THE IMPACT OF DEFICIT IRRIGATION STRATEGIES ON SWEET CHERRY (*PRUNUS AVIUM* L) PHYSIOLOGY AND SPECTRAL REFLECTANCE

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of ALEJANDRO JOSE ANTUNEZ BARRIA find it satisfactory and recommend that it be accepted.

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Abstract

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Prudent irrigation management of sweet cherry trees (*Prunus avium* L.) requires periodic monitoring of plant water status to avoid physiological stress. In recent years, on several fruit crops, various deficit irrigation techniques have been shown to be effective for saving water resources and having little impact on yield and quality. However, to date, little research has been conducted on sweet cherry water management.

The purpose of this research was to investigate the physiological response of sweet cherry to deficit irrigation strategies that varied in placement of water. Additionally, correlations between sweet cherry canopy water status and leaf spectral reflectance were investigated. Lastly, a weighing lysimeter system was designed and utilized to accurately estimate transpiration of young potted trees, in real time.

In the field, sweet cherry trees were subjected to three season-long irrigation strategies: control (C) receiving 100% weekly replacement evapotranspiration (ET), deficit irrigation (DI) receiving 50% replacement of ET to the entire rootzone, and partial root-zone drying (PRD) receiving 50% ET to half of the rootzone per irrigation. In general, there were subtle treatment effects on leaf net photosynthetic rate (P_n), stomatal conductance (g_s), leaf evaporation (E), water use efficiency (WUE), chlorophyll fluorescence and assimilation response parameters to carbon dioxide (CO_2) and photosynthecic active radiation (PAR). However soil water was conserved by PRD and DI vs. C by approximately 20%, we found no clear physiological benefit to PRD.

Leaf spectral reflectance shows promise as a tool to estimate plant water stress. Close correlations were found between reflectance and stem water potential (ψ_{stem}) in the visible range. The best correlation model predicting ψ_{stem} used six wavelengths between 550 and 710 nm.

Throughout the 2004 growing seaon, transpiration from C trees was 846 mm m^{-2}, and deficit-irrigated trees exhibited 31% to 35% less transpiration. Stomatal conductance was reduced by irrigation treatments and correlated with vapor pressure deficit and air temperature.

Overall, sweet cherries show tolerance to mild water stress and there is a potential to adopt deficit irrigation strategies in commercial production systems.

TABLE OF CONTENTS

CHAPTER 1.

CHAPTER 2.

CHAPTER 3.

CHAPTER 4.

LIST OF ABBREVATIONS

$\Delta\psi_{soil-stem}$	soil-stem water potential gradient
$\Delta\psi_{stem-leaf}$	stem-leaf water potential gradient
1^{st}STR	numerical solution of the first derivative of the raw reflectance data
2^{st}STR	numerical solution of the second derivative of the raw reflectance data
ABA	abscisic acid
A-C_i	assimilation response to CO_2 concentration
A_{max}	maximum photosynthetic rate at saturating PAR
A-Q	assimilation response to PAR radiation
C	control irrigation treatment
C : N	carbon to nitrogen ratio
C_i	internal CO_2 concentration
CLA	canopy leaf area
CO_2	carbon dioxide
CV	cross validation
DOY	day of the year, January 1 = 1
DI	deficit irrigation
E	leaf evaporation or transpiration rate
e_s	saturation vapor pressure
ET	evapotranspiration
ET_{crop}	crop evapotranspiration
ET_o	reference crop evapotranspiration
ET : ET_o	Tree evapotranspiration (leaf area base) to crop reference ratio
FC	field capacity
F_v/F_m	maximum yield of PSII
g_s	stomatal conductance for water vapor

g_a	aerodynamic conductance for water vapor
GPS	global positioning system
J_{max}	potential rate of electron transport
K_c	crop coefficient
LC_{point}	light compensation point
LHCII	Light harvesting complex
LMA	leaf mass per area
LSE_{sat}	light saturation estimated point at saturation
MSI	moisture stress index
N	leaf nitrogen concentration
NDVI	normalized difference vegetation index
NDWI	normalized difference water index
NIR	near-infrared spectra
NPQI	normalized phaeophytinization index
PAR	photosynthetic active radiation
PAWS	Public Agriculture Weather System
PLS	partial least squares
P_n	net photosynthetic rate
PRD	partial rootzone drying
PRI	photochemical reflectance index
PSII	photosystem II
PWP	permanent wilting point
QE	quantum efficiency
r	Pearson's correlation coefficient
r^2	regression coefficient
RawSTR	raw reflectance data with no further transformation
Raw1stDer	first derivative of the raw reflectance data
Raw2ndDer	second derivative of the raw reflectance data
R_d	mitochondrial respiration in the light

RDI	relative drought index
R_n	mitochondrial respiration in the dark
$r_{soil\text{-}stem}$	resistance to water flow between the soil and the stem
RWC	relative water content
Rλ	relative reflectance at a specific wavelength
SEP	squared error of prediction
SG1stDer	first derivative of the Savisky-Golas smoothed data
SG2ndDer	second derivative of the of the Savisky-Golas smoothed data
SIPI	structural independent pigment index
SLA	single leaf area
SR	simple ratio of reflectance
T_{max}	maximum daily temperature
θ	volumetric soil water content
V_{cmax}	maximum rate of carboxylation
VI	vegetation indices
VIP	variable importance for projection
VPD	vapor water deficit
W_c	carboxylation activity of Rubisco
WI	water index
W_j	electron transport limiting photosynthesis by regeneration of Ribulose-biphosphate
W_p	regeneration of inorganic phosphate limiting photosynthesis
WUE	instantaneous water use efficiency
X^a	photosynthetic parameter derived from A-Ci data, area based
X^m	photosynthetic parameter derived from A-Ci data, mass based
Y^a	photosynthetic parameter derived from A-Q data, area based
Y^m	photosynthetic parameter derived from A-Q data, mass based
ΦPSII	quantum yield of electron transport at Photosystem II
ψ	water potential

ψ_{leaf}	leaf water potential
ψ_{soil}	soil water potential
ψ_{stem}	stem water potential

LIST OF TABLES

LIST OF FIGURES

Dedication

Dedicated to my parents

and to the memory of my elder brother Raúl Hernán.

His example overcoming difficulties will be a lifetime inspiration.

CHAPTER 1

GENERAL INTRODUCTION

The purpose of this study is to investigate soil plant water relations in sweet cherry trees, studying irrigation management and water stress indicators and understanding the underlying responses related with the plant physiology. The research program include experiments to elucidate the link between environmental conditions and plant physiological status related with irrigation strategies to validate relations that support decisions about water management on sweet cherries.

Sweet cherry (*Prunus avium* L.) is the first deciduous tree fruit crop to mature each summer, the earliest-maturing varieties needing only two months from flowering to develop their fruits to maturity (Hanson and Proesbting, 1996). The lack of applied research focused on water stress and deficit irrigation in sweet cherry might be related to the recent increase in plantings, by the short time elapsed between bloom and harvest and, in the Pacific northwest, water laws which do not promote conservation. Consequently, the economic opportunity for practice deficit irrigation might be limited for sweet cherries compared with other fruit crops.

In commercial orchards, irrigation practices are designed to avoid reduction in crop yield and/or quality while minimizing water application. The state of Washington produces about 40% of the USA sweet cherries and it ranks as the first state for sweet cherry production (Smith and Kupferman, 2003). The importance of the crop in the state's economy encourages research on the development of tools to control cultural practices in the orchard such as canopy management, rootstock selection, orchard density and irrigation practices.

Although sweet cherry is usually considered a low drought tolerance specie (Hurvitz, 1958, cited by Mckersie and Leshem, 1994), the lack of scientific base guiding irrigation practices is common within cherry growers. In the Yakima Valley, WA State, growers often have the perception that irrigation is needed independently of the amount of precipitation recorded during the season. Orchards usually receive water in a regular schedule once every eight to ten days, especially during May and June, and the frequency tends to decline after harvest. Modern systems replacing furrow irrigation are designed for a 24–hr event using micro sprinkler irrigation. A limited number of growers use the program WISE (Washington Irrigation Scheduling Expert) developed by Leib *et al.* (2001) at Washington State University (WSU) IAREC (Irrigated Agriculture Research and Extension Center) to schedule irrigation events in the region, while a significant number of orchadists use weather date from the Public Agricultural Weather System (PAWS) to guide irrigation decisions. WISE uses meteorological data from PAWS stations to estimate crop evapotranspiration (ET_{crop}) using the Penman-Monteith equation and crop coefficients. WISE is designed to accept soil data moisture to refine irrigation scheduling recommendations.

Even though the water available for agriculture is expected to decrease in the Yakima Valley of Washington State, irrigation will continue to be the main consumptive use of the resource (EPA, 2004). Several issues and projects are compelling changes in the Yakima Valley: indian treaty rights; reservoir and irrigation development; increasing demand for wildlife and resident fish; water-quality of the streams and ground-water, and the interaction of ground-water and streamflow. These factors, associated with periodic droughts and limited annual storage from reservoirs, are expected to stimulate growers to improve irrigation efficiency.

An estimation based on the last 15 years indicates that reference crop evapotranspiration (ET_o) between March and September in a normal year for the area is equivalent to approximately 1092 mm, while ET_{crop} for sweet cherry is approximately 1165 mm for the same period. Deficit irrigation techniques such as regulated deficit irrigation (DI) or partial rootzone drying (PRD) implemented at the end of May in a sweet cherry orchard would represent the application of only 790 mm and water savings of 32%, equivalent to 3750 m^3 ha^{-1}. Thus deficit irrigation techniques might become an important tool either to increase the irrigated surface or to save water for other uses in the valley. Although from a water-saving perspective the benefits are clear, more research is needed to reveal the impact of deficit irrigation practices on the vegetative and reproductive growth of sweet cherry trees.

Remote sensing linked with global positioning systems (GPS) permit promising applications in agriculture, including irrigation scheduling as well as potential yield, vigor and nitrogen deficit in orchards with applications that support decisions.

Water management in a fruit orchard requires periodic monitoring of plant water status (Shackel *et al.*, 1977). The measurement of water status requires trained personnel, purchase and maintenance of equipment and is usually labor consuming. Therefore, simplifying field measurement of water status to control irrigation scheduling in orchards becomes relevant.

Specific aspects of this research are related with: the physiological response to deficit irrigation strategies that vary in placement of water studied at orchard level; plant transpiration in potted trees evaluated in real time by weighing lysimeters specifically designed for this purpose; and correlations between water status and spectral reflectance investigated in a sweet cherry orchard.

1.1. Plant water stress and water management

1.1.1. Measuring plant water status

Plant water status is the quantification of the condition of water in a plant relative to its requirement. The concept integrates the effects of available soil water, evaporative demand, and the hydraulic fluxes within the soil-plant-atmosphere continuum (Chalmers *et al.*, 1983; Spomer, 1985). Plants experience water stress by a limitation of water supply (water deficit) and by enhanced transpiration (Frensch, 1997).

It is complicated to define one single measure that can be correlated with all the numerous effects of water deficit. In the literature it is prevalent the use of a two parameters of plant water status: tissue water content, usually normalized to relative water content (RWC); and water potential (ψ), or the components of water potential (Turner, 1986). Other measurements include soil water potential and canopy temperature, though these are considered to indirectly estimate plant water status (Andrews *et al.*, 1992). The disadvantages of these indirect estimations of plant water status are related with the dynamic of the hydraulic resistances within the plant, the difficulty in the estimation of root distribution, the heterogeneity of the hydraulic properties of the soil, and the different response of the canopy temperature to the humidity of the environment, respectively (Chaves, 1991; Andrews *et al.*, 1992).

Initially, RWC was adopted as standard to detect plant water status because of the simplicity of the measurement: the sample is weighted fresh and dry (48 hours at 60º C oven dry). However, the diurnal and seasonal variations in dry weight make comparisons of water content at different times of the day difficult and unreliable. Water potential expresses the absolute availability of water in the plant and it is a measure of the free energy of water per unit volume and it is usually expressed in $J\ m^{-3}$ or in Pascal (Pa) which is an equivalent unit (Hsiao, 1973; Spomer, 1985; Turner, 1986; Taiz and Zeiger, 1998.).

The water potential measured in the stem (ψ_{stem}, also called xylem water potential) and in the leaves (ψ_{leaf}), has been traditionally the most common and fundamental indicator of physiological water stress in plants (Hsiao, 1973; Medrano *et al.*, 2002). Methods to determine plant water potential include the Peltier thermocouple psychrometer and the Scholander pressure chamber. The latter is a relatively quick method for estimating the water potential of whole leaves and shoots that is currently utilized by commercial fruit growers. The pressure chamber measures the tension (negative hydrostatic pressure) that exists in the organs of most of the plants. When a leaf (or a stem) is cut off a plant, the sap is sucked back into the xylem, since it is under tension. The detached leaf is therefore sealed in a steel chamber with only the cut end (petiole) protruding out. Pressure is applied to the chamber from a pressure source such as a compressed nitrogen cylinder. When the sap meniscus appears at the xylem surface the pressure is recorded and taken as the stem (leaf water) potential. Typical ψ_{leaf} of live transpiring leaves range from about −0.3 MPa to −2.5 MPa (PMS Instrument Company, 2005).

The components of the total water potential are the osmotic potential, pressure potential, matric potential and gravitational potential. However in most of the cases the osmotic potential is negligible in absolute values, varying widely with changes in solute concentration. Thus, a given level of osmotic potential has less physiological significance than a given level of pressure potential (Kramer, 1983). Similarly matric potential has no meaning in plant cells and gravitational potential might be considered negligible since the difference in height between plant organs is usually less than a couple of meters. Consequently, and because the xylem is in intimate contact with most cells the ψ_{stem} is assumed to be fairly close to the average water potential of the whole organ (Shackel *et al.*, 1997). In the last decade, researchers have concluded that ψ_{stem} is a more stable and accurate parameter to measure rather than ψ_{leaf} that seems to have a wider variability (PMS Instrument Company, 2005). Indeed, leaf water potential has been criticized as a water stress indicator because it varies from point to point in the plant (Jackson, 1982). Among leaves on a given plant, the variability in ψ_{leaf} depends mainly upon the age and orientation of the leaf (Turner, 1986; Shackel *et al.*, 1997) adopting the ψ_{stem} as a primary parameter for detecting water stress in plants.

Several indicators have been used to measure plant water stress status: changes in water potential, trunk diameter variations, and canopy temperature. Some of the repercussions of water deficit and its physiological consequences have evolved to describe the stress becoming indicators in plants. For example the decrease on stomatal conductance (Chapman, 1970) and the rise of abscisic acid (ABA) concentration affecting the stomatal aperture (Bensen *et al.*, 1988) is usually considered as water stress indicator; while the daily fluctuation of trunk diameter provides information regarding to the water status of the plant.

Stomatal conductance is usually measured directly at leaf level or whole canopy using porometers and infrared gas analyzers. Abscisic acid has been measured in leaves and linked with water stress throughout its recognized influence on the stomatal conductance. Trunk diameter variations can be monitored continuously using electronic calipers and related with the change in water status in the tree. Canopy temperature has been linked to water stress assuming that the lack of transpiration affects the dissipation of heat from the canopy. Among these parameters, leaf and stem water potentials have been traditionally the most common and fundamental indicator of physiological water stress in plants (Hsiao, 1973). The ψ_{stem} is assumed to be fairly close to the average water potential of the whole xylem of the plant (Taiz and Zeiger, 1998). The measurement of ψ_{stem} for field studies using the pressure chamber method developed by Scholander *et al.* (1964), is rapid and does not require delicate instrumentation or elaborate temperature control, and it has been widely used in the literature.

1.1.2. Physiological parameters associated with stress

A plant's response to water deficit is often triggered not by the chemical potential of water per se, but rather by other factors varying in concert with leaf or root water potential such as the increased concentration of chemical compounds coming from the roots (Chaves, 1991). For example, increased concentration of ABA in leaves is considered one of the initial signals though that initiates the cascade of events to cope with water stress (Gao *et al.*, 2004).

It is understood that the physiological response of the plant to water stress in the soil is linked by ABA mobilization from roots to leaves through the xylem

(Davies and Zhang, 1991). The rise of ABA concentration in the mesophyll causes stomata closure. Downton *et al.* (1988) report stomata closure for grapevine and sunflower leaves in concentrations of 6×10^{-5} M and 7×10^{-6} M respectively. In some cropped plants, xylem ABA concentration of 25 and 50 fold have been reported to mild soil drying (Davies and Zhang, 1991). The ultimate response however is also modulated by the leaf water status. Consequently, the first symptom of water deficit/stress is a decrease in leaf stomatal conductance (g_s). This reduction is followed by a decline in the concentration of CO_2 available at chloroplast level (C_i). The decrease in C_i causes a decrease in photochemical yield of open photosystem II (PSII) centers, leading to a reduction in net photosynthesis (P_n).

A derived parameter from the photosynthetic response is the water use efficiency (WUE) defined as the ratio between the carbon assimilated and the water losses in the plant, and it is usually expressed in mol units (mol CO_2 mol^{-1} H_2O) or in mass units (g g^{-1}). The ratio describes the efficiency of photosynthesis in relation to water losses through the stomata and it is particularly useful for describing the adaptation/acclimation of plants to drought or to a dryland environment. Water use efficiencies for mature leaves over a day range usually between 1 to 2 μmol CO_2 per mmol H_2O for C3 plants (Nobel, 1999). It is a dynamic ratio that changes in the same leaf or plant during a day and within species. WUE is maximized by minimal stomatal opening and usually have maximum values early in the morning and decreases during the day as temperature and water demand increase (Jones, 2004).

1.1.3. Deficit irrigation strategies

Maintaining a moderate level of water stress in an orchard can be an effective tool for saving water without negatively affecting fruit quality (Lotter *et al.*, 1985; Mills *et al.*, 1996; Behboudian and Millas, 1997). In general, vegetative growth (shoot growth) is more sensitive to water stress than fruit growth (McCarthy *et al.*, 2002). Consequently, deficit irrigation strategies are usually designed for a particular combination of specie, environment and crop management. The difficulty is in finding a satisfactory balance where, using a controlled stress water management, yield is optimal and fruit quality is maximal. However, very little research has evaluated stress indices for predicting the vegetative or reproductive growth in response to water deficit.

In general, deficit irrigation is a strategy that allows a crop to sustain some degree of water deficit when water is scarce in order to reduce irrigation costs and potentially increase revenues. Deficit irrigation strategies use water stress to control vegetative and reproductive growth. The term ‘deficit irrigation’ (DI) usually refers to an irrigation strategy that imposes water stress during a certain stage of crop development. It was initially applied in peach and pear orchards to control growth by imposing water stress at key stages of fruit development (Shock and Feibert, 2002). The impact of a deficit irrigation technique is strongly dependent on the timing of the water deficit. In apples, Caspari and Neal (2004) reported that using PRD saved approximately 360 mm per season (approximately 50% of the water applied) with no differences in fruit size, yield and quality. There is no published research evaluating DI in sweet cherry. In addition, comparing among published studies is difficult because of variability in climate, soil, plant genotype and general management (Behboudian and Mills, 1997).

Partial rootzone drying is a relatively new irrigation technique that strategically creates a drying a portion of the root system while keeping the remainder well watered. The technique has been applied on grapevines (Dry *et al.*, 2000; Stoll *et al.*, 2000; McCarthy, 2002; De Souza *et al.*, 2003); and lately on fruit crops like apple (Caspari *et al.*, 2004; Leib *et al.*, 2004), pears (Kang and Zhang, 2004), olives (Fernandez *et al.*, 2003) and raspberries (Stoll *et al.*, 2002). The response of any crop to PRD would be influenced by the sensitivity of roots to synthesize ABA under soil drying conditions (Quarrie, 1983). Loveys *et al.* (2004) hypothesized that different species have different strategies to cope with soil water deficit: increasing compatible solute concentrations in the leaves (such as sorbitol) allowing the plant to maintain photosynthesis under relatively drying conditions; or controlling stomatal conductance by ABA signaling from the root system. These different strategies of plants facing soil water deficit might help to predict that certain crops would respond poorly to PRD. For example, Ranney *et al.* (1991) reported the increase of compatible solutes associated with the maintenance of turgor sweet cherry trees under soil water deficit. In apricot

(*Prunus armeniaca* L.), Loveys (1987) reported an increase in compatible solutes (mainly sorbitol) in parallel with a slight increase on ABA concentration in xylem when trees were under soil water deficit that would predict a poor response of the crop to PRD. On the other hand, the same author reported that the success of PRD on grapevines might be explained by the sensitivity of the root system to soil water deficit and the subsequent synthesis of ABA that controls stomata aperture.

1.2. Plant water stress and remote sensing

The early detection of water stress in any crop would be advantageous for water management decisions. Orchadists would often irrigate their trees using fixed schedule with little regard to tree or soil water status. More empirical methods of scheduling irrigation are also used, including estimation of daily evapotranspiration and monitoring soil water content to determine irrigation volume and interval. More recently, progress has been made in the early detection of water stress using sensors for continuous measurement of soil water status (Intrigliolo and Castel, 2004); infrared thermometry techniques to monitor canopy temperature (Chauki *et al.,* 2003; Wanjura *et al.,* 2004); and electronic transducers to measure the trunk diameter variations in fruit trees (Goldhamer and Fereres*,* 2001; Fereres and Goldhamer*,* 2003; Intrigliolo and Castel, 2004).

The majority of the applications of remote sensing in agriculture are based on observing canopy reflectance in distinct areas of the electromagnetic spectrum, particularly in the visible and infrared spectrum regions (Table 1.1).

Reflectance is the proportion of the incident luminous flux upon a surface that is reradiated, frequently expressed in percent (%) or as a fraction (Jensen, 2000).

Plant reflectance is governed by leaf surface properties and internal structure, as well as by concentration and distribution of biochemical components (Peñuelas and Filella, 1998). Intensive research relating leaf chemical composition with reflectance at specific wavelengths in the 0.4–2.4 µm spectral region has been undertaken. Curran (1989) summarizes forty-two absorption features that have been related to particular foliar chemical concentrations including chlorophyll a and b, cellulose, starch, lignin, protein, nitrogen, oil and water. The spectrum around 1.45 µm and above 1.94 µm should be avoided due to atmospheric absorption and rapid decrease in signal to noise ratio of sensors limiting the practical application of this area of the spectra.

Water and photosynthetic pigments (e.g., chlorophyll and carotenoids) are the most significant components of vegetative tissue that influence its reflectance properties. For example, sweet cherry leaves usually contain around 45% of water, fresh mass base. Unfortunately, the majority of the literature available has reported experiments on dried leaves detached from the plant (Yu *et al.*, 2000; Min *et al.*, 2004).

1.2.1. Reflectance patterns and vegetation indices

The reflectance spectra of all types of vegetation in the 400–2400 nm spectral region are remarkably similar and typical spectral reflectance characteristics for healthy green vegetation, dead or senescent vegetation, and dry soil are well defined (Datt, 1999). Healthy green vegetation generally reflects 40 to 50% of the incident near-infrared energy (700 to 1100 nm), with the chlorophyll in the plant absorbing approximately 80 to 90% of the incident energy in the visible (400 to 700 nm) part of the spectrum. Dead or senescent vegetation reflects a greater amount of energy than healthy green vegetation throughout the visible spectrum. Conversely, it reflects less energy than green vegetation in the reflective infrared region. In the visible region of the spectrum, dry soil generally has higher reflectance than green vegetation and lower reflectance than dead vegetation whereas, in the near-infrared, dry soil generally has lower reflectance than green or senescent vegetation. Most vegetation indices are based on the differences in the shape of these three curves (Jensen, 2000).

For the diagnosis of plant physiological status, several indices have been defined at leaf and canopy level, with different purposes such as the estimation of:

- plant cover, green plant biomass, leaf area index , crop yields and end-of-season above ground dry biomass using simple ratio (Gao, 1996; Peñuelas and Filella, 1998; Hunsaker *et al.*, 2003):

  $$\mathrm{SR} = \frac{\mathrm{NIR}}{\mathrm{red}}$$

  and the normalized difference vegetation index:

  $$\mathrm{NDVI} = \frac{(\mathrm{NIR} - \mathrm{red})}{(\mathrm{NIR} + \mathrm{red})}$$

- chlorophyll content, using the position of the local maximum of the first derivative of foliar spectra, at a wavelength of about 0.7 µm or ‘red edge’ (Curran, 1989). Commercial instrumentation to measure the amount of leaf chlorophyll has been developed. Based on this concept other devices calculate Chlorophyll content based on the amount of light transmitted by the leaf in two wave bands regions in which the absorption of chlorophyll is different: red area (0.65 µm) where absorption is high and unaffected by carotene and the infrared area (0.94 to µm), where absorption is extremely low.
- carotenoids to chlorophyll *a* ratio using the structural independent pigment index (Peñuelas and Filella, 1998):

$$\mathrm{SIPI} = \frac{(\mathrm{R800} - \mathrm{R445})}{(\mathrm{R800} - \mathrm{R680})}$$

- chlorophyll degradation using the normalized phaeophytinization index (Peñuelas and Filella, 1998):

$$\mathrm{NPQI} = \frac{(\mathrm{R415} - \mathrm{R435})}{(\mathrm{R415} + \mathrm{R435})}$$

- photosynthesis rate and efficiency through a correlation with chlorophyll fluorescence photochemical reflectance index (Peñuelas and Filella, 1998; Evain *et al.*, 2004; Filella *et al.*, 2004):

$$\mathrm{PRI} = \frac{(\mathrm{R531} - \mathrm{R570})}{(\mathrm{R531} + \mathrm{R570})}$$

- plant water concentration using the water index (Evain *et al.*, 2004; Filella *et al.*, 2004):

$$\mathrm{WI} = \frac{\mathrm{R}900}{\mathrm{R}970}$$

used for drought assessment in natural communities and for defining fire risk (Peñuelas *et al.*, 1997, Peñuelas and Filella, 1998) and the normalized difference water index (Gao, 1996):

$$\mathrm{NDWI} = \frac{\mathrm{R}860}{\mathrm{R}1240}$$

Spectral analysis of fresh leaves is complex and dominated by features related to absorption peaks of water and photosynthetic pigments that interfere with the reflectance located in the infrared and visible area, respectively. In addition, a layer of wax on the upper side of the leaf may actually contribute to the reflectance response of the leaf distorting the results (Vanderbilt, 1985; Ourcival *et al*., 1999).

Most of the research linking plant physiological status and visible and near-infrared reflectance investigate wavelengths related with water stress and risk of fire of natural grasslands, pine forests and other extensive forests areas. Carter (1991), working on aquatic species, tree species and coniferous trees, discovered that the sensitivity of reflectance to water content was greatest in the water absorption bands near 1450, 1940 and 2500 nm. The author also reported that the maximum sensitivity also occurred between 400 and 720 nm, indicating a decrease on the absorption by pigments.

Early research reported wavelengths related with oxygen-hydrogen bond related with water content: 0.97 µm, 1.20 µm, 1.40 µm and 1.94 µm, detected mainly on dry and grinded leaves (Curran, 1989).

A large amount of the research on water stress has focused on laboratory spectra investigations, with little work linking reflectance and plant water status on irrigated crops. Ripple (1986), working on snapbean (*Phaselous vulgaris* L) leaves, found significant correlations between leaf water potential and both middle infrared and red reflectance. Baldini e*t al.* (1997) reported that reflectance from peach and olive leaves measured from the adaxial and abaxial surfaces was well correlated with water content measured from fresh to progressive dehydrated leaves, with remarkable correlation reported in the PAR and NIR region of the spectra.

Carter (1991) described two effects of leaf water content on reflectance: a primary effect that depends solely on radiative properties of water; and a secondary effect that cannot be explained by the absorption properties of water. Absorption by water is relatively weak between 700 and 1300 nm. However, most researchers have found not only an increase on reflectance in plant tissue in the range between 1300 and 2500 nm range but also an increase in the 400–1300 nm range. Diffuse reflectance in the 400–700 nm is typically low, explained by the fact that chlorophyll and other accessory pigments absorb strongly within that range.

Gaussman and Quisenberry (1990) stated that reported research shows that water stress in orange leaves, soybean, corn and cotton causes an increase in leaf reflectance in the range 500 nm to 2600 nm, but particularly in the 1300 nm to 2600 nm wavelength range. Reflectance wavelengths around 1650 or 2200 nm, and wavelengths 540, 850, 1450, 1640 and 1950 nm were increased by water stress while wavelengths such as 1100 nm and 2200 nm showed small changes with water stress.

Carter (1991) reported substantial increases in reflectance sensitivity to water stress at visible, far-red and very-near infrared wavelengths. Carter (1993), working on switchcane (*Arundinaria tecta* [Walt.] Muhl), found that wavelengths between the ranges 506–519, 571–708 and 1119–2508 nm were significantly related to dehydration. The author concluded that increased reflectance in the visible spectra is the most consistent leaf reflectance response to plant stress. In contrast, Moran *et al.* (1989) found that water stressed canopies of alfalfa exhibit lower spectral reflectance in the NIR and red wavebands compared with unstressed canopies. Shakir and Girmay-Gwahid (1998) showed that stressed plots of alfalfa showed lower reflectance than unstressed plots in the range of 850–1150 nm. However, the same authors found that the reflectance of stressed plots was higher above 1150 nm. AccoDIng to Hendry *et al.* (1987), evidence of turnover of chlorophyll in healthy plants is well documented. At the tissue or plant level, diurnal fluctuations in the concentration of chlorophyll, seasonal fluctuations in perennial leaves and fluctuations in response to change in light quality and quantity have been reported. Several perturbations in the environment affect chlorophyll destruction as well as synthesis (Carter, 1993).

1.3. Lysimeters for evapotranspiration measurements

Because of its critical physiological role and singular importance in irrigation scheduling, plant transpiration has been widely studied (Allen, 1998, Remorini and Massai, 2003). Techniques for measuring transpiration have evolved from rudimentary porometers (Levy, 1964) to sophisticated drainage and weighing lysimeters (Phene *et al.* 1991, Martin *et al.*, 2001), heat pulse and balance gauges (Steinberg *et al.*, 1989; Shackel *et al.*, 1992), and infrared gas analyzers coupled to whole-canopy chambers (Perez Peña and Tarara, 2004).

Several reports have concluded that weighing lysimeters are the most reliable method to measure evapotranspiration from a vegetated area (Martin *et al.*, 2001). The basic principle of operation of weighing lysimeters is the measure of variation of soil water content by difference of weight, in a given period of time. Weighing lysimeters are commonly divided in two types: continuous weighing and intermittent (also called weightable) lysimeters (Howell *et al.*, 1991). Continuous weighing lysimeters can be designed to measure and record weights as often as at one minute intervals, while in weightable lysimeters the time interval between measurements is generally one day or longer.

Weighing lysimeters allow more accurate measurement of evapotranspiration compared with those made in the open field by other means (Ayars *et al.*, 2003). With lysimetry, the different terms in the soil water balance equation can be determined with great accuracy (Allen *et al*. 1998). Moreover, with precision weighing lysimeters, evapotranspiration can be determined with accuracy over short time periods. Another advantage of lysimetry is that the plant is growing under little or negligible disturbance. Lysimeters designed to estimate evapotranspiration often use expensive and sophisticated equipment, but lack the ability to isolate plant transpiration from system evapotranspiration (e.g., Phene *et al.*, 1991).

1.4. References

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Table 1.1: Regions within the visible and infrared spectrum (adapted from Campbell and Norman, 1998).

Spectrum	Region	Wavelength range (μm)
Visible	Violet	0.40 - 0.43
	Blue	0.43 - 0.49
	Green	0.49 - 0.56
	Yellow	0.56 - 0.60
	Orange	0.60 - 0.63
	Red	0.63 - 0.70
Infrared	Near-infrared	0.70 - 3.00
	Thermal-infrared	3.00 - 14.0
	Far-infrared	14.0 - 1000

CHAPTER 2

PHYSIOLOGICAL RESPONSE OF SWEET CHERRY TO MODERATE DEFICIT IRRIGATION STRATEGIES THAT VARY IN PLACEMENT OF WATER

2.1. Abstract

Twelve and thirteen-year old ‘Bing’/Mazzard sweet cherry trees were subjected to three season-long irrigation strategies that varied in both the volume of water applied and the placement of the water. Irrigation treatments were comprised of a control (C) receiving 100% replacement of weekly evapotranspiration (ET), deficit irrigation (DI) receiving 50% replacement of weekly ET to the entire rootzone, and partial root-zone drying (PRD) receiving 50% weekly ET to half of the rootzone per irrigation. Soil water potential (ψ_{soil}), stem water potential (ψ_{stem}) leaf water potential (ψ_{leaf}), vapor pressure deficit (VPD), net photosynthetic rate (P_n), stomatal conductance for water vapor (g_s), leaf evaporation (E), instantaneous water use efficiency (WUE) and internal CO_2 concentration (C_i) were measured periodically during the seasons 2003 and 2004. Assimilation responses to CO_2 concentration (A-C_i) and to PAR radiation (A-Q) and maximum yield of PSII (F_v/F_m) and quantum yield of electron transport at Photosystem II (ΦPSII) were analyzed to investigate potential effects of irrigation treatment on the photosynthetic kinetics. Additionally, leaf nitrogen (N)

and carbon (C) concentration was measured and related with leaf area and photosynthesis parameters derived from A-Q and A-C_i data.

In general ψ_{stem} was positively related with ψ_{soil}. Net carbon exchange rate was not affected by irrigation treatment. Parameters estimated from derived A-C_i curves including Rubisco capacity (V_{cmax}), potential rate of electron transport (J_{max}), and mitochondrial respiration in the light (R_d) were unaffected by irrigation treatment on a leaf area or leaf mass basis. Similarly, parameters derived from A-Q curves such as dark respiration (R_n), quantum efficiency (QE), light compensation point (LC_{point}), and light saturation estimated point at saturation (LSE_{sat}) were not affected by treatment. Only maximum photosynthetic rate at saturating PAR (A_{max}) per unit leaf area was statistically reduced by 25% in the PRD treatment. The carbon assimilation for all the treatments were controlled by the carboxylation activity of Rubisco (W_c) in most of the range of C_i, with a limited upper portion controlled by electron transport limiting photosynthesis by regeneration of ribulose-bisphosphate (W_j).

Soil water was conserved by PRD and DI vs. C by approximately 20%. Even though this field experiment demonstrates that it is possible to save water using deficit irrigation techniques, the putative advantages of the PRD irrigation over the DI technique were not evident which was evidenced by few significant differences between treatments. The lack of significant differences between the irrigation regimes might be also attributed to the difficulty of managing PRD strategy on vigorous rootstocks such as Mazzard, where part of the root system cannot be easily isolated and water stressed.

2.2. Introduction

Irrigated agricultural systems provide about 40 percent of the world’s food supply from about only 17 percent of the world’s agricultural land. In developed countries, irrigated agriculture uses more than 70 percent of the water withdrawn from rivers; while in developing countries the proportion exceeds 80 percent. (Kirda, 2002). Worldwide, societies understand that the development of industries and urban cities will require growing amounts of clean water that competes with traditional agriculture. Concepts related with environmental impact and food security are impacting urban societies nowadays. Irrigation strategies that conserve water resources will become part of the solution on assuring the production of safe food and the protection of the environment.

Much research has investigated water relations and the potential for reduced input irrigation strategies for agronomic crops such as corn, rice, potato, etc. (citations of reviews here). Much less has been reported for high value specialty crops such as tree fruit despite their being grown often in close proximity to urban centers (Proebsting et al., 1981). Sweet cherry (Prunus avium L.) is a important tree fruit species grown predominantly in the western states. Acreage has doubled in the past 10 years in the Pacific Northwest as prices for fruit are high. Fundamental water and nutrient management and drought response of sweet cherry research has been conducted (Hanson and Proesbting, 1996; Proebsting *et al.,* 1981). More recent studies have examined the physiological effects of water stress and carbon dioxide concentration on sweet cherry seedlings (Centritto *et al.,* 1999; Centritto, 2005) and the effects of the microclimate on transpiration and photosynthesis of seedlings by Massaci *et al.* (2000). In addition, Goncalves *et al.*(2003) studied the effect of pruning and tree density on the sweet cherry tree water status, concluding that scion-rootstock interaction was important.

Deficit irrigation strategies have been studied and utilized, with varying success, to manage fruit yield and quality and control vegetative growth, in species such as grapes (*Vitis* sp.) (Escalona *et al.,* 1999; Stoll *et al.,* 2000, De Souza *et al.,* 2003), apples (Malus domestica Borkh.) (Lotter *et al.,* 1985; ; Ebel *et al.,* 1995; Mills *et al.,* 1996; Mpelasoka *et al.,* 2000; Ro, 2001; Caspari *et al.,* 2004; Leib *et al.,* 2004; Lombardini *et al.,* 2004.), pears (Pyrus communis L) (Caspari *et al.,* 1993; Kang *et al.,* 2002), plums and peaches (Mitchell and Chalmers, 1982; Natali *et al.,* 1996; Girona *et al.,* 2002; Girona *et al.,* 2005). Most research showed potential for manipulating irrigation to manage vigor without negative impacts on fruit yield and quality.

