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REGULATED DEFICIT IRRIGATION TO MAINTAIN YIELD AND IMPROVE QUALITY AND SHELF LIFE OF HIGHBUSH BLUEBERRY (*V. corymbosum*) FRUITS

DOCTORAL THESIS IN FULFILLMENT OF THE REQUERIMENTS FOR THE DEGREE DOCTOR OF SCIENCES IN NATURAL RESOURCES

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Regulated deficit irrigation to maintain yield and improve quality and shelf life of highbush blueberry (*V. corymbosum*) fruits

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Summary and outline of this thesis

Highbush blueberry (*Vaccinium corymbosum* L.) was introduced to Chile in the decade of the 70's. The planted area increased almost tenfold between 2000 and 2010, and at present exceeds 14,000 hectares (ODEPA-CIREN, 2015). Concurrently, the expansion in plantings brought an even greater productivity as fields were reaching full production stages. Thanks to a greater demand and competitive attributes such as being a country free of many quarantine pests and supplier of quality fruit, the production increased at an annual average of 35% between 2005 and 2010. More than 90% of the Chilean fresh blueberry production is destined to northern hemisphere markets. The Chilean blueberry industry is the largest exporter of this crop in the world (ODEPA, 2013). In recent years, the relative importance of blueberry within the Chilean fruit sector in planting, employment and income generation has brought great interest in local producers and associated companies to increase exports. But the increased fruit supply coming from Chile, Peru, Argentina and other Southern Hemisphere countries has raised the quality standards demanded by importing countries. Higher fruit quality remains a challenge, as every year the rejection rates increase in destination countries mainly due to loss of condition during transport. This is in large proportion due to low berry firmness, increased fruit dehydration and post-harvest fungal decay (ASOEX, 2009).

In addition to yield reduction, water stress conditions during development typically result in reduced fruit size. The occurrence of drought conditions during production of fruit and vegetable crops is becoming more frequent with climate change patterns (Mishra and Singh, 2010). Water stress down-regulates photosynthesis in many fruit crops, reducing their productivity (Flexas et al., 2009). Climate change has brought increased water shortage in various growing areas around the world, creating the need for studying the effects of different water management techniques on several crops. Regulated deficit irrigation (RDI) is a practice that has

been successfully used in several crops. In RDI there is a reduction in the amount of applied water at phenological stages which are less sensitive to water deficit. RDI imposes plant water stress in a controlled manner, without affecting plant performance and development, and in some instances RDI can increase fruit quality. Blueberries possess a high sensitivity to water deficit due to a shallow root system and lack of root hairs. Stomatal conductance is rapidly affected during water shortage, photosynthetic rates are reduced later on and this ends up having a negative impact on yield. In the current study, mature 'Brigitta' blueberry plants expressed high sensitivity to RDI treatments. Growth and yield were affected in the most severe treatment (supply of water equivalent to 50% of actual evapotranspiration (ETa)) due to reduced photosynthetic and transpiration rates. Mild deficit treatment (75% ETa), achieved similar fruit quality as control plants but the water productivity (WP) was improved. Antioxidant capacity was higher in fruit from control plants followed by those from the mild deficit treatment, although fruit oxidative damage increased proportional to water stress level. During the post-harvest stage, water treatments did not affect fruit condition, but altered its quality. Weight loss was decreased by water deficit treatments. Applying 25% less water to highbush blueberries did not change fruit quality or their level of antioxidants.

Considering that most shipments of Chilean blueberries to foreign markets are done by boat, further perspectives for the application of RDI should focus on aiming at increased postharvest life to reach at least 60 days (Moggia et al., 2009). Furthermore, it is important to develop and adapt a model-based approach to establish fo a given situation the deficit irrigation level that provides the best results. Studies on irrigation have generally aimed at full irrigation to maximize blueberry production. However, more attention should be placed on defining the optimum water requirement for each variety and location in order to ensure efficient water use and avoid wastage, while maintaining fruit quality and postharvest life.

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Chapter I

General introduction

1.1 Introduction

Crop production is affected through direct and indirect environmental effects (Chai et al., 2016). One of the most dramatic consequences of climate change is the alteration of water availability for irrigation and increased water demand by crops. Increased water scarcity in the future is due to higher potential evapotranspiration related to an increase in air and soil surface temperatures. This phenomenon is even greater in dry areas and in low-precipitation seasons. In the near future, an increase in the number of regions with reduced soil moisture is forecasted, resulting in direct losses in crop production capacity and its potential productivity (Houghton et al., 2001).

Water deficit is the most unfavorable condition among the various abiotic stresses (Araus et al., 2008). Drought periodically reduces crop yields in virtually all agricultural regions. About one-third of the world's arable land undergoes chronically insufficient water availability (Howell, 2001). While around 80% of the world's useable water resources are currently utilized by agriculture (Condon et al., 2004), in the near future is expected that the expanding global population will soon require more water for domestic and agricultural needs and this trend should accentuate due to global climatic change (Hamdy et al., 2003).

While the effects of drought on crop physiology and production have been studied extensively, there are limited experiences on the impact of pre-harvest water stress on the quality and shelf life of produces (Toivonen and Hodges, 2011). In many fruit crops water stress down-regulates photosynthesis, resulting in reduced productivity due to decreased CO_2 diffusion from the atmosphere to the carboxylation sites (Flexas et al., 2009). In addition to yield reduction, it has been observed that water stress during fruit development typically results in reduced fruit size in peaches (Alcobendas et al., 2013); strawberries (Liu et al., 2007) and wine grapes (Romero et al., 2013). During post-harvest storage of most fruits and vegetables a loss of only 5–10% moisture is sufficient to render the product unsalable (Kays, 1999; Robinson et al., 1975).

In general, growers attempt to manage water to minimize stress and optimize photosynthesis, plant growth and harvestable yield. Although irrigation systems and protocols vary, growers irrigate based on evapotranspiratory demand, well before severe stress conditions occur.

Deficit irrigation (DI) is the application of water below the evapotranspiration (ET) rate and can be performed in several ways. Plants are exposed to lower water availability at a constant fraction of potential ET throughout the season or at specific phenological stages when plants are less sensitive to water deficit. Regulated deficit irrigation (RDI) is a technique which could both reduce water usage, and increase water use efficiency, as well as fruit quality in several horticultural crops. The reduced stress imposed to the plant is expected to have minimal effect on yield, as found among some fruit crops like citrus trees (Gasque et al., 2010); blueberries (Keen and Slavich, 2012) and grapevines (Acevedo-Opazo et al., 2010). RDI is becoming a common practice in several of fruit crops to reduce water applied through irrigation without affecting plant performance and development (Behboudian and Mills, 1997). Control of soil water content (SWC) and plant water status depends on the appropriate use of localized irrigation techniques which strongly affects RDI success. Moreover, the efficient use of irrigation water is necessary for appropriate water and nutrient uptake by the root system (Clothier and Green, 1994).

Studies on RDI in fruit crops have concentrated on effects up to harvest. Fruit culture in Chile has a major focus on post-harvest development because of the long journeys that the fruit has to withstand in order to reach the northern hemisphere markets in Asia and Europe.

Thus, research is needed on the impact on post-harvest quality in order to ensure that Chilean fruit will reach target export markets with proper quality and condition (Moggia et al., 2009).

1.2 Hypothesis

Considering that regulated deficit irrigation has provided a solid alternative to establish water saving strategies on several fruit crops planted in areas facing water deficits, the hypothesis of this work is:

- Mild pre-harvest regulated deficit irrigation does not significantly down-regulate photosynthesis, and thus is able to maintain yield and improve both quality and post-harvest life of *V. corymbosum* fruits compared to severe water deficit.

1.3 General objective

- To evaluate the effects of pre-harvest regulated deficit irrigation on crop water use, as well as on yield, quality, and postharvest life of *V. corymbosum* fruit.

1.4 Specific objectives

- To evaluate the effect of pre-harvest RDI management on crop water use, as well as on physiological parameters, yield and quality at harvest of *V. corymbosum* fruits.
- To evaluate post-harvest quality and condition in *V. corymbosum* fruits under pre-harvest RDI management.

Chapter II

Literature review

2.1 Crop irrigation

For adequate growth and development, crops require water. This resource is supplied by nature in the form of precipitations. When water availability is insufficient to satisfy plant demands, it is necessary to supply it artificially through irrigation. The selection of a given irrigation method depends on water availability, land topography, soil characteristics, crop characteristics and associated costs, among other factors (Holzapfel et al., 2009).

The most common method of applying water to crops is through surface irrigation. Even though there are various surface irrigation techniques employed in horticulture, all share an essential limitation; the depth of applied water is determined by the infiltration rate of the soil (Fereres et al., 2003). Among the most commonly used pressure type irrigation systems are: sprinkler, micro-pray and drip irrigation.

Sprinkler systems are relatively simple to install and maintain, but a proportion of water is applied between rows, where it is unavailable to the crop. Furthermore, sprinkler irrigation use can be problematic in orchards due to canopy interception of the spray patterns resulting in poor distribution uniformity. They can also increase problems with pathogens by boyh washing off pesticides and providing conditions for their growth (Bryla, 2008).

A micro-spray irrigation system is a hybrid between drip and surface spray irrigation. It retains some advantages and some disadvantages of each type of irrigation. It consists of micro tubing pipes connected to a series of nozzles attached to risers. These risers may be fixed or designed to pop-up. As in the case of drip irrigation, micro-spray is considered a type of low-pressure irrigation (Holzapfel et al., 2009).

Drip irrigation consists of plastic pipes in which emitters have been inserted to localize the water placement near individual plants using high frequency application and low discharge rates. It offers the potential for precise water management and divorces irrigation from the engineering and cultural constraints that complicate furrow and sprinkler irrigation (Hartz, 1996).

2.2 Drought stress

2.2.1 Short term responses

During a short period of mild water deficit, RDI may induce plants to significantly lower leaf water content or leaf water potential (Liu et al., 2006; Pérez-Pastor et al., 2014). Reduced leaf water potential acts as a hydraulic signal, triggering a partial closure of stomata and a reduction in leaf area expansion (Shahnazari et al., 2007).

When suddenly encountering drought, it is very important for plants to respond as quickly as possible. A faster response under drought conditions means that less water is lost and the survival rate of the plants is increased. Stomatal closure has been identified as the most important quick response. Among the most common physiological adjustments to drought stress associated with RDI are stomatal characteristics, such as stomatal opening and closing rhythms, guard cells size, and stomatal density (Schroeder et al., 2001).

Water stress can reduce transpiration, CO_2 and nutrient uptake as a result of changes in metabolic processes including photosynthesis and respiration (Xiong and Zhu, 2002). Some of these changes can lead to oxidative damage. Plants exposed to mild deficit due to RDI often express different levels of response in photosynthesis and respiration, mainly because RDI is implemented with different degrees of severity and at different growth stages. The photosynthetic rate can be substantially reduced in plants grown under severe water stress. The main cause of lower photosynthetic rate under mild to moderate water deficit is a decrease in the diffusion of atmospheric CO_2 to the site of carboxylation in leaves (Chaves et al., 2009). However, the magnitude of the photosynthetic response to RDI may vary among species

(Romero et al., 2013).

2.2.2 Long term responses

When water shortage occurs, plants produce and accumulate compatible solutes such as polyols, sugars and amino acids in order to reduce cell osmotic potential, which will ease water absorption and retention (Xiong and Zhu, 2002). Osmotic adjustment is a physiological phenomenon where the osmotic potential of a certain tissue decreases as a result of the progressive net accumulation of solutes in the cell (Morales et al., 2013). Monosaccharides (sugars), amino acids, organic acids, and ions accumulate in the leaves and roots in response to water deficits (Chaves and Oliveira, 2004). Under water stress, soluble sugars act as signaling molecules, interacting with hormones, and modifying the expression of genes involved in the photosynthetic process – generally resulting in a reduction in source activity and photoassimilate export and in an increase in sink activity with greater production of lipids and proteins (Chaves et al., 2009).

A number of studies have shown that plants typically produce phytohormones in response to RDI. These molecules regulate processes in targeted cells, control the formation of flowers, stems, and roots, and adjust the abscission of leaves and fruits. More importantly, these phytohormones act as signaling molecules, regulating a number of biochemical processes in plants and helping to lessen the potential damage caused by RDI-induced water stress. Among the phytohormones involved, abscisic acid (ABA) is a well-known plant growth regulator. Since ABA was discovered in the 1960s, it has been studied extensively in many crop species (Sauter et al., 2001). The importance of ABA as a root-generated signal, transported via the xylem and implicated in stomatal regulation during moisture stress has been highlighted in several studies (Dodd et al., 2006).

