v/v ethanol. This step was repeated one more time, resulting in a total of three extractions, based on preliminary experiments conducted with one, two, three, and four extractions (see appendix; Figure A3.2). Three extractions yielded about 95% of the ss in the leaf tissue. The insoluble fraction remaining in the microtube was frozen at -80°C for later starch extraction and analysis. For starch extraction, 1 mL of 0.1 M KOH was added to the insoluble fraction. Microtubes were placed in a 100°C water bath for 1 hour to gelatinize

the starch. Then microtubes were cooled to room temperature, and alkali was neutralized with 75 µL of 1 M acetic acid to a pH of 6.6 to 7.5, checked against paper type pH indicators. An aliquot of 200 µL of heat tolerant α-amylase (EC 3.2.1.1, Sigma A3403) was added and microtubes incubated in a water bath at 85ºC for 30 min. Microtubes were then cooled to room temperature and pH was lowered to 5 by adding 100 µL acetic acid, checked against paper type pH indicators. Microtubes were then mixed by vortex mixer, emptied into 15 mL tubes, and 1 mL of amyloglucosidase was added (EC 3.2.1.3, Sigma A3042). Tubes were incubated in a water bath at 55ºC for 60 min to complete starch hydrolysis. Tubes were then immersed in boiling water for 5 min to stop starch digestion and centrifuged at 3000 x *g* for 10 min to remove particles from solution.

3.3.3.2. Soluble sugars (ss) and starch analysis

For the analysis of ss, a sequential enzymatic degradation method (Hendrix, 1993) for determining glucose, fructose, and sucrose was modified because manufacturing of the reagent used in the original method was discontinued. Thus a different reagent for glucose determination was used, and the original wavelength at which samples were read by the spectrophotometer was changed. From the 15 mL tubes after ss extraction, an aliquot of 0.5 mL was pipetted and mixed with about 10 mg of activated charcoal (Sigma C3345) in a 0.45 µm cellulose acetate microfilter tube assembly (Costar 8163-Corning) and centrifuged at 2200 x *g* for 3 min (Eppendorf centrifuge 5415 D) to produce a clear extract. An aliquot of 20 µL of the filtered extract was pipetted into a microplate well and oven-dried at 55ºC to concentrate the sample.

Once the samples were dried, 20 µL of water were added to each microplate well. An enzymatic glucose assay kit (G2020, Sigma) was used for the analysis and prepared according to the manufacturer's instructions. An aliquot of 100 µL of the prepared reagent was added to each microplate well to be analyzed. The microplate was then incubated for 15 min at 37°C and read by a microplate spectrophotometer (Spectramax® Plus384, Molecular Devices Corp., Sunnyvale, CA) at 340 nm to determine glucose. To determine fructose, 10 µL of a phosphogluco-isomerase solution (EC 5.3.1.9, Sigma P5381) was pipetted into the microplate wells with the samples. The microplate was incubated for another 15 min at 37°C and read again at 340 nm. The difference between the two readings was proportional to the sample's fructose concentration. For sucrose determination, 10 µL of an invertase solution (EC 3.2.1.26, Sigma I4504) was then pipetted into the wells with the samples, incubated for another 15 min at 37°C, and read again at 340 nm. The difference between the second and third readings was proportional to the sample's sucrose concentration.

Ten microplate wells were filled with glucose standards and their readings were used to build calibration curves from which concentrations of the samples were calculated. After several analyses using 10 standards, and due to the small variability among readings, the number of standards was reduced to six wells. The highest and lowest glucose standard concentrations were 111 µg mL^{-1} and 0 µg mL^{-1} respectively. Another six wells of the microplate were filled with the glucose, fructose, and sucrose controls, and their concentrations were equivalent to the glucose concentration of the highest standard used. The controls indicated whether the enzyme preparations were

still active (enzyme solutions once prepared could lose some of their activity with storage time). Glucose, fructose and sucrose concentrations in leaf tissue were summed and expressed as total soluble sugars.

An aliquot of 20 µL from the supernatant after starch extraction was used for the starch analysis, following the same procedure used for glucose determination. If the samples' concentrations were over those of standards, samples were diluted. The same standards used in the ss analysis were used for starch determination. Corn starch at about 111 µg g^{-1} was used as control; it was digested and analyzed as a sample. Both ss and starch results were expressed in mg g^{-1} fresh weight (FW). Based on starch and ss concentrations, NCE_{SL}, and time elapsed between first and last sample of the day, leaf export rates were calculated by:

$$\textbf{Export rate} = \textbf{NCE}_{\textbf{SL,fl}} - \frac{(\textbf{starch} + \textbf{ss})_{\textbf{l}} - (\textbf{starch} + \textbf{ss})_{\textbf{f}}}{\textbf{t}_{\textbf{fl}}} \textbf{ x F} \qquad \textbf{(3.3)}$$

where export rates and $NCE_{SL,fl}$ (mean of first ($NCE_{SL,f}$) and last ($NCE_{SL,l}$) measurement of the day) are expressed in µmol m^{-2} s^{-1}, t_{fl} is the time (h) elapsed between the first and last sample, and F is a unit conversion factor (2.0568 µmol m^{-2} s^{-1} g mg^{-1} h; see appendix 3).

3.3.4. Statistical analysis

The data were analyzed using the SAS statistical package (V8(2); SAS Institute, Cary, NC). Procedure Univariate was used to test for normality and the Brown-Forsythe

test for homogeneity of variance. The general linear model procedure (proc GLM) was used for the analysis of variance (ANOVA). The Tukey-Kramer method at a significance level of $p \leq 0.05$ was used for mean comparisons, proc CORR procedure was used for correlation analysis, and proc REG for regression analysis.

3.4. Results

3.4.1. Meteorological and irrigation summary

Air temperatures (1.5 m) recorded between DOY 91 and DOY 304 (April 1 - October 31) by the Alderdale PAWS station were similar for both growing seasons (Figure 3.2). In both years maximum temperatures exceeded 40°C only briefly during July. Differences between daily maximum and minimum temperatures during the period of berry growth (DOY 175 - DOY 262) were on average about 16°C for both seasons. Total global irradiance (April 1 - October 31) was 4731 MJ m^{-2} in 2002 and 4808 MJ m^{-2} in 2003. The sum of degree days (April 1 - October 31) was higher in 2003 (1857°C) than 2002 (1669°C), due mainly to warmer temperatures in 2003 during August, September and October (Figure 3.3). Bloom and veraison occurred about one week earlier in 2003 than 2002. Annual rainfall (January 1 - December 31) was below average (211 mm; 1991-2003), totaling 160 mm in 2002 and 148 mm in 2003. Although rainfall during the growing season was similar in both years, more rain was recorded in the winter prior to the 2003 growing season (Table 3.1). Rainfall was more evenly distributed in 2002 than in 2003, with no recordable rain in 2003 between the beginning of June (pre-bloom) and the beginning of August (pre-veraison; see appendix figure A3.3). Total ET_o during the growing season (April 1 - October 31) was similar in

both years and above average (Table 3.1). Maximum daily ET_o was about 14 mm in 2002 and about 11 mm in 2003, whereas the average maximum daily ET_o (April 1-October 31; 1999 to 2003) is 12.3 mm (see appendix figure A3.4).

Cumulative irrigation applied during the RDI regimens (i.e., post fruit set) was 295 mm for S, 225 mm for V, and 185 mm for E during 2002, and 162 mm for S, 122 mm for V, and 92 mm for E during 2003 (Figure 3.4; see also appendix figure A3.5). Differences in irrigation applied between years were due to different crop coefficients, rainfall distribution, and ET_o.

3.4.2. Canopy leaf area

Total leaf area per vine was between 6 and 10 m^2 (Table 3.2). No differences in canopy sizes were recorded between years at pre-veraison, while at pre-harvest canopies were about 27% smaller in 2003 than in 2002. In general, vines under E tended to have less leaf area per vine than V in both years, but about the same leaf area as S vines. Canopy differences were evident in aerial photographs of the plots (Figure 3.5) and also were confirmed by dormant pruning weights (Chapter 5, this volume).

3.4.3. Total daily canopy net CO_2 exchange ($NCE_{WV,d}$, $NCE_{WL,d}$)

Because of technical difficulties with the whole-canopy system, data collected during the fruit set stage of 2002 were not included in the analysis. However, trends will be discussed for both seasons, and illustrative data presented from the more comprehensive 2003 field campaign. Although some differences were recorded in leaf area per vine among the irrigation regimens, these differences were not large enough to

change the trends found between treatments in $NCE_{WV,d}$ when $NCE_{WL,d}$ was computed, so canopy size was not the main determinant of treatment response. Because significant interactions were found between treatment and phenological stage, irrigation regimens were compared within stages, using three days' data per measurement run (Table 3.3). Significant interactions between treatment and DOM usually were due to the confounding effects of cloudy skies, rainfall, and concurrent irrigation application. Between one and four irrigation sets occurred during each of the seven to nine day measurement runs, thereby superimposing a dry-down and re-wetting cycle on the general deficit response. Because of the different RDI coefficients in each regimen, not all plots were irrigated simultaneously. Separate analyses were performed using only DCS to segregate irrigation effects from those of weather (Tables 3.4 and 3.5).

In both years $NCE_{WV,d}$ was higher at pre- and post-veraison than at pre- and post-harvest, with no interaction between year and phenological stage (Tables 3.3, 3.4, and 3.5). Furthermore, in 2003, $NCE_{WV,d}$ was higher around veraison than during the fruit set stage, although the difference between stages was less dramatic than late in the season. Across all treatments, $NCE_{WV,d}$ was 39% less at pre-harvest and 47% less at post-harvest with respect to the pre- and post-veraison stages (Table 3.4). When all vines were watered identically (fruit-set and post-harvest periods), there were no differences among regimens in $NCE_{WV,d}$ whether all measurement days were considered or only those with clear skies (Tables 3.4 and 3.5). A reduction in net CO_2 fixation was observed due to the additional water deficit in E vines before veraison (43% in 2002; 46% in 2003) and in V vines after veraison (33% in 2003), while in 2002 after veraison,

$NCE_{WV,d}$ in E vines was lower than in S or V vines. Although V vines were under additional deficit during the pre-harvest interval, no differences among regimens were observed, and all vines fixed less CO_2 (60% reduction) compared with the measurements recorded three weeks earlier. At post-veraison, E vines fixed about 29% less CO_2 than S vines in both years, suggesting a delayed recovery by the E vines after they were returned to standard RDI. Total CO_2 potentially fixed was calculated for each of the RDI regimens during each period when measurements were collected based on the $NCE_{WV,d}$ values for days with clear skies in 2003 by linear interpolation between stages (Table 3.6). Because the E regimen lasted longer than V and occurred during the period of highest net carbon exchange, the season-long reduction in total carbon fixed was higher for E than for V vines.

3.4.4. Diurnal trends in canopy net CO_2 exchange rate (NCE_{WL})

Irrigation regimens, weather, and timing of irrigation sets all influenced the diurnal pattern of NCE_{WL}. The NCE_{WL} daily curves varied with photosynthetic photon flux density (PPFD), temperature, and ET_o, and time elapsed between irrigation and measurement collection. The NCE_{WL} was low throughout the day on DWC, following the PPFD curve at the beginning and end of the day. At fruit set, when all vines were irrigated equally, vines in all regimens typically followed the same diurnal pattern and fixed CO_2 at similar rates. Small variations are evident due to slightly different timing of irrigation sets (Figure 3.6). For example on DOY 178, while irrigation was being applied to all plots, maximum values of NCE_{WL} were about 9.5 μmol m^{-2} s^{-1}, between 0900 h and 1500 h (Figure 3.6-A). By DOY 181 (Figure 3.6-B), three days had passed since an irrigation set, and

while maximum values of NCE_{WL} approached 8 µmol m^{-2} s^{-1} in the morning (ca. 0800 h) they gradually declined to 4 µmol m^{-2} s^{-1} by mid-afternoon (ca. 1400 h). Clouds on DOY 183 (Figure 3.6-C), lower air temperature, and no irrigation of E plots resulted in some divergence among treatments and a modified sinusoidal pattern of NCE_{WL} during that day.

At pre-veraison, while E vines were under extra water deficit, NCE_{WL} at midday of DCS was up to 57% lower than in vines under standard RDI (Figure 3.7-C). Under clouds, lower temperature, and lower VPD, differences among irrigation regimens were smaller because S and V vines fixed CO_2 at lower rates than on days with clear skies and/or an irrigation set (Figure 3.7-B).

During the post-veraison measurements one would have expected a reversal of the E and V NCE_{WL} from the pre-veraison period and this was generally the case on DCS (Figure 3.8-B). Field scheduling resulted in an irrigation set applied on DOY 239 to S and V vines, but not to E vines resulting in a convergence of NCE_{WL} between E and V, although E vines were no longer under additional deficit. Rain (2.3 mm) on DOY 234 resulted in low NCE_{WL} overall and acted as a relief on the V deficit. Maximum NCE_{WL} recorded during DOY 238 (12 µmol m^{-2} s^{-1}; Figure 3.8-B) and DOY 240 (14 µmol m^{-2} s^{-1}; Figure 3.8-C) were the highest of the season.

At pre-harvest and post-harvest, PPFD, air temperature, and ET_o were considerably lower than previously. The light and temperature dependent responses of photosynthesis are evident when comparing the pre-harvest period to the preceding set of measurements. There was a marked deficit effect in V vines on DOY 256

(Figure 3.9-A), although this effect was less pronounced during the other two measurement days because of intermittent clouds (Figure 3.9-B, C). Consequently, the composite data suggested no difference in $NCE_{WV,d}$ or $NCE_{WL,d}$ between S and V during this period (Table 3.3). Maximum air temperature was as much as 10 to 12ºC lower than during the post-veraison measurement period. After harvest all vines were irrigated at FVET, but slightly higher NCE_{WL} during mid-day hours were observed in the S vines (Figure 3.10-A, B). Cloudy days damped this apparent difference (Figure 3.10-C). Maximum NCE_{WL} recorded on S vines were not different among the phenological stages (Table 3.7), while E and V vines only differed from S at the times when they were under their respective additional deficits.

3.4.5. Single-leaf net CO_2 exchange rate (NCE_{SL}) and stomatal conductance (g_s)

The effects of additional water deficit on NCE_{SL} and g_s were evident in both years and in general confirmed NCE_{WL} results (Figure 3.11 and 3.12). Maximum values of NCE_{SL} were recorded at pre-veraison and post-veraison ($\approx$17 μmol m^{-2} s^{-1} in 2002; $\approx$15 μmol m^{-2} s^{-1} in 2003) with the lowest ones during pre-harvest in both years. Daily maximum values were usually recorded during mid-morning, with a decline in NCE_{SL} during the afternoon. In both years during pre-veraison, NCE_{SL} was lower in the E vines than in S and V at all measurements during the day. During post-veraison, as with whole-canopy measurements, E and V vines had similar values of NCE_{SL} in 2002, while in 2003, NCE_{SL} was lower in V vines than either S or E. At pre-harvest, NCE_{SL} was lower in V than in S or E, while during post-harvest NCE_{SL} was similar in all treatments. Hourly

whole-canopy data were used to evaluate the relationship between NCE_{WL} and NCE_{SL}. Although significant, correlation was poor (r^2=0.38; Figure 3.13). Except for some lower NCE_{SL} in the additional deficit vines, most NCE_{SL} values were generally higher than those of NCE_{WL}. Less dispersion in the whole canopy data is probably due to more measurements per vine collected per hour (n=5) than by the single leaf instrument thereby buffering extreme values in the whole-canopy measurements. Using 2003 data, means for NCE_{WL} and for NCE_{SL} were calculated for days and between times when both types of measurement were taken simultaneously. Except at fruit set when NCE_{WL} was higher, NCE_{WL} in general was lower than NCE_{SL} (Figure 3.14).

3.4.6. Non-structural carbohydrates

In 2002 there was no clear pattern in soluble sugars among treatments according to the time of day the samples were collected (see appendix; Figure A3.9). Thus, in 2003 only the leaf samples corresponding to the first and the last sample time of the day were analyzed, and only for days with clear skies that were preceded by a day with clear skies. Soluble sugars concentrations during 2002 ranged from ≈10 to 20 mg g^{-1} FW, while starch concentrations ranged from 10 to 35 mg g^{-1} FW (Figure 3.15). In 2002 no significant differences in soluble sugars among irrigation regimens were observed at any phenological stage. Starch concentrations were significantly different among regimens only in the first sample of the day at pre-veraison, with leaves of E vines having lower starch concentration than leaves of S or V vines (Figure 3.16). Soluble sugars concentrations in leaves during 2003 ranged from ≈6 to 9 mg g^{-1} FW, while starch concentrations ranged from ≈12 to 33 mg g^{-1} FW. Higher concentrations of soluble

sugars were observed in the first sample of the day (Figure 3.17-A) than in the last one (Figure 3.17-B). As in 2002, no significant differences among regimens were recorded in soluble sugars at any of the phenological stages. Starch concentrations were not different among regimens for the first sample of the day (Figure 3.18-A). In the last sample of the day at pre-veraison and post-veraison, leaves from vines under additional water deficit had lower starch concentration than those under standard deficit (Figure 3.18-B). Based on NCE_{SL} and leaf non-structural carbohydrates, net export rates were estimated. No significant differences were found in net export rates among irrigation regimens (Table 3.9).

3.5. Discussion

Canopies of grapevines under more severe water deficit during berry growth fixed overall lower amounts of CO_2 compared to those under standard RDI, although the timing and duration of irrigation sets (3 to 4 per week) influenced the day-to-day dynamics of this difference among irrigation regimens, as did weather. The influence of additional deficit was most evident during the driest days of the weekly irrigation cycle, leading to a marked decline in photosynthesis from a mid-morning daily maximum, although the diurnal curve of NCE_{WL} in all vines varied between the “wet” and “dry” days of the weekly cycle. Similar patterns in NCE_{WL} have been observed under drying soil conditions elsewhere (Intrieri et al., 1998; Ollat and Tandonnet, 1999; Poni et al., 2003). Given a plant available water (PAW) of 27.7 mm (see calculation in appendix 3) and a daily FVET of about 8 mm at our site, a water deficit could have been generated within two days, with more than half of the available soil water consumed. Rapid drying and

re-wetting at our site was facilitated by the rapid infiltration rate and low water holding capacity of the sandy soil. For a given soil type, irrigation amount and distribution in time both can influence the rate and duration of NCE_{WL} recovery due to rewatering.

All vines, whether under standard or under additional water deficit, responded within a day to irrigation events, via higher $NCE_{WL,d}$, and the diurnal course of NCE_{WL} following the diurnal pattern of irradiance. After E vines were again irrigated at standard RDI, CO_2 fixation recovered to about the same levels as before imposition of the additional deficit. The rapid recovery is an indication that no severe damage to the photosynthetic machinery had occurred; grapevines have been reported to be quite resistant to photoinhibition (Chaumont et al., 1997; Flexas et al., 1998; Gamon and Pearcy, 1990), with photorespiration being indicated as an important process of energy dissipation (Medrano et al., 2002). The general decline in CO_2 fixation of vines in all irrigation regimens towards the end of the season (pre- and post-harvest) and the reduction in atmospheric water demand makes it difficult to evaluate the recovery of vines that were under additional water deficit between veraison and harvest (V vines). The similarity among CO_2 fixation rates after harvest, when all vines were irrigated at FVET, suggests that the capacity of those canopies to fix CO_2 was not permanently impaired by the degree or timing of the additional deficits that were applied. Within the standard deficit, maximum CO_2 fixation rates were similar across the season, as previously reported in field grown vines (Ollat and Tandonnet, 1999). Expected reductions in CO_2 fixation rates by canopy aging towards the end of the season (Bertamini and Nedunchezhian, 2003; Kriedemann et al., 1970; Schultz et al., 1996)

may have been compensated by lower evapotranspiration demand that could have favored higher NCE_{WL}. Canopy size reduction due to normal loss of some leaves by the post-harvest measurement may also have contributed to reductions in total daily CO_2 fixation. For example a reduction in canopy size of 16% in S vines from the post-veraison to the pre-harvest measurement was accompanied by a reduction of about 35% in $NCE_{WL,d}$, which indicates that other factors (i.e., lower irradiance, temperature, canopy age, and day length) apart from canopy size affected $NCE_{WV,d}$.

Although the additional two water deficits imposed were of similar magnitude (equal reduction in crop coefficient used and soil moisture targeted), their effects on CO_2 fixation and partitioning were considerably different. The large reduction in CO_2 fixed, particularly in E vines, without a consistent effect on yield, berry composition or size in either of the two years (chapter 5, this volume), must have affected other organs or reserves of the vine. Reduction in berry size is a common objective of RDI. Elsewhere, water deficits imposed during the berry growth period have reduced berry size, with deficits imposed during phases I and II achieving the largest effect (Matthews and Anderson, 1988; Matthews and Anderson, 1989; McCarthy, 1997). After fruit set, the berries become a strong carbon sink (Hale and Weaver, 1962), and may compete for the limited source of carbohydrates that results from imposed water deficit. In the current work, irrigation applied to the early deficit treatment after bloom and until treatments started to receive different amounts of water (DOY 192) could have ameliorated the reduction in berry size that would have been obtained if the deficit in the early treatment has been prolonged.

Based on estimations of total CO_2 fixed between fruit set and harvest for S vines (9.43 kg; Table 3.6), maximum yield recorded in 2003 (4.7 kg $vine^{-1}$; chapter 5, this volume), and assuming that berries contain 25% dry matter (Winkler et al., 1974) with 44% carbon content, such yield would have required about 1.95 kg of CO_2 to have been fixed, leaving more than 80% of the estimated total carbon fixed to have been directed to leaves, canes, trunk, and roots. During the same period E vines fixed 5.81 kg of CO_2, leaving about 40% of the equivalent amount of CO_2 fixed by S vines for distribution among other organs, whereas fixation of CO_2 in V vines was only 10% lower than S vines. By pre-harvest, the fruit already had high levels of soluble solids, and the fruit sink strength may have declined. This together with the fact that standard RDI vines fixed after harvest only about 15% of the total CO_2 fixed from fruit set to leaf fall, indicates that root growth and replenishment of reserves could have been severely compromised by the extra water deficit. Thus, it is reasonable to assume that vines under early additional deficit directed less carbohydrate to roots and permanent structures, and ended the season with less root biomass and reserves than those under standard and under late additional deficit. Roots have been reported to be a low priority sink during fruit ripening (Candolfi-Vasconcelos et al., 1994) for both growth and replenishment of reserves in permanent structures that will be used for next years' budbreak and initial shoot growth (Quinlan and Weaver, 1970; Stoev and Ivantchev, 1977; Winkler and Williams, 1945). In grapevines, the ratio between below- and above-ground biomass was reported to be constant (Buttrose, 1965; Petrie et al., 2000), but with insufficient fruit, excess carbohydrates were reported to be channelled to roots, increasing that ratio (Bota et al.,

2004; Buttrose, 1965).

During pre- and post-veraison afternoons, lower total non-structural carbohydrates concentrations (starch plus soluble sugars) in vines under additional water deficit were due to lower leaf starch concentration associated with lower NCE_{SL} (Figure 3.12). Previous research also showed reductions in total non-structural carbohydrates due to lower NCE_{SL} (Chaumont et al., 1994; Chaves, 1991; Rodrigues et al., 1993), although the magnitude of this reduction depended on the severity of the water deficit (Patakas et al., 2002; Patakas and Noitsakis, 2001). We did not record in our single-leaf measurements complete stomatal closure due to additional water deficits imposed. Allocation between ss and starch in grapevine leaves has been shown to be affected by water deficits (Patakas, 2000; Quick et al., 1992; Rodrigues et al., 1993). Although we expected to detect a lower starch concentration in the morning in vines under additional water deficit, this only happened in 2002. It is possible that due to the time at which samples were collected (i.e. earliest was 0707 h on DOY 183 at fruit set, while sunrise was at 0412 h), leaves may have had enough time accumulate some starch. At the beginning of the day, daily curves of NCE_{WL} were usually similar among regimens (e.g., Figures 3.7 and 3.8). Thus, there was some time during which leaves under additional water deficit may have been able to replenish some of the starch utilized during the night. At the last sampling time, starch concentration was that at the beginning of the day plus what has been accumulated by CO_2 fixation until time of the sampling. Regardless of irrigation regimen, and although NCE_{SL} were lower in the afternoon, starch concentrations were always higher than in the morning, in agreement

with previous reports where daily starch levels displayed an inverse relationship with CO_2 fixation rates (Hunter et al., 1995). The same study also showed that root starch concentrations increased from morning to afternoon, following the trend observed in leaves. Our calculations showed that leaves under additional water deficit were able to export carbohydrates. It has been suggested that water deficit affected source activity more than phloem translocation (Bota et al., 2004). We do not know the dynamics of carbohydrate concentration during the night in our experiment.

Single-leaf measurements were able to detect differences among irrigation regimens, but in general NCE_{SL} overestimated NCE_{WL}, supporting the idea that single-leaf measurements can be misleading if extrapolated to the whole canopy (Edson et al., 1995; Intrieri et al., 1997; Katerji et al., 1994). Single-leaf measurements provide little evidence of the relationships between sources and sinks, and vineyard-scale CO_2 fixation. Extreme values of NCE_{SL} are buffered by the whole canopy because the latter represents leaves that are under more heterogenous water status conditions (sunlit vs. shaded, east side vs. west side of the canopy, top vs. bottom of canopy). At relatively high NCE_{SL}, one might expect lower NCE_{WL}, because of numerous shaded leaves drawing down whole-canopy average. On the contrary, when NCE_{SL} are relatively low (i.e., water deficit), one might expect comparatively higher NCE_{WL} because numerous leaves are under more favorable water status. To estimate the amount of CO_2 fixed by a vine per day, single-leaf measurements would have to be temporally and spatially extensive to capture the same information as a whole-canopy measurement. With our whole-canopy system we were able to collect simultaneously from six vines, 120 data

points for CO_2 fixation per vine per day, providing information that can not be provided by single leaf measurements.

In eastern Washington where the period between harvest and leaf fall is short, water deficits imposed during berry growth could compromise root growth and replenishment of reserves for the following season. In viticulture regions in lower latitudes with longer periods and day lengths between harvest and leaf fall imply that a balance among sources, sinks, and reserves replenishment may not be as critical as in northern latitudes, especially when additional water deficits are imposed. Although we observed differences in canopies (e.g., pruning weights), no consistent effect was observed on yield, berry size or berry composition due to additional water deficit (chapter 5, this volume). In a separate experiment in the same vineyard, a higher number of berries per cluster and higher yield were achieved by two manipulations: applying more irrigation than 70% FVET around bloom and by removing shoot tips (hedging; R. Smithyman, unpublished data). More irrigation around bloom may have raised CO_2 fixation rates by partly relieving water deficits and increasing stomatal conductance. Hedging might have reduced transpiring area, improved vine water status and increased CO_2 fixation rates. Moreover, at bloom, flower clusters are a relatively weak sink (Hale and Weaver, 1962) and by removing the shoot tips, the competition for photo-assimilates between shoots tips and flower clusters may have been reduced (Coombe, 1959; Quinlan and Weaver, 1970). It is possible that low fruit set in our experiment (data not shown) was a consequence of low carbohydrate reserves and low CO_2 fixation rates due to water deficit. Our measurements were conducted during the

last two years of a five year experiment. The high leaf area:fruit ratio across all irrigation regimens (more than 15 cm^2 leaf area per g of fruit, chapter 5, this volume) compared to those cited as optimum for well-watered vines (Kliewer and Dokoozlian, 2000), might suggest a sink limited situation. For vines under water deficit, optimum leaf area:fruit ratios may need to be higher to offset lower CO_2 fixation rates. Considering that there was not a consistent effect of additional water deficits on yield or berry composition in either of the two years (chapter 5, this volume), one of the goals of RDI, water savings, was achieved. But water savings were achieved at the expense of reduction in CO_2 fixation, especially in the early deficit regimen.

3.6. Conclusion

Vines managed by RDI but under additional water deficit during berry growth fixed less CO_2 than vines solely under standard RDI both at the whole canopy and single leaf levels. Estimated total CO_2 fixed during the entire berry growth period was considerably less in vines under the additional water deficit between fruit set and veraison, than in those under additional water deficit between veraison and harvest. Small but significant differences in canopy size were recorded between vines that were under more severe water deficit after fruit set than those that were under water deficit after shoot growth had stopped.

Leaves of vines under an additional water deficit had lower starch concentrations during the afternoon than vines under standard RDI. However, leaf starch concentration in all vines increased from morning to afternoon, contrary to the trend in CO_2 fixation rates, which in general decreased from morning to afternoon. Although vines under

additional water deficit may still accumulate starch in leaves during the afternoon, rates of starch accumulation were lower as were export rates. Low levels of total daily CO_2 fixation were recorded at the end of the season across all irrigation regimens. However, similar daily maximum values of NCE_{WL} were recorded during the entire season under standard RDI. Single leaf measurements of net CO_2 exchange in general overestimated those of the whole canopy. A poor correlation between whole-canopy and single-leaf net CO_2 fixation was found, confirming previous research that results from single leaf measurements have limited usefulness for estimating CO_2 fixation of entire plants. However, single leaf measurements reflected differences among irrigation regimens and provided information on carbohydrate allocation in the leaf. The absence of severe symptoms of water deficit, like premature senescence or yellow leaves, indicated that targeted soil moisture levels were probably low enough to reduce photosynthesis and growth, but not to reduce berry size or cause leaf abscission. Depending on severity, water deficits imposed between fruit set and veraison may compromise root growth and carbohydrate reserves in permanent structures of the vines. The increase observed in number of berries per cluster and yield by less restrictive irrigation and hedging around bloom, might suggest that water deficits promote an imbalance between sources and sinks, particularly if imposed at bloom when flower clusters are relatively weak carbon sinks.

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Table 3.1: Meteorological summary for the experimental site.

Year	Precipitation		ET_o: Reference evapotranspiration (grass)	Growing degree days
	November 1-March 31	April 1-October 31	April 1 -October 31	
	mm	mm	mm	°C
2001/2002	111	46	1289	1669
2002/2003	147	59	1218	1857
Long term mean	116	101	1119	1624

Long term mean (1991-2003) is from the Paterson, Washington PAWS Station (Latitude: 45.93°, longitude: 119.48°, elevation: 109 m) located 18 km east of site. The 2002 and 2003 data are from Alderdale, Washington PAWS Station (Latitude: 45.8°, longitude: 119.8°, elevation: 224 m) located 10 km west of site, Washington, which is closer to the experimental site than Paterson, but has not existed long enough to provide a meaningful long-term average.

Table 3.2: Effect of RDI regimens on leaf area per vine.

Phenological Stages	2002 Irrigation regimen				2003 Irrigation regimen				Year
	S	E	V	p value[y]	S	E	V	p value[y]	p value[v]
	---------- (m^2 $vine^{-1}$) ----------				---------- (m^2 $vine^{-1}$) ----------				
Fruit set[z]	nc[u]	nc	nc		8.8 ab	8.2 b	9.6 a	0.009	nc
Veraison	9.3 ab	7.5 b	10.7 a	0.004	8.4 b	8.1 b	9.7 a	0.009	0.306
Pre-harvest	10.1	8.4	10.6	0.155	7.0 b	6.7 b	8.7 a	0.001	0.005
Post-harvest	nc	nc	nc		6.0 b	6.2 b	7.5 a	0.001	nc
Stage (p value)		0.325[x]		0.378[w]		0.001[x]		0.586[w]	

S: standard RDI; E: early deficit (fruit set to veraison); V: late deficit (veraison to harvest). Values with different letters within years and within stages are significantly different at $p \leq 0.05$ by Tukey-Kramer.
[z] Data were collected before RDI regimens were imposed.
[y] Effect of RDI regimens within year and within stage.
[x] Effect of phenological stage within year.
[w] RDI regimen x stage interaction within years.
[v] Effect of RDI regimens between years.
[u] nc: data not collected.

Table 3.3: Effect of RDI regimens on net CO_2 fixed per vine ($NCE_{WV,d}$) and per unit leaf area ($NCE_{WL,d}$) per day in 2003. Data are means of six vines averaged across all measurements days.

Phenological Stages	Sampling days (DOY)	Irrigation regimen			p value			Day Length	Global Irradiance
		S	E	V					
		----- g CO_2 d^{-1} $vine^{-1}$ ----			treat	DOM	treat*DOM	h	MJ m^{-2} d^{-1}
Fruit set[z]	177-184	101	94	113	0.168	0.001	0.532	15.7	30.6
Pre-veraison	212-219	125 a	67 b	131 a	0.001	0.001	0.048	14.6	21.9
Post-veraison	233-241	127 a	90 b	85 b	0.001	0.001	0.001	13.6	16.5
Pre-harvest	255-262	55	37	33	0.058	0.001	0.146	12.5	18.3
Post-harvest	274-281	51	52	45	0.405	0.082	0.484	11.4	13.7
Stage (p value)			0.001		0.001[y]				0.068[x]

Phenological Stages	Sampling days (DOY)	Irrigation regimen			p value		
		S	E	V			
		g CO_2 d^{-1} m^{-2} leaf area			treat	DOM	treat*DOM
Fruit set[z]	177-184	11.5	11.5	11.7	0.948	0.001	0.730
Pre-veraison	212-219	14.9 a	8.4 b	13.4 a	0.001	0.003	0.151
Post-veraison	233-241	15.3 a	11.0 b	8.8 b	0.001	0.001	0.007
Pre-harvest	255-262	7.8	5.6	3.8	0.075	0.001	0.259
Post-harvest	274-281	8.7	7.1	6.9	0.294	0.174	0.935
Stage (p value)			0.001		0.001[y]		

DOY: day of year; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest); DOM: day of measurement. Values with different letters within phenological stages are significantly different at $p \leq 0.05$ by Tukey- Kramer.

[z] Data were collected before RDI regimens were imposed.

[y] RDI regimen x phenological stage interaction.

[x] p value for global irradiance across phenological stages.

Table 3.4: Effect of RDI regimens on net CO_2 fixed per vine per day ($NCE_{WV,d}$). Data are means of two vines from days with clear skies.

Phenological Stages	Sampling day (DOY)	Irrigation regimen			p value	Global Irradiance	2002-2003[z]
		S	E	V			
		----- g CO_2 d^{-1} $vine^{-1}$ ----			treat	MJ m^{-2} d^{-1}	
---------- 2002 ----------							
Fruit set[y]		nc[x]	nc	nc			
Pre-veraison	218	124 a	70 b	131 a	0.015	24.1	
Post-veraison	235	117 ab	73 b	135 a	0.028	23.6	
Pre-harvest	256	72	72	64	0.905	20.5	
Post-harvest	279	48	37	55	0.341	16.0	
Stage (p value)			0.001		0.032[w]		
---------- 2003 ----------							g CO_2 d^{-1} $vine^{-1}$
Fruit set[y]	178	111	120	141	0.402	31.4	nc
Pre-veraison	218	140 a	60 b	149 a	0.002	27.5	112 a
Post-veraison	238	145 a	124 a	60 b	0.001	24.2	109 a
Pre-harvest	256	81	66	49	0.197	21.7	67 b
Post-harvest	278	54	59	57	0.462	15.3	52 c
Stage (p value)			0.001		0.001[w]		0.001

DOY: day of year; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters within phenological stages are significantly different at $p \leq 0.05$ by Tukey-Kramer.

[z] Effect of RDI regimens on $NCE_{WV,d}$ across phenological stages with pooled data of 2002 and 2003. No significant interaction was found between year and phenological stage ($p \leq 0.4989$).

[y] Data were collected before RDI regimens were imposed.

[x] nc: data not collected in 2002.

[w] RDI regimen x phenological stage interaction.

Table 3.5: Effect of RDI regimens on net CO_2 fixed per unit leaf area per day ($NCE_{WL,d}$) for 2003. Data are means of two vines from days with clear skies.

Phenological Stages	Sampling day (DOY)	Irrigation regimen			p value
		S	E	V	
		---g CO_2 d^{-1} m^{-2} leaf area --			treat
Fruit set[z]	178	13.2	14.6	14.8	0.704
Pre-veraison	218	17.1 a	7.4 b	15.7 a	0.015
Post-veraison	238	17.9 a	14.5 a	6.2 b	0.015
Pre-harvest	256	11.7	10	5.5	0.179
Post-harvest	278	9.4	9.0	7.8	0.340
Stage (p value)			0.001		0.001[y]

DOY: day of year; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters within phenological stages are significantly different at $p \leq 0.05$ by Tukey-Kramer.

[z] Data were collected before RDI regimens were imposed.

[y] RDI regimen x phenological stage interaction.

Table 3.6: Effect of RDI regimens on total net CO_2 fixed per vine ($NCE_{WV,d}$) and per unit of leaf area ($NCE_{WL,d}$) per day between fruit set and post-harvest of 2003.

Phase	Duration (days)	$NCE_{WV,d}$ [z]			Total NCE_{WV} during phase [y]			*vs.* S	
		S	E	V	S	E	V	E	V
		g CO_2 d^{-1} $vine^{-1}$			kg CO_2 $phase^{-1}$ $vine^{-1}$ (% Total)			%	%
Fruit set to Veraison [x]	46	126	60	145	5.79 (54)	2.77 (40)	6.68 (71)	48	115
Veraison to harvest [w]	32	114	95	55	3.64 (34)	3.04 (43)	1.75 (19)	83	48
Harvest to post-harvest [v]	19	68	63	53	1.29 (12)	1.19 (17)	1.01 (11)	92	79
Total	97				10.73 (100)	7.01 (100)	9.45 (100)	65	88

Phase	Duration (days)	$NCE_{WL,d}$ [z]			Total NCE_{WL} during phase [y]			*vs.* S	
		g CO_2 d^{-1} m^{-2}			kg CO_2 $phase^{-1}$ m^{-2} (% Total)			%	%
Fruit set to Veraison [x]	46	15	7	15	0.700 (50)	0.344 (37)	0.704 (69)	49	101
Veraison to harvest [w]	32	11	12	6	0.476 (35)	0.396 (43)	0.188 (19)	83	40
Harvest to post-harvest [v]	19	14	10	7	0.201 (15)	0.184 (20)	0.127 (12)	91	63
Total	97				1.38 (100)	0.924 (100)	1.02 (100) 6	67	74

S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest).
[z] Based on NCE_W recorded on days with clear skies of 2003.
[y] Calculated as the product of duration (days) x $NCE_{WV,d}$ (g CO_2 d^{-1} $vine^{-1}$) or x $NCE_{WL,d}$ (g CO_2 d^{-1} m^{-2}).
[x] Values for S and V are means of $NCE_{WV,d}$ or $NCE_{WL,d}$ between the fruit set and pre-veraison measurements, while for E is the $NCE_{WV,d}$ or $NCE_{WL,d}$ at pre-veraison.
[w] Values for all RDI regimens are means of $NCE_{WV,d}$ or $NCE_{WL,d}$ between the post-veraison and pre-harvest measurements.
[v] Values for all RDI regimens are means of $NCE_{WV,d}$ or $NCE_{WL,d}$ between the pre- and post-harvest measurements.

Table 3.7: Effect of RDI regimens on daily maximum NCE_{WL} recorded during 24 h. Data are means of two vines from days with clear skies.

Phenological Stages	Sampling day (DOY)	Irrigation regimen			p value
		S	E	V	
		--------- µmol m^{-2} leaf area s^{-1} ---------			
Fruit set[z]	178	10.1	11.9 ab[y]	10.5 a[y]	0.365
Pre-veraison	218	12.1 A[x]	7.1 c B	11.3 a A	0.022
Post-veraison	238	12.8 A	11.7 ab A	7.7 bc B	0.014
Pre-harvest	256	11.6	12. a	6.9 c	0.323
Post-harvest	278	11.5	9.2 bc	9.7 ab	0.290
Stages (p value)		0.901	0.005	0.006	0.029[w]

DOY: day of year; S: standard deficit; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest).
[z] Data were collected before RDI regimens were imposed.
[y] Values with different letters (a, b, c) within columns are significantly different at $p \leq 0.05$ by Tukey- Kramer.
[x] Values with different letters (A, B, C) within phenological stages are significantly different at $p \leq 0.05$ by Tukey- Kramer.
[w]RDI regimen x phenological stage interaction.