In recent years, the novel deficit irrigation strategy partial rootzone drying (PRD) has been reported to have horticultural advantages beyond simpler deficit irrigation techniques (e.g., regulated deficit irrigation) (McCarthy *et al.,* 2002). The advantages of PRD are purportedly linked to the synthesis of abscisic acid (ABA) in roots functioning in the drying zone and its subsequent effect on leaf g_s

(Downton *et al.,* 1988) and gas exchange. PRD has been proven effective mainly in grapevines, with clear advantages over other irrigation regimes (Loveys *et al.,* 2004). Leib *et al.*(2004) in a three-season research project on apples concluded that PRD is preferably to DI when a limited water supply is imposed though, the increased costs of the PRD system makes the decision less clear. To date, however, there are no reports of PRD for stone fruit.

Critical to the investigation of any new irrigation strategy is documenting the crop's physiological response vis-à-vis its effect on yield, quality, and growth. Net CO_2 exchange rate (NCER) is related negatively to stem/leaf water potential (citation) via stomatal closure (decreasing g_s), and associated decrease in intercelullar carbon dioxide concentration (C_i). During increasing water deficit, a decline in C_i is followed by inhibition of ribulose biphosphate (RuBP) regeneration. Nonstomatal limitations to NCER also occur via impacts on light reaction components as well as specific effects on Rubisco activity like V_{cmax} and J_{max} (Nilsen and Orcutt, 1996; Hsiao and Jackson, 1999). Specifically, reversible phosphorylation of the light harvesting complex II (LHC II) is an established mechanism for redistribution of absorbed light energy between Photosystem II (PS II) and Photosystem I (PS I). Phosphorylation of LHC II is triggered by conditions where the plastoquinone pool of the photosynthetic electron transport chain becomes reduced (Nilsson et al., 1997). However, some experimental evidence suggests that when plant water deficit is imposed gradually, stomatal aperture and, therefore, gas exchange are unaffected.

The relation reported in the literature between ψ_{leaf} and g_s depends on the species, orchard water relations, and environmental conditions. In olive (*Olea europaea* L), leaf photosynthesis was affected under severe water stress that lowered the ψ_{stem} to −8.0 MPa, while other crops under water deficit can be affected at fairly mild depletion of water status (Moriana *et al.,* 2002).

Initial stages of water stress usually stimulate respiration that might be related with starch mobilization, followed by a decrease of photosynthesis, reducing the carbon gain of the leaves. In parallel, inhibition of nitrogen accumulation might occur related with inorganic nitrogen pools that build up in tissues following a water stress. Concurrently leaf nitrogen is a major determinant of photosynthesis capacity. Therefore, leaf nitrogen concentration, photosynthesis and water stress are considered to be linked (Nilsen and Orcutt, 1996; Grassi *et al.,* 2002). In addition, components of chlorophyll fluorescence, particularly the maximum yield of ratio between variable fluorescence and maximum fluorescence (F_v/F_m) and the quantum yield of electron transport at Photosystem II (ΦPSII) are indicative of physiological stress (Genty *et al.,* 1989). The objective of this research was to investigate the effect of various deficit irrigation strategies on sweet cherry water status and the tree's physiological response.

2.3. Materials and methods

2.3.1. Orchard Management and Irrigation Treatments

Experiments were conducted during the summers of 2003 and 2004 in a 12– and 13–year-old ‘Bing’/Mazzard sweet cherry orchard at the Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, U.S.A., (256 m.a.s.l; latitude 46º 15’ N, longitude 119º 45’ W). Tree spacing was 2.4 x 4.9 m (850 trees/ha). Trees were trained to a multiple leader, open center architecture. The orchard was irrigated weekly by under-tree micro sprinklers with 12 gph nominal output.

Three season-long irrigation treatments were established by varying volume of water applied and the location of its application. Control (C) received irrigation sufficient to maintain soil water content near estimated field capacity. Deficit irrigation (DI) received about half the volume of the C trees by applying shorter irrigation events than those for C. Partial rootzone drying (PRD) was established by irrigating alternate orchard alleyways with a volume similar to that of the DI treated trees. Switching irrigation between alternate alleyways was accomplished by rotating microsprinklers (half-circle pattern) manually every 2 to 3 weeks. Irrigation treatments were imposed approximately 30 days after bloom (approximately DOY 100). During this period, the orchard was also kept well and equally watered to decrease the risk of frost damage.

Soil water content was maintained near the field capacity level by applying 60 to 70% of the Public Weather Agricultural Weather System (PAWS) estimated reference crop evapotranspiration (ET_o) for C treatment. Irrigation volume was determined weekly based on soil water content and estimated crop evapotranspiration. Soil water content was estimated using a neutron probe (CPN model 503, Campbell Pacific Nuclear Corp., Pacheco, CA, USA). Access tubes were positioned between rows, equidistant to trees. Readings were taken before each irrigation event, at approximately 15, 30, 45, 60 and 80 cm depths as inches of water per foot. These data were later transformed to volumetric water contents (cm^3 water cm^{-3} soil) which were subsequently converted to soil water potential (kPa) using a soil moisture retention curve developed for silt loam soil in Washington (Appendix 2, Figure A2.1 from Mittelstadt, unpublished, cited by Leib *et al.,* 2003) and fitted to a model (Campbell and Norman, 1998) as follows:

$$\psi_{soil} = \psi_e \left(\frac{\theta}{\theta_s} \right)^{-b} \tag{2.1}$$

where ψ_{soil} is the soil water potential in kPa, θ is the volumetric water content ($cm^3\ cm^{-3}$), θ_s is the volumetric water content at saturation (0.46 $cm^3\ cm^{-3}$). The constants, ψ_e and b are air entry water potential equal to 2.52 kPa and the fitting parameter equivalent to 4.19 (recomputed from Campbell and Norman, 1998), respectively**.**

Meteorological data and reference crop evapotranspiration (ET_o) were collected by a weather station located within 500 m of the experimental orchard. Maximum vapor pressure deficit (VPD_{max}) was estimated as the difference between the saturation vapor pressure (e_s) at the minimum daily dew point temperature (T_{min_dew}) and estimated e_s for the maximum daily temperature (T_{max}):

$$VPD = e_s\ (T_{max}) - e_s(T_{min\_dew}) \qquad (2.2)$$

Saturation vapor pressure was estimated using Tetens formula (Buck, 1981):

$$e_s(T) = a \exp\left(\frac{bT}{T + c}\right) \qquad (2.3)$$

where T is the Celsius temperature, and the constants a = 0.611 kPa, b = 17.502, and c = 240.97 ºC (Campbell and Norman, 1998).

The orchard soil was a Warden silt loam soil (Coarse-silty, mixed, mesic, Xerollic Camborthids), with particle size distribution of 44% sand, 53% silt, and 3% clay and bulk density ranging from 1.45 and 1.6 kg m^{-3}. Field capacity (FC) was estimated as 0.31 $cm^3\ cm^{-3}$ corresponding to 30 kPa of tension while permanent wilting point (PWP) was estimated to be 0.10 $cm^3\ cm^{-3}$ at 1500 kPa (Appendix 2, Figure A2.1).

2.3.2. Tree water status

Tree water status was characterized from measurements of midday ψ_{stem} and ψ_{leaf}. These were measured in situ using a pressure chamber (Model 610, PMS, Corvallis, OR, USA) throughout the season on three fully sunlit leaves per tree within 1 hr of solar noon. Stem water potential was measured on leaves enclosed for at least 2 hr within a sleeve of aluminum foil within a zip-lock plastic bag (stem water potential bags, PMS Instruments, Corvallis, OR). Leaf water potential was measured on leaves from the same branch within minutes of stem measurements. Measurements were taken on 12 and 19 dates in 2003 and 2004, respectively. In addition, data were collected every three hours on August 13th, 2002 to determine the diurnal cycle of ψ_{leaf}.

Soil-stem water potential gradient ($\Delta\psi_{soil\text{-}stem}$, J kg^{-1}) was calculated according to Begg and Turner (1970) as following:

$$\Delta\psi_{soil-stem} = \psi_{soil} - \psi_{stem} \tag{2.4}$$

Stem-leaf water potential gradient ($\Delta\psi_{stem\text{-}leaf}$) was calculated similarly.

Resistance to water flow between the stem and the leaf ($r_{stem\text{-}leaf}$, m^4 s^{-1} kg^{-1}) can be estimated from an extension of Equation 4 (Begg and Turner, 1970):

$$\Delta\psi_{soil-stem} = \frac{r_{stem-leaf} * E * 10^6}{M_w} \tag{2.5}$$

where E (mmol m^{-2} s^{-1}) is the net transpiration rate derived from measurements of leaf gas exchange and M_w is the molecular mass of water (18 g mol^{-1}). It is possible to subsequently estimate $r_{stem\text{-}leaf}$, by the following manipulation of Eq. 5:

$$r_{soil-stem} = \frac{\Delta\psi_{soil-stem}}{E} \tag{2.6}$$

2.3.3. Leaf gas exchange

Net CO2 exchange rate (P_n), net transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were estimated from intact, recently fully-expanded leaves on 2–year-old fruiting spurs using a portable infrared gas analyzer (CIRAS-2, PP Systems, Hitchin, UK) fitted to a broadleaf cuvette (2.5 cm^2 aperture). Readings in 2003 were made under ambient radiation. In 2004, supplemental, saturating radiation (1000 µmol photons PAR m^{-2} s^{-1}) was provided by a white halogen lamp. For all measurements, reference CO_2 concentration was set at 370 µmol mol^{-1}. Leaf gas exchange data were recorded after steady state conditions, usually ca. 90 s post-enclosure, with cuvette flow rates between 190 and 200 cm^3 min^{-1}. The measurements were done throughout the season on three leaves per tree within 1 hr of solar noon.

2.3.4. Photosynthetic capacity

Assimilation response curves to PAR (A-Q) and [CO_2] (A-C_i) were measured on fully expanded leaves in early September 2004, using the same instrumentation. However, cuvette temperature was maintained at 25ºC and relative humidity at 40–50% during the measurements.

A-C_i data was measured at 1000 µmol (photon) m^{-2} s^{-1} of PAR radiation by step changes in the CO_2 concentration within the assimilation chamber. Carbon dioxide assimilation rates were measured first at a concentration near zero and subsequently at 50, 100, 200, 350, 500, 650, 800 and 1000 µmol CO_2. Gas exchange rates were recorded at each step following a 5 - 10 min equilibration period at each CO_2 concentration. A mechanistic analysis of A-C_i data was performed to estimate photosynthetic Rubisco capacity ($V_{cmax}{}^a$), potential rate of electron transport ($J_{max}{}^a$), and mitochondrial respiration in the light ($R_d{}^a$), per unit leaf area. The analyses followed the models of Farquhar *et al.* (1980) with subsequent modifications (Farquhar *et al.,* 1980; Harley and

Sharkey, 1991; Harley *et al.,* 1992; Sharkey, 1985), to obtain an iterative estimate of these parameters of photosynthesis.

The A-Q response curve was acquired by recording gas exchange at incremental levels of PAR. The CO_2 flux was adjusted to maintain an intra-chamber concentration of 400 µmol mol^{-1}. The light source was programmed to decrease at 5 min intervals as follows: 2000, 1500, 1000, 500, 200, 100, 50, 10 and 0 µmol photon m^{-2} s^{-1}. The response of photosynthesis to light was analyzed using the standard method of Prioul and Chartier (1977). This permits estimation of mitochondrial respiration in the dark, per unit leaf area (R_n^a), quantum efficiency (QE^a), maximum photosynthetic rate at saturating PAR (A_{max}^a), light compensation point (LC_{point}^a), and light saturation estimated point at saturation (LSE_{sat}^a). The analyses of A-C_i and A-Q data were performed using Photosyn Assistant program (Parson and Ogstone, 1999).

2.3.5 Chlorophyll fluorescence

Parameters of extinction of chlorophyll fluorescence, maximum yield of ratio between variable fluorescence and maximum fluorescence (F_v/F_m, maximum efficiency of PSII) and the quantum yield of electron transport at Photosystem II (ΦPSII) were measured on the same leaves used for water status and gas exchange. Chlorophyll fluorescence was measured using a modulated fluorimeter (Hansatech Fluorescence Monitoring System, Hansatech Instruments, Norfolk, UK). A saturating light pulse of 18,000 µmol m^{-2} s^{-1} was applied for one second and F_v and F_m were measured for the calculation of F_v/F_m and ΦPSII.

2.3.6. Nitrogen and carbon content in leaves

Nitrogen and carbon concentration were assessed on dried and ground leaf tissue samples taken on early September 2004, upon completion of A-C_i/Q curves. The tissue was dried (60ºC) to a constant weight, ground to 40 mesh, and analyzed for total carbon (C^m) and nitrogen (N^m) concentration (mg element g^{-1} dry tissue) by dry combustion (Bremner, 1996) using a CNS Analyzer (LECO, St. Joseph, MI). Prior to analysis, the laminar surface area of each leaf was determined using a laser leaf area meter (LICOR LI-3000, LICOR Inc., Lincoln, Nebraska, USA).

To calculate the leaf mass basis of photosynthetic parameters, single leaf area (SLA) data was measured and used (X^m, µmol g^{-1} s^{-1}) using the following equation:

$$X^m = X^a \times \mathrm{LMA} \tag{2.7}$$

where X^a (µmol m^{-2} s^{-1}, area based) represents each of the photosynthetic parameters derived from A-C_i ($V_{cmax}{}^m$, $J_{max}{}^m$ or $R_{day}{}^m$) and from A-Q ($R_{esp}{}^m$, QE^m, $A_{max}{}^m$, $LC_{point}{}^m$ or $LSE_{sat}{}^m$) expressed and LMA (g m^{-2}, leaf mass per area) is the average mass divided by single leaf area divided of each tree.

To transform the carbon and nitrogen content in area base (Y^a), the following relation was used:

$$Y^a = \frac{Y^m \times \text{LMA}}{1000} \qquad (2.8)$$

where X^m is the element concentration (mg g^{-1}, C^a or N^a) derived from the laboratory analysis and LMA (in g m^{-2}, leaf mass per area) as above defined.

2.3.7. Canopy leaf area

Canopy leaf area was determined by counting all the leaves of each tree and extrapolating from measurements of the area of every 50th leaf following the procedure described by Whiting and Lang (2001).

2.3.8. Statistical analysis

The general linear model procedure (PROC GLM) was used for the analysis of variance (ANOVA) using SAS software (V.8.2; SAS Institute, Inc., Cary, NC, USA). For the gas exchange measurements and plant water potential measurements, significant differences were evaluated for the same date, statistically explained considering the interaction effect between irrigation treatment and the day of measurement (DOY).

The Tukey-Kramer method at a significance level of $p = 0.05$ was used for mean comparisons among irrigation regimens, and the PROC REG procedure and PROC STEPWISE were used for simple and multiple regression analysis and stepwise regression respectively. The data of ψ_{stem}, P_n and g_s from runs of 2003 and 2004 and individually per treatment, was regressed against environmental (ψ_{soil}, VPD) and physiological variables (ψ_{stem}, ψ_{leaf}, g_s, E) and time (DOY) variables with simple and multiple stepwise models at single-leaf level.

2.4. Results and Discussion

2.4.1. Weather patterns

Considering 15–year mean (1990–2004), the weather data for the area reveals that the evapotranspiration peak for the reference crop evapotranspiration (ET_o) occurs typically during the middle of July, with a 10–day average peak of 7.0 mm d^{-1} occurring during early July. There is a characteristic lack of precipitation to satisfy the demand by the crop's evapotranspiration in the area with an average precipitation of 228 mm for 1990–2004 in a normal year, and an ET_o representing an average of 1244 mm for the same period. This fact explains the need of irrigation for fruit trees in the lower Columbia River Basin, emphasizing the importance of soil-plant-water relations and techniques leading to save water resources in the area (Appendix 2, Figure A2.1).

Although the precipitation (mostly rain) of both years 2003 and 2004 are close to the 15 yr-average, the distribution of the rainfall and ET_o are significantly different. Considering the growing season (April to September), the precipitation for 2003 was equivalent to 41.6 mm representing 22% of the annual precipitation. During 2004, the rainfall recorded during the growing season was equivalent to 97.47 mm representing 53% of the annual precipitation. Similarly, the average ET_o for the growing season 2003 was 5.7 mm per day with a peak of 9.7 mm per day while the average ET_o was 5.2 mm per day with a peak of 9.0 mm per day occurring for 2004. A similar phenomenon occurred with the daily maximum vapor pressure deficit (VPD). During the growing season (Appendix 2, Figure A2.2), an average of 2.9 was recorded during 2003 with a peak of 6.6 occurring at the end of July (DOY 209), while during 2004 the maximum VPD registered was 2.7 with a peak of 5.5 occurring approximately on the same day (DOY 206).

During 2004, late spring and early summer showers and abundance of overcastted days, affected the distribution pattern of VPD evidenced by a flat dome shape shifted to the second semester of the year. In our experiment, the occurrence of precipitation during the growing season and the lower atmospheric demand during 2004 reveals that the difficulty to reach the same level of water stress in the orchard than during 2003.

2.4.2. Soil water content

The treatments were imposed according to Behboudian and Mills (1997) that suggest avoiding the development of water stress imposed during flowering and fruit set (stage I of fruit development) when applying deficit irrigation as a management tool.

The seasonal trends in θ and soil water potential (ψ_{soil}) are shown in Appendix 2 Figures A2.3 and A2.4, respectively. At the beginning of the growing season 2003, volumetric water content of the three treatments was similar and ca. 0.22 to 0.23 cm^3 cm^{-3}. Treatment differences in θ did not appear until first week of May 2003. Soil water potential on DI was more affected than PRD.

Season 2004 started with a volumetric water content that fluctuated in a range between 0.21 to 0.24 $cm^3\ cm^{-3}$. Treatment differences in θ were evident at the middle of June for 2004. During 2004 ψ_{soil} water deficit treatments were kept within a closer range of water content. C treatment was successfully kept in a range between 0.22 and 0.25 $cm^3\ cm^{-3}$ of volumetric water content while DI and PRD treatments were held in average at 0.19 $cm^3\ cm^{-3}$ during 2004 (Appendix 2, Table A2.1).

The deficit irrigation treatments showed an average of 9% of water saving during 2003 and 20% during 2004 (Table A2.1). Although DI was more affected than PRD during 2003, the treatments were similarly affected during the second year of the experiment. The difference between the years might be attributed to improvements on the irrigation scheduling and switching the wet side for the PRD treatment.

2.4.3. Plant water status

2.4.3.1. Diurnal trend of water status

The diurnal trend of ψ_{stem} and ψ_{leaf} was measured in August 2002, from measurements taken every three hours (Figure 2.1). Volumetric soil water content was 0.23 $cm^3\ cm^{-3}$ for the C treatment and 0.19 $cm^3\ cm^{-3}$ and 0.18 $cm^3\ cm^{-3}$ for the DI and PRD treatment respectively. The diurnal trend was characterized by a decreasing predawn ψ_{leaf} in parallel with an effect of the irrigation regimen evidenced by the separation of the curves for DI and PRD from the C treatment. The differences on ψ_{stem} and ψ_{leaf} between the treatments remained throughout the day. Minimum values were observed shortly after solar noon for each treatment. Leaf and stem water status recovered late in the afternoon and evening (higher ψ_{stem} and ψ_{leaf}). The overall diurnal trend was similar among treatments but DI consistently had the lowest values during the day. However, there were only slight differences among treatments at pre dawn. This behavior was comparable with experiences reported by Tardieu *et al.* (1996) on field-grown sunflower plants growing in soils with contrasting water availabilities.

The diurnal cycles in Figure 2.1 resemble the ‘anisohydric’ behavior described by Tardieu and Simmonneau (1998) that has been discussed to be strongly linked to stomatal control (Tardieu and Davies, 1993). Isohydric behaviour is thought to be linked through an interaction between hydraulic and chemical information, such as hormonal signals mainly attributed to ABA.

2.4.3.2. Midday water status

Midday water status was affected by irrigation treatment in both years though mostly after harvest. The general seasonal trend of both ψ_{stem} (Figure 2.2) and ψ_{leaf} (Figure 2.3) was characterized by a steady decline and it was more marked during 2004. This general seasonal decline in water potential is thought to be related to the suberization of young fine roots that account for most of water uptake, or the late season cavitation of wide xylem elements (Yoon and Ritcher, 1991).

In 2003, ψ_{stem} of DI declined by 0.4 MPa and PRD declined by 0.15 MPa both in average over the season. The drop on ψ_{stem} was expected to occur in response to the irrigation treatments imposed. During 2004, water stressed treatments were 0.13 MPa and 0.08 MPa lower in average than C for DI and PRD respectively over the season with significant differences among treatments beginning in July (DOY 180). From these subtle differences in water potential among treatments, it appears that the deficit irrigation strategies imposed only a mild water deficit (McCutchan and Shackel, 1992). The reduction on ψ_{stem} above mentioned were clear between DOY 178 and DOY 198 (end of June - middle of July), change that occurred in parallel with the decrease on soil water content. Differences between PRD and DI were slight but PRD exhibited consistently lower water stress, suggesting that PRD slight advantage over DI.

Similarly, significant differences in ψ_{leaf} were detected among the treatments with no apparent seasonal trend. (Figure 2.3). According to Chaves (1991) many plant responses to mild water stress are regulatory in nature rather than stress-induced damage. For example even before water stress started, plants would regulated their stomata aperture in response to drops in water vapor pressure, avoiding further damage on tissues or photosynthetic structures. It is likely that the mild water deficit induced in the current trial induced only self regulation of the plant's ψ_{leaf}. This has been accomplished in other species via the synthesis of polyols to promote osmoregulation or through the regulation of g_s, mediated by ABA signaling.

In both 2003 and 2004, ψ_{stem} correlated well to ψ_{soil} and ψ_{leaf} ($r^2 = 0.70$ and $r^2 = 0.80$ respectively, see Appendix 2 Table A2.3). The lack of stronger relationship may be attributed to mechanisms of modulation of water stress related with the effect of conductances within different tissues of the plant: soil-root, root-stem and stem-leaf. However, our results show that ψ_{stem} reflects consistently the status of the soil water supply, resulting on a physiological based parameter that integrates evaporative demand and soil water supply. Although Xiloyannis *et al.* (1988) proposed ψ_{leaf} as a plant water status indicator for annual species, Shackel *et al.* (1997) concludes that ψ_{leaf} is not a reliable index of plant water status in deciduous fruit trees whereas ψ_{stem} is well correlated with short and medium term plant stress responses such as g_s and shoot growth. More recently, ψ_{stem} has been reported as a sensitive indicator of grapevine water status (Chone *et al.,* 2001). The results presented herein (Appendix 2, Table A2.4) further support the conclusion that ψ_{stem} is a better indicator of tree water status than ψ_{soil} and ψ_{leaf}. Moreover, these results agree a reported close relation between ψ_{stem} and g_s (Shackel *et al.,* 1997).

There are other factors that influence tree water status, though little research has been conducted on sweet cherry. Rootstock may play an important role via effects on vascular connectivity with the scion (Goncalves *et al.,* 2003). Rootstock influences tree growth, the ratio between leaf and root surface areas and specific leaf area, and net assimilation of grafted plants (Ranney *et al.,* 1991). Furthermore, vigorous rootstocks likely access a greater volume of soil and soil water. Recent research in sweet cherry has shown that ψ_{stem} is influenced by rootstock with significant differences causing a better water status reflected on lower ψ_{stem}, with differences of approximately 0.3 MPa (Schmitt *et al.,* 1989; Goncalves *et al.,* 2003). Mazzard rootstock, used in the current trial, is particularly vigorous and therefore likely to have extensive root area with which to access soil water.

Tress under DI treatment showed a negative relationship between plant water status and atmospheric demand (Figure 2.8), although no strong overall relationship was found between ψ_{stem} and vapor pressure deficit. Our data fell below the upper limit proposed by McCutchan and Shackel (1992) for well-watered prune trees, suggesting common physiological adjustment to water stress between both crops. For our well-irrigated trees, a linear correlation was established between ψ_{stem} and vapor pressure deficit (r^2= 0.79, Figure 2.8).

2.4.4. Leaf gas exchange

During 2003 and 2004, significant differences were detected on P_n, g_s and E within the same date. Based on the results of leaf gas exchange, it appears that trees were less affected by water stress during 2004 than 2003. This is probably related mainly to adaptation to water stress in 2003 to better deal with it in 2004 and secondary to the lower atmospheric (ET) demand that occurred during the second year of the trial (Figures 2.4, 2.5 and 2.6).

During middle of May 2003, g_s showed significative differences between C and water stressed treatments (higher by 60%), while during the rest of the season, g_s on C and PRD treatment was significantly higher during middle of July and during the first week of August by 50% compared with RDI. Stomatal conductance at the beginning of the season 2004 (middle of April) was again higher by 80% on C treatment compared to both DI and PRD. Throughout the season g_s on DI and PRD treatment were significantly different being g_s on DI higher by 30% than PRD (middle of May 2004).

Throughout 2004, g_s was generally higher by 30% than during 2003. In 2003, DI trees exhibited g_s levels 40% lower than those of C trees. The difference on g_s between years is most probably related with the different degree of water stress achieved during 2003 and 2004. During 2003, the highest values of g_s were before harvest for the C treatment, decreasing after harvest and showing a tendency to recover and increase at the end of the season (Figure 2.5a). Although C treatment showed the higher g_s during 2004 (Figure 2.5b), the values did not show a clear trend as the previous year. The fall of g_s after harvest observed during 2003 was not detected during 2004 where all the irrigation treatments were able to maintain g_s in values relatively similar to pre-harvest.

In terms of seasonal means, for C treatment g_s decreased after harvest during 2003 and 2004. The difference between the deficit irrigation treatments and the C were more noticeable during preharvest of 2003 where the means of PRD and DI were 28% and 22% lower than the mean of the C, respectively. In postharvest, DI showed a strongly affected with g_s values about 44% lower than the C, while PRD showed a moderate depletion of g_s with values about 12% lower than the C.

During 2004 in PRD the preharvest mean is not as markedly lower compared to the C as the previous year, but the differences is still noticeable (22% lower) for the DI treatment. In postharvest 2004, the differences are not marked between treatments.

The differences of g_s within the season are explained basically for the demand of assimilates from the fruit that is competing for carbohydrates and stimulating the photosynthetic machinery of the plant. However, the aperture of the stomata cannot be accomplished effectively with poor plant water status as we intended to achieve through the irrigation treatments.

Initial lower values of g_s detected during 2004 may be due to immature stomata of young cherry leaves (Roper and Kennedy, 1986). The seasonal trend of g_s is likely related to fruit growth and changes in osmotic potential linked to synthesis and accumulation of compatible solutes in the leaves (Ranney *et al.,* 1991). This hypothesis is further supported by recent research showing a clear role of sorbitol in improving water status of cherries (Centritto, 2005). During 2003, when the trees were under a high atmospheric water demand, g_s decreases after harvest (DOY 170). This is consistent with the results reported by Yoon and Richter (1990). The same authors imply that high competition for assimilates between leaf growth, fruit growth and flower-bud differentiation, all processes occurring at the same period in sweet cherries, may induce higher *gs*.

Similarly Blanke (1997) concluded that fruiting significantly increased net photosynthesis in parallel with the increase of g_s on apple trees due to sink strength. In contrast, during 2003 the effect of immature leaf on g_s was not evident since the period of measurement started on DOY 162, when leaves were more developed.

During 2003, g_s was related with ψ_{stem} on the deficit irrigation treatments with r^2 of 0.65 and 0.58 for DI and PRD respectively (Appendix 2, Table A2.6). The linear relationship is attributable to feed-back to ψ_{stem}, especially after certain threshold was reached with values of about −1.5 MPa, resulting en very low g_s (about 70 mmol m^{-2} s^{-1}). Since in all treatments during 2004, the mentioned threshold was not reached the relationship between g_s and water status was not clear, evidenced by low regression coefficients (Appendix 2, Table A2.3). Thus, plant water status did not appear to control g_s until extremely low ψ_{stem} were attained. This tendency was described for apple (*Malus domestica* Borkh) trees with a reported threshold of about −2.0 to −2.5 MPa of ψ_{leaf} to cause a direct effect on g_s (Warrit *et al.,* 1980). Similarly, a threshold of ψ_{leaf} below −2.8 to −2.6 MPa has been reported in peach leaves to correlate with stomatal aperture (Andersen and Brodbeck, 1988). This is partially coincident with Centritto *et al.* (1999) that found g_s of cherry seedlings highly correlated with water status. Similarly, Naor (1998) found that ψ_{stem} was correlated better with g_s than ψ_{leaf} and Garnier and Berger (1987) that reported a strong correlation between g_s and ψ_{leaf} and VPD.

A second implication of the relatively weak effect of the irrigation treatment on g_s is that the experiment did not clearly show the attributed g_s control of the PRD regimen. As mentioned earlier, the proposed mechanism of action of PRD is the regulation of the stomatal aperture by ABA synthesis and transport from the root system to the leaves. The effect on described on this paper for sweet cherries might not be as evident as reported on *Vitis vinifera* L. that is well known for being fairly sensitive to ABA synthesis under water stress (Loveys *at al*., 2004). For *Prunus* species, response to water stress appears to be more related with the synthesis of compatible solutes (Ranney *et al.,* 1991). The results of the trial reinforce this hypothesis because sweet cherries leaves have an anisohydric behavior, similar to other related species (e.g., almonds and peaches). According to Tardieu and Simmonneau (1998) isohydric species have a markedly increase of [ABA] in response to water stress that is linked to the effects of PRD and controls g_s in the leaves. *Vitis vinifera* L. and *Zea Mays* L. are two isohydric species where most of the benefits of PRD have been extensively and fully reported (Medrano *et al.,* 2002).

In 2003, P_n and g_s conductance of DI were significantly and linearly correlated (r^2=0.66, Figure 2.9). No relationship existed for PRD or C treatments in either year. This suggests that photosynthesis was limited by g_s only when the water deficit exceeded a certain threshold value as suggested by Hsiao (1973). Similarly to the effect of ψ_{stem} on g_s, it results evident that water deficit impacted P_n only below certain threshold of about −1.5 MPa of ψ_{stem}.

Several reports have shown that in the field, water stress often occurs simultaneously with high light or heat, which predisposes the plants to photoinhibition, and leads to a decrease in mesophyll photosynthesis (Chaves, 1991). In our trial during 2004, P_n of DI and PRD were correlated with ψ_{stem}, and with ψ_{stem} and ψ_{leaf} respectively (r^2 = 0.52 for DI and r^2 = 0.90 for PRD, Appendix Table A2.8). The decrease of both ψ_{stem} and ψ_{leaf} affected P_n to a degree similar to that described by Bjorkman *et al.* (1980) and Gollan *et al.* (1985) in Oleander (*Nerium oleander* L), a mediterranean perennial bush, demonstrating a negative effect of the water stress on the photosynthesis level.

The effects of water stress on photosynthesis and g_s have been reported for many fruit trees (Hsiao, 1973; Goldhamer *et al.,* 1999). In general, reductions in P_n and g_s occur only when water deficits approach moderate to severe levels. Andersen and Brodbeck (1988) evaluating 9–yr old peach (P. persica L.) found that g_s was not affected by reductions in ψ_{leaf} to −2.4 MPa. Moreover, the authors reported that a moderate level of water stress can reduce vegetative growth without affecting carbon assimilation. Lakso (1979) also reported that a significant reduction of apple leaf P_n did not occur until ψ_{leaf} fell below −3.0 MPa. In contrast, Besset *et al.* (2001) reported a significant decrease in leaf photosynthesis for peach trees grown in pots when radiation was above 1000 umol m^{-2} s^{-1} with no changes on vegetative growth. Similarly, Lampinen *et al.* (2004) found that P_n and g_s decreased when French prunes (*P. domestica* L.)

were water stressed to a ψ_{stem} equal to −1.5 and −2.0 MPa by harvest. (Flexas *et al.,* 2004).

Net photosynthesis rates did not show a clear trend throughout the experiment with the exception of the middle of May 2003 were P_n showed significative differences between C and DI (higher by 30%) without significant differences with PRD treatment. In synthesis, in this research P_n was only affected during preharvest 2003 for DI treatment, when g_s varied more than 30% among the treatment and the C. In postharvest when the sink competition for assimilates decreases there was no evidence of depletion on P_n. The depletion on g_s recorded during 2003 was achieved with the depletion of ψ_{stem}, throughout the control of the water available in the soil facilitated by the weather conditions.

On both years 2004 and 2005, the overall trend was characterized by the increase of the E over the course of the season, in parallel with a moderate increase on g_s, phenomena that is related with the aperture and regulation of stomata on mature leaves, which has been described on sweet cherries (Roper and Kennedy, 1986). During middle of May 2003, E showed significant differences between C and water stressed treatments (higher by 65%), while at the end of the season (first week of August 2004), E on C and PRD treatment was significantly higher than DI by 90%. Significant differences on E were found between the treatments during certain days of 2003 and 2004. In the majority of the days with significant differences, the E of the leaf was affected on the PRD treatment, while DI showed an intermediate response compared to the C. During

2003 and 2004 E was clearly depleted on the DI and PRD to values in a range around 1.33 mmol m^{-2} s^{-1} to 2.95 mmol m^{-2} s^{-1} during early July 2003 (DOY 190), and May 2004 (DOY 145, Figure 2.6). The observed trend was similar to the trend observed on g_s that reached a lower value (around 50 mmol m^{-2} s^{-1}), which implied that stomatal closure induced by the water stressed treatment was the main factor controlling water losses from the leaf.

The better control of the E demonstrated on the PRD respect to DI treatment implied again a better performance of PRD when trying to cope with water stress, resulting on a water-saving acclimation.

2.4.4.1. Water use efficiency

Water use efficiency is a useful parameter that relates both fluxes of carbon dioxide fixed and water transpired by the leaf. In gas exchange literature is usually expressed in a mole basis, as the ratio between mol CO_2 fixed and mol of H_20 transpired (Nobel, 1999). In the experiment, during 2003 the maximum WUE of 4.09 (all the values expressed in a mole basis) was observed in DI before harvest (DOY 170) and the minimum was equivalent to 0.22 on PRD treatment at the end of July (DOY 212). During 2004, the maximum WUE was 3.60 for DI before harvest (DOY 159) with a minimum of 1.25 at the end of August (DOY 243). A general trend to decrease through the season was observed on WUE, becoming lower after harvest (Figure 2.7). This is likely to be related with the smooth depletion of P_n and the concurrent slight raise of E during the season. Simultaneously, plants under deficit irrigation might have reduced their leaf area or adjusted g_s to reduce transpiration demand, while maintaining certain ψ_{leaf} as an acclimation to the mild water stress.

2.4.5 Water potential gradients

Water potential gradients have important physiological implications for transpiring leaves since the value is linked with g_s in leaves. Therefore, changes on water potential gradient may enable stomata to respond to the change in evaporative demand, before a large change of water potential occurs in the plant (Shackel and Brinckmann, 1985). It has been proved that water potential gradients have implications on the development of xylem cavitation that leads to xylem embolism initiating stomatal closure (Cochard *et al.,* 2002). Therefore, the correlation between g_s and stem-leaf water potential gradient is considered to be an indicator of xylem cavitation induced by water stress.

During 2003 the general trend of the stem-leaf water potential gradients ($\Delta\psi_{stem\text{-}leaf}$) was characterized by a depletion of the values until harvest with a partial recover at the end of the season. This behavior was not observed during 2004, attributed to the milder water stress achieved during the second year of experiment (Figure 2.10 and 2.11).

Resistance to water flow between the soil and the stem ($r_{stem\text{-}leaf}$) was estimated from $\Delta\psi_{stem\text{-}leaf}$ and E. PRD trees exhibited approximately 10% lower resistance during 2004 and 2005, while DI decreased the resistance only during 2003 (Figure 2.12). Interestingly, DI and C were similar and exhibited higher $r_{stem\text{-}leaf}$, compared to PRD.

Combined, the analyses of water potential gradients and stem to leaf resistance suggest that PRD was preferable to DI by maintaining tree water status in the face of mild water stress.

2.4.6. Leaf area and carbon and nitrogen analysis

Carbon, nitrogen and carbon/nitrogen ratio were measured from leaves of each irrigation treatment by dry combustion using a CNS Analyzer (LECO, St. Joseph, MI, USA). These data were subsequently transformed to leaf area units using Equation 2.5. Although N^a and C^a, N^m and C^m and C:N ratio, were observed to be 10% to 20% lower on the DI and PRD treatments with respect to the C, the differences were not statistically significant among irrigation treatment at 95% of confidence ($p= 0.05$) (Appendix 2, Table A2.2). Leaf N concentrations of 1.4 to 1.8% were within the ranges of values reported for healthy *P. avium* L by Grassi *et al.* (2003), but lower than the range reported for sweet cherry seedlings (Grassi *et al.,* 2002), and under the recommended values by Hanson and Proebsting (1996).

Leaf N concentration is strongly linked to chlorophyll content and related positively to leaf P_n. In this experiment, each treatments showed a similar balance between C and N (C:N ratio). Therefore, it appears that there was no depletion of the photosynthetic machinery resulting from the deficit irrigation practices.

Control trees had 6% to 10% higher single leaf area, leaf mass per area and leaf area at canopy level than the deficit irrigation treatments. However, the parameters were not statistically affected by the irrigation treatment at 95% of confidence. The higher leaf area of C treatment may be linked to higher sun harvesting potential that can potentially result on higher carbon assimilation, which was not measured on this experiment.