Plants under RDI can modify their cellular metabolism and trigger diverse defense mechanisms. Exposure of plants to unfavorable conditions causes oxidative stress, which affects plant performance due to the production of reactive oxygen species (ROS) (Mittler et al., 2004). These ROS can induce severe damage to DNA, membranes and proteins (Bai et al., 2010). Plants have evolved a complex antioxidant system to cope with ROS, which includes both activation of enzymes (superoxide dismutase, catalase and glutathione peroxidase) (Johnson et al., 2003) and production of non-enzymatic antioxidants (flavones, anthocyanins, carotenoids and ascorbic acid) (Nayyar and Gupta, 2006). The ability of blueberry bushes to up-regulate the enzymatic antioxidant system might be an important attribute linked to drought tolerance, which could limit the cellular damage caused by ROS during moisture stress. A major defense mechanism is the increased activity of antioxidation enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, lipoxygenase, among others (Guidi et al., 2008; Sajedi et al., 2011; Sofo et al., 2004). Another water saving strategy known as partial root-zone irrigation (in which a portion of the substrate is irrigated) causes mild water deficit stress, and in some plant species this localized irrigation maintains or increases the activities of the above-mentioned enzymes in leaves and roots (Hu et al., 2010). Usually, a higher degree of enzyme activity is needed to improve the protection against pronounced oxidative stress.

Under water stress, the activities of antioxidant enzymes vary among plant species and cultivars (Chaitanya et al., 2009). The level of the enzymatic response by plants depends on intrinsic genetic traits. In some woody species, such as olives (*Olea europaea*), enzymatic activities in leaves and roots decreased under RDI-induced moisture stress (Sofo et al., 2004). Another important defense mechanism triggered under RDI is the production of non-enzymatic compounds, including low-molecular-weight substances such as soluble sugars, proline (Mansouri-Far et al., 2010), and malondialdehyde in leaves (Sofo et al., 2004) and roots (Hu et al., 2010). These substances regulate plant osmotic potential by the law of mass action in order to reduce osmotic stress and enhance plant water holding capacity.

Water shortage can change plant metabolism and normal development. Leaf (Morales et al., 2013), shoot (Pérez-Pastor et al., 2014) and root growth can be affected. Depending on the severity of water deficit, plants can modify their root architecture in order to reach deeper into the soil looking for humidity. The plant growth stage plays in which RDI is implemented has an important role in the expression of enzymatic activities in leaves and roots. For instance, it has been found that tomato plants under RDI rapidly increased the peroxidase activity in cell walls during the initial phase of fruit setting, with enzymatic activity reaching its peak at the end of fruit ripening (Savić et al., 2008).

2.3 Blueberry water relations and use of RDI

A goal of crop irrigation management is to balance vegetative and reproductive growth. Excessive vegetative growth can delay maturity and reduce final yield. The RDI has been shown to reduce vegetative growth, maintain yield and reduce water use, leading to improved water use efficiency (WUE) (Loveys et al., 2000).

Severe water stress results in increased sunburn damage of fruits, irregular ripening (pears), rough and leathery texture (peaches), and incomplete kernel development in nuts (Goldhamer and Viveros, 2000). Moderate water stress has been reported to reduce fruit size and increase acidity and concentrations of soluble solids, and ascorbic acid. On the other hand, excessive water supply to fruit crops results in fruit cracking (cherries, prunes, and tomatoes); under this condition, excessive turgor leads to increased susceptibility to physical damage, reduced firmness, delayed maturity, and reduced soluble solids content (Abdel-Razzak et al., 2016; Khadivi-Khub, 2014; Sams, 1999).

The amount of water applied and its distribution within the soil have a significant effect on blueberry fruit production. Therefore, irrigation management is an important factor in blueberries, especially because the roots of this crop are shallow and lack root hairs (Estrada et al., 2015). Under these conditions, water deficit applied in any growth period reduces both the potential yield and the vegetative growth, especially in highbush blueberry (Holzapfel et al., 1994; Holzapfel and Hepp, 1997), since rabbiteye blueberries have been reported to be more drought tolerant (Davies and Johnson, 1982; Erb et al., 1991; Haman et al., 1997).

Irrigation in highbush blueberries is critical throughout the production season, especially in the fruit development stage. Significant differences in the dry weight, thickness, and length of shoots, as well as flowering and fruit production were observed in ‘Bluecrop’ highbush blueberry plants under water stress (Abbott and Gough, 1986). Early symptoms of water deficit in blueberries are stomatal closure and reduced transpiration, which in combination restrict water loss and prevent the occurrence of embolism due to a reduction of the water potential (Améglio et al., 2000). When water deficit occurred, it was observed that ‘Jersey’ and ‘Bluecrop’ highbush blueberries rapidly reacted by reducing leaf growth due to lower gas exchange and photosynthetic performance, but even after water supply is replenished, depending on water shortage severity, vegetative growth and yield can be diminished (Cameron et al., 1989). Recovery depends on the level, opportunity and length of the stress period; thus, water stress during flower induction reduced the number of flower buds in the following harvest season, although photosynthetic performance was not affected; thus the fruit produced, albeit less numerous, were significantly larger (Mingeau et al., 2001).

Proper irrigation improves fruit quality and yield in blueberries (Holzapfel et al., 2004). Growers often tend to over-irrigate higher density plantings reducing root function and originating potential problems with pathogens such as *Botrytis cinerea* Pers. and *Phytophthora cinnamomi* Rands (Bryla and Linderman, 2007). Little is currently known about water relations of this crop, particularly regarding differential effects due to genotype or agronomic practices (Bryla and Strik, 2006). It was established that 'Bluecrop', 'Duke' and 'Elliott' highbush blueberry cultivars differ on their water requirements. During short water stress periods in high-density plantings, peak water use occurred during fruit filling and ripening but it declined markedly after harvest, having the highest water use with 'Duke' and lowest with 'Elliott' (Bryla and Strik, 2007).

So far, RDI use on blueberries has not been extensive. Keen and Slavich (2012) working with 'Star' blueberry bushes found statistical differences for water productivity when comparing a severe water deficit treatment (50% ETa) to fully irrigated plants (100% ETa). No statistical differences were found for yield, midday water potential or stomatal conductance parameters.

2.4 RDI and water productivity (WP)

Shortage of water at different scales leads to drought with all its agricultural impacts. Climate change has changed all the scenarios and made the situation worse by reducing the amount of precipitations and hence, the amount of water available for agriculture (Morison et al., 2008).

Water productivity (WP) is defined as the ratio of yield (measured as biological or economic output) to crop evapotranspiration (ET) (Fereres and Soriano, 2007). Crop WP or water use efficiency (WUE), is a key term in the evaluation of DI strategies (Geerts and Raes, 2009). Water productivity is a concept employed to express the value or benefit derived from the use of water and evidences the importance of water management in food production in arid and semi-arid regions (Singh et al., 2006). Water scarcity requires the improvement of WP as a critical goal. One of the most promising techniques aiming to attain this objective is the use of RDI (Ruiz Sánchez et al., 2010).

Deficit irrigation management has the potential to optimize WP in horticulture. Nevertheless, the effects of deficit irrigation on yield or produce quality are crop-specific (Costa et al., 2007). Knowledge of how different crops endure moderate water deficits is the basis for a successful practical application of deficit irrigation. Nowadays, DI is a usual practice in many areas of the world, especially in dry regions. In these locations it can be more profitable for a grower to maximize crop WP than to maximize the yield per land unit. The water that has been saved can be used for other purposes. Maximum WP might not often coincide with farmers' interests, whose aim is to maximize land productivity and/or economic profitability (Geerts and Raes, 2009).

Crop WP can be increased significantly if irrigation is reduced. Climate change will impact future temperature and rainfall conditions. In regions with decreased precipitation, the amount of water applied through irrigation will have to be increased for optimal crop growth and production, but this might decrease crop WP. Therefore, it will be a great challenge to increase crop WP at all levels. Increasing WP implies either to produce the same yield with less water resources or to obtain higher crop yields with the same available water (Zwart and Bastiaanssen, 2004), or both.

For better use of water in water-limited agricultural environments, efforts must come from different research disciplines: plant breeders, plant physiologists, agronomists, plant biotechologists, and water engineers. Achieving higher productivity in order to resolve the water crisis will require great efforts. Accomplishing this task is possible, especially in developing countries, where water productivity is far below potential (Hamdy et al., 2003).

Chapter III

Pre-harvest regulated deficit irrigation effects on physiological parameters, yield, fruit quality and antioxidants of Vaccinium corymbosum plants cv. Brigitta.

Sent to Agricultural Water Management

Abstract

Under water scarcity, irrigation management is critical in highbush blueberries throughout the growing season, especially during fruit development. Regulated deficit irrigation (RDI) is a management tool that has been used in many fruit crops to conserve water without reducing yield, and potentially increasing fruit quality. In this study, three irrigation treatments based on actual evapotranspiration (ETa): 50, 75 and 100% (control) were applied to six-year-old ‘Brigitta’ highbush blueberries in Colbún, Maule Region (Chile, 35° 41' 12.99" LS; 71° 25' 8.75" LW) for two seasons (2013-14 and 2014-15; seasons one and two) and to 26-year-old ‘Brigitta’ plants in South Haven, Michigan, USA (42° 21' 16.26" LN; 86° 12' 48.88" LW) during season 3 (2014). The aim of this study was to determine the effects of RDI on yield, plant water relations, and fruit quality at harvest. The results showed that the severe deficit treatment (50% ETa) decreased the photosynthetic rate, vegetative growth (except for season one), and fruit quality i.e. size, titratable acidity, soluble solids and berry weight, and increased oxidative stress (except season three). The 50% ETa treatment only reduced berry yields relative to 100% ETa treatment in Colbún in season 2. In contrast, mild water stress (75% ETa) resulted in fruit yields and quality (firmness, fruit size, titratable acidity, soluble solids and berry weight) similar to the 100% ETa treatment, but with higher water productivity (WP) and intermediate antioxidant capacity.

Keywords: abiotic stress; antioxidants; berries; fruit quality; water management; yield.

3.1 Introduction

Water is the most limiting resource in crop production, and thus irrigation allows high and consistent yield and quality (Araus et al., 2008). Agriculture is the largest and most inefficient water consumer (Costa et al., 2007). Regulated deficit irrigation (RDI), is a strategy that can reduce water use because crops are irrigated below plant water needs (evapotranspiration). Since in the near future water availability is expected to decline in many regions (Ruiz Sánchez et al., 2010), irrigation needs to be scheduled to maximize water productivity (production per unit of water consumed) (Fereres and Soriano, 2007). The use of RDI to conserve water while optimizing yield and quality has been extensively studied in different annual crops and fruit trees (Ortega-Farias et al., 2012). Results have demonstrated that restricting water application altered physical and chemical parameters, which may improve fruit quality (Bacelar et al., 2007; Liu et al., 2001; Zarrouk et al., 2012), in grapevines (Santesteban et al., 2011), raspberries (Morales et al., 2013) and strawberries (Bordonaba and Terry, 2010). RDI has shown positive results in many perennial fruit crops such as peach (Boland et al., 2000), pear (Cui et al., 2008), citrus (García-Tejero et al., 2010), almond (Goldhamer et al., 2006), plum (Intrigliolo and Castel, 2010) and apple (Mpelasoka et al., 2001). In highbush blueberries, water availability during the growing season is critical, especially when fruit are developing (Holzapfel et al., 2015). Blueberries are sensitive to water deficits, causing rapid stomatal closure and reduced transpiration, which restricts water loss and prevents embolism (Améglio et al., 2000; Mingeau et al., 2001). Proper irrigation improves fruit quality and yield in blueberries (Holzapfel et al., 2004; Bryla et al., 2009).

Blueberries contain bioactive compounds such as flavonoids, anthocyanins, and others, which contribute to higher antioxidant capacity in foods and its consumption by humans helps reduce the risk of important diseases including various cancers (Concha-Meyer et al., 2015). However, lack of knowledge regarding changes in fruit quality and antioxidant activity under pre-harvest water stress is still present (Ehret et al., 2012). Crop antioxidant capacity can be influenced by pre-harvest conditions, genetic background plays a key role but cultural practices (Zheng et al., 2003), such as drought stress can induce oxidative stress and membrane lipid peroxidation depending on the severity of the water stress (Guo et al., 2006). Studies are needed to identify the minimum amount of water necessary to optimize both plant performance and fruit quality. Our objective was to evaluate the effect of pre-harvest RDI management on physiological parameters, yield, fruit quality and bioactive compounds of mature *V. corymbosum* plants.