Table 3.8: Effect of RDI regimens on daily mean NCE_{SL}. Data are from days with clear skies of 2003 and are means of 6 to 9 vines.

Phenological Stage	Sampling days (DOY)	Irrigation regimen			p value	
		S	E	V		
		-------- µmol m^{-2} leaf area s^{-1} --------			treat	[z] µmol m^{-2} leaf area s^{-1}
Fruit set[y]	178, 183	7.9	6.9	8.7	0.174	7.8 c
Pre-veraison	213, 218	13.5 a	8.3 b	12.8 a	0.001	11.5 a
Post-veraison	237, 238, 239	12.8 a	11.4 a	6.8 b	0.001	10.4 b
Pre-harvest	255, 260	8.2 a	7.4 ab	6.5 b	0.050	7.3 c
Post-harvest	275, 276	10.3 b	9.4 b	9.9 b	0.184	9.9 b
Stages (p value)			0.001		0.001[x]	0.001

DOY: day of year; S: standard deficit; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters within phenological stages are significantly different at $p \leq 0.05$ by Tukey- Kramer.

[z] Values with different letters within columns are significantly different at $p \leq 0.05$ by Tukey- Kramer.

[y] Data were collected before RDI regimens were imposed.

[x] RDI regimen x stage interaction.

Table 3.9: Effect of RDI regimens on leaf carbohydrate export rates (CO_2 equivalents) and NCE_{SL} in 2003.

Phenological Stages	Irrigation regimen			
	S	E	V	p value[y]
	μmol m^{-2} s^{-1}			
Fruit set[z]	3.2	5.0	5.1	0.062
Pre-veraison	9.9	8.5	10.5	0.497
Post-veraison	7.9	11.4	6.1	0.120
Pre-harvest	9.1	10.7	5.3	0.185
Post-harvest	nc[x]	nc[x]	nc[x]	

[z] Data were collected before RDI regimens were imposed.
[y] p value within phenological stages
[x] nc: data not collected.

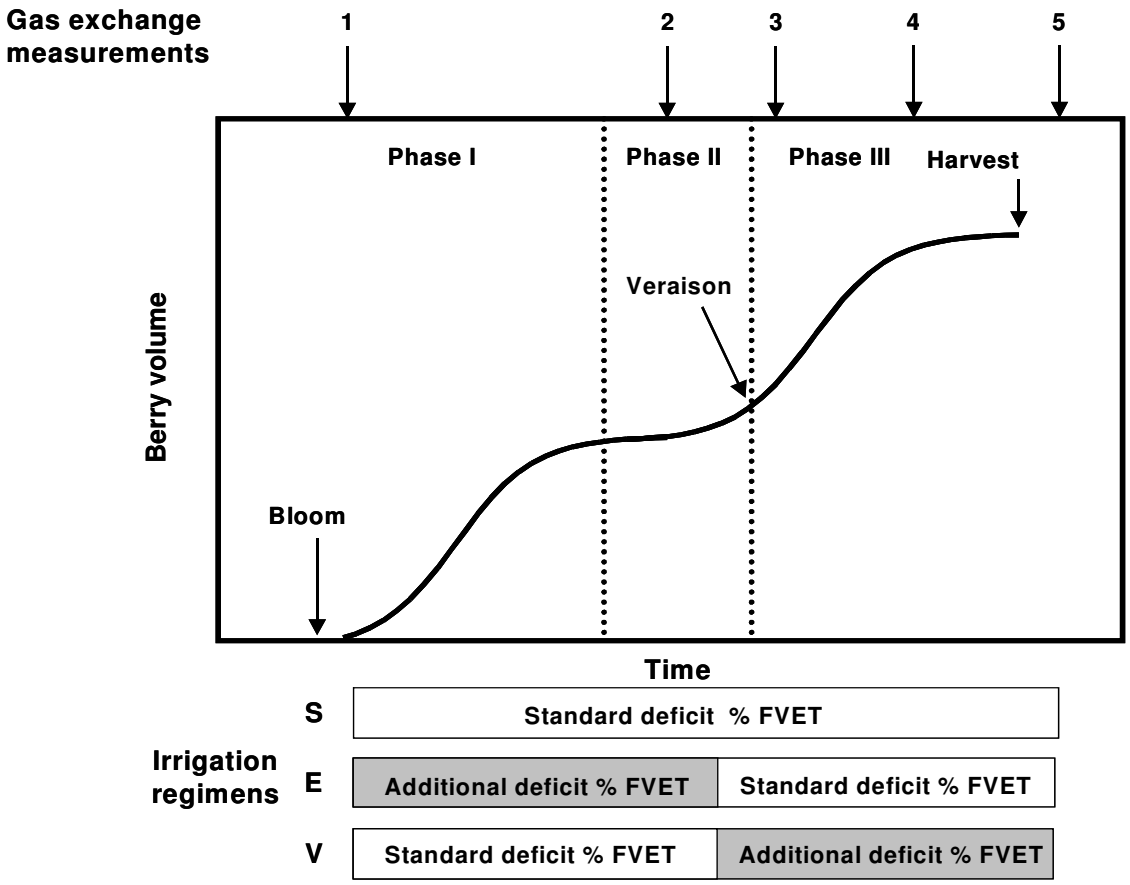


Figure 3.1: Experimental calendar and irrigation schedule superimposed on theoretical curve of berry growth. Arrows at top indicate approximate periods of whole-canopy and single-leaf gas exchange measurements: 1-fruit set, 2-pre-veraison, 3-post-veraison, 4-pre-harvest, 5-post-harvest. Bars at the bottom indicate the duration and scheduling of irrigation regimens. FVET: full vine evapotranspiration; standard deficit = 70% FVET (2002) or 60% FVET (2003); additional deficit = 35% FVET (2002) or 30% FVET (2003).

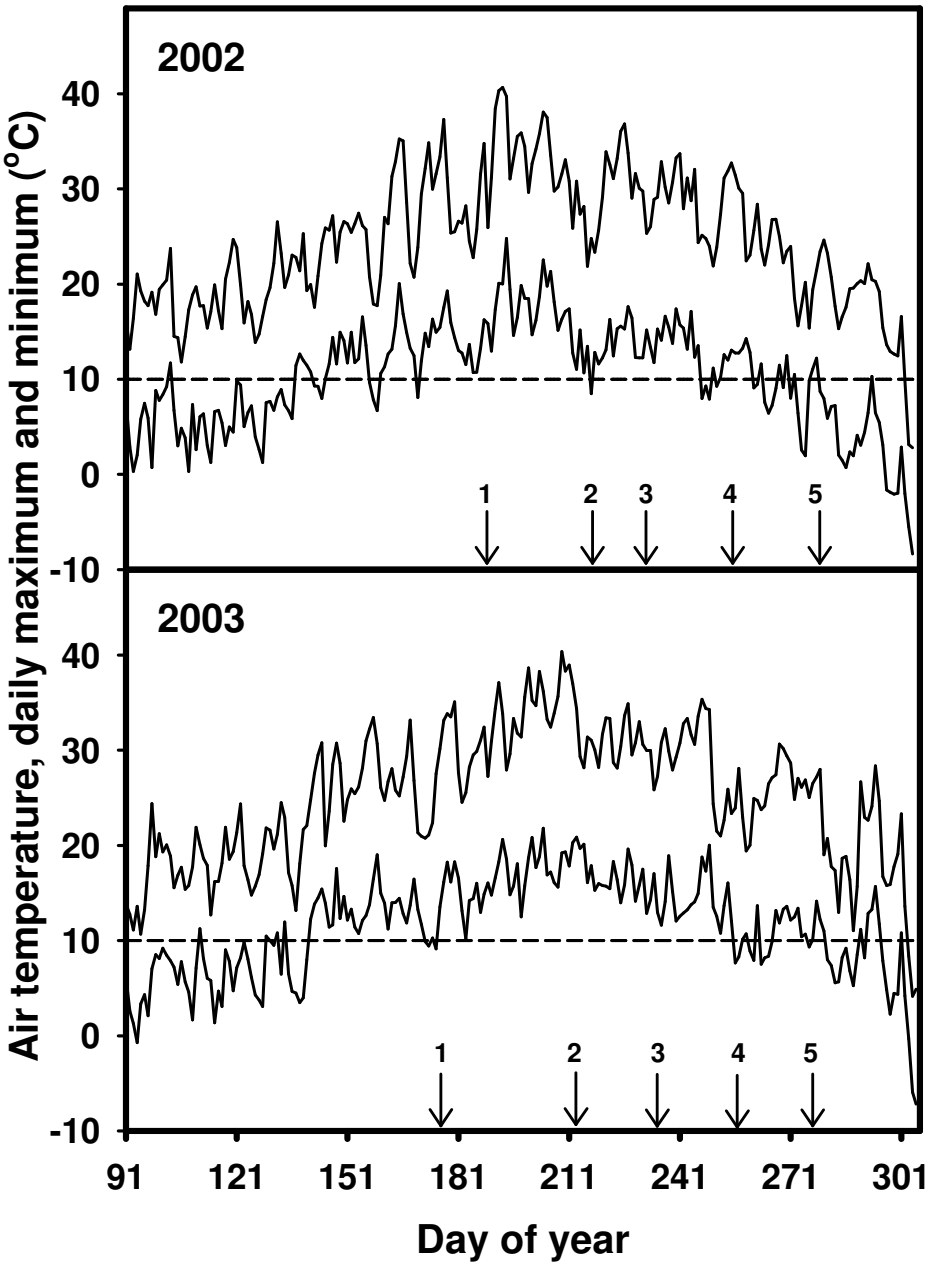


Figure 3.2: Daily maximum and minimum air temperatures (1.5 m) recorded at the Alderdale PAWS station (10 km west of site) during 2002 and 2003. Dashed line is 10°C, the conventional "base temperature" for grapevine growth and accumulation of GDD. Arrow numbers indicate gas exchange measurements: 1-fruit set, 2-pre-veraison, 3-post-veraison, 4-pre-harvest, 5-post-harvest.

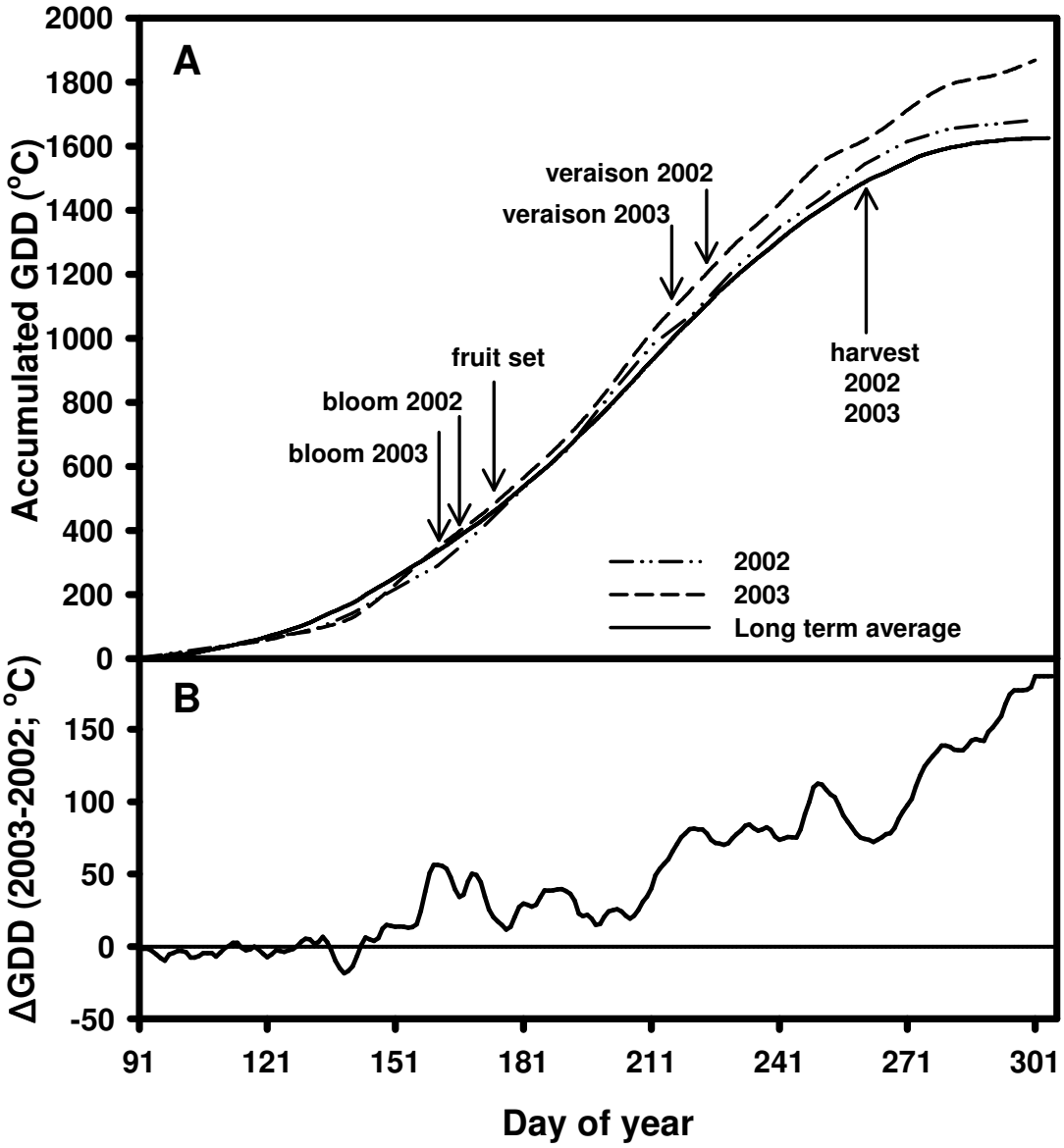


Figure 3.3: A. Growing degree days (GDD; base 10 °C) for 2002 and 2003 measured at Alderdale PAWS station (10 km west of site). Main phenological stages are indicated. Long-term average (1991-2003) is from Paterson PAWS station (18 km east of site) because Alderdale PAWS station has not existed long enough to provide a meaningful long-term average. B. Difference in accumulated GDD between the two seasons.

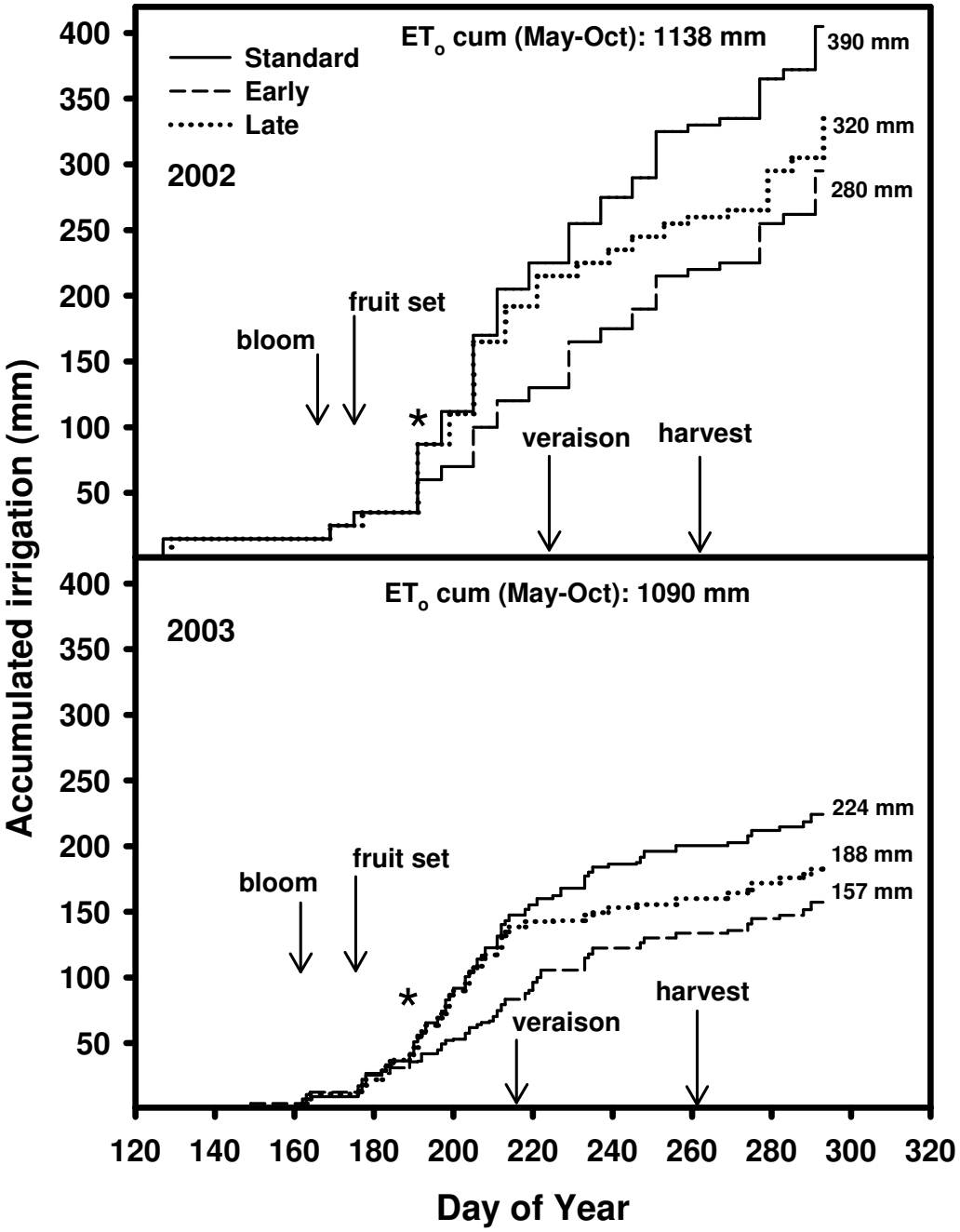


Figure 3.4: Accumulated irrigation applied during growing seasons from May to October (leaf fall), 2002 and 2003. Main phenological phases are indicated. Asterisks denote beginning of RDI regimens.

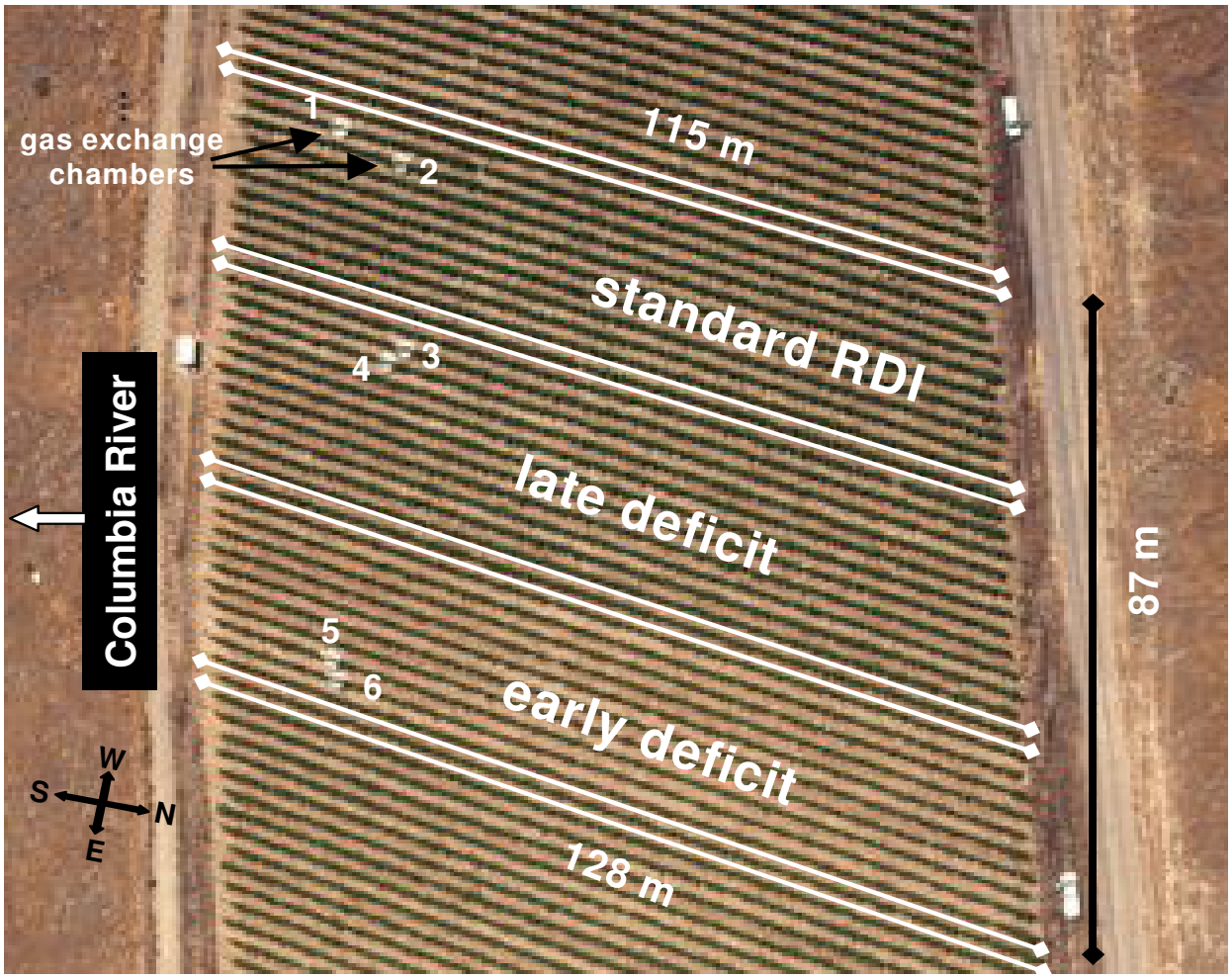


Figure 3.5: Aerial photograph of the experimental site on DOY 216, 2003 (pre-veraison). Effects of the early deficit RDI regimen on canopy size can be distinguished by comparing row thicknesses among irrigation regimens (i.e., early deficit vs. standard RDI or late deficit).

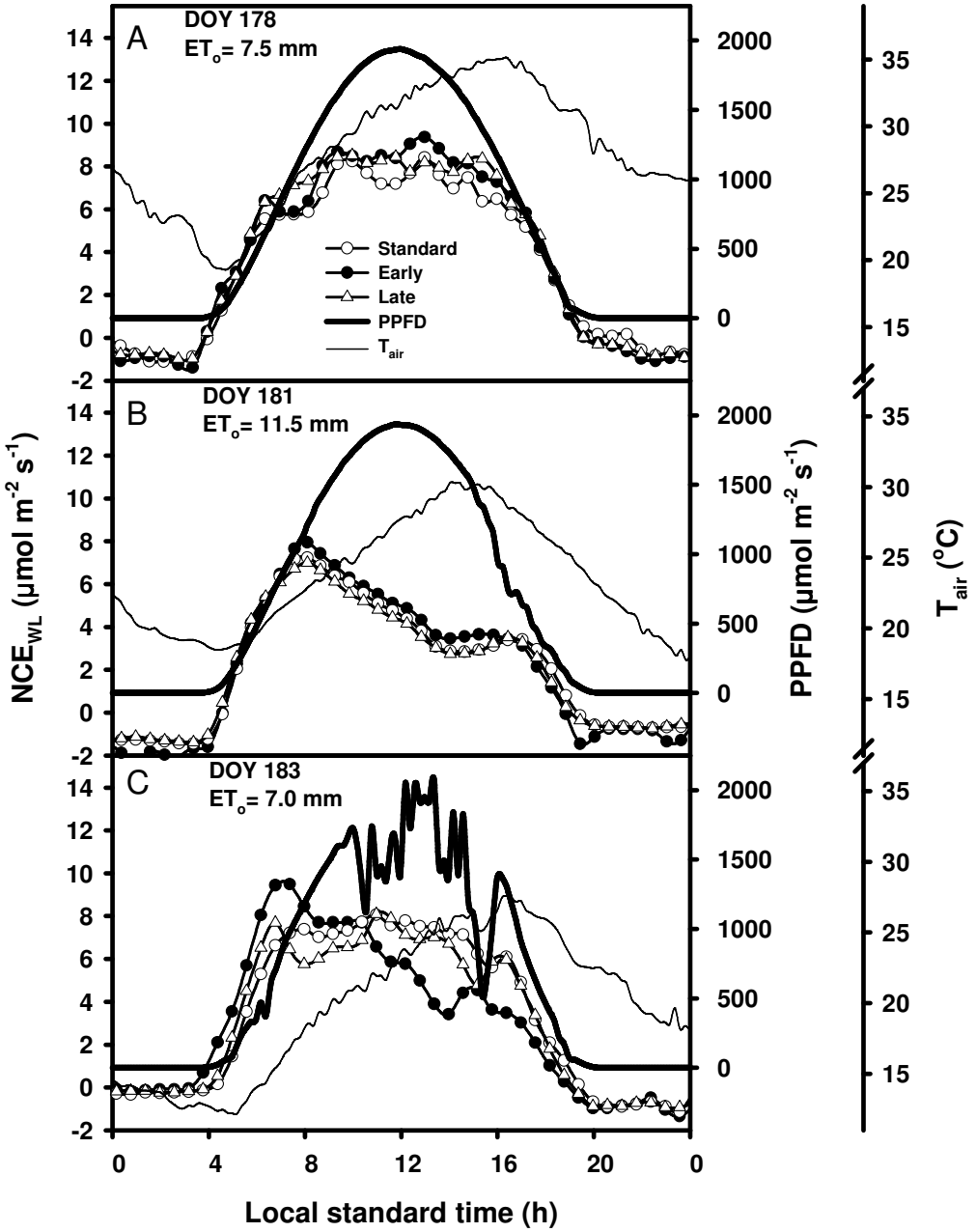


Figure 3.6: Whole-canopy net CO_2 exchange rate (NCE_{WL}), photosynthetic photon flux density (PPFD) and air temperature (T_{air}) during fruit set, 2003. An afternoon depression in NCE_{WL} is evident on DOY 181, after two days of no irrigation, while on DOY 178 irrigation was being applied. Intermediate responses were observed on DOY 183, following irrigation (DOY 182), but confounded by passing cloud. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

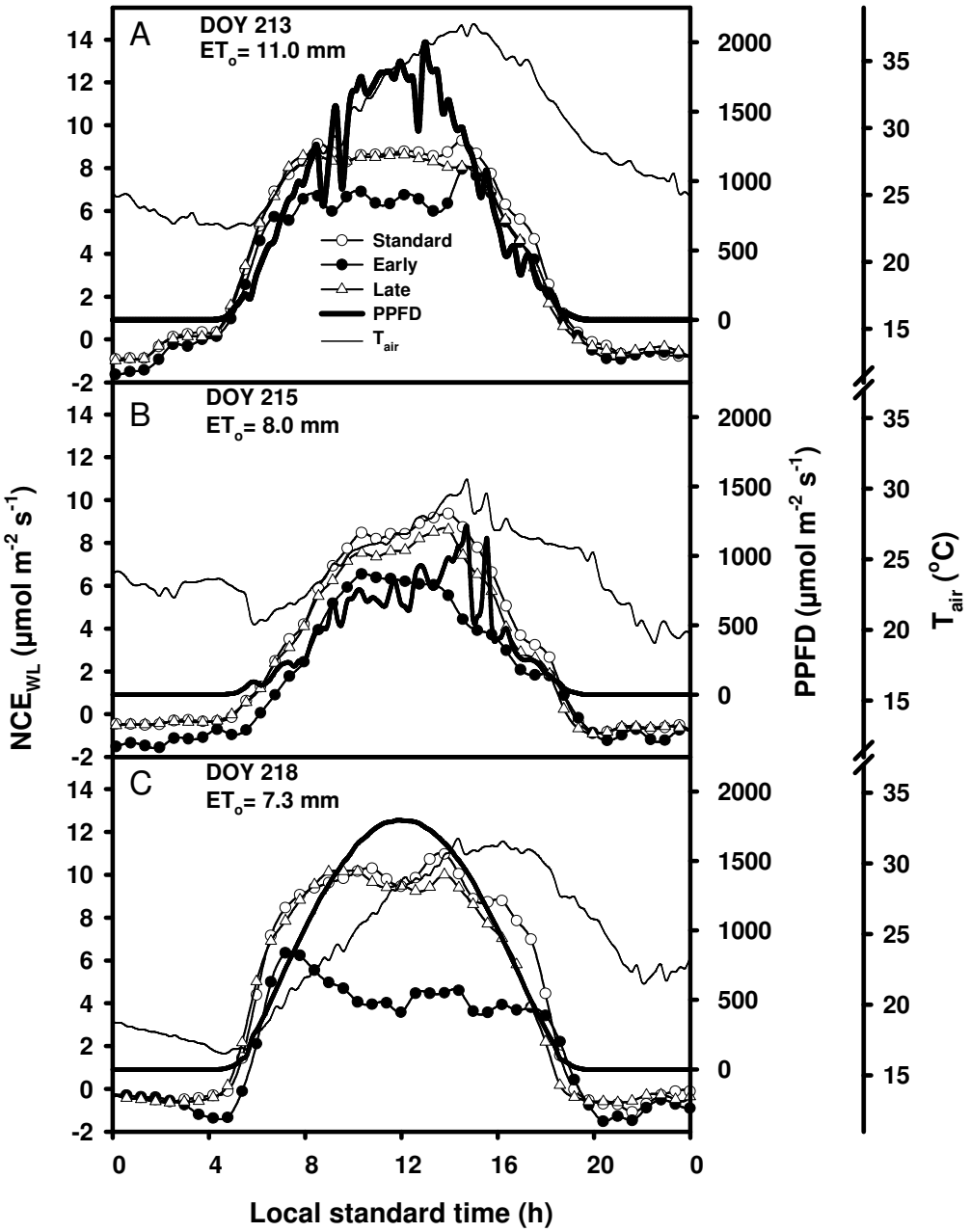


Figure 3.7: Whole-canopy net CO_2 exchange rate (NCE_{WL}), photosynthetic photon flux density (PPFD), and air temperature (T_{air}) during pre-veraison, 2003. Panel B demonstrates a smaller difference in NCE_{WL} between extra water deficit (E) and vines under standard RDI regimen (S and V) due to lower PPFD and lower air temperature. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

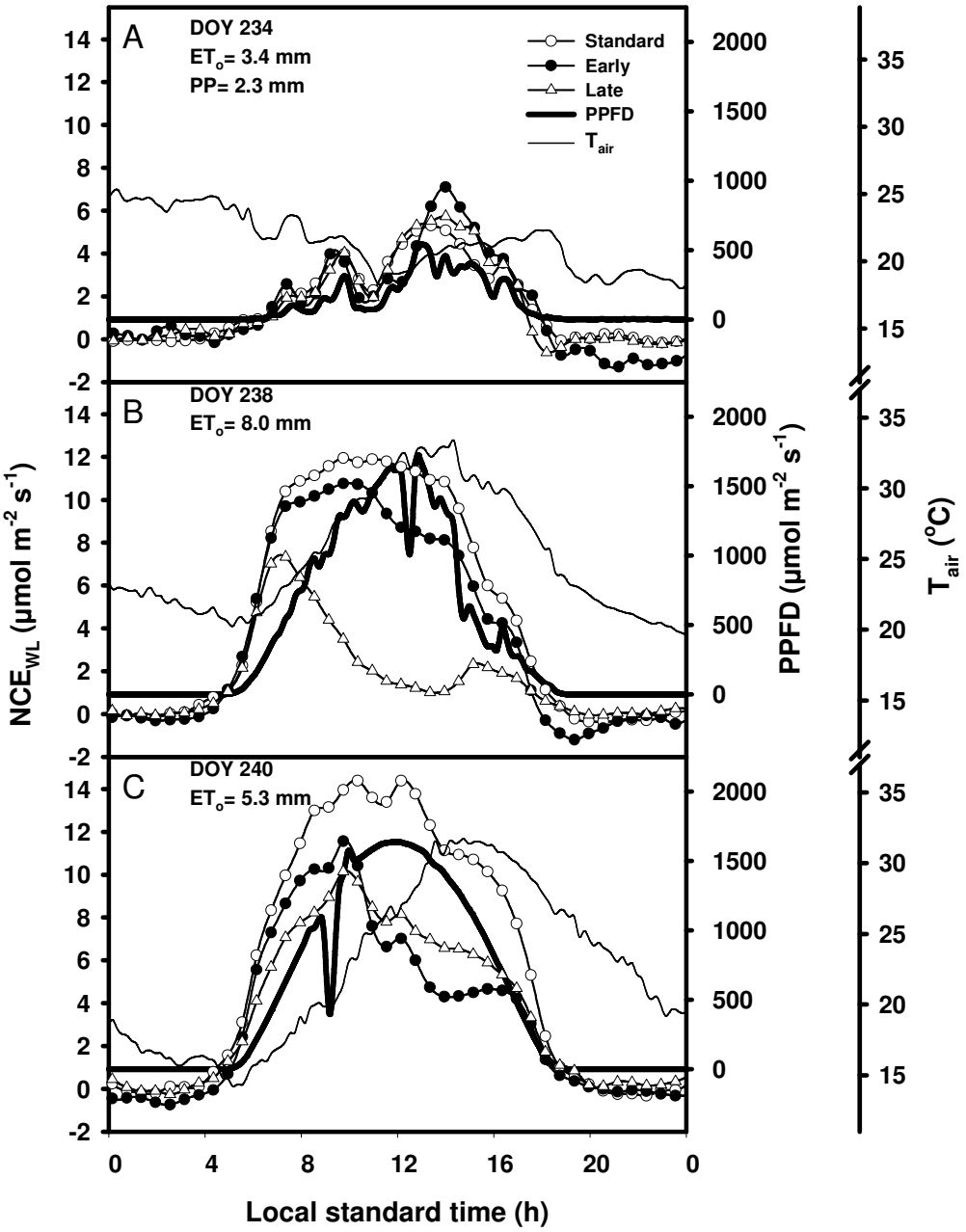


Figure 3.8: Whole-canopy net CO_2 exchange rate (NCE_{WL}), photosynthetic photon flux density (PPFD), and air temperature (T_{air}) during post-veraison, 2003. V vines were under extra water deficit, S and E vines were under standard RDI regimen. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) and precipitation (PP) is from the Alderdale PAWS station (10 km west of site).