2.4.7 Chlorophyll fluorescence

Chlorophyll fluorescence has been related with a fine measurement of the magnitude of depletion of photosynthesis of the leaves coping with environmental stress. In the trial, the maximum efficiency of PSII (F_v/F_m) throughout 2003 season showed significant differences among irrigation treatments on five DOY: 168, 178, 206, 213 and 227. In general, F_v/F_m decreased through the season (Appendix 2, Figure 2.5). This suggests that an increasing proportion of PSII reaction centers become closed, likely via photoinhibition (Genty *et al.,* 1989). C and PRD were similar and were the close to the theoretical maximum of 0.8. However, on four days (DOY 168, 175, 178, 184) F_v/F_m of DI was significantly 10% to 20% lower than C and PRD. This reported depletion on photosynthesis activity is linked with low soil water content measured on the DI plot during the period.

In general, quantum yield of photosystem II (ΦPSII) decreased throughout the pre-harvest interval (approximately DOY 185) representing a decrease on the overall efficiency of PSII reaction centers in the light probably linked with natural senescence of the leaf with the detriment on the reaction centers. Following harvest, ΦPSII increased for each treatment (Appendix 2, Figure 2.6). ΦPSII fluctuated throughout season 2003 with four days showing significant differences within the treatments. C treatment showed the highest values particularly after harvest compared with the deficit treatments.

2.4.8. Photosynthetic capacity

Mitochondrial respiration in the light (R_d^a), the potential rate of electron transport (J_{max}^a), and the photosynthetic Rubisco capacity at leaf level (V_{cmax}^a), were not affected by irrigation treatment (Table 2.1). The null response of the parameters derived from the A/C_i curves to water stress are similar to those reported by Gunasekera and Berkowitz (1992) and suggest that in the experiment the relatively gradual imposition of the water deficit did not affect CO_2 uptake. V_{cmax}^a, J_{max}^a and R_{day}^a evaluated from the A-C_i data are in the lower range of values reported by Centritto (2005) for sweet cherry seedlings. This fact might be attributed to the physiology of the perennial commercial oriented orchard where our experiment took place that might differ from the physiology of young seedlings under controlled conditions studied by the author.

A strong linear correlation ($r^2 = 0.98$, Appendix 2, Figure A2.7) was found between maximum rate of carboxylation (V_{cmax}^a) and electron transport (J_{max}^a) from A-C_i data. This supports previous results reported in species such as cotton (*Gossypium* sp.) and Douglas-fir (*Pseudotsuga menziesii* Franco) (Harley *et al.,* 1992; Medlyn et al., 2002; Manter et al., 2003).

Mitochondrial respiration in the dark (R_n), quantum efficiency (QE), light compensation point (LC_{Point}) and light saturation estimated point at saturation

(LSE_{sat}) were not affected by the irrigation treatment, either at leaf mass nor leaf area basis (Table 2.2). Maximum photosynthetic capacity at saturating PAR (A_{max}^{a}) was significantly lowered in the PRD treatment (reduced by ~ 25%) but statistically similar to DI when expressed on a leaf area basis, but affected on a mass area basis. In general, the values of A_{max}^{a} measured in this experiment were lower than those reported by Centritto *et al.* (1999) under well-watered conditions, but similar to those under progressive water stress condition. Differences may be related to plant material (i.e., seedlings vs. mature trees).

Only subtle differences among treatments in components of light response (P_n, gs, E, C_a and WUE) to photosynthetically active radiation (400 – 700 nm, PAR) (Figures 2.13a, 2.14a, 2.15a, 2.16a and 2.17a; *A*-*Q* data) and to internal CO_2 concentration (Figures 2.13b, 2.14b, 2.15b, 2.16b and 2.17b; A-C_i data) were discovered.

Leaf P_n under increasing PAR was about 20% higher for C compared to DI and PRD, evidencing the decrease on the leaf capability to harvest sunlight under water stress, irrespective of the type of the irrigation regime. Rates of net photosynthesis were saturated at approximately 500 µmol m^{-2} s^{-1}, irrespective of treatment (Figure 2.13a)

Stomatal conductance and E were about 20% lower in DI and PRD, compared to C at all PAR levels. Following an initial rapid increase with increasing PAR, E and g_s tend to remain relatively stable at PAR levels between 50 and 2000 µmol m^{-2} s^{-1}. Lower g_s in PRD and DI led to slight improvements (about 8%) in instantaneous WUE compared to C. C trees showed lower WUE but followed the same pattern. There was a modest (about 0.5%) difference between C_a and PAR between C and water stressed plants (Figure 2.17).

Leaf carboxylation efficiency under increasing internal CO_2 concentration (C_i) was higher on the deficit treatments, though saturation of P_n occurred at lower C_i (55–70 μmol mol^{-1}) for DI and PRD compared to C (Figure 2.13b).

It appears from a mechanistic analysis of A-C_i (see Appendix 2, Figure A2.8) data that assimilation rate for each treatment was limited by the carboxylation activity of Rubisco (W_c limited) for most of the range of C_i. Also, at high Ci, assimilation of CO_2 was limited by regeneration of ribulose-biphosphate (W_j limited). As in most species, the regeneration of inorganic phosphate (W_p or TPU limitation) did not limit carbon dioxide assimilation (Lambers *et al.,* 1998). The curves in Figure A2.9 showed the characteristic limitations of A-C_i for different species.

At most C_i, g_s and E of DI and PRD were about 10% lower than C. Evaporation and g_s were relatively stable under increasing C_i, (Figure 2.14b and 2.15b). There was little difference (about 5%) of C_a in the range between 40 and 80 Pa C_i concentrations between C and water stressed plants. C_i concentrations decreased under DI only at high C_a (70–100 μmol mol^{-1}) demonstrated a mild increase in stomatal limitation of photosynthesis (Figure 2.16b).

Leaves from both deficit irrigation treatments showed a marginal improved WUE across the range of C_i and PAR compared to C, differences attributed to the increment of P_n with controlled increase on E reached on the C experiment. These results are similar to those reported on the effects of carbon dioxide (CO_2) on WUE by Hsiao and Jackson (1999). Consequently, it appears that leaves from trees facing even mild water deficit, from different positions within the canopy and under the dynamic of natural radiation occurring during the day, might achieve a greater WUE than leaves from a well watered sweet cherry tree.

2.5. Conclusions

The magnitude of depletion of soil water potential from the well watered treatment evidences that after the treatments were imposed, only a mild water deficit was achieved on the orchard. The occurrence of precipitation and overcast days during the growing season and the lower atmospheric demand on 2004 complicated the practicability to reach the same level of water stress in the orchard than during 2003.

The results show that in general ψ_{stem} was positively related with ψ_{soil} demonstrating that it is an effective parameter to describe plant water status. Although the application of irrigation water was reduced for DI and PRD treatments, differences on plant water status were only restricted to few runs, while net carbon assimilation was not affected at single leaf level.

In the experiment, no statistically significant differences on physiological parameters consistent throughout the seasons 2003 and 2004 were found between DI and PRD treatments. This lead to the hypothesis that the evident advantages of PRD that have been reported on vineyards might not be common with fruit crops like sweet cherries. Simultaneously, the results question the viability of imposing partial drying of the root system on a *Prunus* orchard planted on vigorous rootstock such as Mazzard. It resulted evident from the physiological parameters that trees under deficit irrigation suffered acclimation to the restricted water supply. Therefore, additional research is needed to recommend the adoption of a deficit irrigation strategy on commercial orchard of sweet cherries. Additional investigations are also need to determine the effect of irrigation on dwarfing rootstocks such as Gisela 5, keeping in mind the economic viability of the proposed solution and the effects on yield and quality.

On the other hand, commercial sweet cherries grown in the state of Washington offer a limited time frame to develop soil water deficit enough to affect the tree physiology. Practices like storing water in the soil at the end of the previous season, preventing frost damage using abundant water and the short period between bloom and harvest make deficit irrigation practices complicated to implement without detriment to the fruit yield and quality.

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Table 2.1: Photosynthetic parameters derived from the mechanistic analysis of A-C_i data for C, DI and PRD irrigation treatments.

Parameter	C	DI	PRD	average
$V_{cmax}{}^{a}$ (µmol m^{-2} s^{-1})	50.42 a	49.13 a	36.43 a	45.33
$V_{cmax}{}^{m}$ (µmol g^{-1} s^{-1})	0.51 a	0.56 a	0.39 a	0.49
$J_{max}{}^{a}$ (µmol m^{-2} s^{-1})	163.33 a	153.17 a	102.60 a	139.70
$J_{max}{}^{m}$ (µmol g^{-1} s^{-1})	1.66 a	1.76 a	1.09 a	1.51
$R_d{}^{a}$ (µmol m^{-2} s^{-1})	1.91 a	2.07 a	1.56 a	1.84
$R_d{}^{m}$ (µmol g^{-1} s^{-1})	0.02 a	0.02 a	0.02 a	0.02

V_{cmax} (photosynthetic Rubisco capacity), J_{max} (potential rate of electron transport), R_d (mitochondrial respiration in the light). All values ± S.E.M., *n* = 8 -11; letters indicate significant differences at $p < 0.05$ in the same row.

Table 2.2: Photosynthetic parameters derived from the analysis of *A*-*Q* data of C, DI and PRD irrigation treatments.

Parameter	C	DI	PRD	average
R_n^a (µmol m^{-2} s^{-1})	−0.95 a	−0.98 a	−0.81 a	−0.92
R_n^m (µmol g^{-1} s^{-1})	−0.01 a	−0.01 a	−0.01 a	−0.01
QE^a (µmol m^{-2} s^{-1})	0.05 a	0.04 a	0.04 a	0.04
QE^m (µmol g^{-1} s^{-1})	0.0005 a	0.0005 a	0.0004 a	0.0005
A_{max}^a (µmol m^{-2} s^{-1})	16.37 a	14.22 ab	12.20 b	14.26
A_{max}^m (µmol g^{-1} s^{-1})	0.17 a	0.16 a	0.13 a	0.15
LC_{point}^a(µmol m^{-2} s^{-1})	20.71 a	23.74 a	19.60 a	21.35
LC_{point}^m(µmol g^{-1} s^{-1})	0.21 a	0.27 a	0.21 a	0.23
LSE_{sat}^a(µmol m^{-2} s^{-1})	376.18 a	368.65 a	314.80 a	353.21
LSE_{sat}^m(µmol g^{-1} s^{-1})	3.83 a	4.20 a	3.35 a	3.79

R_n (mitochondrial respiration in the dark, QE (quantum efficiency), A_{max} (maximum photosynthetic rate at saturating PAR), LC_{Point} (light compensation point), and LSE_{sat} (light saturation estimated point at saturation). n = 3, letters indicate significant differences at $p < 0.05$ in the same row.

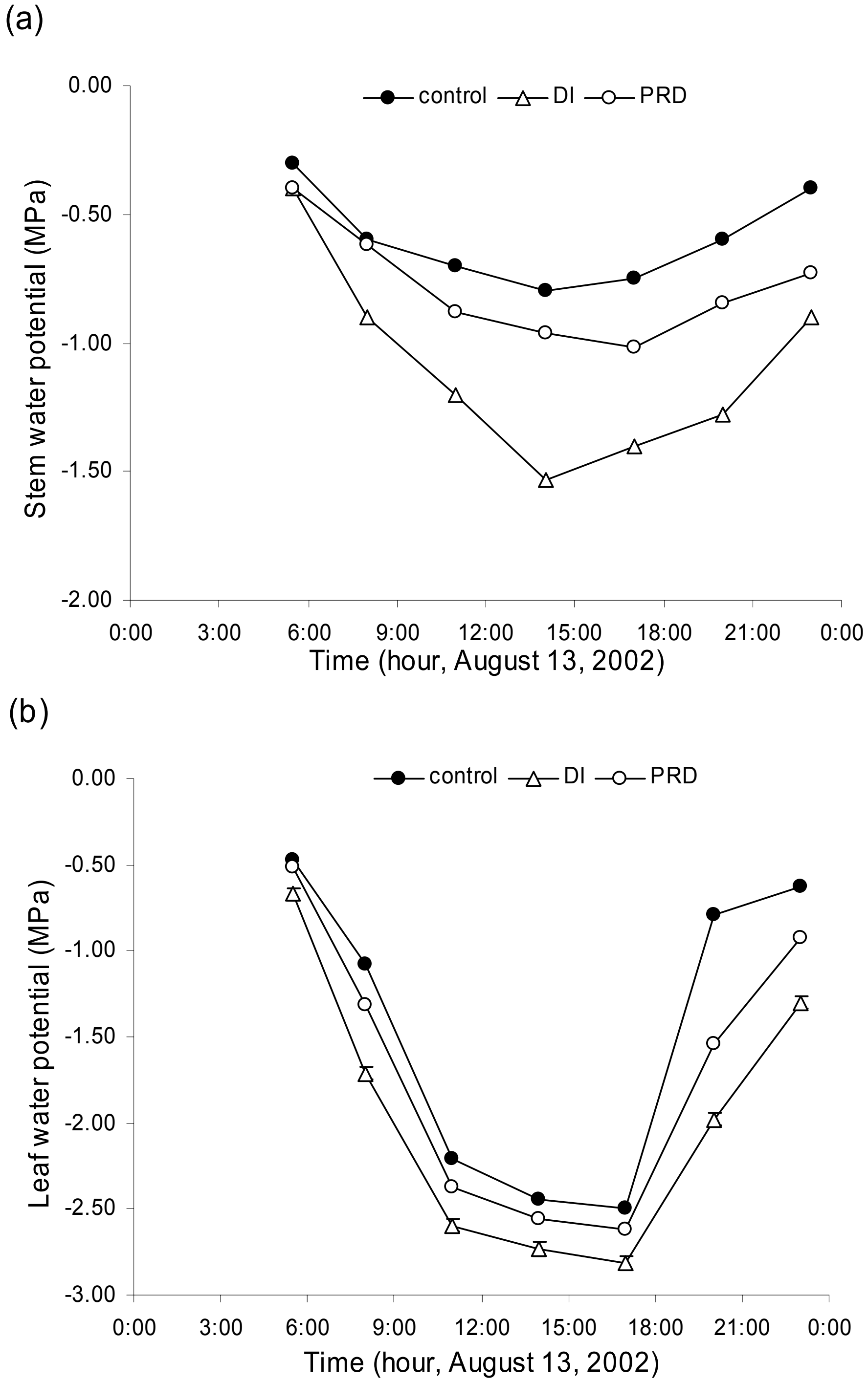


Figure 2.1: Daily cycle of the stem (a) and leaf water potential (b) for C, DI and PRD treatment, measured on August 13, 2002 (n = 3).

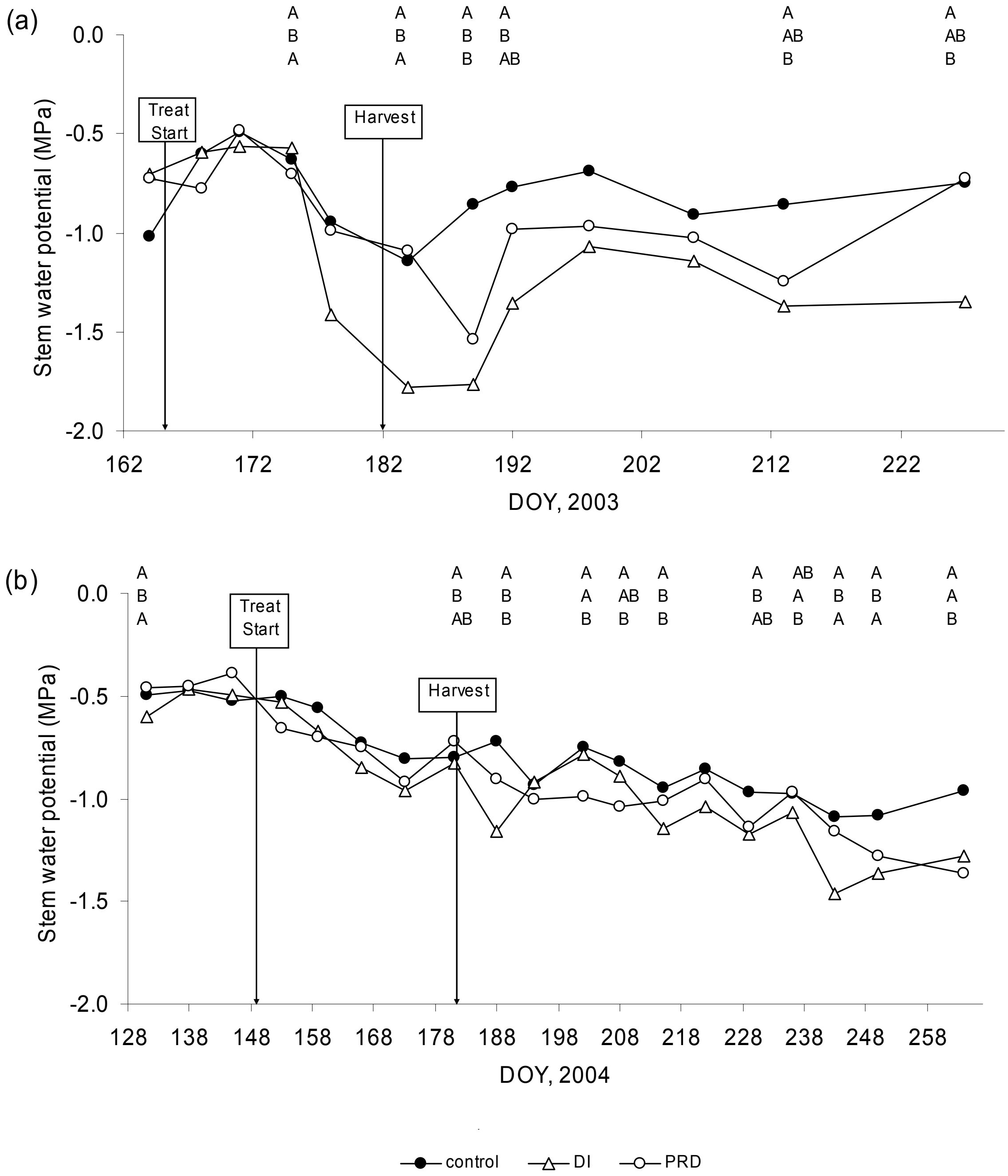


Figure 2.2: Evolution of the stem water potential (ψ_{stem}) measured at midday during 2003 (a) and 2004 (b). Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

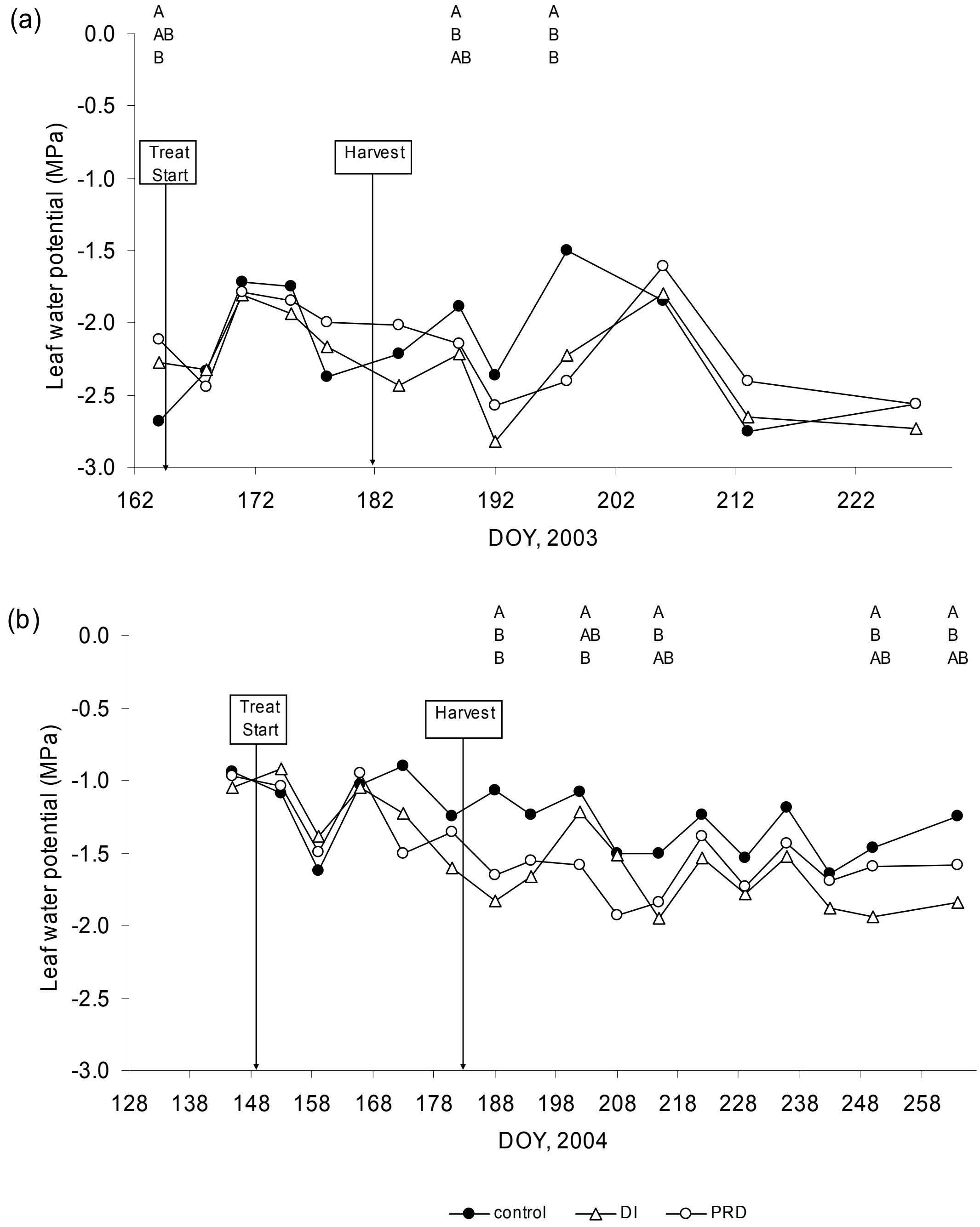


Figure 2.3: Evolution of the leaf water potential (ψ_{leaf}) measured at midday during 2003 (a) and 2004 (b). Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

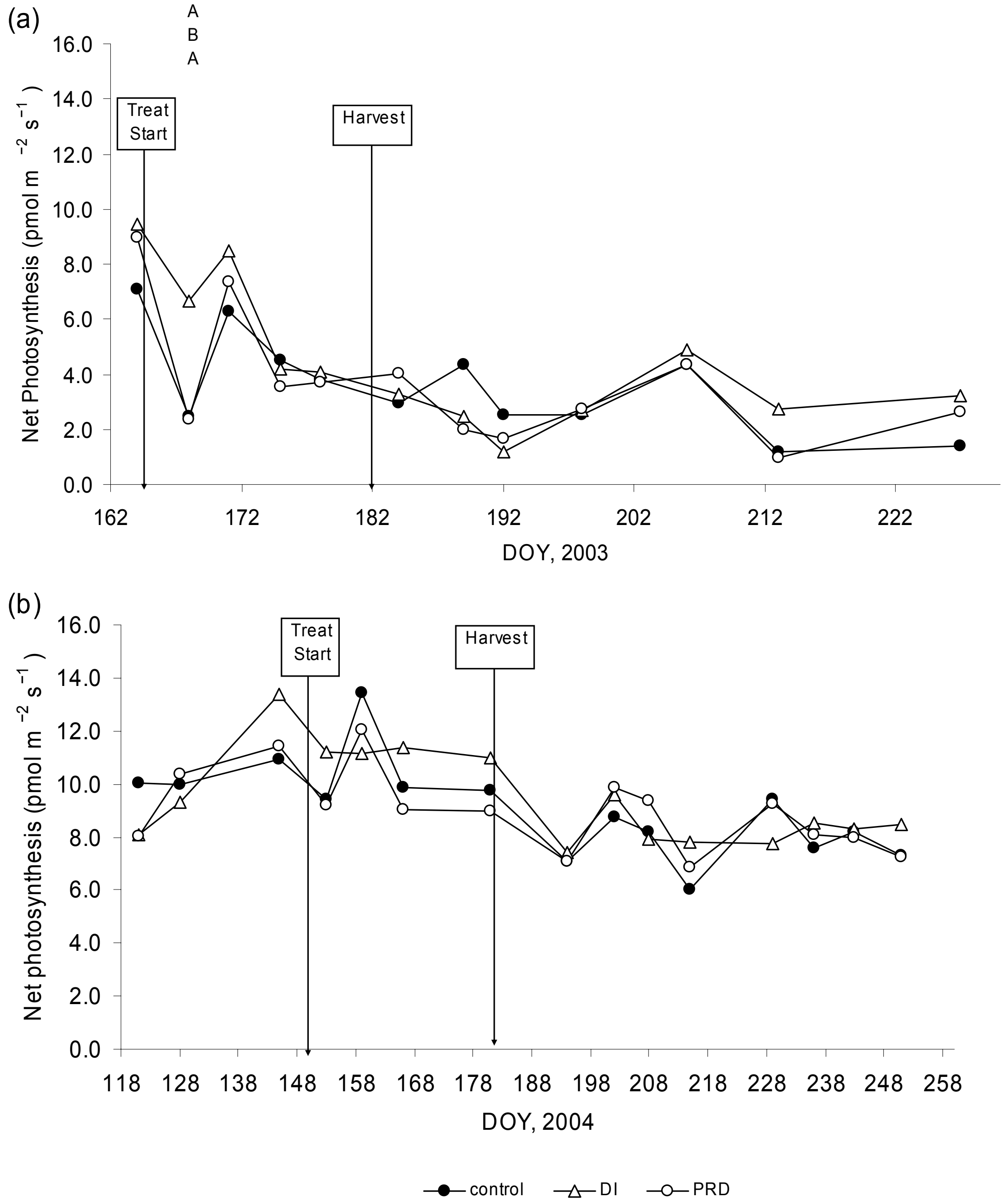


Figure 2.4: Evolution of net photosynthesis (P_n) measured at midday during 2003 (a) and 2004 (b). Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

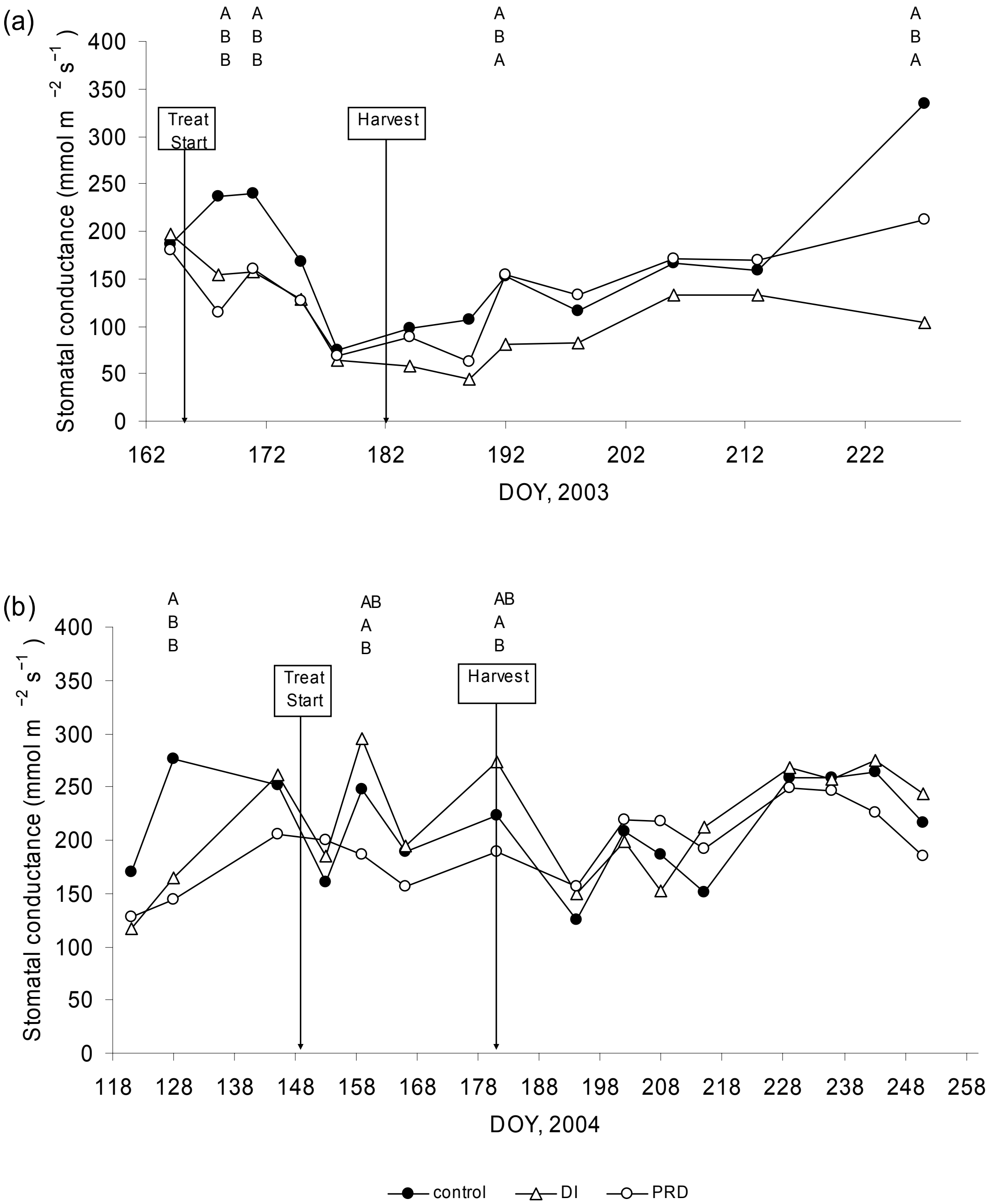


Figure 2.5: Evolution of stomatal conductance (g_s) measured at midday during 2003 (a) and 2004 (b). Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

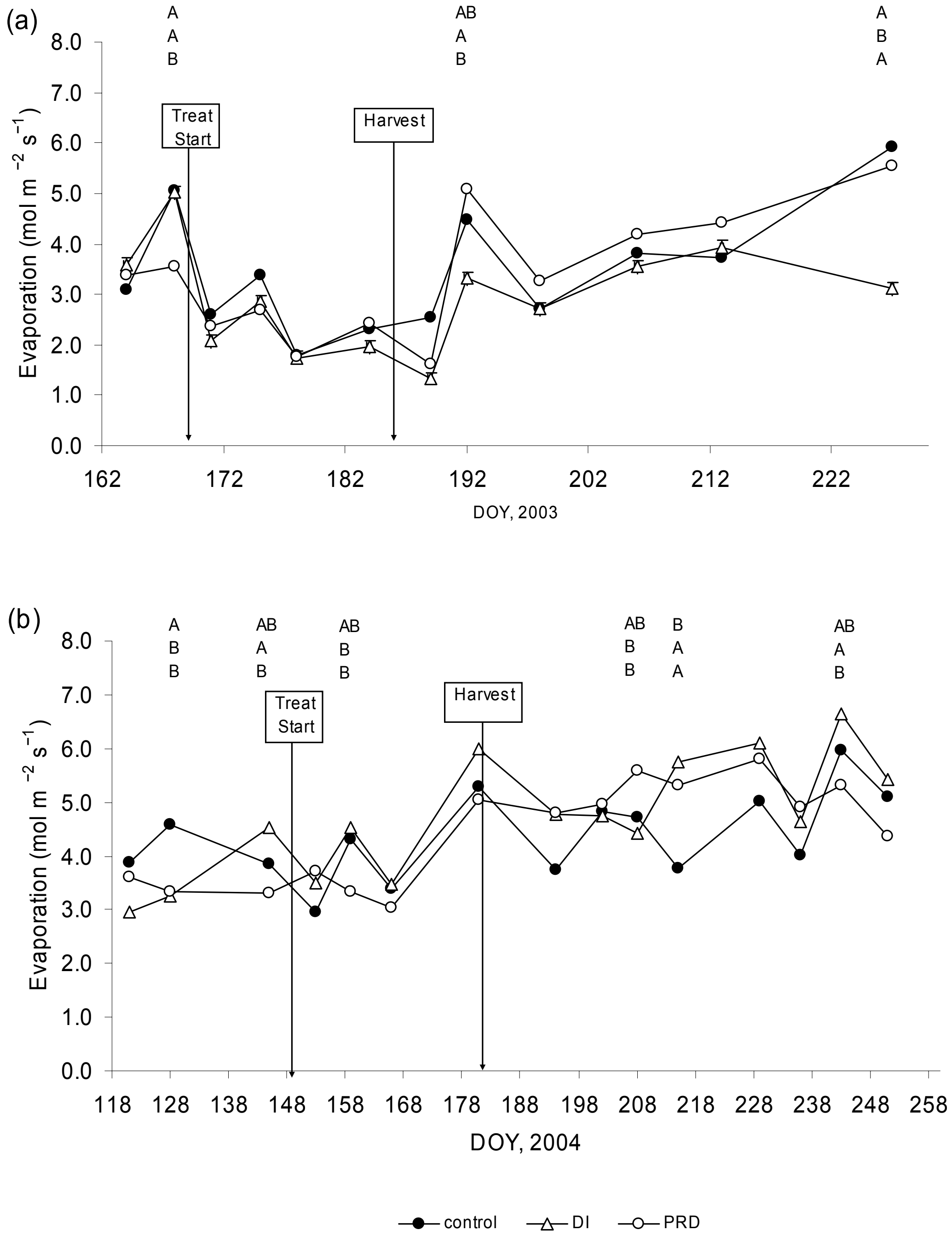


Figure 2.6: Evolution of evaporation (E) measured at midday during 2003 (a) and 2004 (b). Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

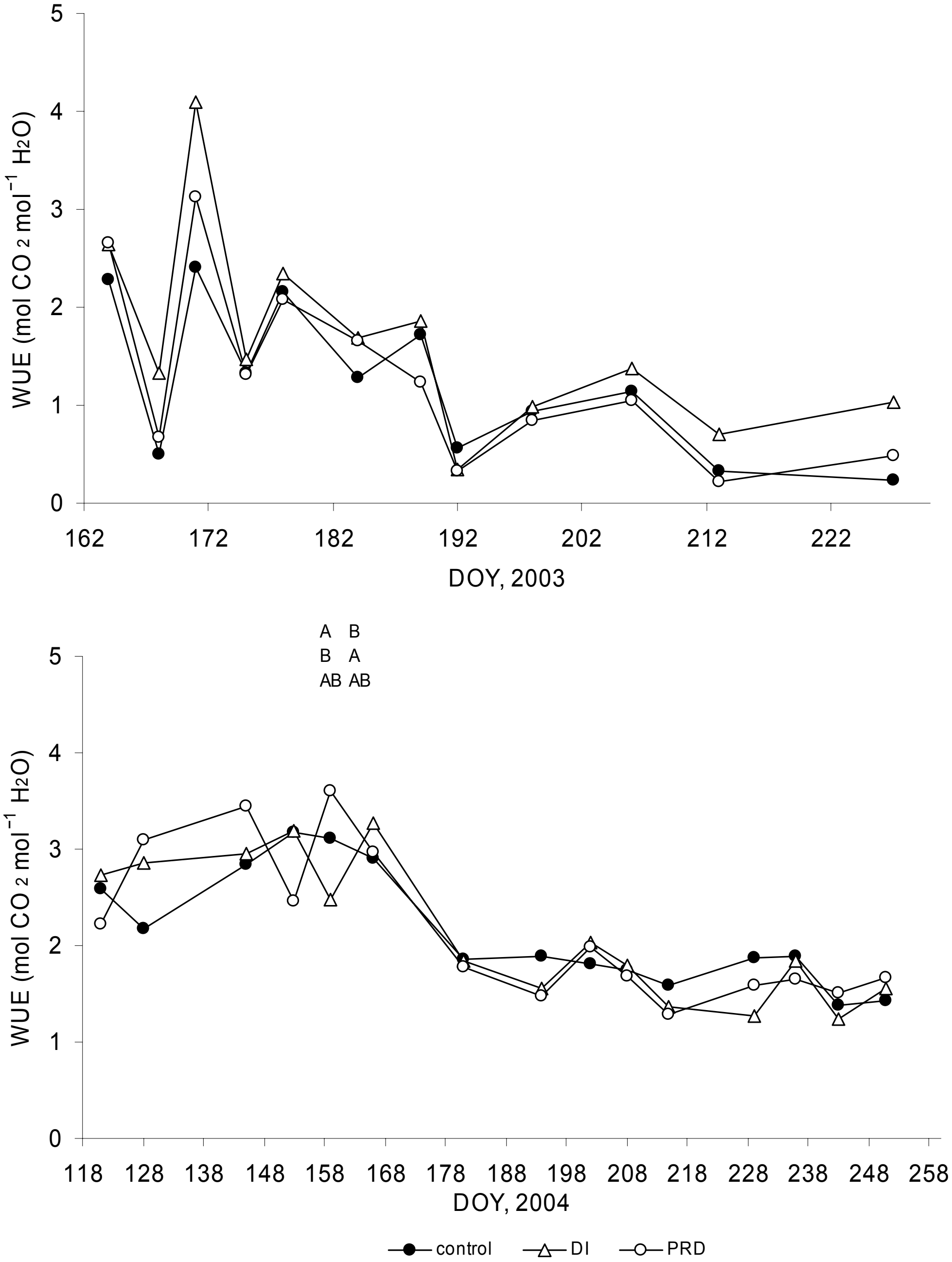


Figure 2.7: Evolution of the WUE from the leaf measured at midday during 2003 (a) and 2004 (b). Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

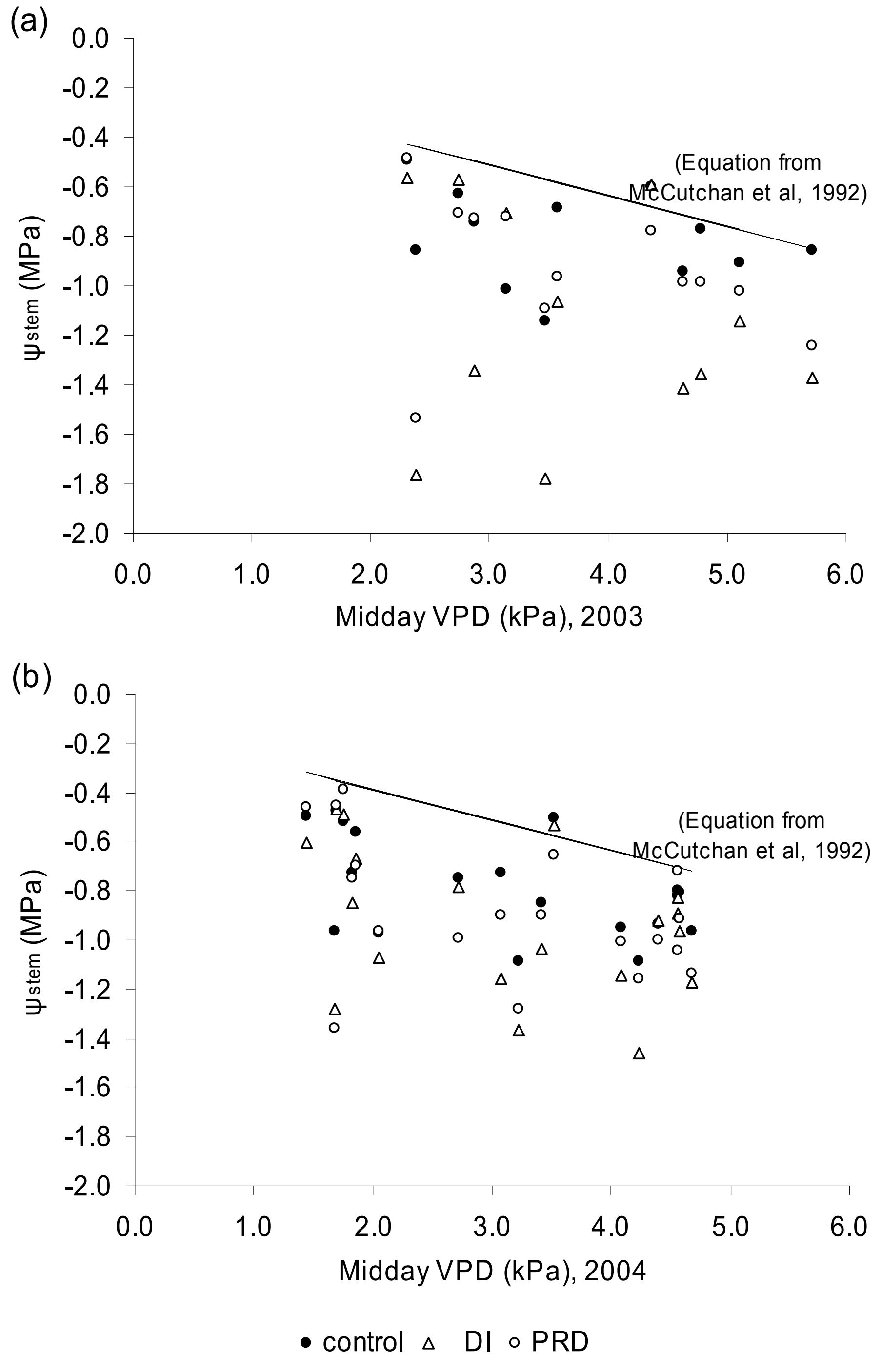


Figure 2.8: Relation between stem water potential (ψ_{stem}) to midday vapor pressure deficit observed in the experiment during 2003 (a) and 2004 (b), and a linear function described by $\psi_{stem} = -0.1235 \times VPD - 0.417$ from McCutchan and Shackel (1992). Each symbol represents the mean for each day and treatment.