3.2 Materials and methods

3.2.1 Experimental site

3.2.1.1 Field experiment 1

The first trial was conducted in Colbún, Maule Region, Chile (35° 41' 12.99" LS; 71° 25' 8.75" LW) during the 2013/14 and 2014/15 seasons. Bushes cv. Brigitta had been planted in 2007 at 3 x 1 m. The soil was a Colbún series Mollisol with a silty clay loam texture. Plants were managed by standard practices (Retamales and Hancock, 2012), including pest and disease control. Nutrients were applied during the season through fertigation (annual doses of N:22; P_2O_5:17; K_2O:75). Plants were irrigated according to the actual evapotranspiration (ETa = ETo * Kc, where ETo is reference evapotranspiration and Kc is the crop coefficient) and soil water content (Allen et al., 1998). Crop coefficient (Kc) ranged from 0.50 to 0.52 during the season (constructed and adapted from Food and Agriculture Organization (FAO) 56 guidelines).

3.2.1.2 Field experiment 2

The second trial was conducted in 2014 on a commercial farm in South Haven, Michigan, USA (42° 21' 16.26" LN; 86° 12' 48.88" LW), using ‘Brigitta’ plants established in1988 at a 3 x 0.9 m spacing. The soil was an Alfisol in the Thetford series with a loamy sand texture. Plants were fertigated throughout the season with annual doses of N:38; P_2O_5:0; K_2O:21. Plants were irrigated according to the actual evapotranspiration and soil water content, as explained above for the Colbún location. Crop coefficient (Kc) in South Haven ranged from 0.21 to 0.49 during the season.

3.2.2 Experimental treatments

Three irrigation treatments were applied for both locations (Table 3.1): 50, 75 and 100% ETa. In Colbún the irrigation system consisted of one Netafim (Netafim Ltd, Tel Aviv, Israel) irrigation line per row with either 1.2 L/h emitters spaced at 50 and 33 cm for the 50 and 75% ETa treatments, respectively, or 2 L/h emitters at 50 cm spacing for the 100% ETa treatment. During the first season, deficit irrigation began on October 17th, 2013 in the early green fruit growth stage (one week after full bloom) and finished on January 31st, 2014 (two weeks after harvest concluded). In the second season, irrigation began on September 23th, 2014 one week before full bloom and ended on January 16th, 2014 two weeks after harvest was finished.

The same treatments and field setup as in the first site were used in South Haven (Table 1). A Netafim irrigation system was also used, consisting of one drip irrigation line with 2 L/h emitters for the 50 and 75% treatments (spaced at 56 and 85 cm, respectively) and 4 L/h emitter for the 100% treatment (spaced at 85 cm). Deficit irrigation began two weeks before full bloom (May 5th, 2014) and finished after harvest was completed (August 15th, 2014). Irrigation frequency at both locations changed weekly according to weather conditions and evapotranspiration (Table 3.2).

In both locations ETo was estimated using the Penman-Monteith model that uses air temperature, relative humidity, solar radiation and wind speed as inputs. In each location, an automatic weather station Adcon Telemetry A733 GSM/GPRS (Adcon Telemetry, Klosterneuburg, Austria) was installed over a reference grass next to the blueberry field.

3.2.3 Experimental design

A completely randomized block experimental design with three water treatments and four replicates per treatment was established in each site (Table 3.1). Each experimental unit consisted of four blueberry plants, giving a total of 16 bushes per treatment. The fruit was harvested with >90% blue coating color with subsequent storage at 0-2 ºC for 1-3 hours in order to lower fruit temperature until it was analyzed.

3.2.4 Experimental measurements

3.2.4.1 Shoot growth.

Twenty-four randomly selected shoots per plot were tagged and measured in length 30, 45 and 60 d after the treatments started.

3.2.4.2 Soil water content.

Soil water content was determined once a week until the first harvest using a portable time-domain reflectometer (TDR) sensor. A Soilmoisture Mini Trase kit was used in Colbún, while a Soilmoisture 6050X1 TRASE System I (Soilmoisture Equipment Corp., Goleta, CA, USA) was used in South Haven. Four probes (two probes per replicate) were installed in each treatment within the root zone at a depth of 45 cm from the base of the plant.

3.2.4.3 Yield, yield components and water productivity (WP).

Mature fruits were harvested by hand every 3-5 d depending on the ripening rate. After picking, fruit was weighed and a 100 fruit subsample was counted to determine yield components (fruit number and weight). Average fruit weight was determined by weighing in each harvest a sample of 100 randomly picked fruits per plot. Water productivity was calculated per bush as the harvested blueberry yield (kg) per unit of water used (m^3) per bush (Howell, 2001).

3.2.4.4 Gas exchange.

Intact leaves which were fully expanded, attached, well exposed, from the upper second third of the canopy, were used to measure carbon dioxide (CO_2) assimilation, stomatal conductance, and transpiration, using a portable photosynthesis system (LI-6400 with a light source, LI-COR Inc., Lincoln, NE, USA). The equipment was configured as described by Reyes-Díaz et al. (2011) for temperature, radiation, humidity and CO_2. Measurements were made at mid-day at the end of the harvest period, one week after all fruit was harvested. A total of 32 leaves per treatment were used.

3.2.4.5 Midday water potential (Ψs).

Sunlit mature leaves were selected to measure Ψs with a pressure chamber (PMS Instrument Co., Model 600, Corvallis, OR, USA). Stem tips with two or three leaves were wrapped with plastic transparent film and aluminum foil at least 2 h before the measurements, allowing an equilibrium between leaf and plant xylem water status to be achieved (Choné et al., 2001; Jara-Rojas et al., 2015). For both locations, seven measurements were done once per week during the pre-harvest period until the first harvest.

3.2.4.6 Fruit quality.

For both sites, fruit size (equatorial diameter) was measured simultaneously with firmness on 50 fruits per replication with a Firmtech II fruit firmness tester (BioWorks, Wamego, KS, USA). The equipment was set up with maximum and minimum compression forces of 200 g (1.96 N) and 15 g (0.15 N), respectively, and a piston speed of 6 mm s^{-1}.

Average TSS was measured with a digital thermos-compensated refractometer (Hannah Instruments HI96811, Woonsocket, RI, USA) which was previously calibrated with distilled water. Measurements were done in the juice obtained after crushing 15 fruits per replicate.

For both locations, the TA was determined on five fruits per replicate through the potentiometric titration method using a Titroline Easy (SI-Analytics GmbH, College Station, TX, USA) automatic titrator according to methodology described by Castrejón et al. (2008).

3.2.4.7 Membrane lipid peroxidation.

The lipid peroxidation of membranes was determined as an indicator of oxidative stress, using fresh fruit material collected at peak harvest (30 mg per replicate, collected at peak harvest). This parameter was determined using the thiobarbituric acid reacting substance (TBARS) assay according to Du and Bramlage (1992). The absorbance was measured at 440, 532 and 600 nm using an automated plate reader Synergy H1 (Bio-Tek, Winooski, VT, USA).

3.2.4.8 Oxygen radical absorption capacity (ORAC).

ORAC was measured on peak harvest fruit using an automated plate reader Synergy H1 (Bio-Tek, Winooski, VT, USA) for fruit from Colbún. Fruits from South Haven were measured using a Bio-Tek semi-automated plate reader FLX800TBID (Bio-Tek, Winooski, VT, USA). ORAC in fruit (1 g per replicate) from both locations was collected and measured according to the procedure proposed by Prior et al. (2003).

3.3 Statistical analyses.

Analysis of variance (ANOVA) was used to determine significant effects of treatment on horticultural, physiological, and biochemical parameters. Statistically significant differences ($P \leq 0.05$) were evaluated with Tukey HSD-test (SPSS software) after statistical assumptions were met.

3.4 Results

Total amount of water applied during the three seasons of the study (Table 1) varied due to weather conditions and rainfall. In general, the seasonal mean values of average temperature were similar for both seasons in Colbún (Table 2). The highest ETo was observed during December and January for Colbún with greater values for the first season, which lead to higher irrigation levels. South Haven’s season was characterized by higher average temperature compared to seasons in Colbún. Rainfall was greater in South Haven, but with lower ETo (Table 3.2), which lead to lower water application.

3.4.1 Shoot growth.

Statistical differences in shoot growth were found between water treatments only in South Haven and in the second season in Colbún (Table 3.3). The lowest shoot growth was always observed in the 50% ETa plants, while the 100% ETa water regime expressed the largest shoot growth for both the second season in Colbún and the one in South Haven. No significant differences in shoot growth were found between 100% ETa and the mild deficit treatment (75% ETa) during South Haven's season.

3.4.2 Soil water content.

Soil water content differed statistically among treatments during each season (Table 3.3). Average season values of volumetric soil water content at 45 cm depth were nearly constant (Table 3.3), with values ranging from 20.8% (for 50% ETa, first season) to 28.1% (for 100% ETa, third season). Results were directly related to the water volumes applied; thus, the 100% ETa treatment had the highest water content, followed by the 75% and then the 50% ETa treatment.

3.4.3 Yield, yield components and water productivity

Yield was only affected by irrigation treatments during the second season in Colbún. In this case, the fully irrigated bushes (100% ETa) produced the highest yields (3.3 kg/bush), followed by the mild deficit (75% ETa), and the severe deficit treatment (50% ETa) (Table 3.4).

Total fruit number per bush was only affected by treatments during the second season in Colbún (Table 3.4), with the 75% treatment showing an intermediate number and the 50% treatment producing 25% fewer fruits than the 100% ETa plants. Bushes produced less fruit in the second season at Colbún because they were severely pruned in the previous winter.

Average berry weight was affected by treatments during all three seasons (Table 3.4). Severe water shortage (50% ETa) reduced berry weight compared to the mild deficit and fully irrigated plants (75 and 100% ETa respectively) during both seasons in Colbún. The 75% treatment had an intermediate response in the South Haven's harvest season. Berry weight during Colbún's second year was higher than in the first season probably because pruning reduced fruit numbers, but the same tendencies according to water volumes were observed.

Significantly higher water productivity (WP) was found for severe water deficit treatment (50% ETa) as compared to fully irrigated plants (100% ETa) in the first season in Colbún (70% greater) and the one in South Haven (65% greater). Mild deficit treatment (75% ETa) was not significantly different to 100% ETa (Table 3.4).

3.4.4 Gas exchange.

Treatments had significant effects on photosynthetic rate, stomatal conductance and transpiration (Table 3.5). The 50% ETa treatment resulted in the lowest gas exchange levels during all three seasons. The 100 and 75% ETa treatments did not differ in gas exchange measurements, except for photosynthetic rate in South Haven. Stomatal conductance and transpiration did not express statistical differences for the second season in Colbún, while mild deficit treatment (75% ETa) had similar (Colbún's first season) or intermediate (South Haven) values for these variables compared to 100% ETa plants.

3.4.5 Midday water potential.

Treatments affected midday water potential during each season. Values ranged from -1.2 to -0.7 MPa (Figure 3.1). Severe water deficit treatment (50% ETa) had the lowest midday water potential the first season in Colbún, but no differences were found between mild deficit (75% ETa) and the 100% ETa treatment in the other two seasons.

3.4.6 Fruit quality.

Different water volumes expressed statistical differences in fruit quality in all evaluations (Table 3.6). Severe deficit treatment (50% ETa) had the highest fruit firmness while 100% ETa had the lowest readings for this variable, and mild deficit treatment (75% ETa) showed intermediate values in every season.

Irrigation treatments had consistent effects on fruit size during all seasons (Table 3.6). Higher water volumes (100% Eta) produced fruit with larger equatorial diameter while the 50% ETa resulted in smaller fruit size every season. Mild deficit treatment (75% ETa) had intermediate equatorial diameter, except for the first season in Colbún where diameter was equivalent to the 100% ETa treatment.

In South Haven, fruit from the 50% ETa treatment had lower titratable acidity than those from the 75 or 100% ETa treatments. Control treatment (100% ETa) had the highest TA, except for Colbún´s first season were no statistical differences were found between treatments and Colbún's second season where mild deficit treatment was not statically different from fully irrigated plants (Table 3.6).