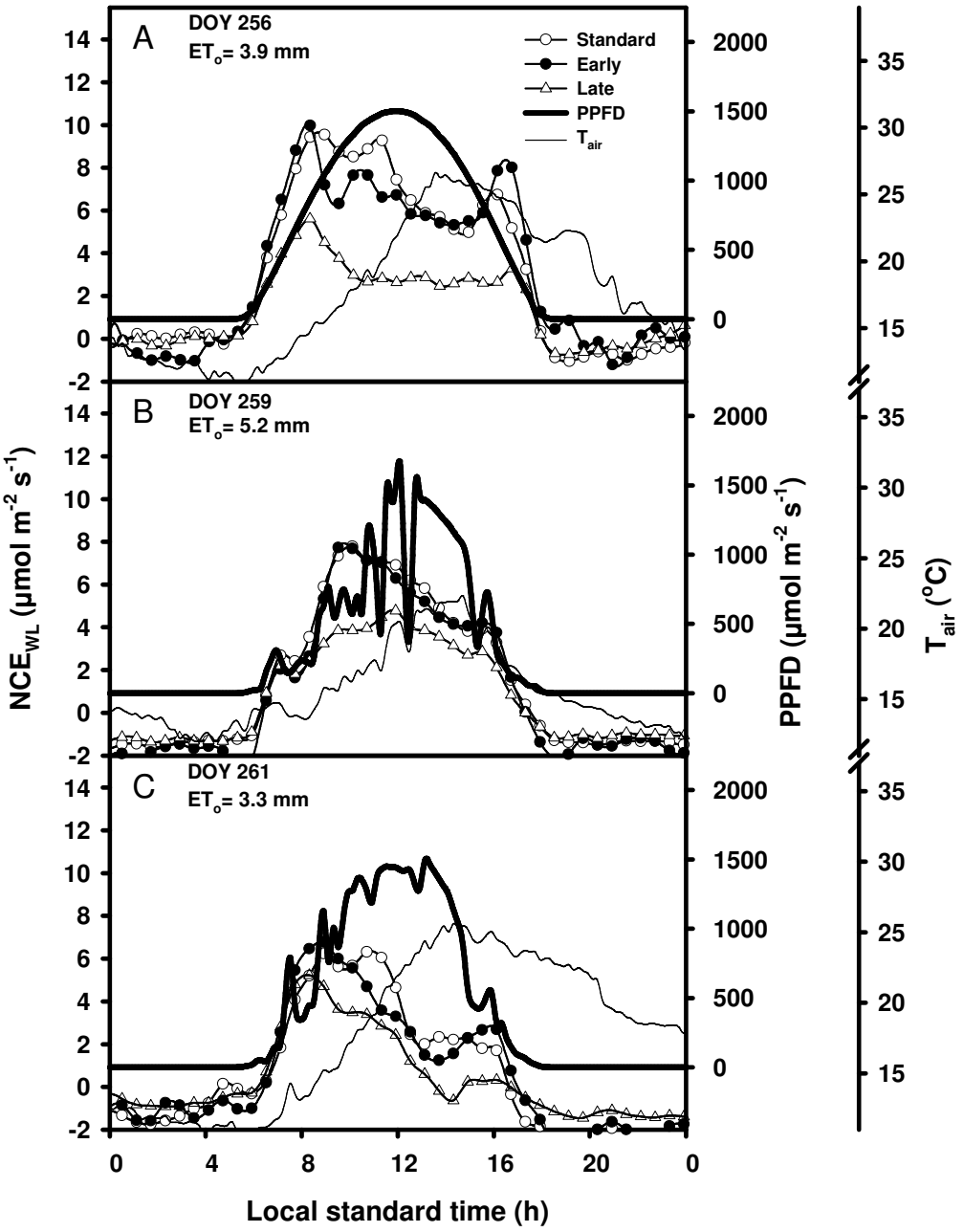


Figure 3.9: Whole-canopy net CO_2 exchange rate (NCE_{WL}), photosynthetic photon flux density (PPFD), and air temperature (T_{air}) during pre-harvest, 2003. V were under extra water deficit, S and E vines were under standard RDI regimen. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

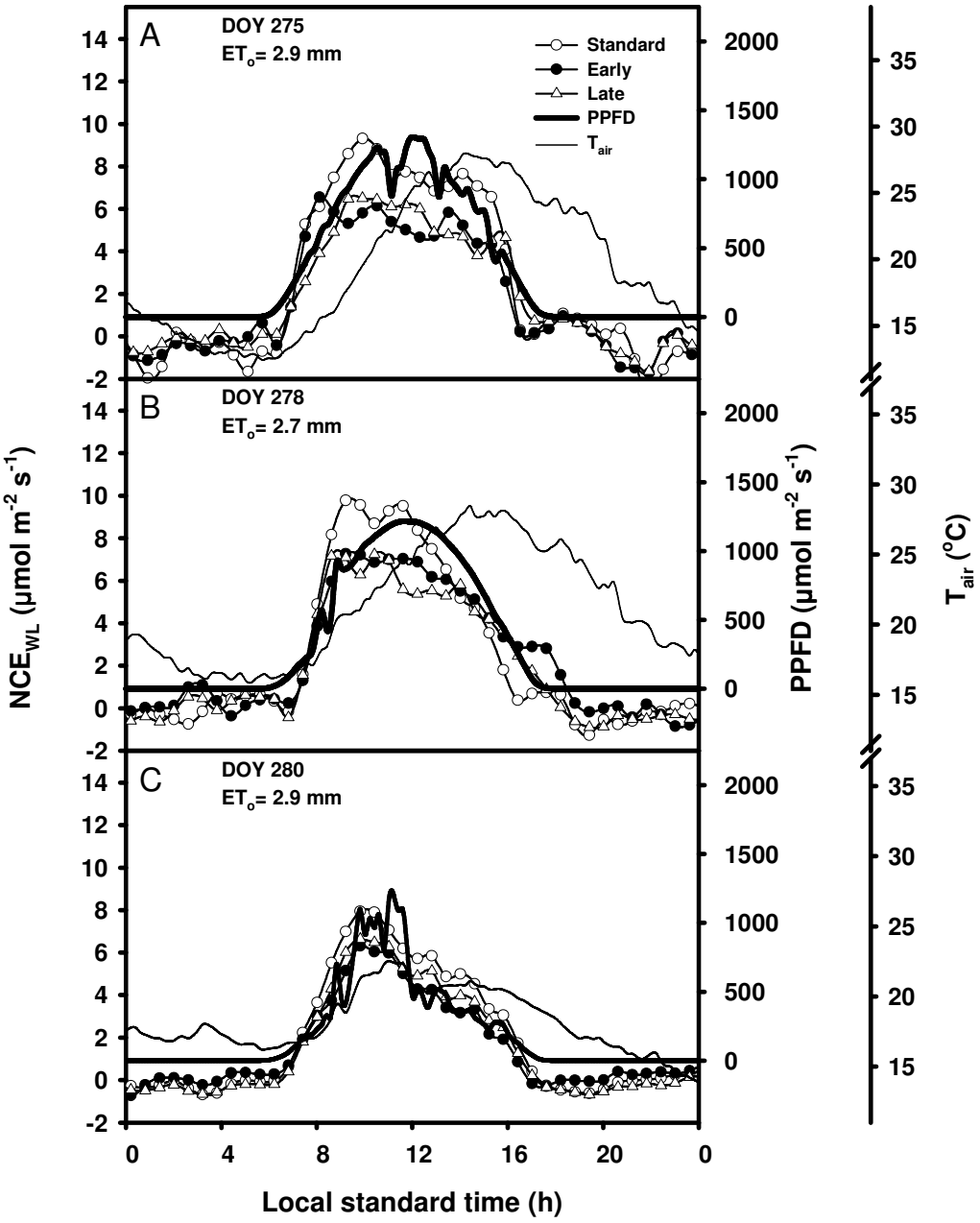


Figure 3.10: Whole-canopy net CO_2 exchange rate (NCE_{WL}), photosynthetic photon flux density (PPFD), and air temperature (T_{air}) during post-harvest, 2003. All vines were irrigated at FVET. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

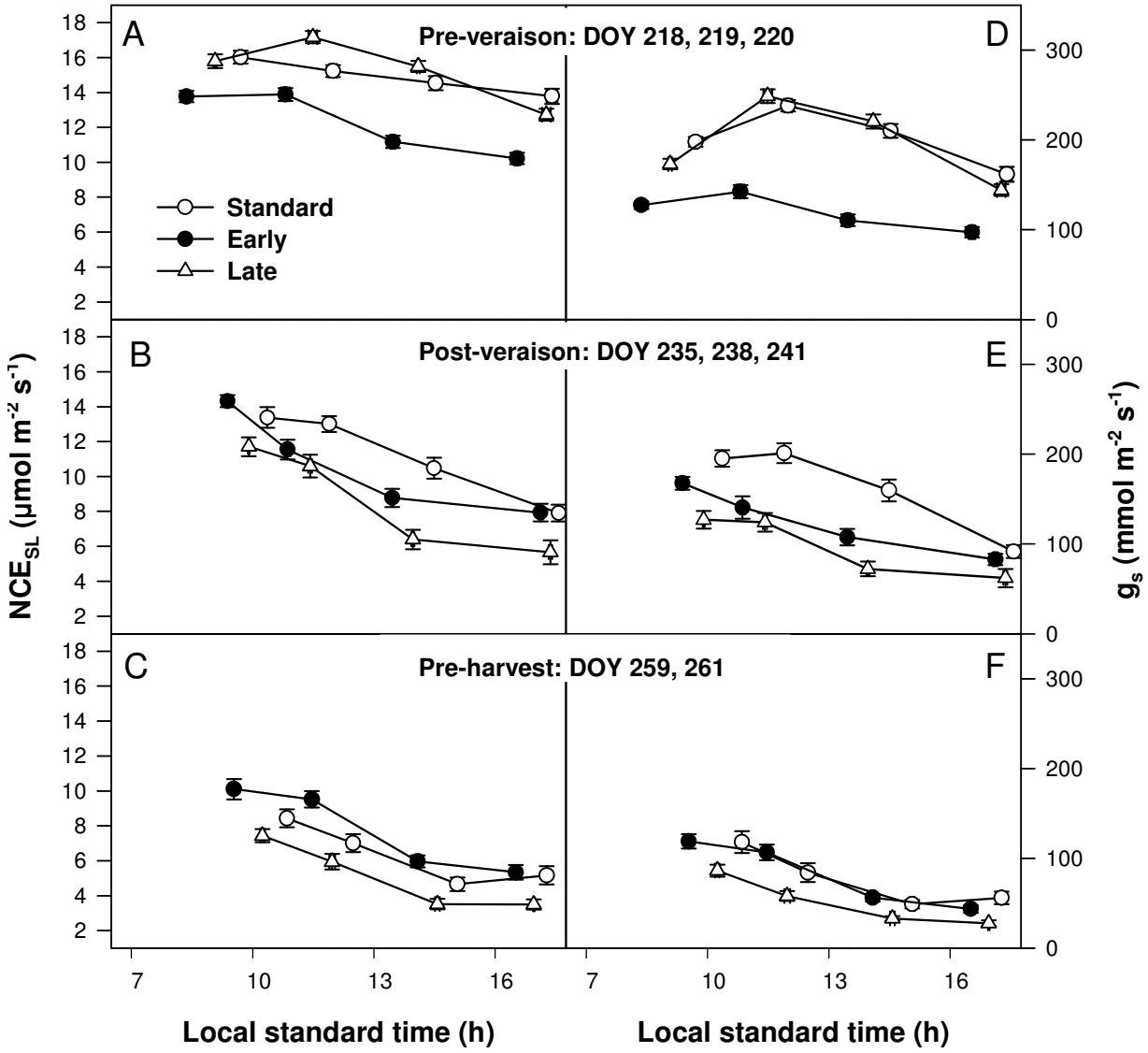


Figure 3.11: Effects of RDI regimens on single-leaf net CO_2 exchange rate (NCE_{SL}; A-C) and stomatal conductance (g_s; D-F), 2002. Mean values are per vine ± SE (n=9; at pre-harvest n=6).

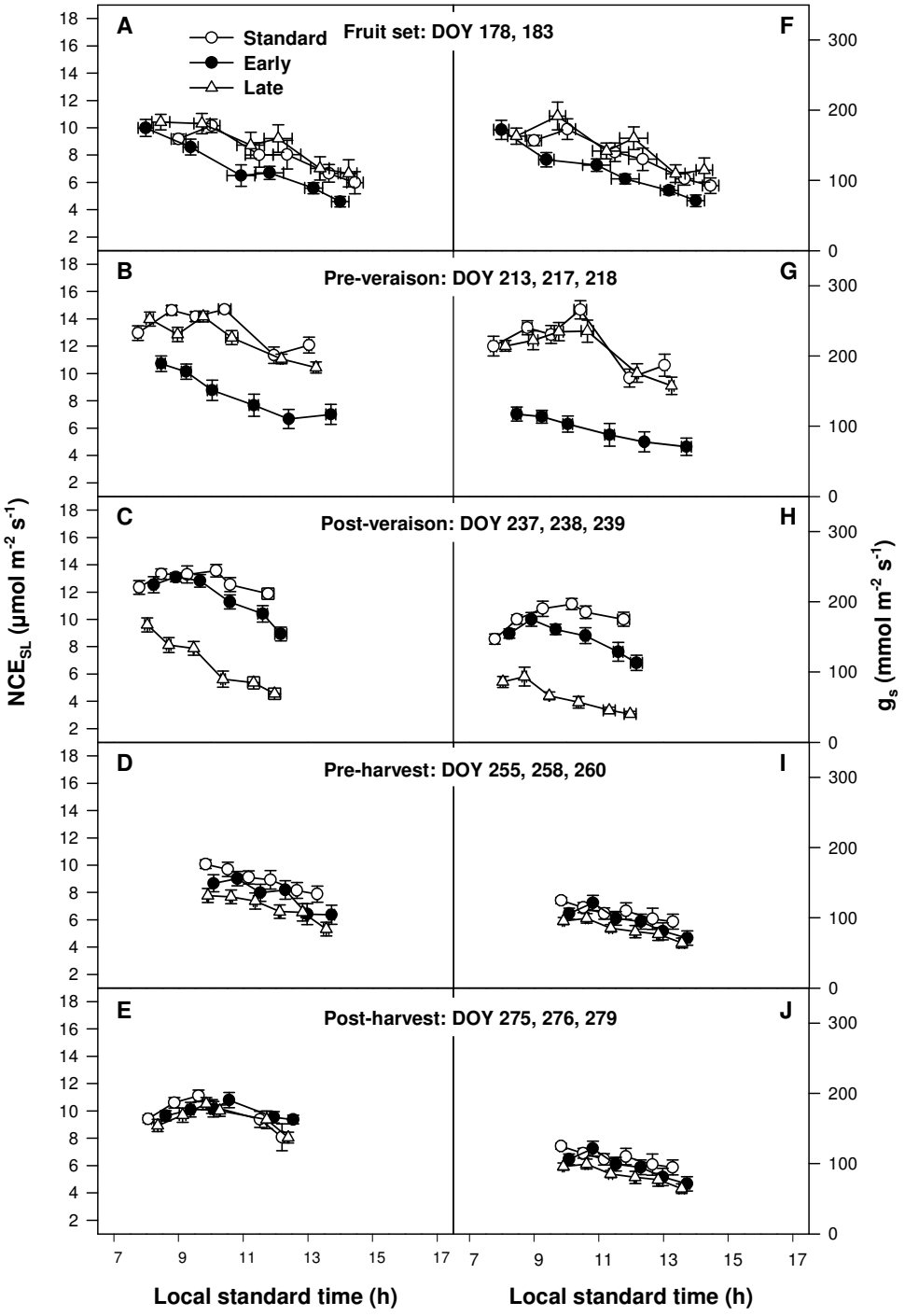


Figure 3.12: Effects of RDI regimens on single-leaf net CO_2 exchange rate (NCE_{SL}; A-E) and stomatal conductance (g_s; F-J), 2003. Mean values are per vine ± SE (n=9; at fruit set n=6).

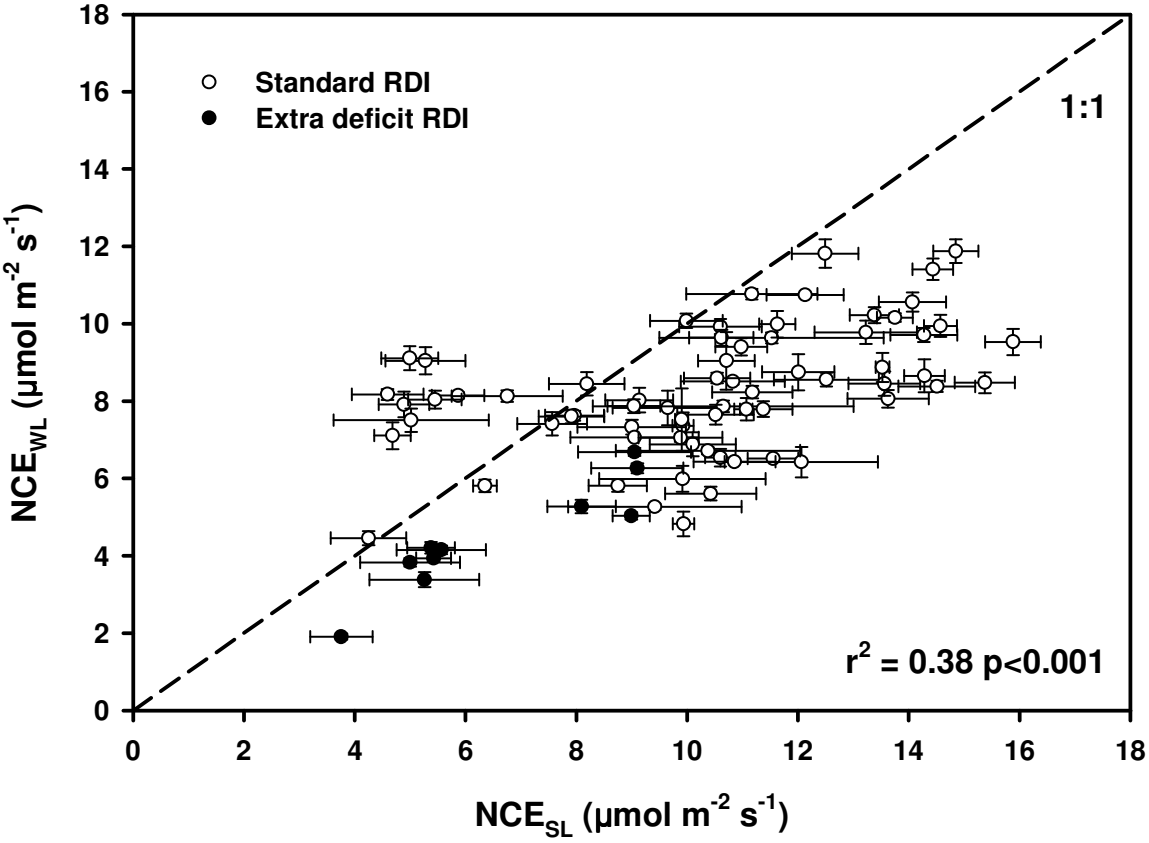


Figure 3.13: Relationship between single-leaf (NCE_{SL}) and whole-canopy net CO_2 exchange rate (NCE_{WL}) in 2003. Values are hourly means per vine ± SE (n=3 to 5 for NCE_{SL} and n=8 to 10 for NCE_{WL}). Data are from all phenological stages except pre-harvest because there was no coincidence in time between NCE_{SL} and NCE_{WL} measurements.

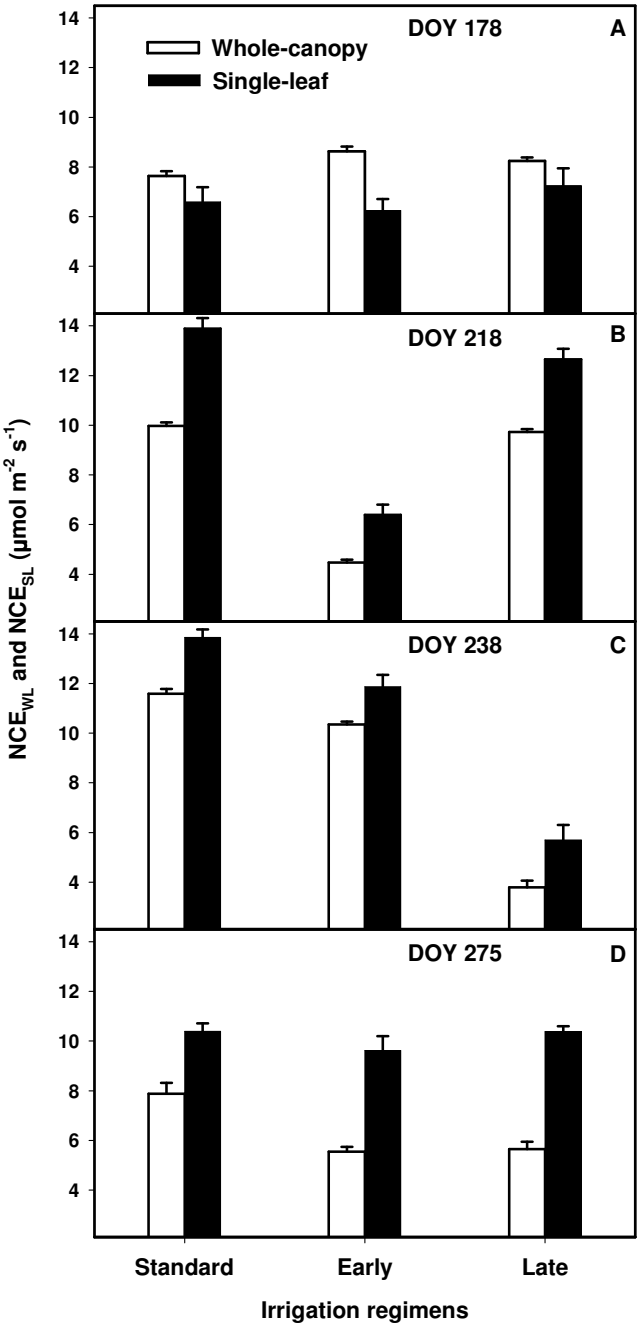


Figure 3.14: Effects of RDI regimens on average whole-canopy (NCE_{WL}) and single-leaf (NCE_{SL}) net CO_2 exchange rate at coincident DOY and time during 2003. Means values are per vine ± SE.

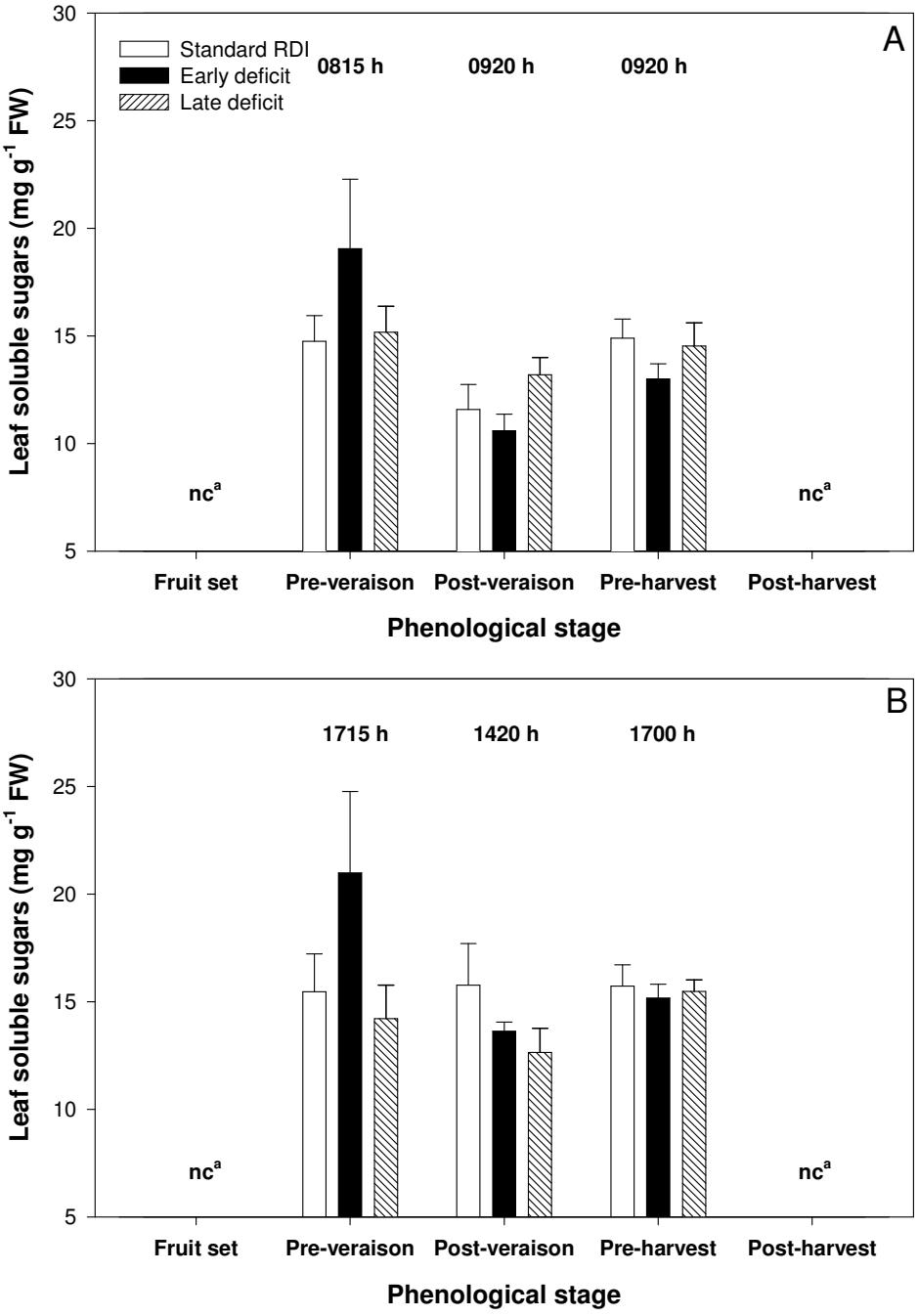


Figure 3.15: Effects of RDI regimens on leaf soluble sugars concentration (glucose+fructose+sucrose). First (A) and last (B) sample times of the day during 2002 on days with clear skies. Sampling times are indicated above bars. Mean values are per vine ± SE (n=9; pre-harvest n=6). [a]nc: data not collected.

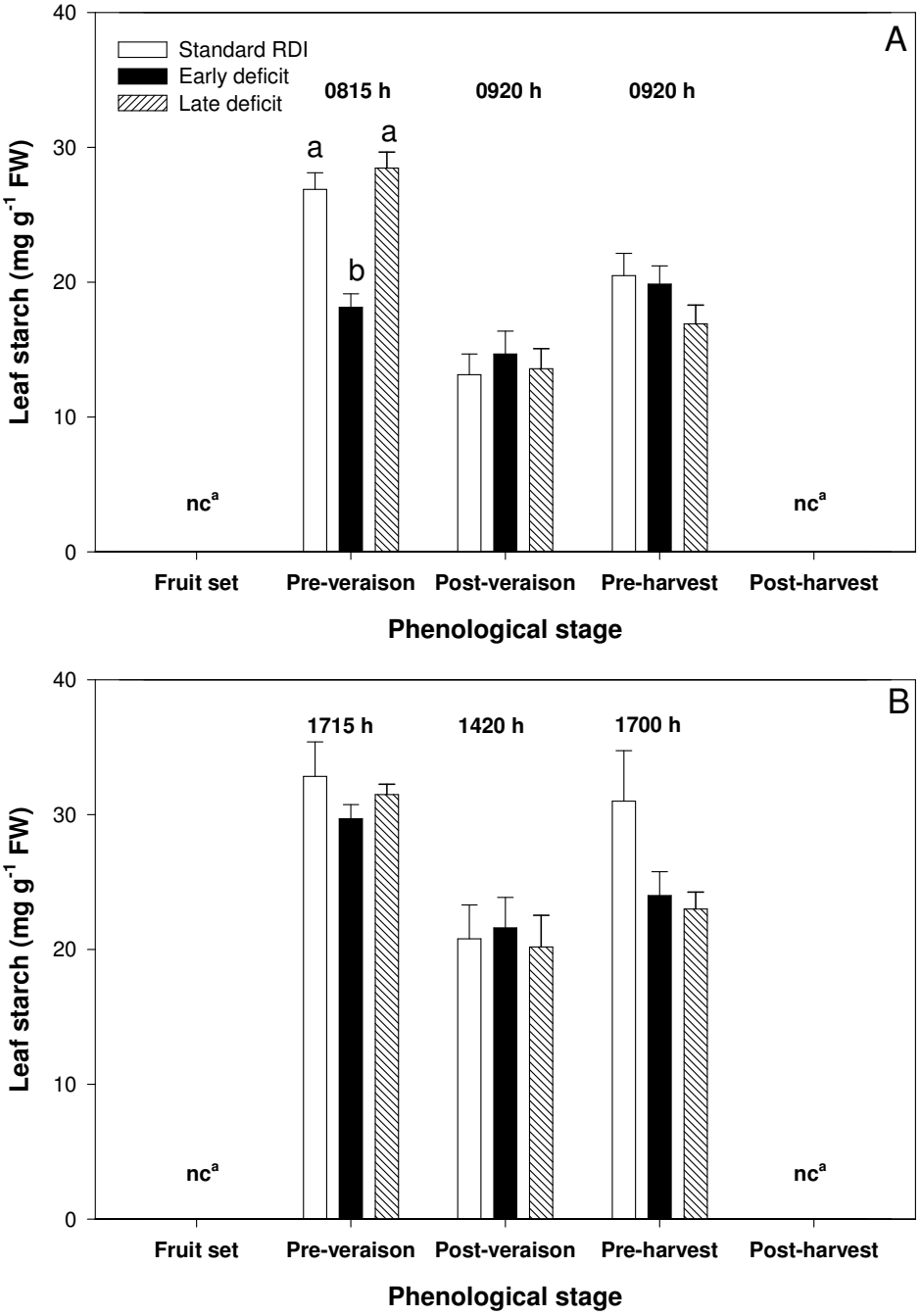


Figure 3.16: Effects of RDI regimens on leaf starch concentration. First (A) and last (B) sample times of the day during 2002 on days with clear skies. Sampling times are indicated above bars. Mean values are per vine ± SE (n=9; pre-harvest n=6). Bars with different letters are significantly different at $p \leq 0.05$ by Tukey-Kramer. [a]nc: data not collected.

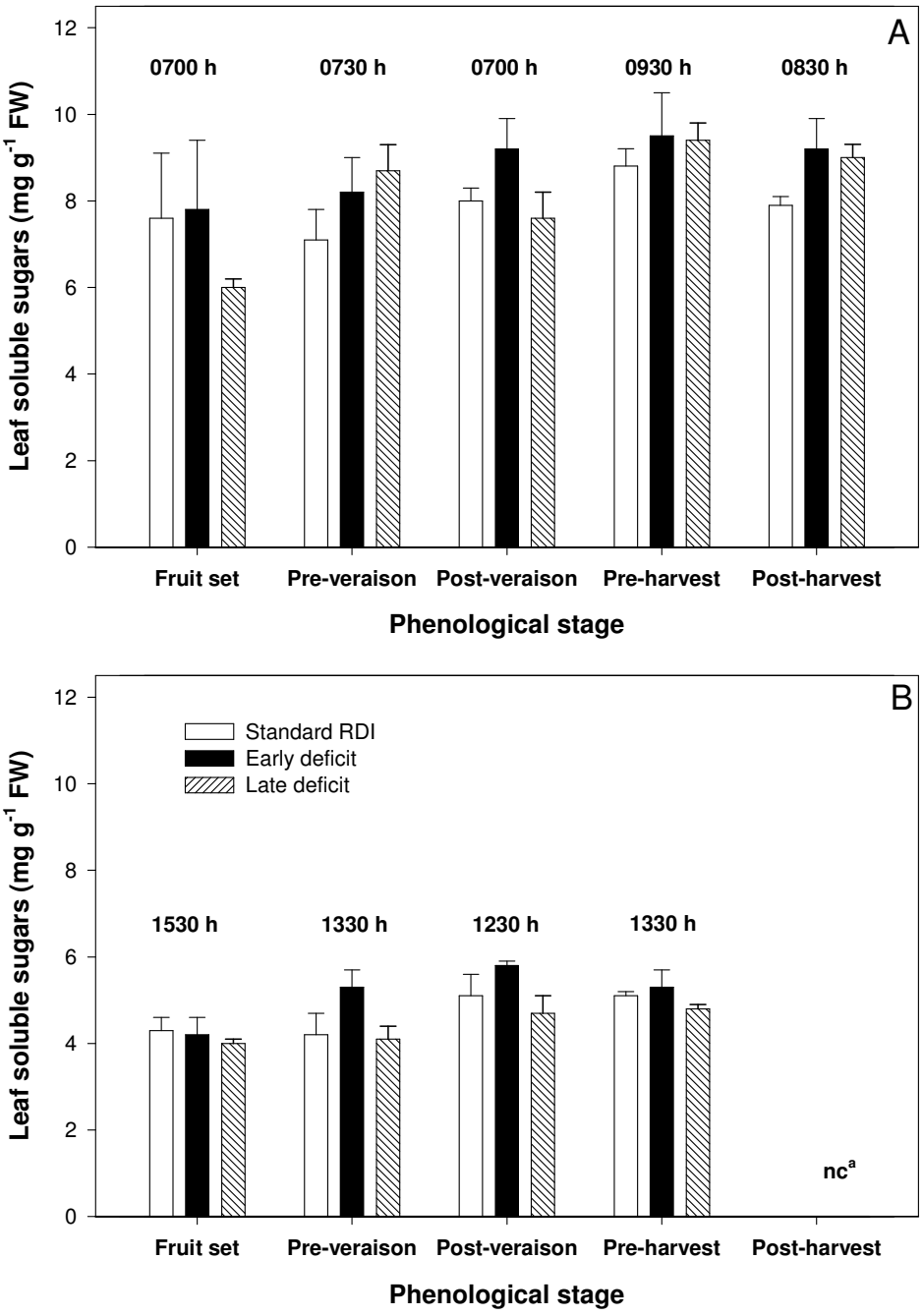


Figure 3.17: Effects of RDI regimens on leaf soluble sugars concentration (glucose+fructose+sucrose). First (A) and last (B) sample times of the day during 2003 on days with clear skies. Sampling times are indicated above bars. Mean values are per vine ± SE (n=3). [a]nc: data not collected.

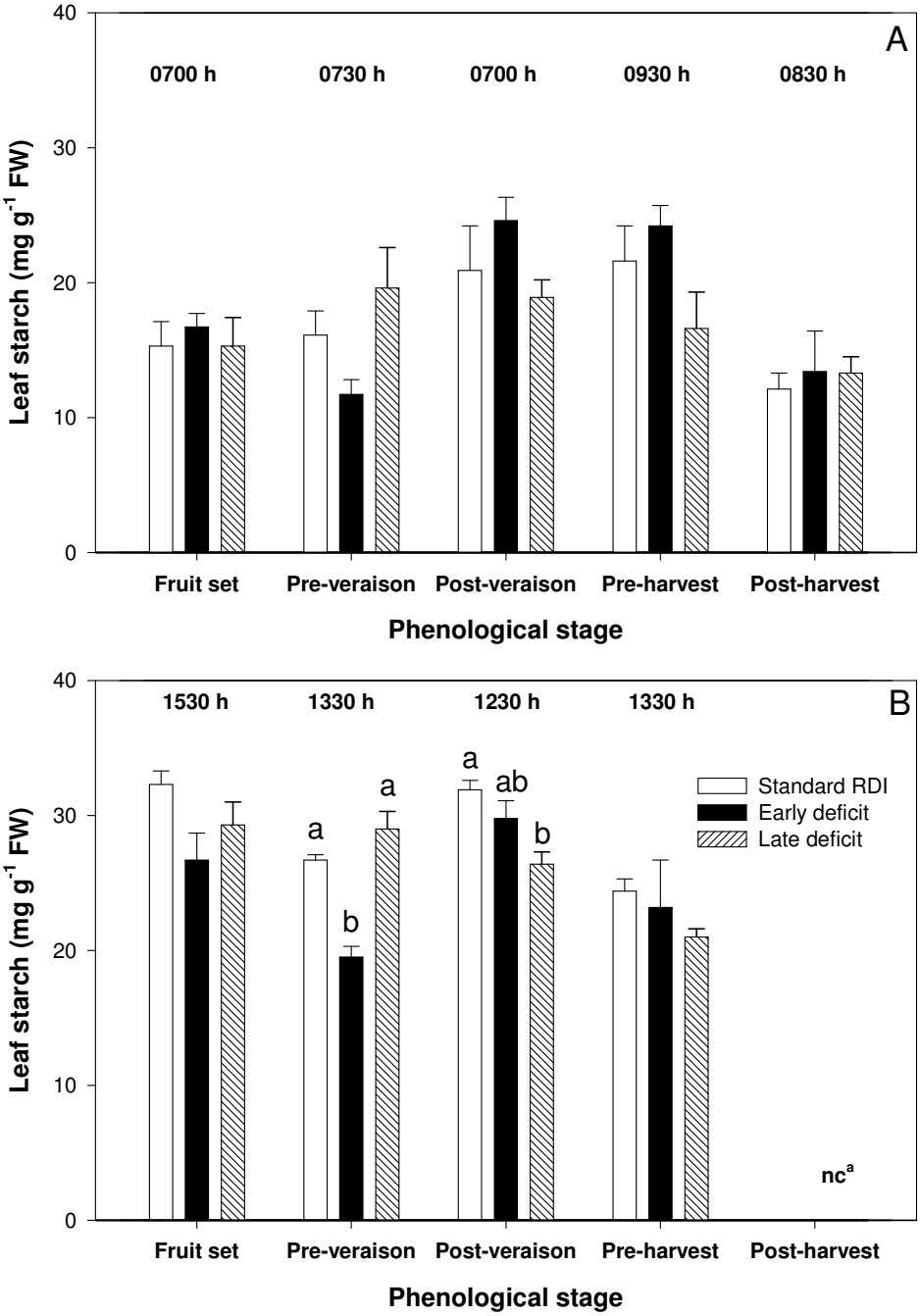
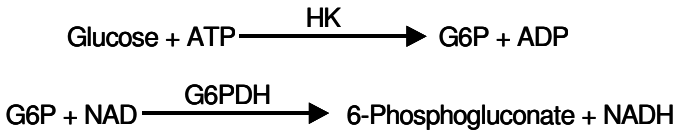


Figure 3.18: Effects of RDI regimens on leaf starch concentration. First (A) and last (B) sample times of the day during 2003 on days with clear skies. Sampling times are indicated above bars. Mean values are per vine ± SE (n=3). Bars with different letters are significantly different at $p \leq 0.05$ by Tukey-Kramer. [a]nc: data not collected.

APPENDIX 3

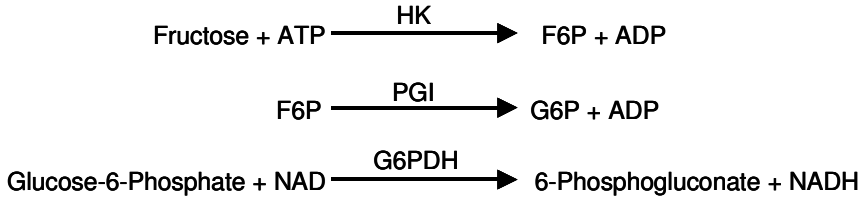
A3.1. Enzymatic reactions for non-structural carbohydrate analysis:

The glucose reagent (G2020, Sigma) used for soluble sugars determination was based on the following enzymatic reactions:



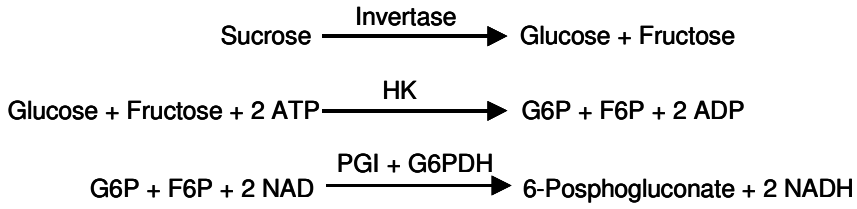
Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). Glucose-6-phosphate (G6P) is then oxidized to 6-phosphogluconate in the presence of nicotinamide dinucleotide (NAD) in the reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). The resulting increase in absorbance at 340 nm is directly proportional to the glucose concentration.

Fructose determination was based on the following enzymatic reactions:



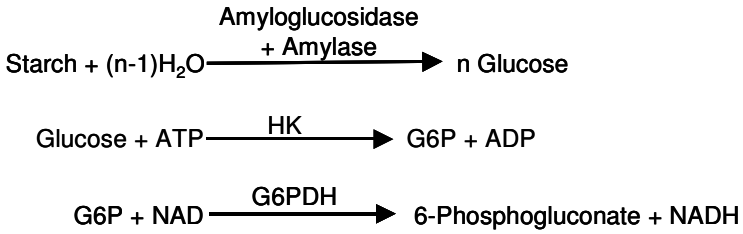
Fructose is phosphorylated by ATP in the reaction catalyzed by HK. Fructose-6-phosphate (F6P) is then converted to G6P by the phosphoglucoisomerase (PGI). The last reaction is the same as in the glucose determination.

The sucrose determination was based on the following enzymatic reactions:



Sucrose is cleaved by the invertase into glucose and fructose, then both hexoses are phosphorylated by ATP and HK. The F6P is converted to G6P in the presence of PGI, and G6P is oxidized by G6PDH.

Starch determination was based on the following enzymatic reactions:



Starch is first cleaved by amyloglucosidase and amylase into glucose which is determined as described above.

A3.2. Conversion factor (F) used in equation 3.3

The area of all 8 leaf discs sampled (2.5 cm^2) was equal to the area enclosed by the cuvette used for single-leaf net CO_2 exchange measurements. The weight of the 8 leaf discs was about 50 mg with very small variation. To transform non-structural carbohydrate concentration change (mg g^{-1} FW h^{-1}) to units of net CO_2 exchange (µmol m^2 s^{-1}) the following conversion factor (F) was applied:

$$F = \frac{44\ C}{100\ DM} \times \frac{100\ CO_2}{27\ C} \times \frac{1\ g\ FW}{50\ cm^2} \times \frac{10000\ cm^2}{1\ m^2} \times \frac{1\ mmol\ CO_2}{44\ mg} \times \frac{1000\ \mu mol}{1\ mmol} \times \frac{1\ h}{3600\ s}$$

where 44 is % of carbon in dry matter (DM) and 27 is % of C in $CO_{2.}$

A3.3. Plant available water (PAW):

$$\mathbf{PAW} = \frac{(\theta_{FC} - \theta_{PWP}) \times \mathbf{sd}}{100} \times \mathbf{\%SW}$$

where θ_{FC} and θ_{PWP} (%) are the volumetric soil water contents at field capacity (14.6%) and permanent wilting point (7.1%), respectively, sd is soil depth to be managed (1m expressed in mm), and %SW is the portion of the total area wetted by drip emitters (37%).

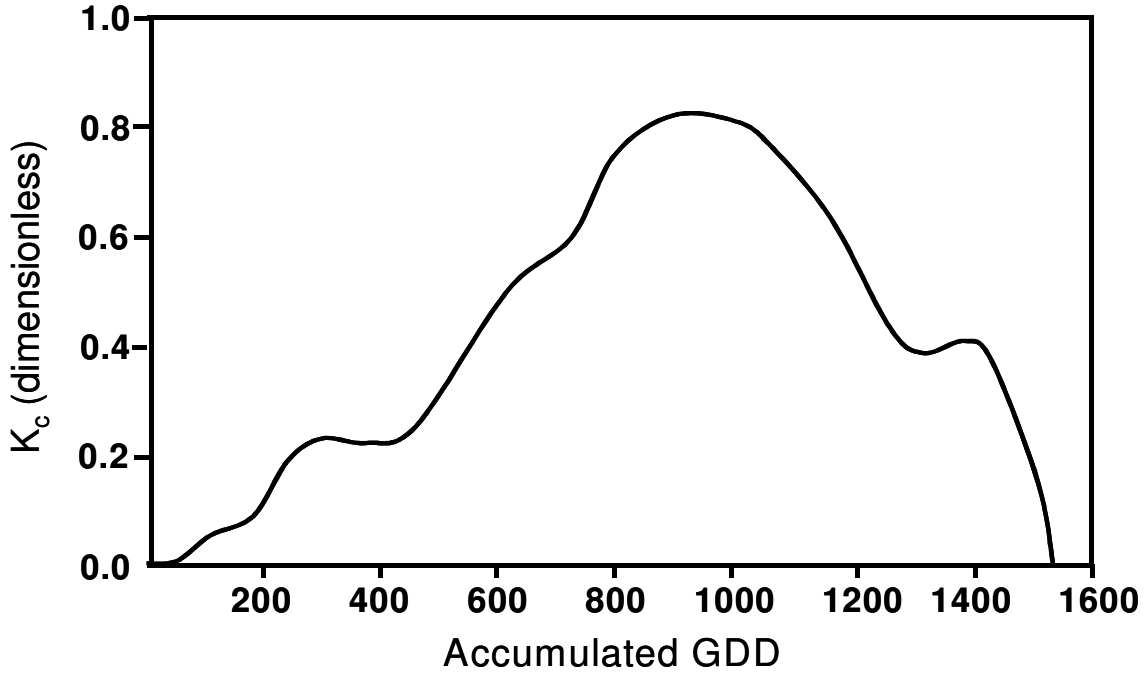


Figure A3.1: Seasonal (April 1-Oct 31) crop coefficient (K_c) for cv. Cabernet Sauvignon in south-central Washington plotted against accumulated growing degree days (GDD, base 10°C). Redrawn from Evans et al. (1993).