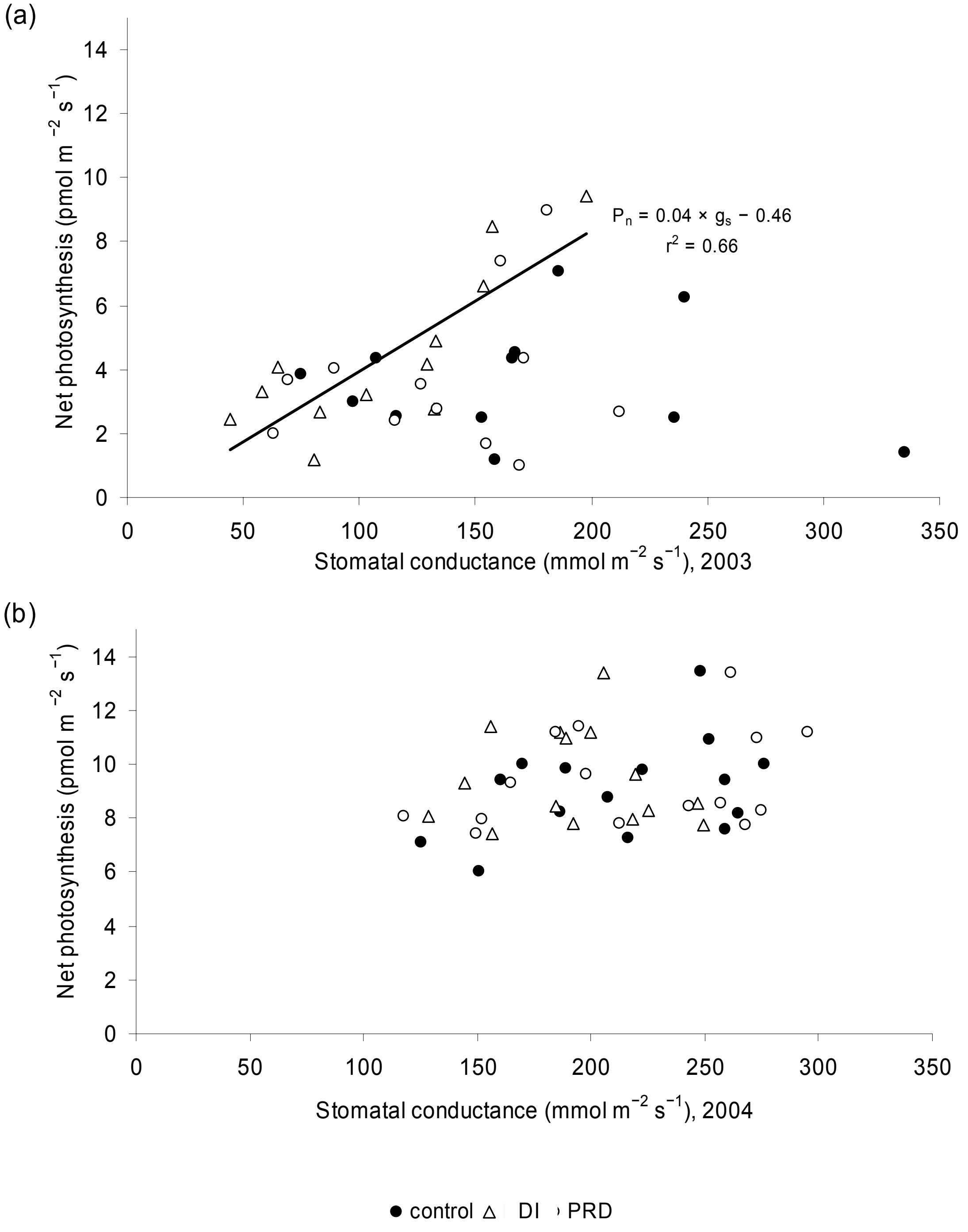


Figure 2.9: Relation between net photosynthesis and g_s during 2003 (a) and 2004 (b). A linear regression ($P_n = 0.04 \times g_s - 0.46$, $r^2 = 0.66$) through the points corresponding to DI treatment during 2003 is shown. Symbols are the average of three measurements per tree and straight lines represent linear regression curve.

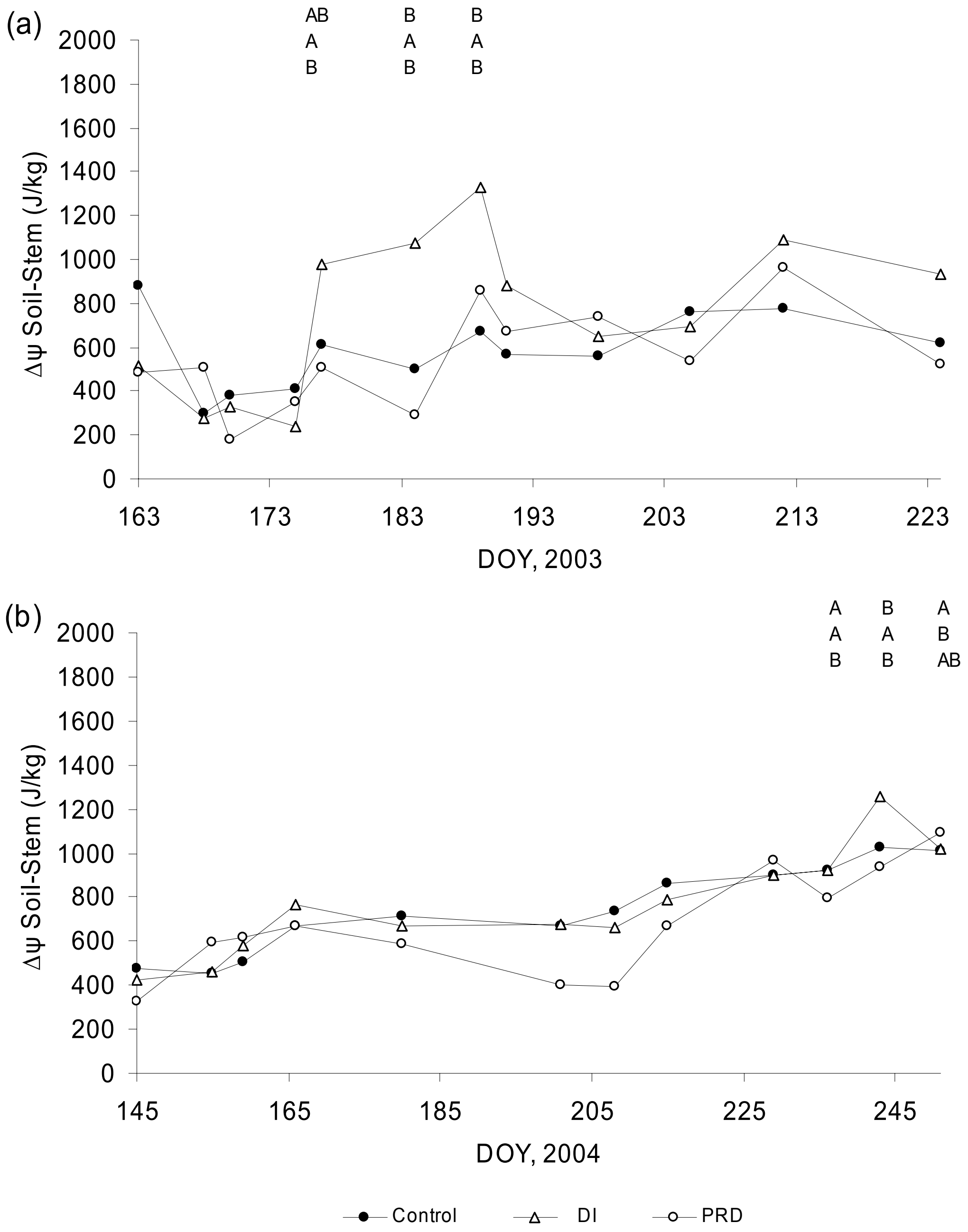


Figure 2.10: Soil-stem water potential gradient ($\Delta\psi_{soil\text{-}stem}$) calculated according to Begg and Turner (1970) for years 2003 (a) and 2004 (b). Symbols are the average of three measurements per tree. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

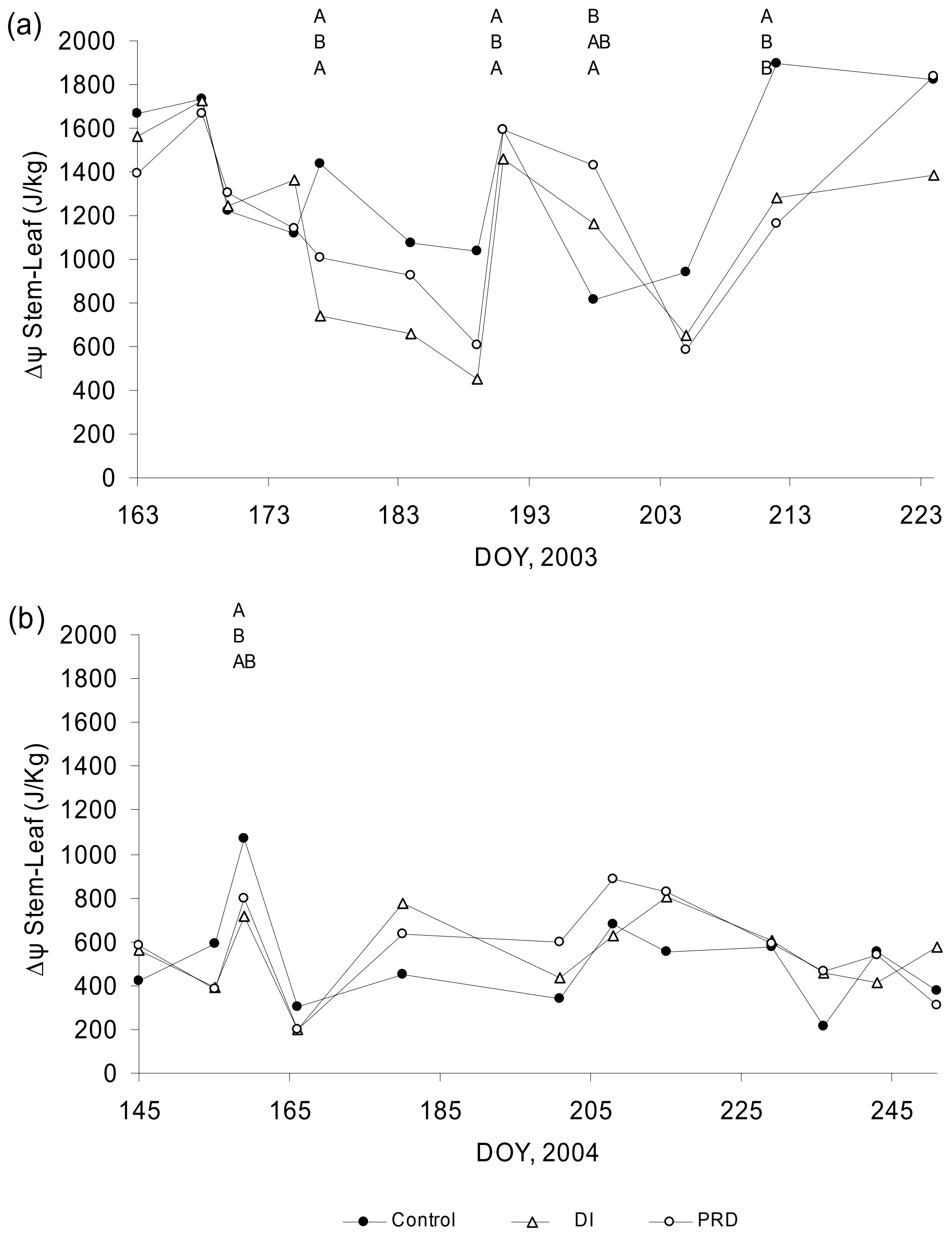


Figure 2.11: Stem-leaf water potential gradient ($\Delta\psi_{\text{stem-leaf}}$) calculated according to Begg and Turner (1970) for years 2003 (a) and 2004 (b). Symbols are the average of three measurements per tree. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

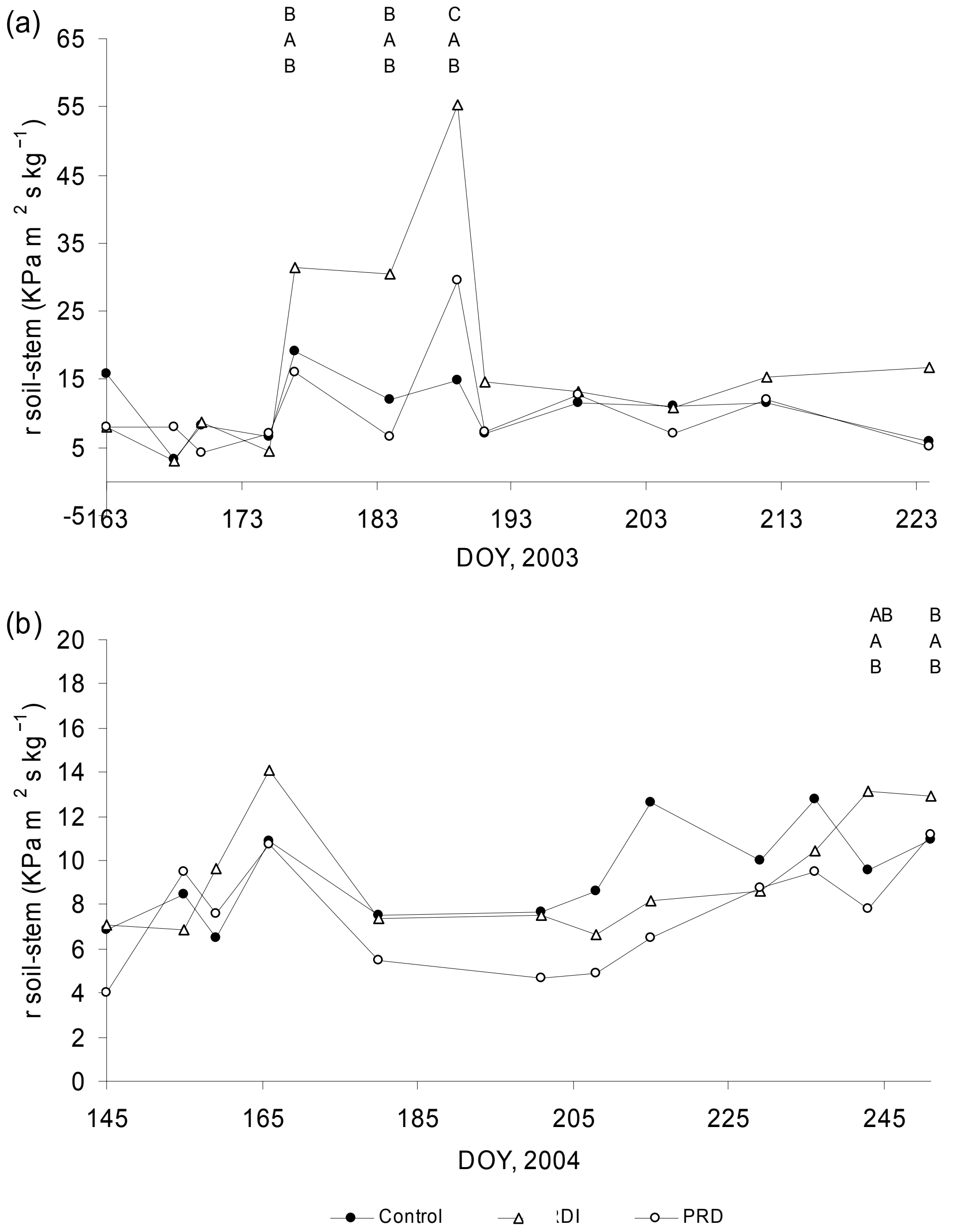


Figure 2.12: Stem-leaf water resistance estimated from $\Delta\psi_{\text{stem-leaf}}$ and E for years 2003 (a) and 2004 (b). Symbols are the average of three measurements per tree. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

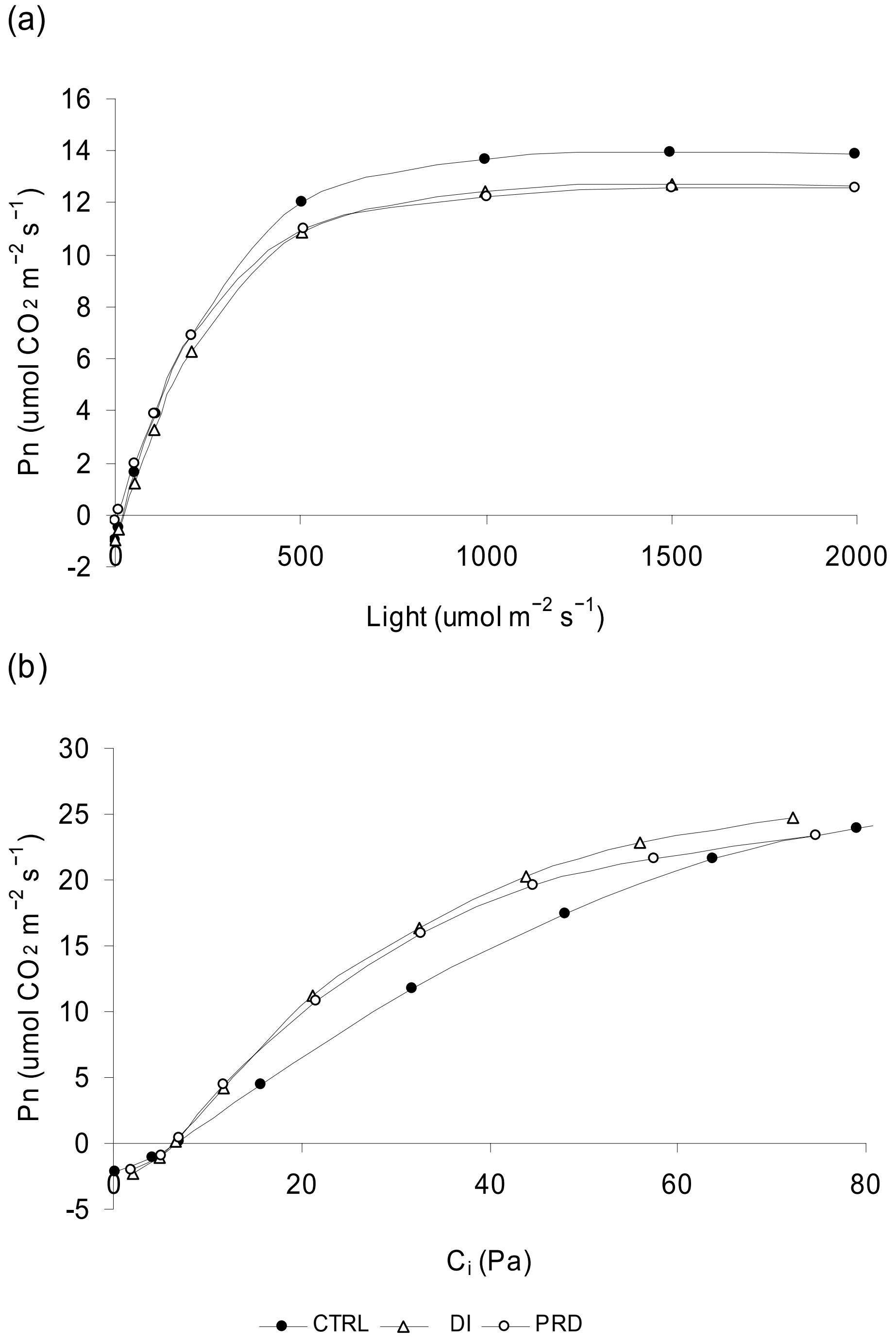


Figure 2.13: Response of net photosynthesis (P_n) to PAR radiation (a; A-Q data, under constant CO_2 concentration of 370 µmol mol^{-1}) and to internal CO_2 concentration (b; A-C_i data, under constant PAR radiation of 1000 µmol photons m^{-2} s^{-1}) of cherry leaves (n = 6) respectively under C, DI and PRD irrigation treatments.

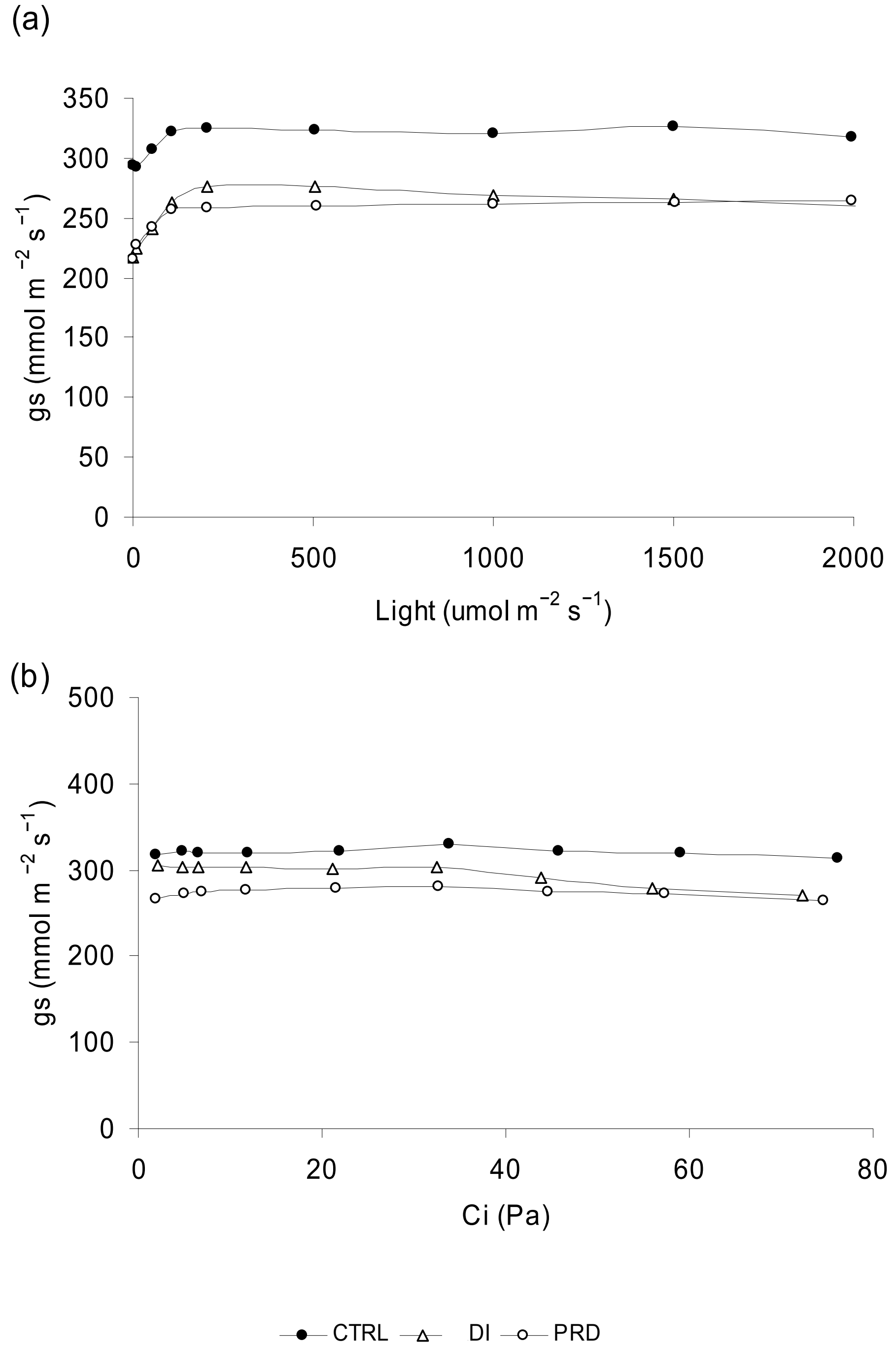


Figure 2.14: Response of stomatal conductance (g_s) to PAR radiation (a); A-Q data, under constant CO_2 concentration of 370 µmol mol^{-1}) and to internal CO_2 concentration (b; A-C_i data, under constant PAR radiation of 1000 µmol photons m^{-2} s^{-1}) of cherry leaves (n = 6) respectively under C, DI and PRD irrigation treatments.

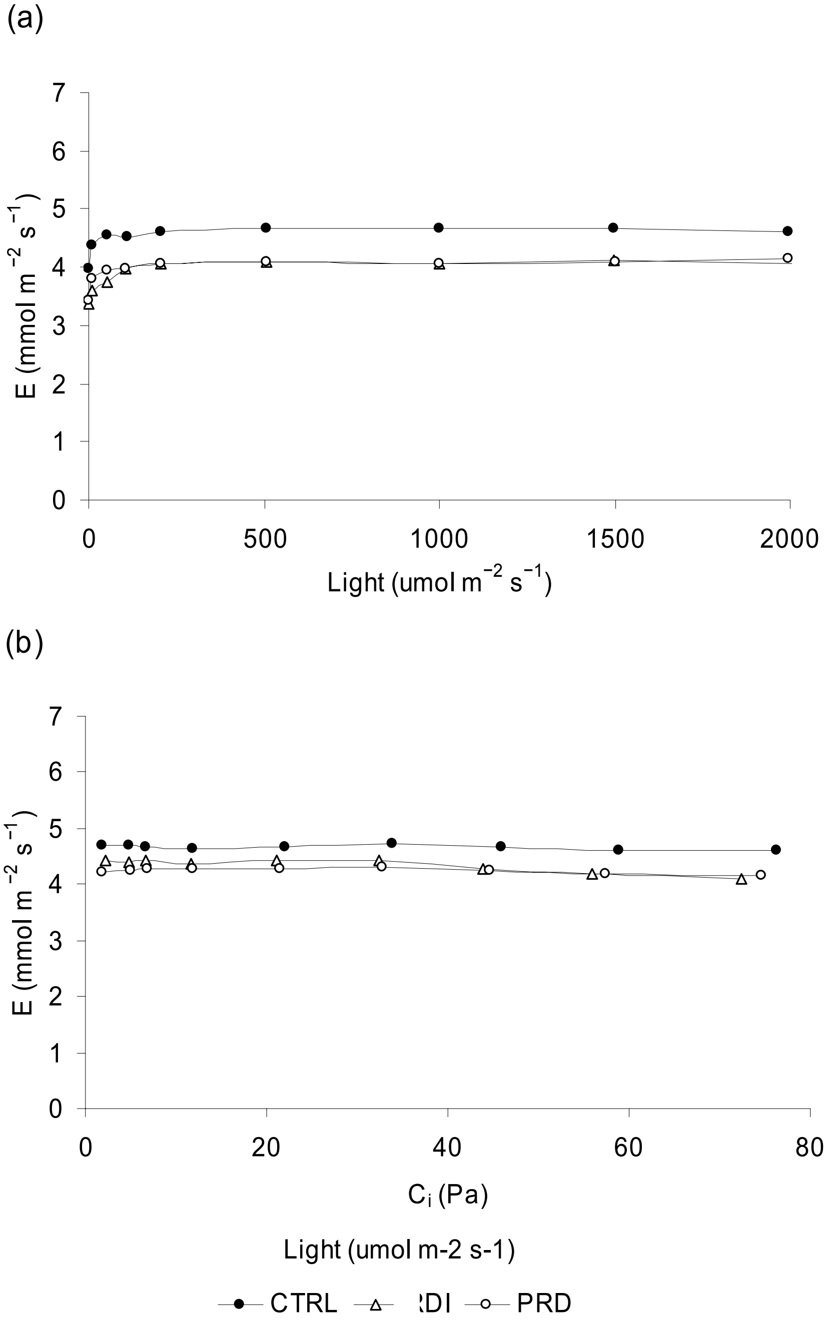


Figure 2.15: Response of leaf evaporation (E) to PAR radiation (a); A-Q data, under constant CO_2 concentration of 370 µmol mol^{-1}) and to internal CO_2 concentration (b; A-C_i data, under constant PAR radiation of 1000 µmol photons m^{-2} s^{-1}) of cherry leaves (n = 6) respectively under C, DI and PRD irrigation treatments.

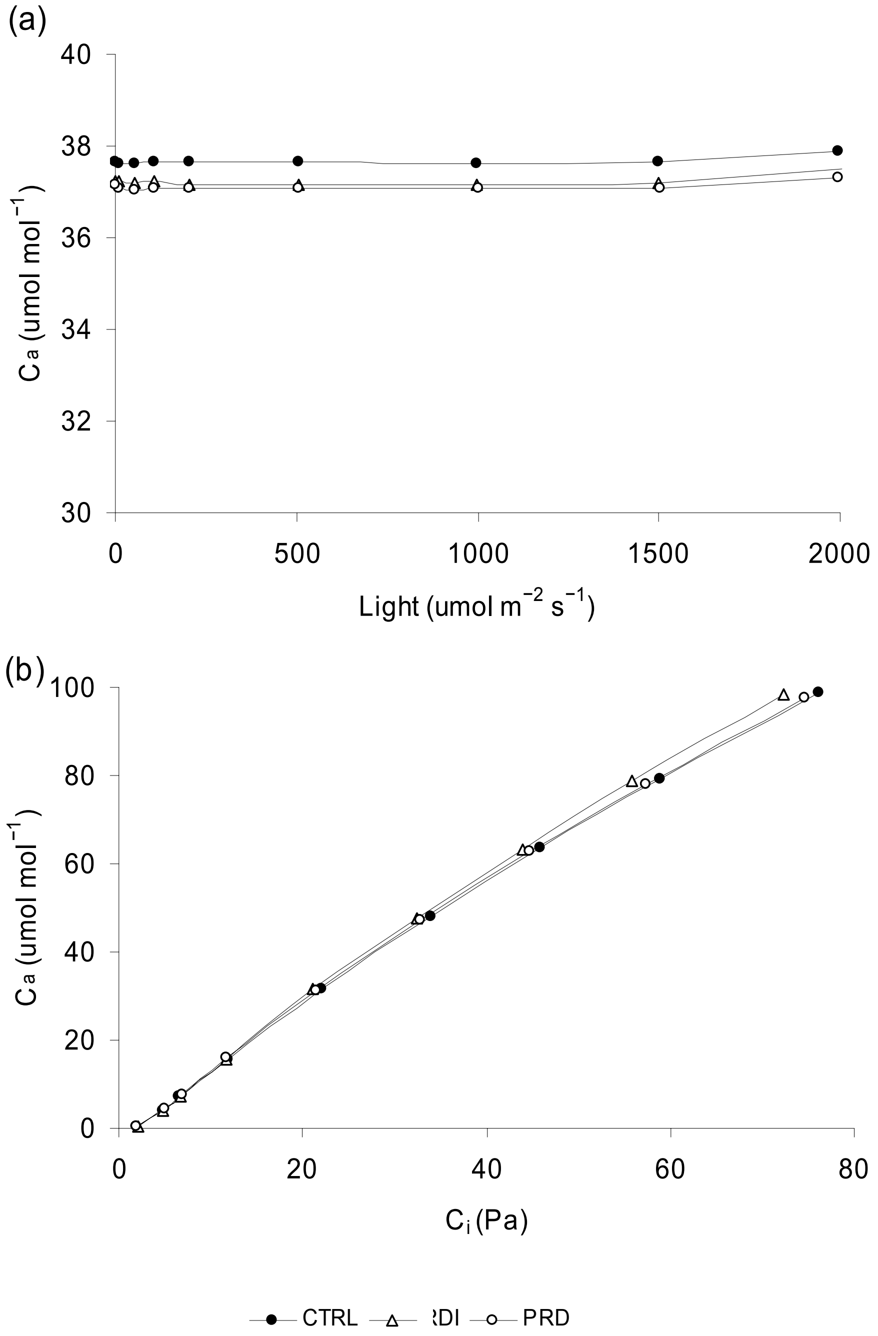


Figure 2.16: Response of C_a to PAR radiation (a); A-Q data, under constant CO_2 concentration of 370 µmol mol^{-1}) and to internal CO_2 concentration (b; A-C_i data, under constant PAR radiation of 1000 µmol photons m^{-2} s^{-1}) of cherry leaves (n = 6) respectively under C, DI and PRD irrigation treatments.