In plants under severe water stress (50% Eta) fruit soluble solids were higher than those in the 100% ETa treatment, except for the first season in Colbún. Mild deficit treatment (75% ETa) only differed statistically from severe deficit treatment (50% ETa) during both seasons in Colbún (Table 3.6).

3.4.7 Membrane lipid peroxidation (TBARS).

Treatments only affected this variable at the Colbún location. Highest TBARS values were observed in the 50% ETa treatment while the lowest ones were detected in the 100% ETa. Mild deficit treatment (75% ETa) had similar low oxidative damage as 100% ETa fruit during the first season in Colbún, but were statistically different during the second season in that location (Table 3.7).

3.4.8 Oxygen radical absorption capacity (ORAC).

Treatments only affected antioxidant capacity during the first season in Colbún (Table 3.7). In this analysis, fruit from severe water stressed treatment (50% ETa) showed significantly lower antioxidant capacity values compared to the other treatments, while mild deficit treatment (75% ETa) had intermediate values. Highest ORAC levels were detected in berries from the 100% ETa treatment.

3.5 Discussion

Water is one of the main factors affecting plant growth and yield (Spreer et al., 2009). Blueberry plant growth and its cultural practices are associated with adequate soil water content throughout the season (Holzapfel et al., 2004). Our results evidenced significant effect of water stress on lateral shoot growth only in South Haven and the second season in Colbún. We also established that lower water availability, negatively affected gas exchange, which may limit the availability of photoassimilates, and plant performance. However, the first season in Colbún did not show statistical differences probably due to plant adaptation to soil moisture conditions compared to South Haven. Mild water deficit treatment (75% ETa) expressed intermediate effects on growth due to a lower negative effect when compared to severe deficit treatment. In contrast, control treatment (100% ETa) made use of full water availability for shoot growth to occur at a normal rate. Depending on the crop studied, RDI has had variable effects on vegetative growth. In the first of a two-year experiment, RDI (40% and 60% ETa treatments after fruit set and enlargement) increased tree top volume on citrus trees compared to control treatment (100% ETa) (Gasque et al., 2010). On the other hand, plant dry mass and annual shoot growth of tomato plants subjected to 50% ETa decreased by about 30% with respect to fully irrigated control treatments (Tahi et al., 2007). Vegetative growth was significantly increased by moderate and heavy irrigation on 'Duke' highbush blueberry compared to non-irrigated plants (Ehret et al., 2012, 2015).

Highbush blueberries may be particularly sensitive to dry soil conditions because they lack root hairs (Cameron et al., 1988). In our experiment, soil water contents were reduced as the applied water volume was restricted (Table 3.3). Water availability was fundamental to understand plant performance. Similar results were found in both 'Duke' highbush (Ehret et al., 2012) and lowbush blueberry plants subjected to water stress (Glass et al., 2005).

Yield levels in our trial were significantly affected by irrigation treatments only in the second season in Colbún, possibly because of the early phenological stage (before bud break) at which deficit irrigation was initiated in that season. Fruit number was only significantly affected during the second season in Colbún, with the water deficit treatments having less fruit than the 100% ETa treatment, but intermediate values with mild deficit treatment (75% ETa), which would suggest a possible impact of deficit treatment on bud induction for the next season (a variable which we did not measure). During water shortage, it is common that plants reduce their transpiration rate affecting the transpiration stream in order to prevent desiccation, with the consequent water translocation to fruits. Berry weight in this study presented significant differences for all seasons, with the 100% and 75% ETa treatments having greater weights due to increased water application compared to 50% ETa treatment (Table 3.4). Working with ‘Star‘ highbush blueberry plants, Keen and Slavich (2012), did not find statistical differences for yield and berry weight when comparing 50% ETa RDI treatment with the control (100% ETa). Ehret et al. (2015, 2012) had increased yields and berry weight in ‘Duke’ highbush blueberry and significant higher bud number during the first and third season when applying higher water volumes per hectare compared to non-irrigated plants. Similar results were reported by Holzapfel et al. (2004) working with ‘Bluetta’ blueberry plants when comparing different water volumes. Spreer et al. (2009) also found less fruit per tree in ‘Chok Anan’ mango with 50% ETa compared to 100% ETa treatment. Although photosynthetic rates and yields generally decline when plants are even subjected to mild to moderate water deficits, water use by the crop is reduced in greater proportion; as a consequence, WP is usually higher under deficit irrigation conditions. Even though achieving better WP sometimes cannot match profit yields, our results indicate that water-saving potentials of 25–70% were achieved by water deficit treatments during the first season in Colbún and the season in South Haven without impacting yield. Keen and Slavich (2012) obtained similar results for WP working with ‘Star’ highbush blueberry plants when comparing 50% ETa to the 100% ETa treatment. Liu et al. (2007) measured better WP with 60% ETa compared to 100% ETa treatment in ‘Honeoye’ strawberry plants, but with significant less yield, which is similar to the findings reported by Spreer et al. (2009) after applying a 50% ETa treatment in ‘Chok Anan’ mango.

Water stress decreases both the photosynthetic rate and the yield in many fruit crops. In most cases, photosynthesis is reduced because stomatal conductance limits CO_2 diffusion from the atmosphere to the site of carboxylation (Flexas et al., 2009). In our trial the application of the different RDI treatments changed stomatal opening and this altered both stomatal conductance and transpiration. Our results showed that the most stressful treatment (50% ETa) had a 20-80% reduction in photosynthetic rate, stomatal conductance, and transpiration as compared to control plants (100% ETa). Berry weight was affected during all the seasons of this study possibly because of the gas exchange reduction, however, yields were only affected during the second season in Colbún. Bryla and Strik (2006) found that, independent of cultivar (Duke, Bluecrop and Elliott), midday stomatal conductance in highbush blueberry plants decreased as plant water potential approached -0.6 to -0.8 MPa. In 'Bluecrop' blueberry grown under greenhouse conditions, net photosynthesis and stomatal conductance were reduced by 30% due to water stress (no irrigation for three weeks at the onset of shoot growth) as compared to daily irrigation (Rho et al., 2012). Net photosynthesis, stomatal conductance, and transpiration were also reduced by 30% in tomato plants for the 50% ETa treatment as compared to the fully irrigated control (Lei et al., 2009; Tahi et al., 2007). Similar reductions in these variables were reported by Perez-Sarmiento et al. (2010) with a 60% ETa treatment applied to apricot trees.

Midday water potential is an indicator of plant water status, and it is essential when planning deficit irrigation practices. RDI has been shown to reduce the diurnal leaf conductance and midday water potential (Sánchez-Blanco et al., 2009). In our trials, the more intense water deficit (50% ETa) had lowest midday water potentials while the mild deficit (75% ETa) showed intermediate midday water potentials during all seasons, except for the first season where values were equivalent to fully irrigated plants. These results suggest that 'Brigitta' blueberry bushes can withstand a mild deficit treatment without affecting their performance too negatively. Similar results were achieved with 40% ETa in grapevines by Niculcea et al. (2013) and in olive trees (rainfed control, 30, 60% ETa) by Bacelar et al. (2007), when comparing values to fully irrigated control treatments. Although leaf water potentials differ among cultivars, measured either at predawn or at midday, the fast decline under deficient soil water content was common among the different highbush blueberry cultivars (Bryla and Strik, 2007).

Water scarcity can influence berry yield and quality (fruit weight, size, firmness, TSS and TA) (Prange and DeEll, 1997). The fruit quality results obtained in our research were proportional to the amount of water applied in each treatment. In general, fruit size is negatively correlated to firmness (Sams, 1999). Tissue density is often higher in smaller berries, causing a greater fruit firmness (Bryla, 2008). In our study, treatments that received more water had larger fruit size and reduced fruit firmness. Although the most severe deficit irrigation treatment (50% ETa) increased fruit firmness, it also resulted in the smallest and lightest berries. Irrigation treatments affected TSS and TA concentrations in fruit. The lower TSS we found in fruit from well-watered bushes may be due to a dilution effect. The moderate deficit treatment (75% ETa), usually behaved similarly to the 100% ETa treatment. Similar effects of water regimes on fruit quality were reported by Bryla et al. (2008) after implementing 50, 100 and 150% ETa treatments to ‘Elliott’ highbush blueberry plants over two years, although yield in their case was significantly affected by irrigation level. The fruit quality parameters obtained in this trial are similar to those reported for the same highbush blueberries varieties by Ehlenfeldt and Martin (2002) and Ribera et al. (2010).

In the last few decades, there has been a marked worldwide expansion in the area planted with blueberries due to greater demand derived from the perceived benefits to human health associated with blueberry consumption (Retamales et al., 2015). Partial stomatal closure is among the effects of drought stress that appear shortly after the onset of the stress on crops. This effect limits the entry of CO_2 for photosynthesis and is followed by the production of reactive oxygen species (ROS) which can cause DNA deterioration, lipid peroxidation (LPO) and oxidation of amino acids (Johnson et al., 2003). The severity of the oxidative damage is commonly determined through malondialdehyde (MDA) which is an end product of membrane lipid peroxidation (Iturbe-Ormaetxe, 1998; Lei et al., 2009). TBARS is a measure of LPO resulting from oxidative stress damaging cell membrane leading to increased electrolyte leakage and compromising its stability. Under drought stress, high correlations have been found in rice between TBARS readings and enhanced electrolyte leakage, resulting in an injury to plasmalemma (Guo et al., 2006). Our results showed that membrane lipid peroxidation was proportional to water stress treatments; the 50% ETa treatment showed the highest oxidative stress for the two seasons in Colbún, enhancing the susceptibility to membrane damage and suggesting negative effects on cellular function that may compromise fruit post-harvest life. Mild deficit treatment (75% ETa) presented damage in a lower scale during the first season in Colbún, which may not adversely affect antioxidant mechanisms in the fruit. Bacelar et al. (2007), Iturbe-Ormaetxe et al. (1998) and Lei et al. (2009) reported that lipid peroxidation effects were proportional to the level of drought stress in olive, pea, and tomato plants, respectively.

ROS are continuously being produced in plants but these organisms have evolved mechanisms to scavenge ROS's and avoid reaching damaging levels. Changes in expression and activities of antioxidant enzymatic and non-enzymatic reactions have been reported in several crops in response to adverse environmental conditions, such as water deficit and other abiotic or biotic factors (Szőllősi, 2014). Over-expression of one or more ROS-scavenging enzymes and antioxidant systems have been shown to decrease oxidative stress (Miller et al., 2010). In blueberries, antioxidant capacity has been correlated with high total phenolic and anthocyanin contents (Zheng et al., 2003). In our trials, antioxidative systems would have been induced in response to drought stress, suggesting that water deficit required an increased activity of protective systems for stress compensation. ORAC values in the first season in Colbún, were inversely related to water status, with the 100% ETa plants having the highest

ORAC activity, suggesting that severity of water stress might have overcome antioxidant capacity leading to an irreversible damage. Drought stress reduced antioxidant activity in olive trees (Bacelar et al., 2007), whereas RDI did not affect ORAC in grapes (Zarrouk et al., 2012). Connor et al. (2002) measured statistical differences in antioxidant activity of blueberries; they found that variation in antioxidant capacity was significant among cultivars across locations and years, as well as within individual years in each location. Ehret et al. (2015) when working with irrigated and non-irrigated 'Duke' highbush blueberry plants measured statistical differences for ORAC values of fruits between seasons. Their results suggest an adaptation of the plants to water deficit after not detecting statistical differences between treatments during the second season that had shown to differ in the first one.

3.6 Conclusions

Blueberry is a shallow-rooted crop that is highly sensitive to water deficits. Shoot growth and yield were negatively affected by the most stressful RDI treatment. Water regimes also influenced fruit quality and gas exchange of 'Brigitta' bushes. The most water-stressed plants (50% ETa) produced smaller but firmer berries than fully irrigated bushes (100% ETa). The results showed that blueberries are highly sensitive to severe water deficit (50% ETa), and by displaying notably by lower stomatal conductance and, in parallel, by a significant reduction in net photosynthesis and transpiration. Promising results for application of RDI in horticultural blueberry management were obtained with moderate deficit irrigation (75% ETa) since these plants had equivalent yield and similar fruit quality (firmness, fruit size, acidity, soluble solids and berry weight) to 100% ETa bushes. Antioxidant capacity was higher in fully irrigated plants, followed by mild deficit treatment (75% ETa). Severe deficit (50% ETa) treatment expressed greater oxidative damage alongside with 75% ETa treatment but with the lowest antioxidant activity. WP was significantly increased by severe water deficit treatments (25-70%) with intermediate values achieved by the 75% ETa treatment. These results evidence that reducing water by 25% could save around 620-680 m^3/ha without sacrificing fruit quality. This should be particularly relevant to fruit production in semiarid areas where water shortage and climate change are demanding reduced use of irrigation water without diminishing fruit quality.