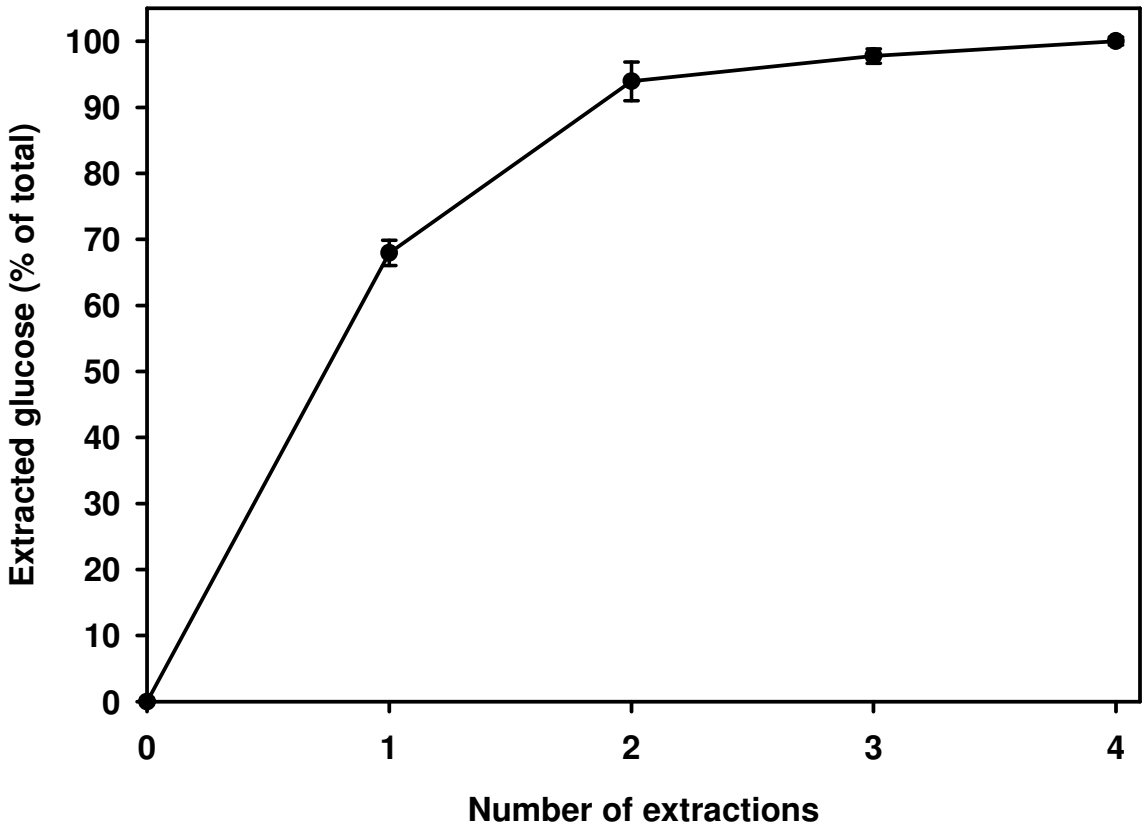


Figure A3.2: Extraction of glucose in relation to number of extractions. Three extractions were enough to extract more than 95% of glucose present in the leaf tissue.

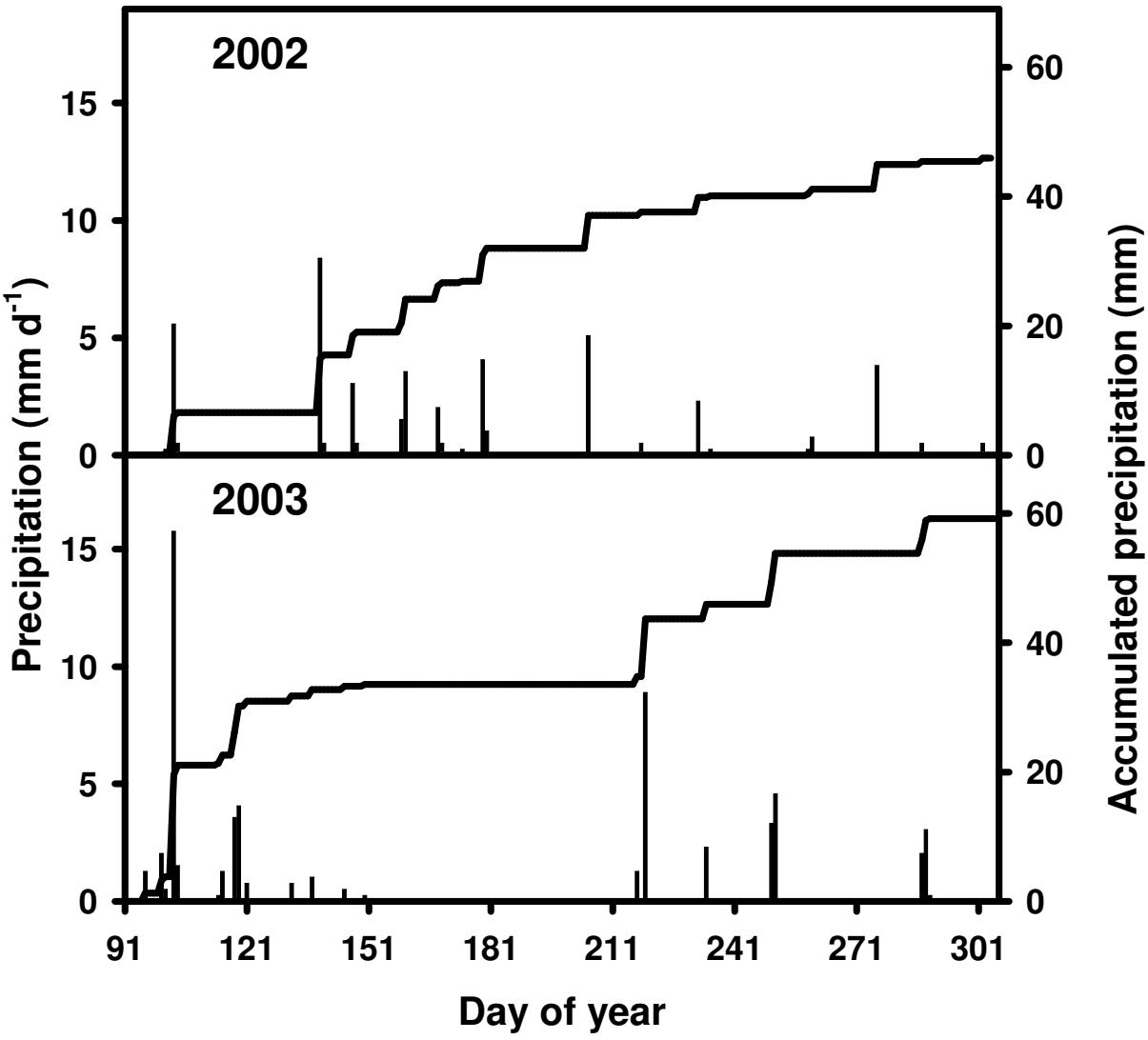


Figure A3.3: Daily precipitation and accumulated precipitation recorded at Alderdale PAWS station (10 km west of site). Bars represent rain events. PAWS: Public Agriculture Weather System, Washington.

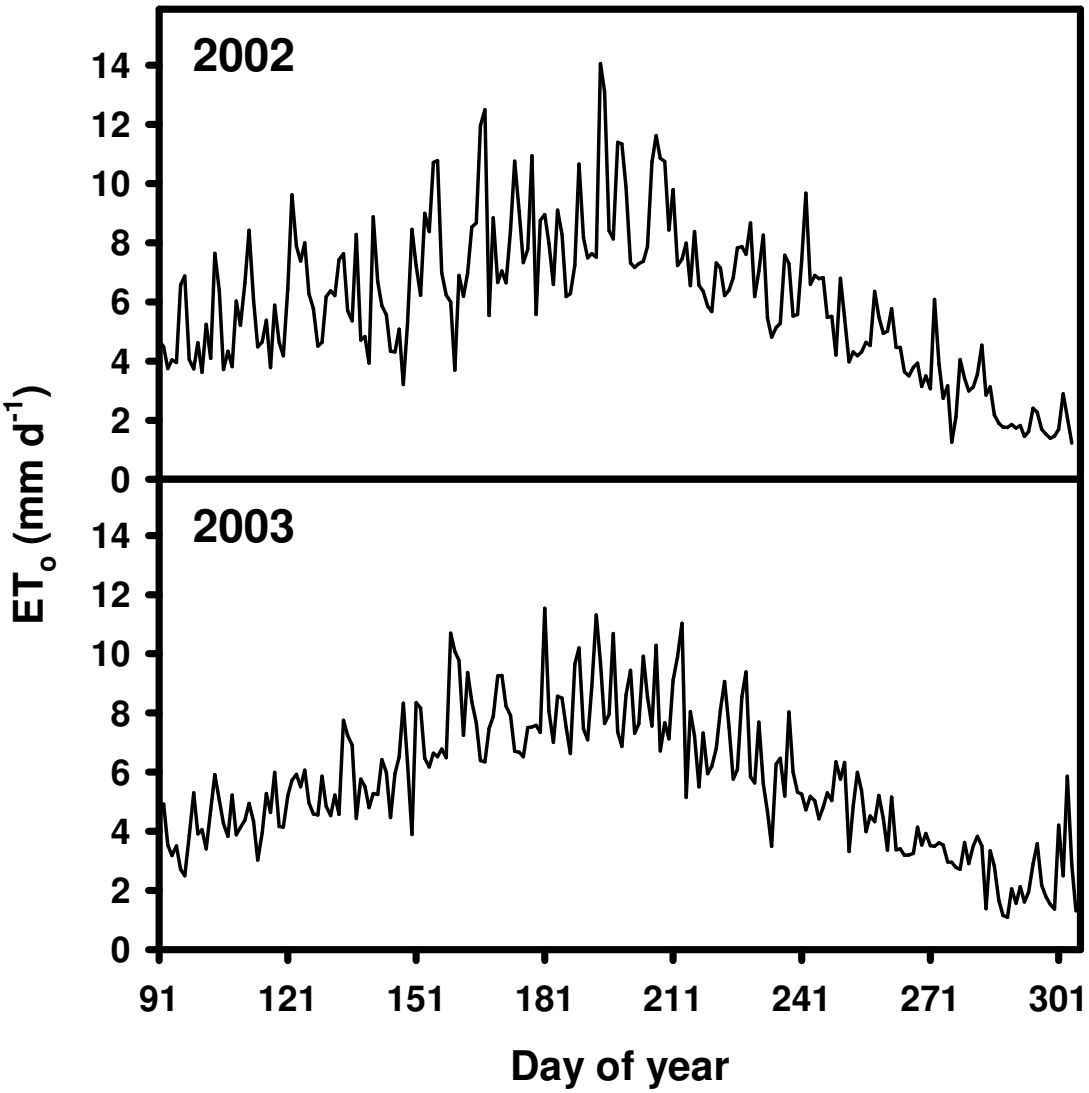


Figure A3.4: Daily ET_o (grass) recorded at Alderdale PAWS station (10 km west of site) during 2002 and 2003. PAWS: Public Agriculture Weather System, Washington.

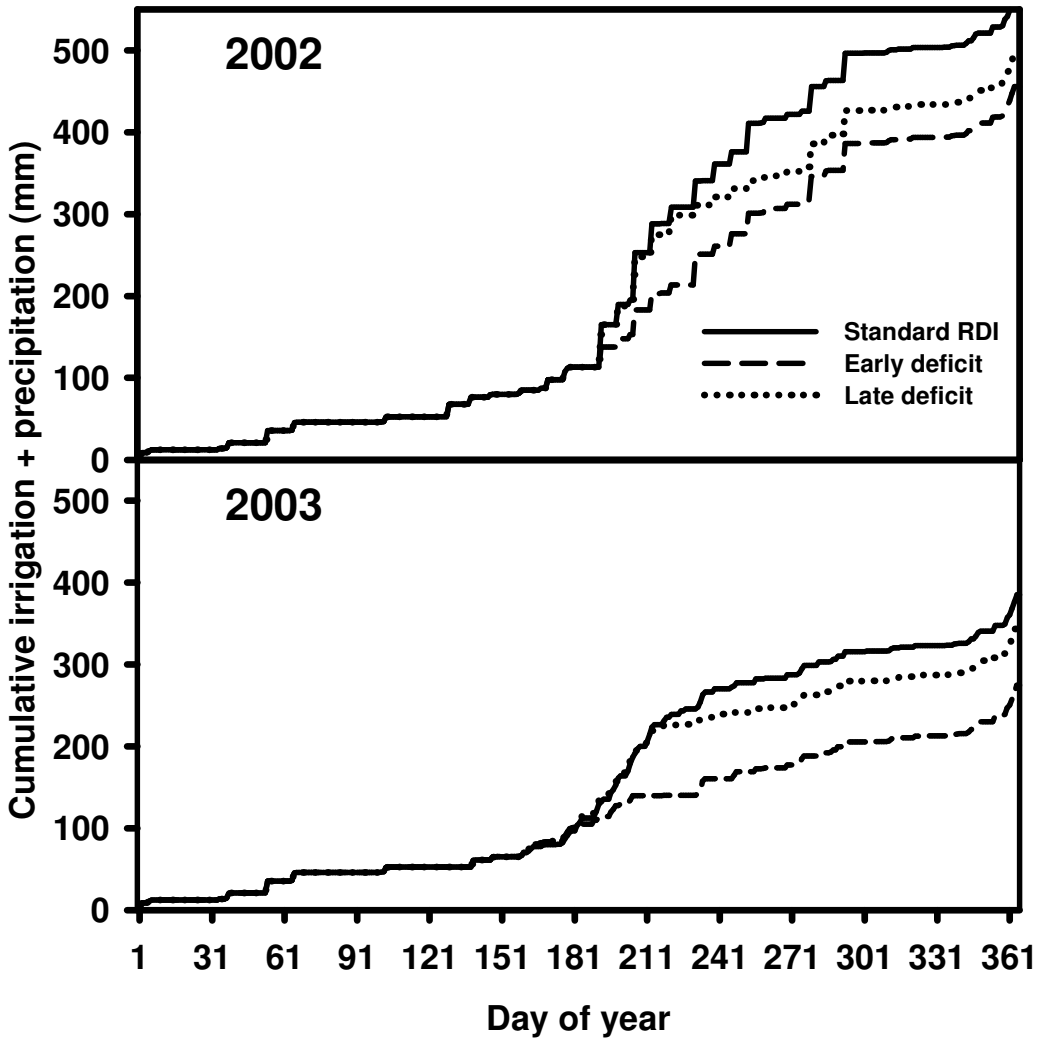


Figure A3.5: Cumulative precipitation recorded at Alderdale PAWS station (10 km west of site) plus cumulative irrigation applied within the RDI regimens at the experimental site during 2002 and 2003. PAWS: Public Agriculture Weather System, Washington.

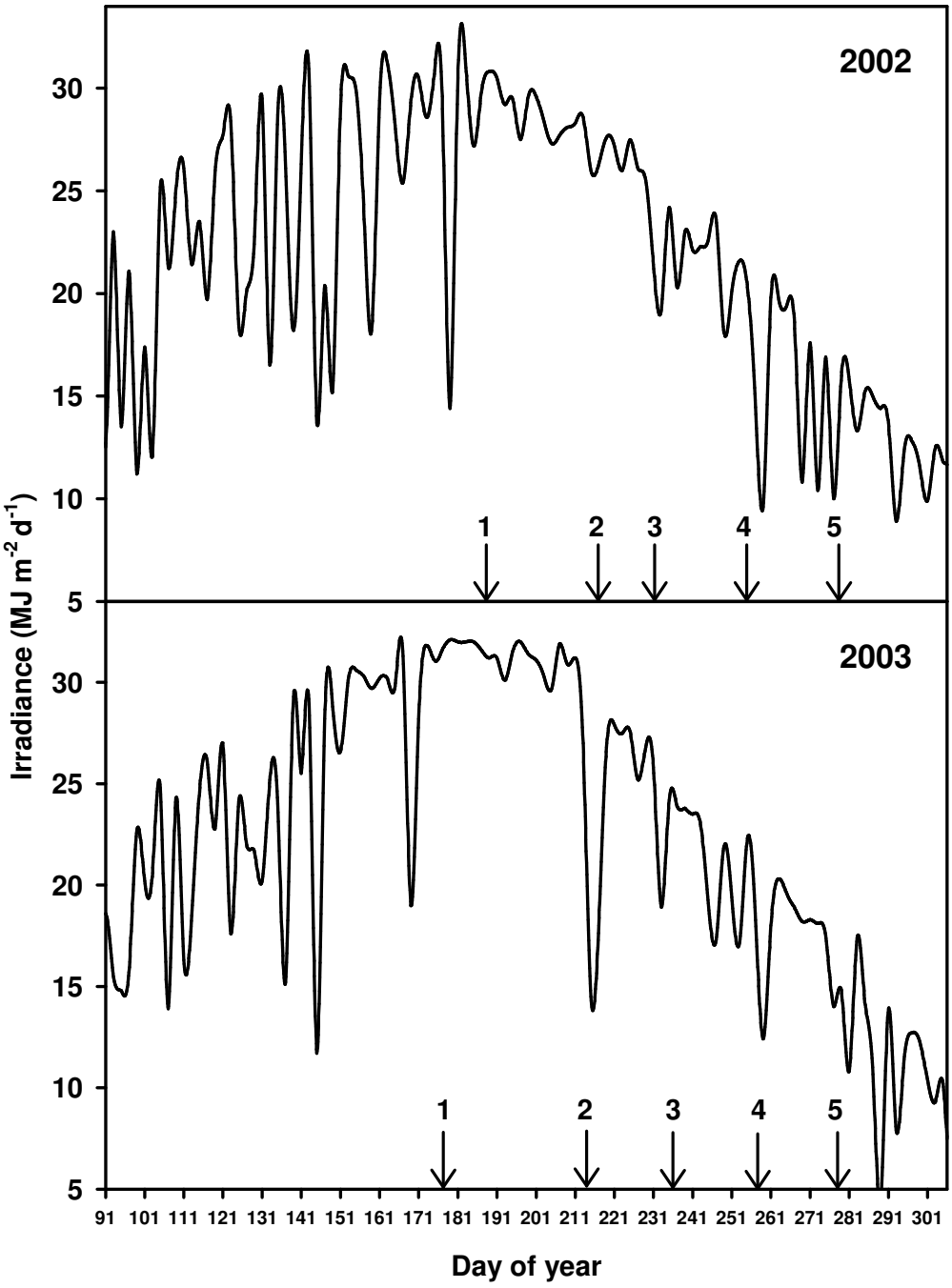


Figure A3.6: Daily irradiance during growing seasons 2002 and 2003 at the Alderdale PAWS station (10 km west of site). Arrow numbers indicate whole-canopy gas exchange measurements: 1-fruit set, 2-pre-veraison, 3-post-veraison, 4-pre-harvest, 5-post-harvest. PAWS: Public Agriculture Weather System, Washington.

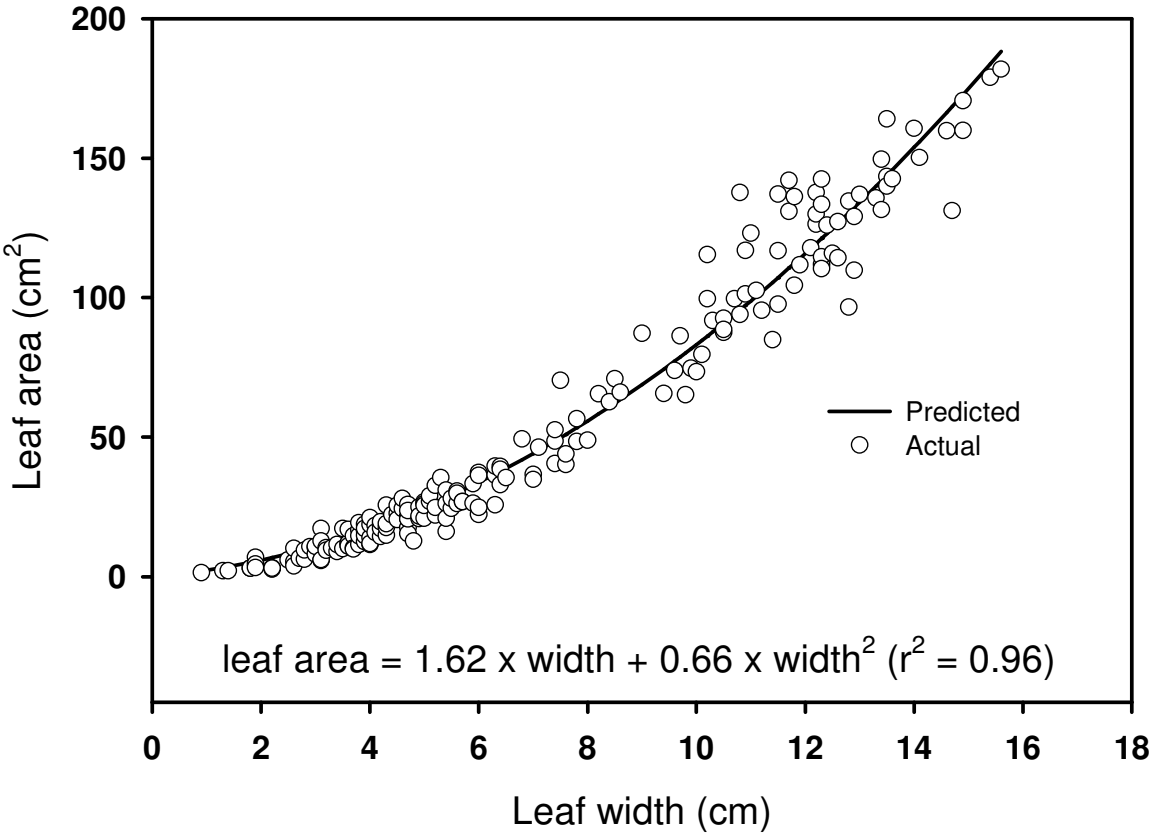


Figure A3.7: Regression model used for vine leaf area estimation in 2002.

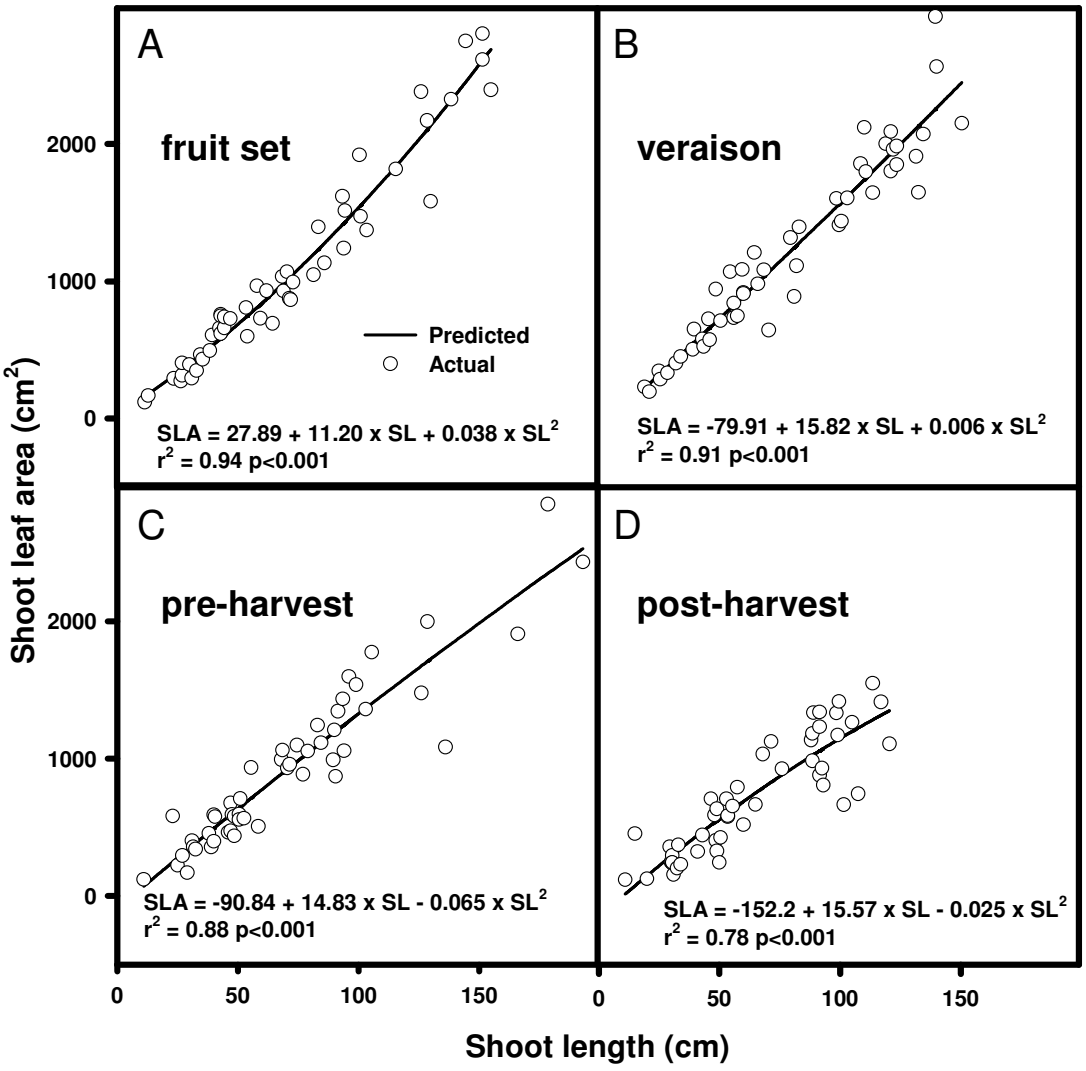


Figure A3.8: Regression models between shoot length and leaf area per shoot during 2003. SLA: shoot leaf area; SL: shoot length. Maximum shoot lengths were around 150 cm, except during the post-harvest phase, where they did not exceed 120 cm because of damage during harvest.

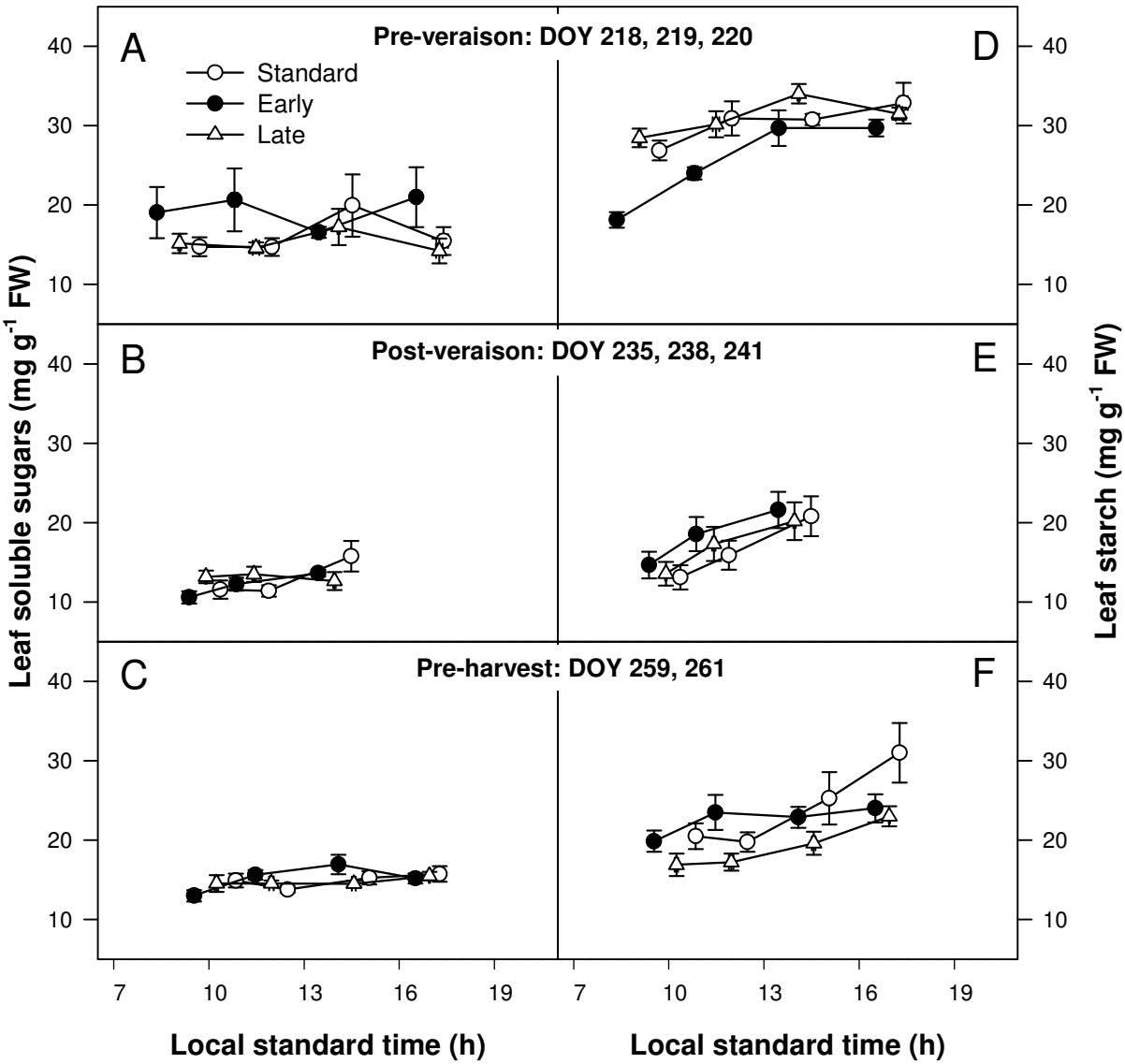


Figure A3.9: Effects of RDI regimens on leaf solubles sugars (A-C; glucose + fructose + sucrose) and starch (D-F) during 2002. Mean values are per vine ± SE (n=9; pre-harvest n=6).

CHAPTER 4

GRAPEVINE TRANSPIRATION UNDER REGULATED DEFICIT IRRIGATION AND ITS RELATION TO NET CO_2 EXCHANGE

4.1. Abstract

In an area like eastern Washington, where commercial viticulture depends totally on irrigation, it is critical to know how much water grapevines transpire and whether it is possible to conserve water. In general it has been reported that well watered vines have lower water use efficiencies (WUE) than deficit irrigated vines. For a better understanding of the relationship between carbon fixation and transpired water, and of the consequences of imposing water deficits in vineyards, whole-canopy transpiration needs to be measured. In 2002 and 2003 whole-canopy transpiration was measured by a six-chamber, mobile field laboratory in field-grown, own-rooted, drip irrigated *Vitis vinifera* L. cv. Cabernet Sauvignon vines that were under three regimens of regulated deficit irrigation (RDI): 1) standard RDI (70% of full vine evapotranspiration, FVET, was replaced weekly); 2) early deficit (35% of vine FVET was replaced weekly between fruit set and veraison); and 3) veraison deficit (35% of vine FVET was replaced weekly between veraison and harvest). Whole-canopy measurements were conducted at fruit set, pre- and post-veraison, and pre- and post-harvest. Maximum cumulative daily transpiration was 20 L $vine^{-1}$ (i.e., 4.1 mm d^{-1}) around veraison. In vines under additional water deficits daily transpiration was about 50% less than vines under standard deficit. Variation in net CO_2 fixation was mainly explained by canopy conductance at the

whole-canopy level and by stomatal conductance at the single-leaf level. No consistent differences in WUE were observed among RDI regimens. A deficit index that ties transpiration to CO_2 fixation based in the monitoring of air vapor pressure deficit and transpiration is proposed.

4.2. Introduction

Recent research in vineyard water management may be explained by the influence of water deficits on grape yield and quality (Araujo et al., 1995; Dry et al., 2001; Jackson and Lombard, 1993; Roby et al., 2004), and by the desire for water-saving methods of irrigation (Loveys et al., 1998). Drip irrigation systems and regulated deficit irrigation (RDI) are among the management practices adopted by Washington grape growers, where commercial vineyards require irrigation because of the combination of low annual rainfall during the growing season (e.g., ≈80 mm, Prosser, WA) and high evapotranspiration (e.g., >1200 mm). Crop coefficients for grapevines in this region have been obtained by lysimetry in vineyards maintained at relatively high soil water contents, and with 100% daily replacement of the water used by the vines (Evans et al., 1993). Mature vines (*Vitis vinifera* L.) near Prosser, WA transpire about 417 mm at yields of 15 Mg ha^{-1}. During the short growing season in this area (158 frost free days), vineyards must be managed to mature fruit, sequester enough carbohydrate reserves for budbreak and initial shoot growth the following year, and 'harden off' the vines before the cold winter. One of the challenges of the Washington wine industry is to determine the timing and amount of RDI to satisfy both requirements.

Regulated deficit irrigation manages soil water supply to impose predetermined

periods of plant or soil water deficit to elicit some desirable responses in plants (Behboudian and Singh, 2001). Commonly water deficits are imposed by reducing crop coefficients or soil water content. For example in vineyards managed under RDI, grapevines are irrigated early in the growing season (ca. April) if winter rains were insufficient to fill the soil profile to field capacity. Excessive canopy development is avoided by applying less water than full vine evapotranspiration (FVET) while shoots are actively growing. Crop load is regulated by pruning and in some cases by cluster thinning. Also within RDI, additional or more extreme water deficits can be imposed during the berry growth period to influence yield and grape composition. Reduced yields due to water deficits have been related to reductions in the degree of initiation of inflorescence primordia, flower differentiation, maintenance of the reproductive organ, and to reductions in fruit expansion (Buttrose, 1974; dos Santos et al., 2003; Matthews and Anderson, 1989; McCarthy, 1997; Poni et al., 1993). Effects of water deficits on berry composition varied whether deficits were imposed between fruit set and veraison, or between veraison and harvest (Kennedy et al., 2002; Matthews and Anderson, 1988; Ojeda et al., 2002), but both have been related to smaller berries (Kennedy et al., 2002; Matthews and Anderson, 1989; Ojeda et al., 2001) with a concomitant increase in the skin:pulp ratio in the must (Freeman, 1983; Matthews and Anderson, 1988; Ojeda et al., 2002). However, changes in anthocyanin concentrations also were reported on the basis of skin surface area (Esteban et al., 2001; Matthews and Anderson, 1988; Ojeda et al., 2002; Roby et al., 2004).

More extreme, or additional water deficits applied within RDI will reduce

photosynthesis and transpiration, the relative reduction of each influencing water use efficiency (WUE; ratio of CO_2 fixed to H_2O transpired). Water deficits imposed on grapevines have increased (Flexas et al., 1998; Flexas et al., 1999; Iacono et al., 1998), decreased (Schultz, 2000), or had no effect on WUE (Chaves and Rodrigues, 1987; Düring, 1987; Medrano et al., 2003). The WUE may vary by time of day and the length of time during which the water deficit was imposed (de Souza et al., 2003; Downton et al., 1987). Water use efficiency may increase with water deficits when defined as the ratio between yield and water applied (dos Santos et al., 2003; du Toit et al., 2003).

In an area like the inland Northwest, USA, where water is limited and commercial viticulture requires irrigation, it is important to know how much water field-grown grapevines transpire, and the effects of water deficits on photosynthesis, transpiration, and WUE. The objective of this experiment was to determine how different timing and severity of water deficits managed via RDI might reduce vine transpiration and influence WUE. More severe deficits were imposed during either the early (i.e., fruit set to veraison) or late (i.e., veraison to harvest) phase of berry growth. The relationship between transpiration and other physiological variables (e.g., canopy conductance, net CO_2 exchange) was explored as were vine responses to environmental variables (e.g., vapor pressure deficit, photosynthetic photon flux density).

4.3. Materials and methods

4.3.1. Site and treatments

The study was conducted during 2002 and 2003 within an experiment that had been initiated in 1999 in a 4-ha block of *Vitis vinifera* L., cv. Cabernet Sauvignon, at the

Canoe Ridge vineyard of Ste. Michelle Wine Estates (45.88° N, 119.75° W, 125 m above mean sea level, west of Paterson, WA). Soil, vine spacing, and other vineyard details are described in chapter 3 (this volume). The vineyard was irrigated once early in the growing season (ca. April) because winter rains did not fill the soil profile to field capacity. Between budbreak and fruit set, irrigation was withheld until shoots were 0.9 to 1.2 m long and the rate of shoot growth was minimal. At fruit set, three irrigation regimens were imposed: standard RDI (S), early deficit (E) and late deficit (V). Full vine evapotranspiration was calculated from reference evapotranspiration (ET_o) using data collected by the PAWS (Public Agriculture Weather System) weather station at Alderdale, WA (10 km west of the site) and a crop coefficient for cv. Cabernet Sauvignon determined in eastern Washington (Evans et al., 1993). Vineyard ET for standard RDI was estimated as 70% of FVET in 2002 and 60% of FVET in 2003. From fruit set to veraison, irrigation was applied within S and V to replenish 70% of FVET weekly (2002; 60% in 2003) with the goal of maintaining soil moisture in the top 1 m at 10% by volume. In the same period, irrigation in E replenished 35% of FVET weekly (2002; 30% in 2003) with the goal of maintaining soil moisture at 8.3%. From veraison to harvest, irrigation was applied in S and E plots to replenish weekly 70% (2002) and 60% (2003) of FVET with the goal of maintaining soil moisture at 10%, while V plots replenished 35% (2002) and 30% (2003) of FVET weekly with the goal of maintaining soil moisture at 8.3%. After harvest, all vines were irrigated to replenish 100% of FVET.

4.3.2. Physiological measurements

During 2002 and 2003, whole-canopy transpiration rates (Tr_{WV}; mmol m^{-2} $vine^{-1}$)

were measured during five periods corresponding to different phenological stages (Appendix figure A4.1): 1) fruit set, before treatment initiation (DOY 183 to 192, 2002, and DOY 177 to184, 2003); 2) pre-veraison (DOY 217 to 224, 2002, and DOY 212 to 219, 2003); 3) post-veraison (DOY 234 to 240, 2002, and DOY 233 to 241, 2003); 4) pre-harvest (DOY 255 to 261, 2002, and DOY 254 to 262, 2003); and 5) post-harvest (DOY 278 to 284, 2002, and DOY 274 to 281, 2003). Cumulative transpiration (24 h) was expressed both per vine ($Tr_{WV,d}$; L H_2O d^{-1} $vine^{-1}$) and per unit leaf area ($Tr_{WL,d}$; L H_2O d^{-1} m^{-2}). Leaf area per vine was estimated twice in 2002 and four times in 2003 by indirect protocol (chapter 3, this volume). Six flow-through chambers of an approximate volume of 8 m^3 were used to enclose one vine each to measure whole-canopy transpiration and net CO_2 exchange (Perez Peña and Tarara, 2004). Details of the gas exchange system are described in chapter 2 (this volume) and details of whole-canopy measurements are in chapter 3 (this volume). Briefly, two chambers were run simultaneously in each of the irrigation regimens for 48 h, moved to replicate vines and the process repeated until a total of 6 vines had been sampled per irrigation regimen. Data from the central 24 h of the 48-h “run” were used in the analysis. The system also included global irradiance and photosynthetic photon flux density (PPFD) sensors that were installed in the vineyard simultaneously with the chambers (described in chapter 2, this volume). A bulk canopy conductance (g_c; Campbell and Norman, 1998) that includes stomatal (g_s) and boundary layer conductances (g_b) and is expressed in mmol m^{-2} s^{-1} was calculated as:

$$g_c = \frac{Tr_{WL}}{VPD} \times P_a \qquad (4.1)$$

where VPD is vapor pressure deficit of air at the chamber inlet (kPa), and P_a is atmospheric pressure (kPa). The VPD was calculated as:

$$VPD = e_s(T_i) - e_a \qquad (4.2)$$

where $e_s(T_i)$ is the saturation vapor pressure (kPa) at the temperature of the air entering the whole-canopy chamber, and e_a is the actual vapor pressure (kPa) of the same air, as measured by infrared gas analyzer (IRGA; CIRAS - DC, PP Systems, Haverhill, MA). At the same phenological stages as whole-canopy measurements were conducted, single-leaf transpiration (Tr_{SL}) and stomatal conductance (g_s) were measured simultaneously with single-leaf net CO_2 exchange rates (NCE_{SL}) on fully expanded leaves (located about 6 to 8 leaves from the shoot tip) from early morning until late afternoon, using a portable infrared gas analyzer (IRGA; CIRAS-2, PP Systems, Haverhill, MA) and leaf cuvette (PLC6(U) - PP Systems). Details of single-leaf measurements are described in chapter 3 (this volume). Vapor pressure deficit between leaf and air (VPD_{la}) was calculated from leaf temperature and air water vapor pressure in the cuvette. Saturation vapor pressure for VPD and VPD_{la} were calculated according to Buck (1981; see appendix 4).