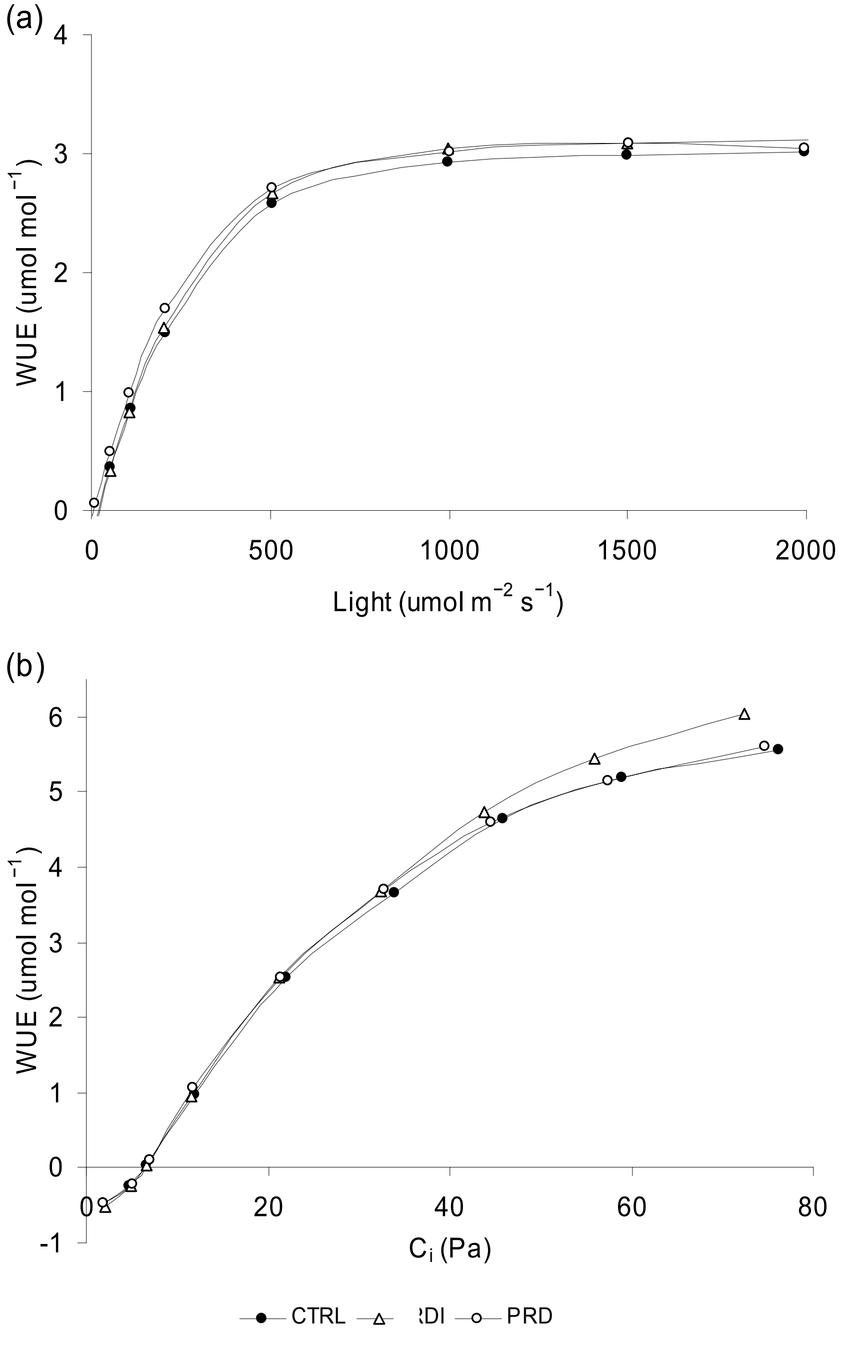


Figure 2.17: Response of WUE to PAR radiation (a); A-Q data, under constant CO_2 concentration of 370 µmol mol^{-1}) and to internal CO_2 concentration (b; A-C_i data, under constant PAR radiation of 1000 µmol photons m^{-2} s^{-1}) of cherry leaves (n = 6) respectively under C, DI and PRD irrigation treatments.

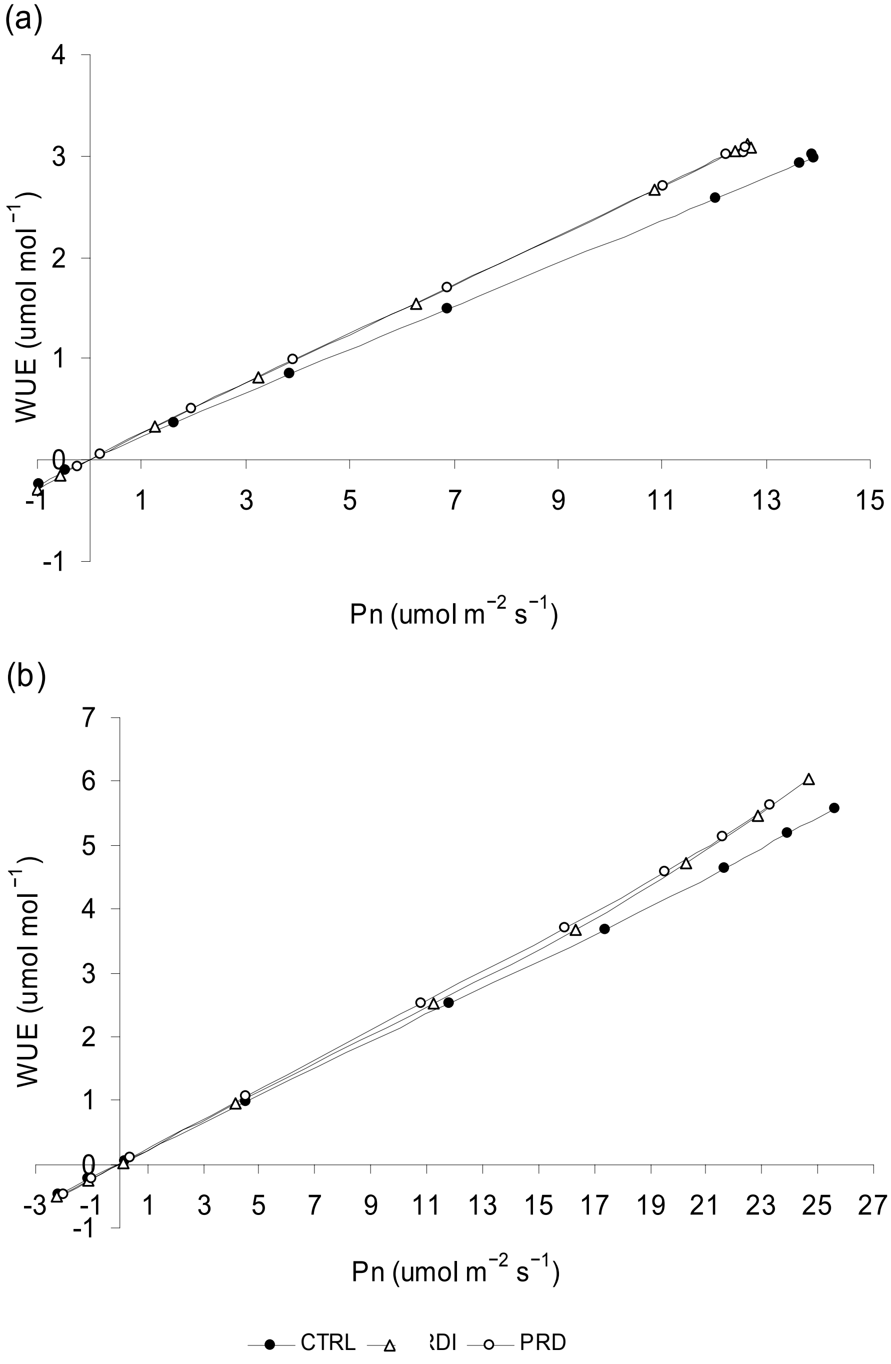


Figure 2.18: Relation between instantaneous WUE and P_n derived from A-Q data (a, constant CO_2 concentration of 370 µmol mol^{-1}) and A-C_i data (b, constant PAR radiation of 1000 µmol photons m^{-2} s^{-1}) of cherry leaves (n = 6) under C, DI and PRD irrigation treatments.

Appendix 2

Table A2.1: Monthly volumetric soil water content (cm^3 cm^{-3}) averaged from weekly measurements for 2003 and 2004.

	season 2003			season 2004		
Month	C	DI	PRD	C	DI	PRD
April				0.24	0.21	0.22
May	0.22	0.23	0.23	0.26	0.21	0.23
June	0.17	0.16	0.17	0.23	0.21	0.21
July	0.17	0.13	0.17	0.22	0.18	0.15
August	0.18	0.13	0.15	0.24	0.17	0.17
September	0.19	0.14	0.18	0.25	0.18	0.19
October				0.24	0.18	0.18
Mean per season	0.19	0.16	0.18	0.24	0.19	0.19

Table A2.2: Nitrogen, carbon, carbon to nitrogen ratio, single leaf area, leaf mass per area and canopy leaf area measured from C, DI and PRD irrigation treatments.

Parameter	C	DI	PRD	average
N^a (g m^{-2})	1.79 ± 0.17 a	1.39 ± 0.10 a	1.53 ± 0.03 a	1.57
N^m (mg g^{-1})	17.92 ± 0.68 a	15.83 ± 1.37 a	16.19 ± 0.39 a	16.65
C^a (g m^{-2})	47.74 ± 2.53 a	42.08 ± 1.09 a	45.73 ± 1.93 a	45.18
C^m (mg g^{-1})	481.00 ± 1.23 a	478.37 ± 4.30 a	484.04 ± 1.90 a	481.14
C : N (ratio)	26.92 ± 1.04 a	30.67 ± 2.59 a	29.93 ± 0.81 a	29.17
SLA (cm^2)	63.86 ± 3.07 a	56.33 ± 3.39 a	59.94 ± 7.46 a	60.04
LMA (g m^{-2})	99.27 ± 5.50 a	88.02 ± 3.04 a	94.45 ± 3.73 a	93.91
CLA (m^2)	49.7 ± 5.64 a	39.4 ± 3.82 a	45.5 ± 5.88 a	44.87

N (leaf nitrogen concentration), C (leaf carbon concentration), C : N (carbon to nitrogen ratio), SLA (single leaf area), LMA (leaf mass per area) and CLA (canopy leaf area). All values ± S.E.M., n = 3; letters indicate significant differences at $p < 0.05$ in the same row.

Table A2.3: Summary table of multiple stepwise regressions with ψ_{stem}, P_n and g_s as the dependent variable and environmental and physiological parameters as independent variables for 2003 and 2004 data set, considering all the data and per treatment.

		season 2003		season 2004	
Data set	Dependent variable	Independent variables	r^2	Independent variables	r^2
all treatments	ψ_{stem}	ψ_{soil}, DOY, $\psi_{leaf,}$ E	0.70	ψ_{soil}, ψ_{leaf}	0.80
	P_n	DOY	0.37	gs	0.32
	gs	DOY, ψ_{stem}	0.47	E	0.27
C	ψ_{stem}	$\psi_{leaf,}$ gs	0.49	DOY	0.84
	P_n	DOY	0.44	DOY	0.35
	gs	ψ_{stem}	0.19	ψ_{soil}	0.07
DI	ψ_{stem}	gs	0.65	ψ_{soil}	0.46
	P_n	ψ_{stem}	0.47	ψ_{stem}	0.52
	gs	ψ_{stem}	0.65	DOY	0.39
PRD	ψ_{stem}	DOY, gs	0.56	DOY	0.87
	P_n	ψ_{stem}	0.27	$\psi_{stem,}$ ψ_{leaf}	0.90
	gs	DOY, ψ_{stem}	0.58	ψ_{soil}	0.03

Table A2.4: Multiple stepwise regressions with ψ_{stem} as the dependent variable and environmental and physiological parameters as independent variables for 2003 data set, considering all the data and per treatment.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
all treatments	Intercept	1.202	0.3552	0.0020	0.70
	ψ_{soil}	0.001	0.0002	0.0002	
	DOY	−0.008	0.0019	0.0004	
	ψ_{leaf}	0.404	0.0982	0.0003	
	E	0.138	0.0349	0.0004	
C	Intercept	−0.5213	0.2194	0.0415	0.49
	ψ_{leaf}	0.2601	0.1000	0.0287	
	gs	0.0017	0.0006	0.0175	
DI	Intercept	−2.0145	0.2053	<.0001	0.65
	gs	0.0079	0.0017	0.001	
PRD	Intercept	0.3145	0.5672	0.5927	0.56
	DOY	−0.0101	−0.0101	0.0127	
	gs	0.0048	0.00477	0.006	

Table A2.5: Multiple stepwise regressions with P_n as the dependent variable and environmental and physiological parameters as independent variables for 2003 data set, considering all the data and per treatment.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
all treatments	Intercept	17.9	3.06013	<.0001	0.37
	DOY	−0.07449	0.0162	<.0001	
C	Intercept	16.1555	4.0780	0.0027	0.44
	DOY	−0.0667	0.0216	0.0115	
DI	Intercept	9.0882	1.5183	0.0001	0.47
	Ψ_{stem}	4.0774	1.2481	0.0085	
PRD	Intercept	8.2519	2.0829	0.0027	0.27
	Ψ_{stem}	4.8514	2.1346	0.0464	

Table A2.6: Multiple stepwise regressions with g_s as the dependent variable and environmental and physiological parameters as independent variables for 2003 data set, considering all the data and per treatment.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
all treatments	Intercept	50.090	76.030	0.5146	0.47
	DOY	1.141	0.425	0.0113	
	ψ_{stem}	130.255	22.777	<.0001	
C	Intercept	330.9997	87.3573	0.0035	0.19
	ψ_{stem}	200.3393	106.0469	0.0882	
DI	Intercept	209.8340	22.8914	<.0001	0.65
	ψ_{stem}	86.3572	18.8179	0.0010	
PRD	Intercept	−60.6392	90.3717	0.5191	0.58
	DOY	1.6651	0.5121	0.0100	
	ψ_{stem}	122.9347	34.4191	0.006	

Table A2.7: Multiple stepwise regressions with ψ_{stem} as the dependent variable and environmental and physiological parameters as independent variables for 2004 data set, considering all the data and per treatment.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
all treatments	Intercept	0.374	0.0908	0.0001	0.80
	ψ_{soil}	−0.004	0.0005	<.0001	
	ψ_{leaf}	0.310	0.0608	<.0001	
C	Intercept	0.1085	0.0949	0.2683	0.84
	DOY	−0.0046	0.0005	<.0001	
DI	Intercept	−0.6093	0.1007	<.0001	0.46
	ψ_{soil}	0.0019	0.0005	0.0012	
PRD	Intercept	0.3582	0.1171	0.0071	0.87
	DOY	−0.0064	0.0006	<.0001	

Table A2.8: Multiple stepwise regressions with P_n as the dependent variable and environmental and physiological parameters as independent variables for 2004 data set, considering all the data and per treatment.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
all treatments	Intercept	−32.3	9.17453	0.0004	0.32
	gs	0.20328	0.04338	<.0001	
C	Intercept	14.2123	1.7918	<.0001	0.35
	DOY	−0.0274	0.0093	0.0115	
DI	Intercept	12.5408	1.0023	<.0001	0.52
	ψ_{stem}	3.6560	1.0190	0.0049	
PRD	Intercept	16.9278	0.8017	<.0001	0.90
	ψ_{stem}	4.5083	1.1004	0.0027	
	ψ_{leaf}	2.1553	0.8512	0.0321	

Table A2.9: Multiple stepwise regressions with g_s as the dependent variable and environmental and physiological parameters as independent variables for 2004 data set, considering all the data and per treatment.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
all treatments	Intercept	215.793	7.568	<.0001	0.27
	E	−1.560	0.377	0.0002	
C	Intercept	296.9389	60.1535	0.0003	0.07
	ψ_{soil}	1.2854	0.8988	0.1763	
DI	Intercept	7.1883	57.1091	0.9018	0.39
	DOY	0.9411	0.2970	0.0074	
PRD	Intercept	229.7335	21.9819	<.0001	0.03
	ψ_{soil}	0.0644	0.0805	0.4380	

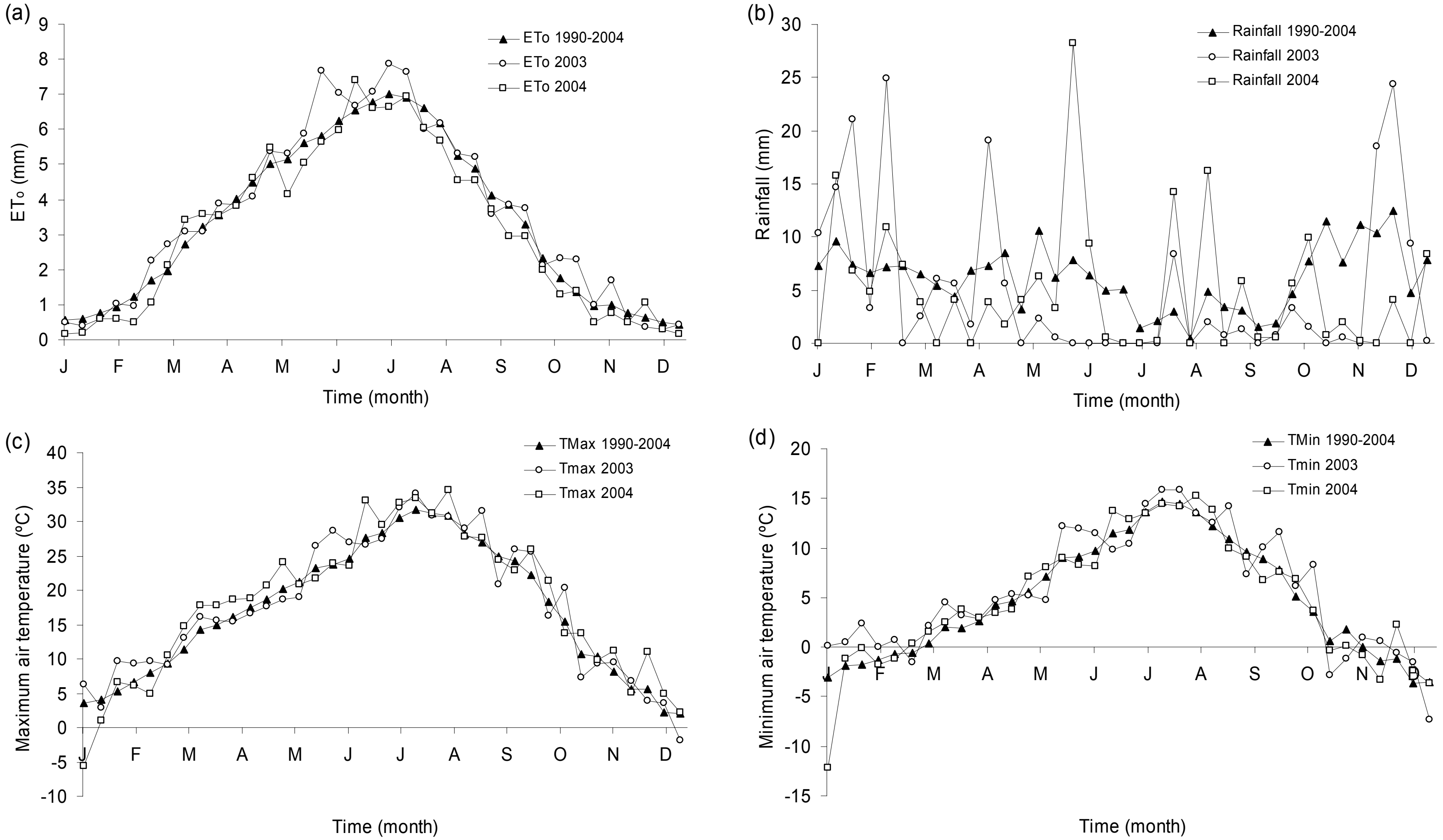
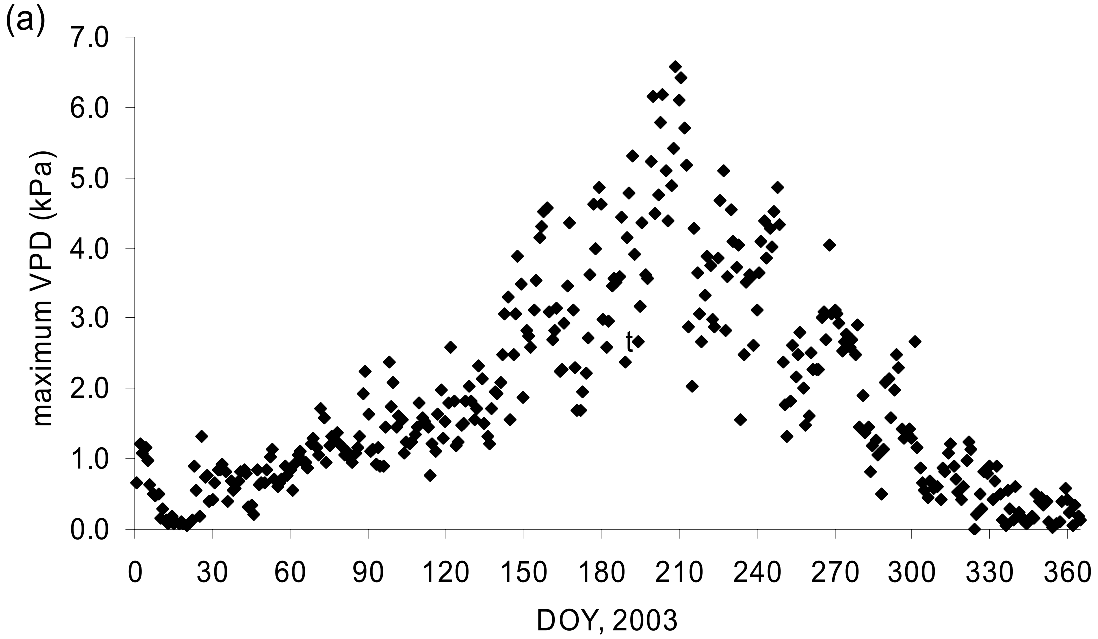


Figure A2.1: Weather patterns of 10–day mean of the reference crop evapotranspiration (ET_o) computed from meteorological data (a), 10–day sum of rainfall (b), minimum (c) and maximum air temperatures (d) compared with their 15–year mean. Roza Field, WSU Experimental Station, years 2003, 2004 and 1990–2004 (online source PAWS, WSU, 2005).



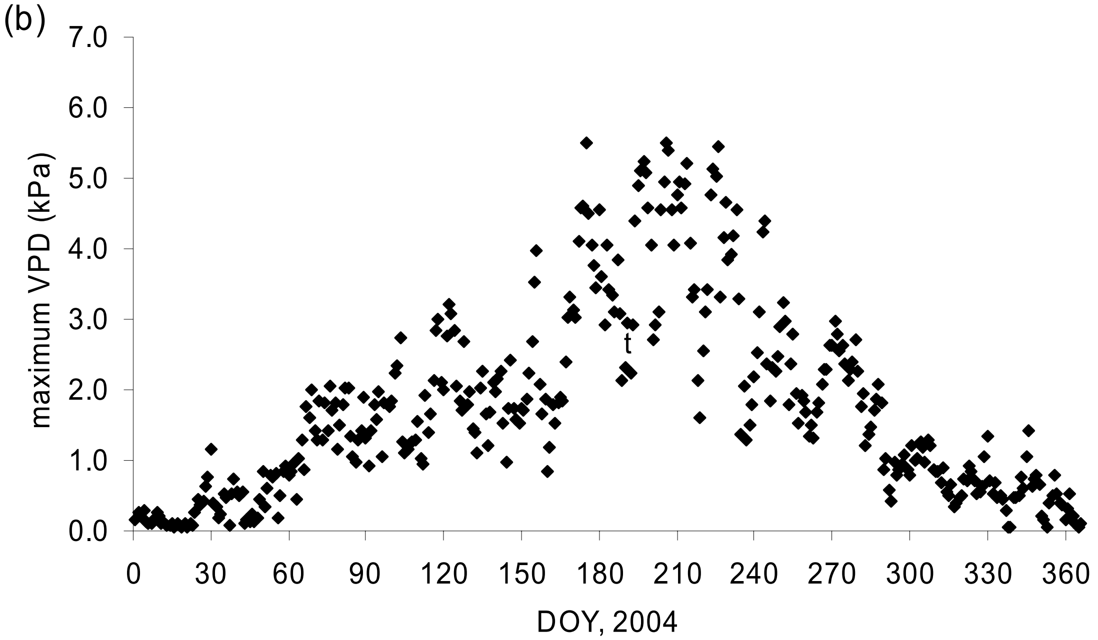


Figure A2.2: Maximum daily VPD (kPa) computed from meteorological data for 2003 (a) and 2004 (b). Online source PAWS, WSU, 2005.

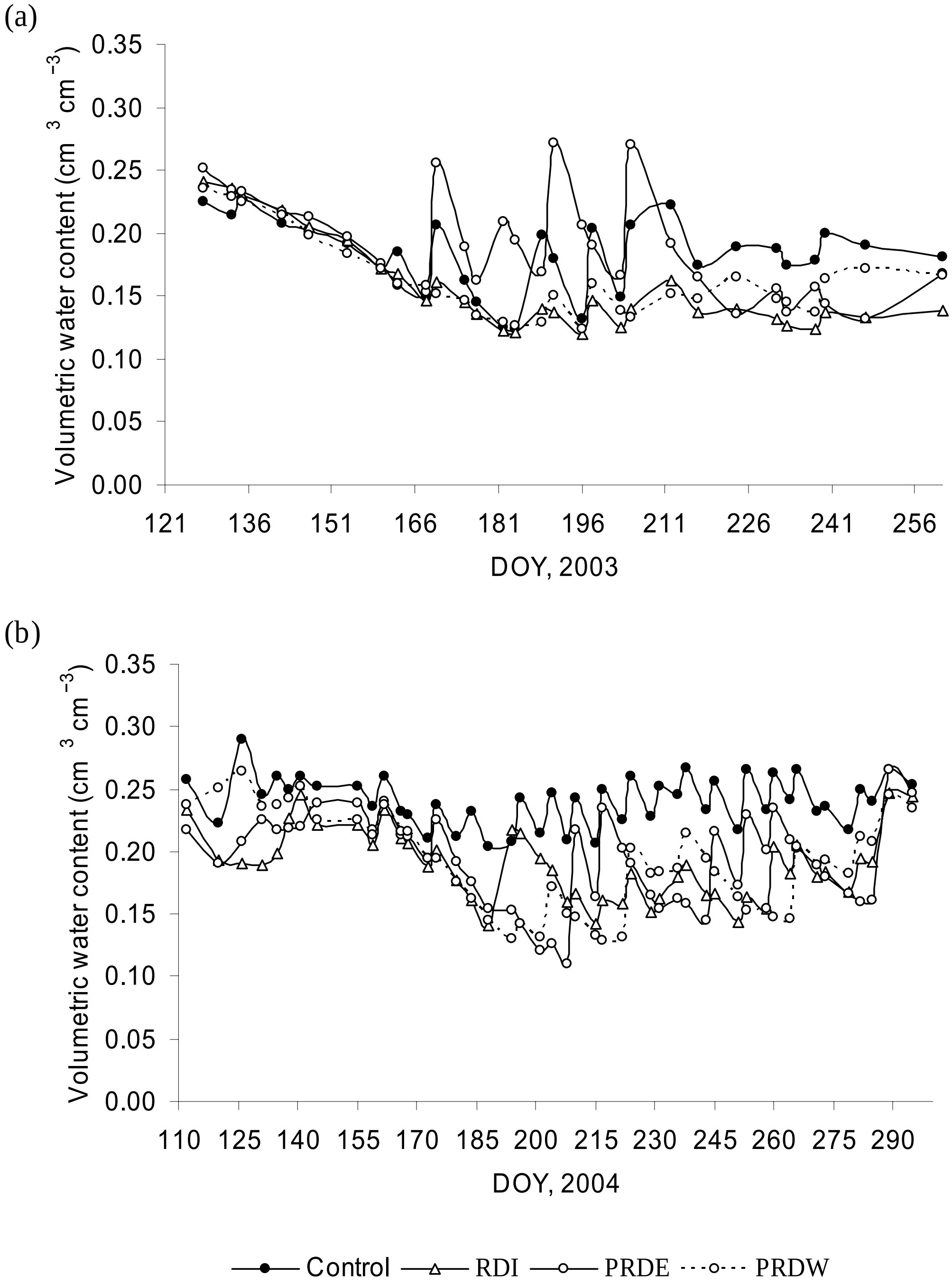


Figure A2.3: Volumetric soil water content measured with weekly measurements using a neutron probe for years 2003 (a) and 2004 (b). The points are the average readings of nine points at four depths. PRD treatment is shown in the graph with two location at the east side (PRDE) and west side of the row (PRDW).

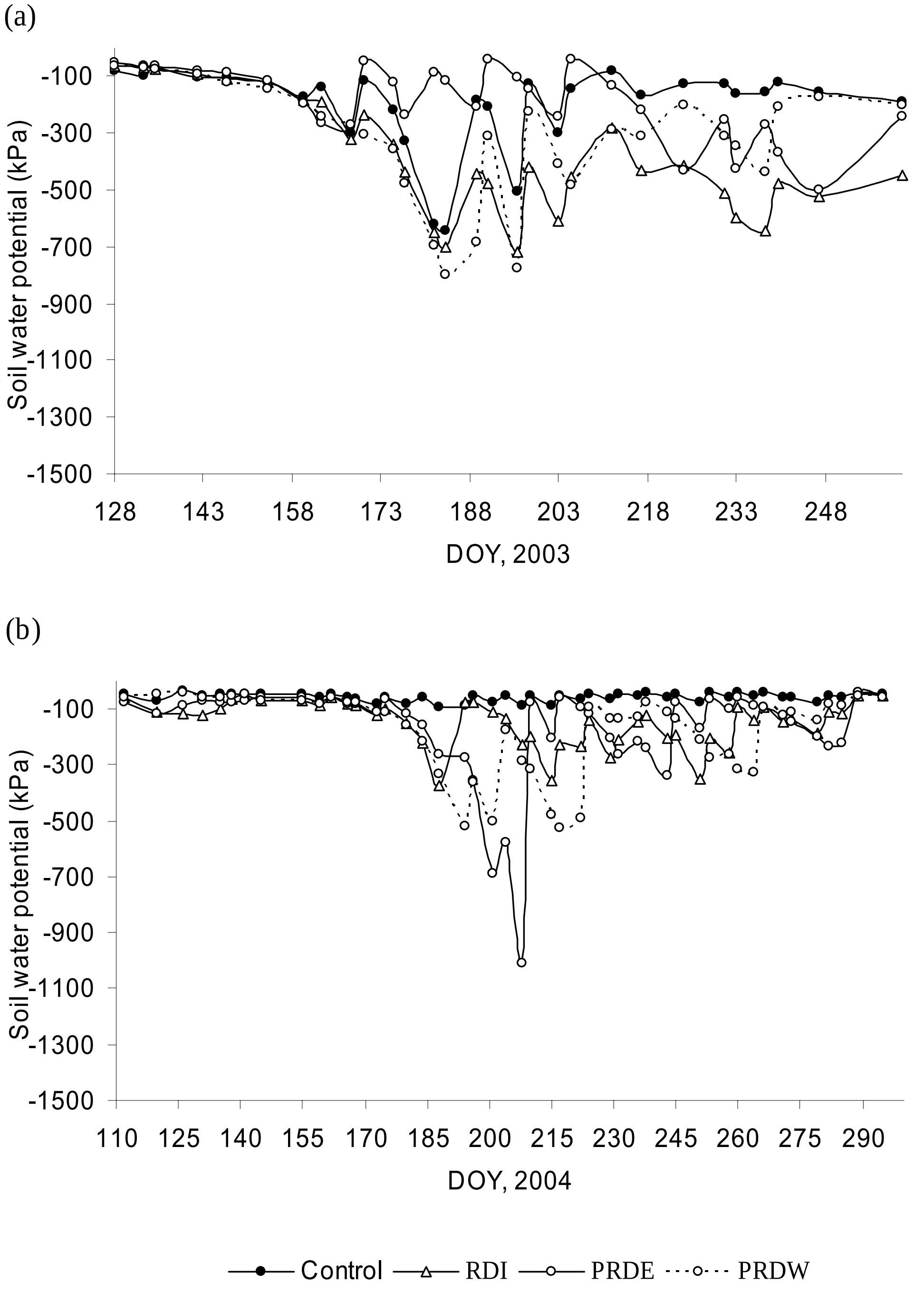


Figure A2.4: Soil water potential (ψ_{soil}) estimated using soil moisture curve from weekly measurements using a neutron probe. The points are the average readings of nine points at four depths. PRD treatment is shown in the graph with two location at the east side (PRDE) and west side of the row (PRDW).

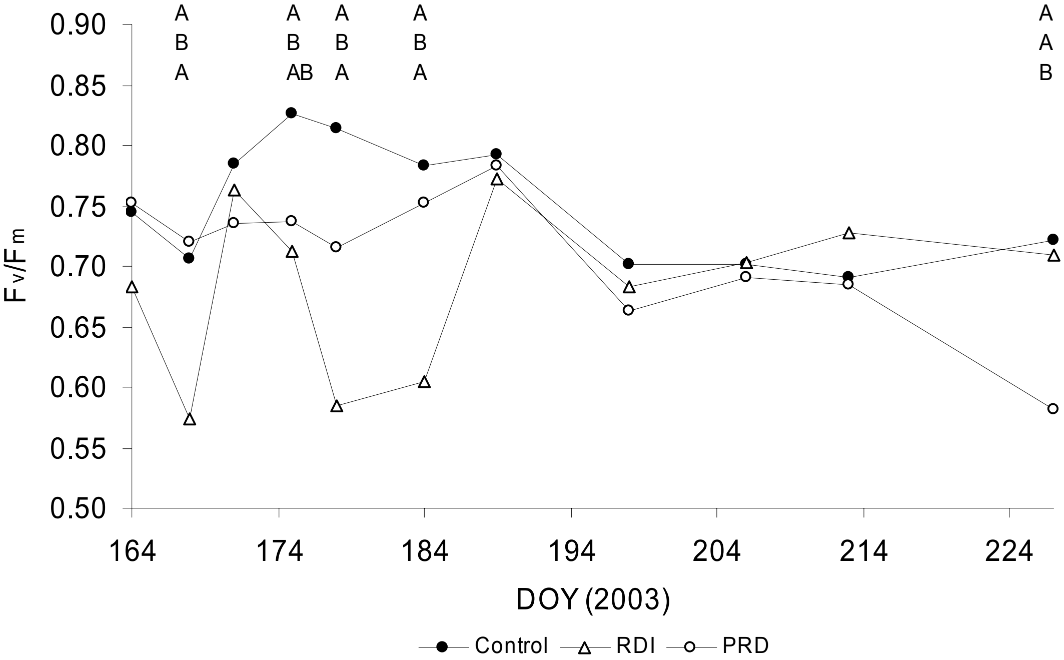


Figure A2.5: Maximum efficiency of PSII (F_v/F_m) measured during 2003 for C, DI and PRD treatments. Symbols are the average of three measurements per tree. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

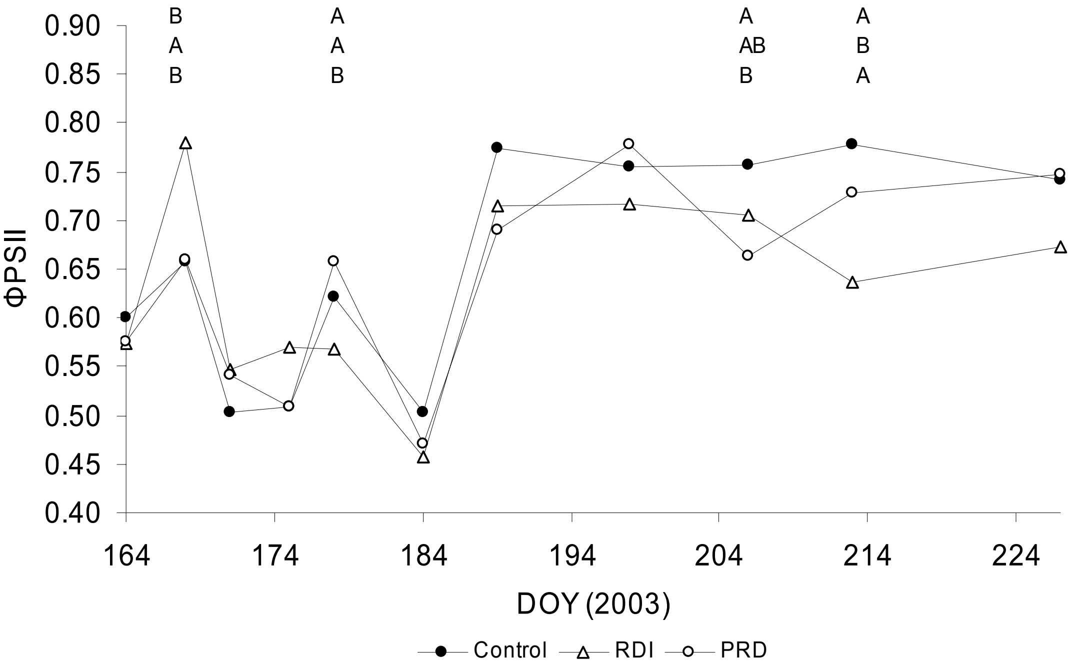


Figure A2.6: Quantum yield of electron transport at Photosystem II (ΦPSII) measured during 2003 for C, DI and PRD treatments. Symbols are the average of three measurements per tree. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

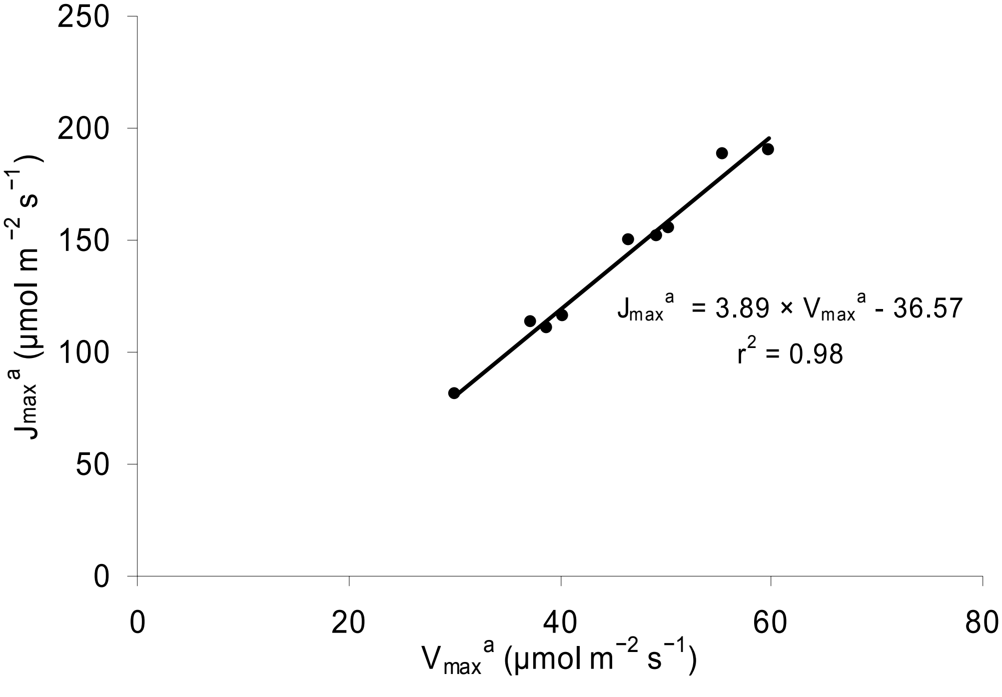


Figure A2.7: Relationship between maximum rate of carboxylation ($V_{cmax}{}^{a}$) and electron transport ($J_{max}{}^{a}$) from A-C_i data. Linear function described by $J_{max}{}^{a}$ = 3.8887 × $V_{cmax}{}^{a}$ – 36.567, r^2 = 0.98, n = 9.