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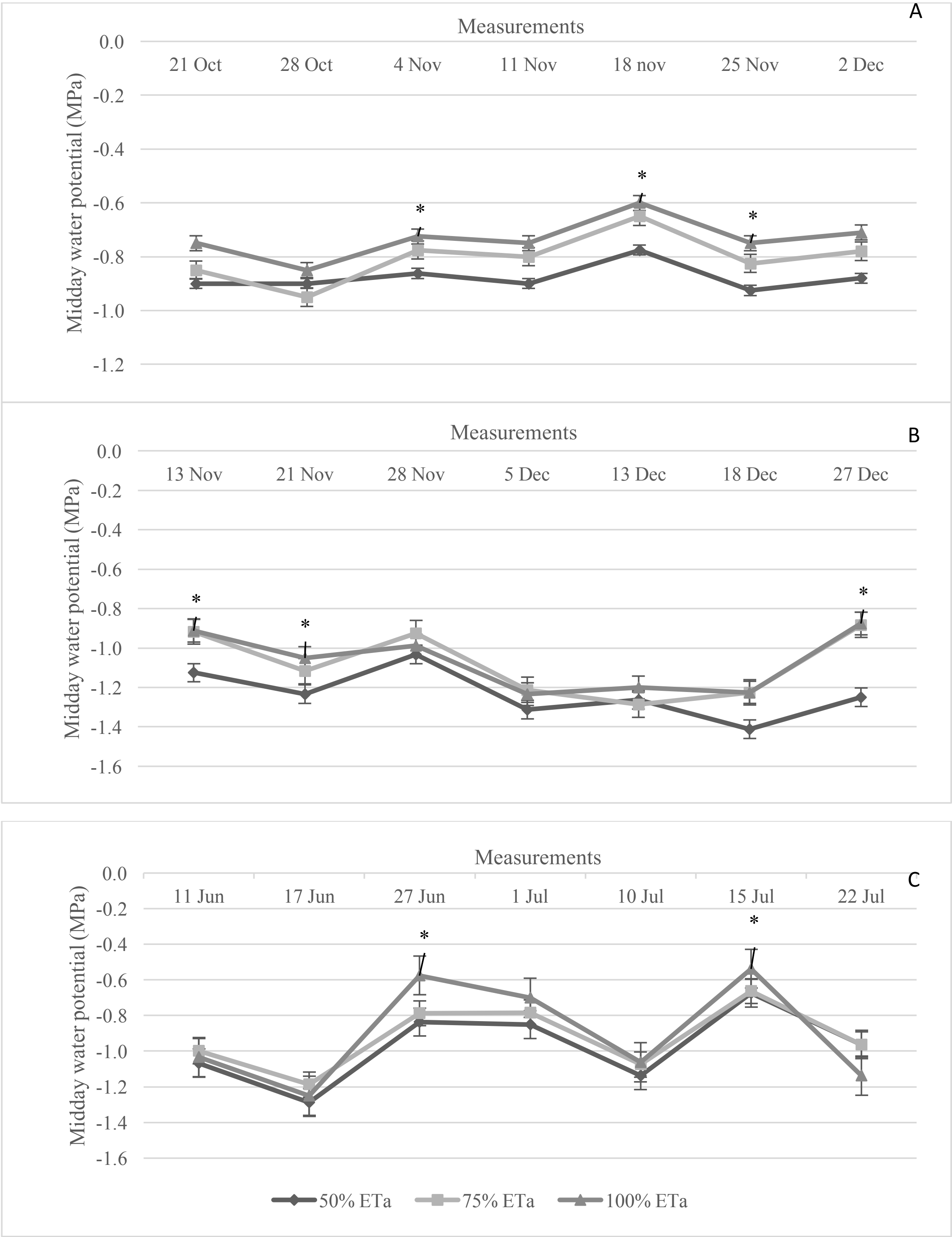

Figure 3.1. Effect of irrigation level (% ETa) on midday water potential for (A) Colbún 2013-14, (B) Colbún 2014-15, and (C) South Haven 2014. *indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Table 3.1. Water applied (mm) for the different treatments and production seasons for Colbún (two seasons) and South Haven (one season)

	100% ETa	75% ETa	50% ETa
	Colbún 2013-14 / 2014-15		
September	0 / 0	0 / 0	0 / 0
October	27 / 27	20 / 20	13 / 13
November	54 / 55	40 / 41	27 / 28
December	81 / 80	61 / 60	41 / 40
January	108 / 82	81 / 61	54 / 41
Total (mm)	270 / 244	202 / 182	135 / 122
	South Haven 2014		
April	0	0	0
May	36	27	18
June	23	17	12
July	23	17	12
August	22	17	11
Total (mm)	104	78	53

Table 3.2. Weather conditions during production season for Colbún (two seasons) and South Haven (one season)

	Average temperature (ºC)	Rainfall (mm)	Effective rainfall (mm)	ETo (mm)
	Colbún 2013-14 / 2014-15			
September	10.7 / 10.9	65.2 / 163.6	41.3 / 60.6	46.5 / 54.7
October	12.3 / 13.4	39.2 / 14.6	20.7 / 0.6	95.8 / 99.2
November	14.8 / 15.2	1.6 / 11.8	0 / 0.3	132 / 133.6
December	18.7 / 17.5	0 / 17.4	0 / 5.1	173.4 / 144.5
January	19.3 / 19.8	2.6 / 0	0 / 0	172.2 / 162.4
	South Haven 2014			
April	7.6	57.6	2.4	59.1
May	13.5	41.4	1.1	76.8
June	19.5	75.7	9.9	78.5
July	18.3	66.0	13.0	84.1
August	20.6	61.2	11.1	72.1

Table 3.3. Effects of irrigation level (% of actual evapotranspiration: ETa) on shoot growth and soil moisture of blueberry plants cv. Brigitta

Season (year)	Treatment (% ETa)	Shoot growth (cm)	Soil water content (%)
Colbún 2013-14	100	10.4	26.7 a
	75	10.7	23.1 b
	50	10.2	20.8 c
Colbún 2014-15	100	20.9 a	28.1 a
	75	17.9 b	26.7 ab
	50	14.8 c	23.7 b
South Haven 2014	100	18.5 a	26.9 a
	75	15.2 ab	25.2 ab
	50	14.6 b	21.7 b

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at $P \leq 0.05$) between RDI treatments.

Table 3.4. Effects of irrigation level (% of actual evapotranspiration: ETa) on yield, total fruits, water productivity (WP) and berry weight of blueberry plants cv. Brigitta.

Season (year)	Treatment (% ETa)	Yield (kg/bush)	WP (kg/m^3)	Total fruits (No./bush)	Berry weight (g/fruit)
Colbún 2013-14	100	4.8	5.9 b	2919	1.6 a
	75	4.8	8.0 ab	3108	1.6 a
	50	4.1	10.1 a	3092	1.3 b
Colbún 2014-15	100	3.3 a	4.6	1674 a	2.0 a
	75	2.7 b	5.0	1403 ab	2.0 a
	50	2.0 c	5.6	1232 b	1.7 b
South Haven 2014	100	4.2	14.8 b	2671	1.6 a
	75	3.8	18.0 ab	2607	1.5 ab
	50	3.5	24.4 a	2482	1.4 b

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at $P \leq 0.05$) between RDI treatments.

Table 3.5. Effects of irrigation level (% of actual evapotranspiration: ETa) on photosynthetic rate, stomatal conductance and transpiration of blueberry plants cv. Brigitta.

Season (year)	Treatment (% ETa)	Photosynthetic rate (µmol $CO_2 m^{-2}s^{-1}$)	Stomatal conductance (mol H2O $m^{-2}s^{-1}$)	Transpiration (mmol H2O $m^{-2}s^{-1}$)
Colbún	100	8.0 a	0.05 a	2.6 a
2013-14	75	7.1 a	0.04 a	2.1 a
	50	3.8 b	0.01 b	0.7 b
Colbún	100	12.5 a	0.09	2.0
2014-15	75	11.0 a	0.09	1.9
	50	8.5 b	0.07	1.6
South Haven	100	9.7 a	0.29 a	3.3 a
2014	75	8.3 b	0.22 ab	2.5 ab
	50	6.3 c	0.15 b	2.2 b

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Table 3.6. Effects of irrigation level (% of actual evapotranspiration: ETa) on fruit quality (firmness, fruit size, titratable acidity and soluble solids) of blueberry plants cv. Brigitta.

Season (year)	Treatment (% ETa)	Firmness (g/mm)	Fruit size (mm)	Titratable acidity (% citric acid)	Soluble solids (ºBrix)
Colbún	100	177.1 b	14.8 a	0.83	14.3 ab
2013-14	75	190.2 ab	14.8 a	0.87	13.7 b
	50	200.4 a	13.4 b	0.85	15.3 a
Colbún	100	150.2 b	16.4 a	0.73 a	12.4 b
2014-15	75	158.0 ab	16.1 b	0.70 ab	12.7 b
	50	163.5 a	14.8 c	0.63 b	13.6 a
South Haven	100	131.5 b	16.0 a	1.08 a	11.4 b
2014	75	134.2 ab	15.4 b	0.99 b	12.6 a
	50	134.9 a	14.9 c	0.87 c	12.9 a

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Table 3.7. Effects of irrigation level (% of actual evapotranspiration: ETa) on membrane lipid peroxidation (TBARS) and oxygen radical absorption capacity (ORAC) of blueberry fruit cv. Brigitta.

Season (year)	Treatment (% ETa)	TBARS (nmol/g FW)	ORAC (µmol TE/g)
Colbún	100	4.7 b	4569 a
2013-14	75	6.1 ab	4242 b
	50	7.7 a	2224 c
Colbún	100	8.0 b	4100
2014-15	75	10.9 a	3849
	50	12.6 a	3766
South Haven	100	7.3	4839
2014	75	7.7	4715
	50	8.7	4677

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Chapter IV

Pre-harvest regulated deficit irrigation management effects on post-harvest quality and condition of V. corymbosum fruits cv. Brigitta

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Abstract

Blueberries are highly sensitive to water stress, especially during fruit development when fruit yields and post-harvest quality can be affected. Regulated deficit irrigation (RDI) can increase fruit quality of many fruit crops without reducing yield. However, the influence of pre-harvest RDI on post-harvest quality of blueberry has not been studied. Our aim was to evaluate the post-harvest quality of *V. corymbosum* fruits cv. Brigitta grown under pre-harvest RDI. Two locations were used for this research: 1.- Colbún, Maule Region, Chile (35° 41' 12.99" LS; 71° 25' 8.75" LW) during the 2013-14 and 2014-15 seasons using six-year-old plants, and 2.- South Haven, Michigan, USA (42° 21' 16.26" LN; 86° 12' 48.88" LW), during the 2014 season, on twenty-six-year-old bushes. Plants were subjected to three irrigation treatments that replaced 50, 75 or 100% (control) of actual evapotranspiration (ETa). Fruits were harvested at > 90 full color and stored for 30 and 60 d at 0-2 ºC + 3 d at 18-20 ºC. Water treatments had no effect on the proportion of sound, dehydrated or decayed berries after storage. However, fruit quality was affected, with mild water deficit (75% ETa) producing similar fruit quality, i.e., firmness, titratable acidity, soluble solids and antioxidant activity as fully irrigated plants (100% ETa), and lower oxidative stress than 50% ETa treatment at 60+3d, but with lesser weight loss. Blueberries are considerably sensitive to water stress, with 50% treatment showing the highest lipid peroxidation and lowest antioxidant activity (ORAC) for both 30+3d and 60+3d, as well as by decreasing fruit quality (high SS and low TA). We have shown that applying 25% less water to highbush blueberries does not reduce fruit quality or levels of antioxidants.