Whole-canopy daily water use efficiency ($WUE_{WV,d}$), the ratio between CO_2 fixed and water transpired, was calculated from net CO_2 fixed per day per vine ($NCE_{WV,d}$; g CO_2 $vine^{-1}$ d^{-1}) and $Tr_{WV,d}$. Whole-canopy daily WUE also was expressed per unit leaf

area ($WUE_{WL,d}$). Leaf-level water use efficiency ($WUE_{SL,d}$) was defined as the ratio between the daily averages of NCE_{SL} and Tr_{SL}. Intrinsic WUE ($WUE_{i,WL}$ and $WUE_{i,SL}$) was defined as the slope of the relationship between NCE_{WL} and g_c (whole-canopy) or NCE_{SL} and g_s (single-leaf). The WUE_i is used to avoid fluctuations in WUE due to variations in VPD or VPD_{la} that may change transpiration without changing conductance (Osmond et al., 1980). Daily sum of degrees (base 10ºC; DSD) was calculated as:

$$\mathbf{DSD} = \sum_{i=1}^{n} (T_{DSD} - 10^{o}C) \qquad (4.3)$$

where T_{DSD} (ºC) is the mean temperature during the 12 min sampling interval and n is the number of intervals in 24 h. Total PPFD per day ($PPFD_d$; mol m^{-2} d^{-1}) was also calculated.

4.3.3. Statistical analysis

Data were analyzed using the SAS statistical package (V8(2); SAS Institute, Cary, NC). Procedure univariate was used to test for normality and the Brown-Forsythe test for homogeneity of variance. The general linear model procedure (proc GLM) was used for the analysis of variance (ANOVA). The Tukey-Kramer method at a significance level of $p \leq 0.05$ was used for mean comparisons among irrigation regimens, and the proc REG procedure was used for simple and multiple regression analysis with the stepwise option. With the data of all runs of 2003, transpiration was regressed against VPD, g_c, NCE, PPFD, and DSD with simple and multiple stepwise models. Similar relationships were also explored at the single-leaf level.

4.3.4. Proposed water deficit index (DI) for RDI

An irrigation index based on a plant variable (Tr) and on a meteorological variable (VPD) is proposed. Both variables should be monitored continuously to calculate their rates of change during the day. Grower-owned or public weather stations can provide data for calculation of VPD (i.e., temperature and relative humidity). Transpiration may be monitored directly by sap flow sensors (Ginestar et al., 1998), or indirectly by diurnal variation in trunk diameter (Escalona et al., 2002). At the single-leaf level, transpiration flux is a function of a driving force (VPD_{la}) and a proportionality factor (g_s). At the whole-canopy level, the driving force is considered VPD and the proportionality factor g_c (Equation 4.1). When stomata are completely open, variations in VPD will be paralleled by variations in transpiration. As stomatal conductance decrease, the stomates exercise some control over Tr; thus variations in VPD will not be paralleled by variations in Tr. The time of day at which changes in Tr diverge from changes in VPD (t_{DI}; h) can be determined graphically, or by a simple program that calculates the ratio between slopes of both curves (see Appendix 4). Thus DI is calculated by:

$$\mathbf{DI} = \mathbf{(1 - \frac{t_{DI} - t_{sunrise}}{t_{sunset} - t_{sunrise}}) \times 100} \qquad \mathbf{(4.4)}$$

where $t_{sunrise}$ and t_{sunset} are local standard times for sunrise and sunset of the corresponding day. The DI ranges from 0 to 100, with 0 indicating that at all times between sunrise and sunset, changes in Tr_{WL} paralleled changes in VPD. The higher the DI, the earlier in the morning Tr diverges from VPD, stomatal started to control over Tr_{WL}.

4.4. Results

4.4.1. Meteorological and irrigation summary

A climatological summary of the 2002 and 2003 growing seasons was presented previously (chapter 3, this volume, Table 3.1). Values described here are for the period April 1 to October 31 (Alderdale PAWS station, 10 km west of site). Briefly, cumulative growing degree days (GDD) were higher in 2003 (1857ºC) than 2002 (1669ºC). Rainfall was 46 mm in 2002 and 59 mm in 2003, while ET_o was 1289 mm in 2002 and 1218 in 2003. Maximum daily ET_o was 14 mm in 2002 and 11 mm in 2003. The cumulative irrigation applied after imposition of RDI regimens at fruit set was 295 mm for S, 225 mm for V, and 185 mm for E during 2002, and 162 mm for S, 122 mm for V, and 92 mm for E during 2003. The smaller amounts of irrigation applied in 2003 were primarily due to lower crop coefficients adopted for that year. Soil moisture was kept about targeted levels within the first meter depth of soil, the managed depth (see appendix 4; Figure A4.2).

4.4.2. Total daily whole-canopy transpiration ($Tr_{WV,d}$ and $Tr_{WL,d}$)

As mentioned in chapter 3 (this volume), because of technical difficulties with the whole-canopy gas exchange system, illustrative data are presented from the more comprehensive 2003 field campaign. Measurements at each phenological stage were conducted during at least 7 days, including days with clear skies (DCS) and those with clouds (DWC). Because of the weekly irrigation cycle, during each measurement “run” two to four irrigation sets were delivered that produced some significant interactions between irrigation treatment and day of measurement (DOM) at pre- and post-veraison

(Table 4.1). Thus, data also are presented for DCS to separate the effects of weather and irrigation frequency.

Maximum $Tr_{WV,d}$ occurred around veraison in all plots, with S vines transpiring on average up to 15 L $vine^{-1}$ d^{-1} across all measurement days (Table 4.1). On days with clear skies, maximum $Tr_{WV,d}$ exceeded 20 L $vine^{-1}$ d^{-1} in 2003 (Table 4.2), while $Tr_{WL,d}$ exceeded 2 L m^{-2} d^{-1} (Table 4.3). Minimum $Tr_{WV,d}$ (between ≈4 and 5 L $vine^{-1}$ d^{-1}) occurred in the pre- and post-harvest period, reflecting shorter days and relatively cool temperatures. At fruit set, before imposition of the additional deficit treatments, there were no significant differences in $Tr_{WV,d}$ among vines. However, vines under more severe RDI (i.e., E or V) transpired up to 50% less per day than S vines, especially during the pre- and post-veraison periods. Differences among treatments were less evident under cloudy skies. Because canopies were of similar size across the irrigation treatments, trends in $Tr_{WL,d}$ were identical to those in $Tr_{WV,d}$. At post-veraison, responses differed between years. In 2002, vines under additional water deficit (V) transpired more than vines that had been under additional deficit between fruit set and veraison (E) but that had been returned to the standard deficit. A similar response was observed in $NCE_{WV,d}$ (chapter 3, this volume). In 2003, on DCS, vines under additional water deficit (V) transpired about 57% less than standard RDI vines, whereas vines that had been under additional water deficit before veraison (E) transpired as much water as S vines. No differences among regimens were observed in $Tr_{WV,d}$ or $Tr_{WL,d}$ during the pre- and post-harvest periods.

4.4.3. Instantaneous transpiration rates: whole-canopy and single-leaf

Whole-canopy transpiration rates varied between days with clouds and days with clear skies at each phenological stage (Figures 4.1, 4.2, 4.3, 4.4, 4.5). Moreover, Tr_{WL} varied by time elapsed since the last irrigation set. Maximum values were above 4 mmol m^{-2} s^{-1} at pre- and post-veraison, and generally occurred under higher soil moisture (i.e., during an irrigation application or immediately after rain; Figures 4.2-A and 4.3-B). In general Tr_{WL} responded rapidly to irrigation. For example between DOY 182 and 183 (before imposition of the irrigation treatments) a total of 10 mm was applied to S and V plots, but not to E plots, resulting in consistently lower Tr_{WL} in E vines that day (Figure 4.1-C). Similarly on DOY 240 (Figure 4.3-C), the unexpected lower Tr_{WV} in E vines (at this time under standard deficit) was due to an irrigation applied to S and V vines, but not to E. Daily irrigation variation at other times during the season (Figure 4.3-C) together with DWC caused significant interactions between treatment and day of measurement. On DCS, differences between vines under standard RDI and vines under additional water deficits were apparent at pre-veraison (Figure 4.2-C), post-verasion (Figure 4.3-B), and pre-harvest (Figure 4.4-A).

Maximum values of Tr_{SL} were over 6 mmol m^{-2} s^{-1} in 2002 and 2003 (Figures 4.6 and 4.7), and usually higher than those of Tr_{WL}. Maximum VPD_{la} reached about 6 kPa both in 2002 and 2003 before veraison. In general Tr_{SL} followed VPD_{la} only during the first part of the day, diverging in the afternoon, similar to the trend found in the Tr_{WL} data. Differences between S vines and those under additional water deficit that were observed at the whole-canopy level were also apparent in Tr_{SL} measurements. At fruit set (2003) there were no differences in Tr_{SL} among irrigation regimens (Figure

4.7-A), but by pre-veraison (2002 and 2003) Tr_{SL} were lower in vines under additional water deficit than in those under standard deficit (Figures 4.6 and 4.7). At post-veraison there were some differences in the responses between years, reflecting those observed in Tr_{WL}. In 2002, as one might expect, S vines had the highest Tr_{SL}, while V vines transpired the least. Vines under additional deficit between fruit set and veraison but that had been returned to standard RDI (E), had intermediate values of Tr_{SL}. However, in 2003 both S and E vines transpired significantly more than V vines during post-veraison measurements. In both years, lower values of Tr_{SL} were recorded towards the end of the season (pre- and post-harvest), similar to the trend in Tr_{WL}.

4.4.4. Canopy conductance (g_c) and vapor pressure deficits (VPD and VPD_{la})

Applying a more extreme water deficit than standard RDI consistently resulted in lower g_c (Figure 4.8). Although g_c recovered overnight, before mid-morning there was a marked discrepancy between treatments. Maximum g_c was $\approx$150 mmol m^{-2} s^{-1} at pre-veraison and pre-harvest. Regardless of irrigation regimen, the highest g_c were recorded around mid-morning similar to the trends observed in g_s (chapter 3, this volume), while maximum Tr_{WL} usually occurred after midday. In general, VPD_{la} was higher than VPD because leaf temperature in the cuvette consistently exceeded bulk air temperature. In 2003, maximum VPD exceeded 4.5 kPa, while VPD_{la} exceeded 6 kPa.

Hysteresis is apparent in the relationship between Tr_{WL} and VPD (Figure 4.9) and its relationship to g_c (Figure 4.10). Significantly more water was transpired in the afternoon than in the morning hours at the same value of g_c, a trend consistent across irrigation treatments and phenological stages. Diurnal hysteresis is due to the two

variables that determine Tr changing in opposite directions during the afternoon. At a given VPD, Tr_{WL} in S vines was consistently higher than Tr_{WL} in vines under an additional deficit, especially when ET_o was high (pre- and post-veraison). When ET was lower, differences were not as obvious (pre- and post-harvest). At fruit set, when no additional deficit was imposed, no differences in Tr were recorded between vines that had been under additional deficit in previous years and those that had always been under standard RDI.

4.4.5. Relationships of NCE with other variables, and WUE

Most of the variation in NCE_{WL} was explained by variation in g_c ($r^2 \approx 0.86$ to 0.96; $p \leq 0.001$; Figure 4.11), as was the case at the single-leaf level, where variation in NCE_{SL} was mostly explained by variations in g_s ($r^2 \approx 0.76$ to 0.93, $p<0.001$; Figure 4.12). All irrigation regimens fell on the same regression line, with the additional deficit vines at the lower end of the scale suggesting that water deficit reduced net carbon fixation through stomatal limitation. This was confirmed by the apparent response of net CO_2 fixation and canopy conductance to PPFD (Figure 4.13); canopy conductance limited NCE_{WL} in S vines and those under additional water deficit. However, when atmospheric demand was low and an irrigation set was applied during the day prior to measurements, NCE_{WL} reached higher levels and followed more closely the PPFD curve (Figure 3.8-C; chapter 3, this volume). Using 2003 data, $NCE_{WV,d}$ was regressed against Tr_{WV}, DSD, and $PPFD_d$ using stepwise procedures. The only variables that remained in the model were $Tr_{WV,d}$ and DSD, while $PPFD_d$ was not included (Table 4.4). The slope of the relationship between $NCE_{WL,d}$ and $Tr_{WL,d}$ was similar to that between $NCE_{WV,d}$ and $Tr_{WV,d}$

(Figure 4.14), reflecting only small differences in canopy size among irrigation regimens. All irrigation regimens fall on the regression line, with those under additional water deficit located on the lower part of the curves. Whether on a per vine or leaf area basis, no evidence of saturation was observed in the relationship between NCE_{WV} and Tr_{WV} or between NCE_{WL} and Tr_{WL}. At the single-leaf level a significant correlation was found between NCE_{SL} and Tr_{SL}, but there was some evidence of saturation in NCE_{SL} (Figure 4.14).

No consistent differences were detected in either $WUE_{WV,d}$, $WUE_{WL,d}$, or WUE_{SL} between vines under standard water deficit and those under additional water deficit in 2002 or 2003 (Tables 4.5, 4.6 and 4.7). On DCS, the highest $WUE_{W,d}$ were recorded at pre-veraison in 2002, and after harvest in 2003. Within each phenological stage, no difference was found in $WUE_{i,WL}$ between vines under standard deficit and those under additional water deficit (Figure 4.11). Similar results were obtained at the single-leaf level (Figure 4.12). Figure 4.15 shows an example of WUE_{WL} calculated at different times of the day (0800 h and 1600 h), that will help to explain in the discussion, a probable reason of why sometimes WUE found in different irrigation experiments differed. Across all irrigation regimens, WUE was higher towards the end of the season due to lower atmospheric demand and lower temperatures.

4.4.6. Proposed water deficit index (DI): an example

Values of of NCE_{WL}, Tr_{WL}, VPD, g_c on a DCS (DOY 218, 2003) for one vine under standard deficit and one under additional deficit (Figure 4.15) were fitted to a 10^{th} order polynomial (Figure 4.16), and used to calculate the slopes of Tr_{WL} and VPD

(Figure 4.17). The t_{DI} was determined and DI calculated. On DOY 218, $t_{sunrise}$ was 0447 h and t_{sunset} 1922 h. In the vine under standard deficit, t_{DI} was 1133 h and DI was 53, while in the vine under additional water deficit, t_{DI} was 0625 h and DI was 88. Values of DI were also calculated for a DWC (DOY 215, 2003) following the same method described above for one vine under standard deficit and one under additional deficit (Figures 4.18 and 4.19). In the vine under standard deficit DI was 41 and in that under additional deficit DI was 71.

4.5. Discussion

Vines under additional water deficit transpired less than those under standard RDI for the entire berry growth period, at both whole-canopy and single-leaf levels. Transpiration reflected the irrigation regimen and the timing of irrigation events within the weekly cycle. Vines responded rapidly to irrigation, increasing Tr_{WV} within 24 h of an irrigation set. The response to soil drying occurred over 2 to 3 days. Some studies report only crop coefficients or weekly irrigation applied without specifying how the application was temporally distributed (McCarthy and Coombe, 1985; Schultz, 2000). For a given amount of irrigation, its distribution during a week can change the response of Tr_{WV} and NCE_{WV} (chapter 3, this volume), as observed in this study. Given sandy soil, low incidence of weeds, and small surface area wetted by the drip emitters, one would anticipate very low evaporation from the soil surface. Thus ET was dominated by Tr_{WV}.

Whole-canopy transpiration rates recorded in this experiment were comparable to other results (e.g., 5 mmol m^{-2} s^{-1}, Katerji et al., 1994; 6.5 mmol m^{-2} s^{-1}, Ollat and Tandonnet, 1999). In general, transpiration rates recorded by single-leaf measurements

were higher than those measured at the whole-canopy level, confirming the difficulty of scaling up from single-leaf to whole-canopy transpiration. Moreover, VPD_{la} values were higher than those of bulk VPD. Whole-canopy measurements integrate sunlit and shaded leaves, the latter having lower temperatures and Tr_{SL} (Schultz, 1993; Schultz et al., 1996), whereas single-leaf measurements were taken on sun exposed leaves at higher temperatures and thus higher VPD_{la}. For better estimation of whole-canopy transpiration with single-leaf measurements, leaves in different canopy positions should be included in the sample.

Reductions in CO_2 fixation in both additional deficits (E and V) were associated with reductions in stomatal and canopy conductances, confirmed by the close relationship between NCE_{SL} and g_s that has been observed elsewhere (Chaumont et al., 1997; Winkel and Rambal, 1993), and between NCE_{WL} and g_c. These results are in agreement with other of field-grown vines, where stomatal limitation seems to be more important than non-stomatal limitation of NCE (Escalona et al., 1999; Flexas et al., 2002) and no evidence of photoinhibition was observed (Chaumont et al., 1997; Chaves, 1991; Delgado et al., 1995; Escalona et al., 1999; Flexas et al., 1998; Flexas et al., 1999; Kliewer et al., 1983). The smaller crop coefficients and the lower soil moisture targeted in vines under water deficit in the current experiment compared to vines under FVET could have generated hydraulic and chemical root signals, like reduced root water potential (van Zyl, 1987; Williams and Matthews, 1990), increased xylem sap pH (Davies et al., 2002; Wilkinson and Davies, 1997; Wilkinson and Davies, 2002) or increased absicic acid (ABA), all known to decrease stomatal conductance (Correia et al., 1995;

Davies and Zhang, 1991; Hartung et al., 2002; Loveys and Düring, 1984; Murillo de Alburquerque and Carbonneau, 1997; Peterlunger et al., 2000; Sauter et al., 2001; Stoll et al., 2000). However, direct application of ABA did not inhibited photosynthesis (Downton et al., 1988; Kriedemann et al., 1975). Reduction of NCE in field-grown grapevines seems to start with a reduction in stomatal conductance due to lower soil moisture, and subsequently, as a consequence of a reduction in CO_2 concentration inside the leaf, an acclimation of the photosynthetic machinery to the lower internal CO_2 concentration (Chaves et al., 2002; Medrano et al., 2002b). Previous research into the so-called 'afternoon depression' of photosynthesis in grapevines suggested the possibility of non-stomatal inhibition (Correia et al., 1990; Downton et al., 1987; Düring, 1991), but non-stomatal effects (i.e., light stress, reduction in Calvin cycle enzyme activity) may appear when water deficits are more severe (Escalona et al., 1999; Flexas et al., 1998) or rapidly induced (Flexas et al., 1998; Flexas et al., 1999; Flexas et al., 1999), situations that are common in potted plants, but rare in irrigated field-grown vines. Although leaf and xylem water potential values may seem very low (R. Smithyman, unpublished data; see appendix 4, Figure A4.3) we did not record in any of our single-leaf measurements complete stomatal closure. On the contrary, g_s generally exceeded 50 mmol m^{-2} s^{-1}.

No consistent differences in WUE were found between vines under standard deficit with those under additional water deficit as observed elsewhere (Chaves and Rodrigues, 1987; Medrano et al., 2003), although other literature reports increases in WUE under additional water deficit (Downton et al., 1987; Ollat and Tandonnet, 1999;

Schultz, 2000; Williams et al., 1994), and still others describe increases in WUE with additional irrigation (Schultz, 2000). Reasons for the inconsistency of the results are differences in experimental conditions and time at which measurements were taken. Comparison of transpiration between different experiments is not easy, especially with large differences between this experiment an others in VPD (Intrieri et al., 1998), light (Boyer et al., 1997), irrigation (Hepner et al., 1985), and canopy size (Edson et al., 1993). The time at which measurements are recorded during the day and the variation in plant-available water during a given measurement period can bias results if plots are not irrigated simultaneously. For example (Figure 4.15) on DOY 218, 2003, instantaneous WUE at 0800 h was about 1% higher in S vines than in those under additional deficit, whereas at 1600 h, vines under the additional deficit had an instantaneous WUE 42% higher than the S vines. However, integrated over the whole day, there was no difference on average between irrigation regimens. Measurements recorded only during the afternoon would have suggested higher WUE_{SL} with additional water deficit, while integrating continuous measurements shows instead that the observed reduction in Tr_{WL} apparently occurred at the expense of CO_2 fixed.

Plants under well-watered conditions will have lower WUE than those under some water deficit, especially under high ET. In this experiment reductions in transpiration were accompanied by reductions in CO_2 fixation. Under standard RDI vines are under some degree of water deficit, and in this study stomata apparently regulated transpiration during most of the day. No differences were found in $WUE_{i,W}$ or in $WUE_{i,SL}$ between vines under standard deficit or under additional water deficit, in agreement with

previous research where $WUE_{i,SL}$ differed only between varieties of different geographic origin, or within a given variety when g_s was below 50 mmol m^{-2} s^{-1} (de Souza et al., 2003; Escalona et al., 1999). Others found even higher $WUE_{i,SL}$ in irrigated vines, explained by higher leaf temperatures in deficit irrigated vines which increased VPD_{la} and thus transpiration per unit CO_2 fixed (Schultz, 2000). The WUE_i ties CO_2 fixation and stomatal aperture, while WUE ties CO_2 fixation to transpiration. Mesophyll photosynthesis and stomatal aperture have been reported to be tightly correlated (Correia et al., 1990; Flexas et al., 1999; Flexas et al., 1999; Jacobs et al., 1996; Jarvis and Davies, 1998). At the beginning of the day, stomatal aperture has been related to an increase in turgor pressure due to the accumulation of K ions inside the guard cells, while later in the day that turgor pressure is maintained by accumulation of sucrose, the connection between photosynthesis and stomatal conductance, thus transpiration (Talbott and Zeiger, 1998). Stomatal closure at the end of the day follows sucrose depletion in the guard cells.

Vines fix CO_2 that is used to manufacture organic phosphates, precursors of all components for growth and development, including sugars and secondary metabolites in the fruit. The proposed water deficit index (DI) intends to tie irrigation management to CO_2 fixation by detecting the time at which stomata exert control over rates of CO_2 fixation, instead of tying irrigation management to Tr or ET. This constitutes an improvement over crop coefficients or soil moisture content as indices for irrigation because a variable related to the vine physiology (g_c) is included in the index and because most of the variation in CO_2 fixation may be explained by g_c. Although leaf or

xylem water potential measured at predawn or midday has been used to estimate plant water status (Williams and Araujo, 2002), the measurement is on a single leaf or at best a few leaves. At the single-leaf level, some photosynthetic parameters (i.e., electron transport rate, carboxylation efficiency) were more strongly correlated with stomatal conductance than with leaf water status (Medrano et al., 2002a; Medrano et al., 2002b). One of the advantages of DI is that it is not necessary to know the exact amount of water transpired to calculate and use it. Nonetheless, one still must monitor continuously Tr_{WV} or a surrogate indicator of Tr. It is the time of the day at which the slope of Tr_{WL} becomes smaller than the slope of the VPD curve that is important. Even the simplest meteorological station provides the necessary data to calculate VPD (i.e., temperature and relative humidity). Values of DI may be vineyard-specific and may vary within phenological stage and latitude. The inclusion of daylength as a variable in the calculation of DI suggests that although $t_{DI} - t_{sunrise}$ could be the same in two different regions, longer days will result in higher DI. A possible reason is because more time will pass after t_{DI} in the region with longer days than in that with shorter days. The DI will not indicate the amount of irrigation to apply like most plant based indices (Jones, 2004), but either soil moisture could be monitored, soil water balance based on irrigation and evapotranspiration could be calculated, or fixed irrigation sets could be applied.

4.6. Conclusion

Additional water deficits beyond standard RDI imposed during the berry growth period reduced grapevine transpiration. Maximum values of transpiration of about 20 L $vine^{-1}$ d^{-1} were recorded around veraison. Variation in canopy conductance

explained most of the variation in NCE_{WV}. Likewise, variations in g_s explained most of the variation in NCE_{SL}, in both vines under RDI and those under additional water deficits. During most of the measurements, WUE and WUE_i did not differ among irrigation regimens, whether measured at whole-canopy or single-leaf level. Under the water deficits applied here (0.7 and 0.35 of FVET), light does not seem to limit NCE_{WV} on DCS. Water savings from the reductions in transpiration were at the expense of reductions in CO_2 fixation. In an area like eastern Washington that has a short growing season and a short period between harvest and leaf fall to replenish carbohydrate reserves, the water savings achieved by imposing additional water deficits should take into account this reduction in CO_2 fixation. The proposed water deficit index (DI) for use under RDI, based on a plant (g_c) and a meteorological variable (VPD), ties irrigation management to CO_2 fixation, which other indices do not. But the index requires more work and more data analysis, plus development of site-specific, variety-specific, and season-specific relationships, as well as other proposed plant based indicators for irrigation.

4.7. References

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Table 4.1: Effect of RDI regimens on transpiration per vine ($Tr_{WV,d}$) and per unit leaf area ($Tr_{WL,d}$) per day in 2003. Data are means of six vines averaged across all measurement days.

Phenological stages	Sampling days (DOY)	Irrigation regimens			p value			Day length	Global irradiance
		S	E	V					
		----- L H_2O $vine^{-1}$ d^{-1} ----			treat	DOM	treat*DOM	h	MJ m^{-2} d^{-1}
Fruit set [z]	177-184	11.6	11.2	13.7	0.176	0.001	0.182	15.7	30.6
Pre-veraison	212-219	14.1 a	7.7 b	15.6 a	0.001	0.001	0.030	14.6	21.9
Post-veraison	233-241	11.6 a	7.8 b	6.0 b	0.001	0.001	0.001	13.6	16.5
Pre-harvest	255-262	6.9	6.2	5.8	0.178	0.003	0.247	12.5	18.3
Post-harvest	274-281	4.2	4.1	5.2	0.087	0.001	0.341	11.4	13.7
Stage (p value)			0.001		0.001[y]				0.067[x]

Phenological stages	Sampling days (DOY)	Irrigation regimens			p value		
		S	E	V			
		- L H_2O d^{-1} m^{-2} leaf area -			treat	DOM	treat*DOM
Fruit set [z]		1.3	1.3	1.4	0.734	0.001	0.202
Pre-veraison		1.6 a	0.9 b	1.6 a	0.001	0.001	0.068
Post-veraison		1.4 a	0.9 b	0.6 c	0.001	0.001	0.001
Pre-harvest		0.9	0.8	0.7	0.058	0.026	0.592
Post-harvest		0.7	0.7	0.7	0.862	0.004	0.753
Stage (p value)			0.001		0.001[y]		

DOY: day of year; DOM: day of measurement; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters are significant at $p \leq 0.05$ by Tukey- Kramer within phenological stages.

[z] Data were collected before irrigation treatment was imposed.

[y] RDI regimen x phenological stage interaction.

[x] p value for global radiation across phenological stages.

Table 4.2: Effect of RDI regimens on transpiration per vine per day ($Tr_{WV,d}$). Data are means of two vines from days with clear skies.

Phenological stages	Sampling day (DOY)	Irrigation regimens S	E	V	p value	Global irradiance	2002-2003[x]
		----- L H_2O d^{-1} $vine^{-1}$ ----			treat	MJ m^{-2} d^{-1}	
---------- Year 2002 ----------							
Fruit set[z]		nc[w]	nc	nc			
Pre-veraison	218	11.3 a	5.4 b	12.3 a	0.001	24.1	
Post-veraison	235	16.6	9.5	16.7	0.088	23.6	
Pre-harvest	256	10.7	8.5	7.3	0.431	20.5	
Post-harvest	279	6.4	5.3	5.2	0.834	16.0	
Stage (p value)			0.001		0.009[y]		
---------- Year 2003 ----------							L H_2O d^{-1} $vine^{-1}$
Fruit set[z]	178	14.1	15.7	19.0	0.400	31.4	nc
Pre-veraison	218	18.3 a	7.8 b	20.7 a	0.002	27.5	12.6 a
Post-veraison	238	18.2 a	16.2 a	7.7 b	0.003	24.2	14.2 a
Pre-harvest	256	8.6	7.5	6.6	0.318	21.7	7.9 b
Post-harvest	278	4.1	4.7	5.9	0.207	15.3	5.3 c
Stage (p value)			0.001		0.042[y]		0.001

DOY: day of year; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters within phenological stages are significantly different at $p \leq 0.05$ by Tukey-Kramer.

[z] Data were collected before RDI regimens were imposed.

[y] RDI regimen x phenological stage interaction.

[x] Effect of RDI regimens on $Tr_{WV,d}$ across phenological stages with pooled data of 2002 and 2003. Significant interaction was found between year x phenological stage x RDI regimen ($p \leq 0.001$).

[w] nc: data not collected in 2002.

Table 4.3: Effect of RDI regimens on transpiration per unit leaf area ($Tr_{WL,d}$) during 24 h for 2003. Data are means of two vines on days with clear skies.

Phenological stages	Sampling day (DOY)	Irrigation regimens			p value	Day length	Global irradiance
		S	E	V			
		-- L H_2O d^{-1} m^{-2} leaf area --			treat	h	MJ m^{-2} d^{-1}
Fruit set [z]	178	1.6	1.9	1.9	0.667	15.7	31.4
Pre-veraison	218	2.2 a	0.9 b	2.1 a	0.023	14.6	27.5
Post-veraison	238	2.2 a	1.9 a	0.7 b	0.011	13.6	24.2
Pre-harvest	256	1.2	1.1	0.7	0.205	12.5	21.7
Post-harvest	278	0.7	0.7	0.8	0.862	11.4	15.3
Stage (p value)			0.001		0.001[y]		

DOY; day of year; S: standard deficit; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters are significant at $p \leq 0.05$ by Tukey- Kramer within phenological stages.

[z] Data were collected before irrigation treatment was imposed.

[y] RDI regimen x phenological stage interaction.

Table 4.4: Multiple stepwise regression with $NCE_{wv,d}$ as the dependent variable and transpiration ($Tr_{wv,d}$), $Tr_{wv,d}^2$, $PPFD_d$, $PPFD_d^2$, DSD, and DSD^2 as independent variables.

Variable	Parameter estimate	Partial R^2	Model R^2	p value
Intercept	-88.52			0.001
Transpiration	6.42	0.701	0.701	0.001
DSD	18.99	0.018	0.720	0.025
DSD^2	-0.73	0.046	0.766	0.001

Table 4.5: Effects of RDI regimens on daily water use efficiency per vine ($WUE_{wv,d}$) calculated as $NCE_{wv,d}/Tr_{wv,d}$ for 2003. Data are from six vines across all measurement days.

Phenological stages	Sampling days (DOY)	Irrigation regimens			p value			Day length	Global irradiance
		S	E	V					
		g CO_2/ L H_2O $vine^{-1}$ d^{-1}			treat	DOM	treat*DOM	h	MJ m^{-2} d^{-1}
Fruit set [z]	177-184	8.7 b	9.1 a	8.3 b	0.009	0.001	0.001	15.7	30.6
Pre-veraison	212-219	9.1	8.9	8.6	0.876	0.083	0.910	14.6	21.9
Post-veraison	233-241	16.7	16.4	16.4	0.918	0.001	0.001	13.6	16.5
Pre-harvest	255-262	7.5 a	5.6 b	5.4 b	0.018	0.001	0.045	12.5	18.3
Post-harvest	274-281	13.7 a	11.0 b	10.8 b	0.022	0.001	0.064	11.4	13.7
Stage (p value)			0.001		0.167[y]				

DOY: day of year; DOM: day of measurement; S: standard deficit; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters are significant at $p \leq 0.05$ by Tukey- Kramer within phenological stages.

[z] Data were collected before irrigation treatment was imposed.

[y] RDI regimen x phenological stage interaction.

Table 4.6: Effect of RDI regimens on $WUE_{wv,d}$ calculated as $NCE_{wv,d}/Tr_{wv,d}$. Data are means of two vines from days with clear skies.

Phenological stages	Sampling day (DOY)	Irrigation regimens S	E	V	p value	Global irradiance	2002-2003[x]
		-- g CO_2/ L H_2O $vine^{-1}$ d^{-1} --			treat	MJ m^{-2} d^{-1}	
---------- Year 2002 ----------							
Fruit set[z]		nc[w]	nc	nc			
Pre-veraison	218	10.9	12.9	10.6	0.047	24.1	
Post-veraison	235	7.1	7.8	8.0	0.726	23.6	
Pre-harvest	256	6.6 ba	8.4 ab	8.8 a	0.042	20.5	
Post-harvest	279	7.4 b	7.0 b	10.6 a	0.007	16.0	
Stage (p value)			0.001		0.009[y]		
---------- Year 2003 ----------							g CO_2/ L H_2O $vine^{-1}$ d^{-1}
Fruit set[z]	178	8.0	7.6	7.4	0.590	31.4	nc[w]
Pre-veraison	218	7.6	7.6	7.2	0.530	27.5	9.5
Post-veraison	238	7.9	7.6	7.8	0.761	24.2	7.7
Pre-harvest	256	9.4	8.7	7.4	0.084	21.7	8.2
Post-harvest	278	13.2	12.7	9.7	0.261	15.3	10.1
Stage (p value)			0.002		0.318[y]		0.001

DOY: day of year; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters within phenological stages are significantly different at $p \leq 0.05$ by Tukey-Kramer.

[z] Data were collected before RDI regimens were imposed.

[y] RDI regimen x phenological stage interaction.

[x] Effect of RDI regimens on $WUE_{wv,d}$ across phenological stages with pooled data of 2002 and 2003. Significant interaction was found between year x phenological stage x RDI regimen ($p \leq 0.001$).

[w] nc: data not collected in 2002.

Table 4.7: Effect of RDI regimens on daily leaf water use efficiency per unit leaf area ($WUE_{SL,d}$) calculated as the daily average of $NCE_{SL}/Tr_{SL,d}$ for 2003. Data are means of three vines on days with clear skies.

Phenological stages	Sampling day (DOY)	Irrigation regimens			p value	$WUE_{SL,d}$[y]
		S	E	V		
		g CO_2/ L H_2O vine^{-1} d^{-1}			treat	g CO_2/ L H_2O m^{-2} d^{-1}
Fruit set [z]	178	3.3	3.1	3.4	0.214	3.3 c
Pre-veraison	213	4.9	4.7	4.5	0.625	4.7 c
Post-veraison	237	7.3	6.5	7.8	0.108	7.2 b
Pre-harvest	255	10.2	8.7	8.3	0.139	9.1 a
Post-harvest	275	10.8	9.0	9.9	0.154	9.9 a
Stage (p value)			0.001		0.065[x]	0.001

DOY: day of year; S: standard RDI; E: early deficit (fruit set - veraison); V: late deficit (veraison - harvest). Values with different letters are significant at $p \leq 0.05$ by Tukey- Kramer within phenological stages.

[z] Data were collected before irrigation treatment was imposed.

[y] WUESL,d at each phenological stage. Values with different letters are significant at $p \leq 0.05$ by Tukey-Kramer within column.

[x] RDI regimen x phenological stage interaction.