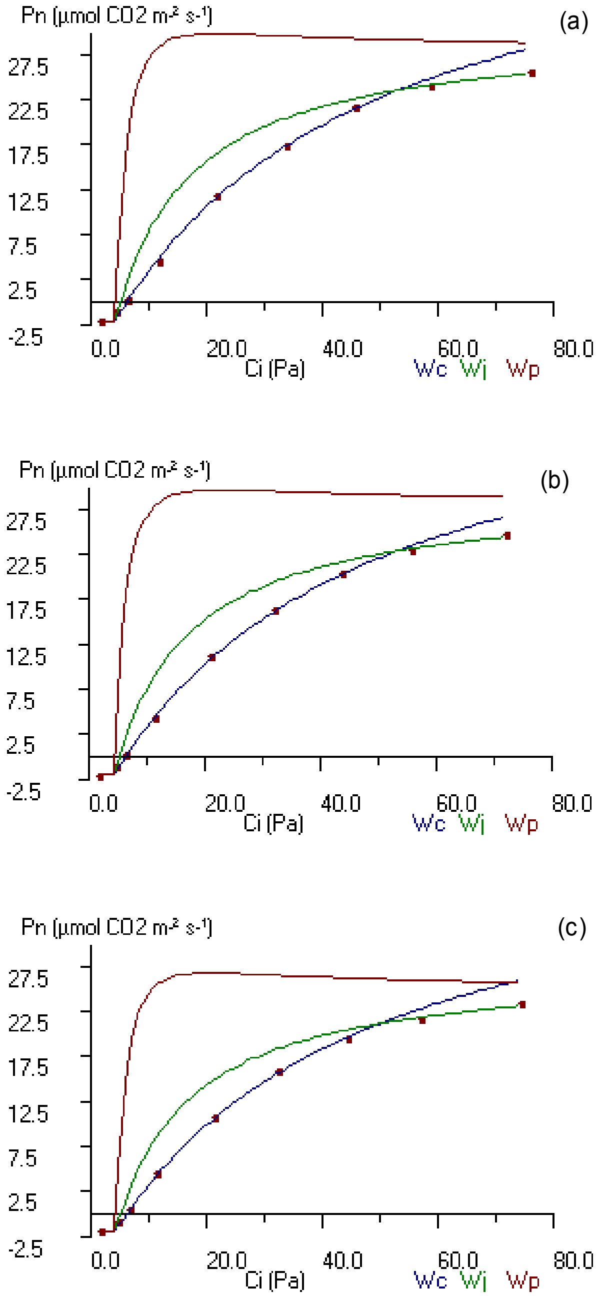


Figure A2.8: Construction of the A-C_i curve. The points represent the values of P_n and C_i recorded values calculated W_c model characterizes the carboxylation activity when the rate of carboxylaton is limited on by activity of Rubisco, W_j model represents the electron transport limiting photosynthesis by regeneration of Ribulose-biphosphate; and W_p model carboxylation is limited by regeneration of inorganic phosphate (TPU limitation). a, b and c represents C, DI and PRD treatments respectively.

CHAPTER 3

SPECTRAL REFLECTANCE AS A MEAN TO SCREEN PLANT WATER POTENTIAL IN SWEET CHERRY TREES

3.1. Abstract

Monitoring plant water status is a practice that helps growers make decisions about irrigation scheduling and managing plant water stress within certain thresholds.

This study was conducted to identify reflectance wavelengths and midday stem water potential (ψ_{stem}) in sweet cherry trees (*Prunus avium* L.) as indicators of plant water status.

In 2003 and 2004 tree water status was characterized as ψ_{stem} and measured using a pressure chamber (Model 610, PMS, Corvallis, OR). Leaf reflectance was measured in the wavelength range between 350 and 2500 nm with a FieldSpec FR spectrophotometer ASD assembled to a leaf clip probe (Analytical Spectral Devices, Inc., Boulder, CO, USA). Pearson's correlation coefficient (r) and partial least squares (PLS) with cross validation (cv) were used to determine the combination of reflectance wavebands (10 nm resolution) that best explains variation in stem water potential.

Manipulation of the raw reflectance data such as smoothing and first and second derivatives did not contribute significantly to a better performance of the regression model. The majority of wavelengths correlated with water status was

in the range of visible light (green, yellow and orange spectra, 400 to 700 nm range). The best PLS model developed to predict ψ_{stem} in sweet cherry used six wavelengths (between 550 and 710 nm) with a regression coefficient (r^2) of 0.75 and a squared error of prediction (SEP) equivalent to 0.14.

Leaf reflectance showed promise for screening plant water status, especially in the water status range commonly observed in commercial sweet cherry orchards, where moderate water stress occurs.

3.2. Introduction

Remote sensing has become an important tool to monitor parameters such as yield (Lambert *et al.*, 2004; Magri *et al.*, 2005), leaf area (Elwadie *et al.*, 2005) and nitrogen deficit (Xue *et al.*, 2004; Schlemmer *et al.*, 2005). Identifying the link between water status and a parameter that can be automatically measured in sweet cherries would help to develop tools that can monitor water status in different areas of the orchard.

Plant water status is the quantification of the condition of water in a plant relative to its requirement. The concept integrates the effects of available soil water, evaporative demand, and the hydraulic fluxes within the soil-plant-atmosphere continuum (Chalmers *et al.*, 1983; Spomer, 1985). Researchers have adopted the ψ_{stem} as a primary parameter for detecting water stress in plants and it has been proposed as the standard measurement of water status for irrigation management (Naor, 2000; Shackel *et al.*, 1997). According to

Shackel (2004), midday is the best time to measure ψ_{stem} because it corresponds to the time of maximum plant water stress or maximum water deficit.

Spectrophotometric detection has been used for sensing several types of plant stress or damage caused by insects, diseases, drought, ozone, mineral toxicity, nutrient deficiency, adverse temperatures, growth regulators and soil salinity (Gaussman and Quisenberry, 1990). Spectroscopy measurements have the advantage of being a low cost, rapid and nondestructive analysis with minimal sample preparation (Sohn *et al.*, 2003). According to Davies *et al.* (2001) the rapid development in the field of micro-spectrometers has made cheap spectrometric sensors a reality. On the other hand, regular monitoring of ψ_{stem} in commercial orchards may be time consuming and require trained personnel. Therefore innovative means for determining plant water status such as spectrometric reflectance have the potential to facilitate the measurements in the orchard avoiding destructive and time consuming techniques.

Unfortunately the majority of the literature available reports on dried and ground leaves with few trials on wilting leaves. In research utilizing drying leaves, the samples are usually detached from the plant, and few controls are performed to standardize the physiological changes in the leaf that can influence the recorded responses (Yu *et al.*, 2000; Min *et al.*, 2004). Most of the research linking plant water status and visible and near-infrared reflectance (700 to 300 nm range) investigate certain wavelengths related with water stress vegetation at risk of fire, with emphasis on natural grasslands, pine forests and other extensive forests areas (Card *et al.*, 1988; Carter, 1997).

Furthermore, a large amount of the research on water stress has been done in the laboratory, while little work inking reflectance and plant water status has focused on field crops. Ripple (1986), working on snapbean (*Phaseolus vulgaris* L) leaves, reported significant correlations between leaf water potential and both near infrared and red reflectance.

Although absorption by water is relatively weak between 700 and 1300 nm (Carter, 1991) most of the research had reported a reflectance increase in the 400–1300 nm range with a proportional increase between 1300 and 2500 nm range. It is known that chlorophyll and other accessory pigments absorb strongly between 400 and 700 nm so that reflectance in this range is typically low. Therefore the changes in reflectance in this range might be explained by the turnover of chlorophyll in healthy plants (Hendry *et al.*, 1987). At the tissue or plant level, diurnal fluctuations in the concentration of chlorophyll, seasonal fluctuations in perennial leaves and fluctuations in response to change in light quality and quantity have been reported.

A review by Gaussman and Quisenberry (1990) concluded that water stress in orange, soybean, corn and cotton leaves caused an increase in the reflectance of the leaf throughout the 500 nm to 2600 nm range, but particularly between the 1300 nm to 2600 nm. Wavelengths around 1650 or 2200 nm, and wavelengths 540, 850, 1450, 1640 and 1950 nm are affected by water stress while wavelengths such as 1100 nm and 2000 nm show small changes with water stress. Baldini *at al.* (1997) reports that, on peach and olive leaves, reflectance measured from the adaxial and abaxial surface was well correlated with water content measured from fresh to progressive dehydrated leaves, with good correlation reported in the PAR and NIR region of the spectra. Carter (1991) described substantial increases in reflectance sensitivity to water stress at visible (400 to 700 nm range) and far-red and very-near infrared wavelengths (650 to 1000 nm range). Carter (1993), working on switchcane (*Arundinaria tecta* [Walt.] Muhl,), found that wavelengths between the ranges 506–519, 571–708 and 1119–2508 nm are related significantly to dehydration. However, the author concluded that increased reflectance in the visible spectra (400 to 700 nm range) is the most consistent leaf reflectance response to plant stress. Alfalfa water stressed canopies showed a lower spectral reflectance in the NIR and red wavebands when compared with unstressed canopies (Moran *et al*., 1989) while for the same crop, Shakir and Girmay-Gwahid (1998) concluded that in the wavelength range of 850–1150 nm the stressed plots showed lower reflectance than unstressed plots. However, the same authors found that the reflectance of stressed plots was higher above 1150 nm.

Researchers have used mathematical smoothing methods to decrease the noise to signal ratio of reflectance data. There are several methods used for this purpose: running mean, Savitzky-Golas smooth, binomial, Fourier and Gaussian (Hruschka, 2001; Sohn *et al.*, 2003). Many past studies have suggested that empirical estimates of leaf biochemisty using first or second derivatives of the data may be useful (Card *et al.*, 1988; Curran, 1989). Although several techniques may give equivalent results (Hruschka, 2001; Sørensen *et al.*, 2005), the optimal smoothing transformation and other pretreatments of spectra must be determined from trials.

There is no standard methodology for the statistical analysis of spectrum reflectance data, and the literature reports the use of stepwise regression solely (Curran, 1989; Jacquemoud *et al.*, 1995) or contrasted with band depths analysis (Curran *et al.*, 2001; Kokaly *et al.*, 1998). Also different multiespectral vegetation indices such as moisture stress index (MSI), water index (WI) (Peñuelas, 1997), NDVI (Aldekheel *et al.*, 2004; Li *et al.* (2001), NDWI (Gao, 1996) and their comparison with generated new indices (Datt, 1999) or relative drought index (RDI) and relative water content (RWC) (Ceccato *et al*., 2001) have been reported in the literature. To analyze the data, different methodologies have been reported such as: canonical correlation (Baldini *et al*., 1997), correlation with a single wavelength (Bowman, 1989), and sensitivity analysis and reflectance change to detect water stress (Carter, 1993).

The objective of this study was to determine correlations between ψ_{stem} and spectral reflectance measured in a sweet cherry orchard. The specific objectives of this study were to:

1. Identify reflectance wavelengths that correlate with ψ_{stem} as an indicator of water status.

2. Evaluate the effect of mathematical smoothing in the selection of wavelengths

3.3. Materials and methods

3.3.1. Site description

The experiment was conducted at the Irrigated Agriculture Research and Extension Center of Washington State University located in Prosser, Benton County, Washington State, U.S.A., at an elevation equivalent to 256 m.a.s.l; latitude of 46º 15’ N and longitude of 119º 45’ W. The soil is Warden silt loam (coarse-silty, mixed, mesic, Xerollic, Camborthid). The experiment is part of a broader study that investigates the physiological response of sweet cherry to various deficit irrigation strategies (see Chapter 2, this volume). The orchard was managed in such a way that we were able to induce water stress to the trees in different degrees, throughout the 2003 and 2004 irrigation seasons.

3.3.2. Water status characterization

Midday stem water potential (ψ_{stem}) characterized tree water status and was measured in situ in three leaves per tree, between 1200 and 1300 hours using a pressure chamber (Model 610, PMS, Corvallis, OR). Stem water potential was measured in the field on eight and twelve dates in 2003 and 2004, respectively, taking a leaf near the stem that had been inside a bag made of foil-laminate material with a zip-lock type opening on the top (stem water potential bags, PMS, Corvallis, OR). The leaf was covered for at least 2 hours (equilibrium period) before the measurement (Scholander *et al.*,1964) (Figure 3.1).

3.3.3. Spectral measurements

Reflectance was measured using a dual-beam scanning field spectrometer UV-Vis-NIR model FieldSpec FR spectrophotometer ASD's assembled to a leaf clip probe (Analytical Spectral Devices, Inc.,Boulder, CO, USA) on the upper side (adaxial side) of the leaf at 1–nm intervals. The samples were taken and measured around ±1 hr solar noon. A total of 540 samples were analyzed (20 times × 3 treatments × 9 leaves per treatment). Reflectance measurements were performed in the wavelength range 350 to 2500 nm at 1 nm intervals. Before each measurement, 10 reference scans were taken on a white ceramic standard and 20 photometric scans were then collected and averaged on each sample (Figure 3.2).

Water potential and spectral reflectance were measured on the same trees in parallel in 1–year old branches.

3.3.4. Spectral transformations and calibration

The original spectra consisted of relative reflectance values measured at 2151 wavelengths between 350 and 2500 nm, inclusive. Data reduction was to decrease the number of wavelengths (independent variable) avoiding overfitting the model, and to decrease time-intensive computations. In order to reduce the data, spectral averaging was performed at intervals of 10 nm from 350 nm to 2500 nm, following the procedure described by Ingleby and Crowe (2000). Thus, the new spectrum consisted of 216 reflectance values at 350, 360, 370,…2500 nm, inclusive. As suggested by Hruschka (2001), the raw reflectance data with no further transformation (RawSTR) was manipulated to obtain the first difference (slope) and second derivative, in this paper referred as the numerical solution of the derivative (Raw1stDer and Raw2ndDer respectively).

Slope from the raw data was calculated using the finite-difference method. For both first and second derivative the interval gap was equivalent to 10 nm. The reduced raw data from RawSTR values was smoothed in order to reduce noise and to evidence patterns that might be covered by the raw spectra. As suggested by Sohn *et al.* (2003), the raw data was smoothed using the algorithm of Savitzky-Golas (SG STR).The first derivative of the Savitzky-Golas smoothed data was evaluated (SG1stDer) and the second derivative of the smoothed data was also calculated (SG2ndDer) (Hruschka, 2001). All the data manipulation and calculations were performed using the software package PeakFit version 4.12 (SeaSolve Software Inc.1999–2003 Richmond, CA, USA).

3.3.5. Statistical analysis

Pearson's correlation coefficient (r) was calculated across all the treatments and sampling dates to determine associations between reflectance measured in narrow wavebands and the midday water potential of the stem. Values for r from the linear relationship between spectral reflectance and leaves were plotted against the individual denominator wavebands. The r value indicates the fraction (0–1) of variability in stem water potential that can be accounted for by plant reflectance features. Linear regression analysis was used to identify relationships between the waveband ratio with maximum r and changes in stem water potential across the different treatments and sampling dates.

Because of the potential for inflation of r^2 values (over-fitting data) when independent variables are autocorrelated, as in the case of spectral data, partial least squares (PROC PLS procedure in SAS) was used to generate a calibration model with a combination of reflectance wavebands (10 nm resolution) that best explained variation in stem water potential. (V.8.2; SAS Institute, Inc., Cary, NC, USA). The model used was a linear equation of the form:

$$\psi_{\text{stem}} = b_0 + b_1R_1 + b_2R_2 + \ldots + b_pR_p \tag{3.1}$$

where:

b_o = regression coefficient for the intercept

b_1, b_2 … = regression coefficients for the b_i wavelength, and

R_1, R_2… =relative reflectance or derivative from reflectance at wavelength 1, 2, etc. (%)

The first step considered 216 wavelengths with their respective “weights” and Variable Importance for Projection (VIP number). Calibration was performed using cross-validation (CV) segments where calibration models are subsequently developed on parts of the data and tested on other parts. One at-a-time (“leave one out”) cross validation method was used, equivalent to the recomputation of a PLS model for each input observation. The number of factors was determined minimizing using the predicted residual sum of squares (PRESS). The model comparison test is based on a rerandomization of the data. The SEED value for the randomization was based on the clock system.

The selection of the most appropriate model was done considering that practical applications such as future development of sensors will favor models with fewer parameters. Further, the model with smaller number of regressors presents advantages over other models. The model development will consider the decrease of regressors from 216 wavelengths (full spectra) to twelve wavelengths and then six wavelengths (regressors). The models will be reduced using the VIP number. According to Wold (1994) if a predictor has a relatively small value of VIP, then it is a prime candidate for deletion. Usually a value less than 0.8 is considered to be "small" for the VIP.

The regressor with higher VIP will be kept and the rest will be deleted from the model. The final decision was made comparing calibration squared error of prediction (SEP) and regression coefficients (r^2) data with different number of regressors and data treatment.

3.4. Results and discussion

3.4.1. Stem water potential

The average ψ_{stem} during 2003 was equivalent to −1.10 MPa while ψ_{stem} for 2004 was approximately equal to −0.93 MPa. The higher ψ_{stem} during 2004 represents a less water stressed status than on the previous year (see Chapter 2 this volume for details). The standard deviations were similar for 2003 and 2004 years (0.27 and 0.26 respectively). Data from year 2003 shows a larger range of stem water potential than 2004 (Table 3.1). The values of ψ_{stem} measured during 2003 fluctuated between −0.69 MPa and −1.71 MPa while during 2004 the values of ψ_{stem} ranged between −0.51 MPa and −1.48] MPa. The series of ψ_{stem} collected were within the range of water potentials reported by Shackel *et al.* (2000) for other *Prunus* species with commercial purposes.

3.4.2. Reflectance spectra data

The measured reflectance spectra for all the samples during 2003 and 2004 showed a common pattern similar to most green vegetation. The spectra shows a relative peak around 500 nm, the characteristic 'red edge', a plateau on the near-infrared band radiation (700 to 1300 nm), and two characteristic regions with peaks around 1600 nm and 2200 nm (Figure 3.3).

The influence of different wavelength regions on the predicted water status can be assessed by correlation plots (Sørensen and Dalsgaard, 2003). Figure 3.4 shows the correlation coefficient (r) spectra of reflectance with ψ_{stem} with the raw data. The correlation was negative in the whole spectra, evidencing that the reflectance of the leaf diminished with the decline on ψ_{stem}. This inverse proportionality is concordant with the literature and it is primarily explained by the decrease of the leaf water content related with the lower leaf water potential, with lower 'water depth' to absorb the emitted radiation (Carter, 1991). The region around 510 nm and 720 nm (green to red colors) was well correlated with ψ_{stem}, with a maximum r equivalent to −0.76 occuring at 600 nm.

Data manipulation such as smoothing and first and second derivatives caused the curve of the derivative of the reflectance data to become flat, with fewer waves caused by the elimination of small peaks after the smoothing treatment (figure not shown). The Savitzky-Golas algorithm smoothed and virtually eliminated small peaks that are related with the plant water stress status, similar to the effect reported by Bolster *et al.* (1996).

3.4.3. Model generation

Six models were generated using PLS regression analysis: three of them using the averaged raw data (RawSTR, Raw1stDer and Raw2ndDer) and three using Savitzky-Golas smoothing algorithm (SGSTR, SG1stDer and SG2ndDer).

Calibration statistics of PLS models for determining of ψ_{stem} in sweet cherry trees including regressor wavelengths, intercept values (b_o), regression coefficients (r^2), standard error of prediction (SEP) for all models evaluated with cross calibration data are listed in Table 3.1. For the six models calibrated using 216 wavelengths averaged every 10 nm in the 350 to 2500 nm range, the r^2 ranged between 0.71 and 0.75 with fairly similar SEP (0.14 and 0.15), independently of the data set used. Using the derivative of raw data (Raw1stDer) the number of factors decreases from four to two, with improved regression coefficients which are beneficial for the model. By using the derivatives of smoothed data the number of factors decreases only when using the second derivative, with lower regression coefficients.

Interestingly, the mathematical treatment to smooth the data did not improve the model performance but increased the number of factors use on the PLS model. The best model to predict ψ_{stem} used the Raw1stDer data and use two factors on the PLS model with a coefficient of regression (r^2) of 0.75 and with a SEP equivalent to 0.14. Using the Savitzky-Golas algorithm to smooth the data resulted in a lack of prediction of the model.

In order to check the robustness of the models, twelve wavelengths (approximately 5% of the regressors) having the highest VIP value were selected representing the most correlated wavelengths obtained by PLS. This statistical treatment is utilized for the development of model using fewer wavelengths (reduced model). Additionally, reduced models might lead to interesting information for the future development of field devices to screen plant water status using simpler software and less costly spectral detectors. Figure 3.5 shows graphically the selection of the highest 12 VIP numbers for the RawSTR data. Similarly, twelve wavelengths with highest VIP were selected for the RawSTR, Raw1stDer, Raw2ndDer, SGSTR, SG1stDer and SG2ndDer.

Using twelve wavelengths, the best model to predict ψ_{stem} was the one using Raw1stDer data, with three factors on the PLS model, resulting on similar coefficient of regression and standard error of prediction than using the whole spectra. Similar to the previous step using 216 wavelengths, the application of the Savitzky-Golas algorithm resulted on a lack of prediction of the model, the increment of PLS factors and the increase of the SEP. In general, using 12 wavelengths it was possible to keep regression coefficients within a reasonable range [0.69–0.75] when using raw data or first derivative. The second derivate for both raw and smoothed data did not show a good correlation coefficient (0.59 and 0.58 for raw and smoothed data respectively) and showed a higher SEP equivalent to 0.18. Also the number of factors decreased when the first derivative of the raw data, was used while SEP was not affected using 12 wavelengths. When using smoothed data the number of factors decreased only when using the first and second derivative, with a parallel detriment on the regression coefficients. Consequently, the smoothing and second derivative treatments did not improve the regression coefficients when dealing with the twelve selected wavelengths.

In order to further reduce the wavelengths used by the model, the six highest VIP values (equivalent to approximately 2.5% of the original spectra) were selected, representing the most correlated wavelengths obtained by the second PLS models. Figure 3.6 shows graphically the selection of the highest six VIP numbers for the RawSTR data. Similarly, six wavelengths with highest VIP were selected for the RawSTR, Raw1stDer, Raw2ndDer, SGSTR, SG1stDer and SG2ndDer.

Table 3.3 shows the third set of models developed using PLS with only six wavelengths. The best model to predict ψ_{stem} was again RawSTR data, using six factors on the PLS model with a coefficient of regression equivalent to 0.75 and standard error of prediction of 0.14. Similarly to the first and second group of models, using the Savitzky-Golas algorithm resulted in a lack of prediction of the model, the increment of PLS factors and the increase of the SEP.

Six wavelengths between 550 and 710 nm (710, 700, 640, 550, 570 and 600 nm) with a regression coefficient (r^2) of 0.75 and a squared error of prediction (SEP) equivalent to 0.14 were selected and used for the PLS model. The regression coefficients for the PLS model followed the order 640 nm > 570 nm > 710 nm >700 nm > 550 nm > 600 nm.

Figure 3.7 shows the linear relationship of the main components of the selected model (wavelengths from the visible range) plotted versus ψ_{stem} showing a fair correlation (r^2) between reflectance and plant water status. However, it is important to stress that only the weighted combination of the six wavelengths obtained by partial least squares explained the variation on ψ_{stem}.

The plotted validation results for estimated and measured ψ_{stem} for the model is shown in Figure 3.8. The selected wavelengths related with stem water potential are in the visible range, evidencing a detriment on the chlorophyll pigments related with water stress. Our data suggest that reduction of reflectance in the visible range occurs when leaves are affected by water stress and that these changes have a stronger statistical relationship than the alteration occurring in the visible and NIR range. This might be related with stress induced chlorophyll loss, and it is coincident with the bands identified by Carter *et al.* (2001) as indicators for leaves affected by different stressors. It is important to consider that destruction of chlorophyll occurs not only in the premature or senescent stages but probably throughout the life span of all plants, including diurnal and seasonal changes (Hendry *et al.*, 1987). This is coincident with Carter (1991) and Carter (1993) who reported the visible spectra to be the most consistent leaf reflectance response to plant stress.

Our results showed better performance than similar a study by Min *et al*. (2004), authors that also correlated leaf water content with spectral reflectance in citrus leaves. This might be explained with the use of in vivo and in situ collection of the leaf reflectance and water status that may eliminate the errors caused by dehydration and discoloration attributed by Min *et al*. (2001) as a possible source of errors.

The conclusions from this report are coincident with Baldini *et al*. (1997), who found a significant relation between PAR and water content measured from progressively dehydrated peach and olive leaves, and are consistent with Moran *et al.* (1989), who found that water stressed canopies of alfalfa have a lower spectral reflectance in the NIR and red wavebands when compared with unstressed canopies.

Mathematical smoothing treatment does not seem to be necessary to estimate water potential in sweet cherry trees. Moreover, using the first and second order differences of the smoothed data weakened the predictive ability of the model. According to Bolster *et al.* (1996), data smoothing offsets the location of constituent absorption peaks and this may affect the correlation when using first or second derivatives.

3.5. Conclusions

Using six wavelengths of the visible and near infrared spectra, it was possible to determine ψ_{stem} through leaf reflectance measurements in a two-season experiment carried out in a sweet cherry orchard. The calibration-prediction model developed using a single sample set showed a good calibration statistics (r^2) of 0.75 and a squared error of prediction (SEP) equivalent to 0.14 MPa.

These results demonstrate that leaf reflectance could be used for screening plant water status, especially in a water potential range commonly observed in commercial sweet cherry orchards, where moderate water stress occurs. These results might lead to the development and design of prototype field devices to screen plant water status using simple software and inexpensive spectral detectors working only in the visible range. Improvements on testing within the range of the selected wavelengths are expected to be required as more locations and varieties are added to the calibration. Additional progress can be achieved evaluating the entire canopy reflectance where leaves are randomly arranged.

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Table 3.1: Calibration statistics for PLS models using 216 reflectance wavelengths between 350 and 2500 nm.

Calibration parameters	Number of factors	SEP	r^2	b_o
RawSTR	4	0.14	0.73	−1.92
Raw1stDer	2	0.14	0.75	−2.95
Raw2ndDer	2	0.14	0.75	−2.29
SGSTR	4	0.15	0.73	−1.99
SG1stDer	5	0.14	0.75	−4.39
SG2ndDer	2	0.15	0.71	−3.89

Table 3.2: Calibration statistics for PLS models using twelve selected wavelengths between 350 and 2500 nm.

Calibration parameters	Number of factors	SEP	r^2	b_o	wavelengths (nm)					
RawSTR	5	0.15	0.70	−2.15	600	590	580	610	570	700
					620	630	560	640	710	550
Raw1stDer	3	0.14	0.75	−2.59	490	480	660	450	650	460
					500	440	640	470	630	720
Raw2ndDer	2	0.18	0.59	−0.82	1720	640	1710	480	670	700
					750	630	740	760	1200	680
SGSTR	5	0.15	0.69	−2.59	620	630	610	640	600	650
					590	660	580	570	670	560
SG1stDer	3	0.15	0.69	−3.17	550	450	770	440	1120	2390
					2380	2330	2400	760	2340	2370
SG2ndDer	1	0.18	0.58	−2.72	400	410	570	560	390	580
					550	420	590	380	540	600

Table 3.3: Calibration statistics for PLS models using six selected wavelengths between 350 and 2500 nm.

Calibration parameters	Number of factors	SEP	r^2	b_o	wavelengths (nm)					
RawSTR	6	0.14	0.75	−1.68	710	700	640	550	570	600
Raw1stDer	6	0.16	0.67	−1.06	490	660	500	480	460	720
Raw2ndDer	6	0.19	0.54	−0.45	1710	1720	640	670	480	630
SGSTR	5	0.16	0.69	−2.61	670	650	560	610	620	640
SG1stDer	2	0.18	0.58	−3.30	550	450	2380	770	2370	2390
SG2ndDer	1	0.18	0.57	−3.76	570	580	560	590	400	550

Figure 3.1: Detail of the pressure chamber with the leaf inserted prior to the measurement of ψ_{stem}.

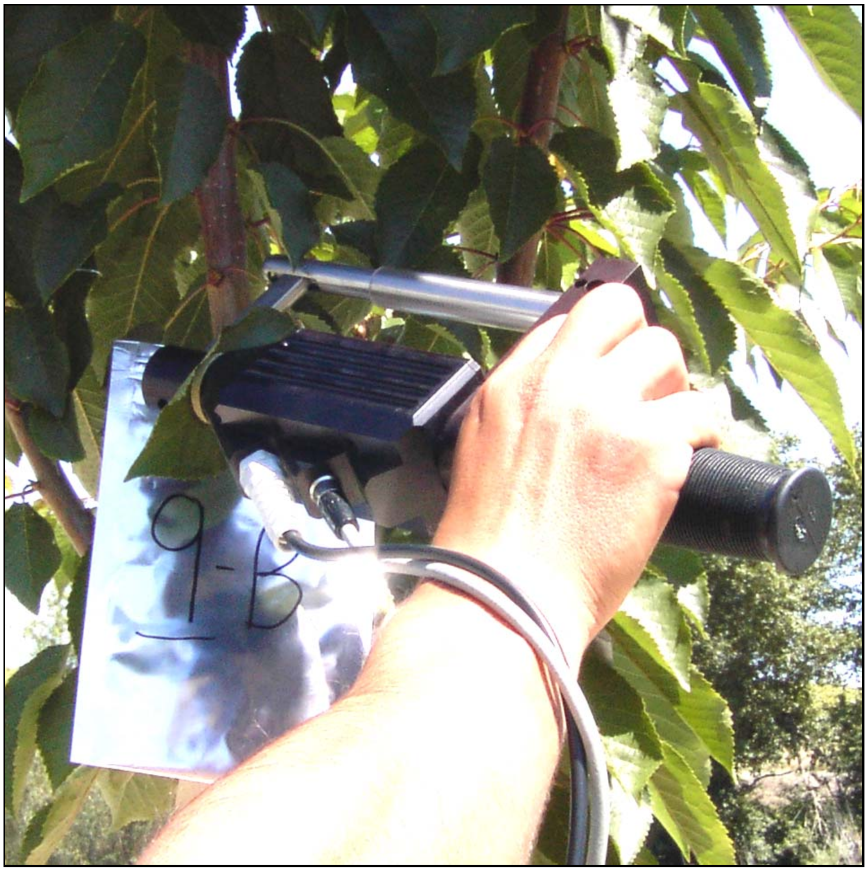

Figure 3.2: Detail of the leaf clip probe sampling the reflectance spectra of a sweet cherry leaf. In the back, aluminum bag used to equilibrate the leaf previous to the measurement of ψ_{stem}.

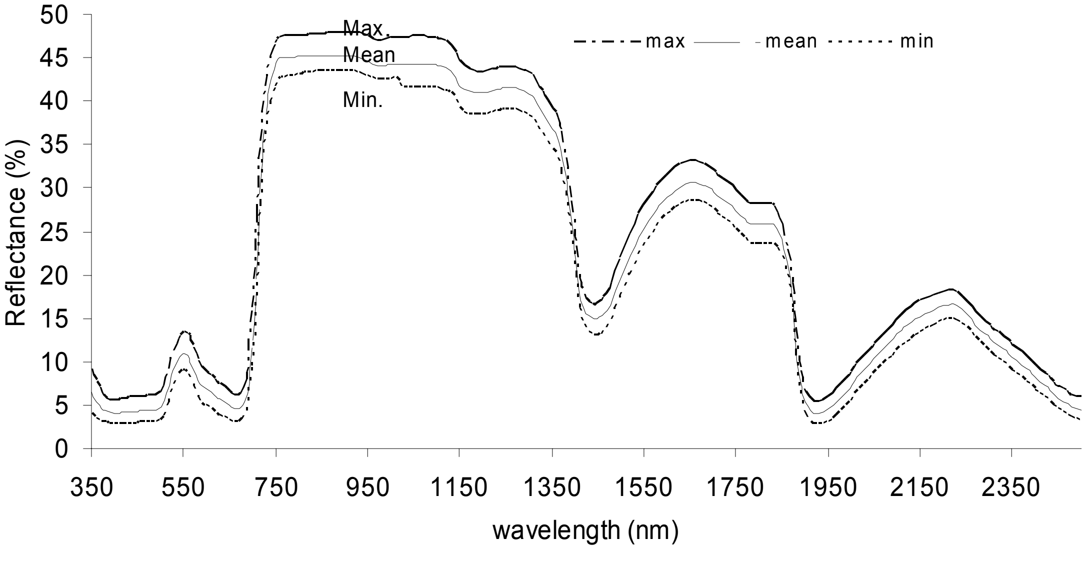

Figure 3.3: Mean, maximum and minimum reflectance curves averaged the raw data (RawSTR) values.

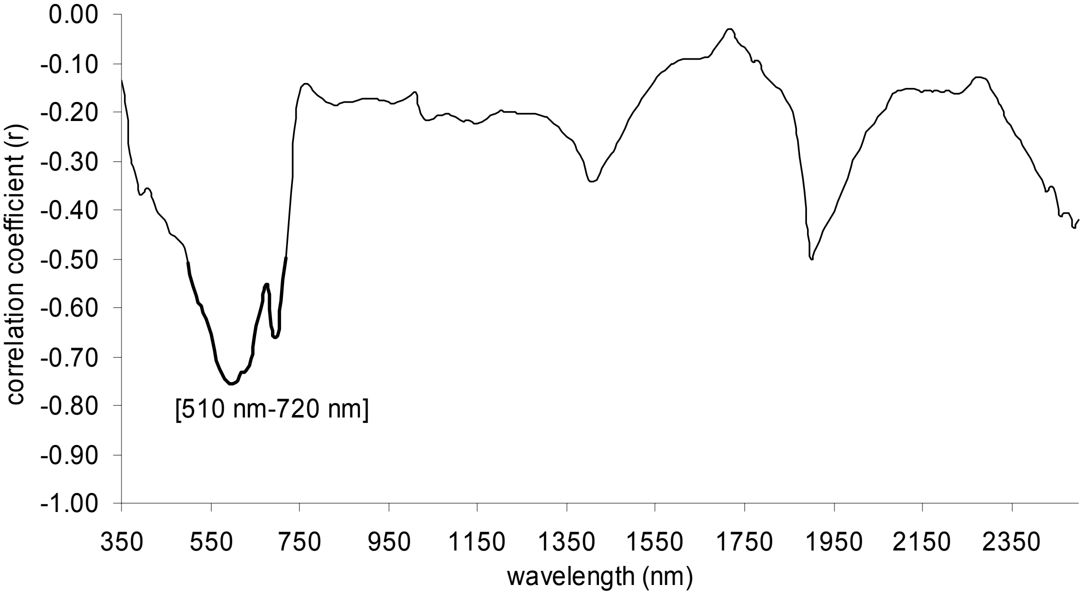

Figure 3.4: Correlogram relating spectra of reflectance with ψ_{stem}, for the raw data. The bold line represents the range between 510 and 720 nm were the reflectance wavelength is highly correlation with water status.