Keywords: abiotic stress; antioxidants; berries; fruit quality; irrigation regimes; shelf life

4.1 Introduction

Drought stress limits worldwide crop production more than any other abiotic stress (Grant, 2012). Abiotic factors can alter fruit and vegetable quality before harvest, thus management decisions during crop production are very important (Kays, 1999). Irrigation should maximize water productivity (production per unit of water consumed) (Fereres and Soriano, 2007) without negatively affecting the post-harvest quality or levels of bioactive compounds in fruit. Regulated deficit irrigation (RDI) is a strategy that attempts to conserve water by limiting irrigation during less drought sensitive growth stages. In various fruit and vegetable crops, adequate soil moisture just prior to harvest is essential to maintain produce quality (Nora et al., 2012). Water stress during the growing season can reduce fruit size and this may lead to soft or dehydrated fruit, increasing the probabilities of physical damage, decay during storage and reduced shelf life (Kays, 1999). Changes in water status alter the condition of the produce in general, with firmness being one of the most critical traits influencing consumer appeal of fresh blueberries (Ehret et al., 2015; Keen and Slavich, 2012; NeSmith et al., 2002). Blueberries tend to soften during the postharvest chain and this reduces their final quality (Ehlenfeldt and Martin, 2002). Many blueberries are rejected when they reach markets because they are too soft to meet retail standards (Paniagua et al., 2013).

Physiological, physical and pathological processes often determine postharvest changes in blueberry quality. Decay, softening and shriveling have been shown to decrease fruit quality during postharvest handling. Fruit moisture loss has an important role in defining firmness changes of blueberries after harvest (Paniagua et al., 2014). Fruit quality is also influenced by harvest practices as well as cultivar, cultural practices, and environmental conditions during the season (Forney, 2009).

There have been numerous recent studies on the factors affecting the antioxidant capacity in fruits and vegetables. Blueberries are a rich source of natural antioxidants, including total phenolics and anthocyanins that have high activity against reactive oxygen species (ROS) (Kim et al., 2013; Wang et al., 2009). Proper water management should prevent moisture stress and oxidative damage, even after harvest, and consequently extend fruit shelf life. Predictions suggest that worldwide water shortages will become more common. Also, blueberries from the Southern hemisphere need to withstand long boat journeys to reach markets in Northern hemisphere countries (Retamales et al., 2015). In this context, the objective of this study was to evaluate the long-term postharvest quality and condition of *V. corymbosum* fruits subjected to pre-harvest RDI management.

4.2 Materials and methods

4.2.1 Experimental site

4.2.1.1 Field experiment 1

The first blueberry field was located in Colbún, Maule Region, Chile (35° 41' 12.99" LS; 71° 25' 8.75" LW). Treatments were applied and measurements were taken in the 2013/14 and 2014/15 seasons. Plants cv. Brigitta had been planted in 2007 at 3 x 1 m. The soil is classified as Mollisol, has a silty clay loam texture and corresponds to Colbún series. Plants were fertigated in both seasons with annual doses of N:22; P_2O_5:17; K_2O:75. Plants were irrigated according to the soil water content and the actual evapotranspiration (ETa = ETo * Kc, where ETo is reference evapotranspiration; Kc is the crop coefficient) (Allen et al., 1998). The crop coefficient (Kc) ranged from 0.50 to 0.52 during the season. Kc values were constructed from the Food and Agriculture Organization (FAO) 56 guidelines through calibration during three years using measurements of midday water potential (> -0.8 MPa).

4.2.1.2 Field experiment 2

The second blueberry field was located in South Haven, Michigan, USA (42° 21' 16.26" LN; 86° 12' 48.88" LW) where measurements were performed only during the 2014 season. ‘Brigitta’ blueberry bushes planted in 1988 at 3 x 0.9 m were used. Plants were grown in a loamy sand soil classified as an Alfisol, corresponding to the Thetford series and fertigated throughout the season with annual doses of N:38; P_2O_5:0 and K_2O:21. Plants were irrigated according to the actual evapotranspiration and soil water content, as explained above for the Colbún location. Crop coefficient (Kc) in South Haven ranged from 0.21 to 0.49 during the season. Kc values used in this location were extrapolated from a similar climate condition found in southern Chile (INIA-Carillanca, Vilcún, Lat. 38° 40’ N). Stem water potential measurements done during the blueberry growth and development period showed values > -0.89 MPa for the 100% ETa treatment, corroborating that those plants were not under water deficit conditions according to Estrada et al. (2015).

4.2.2 Experimental treatments

Three irrigation treatments were imposed in both locations (Table 4.1): 50, 75 and 100% ETa. In Colbún irrigation was applied with one Netafim (Netafim Ltd, Tel Aviv, Israel) drip irrigation line with emitter flow rates of either 1.2 L/h (50 and 75% ETa, drippers spaced at 50 and 33 cm respectively) or 2 L/h (100% ETa, drippers spaced at 50 cm). In the first season, irrigation treatments began on October 17, 2013 in the early green fruit growth stage (one week after full bloom) and finished on January 31, 2014 (two weeks after harvest was finished). Treatments during the second season began on September 23, 2014 one week before full bloom and ended on January 16, 2014 after harvest was completed.

South Haven location used same three treatments as Colbún: 50, 75 and 100% ETa (Table 4.1). Irrigation system consisted of one drip Netafim irrigation line with 2 L/h emitters for the 50 and 75% treatments (spaced at 56 and 85 cm, respectively) and 4 L/h emitter for the 100% treatment (spaced at 85 cm). Irrigation treatments began two weeks before full bloom stage (May 5, 2014) and finished on August 15, 2014 after harvest was completed.

Irrigation frequency for both locations changed weekly according to weather conditions and evapotranspiration. Weather conditions during the trial period for both sites are shown in Table 4.2. In both locations ETo was estimated using the Penman-Monteith model which has as inputs: air temperature, relative humidity, solar radiation and wind speed. In each location, an automatic weather station Adcon Telemetry A733 GSM/GPRS (Adcon Telemetry, Klosterneuburg, Austria) was installed over a reference grass adjacent to the blueberry field.

4.2.3 Experimental design

A completely randomized experimental design with three irrigation treatments and four replicates was used. Each experimental unit consisted of four plants, with a total of 16 plants per treatment. Fruit from peak harvest with >90% blue coating color were harvested and they were subsequently stored at 0-2 ºC. For statistical analysis the experimental unit was composed of 125 g of fruit (clamshell container), using a total of four clamshells per treatment per evaluation date. Fruits were analyzed after 30 or 60 d in cold storage plus 3 d at 18-20 ºC.

4.2.4 Experimental measurements

4.2.4.1 Gas exchange.

Carbon dioxide (CO_2) assimilation, stomatal conductance, and transpiration were measured using a portable photosynthesis system (LI-6400 with a light source, LI-COR Inc., Lincoln, NE, USA). Intact fully-expanded leaves from the upper second third of the canopy which were attached to the plant and well exposed were used. The equipment was configured as described by Reyes-Díaz et al. (2011) for temperature, radiation, humidity and CO_2. Measurements were made early in the morning at the end of the harvest period, one week after all fruit was harvested. A total of 32 leaves per treatment was used.

4.2.4.2 Midday water potential (Ψs).

Mature sunlit leaves were selected to measure Ψs once per week during the pre-harvest period until the first harvest with a pressure chamber in both locations (PMS Instrument Co., Model 600, Corvallis, OR, USA). Shoot tips with two or three leaves were wrapped with plastic transparent film and aluminum foil at least 2 h before the measurements, allowing an equilibrium between leaf and plant xylem water status to be achieved (Choné et al., 2001; Jara-Rojas et al., 2015). For each measurement, 16 shoots per treatment were used.

4.2.4.3 Weight loss.

A precision balance PN-6100A (American Weight Scales, Norcross, GA, USA) was used to determine average fruit weight from Colbún. Fruit weight from South Haven site was determined with a precision balance PT-2100 (Sartorius Corporation, Bohemia, NY, USA). In each case, a sample of 50 randomly-picked fruits were measured per replicate and then individual fruit weight was determined. Weight loss was calculated as the percentage difference between the initial and the final weight of the clamshell containing fruit, after subtracting the weight of the clamshell.

4.2.4.4 Fruit quality

Total soluble solids (TSS) was determined with a digital thermo-compensated refractometer (Hannah Instruments HI96811, Woonsocket, RI, USA), from juices of 15 fruits per replication and treatment. The device was calibrated with distilled water.

Fruit titratable acidity (TA) was determined on five fruits per replicate by the potentiometric titration method using a Titroline Easy automatic titrator (SI-Analytics GmbH, College Station, TX, USA) according to methods described by Castrejón et al. (2008).

A Firmtech II meter (BioWorks, Wamego, KS, USA) was used to determine fruit firmness. The equipment was set up with maximum and minimum compression forces of 200 g (1.96 N) and 15 g (0.15 N), respectively, while the speed of the piston was configured at 6 mm/s. Firmness was measure on 50 fruits per replicate or a total of 200 fruits per treatment.

4.2.4.5 Fruit condition.

Fruit were visually inspected after storage and designated as either sound, dehydrated or decaying, and expressed as percentage in each condition. Dehydrated fruit had visibly wrinkled skin. Decaying fruits had visible mold and/or juice exudation.

4.2.4.6 Membrane lipid peroxidation (TBARS) and Oxygen radical absorption capacity (ORAC).

As an oxidative stress indicator, the lipid peroxidation of membranes was measured from fresh material (30 mg per replicate) stored in a -80 ºC freezer until evaluation date. This variable was determined using thiobarbituric acid reacting substance (TBARS) assay according to the method reported by Du and Bramlage (1992). Absorbance was measured at 440, 532, and 600 nm using an automated plate reader Synergy H1 (Bio-Tek, Winooski, VT, USA) with 96-well plates.

The fruit ORAC value was measured on 1g fruit (fresh fruit kept in a -80 ºC freezer until analysis) per replicate using the procedure described by Prior et al. (2003). In the case of Colbún, an automated plate reader Synergy H1 (Bio-Tek, Winooski, VT, USA) with 96-well plates was used, while for South Haven fruit were measured using a Bio-Tek semi-automated plate reader FLX800TBID (Bio-Tek, Winooski, VT, USA).

4.3 Statistical analyses.

Analyses of variance (ANOVA) were performed on the data. Treatment means were compared at $P \leq 0.05$ using Tukey's Studentized range test (HSD) based on harmonic mean sample size. Statistical analyses were done using SPSS for Windows, version 8.0 (Chicago, IL).

4.4 Results

Weather conditions and rainfall determined the total amount of water applied during the three seasons of study (Table 4.1). Seasonal mean average temperatures were similar for both seasons in Colbún. December and January represented the highest ETo for Colbún with higher values during the first season. Higher average temperatures were registered during South Haven's season compared to the ones in Colbún. Rainfall was greater in South Haven, but with lower ETo (Table 4.2), which lead to lower irrigation needs.

4.4.1 Gas exchange.

Treatments affected photosynthetic rates, stomatal conductance and transpiration (data not shown). The 50% ETa treatment resulted in the lowest gas exchange levels during all three seasons. The 100 and 75% ETa treatments did not differ in gas exchange measurements, except for photosynthetic rate in South Haven. Stomatal conductance and transpiration did not express statistical differences for the second season in Colbún, while mild deficit treatment (75% ETa) had similar (Colbún's first season) or intermediate (South Haven) values for these variables compared to 100% ETa plants.

4.4.2 Midday water potential.

Treatments affected midday water potential during each season. Values ranged from -1.2 to -0.7 MPa (data not shown). Severe water deficit treatment (50% ETa) had the lowest midday water potential the first season in Colbún, but no differences were found between mild deficit (75% ETa) and the 100% ETa treatment in the other two seasons.

4.4.3 Fruit weight loss

Weight loss after 30d of storage was only affected by treatments in Colbún. Fruit from the fully irrigated treatment (100% ETa) lost 10-15% more weight during both seasons than those from the 50% ETa treatment (Table 3). Mild water deficit (75% ETa) had no consistent effect as statistical differences with the 100% ETa treatment were expressed only during the first season in Colbún. After leaving the fruit for three days at room temperature (30+3d), no statistical differences were found. Weight losses increased from 4 to 8 percent during the period the fruit were held at higher temperature (Table 4.3).

For the evaluation after 60d storage, statistical differences were found only for the second season in Colbún; while for the 60+3d evaluation both seasons in Colbún showed significant differences. In both cases, mild and severe deficit treatments reduced fruit weight loss compared to 100% ETa treatment, except for Colbún's second season 60+3d (Table 4.4).