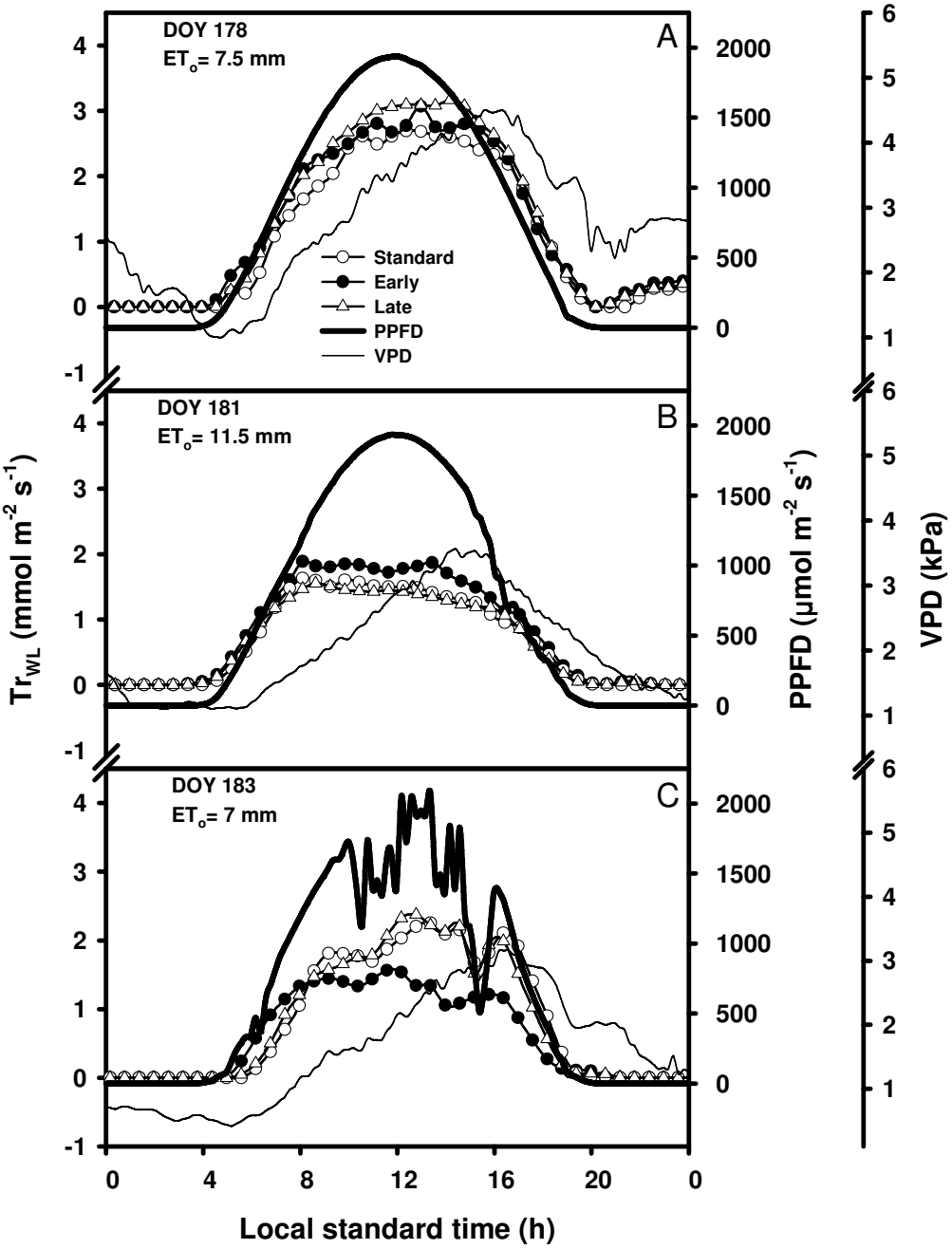

Figure 4.1: Whole-canopy transpiration rate (Tr_{WL}), photosynthetic photon flux density (PPFD) and air vapor pressure deficit (VPD) during fruit set, 2003. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

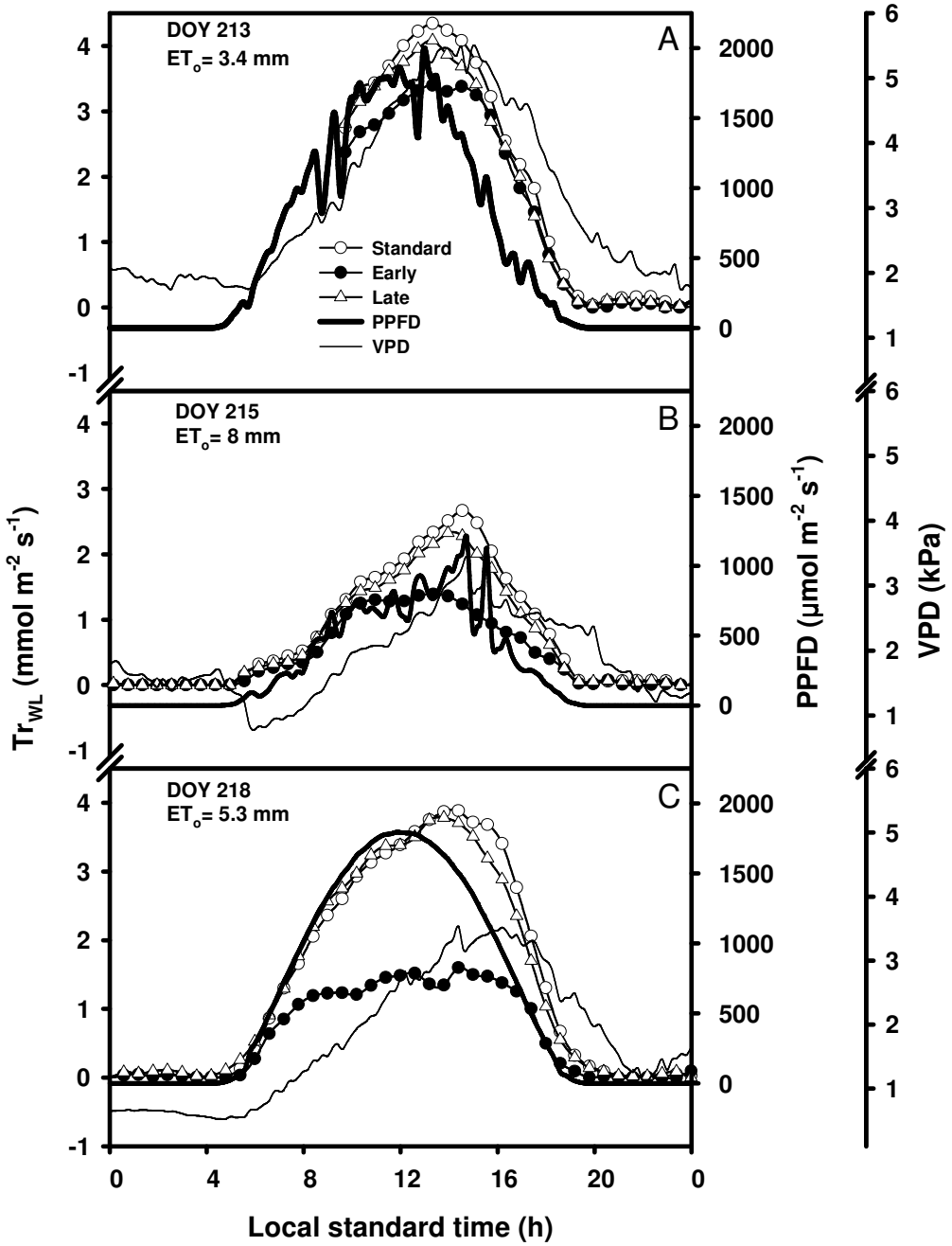

Figure 4.2: Whole-canopy transpiration rate (Tr_{WL}), photosynthetic photon flux density (PPFD), and air vapor pressure deficit (VPD) during pre-veraison, 2003. Panel B demonstrates a smaller difference in Tr_{WL} between extra water deficit (E) and vines under standard RDI regimen (S and V) due to lower PPFD and lower air temperature. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

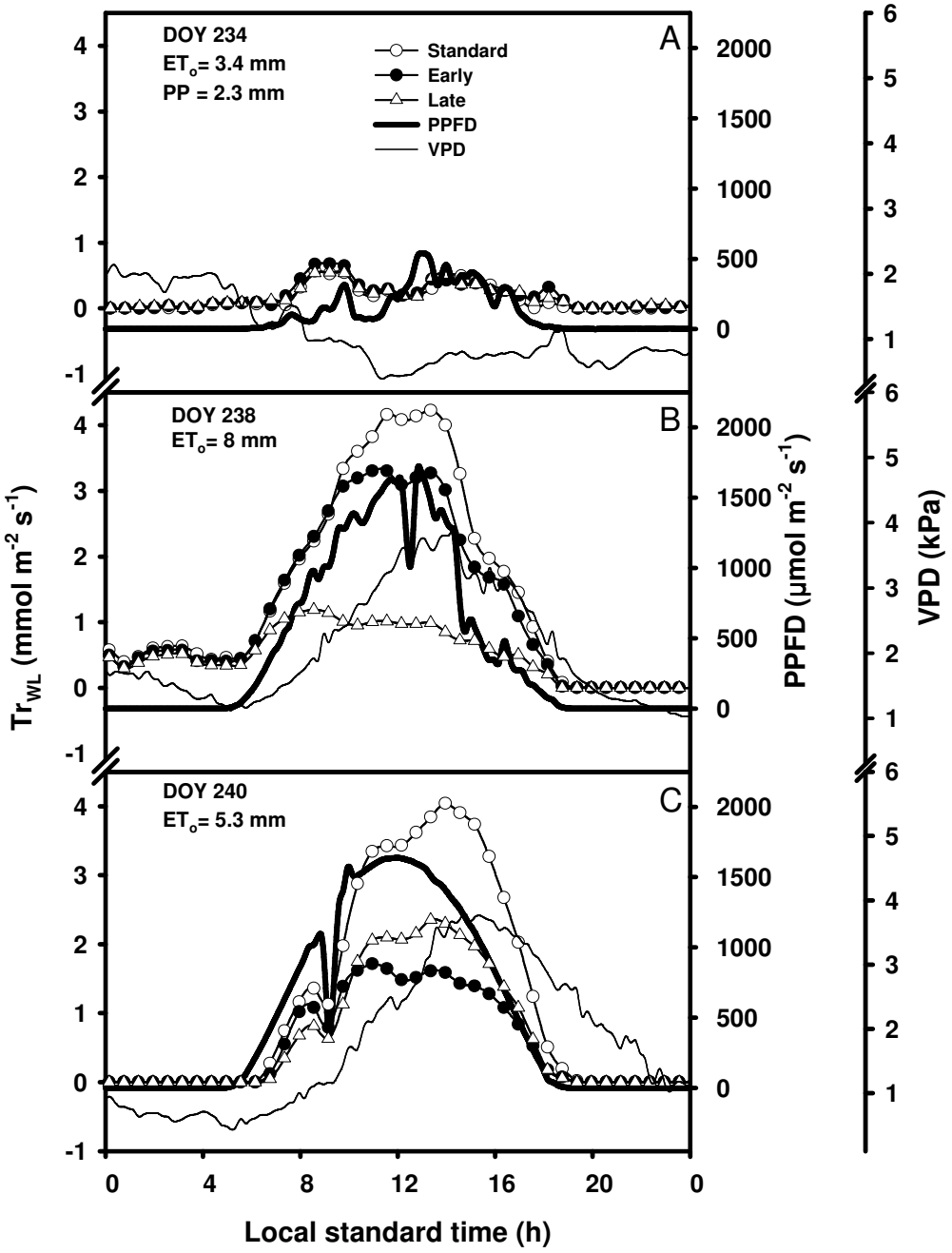

Figure 4.3: Whole-canopy transpiration rate (Tr_{WL}), photosynthetic photon flux density (PPFD), and air vapor pressure deficit (VPD) during post-veraison, 2003. V vines were under extra water deficit, S and E vines were under standard RDI regimen. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) and precipitation (PP) are from the Alderdale PAWS station (10 km west of site).

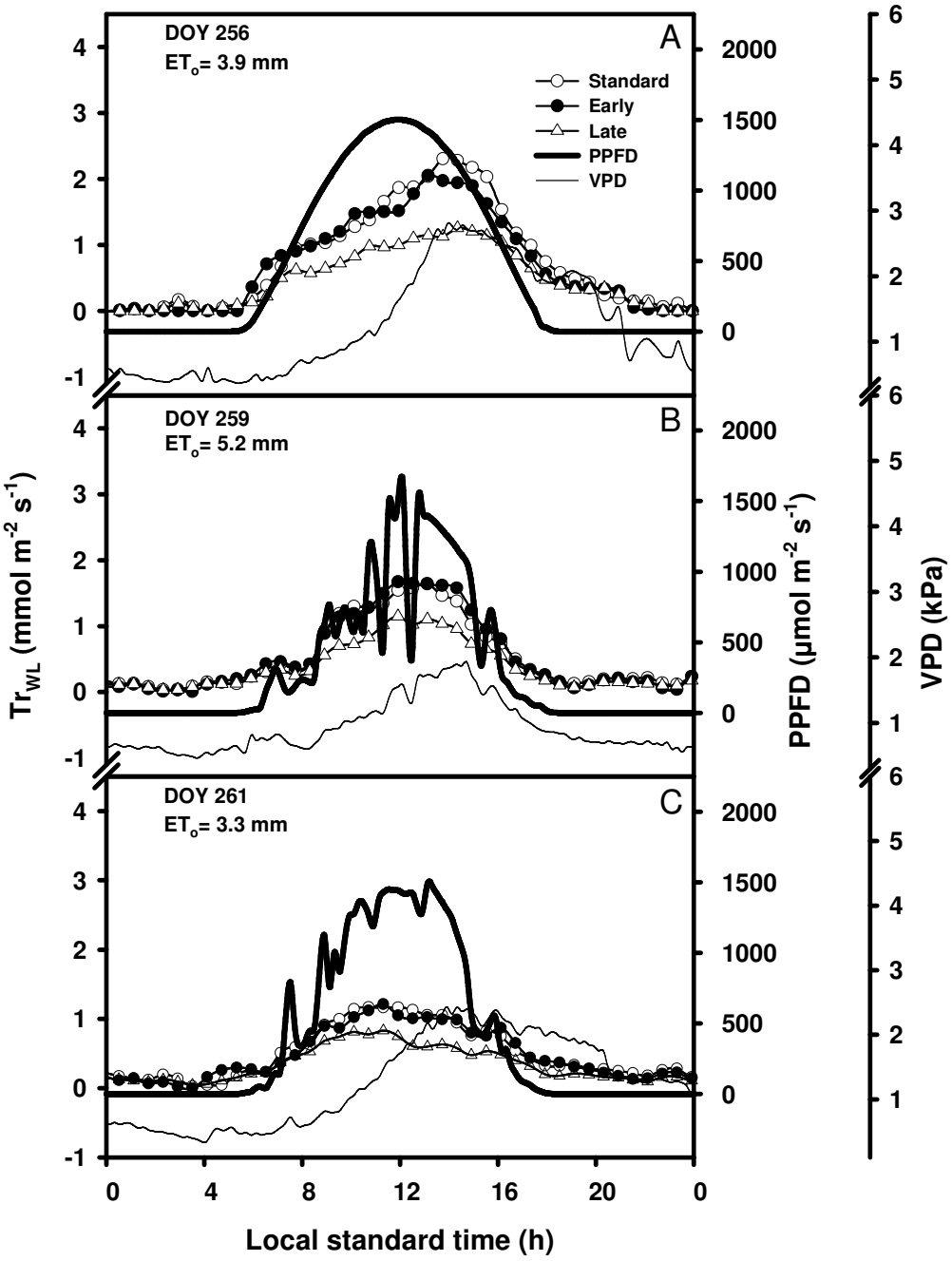

Figure 4.4: Whole-canopy transpiration rate (Tr_{WL}), photosynthetic photon flux density (PPFD), and air vapor pressure deficit (VPD) during pre-harvest, 2003. V vines were under extra water deficit, S and E vines were under standard RDI regimen. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

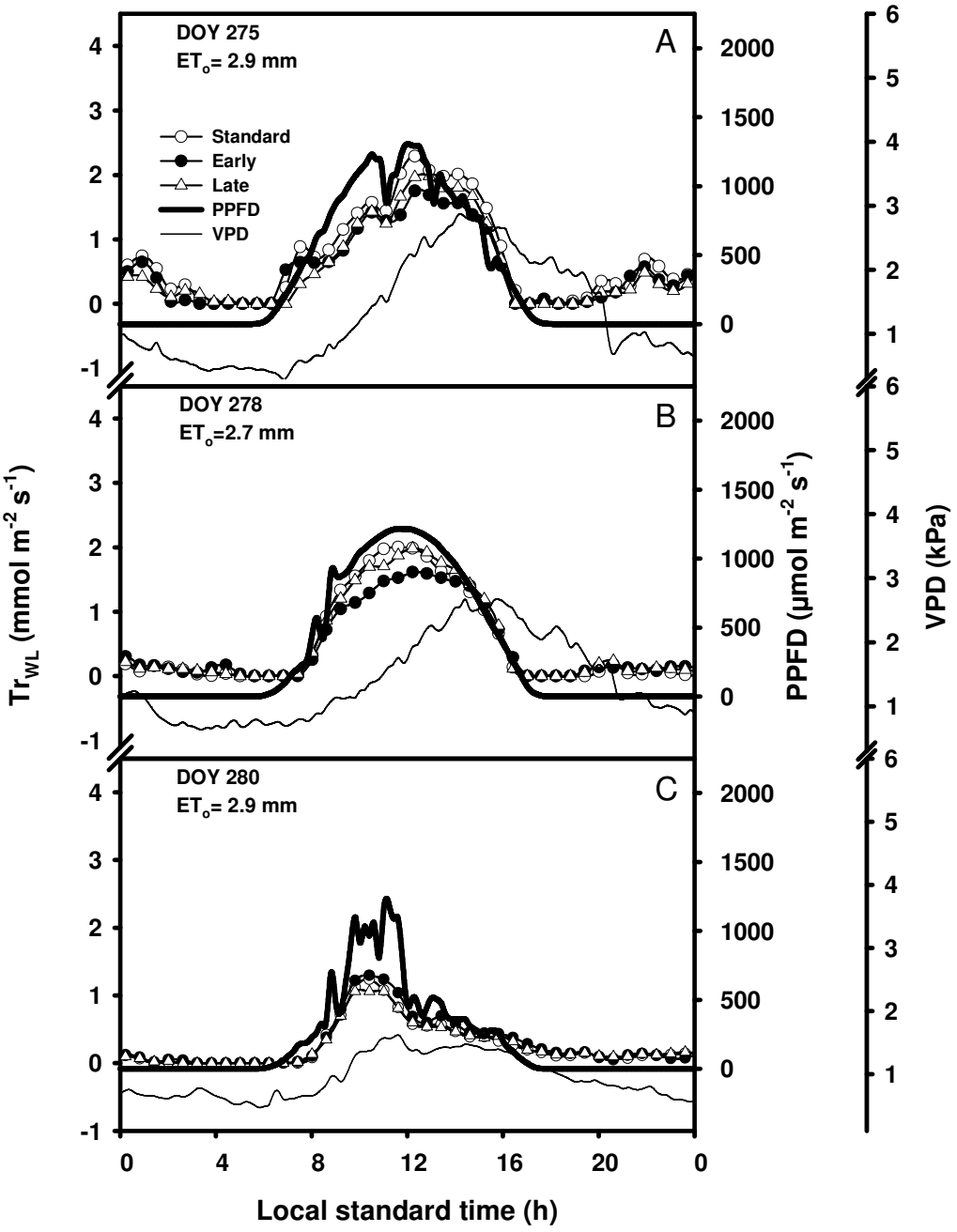

Figure 4.5: Whole-canopy transpiration rate (Tr_{WL}), photosynthetic photon flux density (PPFD), and air vapor pressure deficit (VPD) during post-harvest, 2003. All vines were irrigated at FVET. Data points are means of two chambers operating simultaneously. Daily reference evapotranspiration (grass; ET_o) is from the Alderdale PAWS station (10 km west of site).

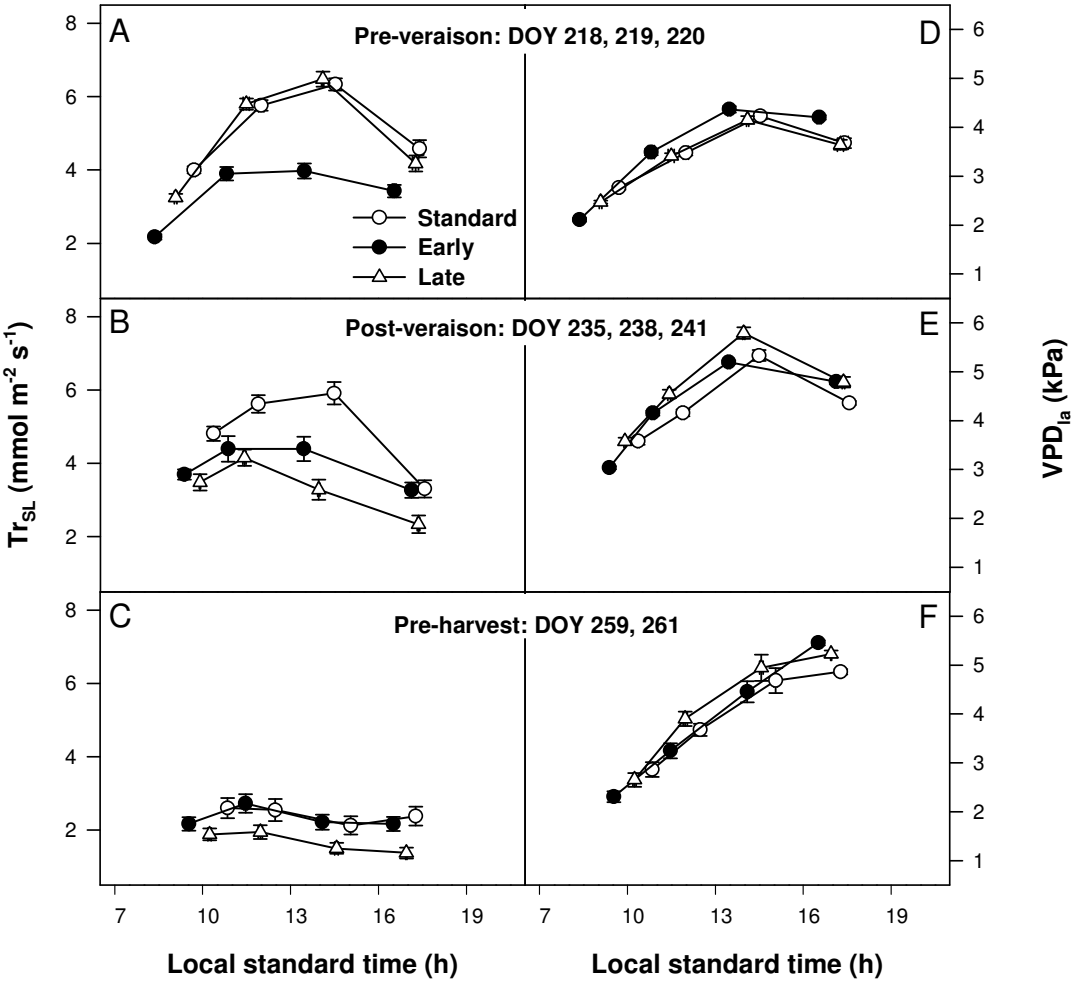

Figure 4.6: Effects of RDI regimens on single-leaf transpiration (Tr_{SL}; A-C) and leaf-to-air vapor pressure deficit (VPD_{la};D-F) at different phenological stages and at different times of the day during 2002. Values are means per vines ± SE (n=9; pre-harvest n=6).

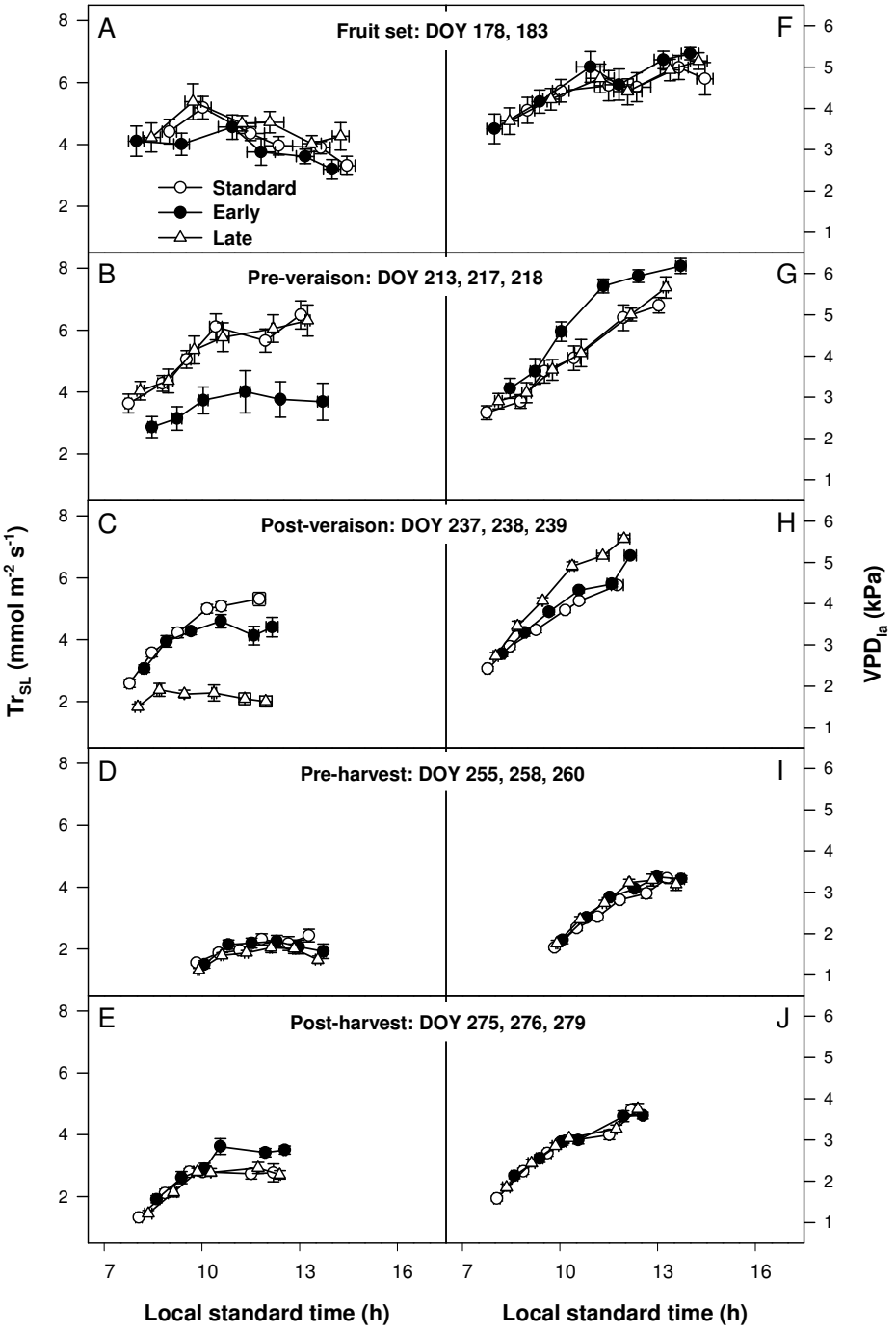

Figure 4.7: Effects of RDI regimens on single-leaf transpiration rate (Tr_{SL}; A-E) and leaf-to-air vapor pressure deficit (VPD_{la}; F-J) at different phenological stages and at different times of the day during 2003. Values are means of vines ± SE (n=9; at fruit set n=6).

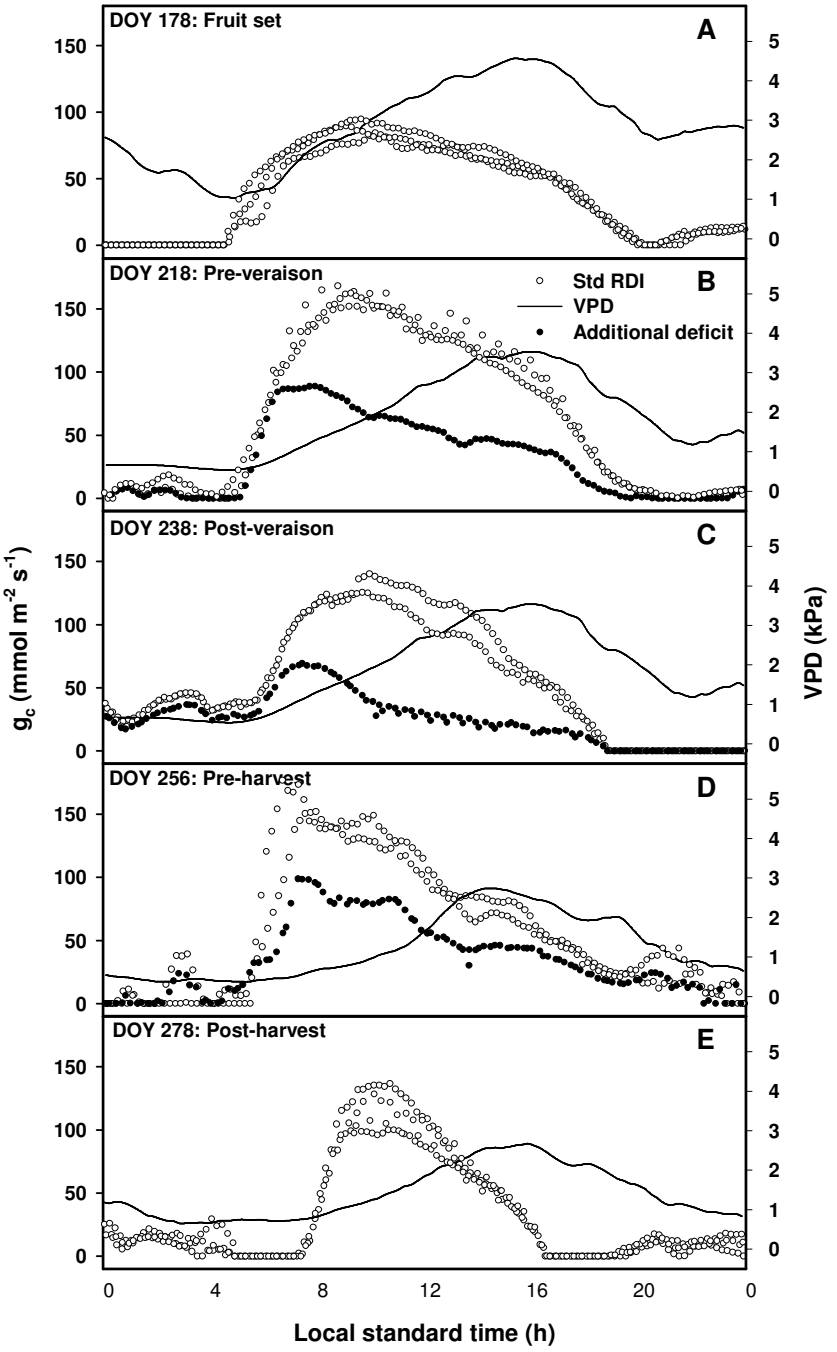

Figure 4.8: Effect of RDI regimens on canopy conductance (g_c) on days with clear skies at five phenological stages during growing season 2003. Data points are means of two chambers operating simultaneously on days with clear skies.

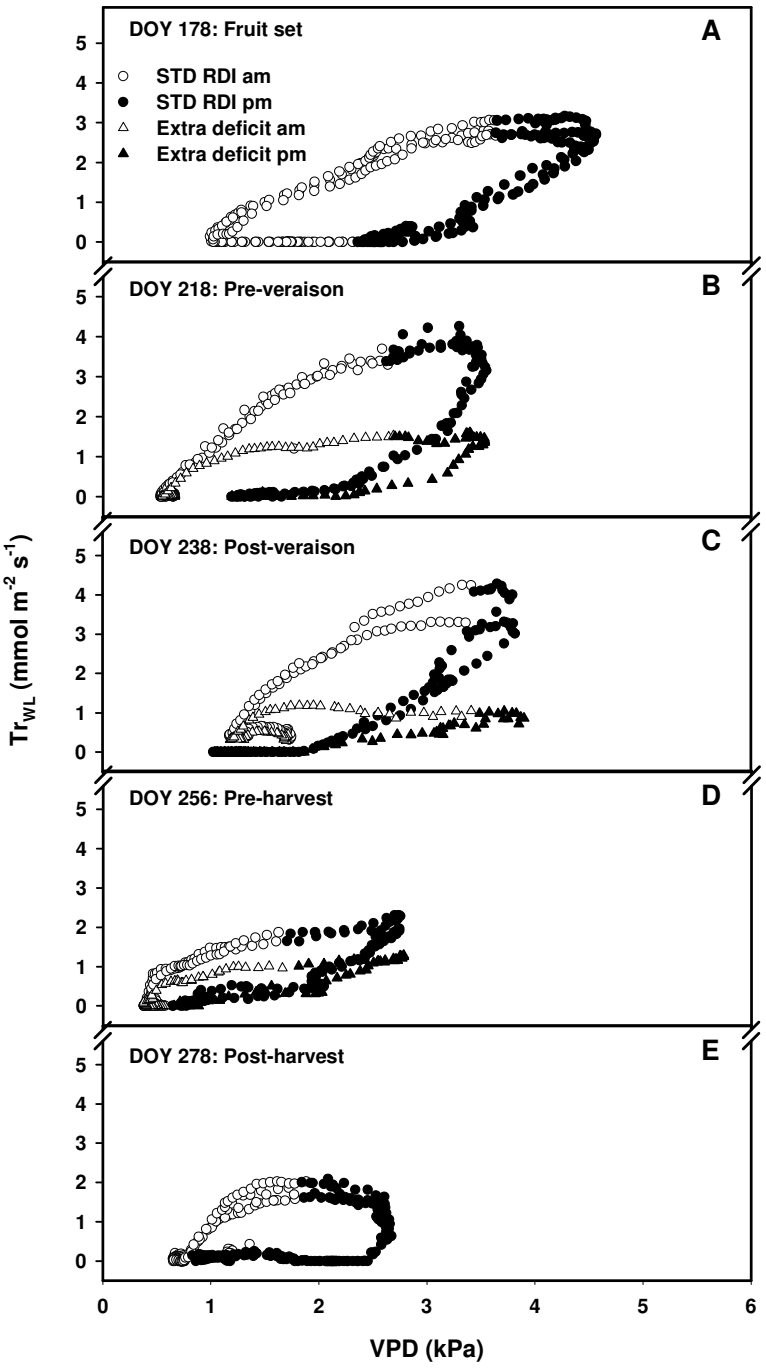

Figure 4.9: Effect of RDI regimens on the relationship between whole canopy transpiration rate (Tr_{WL})and air vapor pressure deficit (VPD) at five phenological stages during growing season of 2003. Data points are means of two chambers operating simultaneously on days with clear skies.

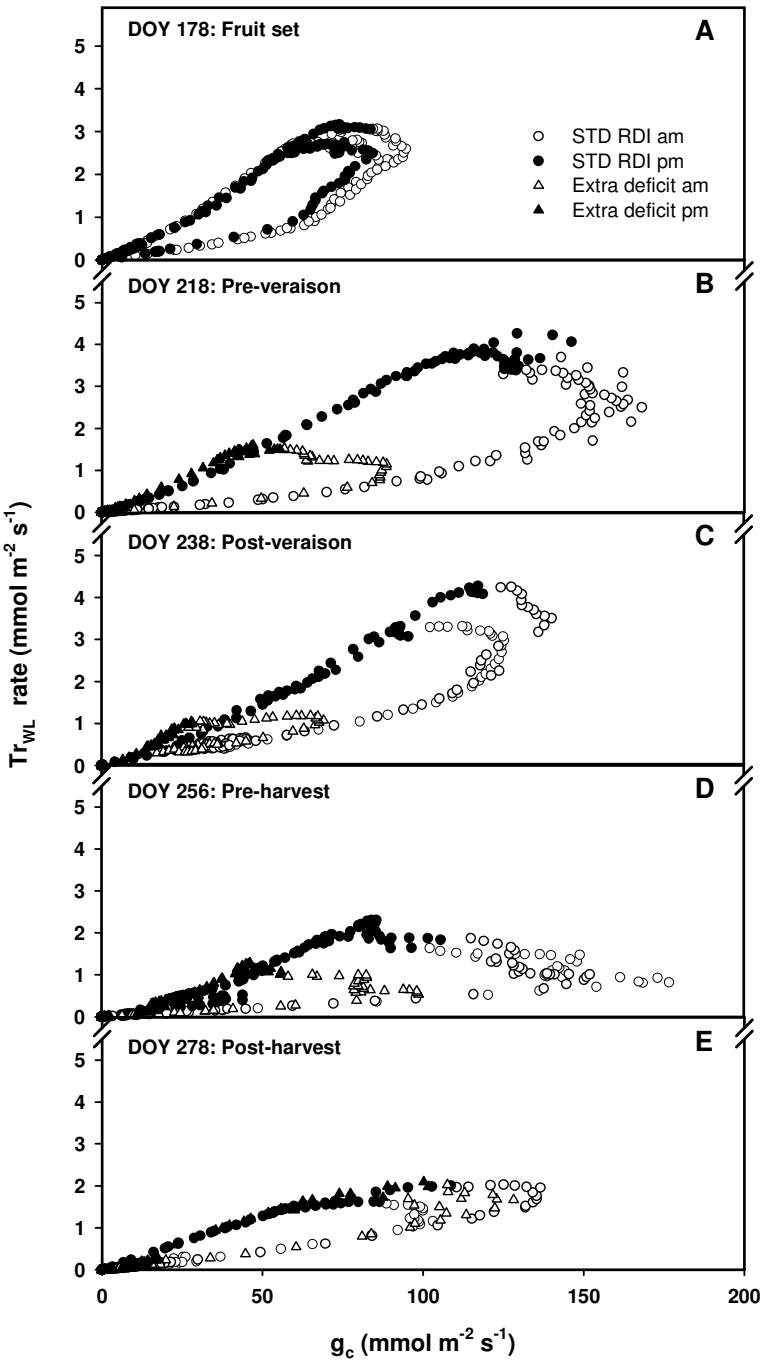

Figure 4.10: Effect of RDI regimens on the relationship between whole-canopy transpiration (Tr_{WL}) and canopy conductance (g_c) at each phenological stage during season 2003. Data are from days with clear skies. Data points are means of two chambers operating simultaneously.

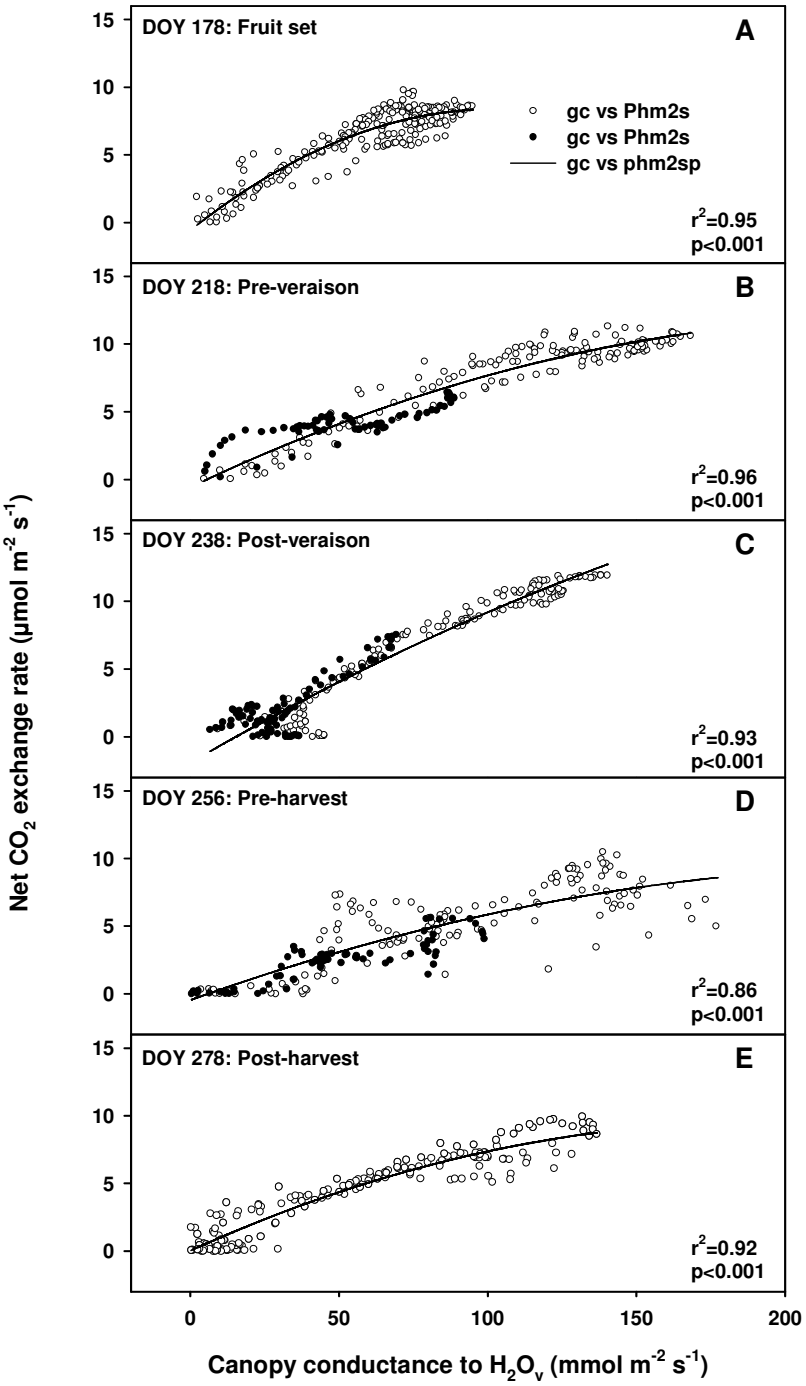

Figure 4.11: Effect of RDI regimens on the relationship between whole-canopy net CO_2 exchange rate (NCE_{WL}) and canopy conductance (g_c) at five phenological stages during growing season 2003. Data points are means of two chambers operating simultaneously on days with clear skies.

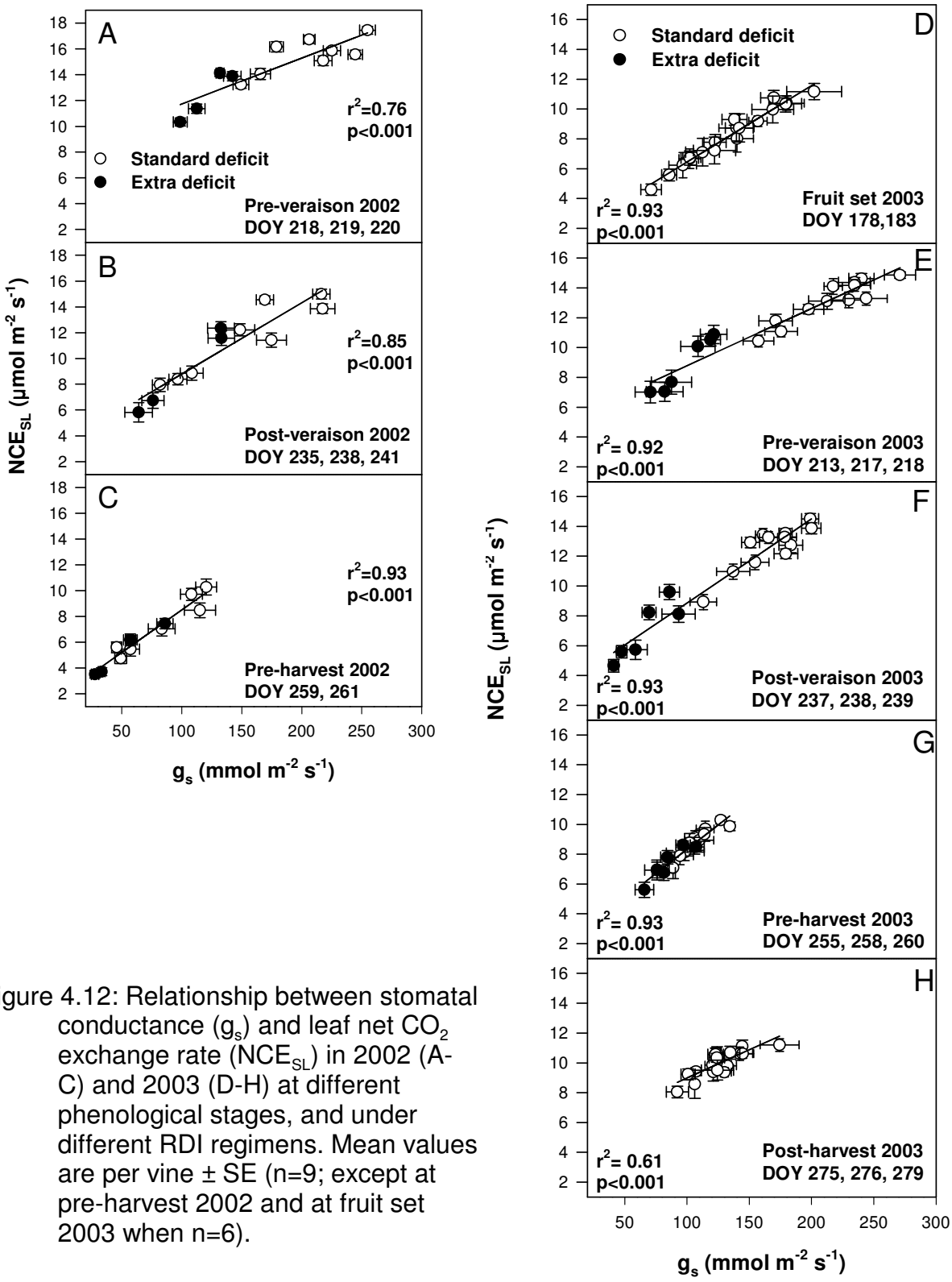

Figure 4.12: Relationship between stomatal conductance (g_s) and leaf net CO_2 exchange rate (NCE_{SL}) in 2002 (A-C) and 2003 (D-H) at different phenological stages, and under different RDI regimens. Mean values are per vine ± SE (n=9; except at pre-harvest 2002 and at fruit set 2003 when n=6).