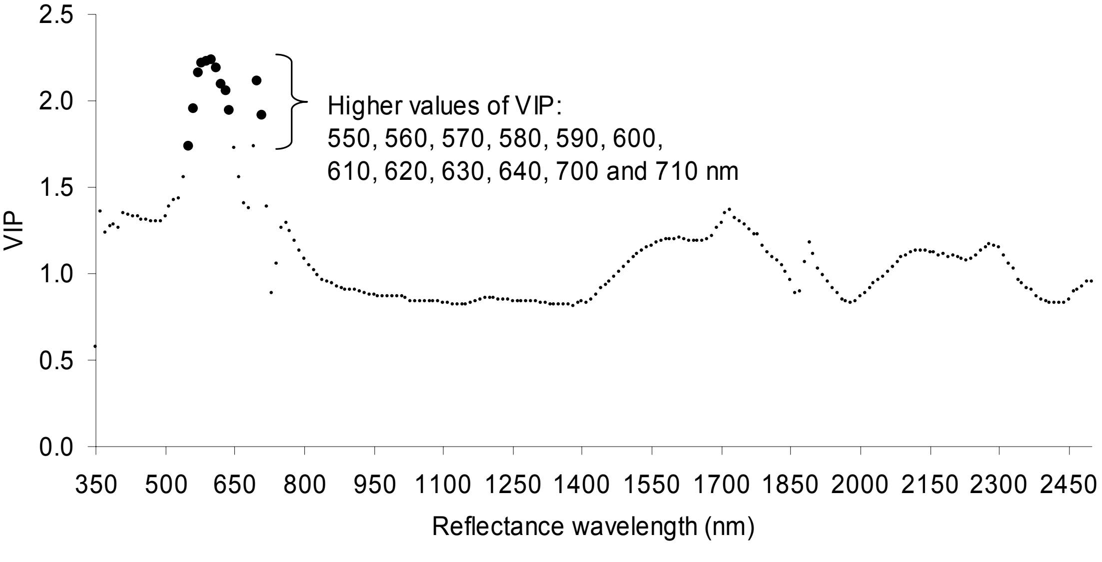

Figure 3.5: Value of importance of prediction (VIP) numbers versus spectra of reflectance for the raw data. The bold numbers represent the twelve highest values of VIP selected for the next step of the model.

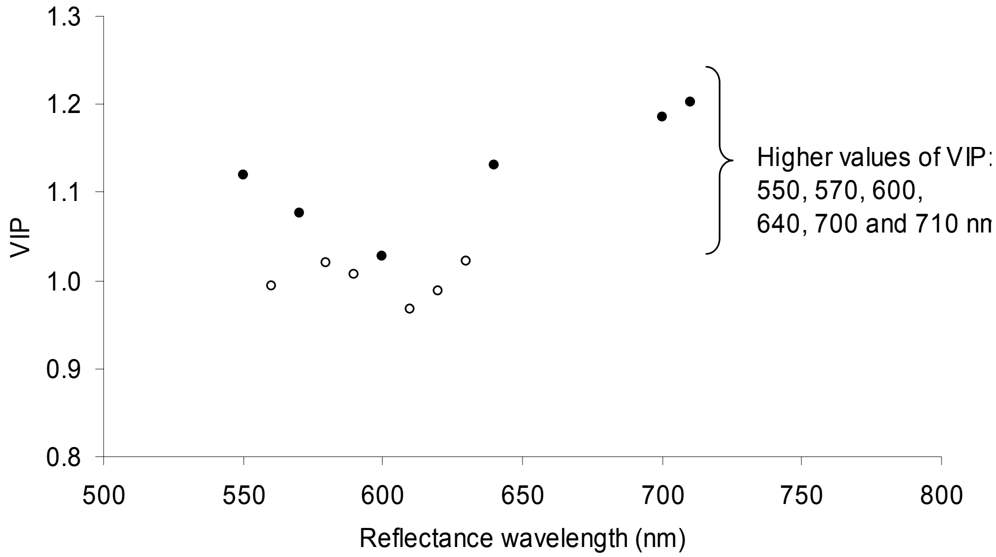

Figure 3.6: Value of importance of prediction (VIP) numbers versus spectra of reflectance for the raw data using only 12 walengths selected from Figure 3.5. The bold numbers represent the six highest values of VIP selected for the next step of the model.

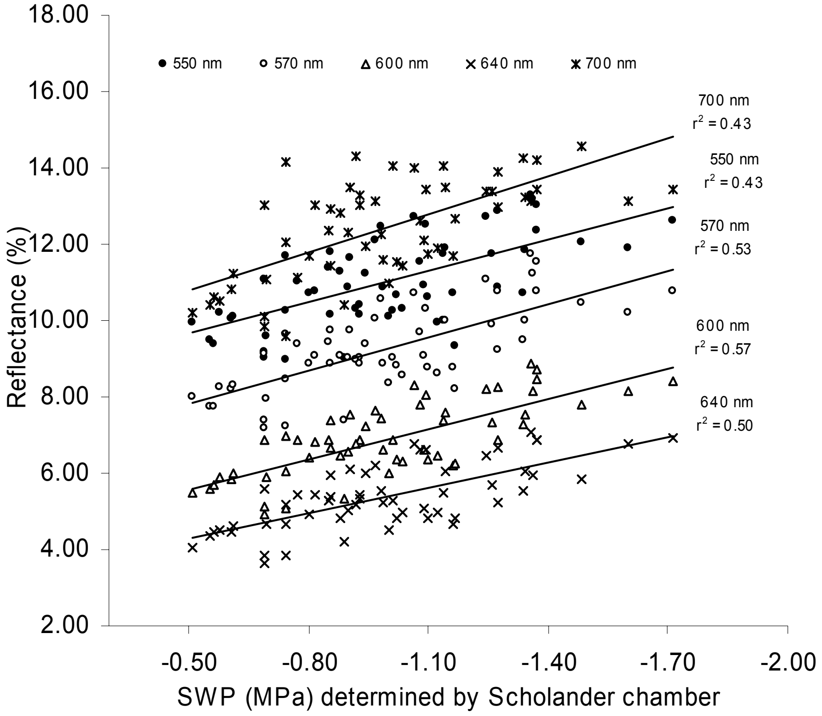

Figure 3.7: Correlation of reflectance at five visible wavelengths as a function of ψ_{stem} in sweet cherry leaves.

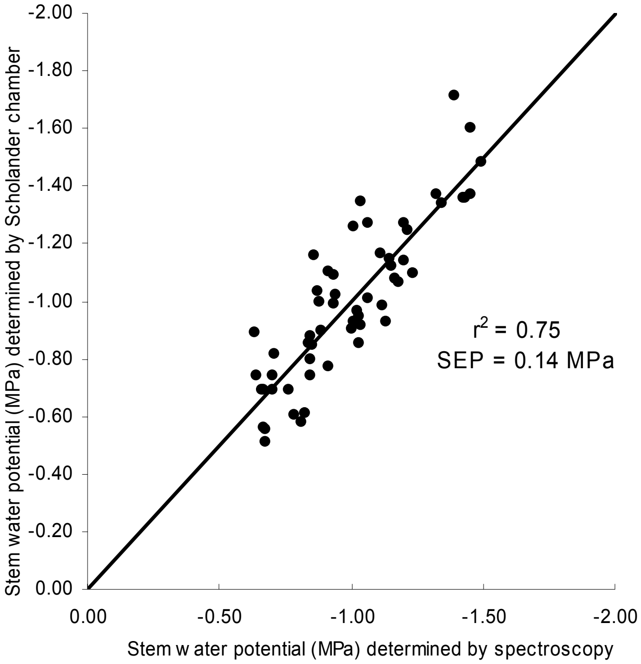

Figure 3.8: Validaton results obtained for ψ_{stem} using the raw data and six wavelengths (RawSTR 6 regressors).

CHAPTER 4

THE EFFECT OF DIFFERENT DEFICIT IRRIGATION STRATEGIES ON TRANSPIRATION OF YOUNG SWEET CHERRY TREES MEASURED USING A GRAVIMETRIC LYSIMETER

4.1. Abstract

An above-ground, load-cell weighing lysimeter was designed, tested, and utilized for estimating transpiration from young sweet cherry trees (*Prunus avium* L.) subjected to three different irrigation regimes (C-control, DI-deficit irrigation and PRD-partial root-zone drying) at Prosser, Washington State, USA. Each system was comprised of a rigid copolymer Polypropylene PP U.V. stabilized bin (ca. 1.21 m × 1.21 m × 0.60 m) and a high precision, 4–load-cell floor scale coupled to a datalogger. Soil evaporation was practically eliminated by sealing the top of the bin with a 5–cm thick polystyrene isolating layer. An opening for the trunk was sealed with closed-cell foam. Bin mass was determined intermittently and tree transpiration was estimated by applying mass balance to the system. Under well-watered conditions during tree establishment, canopy transpiration ranged from 10 to 13 kg m^{-2} day^{-1} (leaf area based) while tree evapotranspiration (ET) ranged between 5 to 6 kg m^{-2} day^{-1} (ground area based). Stomatal conductance was affected by the irrigation treatments and correlated with VPD and air temperature ($p < 0.001$). Over the entire season of 2004, total transpiration from C trees was ca. 846 mm, and deficit-irrigated trees

exhibited between 31% and 35% less transpiration (DI was 585 mm, and PRD was 546 mm respectively). Tree evapotranspiration related to the crop reference evapotranspiration (ET : ET_o ratio) calculated for DI and PRD trees fluctuated between 0.2 starting the season and 0.95, at the middle of August. For C trees, ET : ET_o ratio increased throughout the season from 0.4 to 1.6 (early April to middle August).

4.2. Introduction

Because of its critical physiological role and singular importance in irrigation scheduling, plant transpiration has been widely studied (Allen *et al.*, 1998, Remorini and Massai, 2003). Techniques for measuring transpiration have evolved from rudimentary porometers (Levy, 1964) to sophisticated drainage lysimeters (Phene *et al.*,1991), heat pulse and balance gauges (Steinberg *et al.,* 1989; Shackel *et al*., 1992), and infrared gas analyzers coupled to whole-canopy chambers (Perez Peña and Tarara, 2004).

There are two main types of lysimeters, drainage lysimeters and gravimetric lysimeters. The common concept is the design of a unit to isolate a certain soil/air/water volume from the surrounding (McFarland *et al*., 1983). Crop water use estimates from drainage lysimeters are based on knowing the volume of water applied and determining the drainage output from the unit. In contrast, gravimetric lysimeters, generally considered to be more accurate, may be configured to continuously monitor system weight. With either approach, lysimetry is a powerful technique to investigate the different terms in the soil water balance equation with great accuracy (Allen *et al*., 1998). Moreover, with precision weighing lysimeters, where water loss is measured directly by the change of mass, evapotranspiration can be obtained with accuracy over short time periods with little or negligible disturbance to the soil and plant. However, lysimeters designed to estimate evapotranspiration often use expensive and sophisticated equipment, but lack the ability to isolate plant transpiration from system evapotranspiration (e.g., Phene *et al*., 1991).

Lysimeters have been utilized on wheat (*Triticum aestivum*) and maize (*Zea Mays* L., Liu *et al*., 2002), bell pepper (*Capsicum annum* L., Dalla Costa and Gianquinto, 2002), tomato (*Lycopersicon esculentum* Mill., Mameli *et al*., 2004) and asparagus (*Asparagus officinalis* L., Paschold *et al*., 2004). On tree fruit studies lysimeters has been previously utilized to determine K_c and water requirements in crops like asian pears (*Pyrus pyrifolia* Burm. f. Nak.; Caspari *et al*., 1993; Chalmers *et al*., 1992; Girona *et al*., 2004; Renquist *et al*., 1994), apples (*Malus domestica*; Ro, 2001), citrus trees (*Citrus* spp.; Yang *et al*., 2002), grapevines (*Vitis vinifera* L; Williams, 1999) and peach trees (*Prunus persica*; Worthington *et al*., 1989; Mitchell *et al*., 1991; Ayars *et al*., 2003).

Prudent irrigation scheduling requires actual crop water use data in relation with the reference crop evapotranspiration (ET_o) in each stage of the plant cycle (Ayars *et al*., 2003). In the literature, the relation between actual crop water use (ET) and ET_o has been estimated for scheduling irrigation and it has been also used to compare the effect of irrigation treatments, also called crop coefficient (K_c). Field determination of K_c for different crops has been traditionally estimated by using soil water balances for short periods of time, which is not an accurate method (Fereres and Goldhamer, 1990). Weighing lysimeters are an alternative method utilized most commonly for perennial crops with few reports for tree fruit species. Although K_c estimates from lysimeter experiments are often used to describe tree water use (Boland *et al*., 1993), it has been hypothesized that high values of K_c reported are unreliable and caused by micro-advection, leaf area and as a possible phenomenon unique to lysimeter trees (Stevenson, 1989).

One of the most evident effects of soil water deficit is the decrease on transpiration. This response has been primarily linked to the reduction of stomatal conductance (g_s) mediated by root-leaf signals (Downton *et al.,* 1988; Davies and Zhang, 1991). Deficit irrigation trials in olive showed that g_s limits photosynthesis in trees subjected to moderate water stress, whereas non stomatal factors influenced net carbon assimilation only under severe stress conditions (Angelopoulos *et al*., 1996). In general, because g_s is likely to be at least an order of magnitude smaller than aerodynamic conductance (g_a), tends to dominate the control of transpiration compared with aerodynamic resistance (Landsberg and McMurtrie, 1984).

According to Doorembos and Pruitt (1977) the difference between crop evapotranspiration (ET) and evapotranspiration of the reference crop (ET_o) accounts for the effect of crop characteristics on the transpiration, where water stressed crops may be evidenced by ET values significantly lower ET_o. The wide variations between crops and treatments are explained by the difference on stomatal resistance and adjustment between the different plants and waxy leaf cuticle (Allen *et al.*, 1998). Similarly, different plant architecture (crop height, crop roughness) and ground crop cover may contribute to significant variations in ET. Moreover, the role of irrigation volume (i.e., severity of stress) and the placement of irrigation water (i.e., entire vs. half the rootzone) may influence crop water use.

This paper reports on the design and preliminary testing of a system to measure and model transpiration of sweet cherry trees under three irrigation regimes. Results from the first season include the testing and validation of the methodology. For the second season results of transpiration of the trees under the three irrigation regimes (C, DI and PRD) is presented and compared with the Penman-Monteith ET_o. The purpose of this study was to determine transpiration rates and its correlation with the response of plant to water stress irrigation strategies in young sweet cherry trees in Washington State under controlled conditions. The specific objective of this study was to report crop water use and evapotranspiration of the sweet cherry trees related to the crop reference evapotranspiration for well watered compared with two water stressed treatments that differ in the placement of water, using a gravimetric lysimeter. This paper also investigates on the physiological adaptation to water stress that promotes plant adjustment to imposed water deficit.

4.3. Materials and methods

4.3.1. Site information

Research trials were conducted at Washington State University's Irrigated Agriculture Research and Extension Center (IAREC), Prosser, Washington, at an elevation of 256 m.a.s.l; latitude: 46º 15' N; longitude: 119º 45' W (Figure 4.1 and 4.2).

4.3.2. Container set up

During the winter 2003, nine dormant two year-old 'Bing'/Mazzard sweet cherry trees were planted in rigid copolymer Polypropylene PP U.V. stabilized containers (bins) (ca. 1.21 m × 1.21 m × 0.60 m; Macro Plastics Inc., Fairfield, CA). The bins were filled with sandy loam soil, pH of 6.8, bulk density 1.31 kg m^{-3} and saturated hydraulic conductivity 0.55 m d^{-1}. The dry weight of the soil volume in the containers was 1050 kg ± 45 kg, with a wet weight of 1344 ± 62 kg.

A drip irrigation system including four emitters per tree (3.78 L hr^{-1}), solenoid valves, a controller and a totalizer to record the volume applied was installed. Evaporation from the soil was eliminated by sealing the top of each bin with a 5–cm thick polystyrene insulating layer (Styrofoam^{TM}, DOW Chemical Co.) (Figures 4.3 and 4.4). An opening for the trunk was sealed with closed-cell foam.

4.3.3. Gravimetric scale and recording

A high precision, four-load-cell floor scale (Model RoughDeck^{TM} HP, Rice Lake weighing systems, Rice Lake, WI), with the voltage output coupled to a datalogger (Model CR10X, Campbell Scientifics Inc., Logan, UT), was used to determine mass changes at 10–min intervals. The data acquisition system collected the load cell analog current output equivalent to 4–20 mA.

Since the load cells voltage output is linearly related to load, the output signal was previously calibrated with a known mass in a range between 1155 and 1180 kg. Thirteen increments of calibrated steel laboratory weights (of 1.0 and 2.2 kg) were placed onto the top of each lysimeter.

4.3.4. Calculation of transpiration

Tree transpiration was calculated by applying the mass balance equation to the system, knowing the change of mass (ΔW) within a time period and the water applied by irrigation. The mass of the whole bin included the following:

$$W_{Total} = Pot + Soil_{dry} + Water + Tree \tag{4.1}$$

A mass balance applied to the system accounted for the water added to the system in the form of precipitation and irrigation and the losses due to evaporation from the soil and the transpiration from the leaves of the tree:

$$Precipitation + Irrigation + \Delta W = Evaporation_{Soil} + Transpiration_{Tree} + Drainage \tag{4.2}$$

To eliminate precipitation and evaporation from the system, the top of the bin was covered with a 5–cm thick layer of Styrofoam™.

Discarding precipitation and drainage from Equation 4.2 and rearranging the terms, the change of mass in the bin was equivalent to the gain by irrigation and the loss by transpiration from the tree:

$$\Delta W = Irrigation - Transpiration \tag{4.3}$$

Finally, rearranging terms in Equation 4.3, the transpiration of the tree was estimated as:

$$\text{Transpiration} = \Delta W - \text{Irrigation} \tag{4.4}$$

4.3.5. Irrigation treatments

During 2003, following transplanting, the trees were all watered similarly to promote good root and canopy development. In August 2003, two irrigation regimes were evaluated for their effect on tree water use: 1) daily irrigation configured to replenish transpiration and 2) a drying cycle (i.e., irrigation withheld) to observe the response of the transpiration associated with the decreasing soil water content. These regimes were imposed over a four-week period.

During 2004, two deficits (deficit irrigation, DI, and partial rootzone drying, PRD) and a control C irrigation treatments were imposed (DOY 121 until the end of the season), when treatments were imposed to evaluate the effects of irrigation water volume and placement.

The bins were lined up in two rows in a random design (Figure 4.1). Three containers were weighted continuously and the rest were weighted periodically. A gasoline propelled lift truck H45XM (Hyster, Greenville, NC, USA) was used to move and place the pots over the scales for mass recording.

4.3.6. Tree water status

Tree water status was characterized from measurements of midday ψ_{stem} and ψ_{leaf}. These were measured *in situ* using a pressure chamber (Model 610, PMS, Corvallis, OR, USA) throughout the season on three fully sunlit leaves per tree within 1 hr of solar noon. Stem water potential was measured on leaves enclosed for at least 2 hr within a sleeve of aluminum foil within a zip-lock plastic bag (stem water potential bags, PMS Instruments, Corvallis, OR). Leaf water potential was measured on leaves from the same branch within minutes of stem measurements.

4.3.7. Leaf gas exchange

Stomatal conductance and water use efficiency (WUE) were estimated from intact, recently fully-expanded leaves on 2–year-old fruiting spurs using a portable infrared gas analyzer (CIRAS-2, PP Systems, Hitchin, UK) fitted to a broadleaf cuvette with saturating radiation (1000 μmol photons PAR m^{-2} s^{-1}) provided by a white halogen lamp. For all measurements, reference carbon dioxide (CO_2) concentration was set at 370 μmol mol^{-1}. Leaf gas exchange data were recorded after steady state conditions, usually at least after 90 seconds, with cuvette flow rates between 190 and 200 cm^3 min^{-1}. The measurements were made throughout the season on three leaves per tree within 1 hour of solar noon.

4.3.8. Leaf area

Leaf area was measured by periodically sampling the active growing shoots to record the leaf area increments using a Portable Leaf Area meter CI-203 (CID Inc., Camas, WA, USA). Shoot growth was measured in parallel using a metric tape. Tree transpiration calculated from Equation 4.4 in kg was divided by the corresponding tree leaf area. A regression function relating the day of the year (DOY) and the leaf area of each pot was obtained. The function was then incorporated to the model to estimate transpiration.

4.3.9. Volumetric soil water content

The particle size distribution of the soil is 71% sand, 24% silt, and 5% clay with soil bulk density of 1.3 kg m^{-3}. Field capacity (FC) and permanent wilting point (PWP) were estimated in the laboratory using gravimetric sampling and measuring water activity with a True Psi Psycrometer (Decagon devices, Pullman, WA). The water content at field capacity (FC) and permanent wilting point (PWP) was estimated to be 0.27 and 0.12 m^3 m^{-3}, corresponding to 30 and 1500 kPa respectively. Soil moisture was measured weekly during the season 2004 with a neutron probe CPN model 503 (Campbell Pacific Nuclear Corp., Pacheco, CA, USA) using a PVC access tube (381 mm in diameter) installed by auguring a hole of the same diameter and inserting the access tube. Access tubes were installed in the middle of the two sides of the pots. The readings were taken periodically to decide irrigation events, at approximately 0.15, 0.30 and 0.45 meters from the soil surface and recorded as centimeters of water per meter and transformed to volumetric water content (cm^3 water cm^{-3} soil).

Starting from known initial water content, the loss or gain of mass resulted from transpiration or irrigation respectively. As described in Equation 4.4, to calculate the volumetric soil water content of the day j+1, the mass of water gained or lost on the day j+1 divided by the total volume of the soil was subtracted from the volumetric soil water content of the day j.

$$\theta_v(j+1) = \theta_v(j) - \frac{\Delta W(j)}{V_t} \qquad (4.\ 5)$$

4.3.10. Weather data

Weather variables (solar radiation, temperature, vapor pressure deficit and wind speed) were continuously monitored and recorded by an automatic weather station. These data were used to compare and interpret the measured transpiration with calculated FAO-56 reference crop evapotranspiration (Allen *et al.*, 1998).

4.3.11. Loading test

Load cell output linearity and accuracy were tested by incrementing and decrementing a series of standard weights to each lysimeter and noting the recorded mass change on the data logger. All lysimeter readings were the average of 40 scans taken at a 5 second frequency following the calibration method suggested by Allen and Fisher (1991).

4.3.12. Tree evapotranspiration related to the crop reference evapotranspiration

Weekly evapotranspiration of the sweet cherry trees measured (leaf area base) was related to the crop reference evapotranspiration (ET : ET_o ratio; Chalmers *et al.*, 1992).

4.3.13. Statistical analysis

The general linear model procedure (PROC GLM) was used for the analysis of variance (ANOVA) using SAS software (V.8.2; SAS Institute, Inc., Cary, NC, USA). For the gas exchange measurements and plant water potential measurements, significant differences were evaluated for the same date, statistically explained considering the interaction effect between irrigation treatment and the day of measurement (DOY).

The Tukey-Kramer method at a significance level of $\alpha = 0.05$ was used for mean comparisons among irrigation regimens, and the PROC REG procedure was used for multiple regression analysis. Stomatal conductance data from 2004 was correlated against environmental (VPD and T_{air}) and physiological variables with simple models at single-leaf level.

4.4. Results and discussion

4.4.1. Lysimeter performance

4.4.1.1. System testing

The insulation material (Styrofoam™) placed on the top of the bin was also useful for controlling the temperature inside the bin. Maximum and minimum temperatures inside the container were approximately ± 5 ^{o}C higher and lower than those recorded at a nearby weather station at both 20 and 40 cm depths, respectively. Preliminary findings showed that the insulating layer decreased the ventilation of the soil surface, resulting in a decrease of the water evaporation from the soil surface to negligible values.

The load cells exhibited strong linearity (r^2=0.99) over the tested range for both loading and unloading cycles and did not show evidence of hysteresis (Figure 4.5). Similar results were reported by Allen and Fisher (1990) using a direct load cell-based weighing lysimeter. Our system was able to resolve 0.1 kg changes in mass. On the other hand, errors were generally less than ± 0.05 kg over the loading range of 25 kg equivalent to ± 0.03 mm over a range of 17 mm soil moisture change (Figure 4.6).

The lower boundary of the resolution of the load cells might limit the practical estimating transpiration on a daily basis. However, during periods of high evapotranspiration demand, when potential maximum transpiration may be 10 to 15 kg d^{-1}, the resolution of the scale appears to have been adequate, representing less than a 1% change of the ET_o losses on a daily basis. This sensitivity and the lack of hysteresis are important because the system must account for relatively small weight losses (water losses by transpiration) and/or weight gain (water gained by irrigation) in a relatively heavy (ca. 1200 kg) system including the pot, soil, plant and water.

An example of transpiration recorded hourly for a well-watered tree is shown in Figure 4.7. The transpiration for that day was 2.4 kg m^{-2} day^{-1} (leaf area based). Little signal noise was observed when taking hourly averages of the data logger outputs, similar to the results obtained by Phene *et al*. (1991). The diurnal pattern observed was similar to that reported for apple (Weibel and de Vos, 1994) and peach trees (Shackel *et al*., 1992). Transpiration rates were related to levels of solar radiation. The inhibitory effect of clouds was apparent as dips on the typical parabolic-shaped curve (Phene *et al*., 1991; Shackel *et al*., 1992; Weibel and de Vos, 1994; Allen *et al*., 1998).

At full canopy (late August, leaf area 0.37 ± 0.1 m^2) in 2003, transpiration from a well-watered system was compared to a system in which gradual water stress was applied by withholding irrigation. Two distinct responses were observed in relation to irrigation regime: transpiration remained in a range between 10 to 13 kg m^{-2} day^{-1} (leaf area based) when the volumetric water content was kept at about 0.25 m^3 m^{-3} (i.e., well-watered scenario). Daily tree transpiration and FAO-56 ET_o followed a similar pattern during this period, suggesting that were no limitations from the soil to meet the water demand. In contrast, transpiration decreased slowly when the soil water decreased (from an initial value of 0.27 m^3 m^{-3}), and was no longer correlated with the FAO-56 ET_o, indicating the onset of water stress (Figure 4.8).

The daily increment in tree mass from carbon fixation was considered to be negligible. Whiting and Lang (2004) showed net daily whole tree carbon gains of 200 - 800 g $tree^{-1}$ for 9–year old sweet cherry trees with leaf areas of ca. 35 - 40 m^2 (i.e., 10–fold greater leaf area than the trees on the lysimeters). Thus, estimated biomass gain of the tree in each lysimeter was very small compared to water losses of ca. 10 kg day^{-1}. Therefore, the scale mainly records the weight of the soil plus changes in bin volumetric water content.

The effect of soil water content on evapotranspiration varies with crop and is conditioned primarily by type of soils, water holding characteristics, crop rooting and meteorological factors. It has been shown that under conditions of high evaporative demand, ET is reduced if the rate of water supply to the roots is unable to cope with transpiration losses (Doorenbos and Pruitt, 1977).

The results of our measured transpiration on cherry trees are similar to the response detected on cotton published in the classic paper by Rijtema and Aboukhaled (1975). The authors concluded that within eight days following irrigation, the crop will transpire at the predicted rate. However, as the soils become drier over time, the transpiration rate will decrease, and the decline is greater under high evaporative demand.

4.4.1.2. Costs

The cost of materials and installation to fabricate the lysimeter was approximately \$5,700 including \$50 for the PVC bin, \$2,750 for the scale with four built-in load cells and analog output, \$2,600 for the datalogger and charging power supply, and \$300 for hand labor and miscellaneous items.

Although the cost of lysimeters depends strongly on the size of the container, the design resulted inexpensive compared with similar size reported in the literature. For instance, Allen and Fisher (1991) reported a lysimeter installed at a cost of \$11,000 for a 1 m^2 area by 1.2 deep. McFarland *et al*. (1983) reported a cost of \$10,719 for a 2.44 m diameter by 1.52 m deep lysimeter while Dugas *et al*. (1985) reported a cost of \$14,000 for a 2.0 × 1.5 × 2.54 m unit. However the proposed lysimeter has high sensitivity, it resulted on a relatively modest cost per volume unit (\$6,489 per cubic meter) when compared to similar lysimeters (Table 4.1).

4.4.2. Effect of the irrigation treatments

4.4.2.1. Midday water status

Midday canopy water status was affected by irrigation treatment during 2004 (Figure 4.9). The general trend of both ψ_{stem} and ψ_{leaf} was characterized by a slight decline that is most likely related with the suberization of young fine roots that account for most of water uptake, or the late season cavitation of wide xylem elements (Yoon and Ritcher, 1991). We reported a similar trend for field-grown trees (Chapter 2).

Throughout the season, ψ_{stem} of DI declined by 0.1 MPa and PRD declined by 0.15 MPa, with significant differences among treatments beginning at the end of May (DOY 147). However, only four runs with significant differences in ψ_{stem} between PRD and DI were recorded, DI showed a slightly higher water status compared to PRD (Figure 4.9a).

Similarly, we recorded a progressive seasonal decline in ψ_{leaf} (Figure 4.9b). However, significant differences among treatments were recorded only on four dates, DOY 147, 182, 217 and 231. Apparently, trees under water stress controlled better leaf water status. Interestingly, C trees exhibited significantly lower ψ_{leaf} than both deficit irrigation treatments (DOY 182, 217 and 231). This response may be related with adjustment in osmotic potential linked to synthesis and accumulation of compatible solutes in the leaves throughout the season (Ranney *et al.,* 1991).

4.4.2.2. Stomatal conductance

During 2004, g_s of PRD trees was on average lower, by 25%, than DI and C trees (Figure 4.10a). Interestingly, during June 2004, DI trees exhibited significantly higher g_s than those of C trees evidencing poor control over stomata aperture. Other authors have reported lower g_s on DI during experiments conducted on apple trees (Mpelasoka *et al*., 2001). The different response of the trees in our experiment may be related with the induction of more root growth in water-stressed plants as suggested by Kang and Zhang (2004). Since higher g_s were not achieved until DOY 165, increasing on root density to absorb water more efficiently might have occurred.

Stomatal conductance was affected by the irrigation treatments and correlated with VPD and air temperature (Table 4.2). Our results are similar to those of Moriana *et al*. (2002) who reported a close linear relationship between VPD and g_s in olive trees (*Olea europaea* L). However, the relationship does not follow the trend reported on apples where g_s was less responsive to VPD on water stressed conditions (Flore *et al*., 1985). In the current trial, the correlation explains 50% of the variability on g_s in PRD treatment while in C it only explains 31% with an intermediate response in DI. This suggests that air temperature and vapor pressure deficit have greater control over g_s under deficit irrigation. In the well irrigated control treatment the model explained only 31% of the variation demonstrating that VPD and T_{air} are not controlling g_s as strongly as in the water deficit treatment.

4.4.2.3. Single leaf water use efficiency

In the experiment, the maximum WUE of 5.13 µmol µmol^{-1} was observed in DI on early June (DOY 161) and the minimum was equivalent to 0.96 µmol µmol^{-1} on DI treatment at the middle of June (DOY 168, Figure 4.10b). A general declining trend through the season was observed on WUE, especially after middle July (DOY 188) probably related with a reduction on the assimilation of carbon through photosynthesis (Figure 4.10b). Plants under PRD had significantly higher WUE compared to DI and C irrigation on four sample dates. This agrees with previous reports on the benefits of PRD over other regular deficit irrigation techniques (Kang *et al*., 1998; Kang and Zhang, 2004). Although DI trees showed higher g_s during June, differences on WUE were not clear for the same period, modulated by higher rates of net photosynthesis in the leaf.

4.4.2.4. Tree transpiration

Whole-tree transpiration was assessed for ca. 28 weeks over spring, summer and early fall 2004. Weekly transpiration, in terms of liters of water per leaf area per day, is shown in Figure 4.11. Significant differences between transpiration of C and the deficit irrigation treatments (DI and PRD) were observed after DOY 121, when treatments were imposed.

Mean FAO-56 ET_o varied through the season in response to variation on weather parameters and the differences were smoothed when weekly averages were taken. As expected, weekly tree transpiration increased from middle of March (DOY 80) until middle of August (DOY 227) in parallel with leaf area expansion and increasing ET_o. Although irrigation treatments were applied at the beginning of May (DOY 121), transpiration rate kept rising steadily at similar values until DOY 145 and continue with remarkable differences between C and the deficit treatments throughout the rest of the season.

Total seasonal transpiration of C trees was 846 mm. Deficit irrigated trees exhibited ca. 31% and 35% lower transpiration for DI and PRD, respectively (Table 4.3). Monthly water use, mm month^{-1} (leaf area base) revealed a progressive increase of transpiration throughout the season with a peak in August and September (Figure 4.12). The observed trend in transpiration in our results is similar to results reported on late season peaches in California (Ayars *et al*., 2003). However, climatic differences between San Joaquin Valley and Yakima Valley and phenotypic characteristics may explain the quantity and length of period of active transpiration by the plants.

It is important to keep in mind that trees in the experiment were strictly vegetative. In other fruit crops competition for assimilates between fruits and branches and leaves have been reported, decreasing leaf area expansion and the consequent depletion on transpiration. Declination of transpiration has been resported after harvest by the closure of stomata due to negative feed back regulation from available phosynthetic assimilates (Whiting and Lang, 2004). In the current trial, transpiration rose steadily during the season and declined only after the leaves became senescent and cold damaged.

4.4.2.5. Tree evapotranspiration related to the crop reference evapotranspiration

From the relationship between tree ET (leaf area base) and ET_o the effect of the irrigation treatment on the transpiration is evidenced by ET values significantly lower than ET_o (ET : ET_o ratio lower than 1.0). ET : ET_o ratio calculated for DI and PRD treatment fluctuated in a range between 0.2 (middle of April) and 0.95 at the middle of August. The results agree with data reported by Chalmers *et al*. (1992) on asian pears where ET : ET_o ratio declined when water stress was imposed.

Evapotranspiration of the sweet cherry trees related to the crop reference evapotranspiration for C trended to increase during the season, from 0.4 at the beginning of April to a maximum of 1.6 during the middle of August (Figure 4.13). Our results agree with the effect of deficit irrigation reported on peach (*Prunus persica* L. Batsch) by Boland *et al*. (1993). In their experiment ET : ET_o ratio values reached a maximum within a range between 0.8 and 1.0 near harvest while ET : ET_o ratio for the well irrigated treatment was approximately 1.6. For fruit trees, using gravimetric lysimeters other authors have reported ET : ET_o in peach that fluctuates between 0.7 (Klein, 1983) and 1.3 (Chalmers *et al*., 1983), 1.4 for pecan (Miyamoto, 1983) and in the range between 1.3 to 1.6 in apples (Beukes and Weber, 1982).

Analyzing the 1:1 line relating ET and ET_o (Doorembos and Pruitt, 1977; Figures 4.14, 4.15 and 4.16) C treatment showed a relatively even distribution of values above, DI treatment transpired less than ET_o while PRD treatment shows an intermediate situation, implying a better performance of PRD when coping with a similar degree of water stress. From the graph it is possible to infer that PRD treatment kept open stomata even under water deficit conditions showing an improved response to cope with water stress compared to DI treatment.

4.5. Conclusions

This paper describes a weighing lysimeter that measures transpiration from young trees. The system was able to resolve 0.1–kg changes in mass representing the lower boundary that limits the practical use for hourly transpiration of very young trees but with an adequate precision for daily periods. These data will be useful to evaluate water uptake and transpiration using a relatively inexpensive system.

The observed transpiration of cherry trees correlated well with soil water content changes. The tree's response to decreasing soil water potential is complex.

The differences between the response of g_s to VPD and T_{air} revealed a tighter control of transpiration losses when a deficit irrigation strategy was imposed. However, there was a subtle difference in the degree of control of VPD and T_{air} between PRD and DI. Our results besides testing the advantages of PRD techniques may be considered for scheduling the irrigation on new orchards.

Over the entire season of 2004, total transpiration from C trees was ca. 846 mm, and deficit-irrigated trees exhibited between 31% and 35% less transpiration (DI was 585 mm, and PRD was 546 mm respectively). Although trees under PRD had significantly higher WUE only on four sample dates, compared to C and DI, the results agree with previously reported experiences on the benefits of PRD over other regular deficit irrigation techniques.

Tree evapotranspiration related to the crop reference evapotranspiration (ET : ET_o ratio) calculated for DI and PRD trees fluctuated between 0.2 starting the season and 0.95, at the middle of August. For C trees, ET : ET_o ratio increased throughout the season from 0.4 to 1.6.

4.6. References

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Table 4.1 Comparative reported cost (materials and installation) of ‘small weighing lysimeters’ reported in the literature per unit and per volume unit.

Reference (year)	Crop	Volume (m^3)	Sensitivity (kg)	Reported cost per unit ($)	Cost per volume unit ($ m^{-3})
Proposed design	Young sweet cherry (*Prunus avium* L)	0.88	0.1	5,700	6,489
Dugas *et al*. (1993)	Wheat (*Triticum aestivum* L.)	7.62	60.0	29,000	3,806
Allen and Fisher (1990)	Grass/fescue mixture forage grass	1.20	50.0	11,000	9,167
McFarland *et al*. (1983)	Mature peach trees (*Prunus persica* L.)	7.10	4.5	10,719	1,509

Table 4.2: Multiple regression with g_s as the dependent variable and VPD and T_{air} as independent variables for the three treatments.

Data set	Variable	Parameter estimate	Standard error	p value	r^2
Control	*Intercept*	39.40	65.39	0.5484	0.31
	T_{air}	16.95	4.66	0.0005	
	VPD	−186.95	37.72	<0.0001	
DI	*Intercept*	−21.39	52.87	0.6868	0.46
	T_{air}	3.42	3.41	<0.0001	
	VPD	24.52	24.52	<0.0001	
PRD	*Intercept*	−16.56	66.63	0.8042	0.50
	T_{air}	25.01	4.16	<0.0001	
	VPD	−265.13	30.75	<0.0001	

Table 4.3: Monthly water use of sweet cherry trees measured with a weighing lysimeter expressed in mm month^{-1} expressed in leaf area base (C-control, DI-deficit irrigation and PRD-partial root-zone drying).