4.4.4 Fruit quality

After 30 d of cold storage plus 3 d at room temperature, differences in TA were found from the second season in Colbún and the one in South Haven. The highest TA was found in fruit from South Haven and in those from the second season in Colbún that received 100% ETa (control), followed by 75 and 50% ETa treatments. There were statistical differences for soluble solids in all seasons, with 50 and 75% ETa showing higher TSS concentrations than the 100% ETa, except for the first season in Colbún (Table 4.5). Firmness was altered by the irrigation treatments only in the second season in Colbún; were the firmest fruit were from the most stressful treatment (50% ETa), followed by the 75% ETa treatment, and the softest fruit were from the fully irrigated plants (100% ETa). Fruit quality after 30d storage showed the same trends as 30d+3 with the exception that TA had significant differences between irrigation treatments for all the seasons (data not presented).

For the 60+3d evaluation, titratable acidity was significantly affected by irrigation treatments in both locations; whereas soluble solids and firmness were altered in one season only (Table 4.6). The highest TA was for the 100 and 75% Eta in the first season in Colbún, whereas 75% ETa was intermediate in this variable for the other seasons. The 50 and 75% ETa treatments had the highest SS concentration in the first season in Colbún. Firmness was only altered significantly for the fruit of the second season in Colbún with greater firmness for the 50 and 75% ETa treatments (Table 4.6). Fruit quality for the 60d evaluation presented significant differences for TSS during the first and second season in Colbún with effects being proportional to water deficit. The rest of the quality parameters had similar trends to the 60+3d evaluation (data not shown).

4.4.5 Fruit condition

Post-harvest fruit condition after 30+3d of storage was not significantly affected by treatments. Across all sites and seasons, between 45 to 83% of the fruit were sound, 8 to 52% were dehydrated, and 3 to 10% were decayed. The highest percentage of sound fruit, and the lowest amount of dehydrated fruit, were found during the second season in Colbún, although the proportion of decayed fruit was the highest (9 to 10%) (data in Appendix A).

Treatments did not affect the post-harvest condition of the fruit after 60+3d of storage. Fruit from the second season in Colbún had a higher percentage of sound fruit (66-69%), but also the highest proportion of decayed fruit (17-19%). Fruit condition worsened compared to the measurements after 30+3d storage. Sound fruit from all seasons fluctuated from 34 to 69%, dehydrated fruit ranged from 13 to 62%, and decayed fruit varied from 4 to 18% (data in Appendix A).

4.4.6 TBARS and ORAC

Both membrane lipid peroxidation (TBARS) and antioxidant capacity (ORAC) were affected by the irrigation treatments after 30+3d (Table 4.7) and 60+3d storage (Table 4.8). After 30+3d, fruit from the 100% ETa treatment consistently had the lowest oxidative stress and those from the 75% treatment had intermediate values for the first season in Colbún and the one in South Haven. Fruit from the severe deficit (50% ETa) had the lowest antioxidant capacity (ORAC) during each season except the second one in Colbún, where it was equivalent to 75% Eta (Table 4.7). No statistical differences were found between 75 and 100% ETa treatments regarding antioxidant capacity for the first season in Colbún and the one in South Haven (Table 4.7).

In all seasons, after 60+3d storage the 75% and 100% ETa treatments had lower oxidative stress (TBARS) than the severe water deficit (50% ETa). Except for the second season in Colbún, all ORAC values after 60+3d storage were affected by the irrigation treatments. In South Haven, the 100% ETa and mild stress (75% ETa) treatments had the highest activity while for Colbún´s first season the 50 and 75% ETa treatments were equivalent and different from the control (100% ETa) (Table 4.8).

4.5 Discussion

Water stress decreases both the photosynthetic rate and the yield in many fruit crops. In most cases, photosynthesis is reduced because stomatal conductance limits CO_2 diffusion from the atmosphere to the site of carboxylation (Flexas et al., 2009). Our results showed that the most stressful treatment (50% ETa) reduced the photosynthetic rate by 20-80%, as compared to control plants (100% ETa). This effect on bushes performance affects fruit not only during harvest but also their shelf-life. In 'Bluecrop' blueberry grown under greenhouse conditions, net photosynthesis and stomatal conductance were reduced by 30% due to water stress (no irrigation for three weeks at the onset of shoot growth) as compared to daily irrigation (Rho et al., 2012). Net photosynthesis, stomatal conductance, and transpiration were also reduced by 30% in apricot trees for the 60% ETa treatment as compared to the fully irrigated control (Perez-Sarmiento et al., 2010).

Midday water potential is an indicator of plant water status, and it use is essential when planning deficit irrigation practices. RDI has been shown to reduce the diurnal leaf conductance and midday water potential (Sánchez-Blanco et al., 2009). In our trials, the more intense water deficit (50% ETa) had the lowest midday water potentials and reached a stress point where bushes decreased their gas exchange, while the mild deficit (75% ETa) showed intermediate midday water potentials during all seasons, except for the first season where values were equivalent to fully irrigated plants, leading to similar fruit quality. These results were corroborated in olive trees (rainfed control, 30, 60% ETa) by Bacelar et al. (2007), when comparing values to fully irrigated control treatments. Although leaf water potentials differ among cultivars, measured either at predawn or at midday, the fast decline under deficient soil water content is common among the different highbush blueberry cultivars (Bryla and Strik, 2007).

Fruit quality was affected by irrigation regimes. Irrigation treatments altered weight loss during storage only in fruit from the Colbún location. Increased TSS has been shown to correlate positively with lower weight loss during storage in mandarin subjected to RDI treatments (Conesa et al., 2014). In general, in our study fruit from the mild deficit treatment (75% ETa) lost less weight than those from fully irrigated plants (100% ETa), contrary to what literature reports. Similar results were observed with moderately drought-stressed mandarins (Conesa et al., 2014) and apples (Mpelasoka et al., 2000). However, Ehret et al. (2012) reported opposite trends working with 'Duke' highbush blueberries. In their case, fruit from non-irrigated bushes lost more weight in storage (one week at 4 ºC) than those from moderately or heavily irrigated plants.

In our assay, irrigation treatments affected TSS and TA concentrations in fruit after 30 and 60d cold storage. The lower TSS in fruit we found in well-watered bushes may be due to a dilution effect (Ehret et al., 2012) in comparison to the deficit treatments. Increased irrigation reduced concentrations of flavor-related compounds in strawberry (Terry et al., 2007). The increased TSS values expressed by water deficit treatments may be caused by an osmotic adjustment that increased solute content.

Texture of plant organs can be directly influenced by their water status since water content has a direct influence on cell turgor (Shackel et al., 1991). In general, fruit size is negatively correlated to firmness (Sams, 1999). Fruit size is determined by both cell size and number. Small fruits are usually firmer than large fruit because small fruit would have a similar number of cells as larger fruit, but a higher percentage of their volume is cell wall material, which results in denser tissues and greater firmness (Bryla, 2008). In our assay, firmness after storage was only affected by water deficit treatments during the second season in Colbún. The smaller berries from the severe and mild water deficit treatments were most firm. This effect might also be related to a higher concentration of TSS. Similar to our results, Mpelasoka et al. (2001) found greater firmness of ‘Braeburn’ apples stored for 12 weeks at 0 ºC and 7 d at 20 ºC after early or late deficit irrigation treatments (half irrigation frequency) during the season. On the other hand, ‘Andross' peach fruits managed with regulated RDI during growth stage II (pit hardening) were less firm than fully irrigated control fruits after one-week storage at 0 ºC (Gelly et al., 2004).

Irrigation treatments did not influence fruit condition during storage in our studies. Fruit perishability occurred at a normal rate during the 30-60d storage period. Even though the percentage of sound fruit was less after 60d storage than 30d, water deficit treatment had no significant effect on fruit dehydration or decay, not even for the 30+3d and 60+3d evaluations were there was a large increase in fruit decay at room temperature (18-20 ºC). Paniagua et al. (2014) also found greater decay in ‘Brigitta’ highbush blueberry fruit within a temperature change from 0-4 ºC. They established that all treatments eventually developed gray mold after harvest, as reported by Terry et al. (2007) in strawberries. Nunes et al. (2004) reported that the best temperature for for maximizing storage life in ‘Patriot’ blueberries was 0-1 ºC and that fruit quality decreased as storage temperature increased up to 20 ºC.

Antioxidant capacity is an increasingly important aspect of fruit quality because it is associated with benefits to consumer´s health (Laribi et al., 2013). Normal aerobic metabolism in plants generates reactive oxygen species (ROS) which in plants are maintained at steady state levels through various mechanisms. However, when plant tissues produce excessive ROS during severe abiotic or biotic stress conditions, the ability of cells to regulate levels is no longer sufficient, leading to ROS to damage membranes, DNA, proteins and generate lipid peroxidation (LPO) (H. E. Johnson et al., 2003). During the LPO process, malondialdehyde, and other small hydrocarbon fragments are produced that can react with thiobarbituric acid (TBA) to form coloured products called TBARS (Larkindale and Knight, 2002). Total phenolic and anthocyanin concentrations are highly correlated with antioxidant capacity in blueberries (Zheng et al., 2003). The 75% ETa irrigation treatment did positively influence antioxidant levels in the fruit stored for 30 and 60 d, suggesting that deficit irrigation has the potential to improve health benefits associated to blueberry consumption. Antioxidant capacity for the mild deficit treatment (75% ETa) was usually similar to 100% ETa fruits (30+3d: Colbún's first season and South Haven; 60+3d: South Haven's season), while the severe deficit treatment (50% ETa) always resulted in lower ORAC values probably due to an excessive oxidative damage that might have overcome the capacity of antioxidant systems in the plant. As for the oxidative damage, mild water stress (75% ETa) reduced fruit membrane lipid peroxidation in every location compared to severe stress (50% ETa) at the 60+3d evaluation date, but this did not occur at the 30+3d evaluation, suggesting that this phenomenon requires a longer period to develop, especially considering the potential long storage required for long distance markets. Kalt et al. (1999) mentioned that although no temperature related increase in ORAC was found for blueberries, antioxidant capacity increased in raspberries and strawberries stored at higher temperatures, possibly due to an increase in total anthocyanins and total phenolics concentration.

4.6 Conclusions

In our trials, the most stressful treatments consistently generated the lowest water potential, photosynthetic rate, stomatal conductance, and transpiration in 'Brigitta' highbush blueberry plants. However, pre-harvest RDI management had inconsistent effects on fruit condition during the post-harvest stages. Severe deficit treatment (50% ETa) increased fruit TSS and firmness, but lowered TA. The severe deficit treatment also had the highest lipid peroxidation and lowest antioxidant capacity. A promising result was that plants receiving moderate deficit irrigation (75% ETa) produced fruit with similar quality (firmness, titratable acidity, soluble solids) and antioxidant activity as fully irrigated plants (100% ETa), but with lower oxidative stress and similar weight loss than 50% ETa treatment at 60+3d. These results are interesting for growers from exporting countries located in semiarid areas where climate change has reduced rainfall and the availability of irrigation water is limited, since using RDI they can produce fruit of similar quality and post-harvest life while applying less irrigation water during the season.

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Table 4.1. Water volumes (mm) applied for the different treatments and production seasons for Colbún (two seasons) and South Haven (one season)

	100% Eta	75% Eta	50% Eta
Month	Colbún 2013-14 / 2014-15		
September	0 / 0	0 / 0	0 / 0
October	27 / 27	20 / 20	13 / 13
November	54 / 55	40 / 41	27 / 28
December	81 / 80	61 / 60	41 / 40
January	108 / 82	81 / 61	54 / 41
Total (mm)	270 / 244	202 / 182	135 / 122
	South Haven 2014		
April	0	0	0
May	36	27	18
June	23	17	12
July	23	17	12
August	22	17	11
Total (mm)	104	78	53

Table 4.2. Weather conditions during two production seasons for Colbún and one in South Haven.

	Average Tº (ºC)	Rainfall (mm)	Effective rainfall (mm)	ETo (mm)
	Colbún 2013-14 / 2014-15			
September	10.7 / 10.9	65.2 / 163.6	41.3 / 60.6	46.5 / 54.7
October	12.3 / 13.4	39.2 / 14.6	20.7 / 0.6	95.8 / 99.2
November	14.8 / 15.2	1.6 / 11.8	0 / 0.3	132.0 / 133.6
December	18.7 / 17.5	0 / 17.4	0 / 5.1	173.4 / 144.5
January	19.3 / 19.8	2.6 / 0	0 / 0	172.2 / 162.4
	South Haven 2014			
April	7.6	57.6	2.4	59.1
May	13.5	41.4	1.1	76.8
June	19.5	75.7	9.9	78.5
July	18.3	66.0	13.0	84.1
August	20.6	61.2	11.1	72.1

Effective rainfall: (pluviometric precipitation - 10) x 0.75 (Acevedo-Opazo et al., 2010).