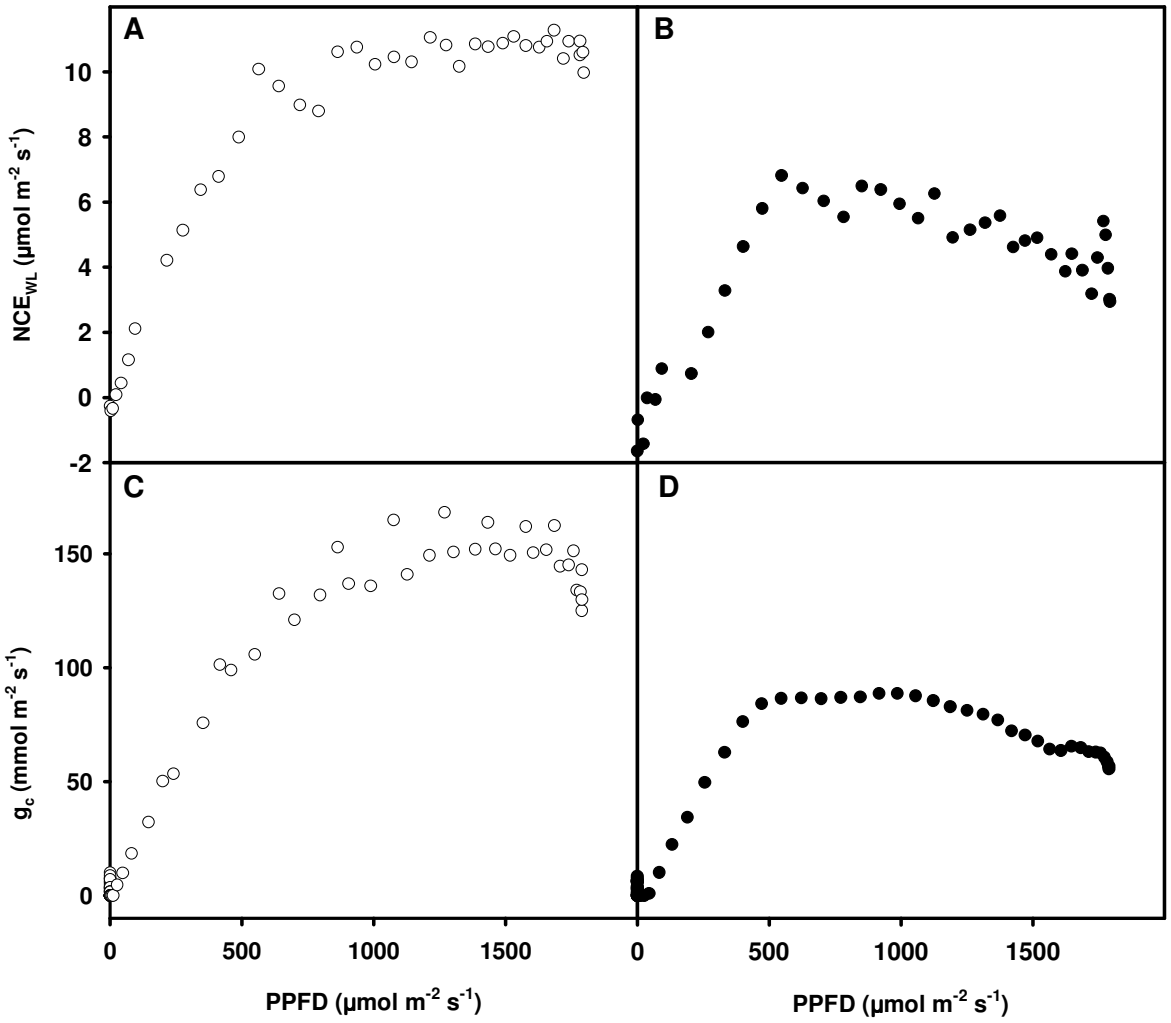

Figure 4.13: Relationships between whole-canopy net CO_2 exchange rate (NCE_{WL}) and g_c with photosynthetic photon flux density (PPFD) at pre-veraison, for two vines under standard RDI (A, C) and under additional water deficit (B, D). Data represent DOY 218, 2003 from 0000 h to 1200 h recorded at 12 min intervals.

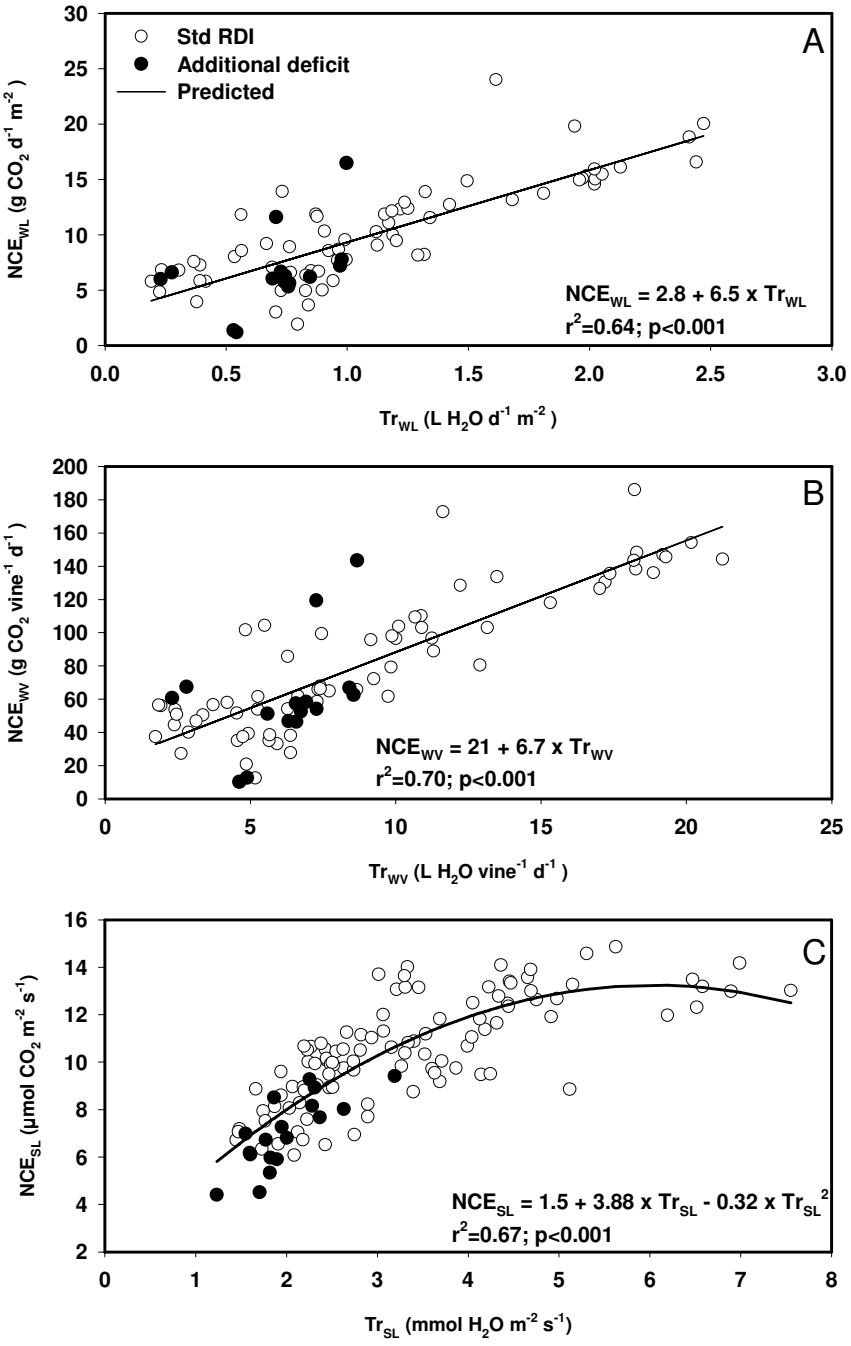

Figure 4.14: A) Relationships in 2003 between A) whole-canopy net CO_2 exchange rate ($NCE_{WL,d}$) and transpiration ($Tr_{WL,d}$) per unit leaf area; B) whole-canopy net CO_2 exchange rate ($NCE_{WV,d}$) and transpiration ($Tr_{Wv,L}$) per vine; C) single-leaf net CO_2 exchange rate (NCE_{SL}) and transpiration (Tr_{SL}).

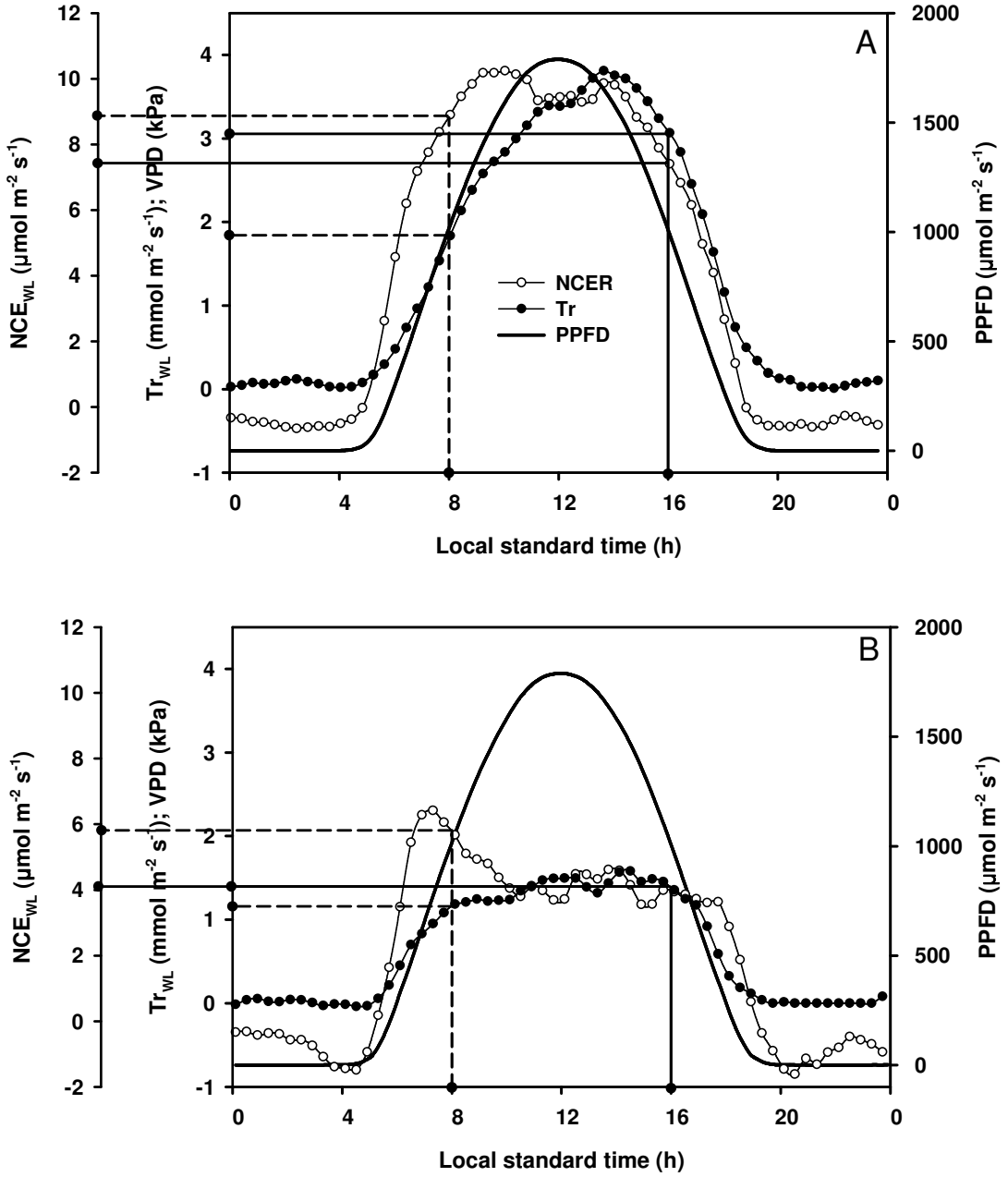

Figure 4.15: NCE_{WL}, Tr_{WL}, and PPFD on DOY 218, 2003 (pre-veraison) for a vine under standard RDI (A) and under additional deficit (B). NCE_{WL} and Tr_{WL} used for WUE_{WL} calculation at 0800 h and at 1600 h are indicated with dashed and solid lines, respectively.

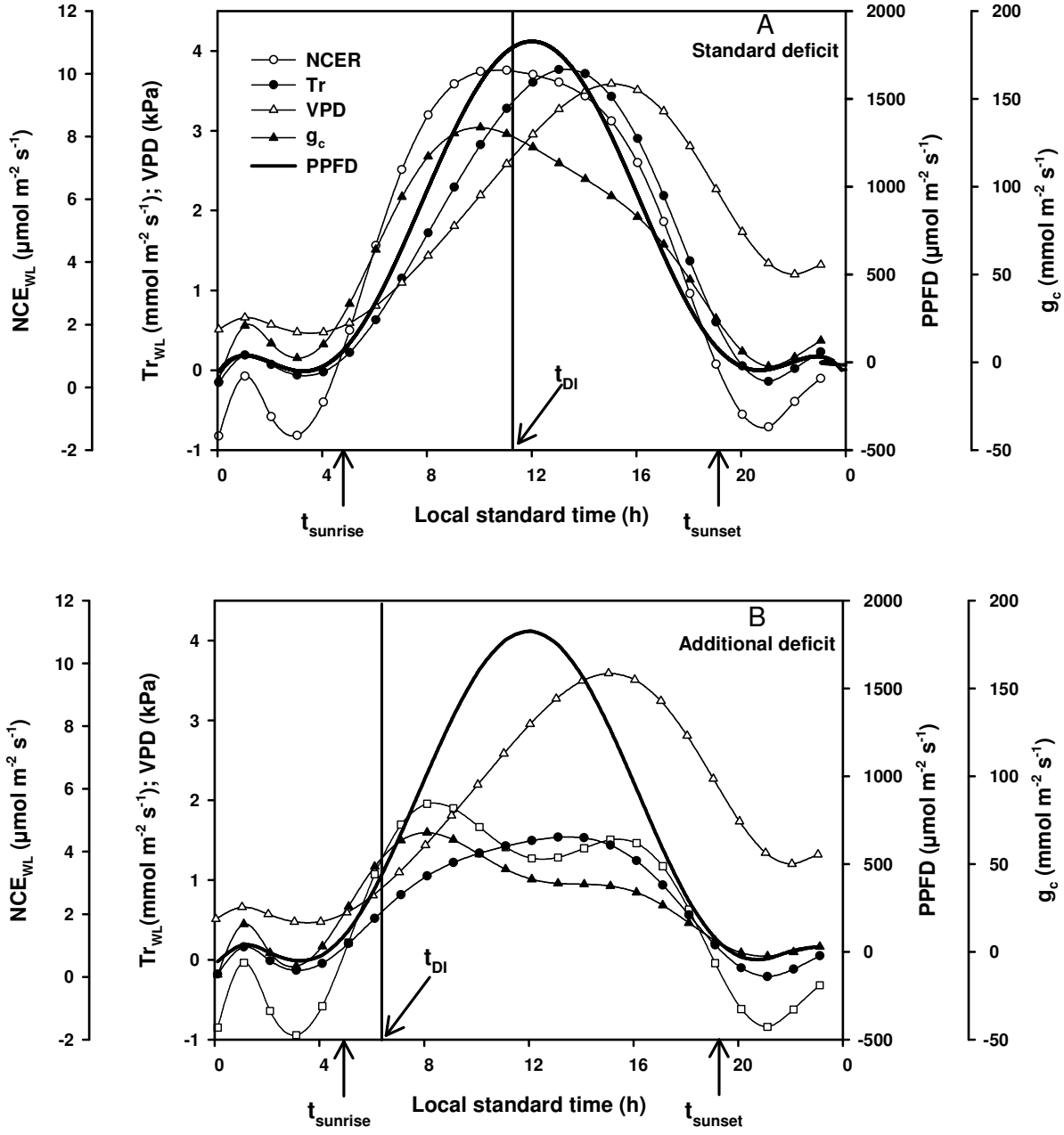

Figure 4.16: Model derived from data of DOY 218, 2003 of a vine under standard RDI (A) and a vine under additional deficit (B) using a 10th order polynomial for describing NCE_{WL}, Tr_{WL}, VPD, and g_c. Sunrise and sunset times are indicated by arrows. t_{DI} indicates the time when slope of VPD exceeds slope of Tr_{WL}.

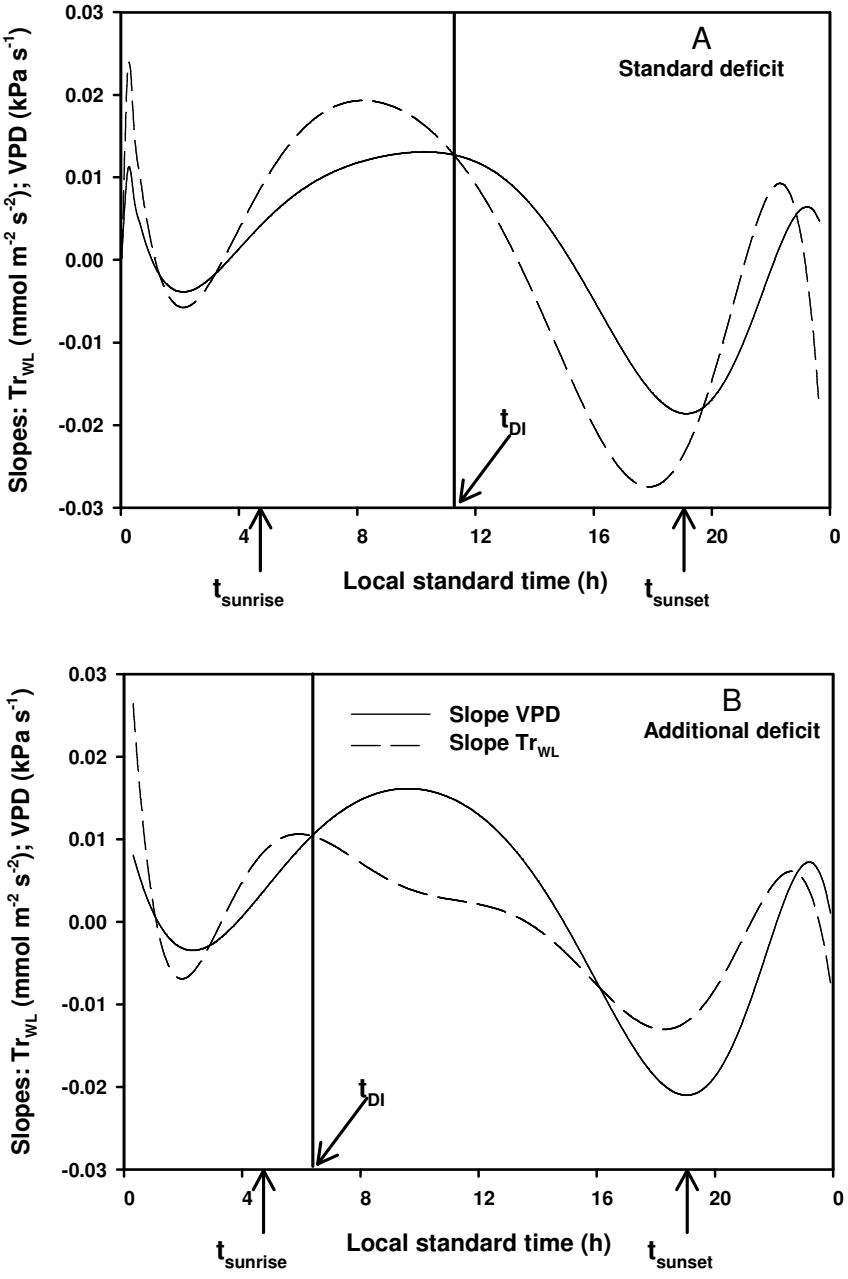

Figure 4.17: VPD and Tr_{WL} slopes derived from models using figure 4.16 data for a vine under standard RDI (A) and a vine under additional deficit (B). Sunrise and sunset times are indicated by arrows. t_{DI} indicates the time when slope of VPD exceeds slope of Tr_{WL}.

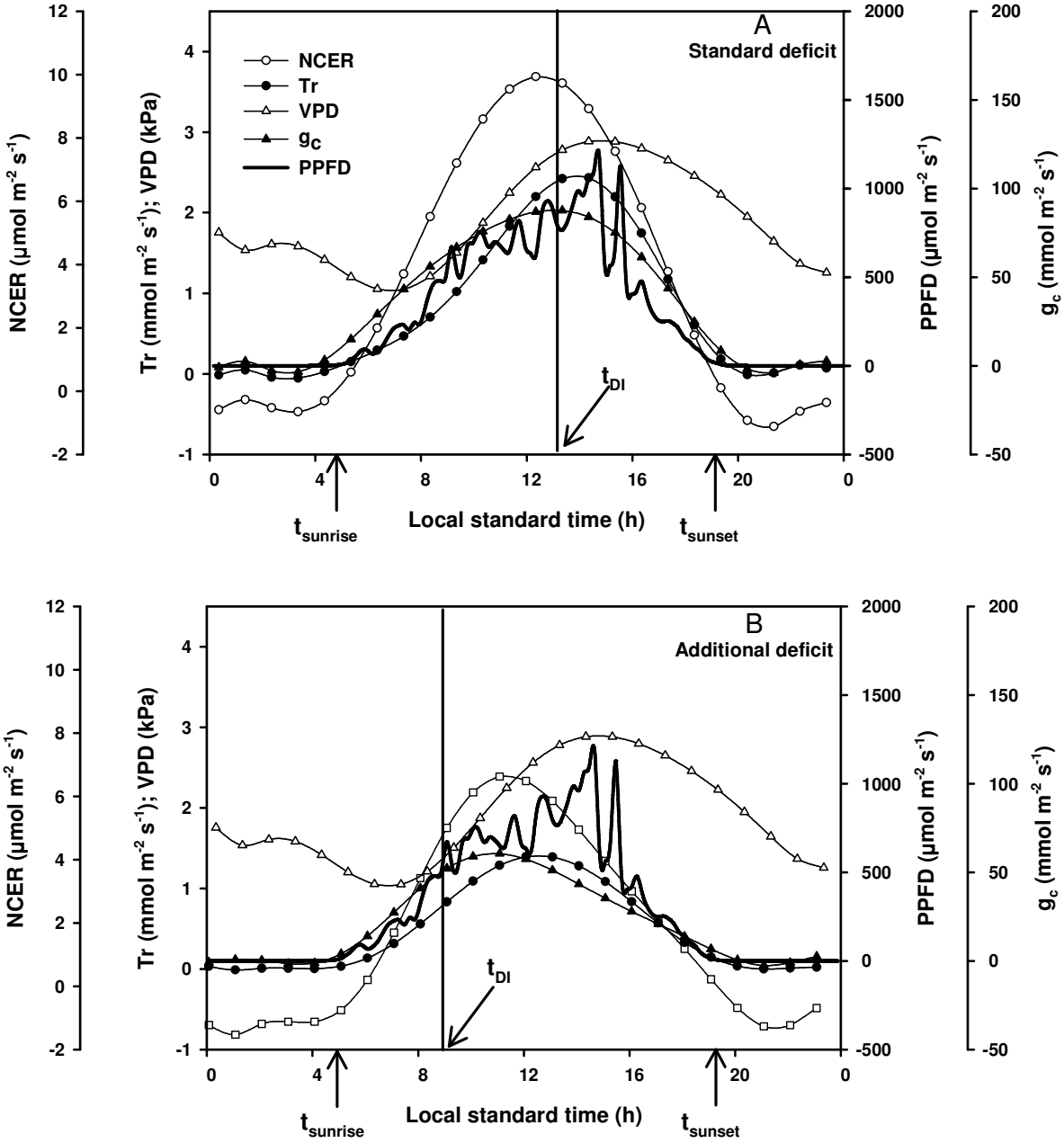

Figure 4.18: Model derived from data of DOY 215, 2003 of a vine under standard RDI (A) and a vine under additional deficit (B) using a 10th order polynomial for describing NCE_{WL}, Tr_{WL}, VPD, and g_c. Sunrise and sunset times are indicated by arrows. t_{DI} indicates the time when slope of VPD exceeds slope of Tr_{WL}.

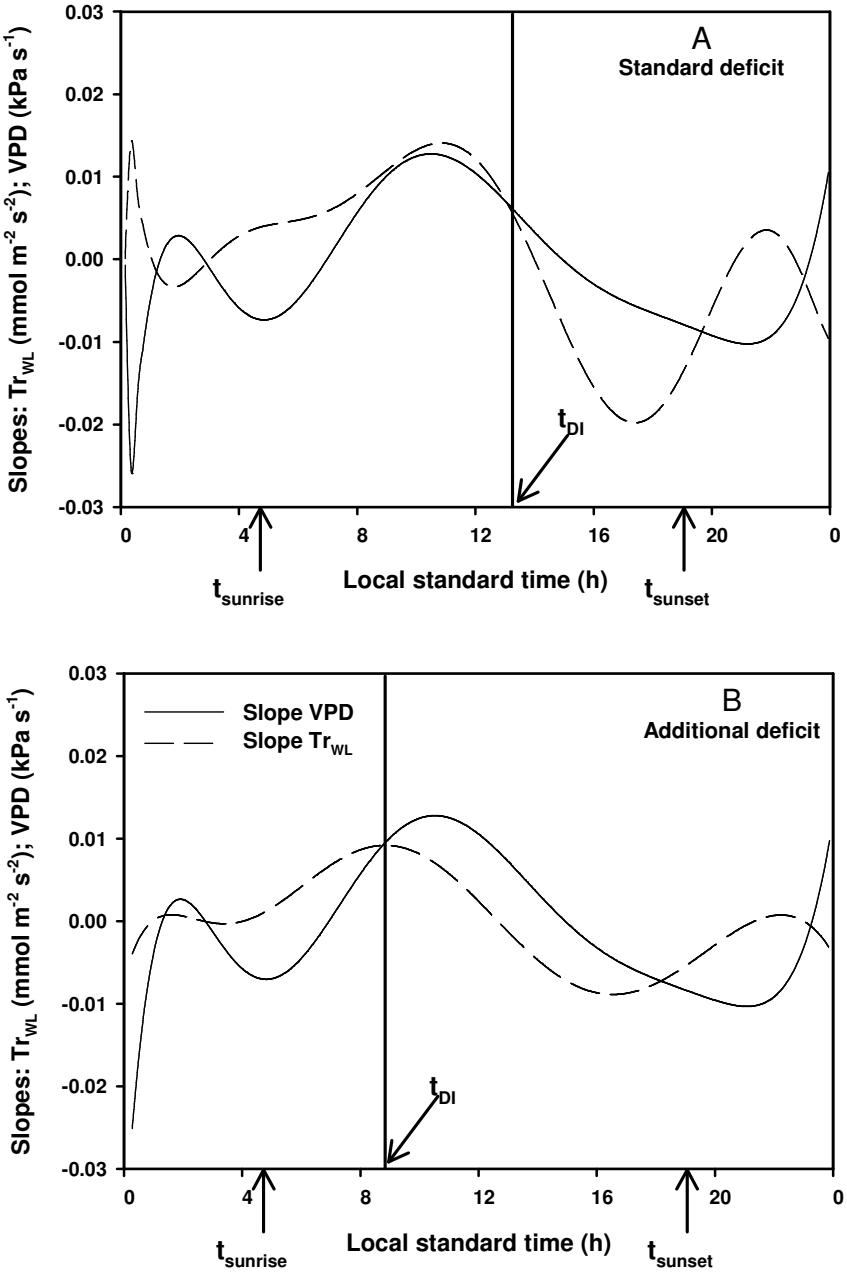

Figure 4.19: VPD and Tr_{WL} slopes derived from models using figure 4.18 data for a vine under standard RDI (A) and a vine under additional deficit (B). Sunrise and sunset times are indicated by arrows. t_{DI} indicates the time when slope of VPD exceeds slope of Tr_{WL}.

APPENDIX 4

A4.1. Saturation vapor pressure (Buck 1981):

$$e_s = 101325 \exp(13.3185 * y - 1976 * y^2 - 0.6445 * y^3 - 0.1229 * y^4)$$

where e_s is the saturation vapor pressure, and y is:

$$y = 1 - \frac{T_s}{T}$$

where T_s (K) is steam temperature and T (K) is temperature of interest.

A4.2. Atmospheric pressure (Campbell and Norman, 1998):

$$P_a = 101.3 \exp\left(\frac{-A}{8200}\right)$$

where Pa is atmospheric pressure (kPa) and A is the altitude (m) above sea level.

A4.3. Calculations of t_{DI}

After adjusting a 10th order polynomial to the Tr_{WL} and VPD data, the ratios between those slopes at each sampling time can be calculated:

$$SR = \frac{Trp_{WL(tn)} - Trp_{WL(tn-1)}}{VPDp_{(tn)} - VPDp_{(tn-1)}}$$

where SR is the ratio between the calculated slopes of the curves of the predicted Tr_{WL} (Trp_{WL}) and predicted VPD (VPDp) at sampling time n (tn) and previous time (tn-1). The time in the day at which the SR becomes below 1 is the t_{DI} used in the calculation of the deficit index DI.

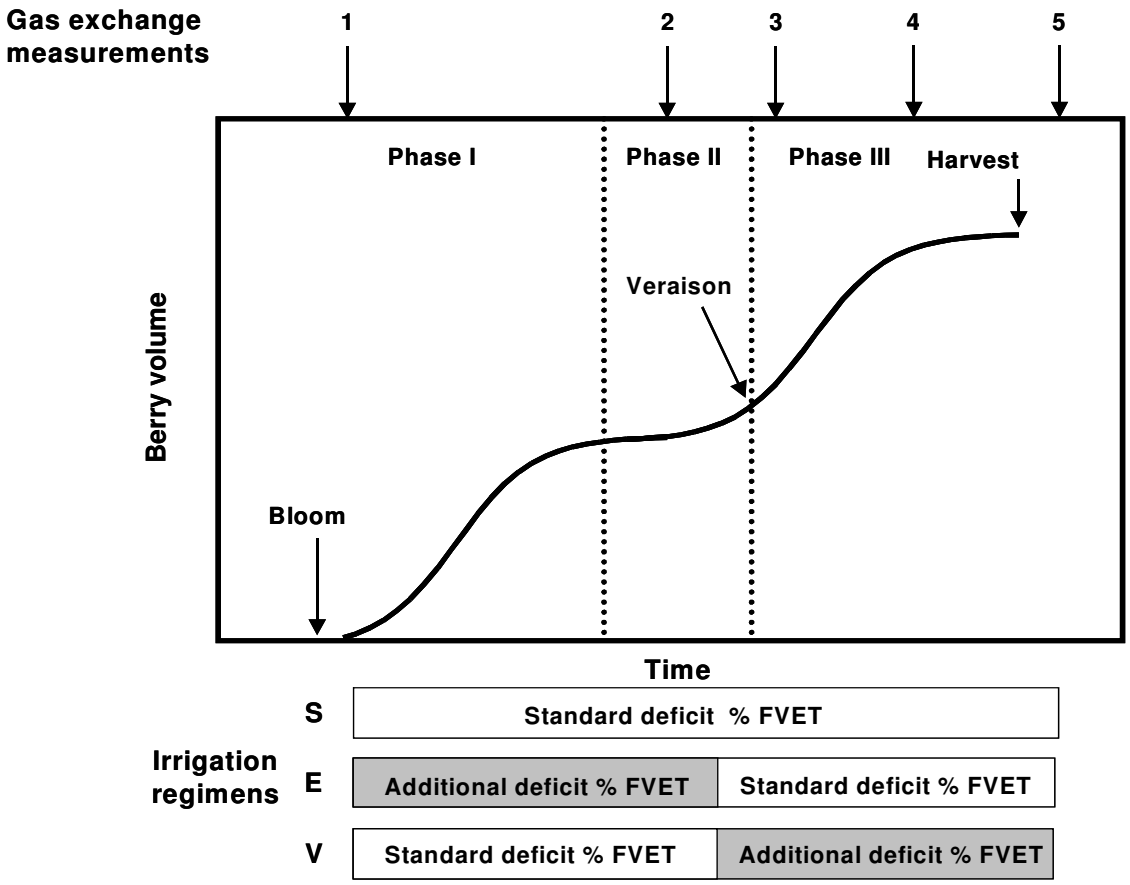

Figure A4.1: Experimental calendar and irrigation schedule superimposed on theoretical curve of berry growth. Arrows at top indicate approximate periods of whole-canopy and single-leaf gas exchange measurements: 1-fruit set, 2-pre-veraison, 3-post-veraison, 4-pre-harvest, 5-post-harvest. Bars at the bottom indicate the duration and scheduling of irrigation regimens. FVET: full vine evapotranspiration; standard deficit = 70% FVET (2002) or 60% FVET (2003); additional deficit = 35% FVET (2002) or 30% FVET (2003).

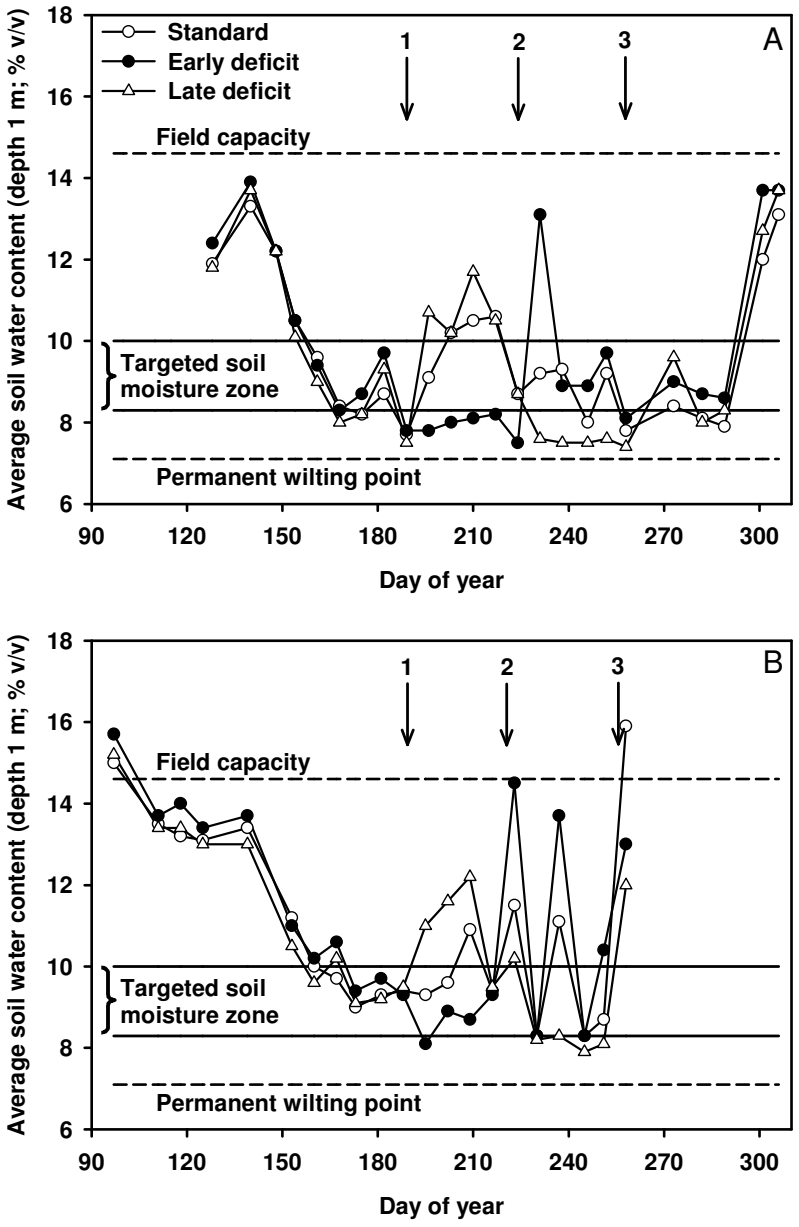

Figure A4.2: Average soil water content in the first 1 m depth of soil for 2002 (A) and 2003 (B) for the three irrigation regimens. Arrows 1 indicate when E regimen was initiated, arrows 2 indicate when E return to standard RDI and V was initiated, and arrows 3 indicate when all regimens started to be irrigated at FVET. Data provided by R. Smithyman - St. Michelle Wine Estates.