Month	Water use (mm m^{-2})		
	C	DI	PRD
April	94	49	56
May	91	83	73
June	122	96	98
July	181	141	106
August	194	126	121
September	163	90	91
Total	846	585	546

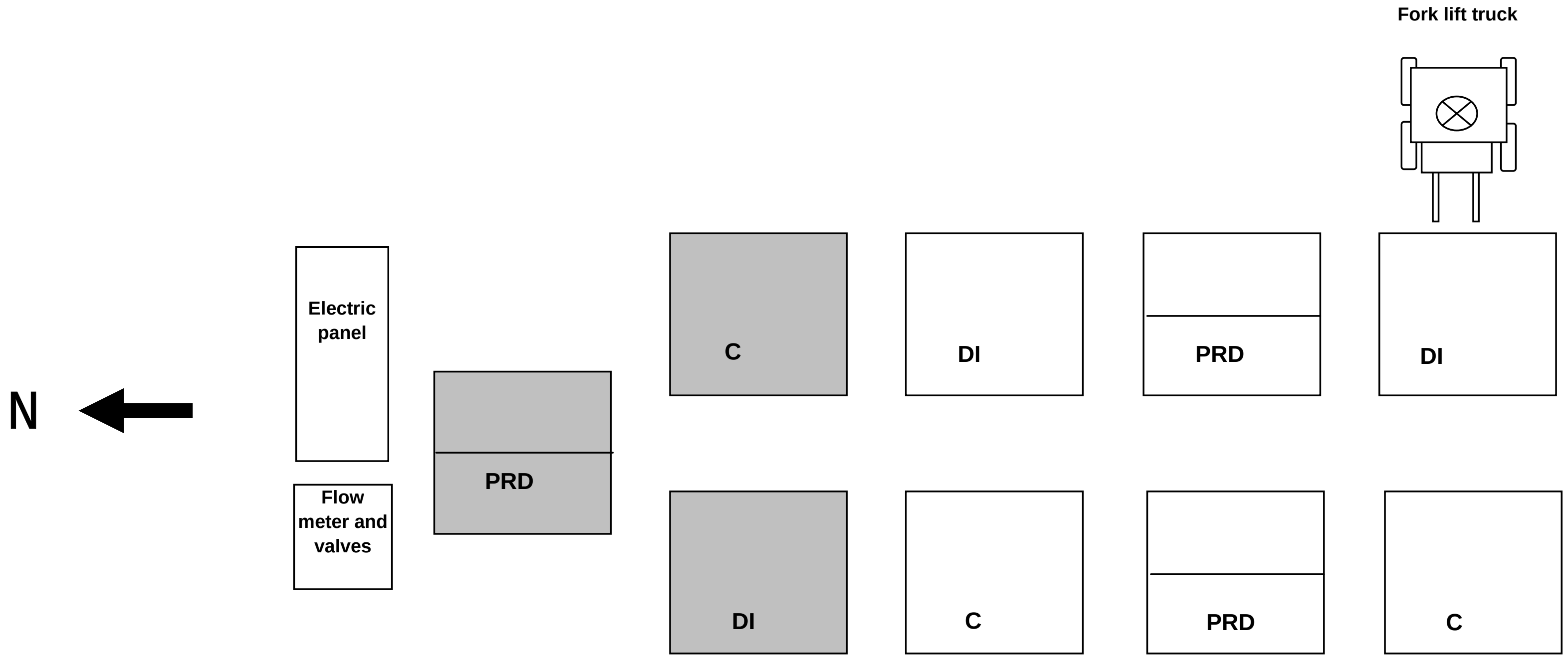

Figure 4.1: Set up of the experimental weighing lysimeter experiment including the electric panel, the flow meter and solenoid valves controlling the irrigation and the nine bins containing the plants. The squares represent the pots lined up in two rows in a random design. The letters inside the squares symbolize the irrigation treatment. The gray squares represent the scale recording the change of mass in the container and the fork lift truck used to move the bins periodically from their position to the scale.

Figure 4.2: Experimental weighing lysimeter experiment at IAREC-WSU, Prosser, WA

Figure 4.3: Design of experimental weighing lysimeter. A ‘Bing’ sweet cherry trees was planted in a rigid copolymer polypropylene bins and placed on a high precision, 4 load-cell floor scale. A layer of Styrofoam™ was placed over the top of the bin to eliminate soil evaporation.

Figure 4.4: Detail of tree's plantation. Top of bin sealed with a 5 cm thick polystyrene isolating layer with an opening for the trunk fasten with closed-cell foam preventing evaporation from the soil.

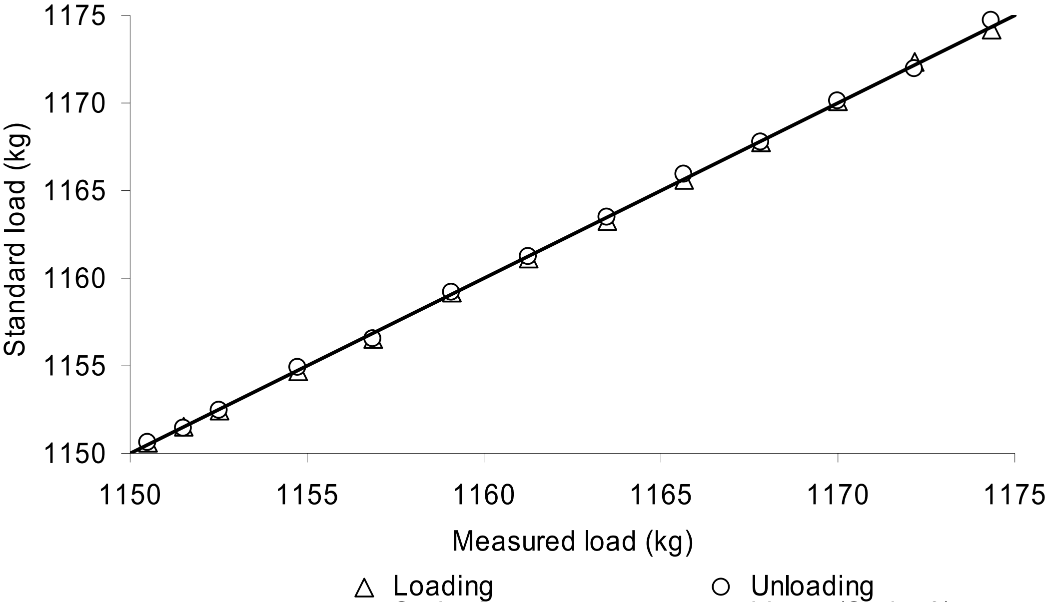

Figure 4.5: Measured readings of lysimeter mass vs. standard weights added and removed during a lysimeter calibration test.

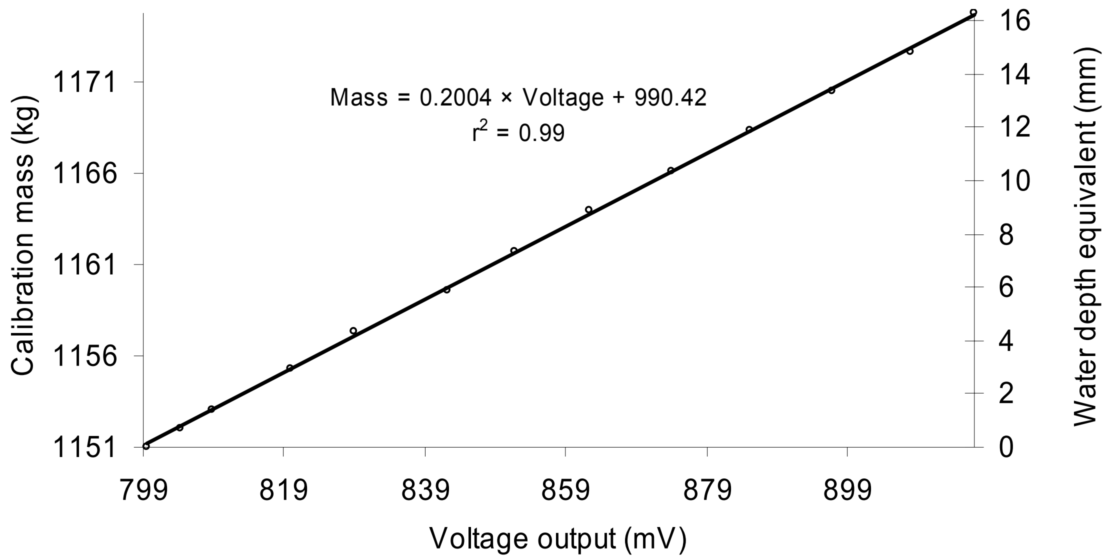

Figure 4.6: Example of lysimeter calibration for one scale lysimeters using standard weights added. The equation in the figure was obtained by linear regression.

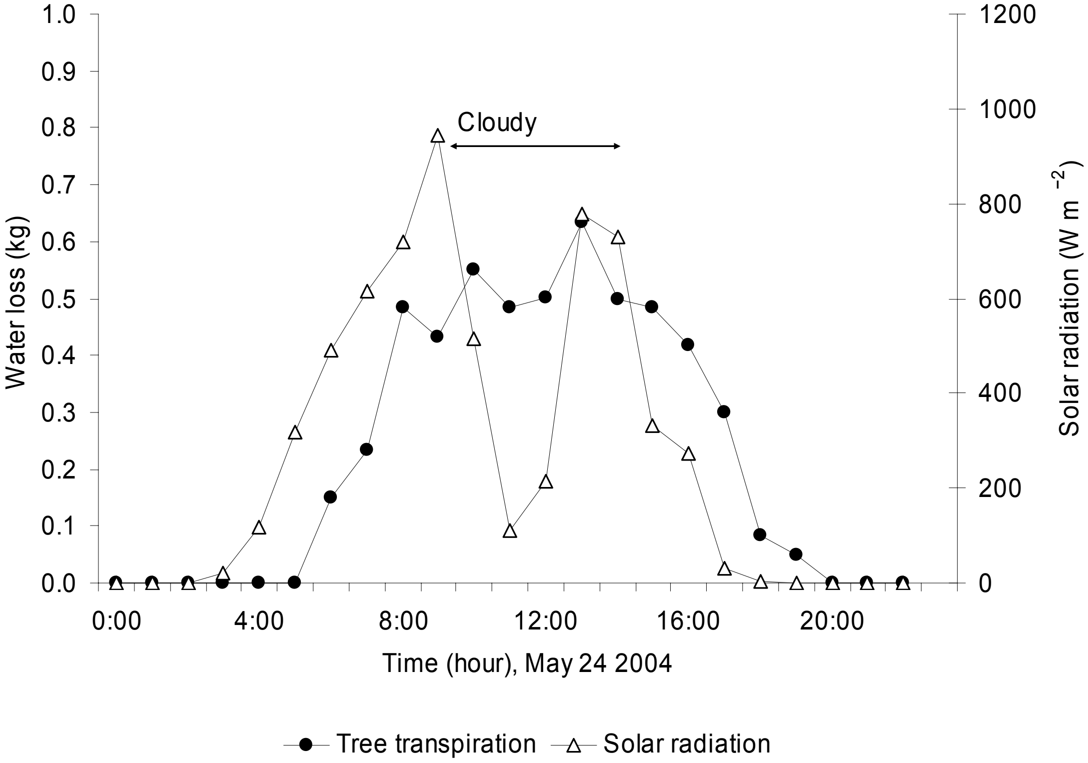

Figure 4.7: Hourly transpiration recorded with the gravimetric lysimeter and total solar radiation (24 May, 2004). The FAO-56 ET_o was equivalent to 3.95 mm with partial.

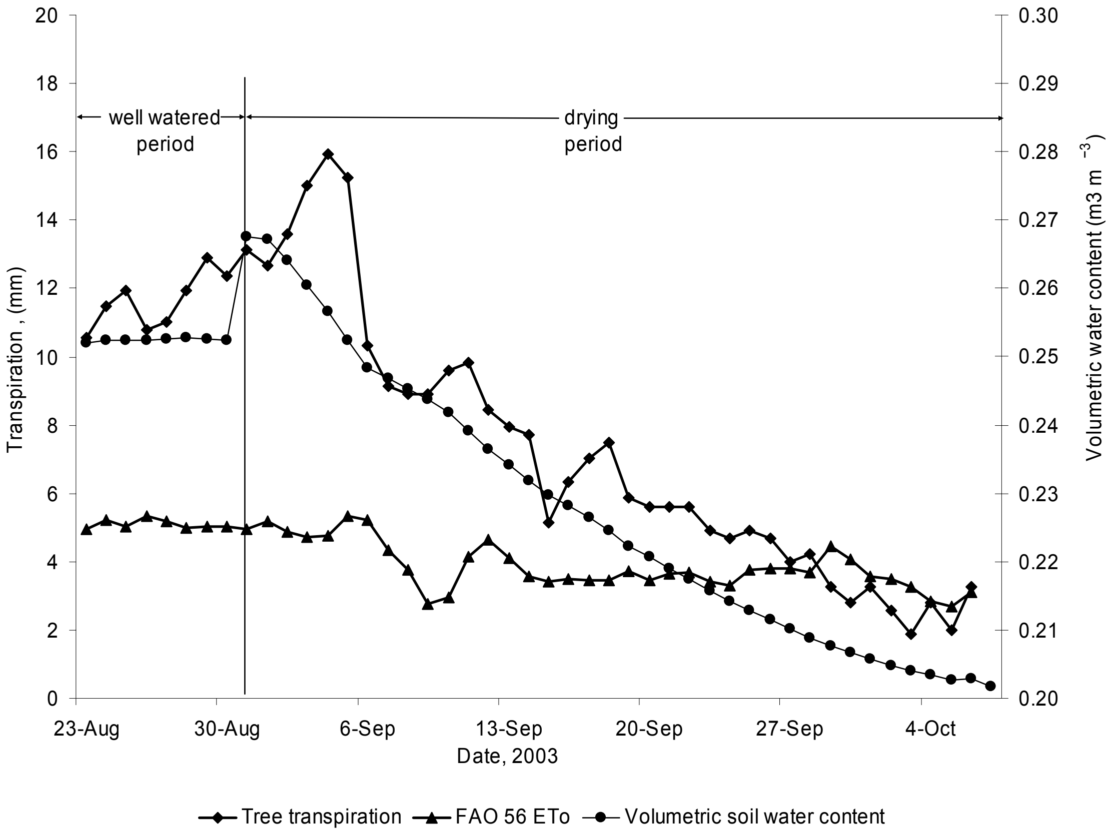

Figure 4.8: Estimated transpiration from 2–year-old ‘Bing’ sweet cherry tree in kg m^{-2} (leaf area basis). Evapotranspiration was estimated using the FAO-56 Penman-Monteith ET_o (mm) and volumetric soil water content (m^3m^{-3}) with daily irrigation scheduling and during a drying period after irrigation.

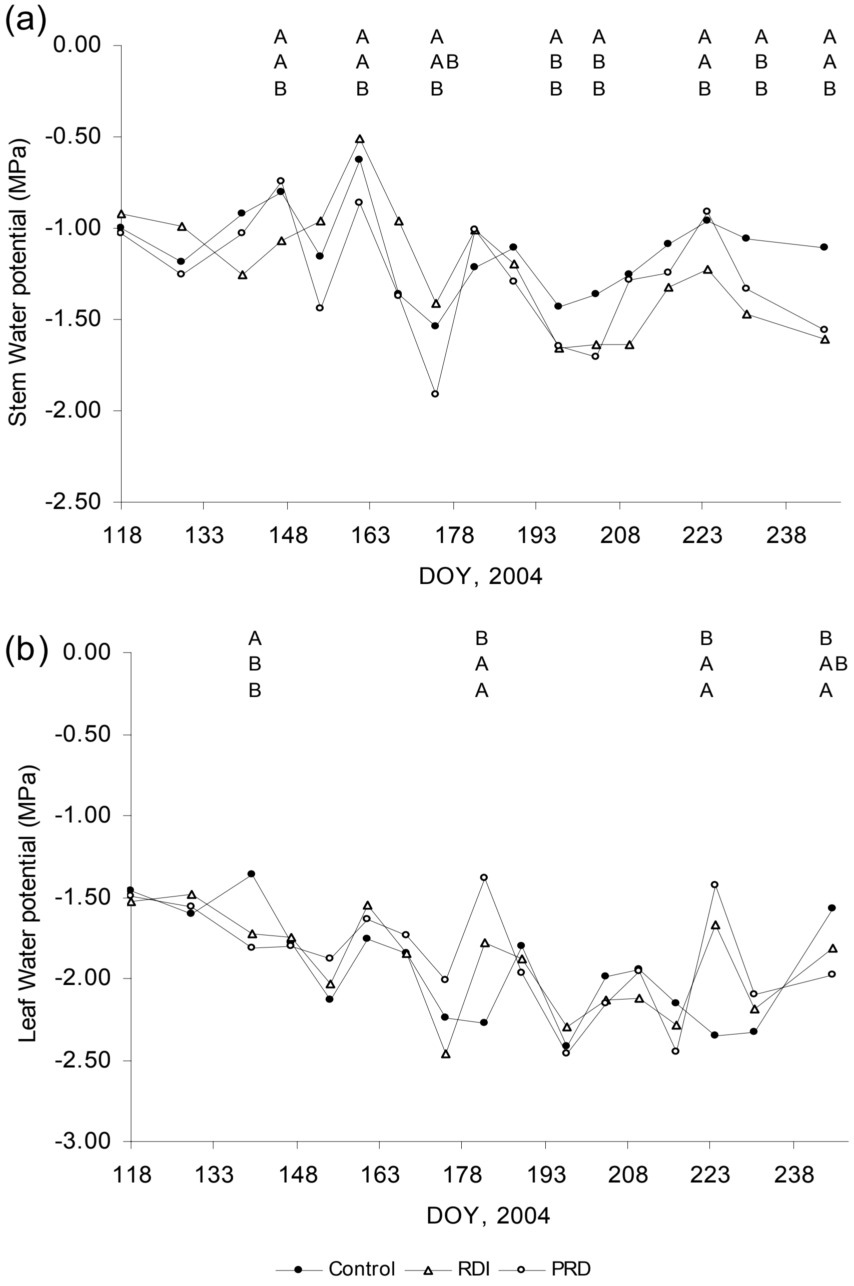

Figure 4.9: Trend in tree water status: stem water (ψ_{stem}, a) and leaf water potential (ψ_{leaf} , b) measured at midday during 2004. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

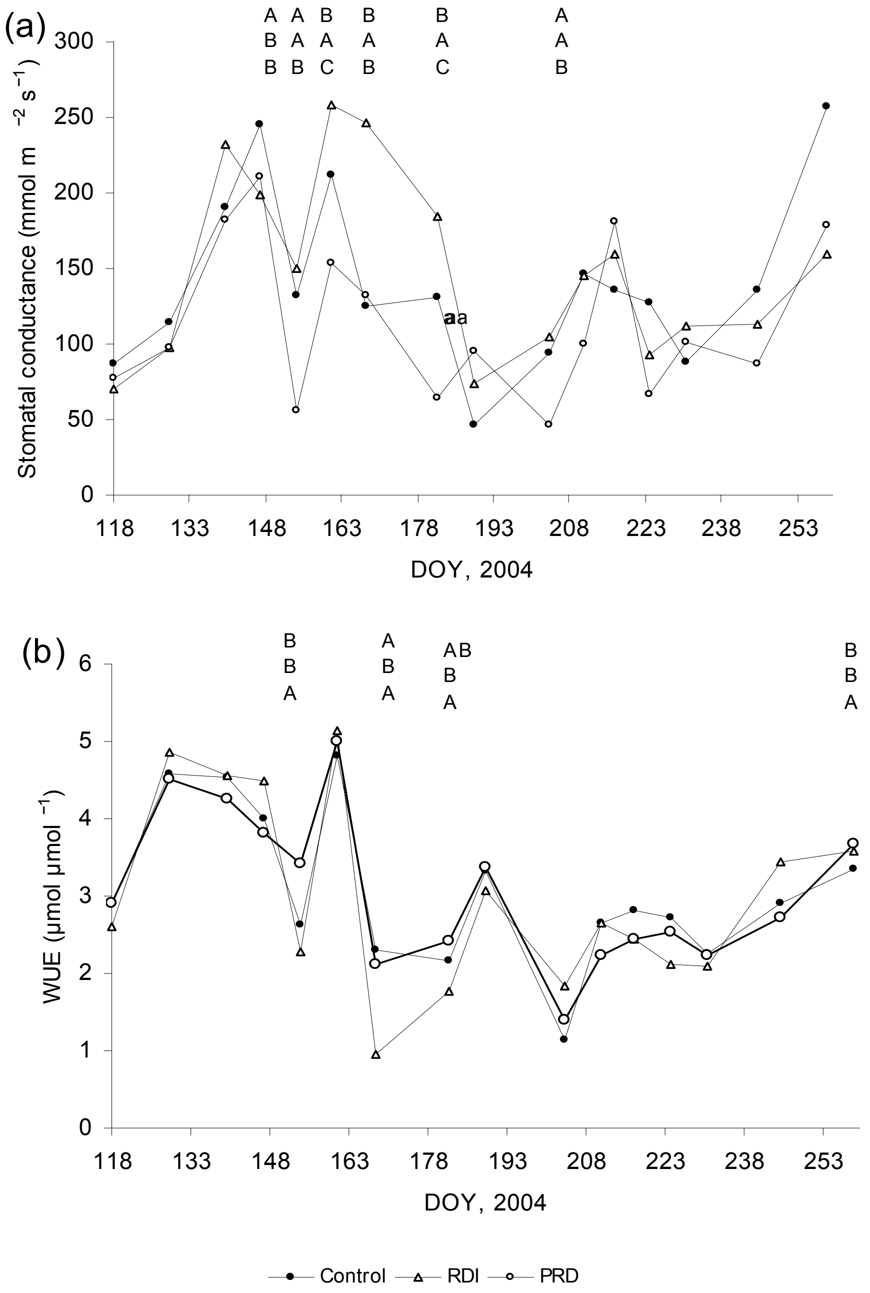

Figure 4.10: Trend in stomatal conductance (g_s, a) and water use efficiency (*WUE*, b) from the leaf measured at midday during 2004. Symbols represent the average per treatment (n = 3). Capital letters are shown when significant differences occur between the mixed effect of irrigation treatment and day of measurement within the same date. The top row refers to C, the middle to DI and the bottom to PRD treatment. The absence of letters indicates that means are not statistically significantly different.

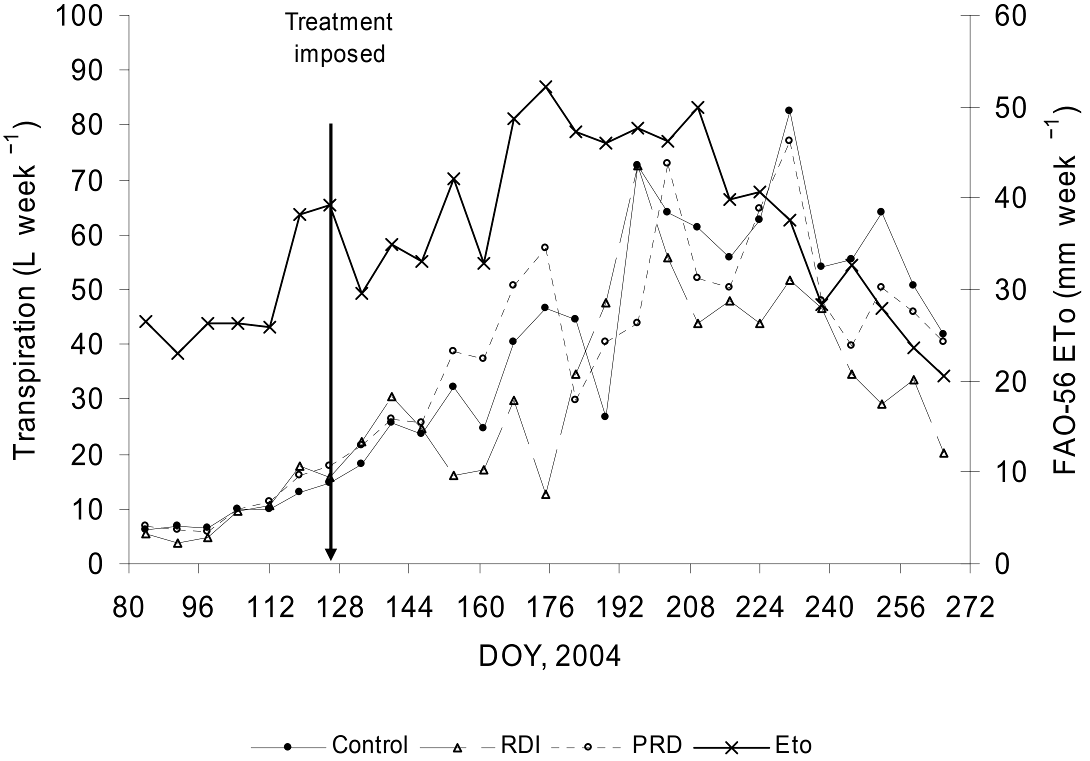

Figure 4.11: Weekly transpiration from 2–year-old ‘Bing’ sweet cherry tree (mm week^{-1}m^{-2}, leaf area base) for control (C), deficit irrigation (DI) and partial rootzone drying (PRD) treatments (ET ET$_o$$^{-1}$) compared to reference crop evapotranspiration (ET$_o$).

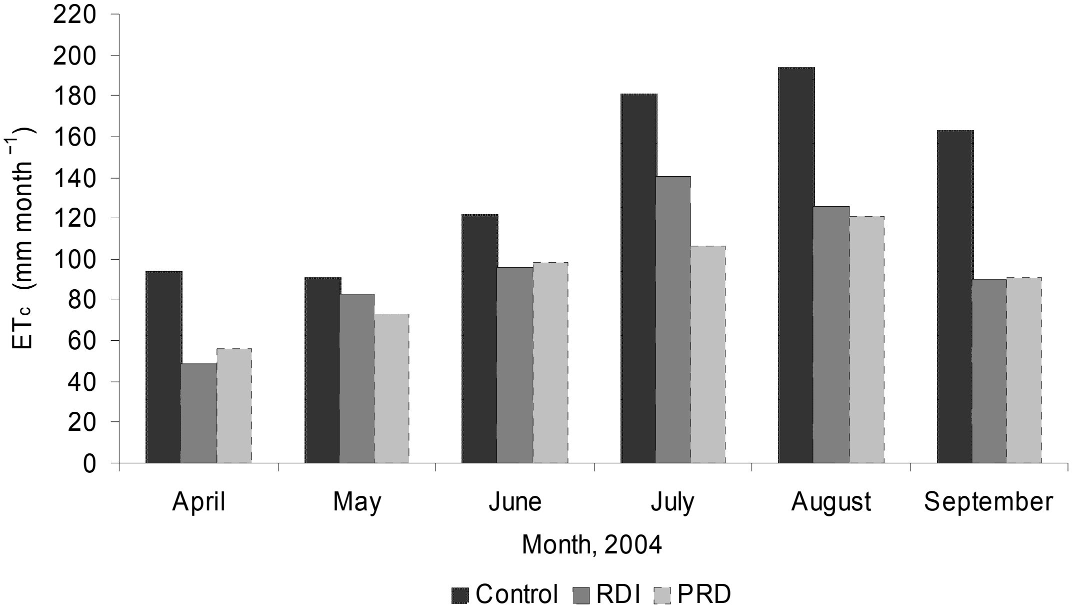

Figure 4.12: Monthly transpiration from 2–year-old ‘Bing’ sweet cherry tree (mm month^{-1})

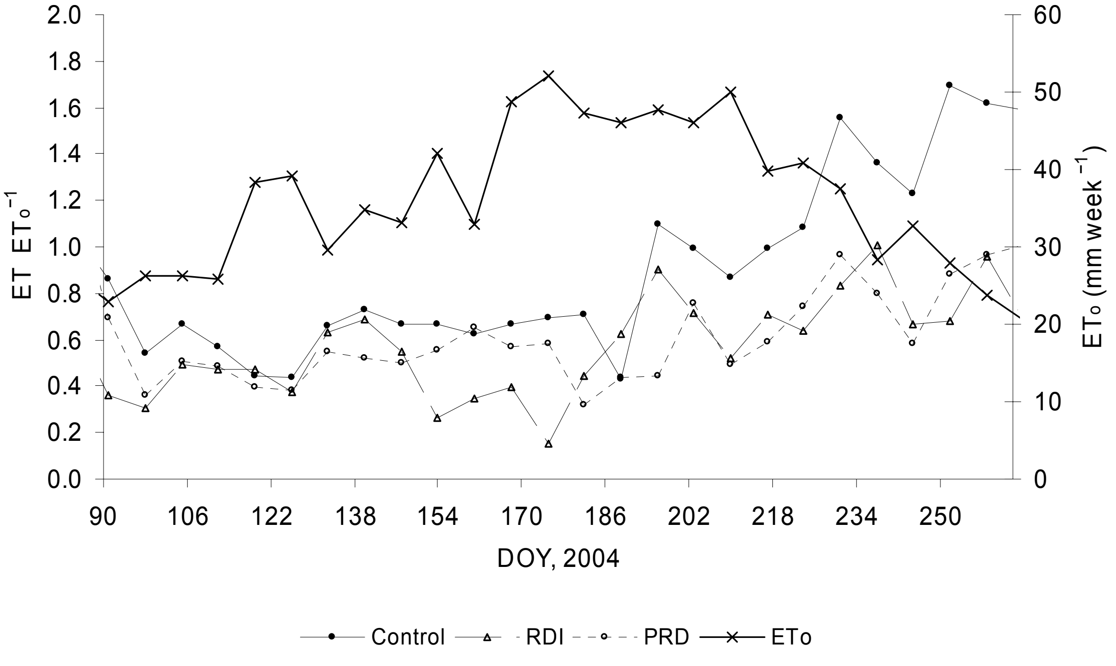

Figure 4.13: Trend on the ratio between ET and ET_o from weekly transpiration data from 2–year-old 'Bing' sweet cherry tree (mm week^{-1}m^{-2}, leaf area base) for control (C), deficit irrigation (DI) and partial rootzone drying (PRD) treatments (ET ET_o^{-1}) compared to reference crop evapotranspiration (ET_o).

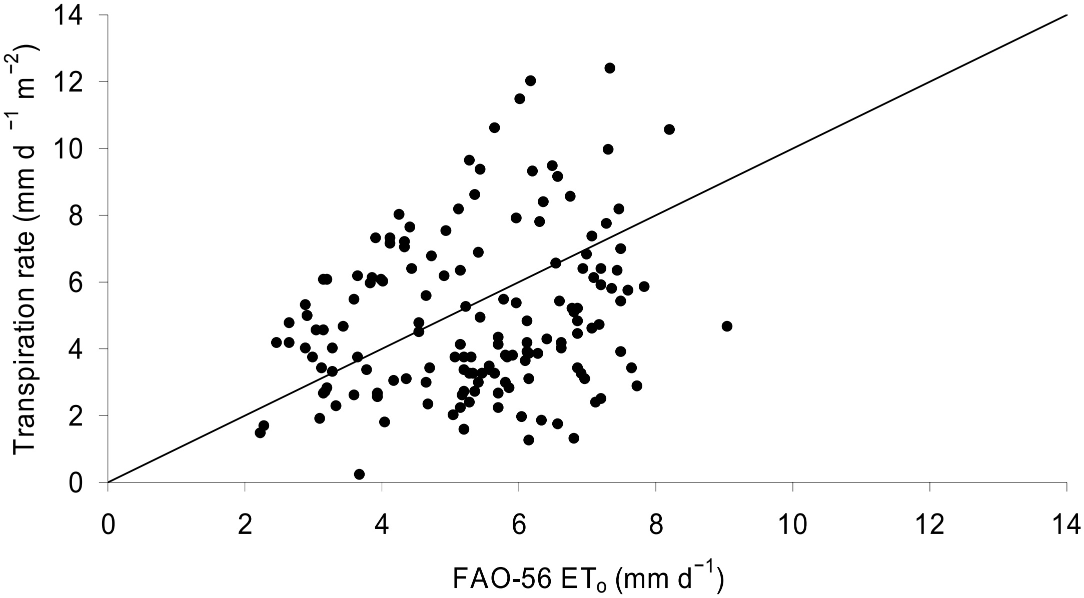

Figure 4.14: FAO-56 ET_o as compared to transpiration rate of the 2–year-old ‘Bing’ sweet cherry tree in mm dm^{-2} (leaf area basis) after the treatments were imposed (DOY 170 to 175) for the control treatment.

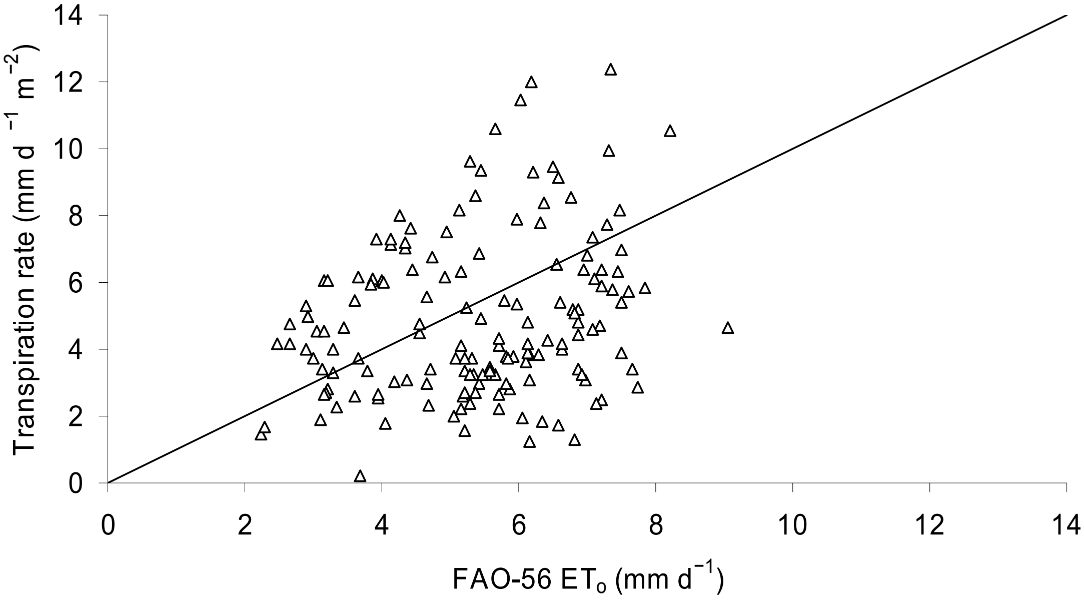

Figure 4.15: FAO-56 ET$_o$ as compared to transpiration rate of the 2–year-old ‘Bing’ sweet cherry tree in mm dm^{-2} (leaf area basis) after the treatments were imposed (DOY 170 to 175) for the DI irrigation treatment.

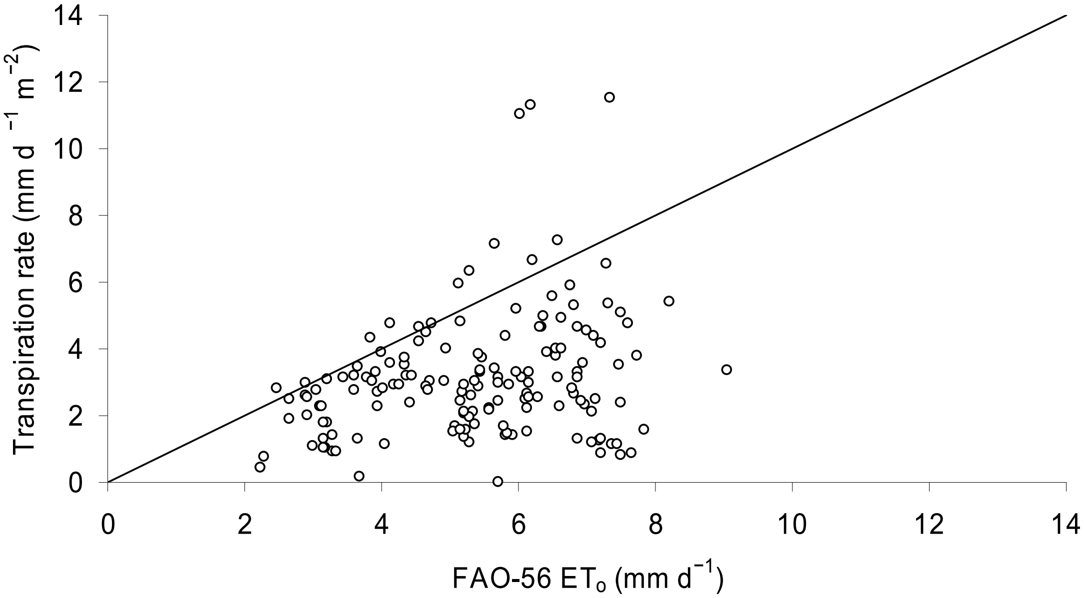

Figure 4.16: FAO-56 ET$_o$ as compared to transpiration rate of the 2–year-old 'Bing' sweet cherry tree in mm dm^{-2} (leaf area basis) after the treatments were imposed (DOY 170 to 175) for the PRD irrigation treatment.