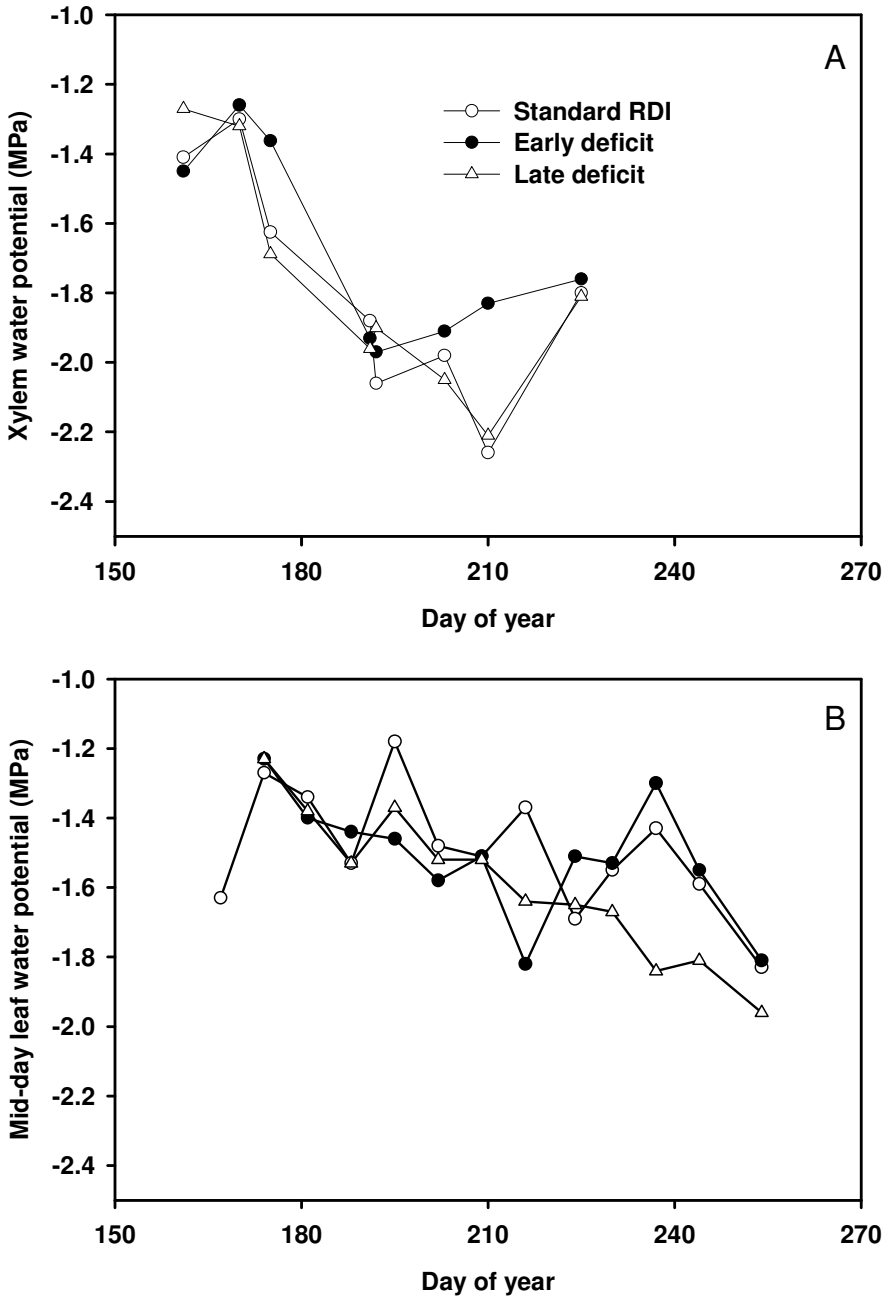

Figure A4.3: Effects of RDI regimens on xylem water potential in 2002 (A) and on leaf water potential in 2003 (B). Data provided by R. Smithyman - St. Michelle Wine Estates.

CHAPTER 5

YIELD, YIELD COMPONENTS, FRUIT COMPOSITION, AND NON-STRUCTURAL CARBOHYDRATES IN CANES UNDER REGULATED DEFICIT IRRIGATION

5.1. Abstract

Regulated deficit irrigation (RDI) is applied in wine grapes mainly for improving fruit quality. The effects RDI on yield, fruit composition, and non-structural carbohydrates in dormant canes were evaluated in 2002 and 2003 on field-grown vines cv. Cabernet Sauvignon that were irrigated during the period of berry growth according to three regimens within RDI: 1) standard RDI (70% of full vine evapotranspiration, FVET, was replaced weekly); 2) early deficit (35% of vine FVET was replaced weekly between fruit set and veraison); and 3) veraison deficit (35% of vine FVET was replaced weekly between veraison and harvest). When not under 35% deficit, vines in scenarios #2 and #3 were irrigated according to standard RDI practice. No consistent effect on yield or berry quality indicators (Brix, berry weight, or color in the must) were observed among regimens, nor on soluble sugars or starch concentrations in canes. Lack of effect may be explained by the low yields (9.8 Mg ha^{-1}) and high leaf area:fruit ratios, suggesting that all vines were under-cropped. However, vines under the standard and veraison RDI deficit regimens had 41% higher pruning weights than the early deficit regimen, thus greater amount of above-ground biomass.

5.2. Introduction

Crop yield is generally limited by more than one environmental factor (Gifford et al., 1984), and water deficit is the one factor that most limits yield (Boyer, 1982). In winegrapes, it is not usually yield alone but a combination of fruit quality and quantity that is the main goal for growers (Jackson and Lombard, 1993). Yield reduction due to deliberate water deficit can be a goal of irrigation management via regulated deficit irrigation (RDI). Effects of water deficits on grapevine yield and fruit quality have been widely studied (e.g., dos Santos et al., 2003; Esteban et al., 1999; Hepner et al., 1985; Kennedy et al., 2002; Roby et al., 2004; Van Zyl, 1984); responses depended on the severity of the water deficit, its timing during the growing season (Matthews and Anderson, 1988; McCarthy, 2000; Myburgh, 2003; Naor et al., 1993), and the vine's source:sink balance (Bravdo et al., 1985; Hepner and Bravdo, 1985; Kliewer et al., 1983; Poni et al., 1994).

Research suggests that a water deficit applied to winegrapes using RDI is a viable practice for controlling excessive vigor, reducing pest populations and disease pressure, and improving wine quality (Williams, 1996). Water deficits applied early in the season reduced shoot growth, leaf area, canopy size, and increased the proportion of sun-exposed clusters (dos Santos et al., 2003; Hepner et al., 1985; Kliewer et al., 1983; Neja et al., 1977; Poni et al., 1993a). Applied during the berry growth period, water deficits usually were associated with smaller berries and sometimes lower yields (Greenspan et al., 1996; Matthews and Anderson, 1989; McCarthy, 1997; Poni et al., 1993a; Van Zyl, 1984). The reduction in berry weight results in an increase in berry skin:flesh ratio and a concentration of skin compounds in the must, especially important

in red varieties. The reduction varies according to the severity and timing of the water deficit imposed. Phase I of berry growth has been reported as the phase where the effect of a water deficit on berry size is greatest (Matthews and Anderson, 1989; Ojeda et al., 2002). Applied between veraison and harvest, the effect on berry weight is less pronounced, while sugar accumulation, flavonol, and anthocyanin production can be affected (Bravdo et al., 1985; Freeman and Kliewer, 1983; Kennedy et al., 2002; Kennedy et al., 2000; Matthews and Anderson, 1988; Ojeda et al., 2002).

Results from water deficit experiments do not always agree because of differences in environment, vine management, and plant material. For example, results vary by use of greenhouses (Correia et al., 1990; Düring, 1998; Rühl and Alleweldt, 1985), potted vines (Hardie and Considine, 1976; Poni et al., 1993b), mature field-grown vines (Bravdo et al., 1985; McCarthy, 1997), young vines (Buttrose, 1965; Dry et al., 2000; Düring, 1998; Hardie and Considine, 1976) and different rootstock-scion combinations (Iacono et al., 1998). Also contributing to the confusion is whether the experiments were conducted in irrigated or non-irrigated regions. In typically non-irrigated districts, under severe natural water deficits, irrigation increased yield and quality (Delgado et al., 1995; Esteban et al., 1999; Nadal and Arola, 1995). In irrigated districts, irrigation can be managed to increase or decrease yields and quality (Bravdo et al., 1985; Myburgh, 2003). For example, without irrigation, fruit had lower titratable acidity, higher pH, and higher sugar concentration than that from well watered vines (Esteban et al., 1999; Matthews and Anderson, 1988). Conversely, moderate irrigation was shown to not affect sugar accumulation (dos Santos et al., 2003), but heavy

irrigation reduced sugar accumulation and produced low quality wines (Bravdo et al., 1985; Freeman, 1983).

The primary goal of the research detailed in this volume was to study in grapevines the response of photosynthesis and transpiration at the whole-canopy level to additional water deficits imposed within RDI during the period of berry growth, in a semi-arid climate, where irrigation management is used to control vigor and increase fruit quality. In this chapter, yield and berry quality parameters are reported for vines under standard RDI and under additional water deficits between fruit set and harvest.

5.3. Materials and methods

5.3.1. Yield, yield components, and pruning weights

Vitis vinifera L. cv. Cabernet Sauvignon was planted in 1992 at 1.8 m x 2.7 m (vine x row spacing) in a north-south row orientation, double-trunked, trained to a bilateral cordon at 1.06 m on the bottom wire of a two-wire sprawl trellising system, and spur pruned with 20 to 23 buds per m of row. The 18 sample vines measured by whole-canopy gas exchange system (chapter 3, this volume) were harvested in both 2002 and 2003 on DOY 262. All other sentinel vines in the 4-ha irrigation study were harvested at the same time by hand. Vines had been irrigated according to three irrigation regimens (see chapter 3, this volume for details): 1) standard RDI (S, 70% of full vine evapotranspiration, FVET, was replaced weekly); 2) early deficit (E, 35% of vine FVET was replaced weekly between fruit set and veraison); and 3) veraison deficit (V, 35% of vine FVET was replaced weekly between veraison and harvest). When not under 35% deficit, vines in scenarios #2 and #3 were irrigated according to standard RDI

practice. Yield and number of clusters per vine were recorded. About 200 berries were collected from each vine to determine mean berry weight, which was then used to calculate the number of berries per cluster. Pruning weights were collected on DOY 43 in 2003 and 2004. Leaf area:fruit ratio (LFR; cm^2 of leaf area g^{-1} of fruit) and fruit:pruning weight ratio (FPR; g of fruit g^{-1} of pruning weight) were calculated (LFR was calculated using leaf area data collected the previous growing season; chapter 3, this volume). Yield and yield components also were recorded from sentinel vines across the 4-ha block (R. Smithyman, unpublished data).

5.3.2. Berry composition

The same 200 berries used to determine mean berry weight were ground whole in a blender for 45 s and then filtered through paper (no. 588, Schleicher and Schuell, Keene, NH). Total soluble solids (Brix), pH, titratable acidity (TA), color density and hue of the filtrate were measured. Brix were measured using an Abbé refractometer (model 10450, American Optical Corp., Buffalo, NY). A pH meter with glass electrode (model 455, Corning, Kennebunk, ME) was used to measure pH. Titratable acidity was measured by titrating the filtrate with sodium hydroxide (0.097 N) to an end point of pH 8.2 (20ºC) and was expressed as g of tartaric acid/100mL. The absorbance of an aliquot of 5 mL of the filtrate diluted to a volume of 25 mL with acidified ethanol (pH 1.0) was read by spectrophotometer (DU 600, Beckman, Irvine, CA) at 420 nm (A_{420}), 520 nm (A_{520}), and 700nm (A_{700}). Color density was calculated as $A_{420} + A_{520}$, and color hue as A_{420} / A_{520}. The A_{700} was used as a control for the filtration step. Color density and hue were expressed as absorbance units per mL of juice.

5.3.3. Non-structural carbohydrates in dormant canes

On DOY 43 in both years, cane samples 2 to 5 cm long were collected above the second node from the base of all shoots of the 18 experimental vines. Cane pieces were divided into 1-cm long segments, dried until constant weight at 60ºC (about 48 h), and then ground in a mill at 14000 rpm (ZM100, Retsch, Haan, Germany) in two steps, first with a screen of 1 mm orifices, and then with a screen of 0.08 mm orifices. About 20 mg per sample of the powder obtained were used. Soluble sugars and starch were extracted and analyzed by the same method as leaf tissue (chapter 3, this volume).

5.3.4. Experimental design and statistical analysis

The irrigation experiment was in a completely randomized design with a two way treatment structure (irrigation and year). Data were analyzed using SAS V8(2) (SAS Institute, Cary, NC). The univariate procedure was used to test for normality and the Brown-Forsythe test for homogeneity of variance. The general linear model procedure (GLM) was used for the analysis of variance and the correlation procedure (Proc CORR) was used for correlation analysis. The Tukey-Kramer method at a significance of $p \leq 0.05$ was used for mean comparisons.

5.4. Results

5.4.1. Yield, yield components, pruning weight, and non-structural carbohydrates

Yields of the 18 experimental vines averaged across both years were 4.8 kg vine^{-1} (9.8 Mg ha^{-1}). In 2002 yields were higher than in 2003 (Table 5.1; Figure 5.1-A), in agreement with yields recorded across the larger plot (Figure 5.1-B).

Berry weight and LFR were similar between the two seasons, but all other yield-related variables differed between years (Table 5.1). A significant interaction was found between irrigation regimen and year in FPR. More shoots and clusters per vine were recorded in 2003, but those clusters were lighter due to fewer berries per cluster than in 2002. The number of flowers per cluster was significantly higher in 2002 than in 2003, with no significant difference between years in percentage fruit set (M. Keller, unpublished data). Fewer flowers per cluster in 2003 could have been due to cold damage to buds during the fall/winter (DOY 304, 2002, -11°C).

Vines under standard deficit had lower yields, lower cluster weights, and fewer berries per cluster than vines under additional water deficits (Table 5.1). No differences in number of clusters per shoot nor in berry weight were recorded among irrigation regimens. Although vines from all irrigation regimens were dormant-pruned to equal numbers of buds per vine, V vines had more shoots and clusters per vine, which partially explained the tendency towards higher yields. Across the larger plot that included the 18 experimental vines, no significant differences were found among treatments (Figure 5.1-B; R. Smithyman, unpublished data). Across the 4-ha experiment, there was no consistent effect of additional water deficits on yield or on berry weight in the five years of the study (Figure 5.1-C; M. Keller, unpublished data).

When correlated against its components with data pooled across both years, yield variation was explained mainly by cluster weight (r^2=0.56; $p \leq 0.001$) and number of clusters per shoot (r^2=0.21; $p \leq 0.001$), with cluster weight mainly explained by number of berries per cluster (r^2=0.87; $p \leq 0.001$). In 2002 yield variations were mainly due to cluster

weight (Figure 5.2-B), itself due to number of berries per cluster (Figure 5.2-E). In 2003, yield variations were mainly explained by cluster weight (Figure 5.2-B) and number of clusters per vine (Figure 5.2-C), the latter being explained by number of clusters per shoot (Figure 5.2-D). Cluster weights in 2003 were lower than in 2002 due to fewer berries per cluster which resulted in lower yields in 2003 although there were more clusters per vine. Because of the lower yields, vines under standard water deficit had higher LFR and lower FPR than those under additional water deficit. Pruning weights of S and V vines were 41% higher than those of E vines. No differences among irrigation regimens were found in non-structural carbohydrates concentrations in dormant canes. Concentrations of soluble sugars were significantly higher in 2002 than in 2003, but there were no significant differences in starch concentrations between years.

5.4.2. Berry composition

In both years vines from all irrigation regimens were harvested on the same day (DOY 262). High levels of soluble solids were achieved across all irrigation regimens (Table 5.2). Soluble solids was the only measured quality attribute that differed between years, although not among irrigation regimens within either year. There was a significant interaction between year and treatment in pH and color density. In 2002 E fruit had higher pH than S, but not different from V, the latter also similar to S, while in 2003 S and E had higher pH than V. In 2002 S and V fruit had higher color density than E, with no differences among regimens in 2003. Vines under additional water deficit between fruit set and veraison (E) had the highest pH in both years, while those under additional water deficit between veraison and harvest (V) had the highest TA, although not different

from S. The E vines had lower color density in 2002, but no differences were found in 2003 among irrigation regimens. No differences were recorded in color hue. Sensory evaluation (S. Spayd, unpublished data) identified no differences among wines produced from grapes harvested in 1999 and 2001 from the same irrigation regimens.

5.5. Discussion

Among the desired effects of imposing RDI in grapevines, water savings and improvement of fruit quality, together with limited growth and fruit exposure, are among the most important (Behboudian and Singh, 2001). The latter sometimes is achieved by a reduction in berry weight that consequently increases the skin:pulp ratio and thus the concentration of skin compounds in the must. In this experiment the lack of a difference in berry weight between the standard deficit and the other two regimens, especially in E vines, contrary to previous results (Hardie and Considine, 1976; Matthews and Anderson, 1989; Van Zyl, 1984; Williams and Matthews, 1990), could be explained by the timing of the water deficit. In 2002 full bloom was around DOY 162, while in 2003 bloom was approximately one week earlier (DOY 155). The additional deficit in E plots began on DOY 192 in both years, 30 d after bloom in 2002 and 37 d after bloom in 2003. All vines continued to be irrigated, but in E vines reduced water amounts were applied following the corresponding deficit coefficient and soil water content of the regimen. The periods from fruit set to veraison (DOY 224 in 2002, DOY 217 in 2003) were about 55 days in both years, thus E vines were under additional deficit for about 32 d in 2002 and about 25 d in 2003. Fruit growth was reported to respond to plant water status (Matthews and Anderson, 1988; Ojeda et al., 2002), and a large role of

shoot transpiration in the pre-veraison water budget of the berry has been indicated (Creasy and Lombard, 1993; Greenspan et al., 1994). But despite the lower transpiration rates at single-leaf and whole-canopy level in E vines in the current experiment, and the low leaf water potential recorded (R. Smithyman, unpublished data. See Appendix 4), final berry size was similar to that of S vines. It was previously reported that before veraison, berries that shriveled during the day when leaf water potentials dropped below -1.4 MPa, regained much of their volume during the night (Greenspan et al., 1994), and after veraison, berries have been shown to continue growing despite low and decreasing leaf water potentials (Creasy and Lombard, 1993; Matthews et al., 1987). It may be possible that although E vines were irrigated with smaller water amounts, those amounts were sufficient to avoid further reductions in berry weight that probably already occurred in the other two regimens (S, V). When irrigation is based in soil water content, and/or ET, effects on plant physiology may be more difficult to predict than when it is based on sensing plant stress (Jones, 2004).

Water deficit usually reduces yield, but in this experiment vines under standard deficit (S) tended to have lower yields than vines under additional water deficit (E, V). The six S vines selected may have had intrinsically lower yields or the south end of the vineyard, where whole-canopy measurements were recorded, may have been less representative of the 4-ha vineyard. However, yield recorded across the plot that included the 18 experimental vines (R. Smithyman, unpublished data) and across the 4-ha block also showed no consistent effects of irrigation regimen on yield.

Reductions in CO_2 fixation have been reported to influence yield or quality

indicators only when source limitation develops (Poni et al., 1993a). The balance between sources and sinks can modulate the effect of water deficit on grape quantity and quality, and some of the compensation processes that occur in vines (i.e., berry weight and berries per cluster, or cluster weight and clusters per vine) depend on that balance. The high LFR and low FPR of all the vines of this experiment compared with those reported in the literature as “optimum” for achieving high levels of soluble solids and coloration (Kliewer and Dokoozlian, 2000) suggest sink limitation, which could explain the apparent lack of effect of the irrigation regimens on yield and berry weight (Candolfi-Vasconcelos et al., 1994; Kliewer and Dokoozlian, 2000; Poni et al., 1993a). For well-watered vines, 6 to 15 cm^2 of leaf area have been reported to be necessary to ripen 1 g of fruit (Intrieri et al., 1997; Kaps and Cahoon, 1989; Kliewer and Dokoozlian, 2000; Kliewer and Weaver, 1971) and above 8 to 10 $cm^2\ g^{-1}$ there was no increase in berry weight (Kliewer and Dokoozlian, 2000; May et al., 1969). An FPR below 8 was suggested as “optimal” for wine quality in well-watered vines (Bravdo et al., 1984), but these figures depended also on variety and trellis systems used (Kliewer and Dokoozlian, 2000). The fact that the vines in this experiment were all under some degree of water deficit but were able to ripen the crop may indicate that they were not overcropped, and it is possible that they could have ripened higher crops.

Reductions in CO_2 fixation due to water deficit can reduce carbohydrate reserves in grapevines (Christensen, 1975; Freeman et al., 1980; Kliewer et al., 1983; Neja et al., 1977). Lower pruning weights in E vines, with no reduction in cane starch concentrations, may suggest lower total carbohydrates in the permanent tissues of the

vines. Differences between years in soluble sugars concentrations in canes probably were not related to minimum air temperatures when cane samples were collected, because in 2003 minimum temperature was -0.9 ^{o}C (at 0700 hr), while in 2004 minimum temperature was -3.3 ^{o}C (at 0400 h). Average minimum temperatures from 5 days previous to sample collection were -1.5^{o}C in 2003 and -2.5^{o}C in 2004.

At fruit set E vines already had smaller canopies (chapter 3, this volume) than S or V, which could have been a carry-over effect of lower carbohydrate reserves from the previous year's deficit. When the additional water deficit was imposed between fruit set and veraison, shoots were still growing, increasing the difference between E vines and those under standard deficit. Root growth and permanent vine structures could have been an alternative vegetative sink for vines under standard deficit (S), which were fixing larger amounts of CO_2 than those under additional deficit. Previous research has shown that when assimilates are in excess of fruit demand, they may be partitioned to the roots (Buttrose, 1965), and that vegetative sinks could replace fruit demand for carbohydrates (Chaumont et al., 1994; Edson et al., 1995; Williams, 1996). In this experiment, we do not know whether water deficit affected below-ground carbohydrate reserves because roots were not collected. It would be reasonable to postulate that the observed reduction in pruning weight could also have been accompanied by a concomitant reduction in root biomass, although it is also possible that because of the water deficit, roots could have been favored as a sink for carbohydrates (Hsiao and Acevedo, 1974; Hsiao and Xu, 2000). Decreases in root fresh weight and starch concentrations were observed in potted Syrah vines under water stress (Smith, 2004); under similar conditions, main

roots were shorter and thinner than those of well-watered Syrah vines (Mapfumo et al., 1994).

Higher Brix in 2003 than in 2002 can be partly explained by lower yields and higher air temperatures before harvest. The lack of effect of the irrigation regimens on soluble solids probably was due to relatively low yields, compared to industry averages ($\approx$14 Mg ha^{-1}) and to LFR, an indication of the source:sink balance. Sugar accumulation was shown to decrease only when LFR was below 6 cm^2 g^{-1} (Intrieri et al., 1997), well below the values in this experiment. Because additional water deficits did not reduce berry size, an expected increase in color density due to smaller berries did not occur. Other studies have shown that red wine flavonoid content increased when water deficits were applied post-veraison, but those effects were predominantly due to reductions in berry weight, and secondarily by modifying biosynthesis (Kennedy et al., 2002). When comparing berries of similar sizes, concentrations of phenolics and anthocyanins were higher in the skins of berries from vines that had experienced water deficit, suggesting that a reduction in berry size by water deficit was not the sole cause of more concentrated musts (Roby et al., 2004).

5.6. Conclusion

Additional water deficit within RDI did not affect yield or berry quality indicators. Relatively low yields compared to industry averages for cv. Cabernet Sauvignon, and the timing of the water deficit between fruit set and veraison were the probable causes of the lack of effect on berry size. High LFR in all vines probably masked the lower CO_2 fixation. Although the latter and leaf water potential were reduced in the vines under

additional deficit, the high LFR buffered those processes from affecting fruit growth and ripening. Decisions on irrigation management should take into account LFR to achieve the desired results of RDI and to understand the physiological effects of this management practice. It seems possible to achieve more water savings under RDI and additional deficits without negatively affecting yield and fruit composition.

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Table 5.1: Yield, yield components, pruning weight, and non-structural carbohydrates in canes of vines cv. Cabernet Sauvignon under standard and additional water deficit within RDI.

	Irrigation regimens			Year		p values		
Variables	**S**	**E**	**V**	**2002**	**2003**	**treat**	**year**	**treat* year**
Yield (kg vine^{-1})	3.8 c	4.9 b	5.6 a	5.4	4.2	0.001	0.001	0.245
No. shoots vine^{-1}	69 b	70 b	84 a	62	87	0.001	0.001	0.129
No. of clusters vine^{-1}	93 b	97 ab	109 a	93	109	0.026	0.005	0.076
No. clusters shoot^{-1}	1.4	1.4	1.4	1.4	1.2	0.731	0.003	0.163
Cluster weight (g)	41 b	52 a	51 a	58	38	0.001	0.001	0.135
Berries cluster^{-1}	43 b	55 a	55 a	59	41	0.002	0.001	0.257
Berry weight (g)	0.95	0.94	0.97	0.98	0.93	0.779	0.124	0.962
Leaf area / fruit weight [z] (cm^2 g^{-1})	23.3 a	15.1 b	18.9 b	18.0	20.4	0.001	0.059	0.688
Pruning weight (kg vine^{-1})	1.1 a	0.8 b	1.1 a	1.12	0.93	0.001	0.001	0.534
Fruit weight/pruning weight	3.3 c	6.5 a	4.8 b	4.9	4.8	0.001	0.691	0.021
Starch (mg g^{-1} DW)	100	105	105	107	99	0.825	0.252	0.823
Sol. sugars (mg g^{-1} DW)	13	13	15	18	10	0.140	0.001	0.269

Values with different letters within rows are significantly different at $p \leq 0.05$ by Tukey-Kramer.
[z] Leaf area data are presented in chapter 3, this volume.

Table 5.2: Fruit composition of vines under standard and under additional water deficit within RDI.

	Irrigation regimens			Year		p values		
Variable	**S**	**E**	**V**	**2002**	**2003**	**treat**	**year**	**treat* year**
Brix	26.4	26.0	26.1	25.6	26.8	0.274	0.001	0.369
pH	3.71 b	3.81 a	3.70 b	3.7	3.7	0.002	0.140	0.017
TA [z]	0.59 ab	0.54 b	0.64 a	0.60	0.58	0.001	0.248	0.057
Color density [y]	16.0	14.9	15.1	15.1	15.6	0.134	0.395	0.020
Color hue [y]	0.20	0.20	0.20	0.20	0.20	0.697	0.104	0.337

Values with different letters within rows are significantly different at $p \leq 0.05$ by Tukey-Kramer.
[z] Expressed as g of tartaric acid per 100 mL of juice.
[y] Expressed as absorbance units per mL of juice.

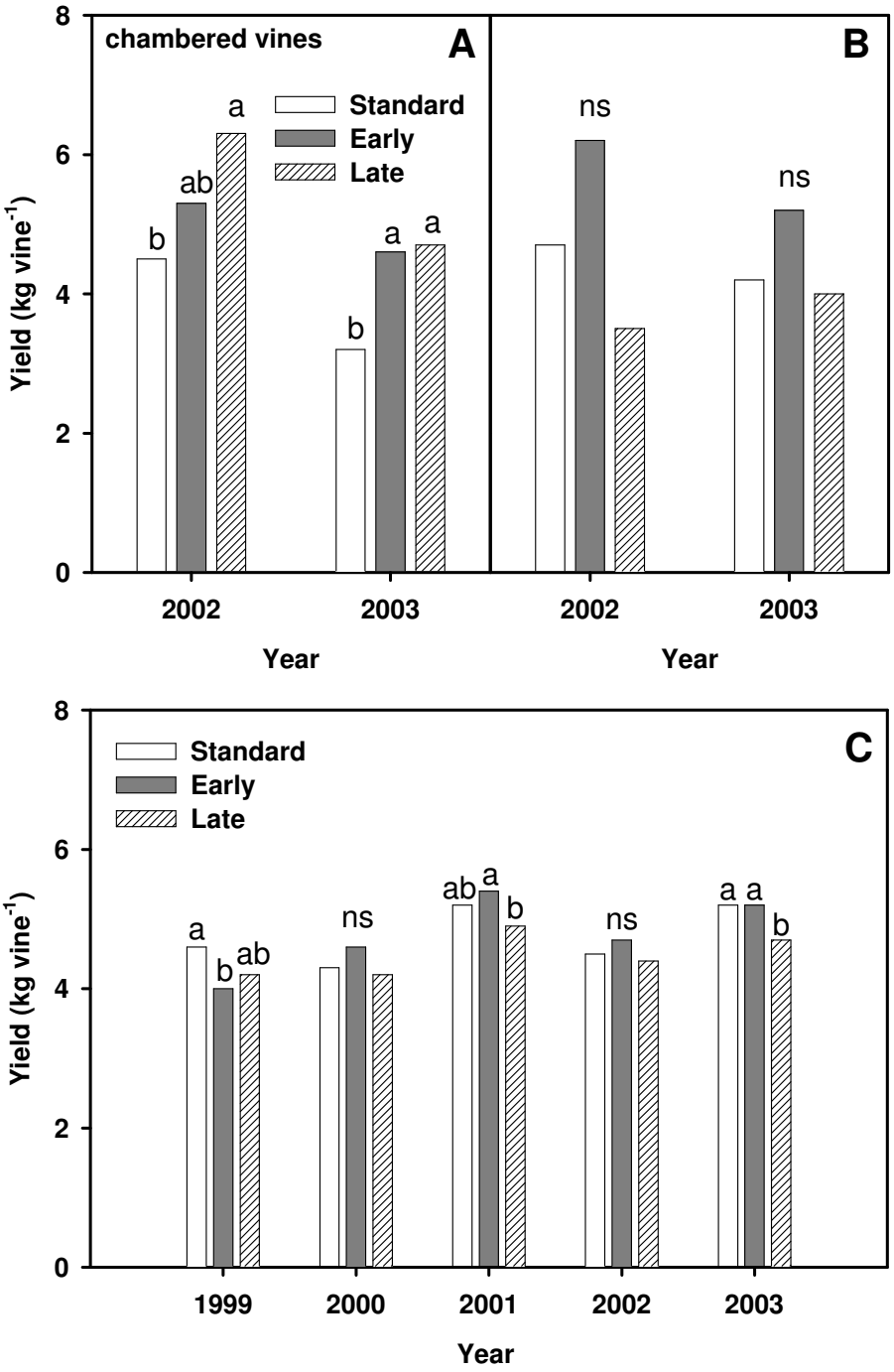

Figure 5.1: A) Effects of irrigation regimens on yield recorded from vines located at the south end of the plot and measured by the gas exchange chambers (n=6); B) yield recorded from all other sentinel vines in the south end of the plot (n=6); C) Effects of irrigation regimens on yield during five seasons on sentinel vines across the 4-ha plot. Bars with different letters within years are significantly different at $p \leq 0.05$ by Tukey-Kramer. ns: no significant difference.

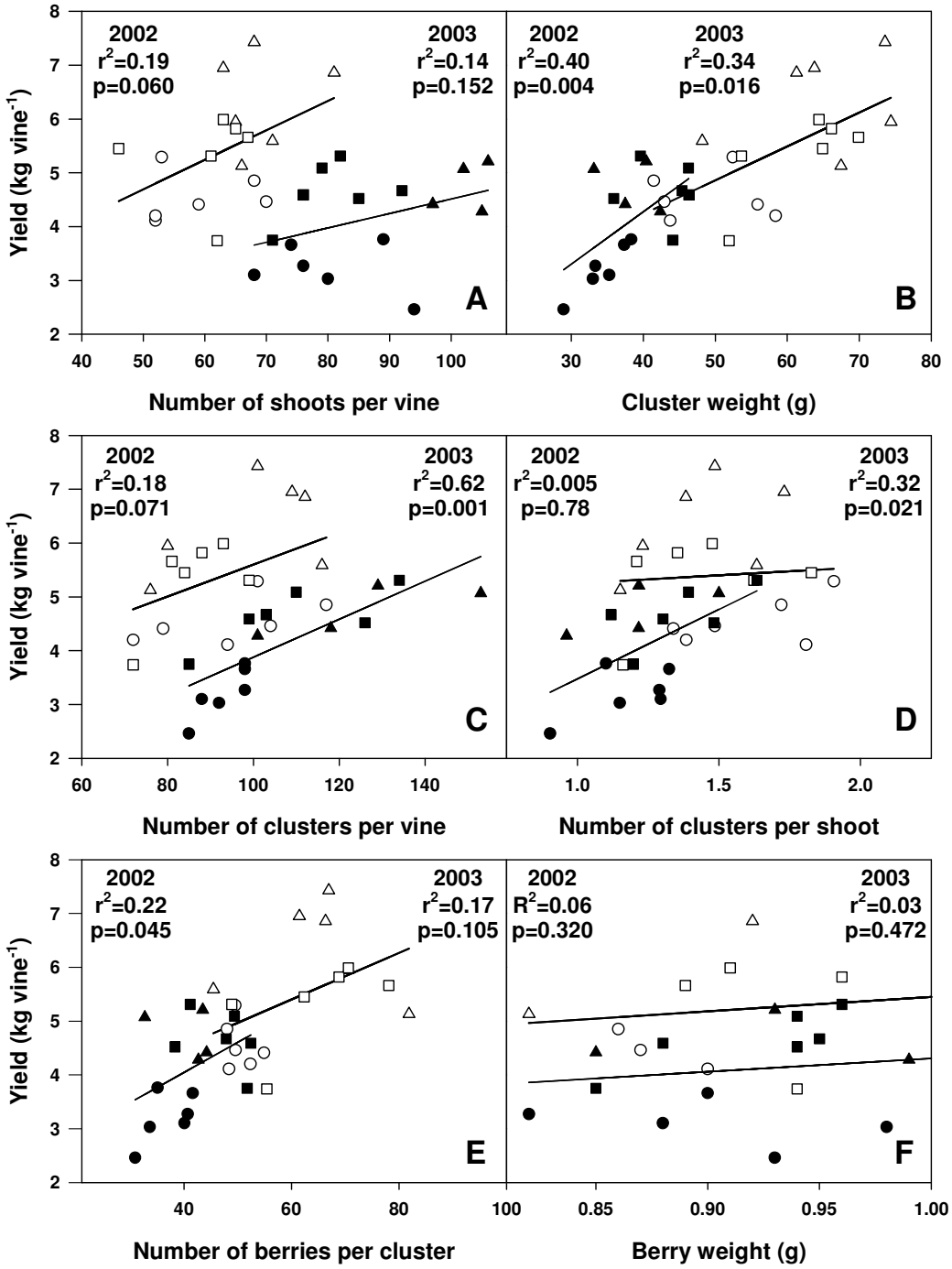

Figure 5.2: Relationships between yield and yield components. The correlations were computed for the 18 experimental vines. (○,●) standard deficit; (□, ■) early deficit; (△, ▲) late deficit. Open symbols 2002, closed symbols 2003.

CHAPTER 6

GENERAL CONCLUSIONS

Previous research has shown that water deficits imposed on wine grapes influence fruit yield and quality. Water deficits occur in vineyards all around the world. In regions where profitable viticulture depends on irrigation, the timing and amount of water applied are among the most important vineyard management decisions. They determine not only grape quality and quantity, but also the condition in which vines will entre dormancy and initiate the following growing season. Particularly in eastern Washington, because of the short growing season, the challenge of growers is to produce grapes with the fruit characteristics demanded by winemakers and end the growing season with vineyards prepared to withstand cold winters. To face this challenge, growers of premium and ultra premium grapes have adopted regulated deficit irrigation (RDI) as their standard irrigation practice. In the search for higher quality and water savings, additional water deficits have been imposed on vines that were already under some degree of water deficit during the berry growth period. Although it is known that water deficits affect yield and quality, it is still debated how to manage those deficits to achieve the desired goals of fruit quality and yield. Only by understanding the physiology affected by water deficits will this be possible.

The physiological process responsible for yield and quality is photosynthesis, thus it is reasonable to study vine photosynthesis under water deficit. Water deficit affects the whole vine, the response being an integration of the responses of all the organs that

constitute the vine. For this investigation, a whole-canopy gas exchange system was designed, built, tested, and used. In an RDI experiment initiated in 1999 in a commercial vineyard, whole-canopy photosynthesis and transpiration were measured during two growing seasons, 2002 and 2003. Three irrigation regimens were imposed within RDI: vines under the standard RDI regimen were irrigated during the berry growth period at about 60 to 70% of FVET. Vines under an early additional water deficit were irrigated between fruit set and veraison at about 30 to 35% of full vine evapotranspiration (FVET), while vines under a late additional water deficit were irrigated between veraison and harvest at about 30 to 35% of FVET. After harvest all vines were irrigated at FVET. At the same phenological stages that whole-canopy measurements were taken, single-leaf measurements of photosynthesis and transpiration were also recorded, together with leaf tissue samples for non-structural carbohydrate analysis.

Additional water deficits induced significant reductions in photosynthesis and transpiration that were associated mainly with reductions in canopy conductance. Lower instantaneous photosynthetic rates during for example the so-called “afternoon depression of photosynthesis” also were related to reduction in canopy conductance. Vine photosynthesis and transpiration responded rapidly to irrigation events, probably enhanced in this experiment by the sandy soil and the small soil volume wetted by the drip irrigation system. Considering the entire period between fruit set and leaf fall, the total amount of CO_2 fixed was considerably reduced by the early additional water deficit, with only small difference between the standard and the late deficit regimens. Differences in carbon fixation were reflected in the lower pruning weights of the vines

under early additional deficit, suggesting that those vines ended the growing season with lower levels of carbohydrate reserves than those from the other two irrigation regimens. Because average yields were low (9.8 Mg ha^{-1}), it is possible that low reserves were not problematic for ripening the fruit or for initial growth the following spring. Originally this experiment set a target crop level of 14.8 Mg ha^{-1}.

Contrary to what was expected, no consistent effects on yield or quality were detected due to additional water deficits imposed during the berry growth period. After fruit set, when the early deficit was imposed, the clusters were a stronger sink then during bloom. Although the early deficit induced large reductions in CO_2 fixation and transpiration, reductions apparently were not severe enough to affect berry size. Alternatively, the early deficit was applied after the main burst of cell division. After fruit set, the number of berries per vine is determined, clusters increase in sink strength, and the sink strength of other organs may decline, leaving them more susceptible to water deficit (e.g., shoots and roots). The high leaf area:fruit ratio of the experimental vines may have buffered the impact of extra deficits on berry size and fruit ripening. Although there were large reductions in CO_2 fixation and transpiration relative to standard RDI vines, absolute amounts of carbon fixed were sufficient to ripen the fruit in all irrigation regimens at these low yields. Non-structural carbohydrates in the leaves were in general similar among irrigation regimens, except for starch concentration in the afternoon in those vines under additional water deficit at times of high evapotranspiration (pre- and post-veraison). Despite those lower starch concentrations, starch was always higher during the afternoon than during the morning, indicating that even vines under additional

water deficits accumulated starch in leaves during the day.

Also contrary to what was expected, vines under additional water deficit were not more efficient in water use than those under standard deficit, unless one computes water use efficiency as the ratio of yield per unit water transpired. Reductions in transpiration were paralleled by reductions in photosynthesis in all irrigation regimens. Intrinsic water use efficiencies (carbon fixed per unit canopy conductance) were similar among irrigation regimens, indicating a tight relationship between conductance and photosynthesis. Thus under deficit irrigation, savings in irrigation water are achieved at the expense of photosynthesis, the consequences of which depend on yield and geography. This could be of less concern in regions with longer growing seasons, but of more concern in eastern Washington, where because of the short growing season there is less time available to ripen the fruit and replenish reserves in the vine.

Photosynthesis measurements from single leaves detected differences among irrigation regimens, but were not well-correlated with whole canopy measurements. Single-leaf measurements are useful to study the response to water deficits of leaves at different locations within the canopy, rather than the integrated canopy response. However, for application to management practices, results from whole-canopy measurements are much more representative and closely related to real vineyard situations. More experiments that study the response to management practices in field grown vines should be undertaken. Whole-canopy chambers and their operation are not much more expensive than those for single leaf measurements, especially considering the usefulness of information provided by them on vineyard responses.

Those growers already applying or planning to adopt RDI with additional deficits should be aware of the reduction in CO_2 fixation that will be imposed in their vines. Moreover, growers should consider the modulating effect that the source:sink balance (e.g., canopy:fruit) can have on the response of the vine, and determine in their vineyards which balance will achieve the desired effect of a certain practice like RDI. The results of this study should be shown to those who govern water allocation issues, which sometimes take into account only crop yield. Under this scenario, vineyards under water deficits may appear to have very high water use efficiencies. If water policy considers only yield, mis-informed policies could remove water allocations from vineyards in absence of enough information about the long term effects of RDI on vines.

Future research based on RDI that includes variation in source:sink balance would provide insight for more informed decisions with respect to water management: 1) Whole-canopy photosynthesis and transpiration of vines under different irrigation regimens from before bloom until fruit set when number of berries per vine is determined, with treatments such as increasing irrigation amounts and or frequencies; 2) Whole-canopy photosynthesis and transpiration of vines under different irrigation regimens from before bloom until fruit set, with the addition of treatments like hedging or removing shoot tips to eliminate competition with clusters; 3) Whole-canopy photosynthesis and transpiration of vines under different irrigation regimens in which a high number of buds have been left at winter pruning, or even under minimal pruning, to indicate the maximum number of viable flowers and berries that a vine can support in an area like eastern Washington; 4) Include in the studies total biomass of the plant (i.e.,

cordon, trunk, and roots); 5) Use of this information to develop irrigation indices or validate the deficit index proposed in this volume, tying irrigation to vine physiology; and 6) Use of decision criteria for RDI that are primarily based on plant water indicators and follow variables like soil water content or ET as complementary information.