Table 4.3. Effect of irrigation level (% of actual evapotranspiration: Eta) on post-harvest weight loss (%) of blueberry fruit cv. Brigitta after 30 d at 0-2 ºC and 30 d+3 d at 18-20 ºC.

Season (year)	Treatment (% Eta)	Weight loss 30d (%)	Weight loss 30+3d (%)
Colbún 2013-14	100	7.5 a	12.7
	75	6.3 b	12.6
	50	6.5 b	11.9
Colbún 2014-15	100	7.2 a	12.2
	75	7.0 a	11.7
	50	6.3 b	11.4
South Haven 2014	100	8.0	14.1
	75	7.8	14.0
	50	7.3	13.9

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at $P \leq 0.05$) between RDI treatments.

Table 4.4. Effect of irrigation level (% of actual evapotranspiration: ETa) on post-harvest weight loss (%) of blueberry fruit cv. Brigitta after 60 d at 0-2 ºC and 60 d+3 d at 18-20 ºC.

Season (year)	Treatment (% ETa)	Weight loss 60d (%)	Weight loss 60+3d (%)
Colbún 2013-14	100	11.0	16.4 a
	75	11.0	15.3 b
	50	10.7	15.6 b
Colbún 2014-15	100	12.4 a	18.8 a
	75	11.0 b	17.0 b
	50	10.5 b	17.8 ab
South Haven 2014	100	12.7	18.1
	75	11.6	17.0
	50	11.7	17.9

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at $P \leq 0.05$) between RDI treatments.

Table 4.5. Effect of irrigation level (% of actual evapotranspiration: ETa) on post-harvest quality of blueberry fruit cv. Brigitta after 30 d of cold storage (0-2 ºC) plus 3 d at 18-20 ºC.

Season (year)	Treatment (% ETa)	Titratable acidity (% citric acid)	Soluble solids (ºBrix)	Firmness (g/mm)
Colbún 2013-14	100	0.72	14.9 b	121.5
	75	0.71	15.3 ab	129.6
	50	0.66	15.6 a	130.7
Colbún 2014-15	100	0.89 a	12.7 b	114.7 b
	75	0.86 b	13.7 a	119.4 ab
	50	0.86 b	13.8 a	122.0 a
South Haven 2014	100	0.83 a	12.9 b	110.4
	75	0.81 ab	14.6 a	110.6
	50	0.80 b	14.6 a	112.0

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Table 4.6. Effect of irrigation level (% of actual evapotranspiration: ETa) on post-harvest blueberry fruit cv. Brigitta quality after 60 d of cold storage (0-2 ºC) plus 3 d at 18-20 ºC.

Season (year)	Treatment (% ETa)	Titratable acidity (% citric acid)	Soluble solids (ºBrix)	Firmness (g/mm)
Colbún 2013-14	100	0.49 a	15.7 b	107.8
	75	0.47 a	16.5 a	111.9
	50	0.42 b	16.5 a	119.3
Colbún 2014-15	100	0.60 a	11.3	104.5 b
	75	0.54 ab	12.5	110.0 a
	50	0.51 b	13.8	113.3 a
South Haven 2014	100	0.79 a	13.8	103.4
	75	0.75 ab	14.4	105.2
	50	0.71 b	14.8	108.2

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Table 4.7. Effect of irrigation level (% of actual evapotranspiration: ETa) on membrane lipid peroxidation (TBARS) and oxygen radical absorption capacity (ORAC) of blueberry fruit cv. Brigitta after 30 d of cold storage (0-2 ºC) plus 3 d at 18-20 ºC.

Season (year)	Treatment (% ETa)	TBARS (nmol/g FW)	ORAC (µmol TE/g)
Colbún	100	5.6 b	5999 a
2013-14	75	7.6 ab	5099 a
	50	9.2 a	3901 b
Colbún	100	10.1 b	4547 a
2014-15	75	14.5 a	3697 b
	50	16.5 a	3664 b
South Haven	100	9.3 b	5335 a
2014	75	10.6 ab	5261 a
	50	12.5 a	4463 b

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Table 4.8. Effect of irrigation level (% of actual evapotranspiration: ETa) on membrane lipid peroxidation (TBARS) and oxygen radical absorption capacity (ORAC) of blueberry fruit cv. Brigitta after 60 d of cold storage (0-2 ºC) plus 3 d at 18-20 ºC.

Season (year)	Treatment (% ETa)	TBARS (nmol/g FW)	ORAC (µmol TE/g)
Colbún	100	9.6 b	5435 a
2013-14	75	10.7 b	3754 b
	50	13.8 a	3414 b
Colbún	100	14.9 b	3380
2014-15	75	18.6 b	3371
	50	23.5 a	3040
South Haven	100	10.2 b	4402 a
2014	75	11.6 b	4386 a
	50	15.5 a	3355 b

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between RDI treatments.

Chapter V

General discussion, concluding remarks and further perspectives

5.1 General discussion

Securing food production for an expanding worldwide population will require closing the gap between potential crop productivity under optimal conditions and the yield achieved by farmers under a changing environment, which is termed agronomical stability (Albacete et al., 2014). This increase must be obtained in a sustainable way without increasing the use of resources (arable land, irrigation water, fertilizers) and at the same time overcoming yield losses due to environmental stresses.

While biotic factors (diseases, pests, and weed competition) account for less than 10% of yield reductions, the remaining percentage is attributed to abiotic constraints such as drought and salinity, that are being exacerbated by climate change (Peleg et al., 2011). Water availability has changed through the last decades due to reduced precipitation and increased water demand caused by higher ambient temperatures. This trend is most likely to continue occurring.

In the present study, while working with cv. Brigitta bushes we have corroborated the high sensitivity of blueberries to water stress already reported by Améglio et al. (2000). Gas exchange measurements showed how different water levels can impact stomatal conductance and transpiration, affecting photosynthesis rate and yield. As mentioned by Yordanov et al. (2003), water stress can result in stomatal closure and reduced transpiration rates, a decrease in the water potential of plant tissues, lowered photosynthesis rate and growth inhibition. Although other fruit crops are less sensitive than blueberries -due to the shallow root system that the latter possess, a common response of these horticultural crops when facing lower water availability is to reduce their transpiration rate in order to protect the plant from dehydration which has a direct impact on yield. However, our study showed that differences in yield and total fruit number were only observed during the season and following a severe pruning, even though unexpectedly WP was not affected. This effect also suggests an effect on bud induction that can affect next season's fruit number, but we did not measure this parameter during our study.

In different scenarios, water deficit treatments should have an effect on vegetative growth, in fact, among other fruit crops, deficit irrigation has been used on apples (Mpelasoka et al., 2001), grapevines (Acevedo-Opazo et al., 2010), olives (Tognetti et al., 2006) and peaches (Dichio et al., 2007), in order to control excessive vegetative growth, improve fruit quality and reduce pruning costs. However, this effect was not consistent in our trials, since a reduction in vegetative growth only occurred during the second season in Colbún and in the only season in South Haven. The occurrence of this growth difference only during the second season could be in part due to an adaptation process triggered by the plant after responding to the first season of water deficit. Nonetheless, the effect observed during the first season in Michigan could be explained by the different environmental conditions in the two production areas where the trials were implemented.

Fruit quality can change due to several factors, being water one of the principal actors triggering modifications within the plant. These alterations will produce changes in source-sink relationships that alter their metabolism and influence the quality of the final produce (Albacete et al., 2014). Fruit would have been affected during our trials in direct proportion to the amount of water applied. First, when the plant had enough available water to satisfy its physiological needs, this would have been sufficient to complete normal cellular division as it has been reported that drought stress during the early cell-division period can reduce fruit set and fruit size, affecting the remainder of the season even if water is fully available later (Morales et al., 2013). This final number of cells might have had greater elongation due to the available water resource, ending with larger fruit size, although this might have caused a dilution effect, which implies that those fruits had lower firmness. This agrees with previous research since it has been reported that fruit size is negatively correlated to firmness and soluble solutes accumulation in blueberries (Ballinger et al., 1973; Bryla et al., 2009; Ehret et al., 2012; Sams, 1999).

Agricultural science has evolved over the years. Abiotic factors have always affected plants but thanks to the development of new equipment and techniques it is now possible to measure the real impact of these stresses on plant metabolism and physiology. Water deficit induces oxidative stress and this may alter the normal rate of ROS synthesis within the plant. These changes in ROS metabolism can end up degrading cell membranes, proteins and DNA (Cruz de Carvalho, 2008). Normal water input can prevent this increased ROS synthesis and maintain the plant within its normal homeostasis. Alternatively plants possess different antioxidant systems that can control a certain build up of ROS, but if the scavenge threshold is exceeded, damage can be irreversible (Laribi et al., 2013). Our study would seem to indicate that applying 75% of the plant water demand can still keep the plant within an oxidative range that would have no deleterious effects on plant metabolism.

As mentioned before, most previous studies have measured the effects of water deficit until harvest (Keen and Slavich, 2012), yet there are multiple responses during the post-harvest period that are still unknown, especially when the produce needs to be exported to distant markets and it needs a long postharvest life in order to reach them.

Our results for the post-harvest stage showed that the amount of water input can also affect fruit quality in this stage. Paniagua et al. (2013) suggested that although moisture loss appears to relate to blueberry firmness depending on the extent of dehydration, demonstration of this relationship and potential moisture loss during storage on firmness changes has not been addressed. Fruit dehydrates during storage and loses water as the respiratory process occurs.

Since blueberries are considered climacteric fruits, this process is affected among others by ambient temperature, air flow and relative humidity gradient during storage (Suzuki et al., 1997). During storage, fruit with higher surface area to volume ratios are likely to lose more weight (Shibairo et al., 1997). In reviewing our measurements, an opposite effect can be seen on fruits from well irrigated plants (100% ETa), which had higher dehydration and weight loss compared with water deficit treatments, although intermediate water deficit treatment (75% ETa) presented an equivalent degree of water loss after 30 and 60 days in storage. Other parameters behaved similar to the quality measurements performed right after harvest, where severe deficit treatments expressed higher fruit firmness and soluble solid concentrations.

After measuring fruit through its cold storage we were able to observe that the pre-harvest RDI management would affect fruit metabolism. Oxidative damage in this fruit was similar to those measured after harvest, with the most severe water deficit treatment producing irreversible damage and decreased antioxidant capacity, but promising results were observed with intermediate stress (75% ETa), which expressed less membrane damage and similar antioxidant capacity to well irrigated plants during this study.

5.2 Concluding remarks

Water deficit is an abiotic stress that is becoming more frequent as climate change affects the various producing regions and crops. Many practices have been developed to control plant water status, maintain turgor pressure, and reduce water loss by plants. A variety of water-saving practices have been studied and adapted by farmers in order to face the critical issue of worldwide water scarcity. RDI has been regarded as a key water-saving approach for the production of horticultural and field crops with the potential of maintaining (and even improving) yield and quality. A mild water deficit can reach similar plant growth and development compared with fully irrigated plants. In particular, a sustained water stress throughout the fruit growth stage can lead to internal physiological adjustments and regulations geared at protecting plants from damage. Some key agronomic management strategies and practices can be used in order to increase the implementation of this technology in fruit production. Thus, RDI practices can be adopted and employed in real-world agricultural systems, although a number of theoretical and technical issues need to be further studied. In order to successfully apply RDI management in large-scale commercial agricultural operations, there is a need to define specific conditions, under which this water saving management can be implemented effectively and efficiently. This study showed that mild regulated deficit irrigation applied to 'Brigitta' highbush blueberry plants, is not only useful as a water saving technique, but also a powerful tool that may modify fruit composition and post-harvest performance, however, more studies need to be performed in order to corroborate the effects we found. Particularly, measuring the effect on other *Vaccinium* cultivars.

5.3 Further perspectives

During recent years, the quantity of water resources available for agriculture have been decreasing rapidly due to increased competition for freshwater with other sectors, as well as a marked reduction in precipitations and a higher evapotranspiratory demand. Although regulated deficit irrigation has provided significant water savings among fruit crops and has increased WUE and WP, this is only one of a very limited number of research efforts done on RDI in this crop. Thus, there is still much work to do in *Vaccinium* species.

In this study, which was performed in two different production areas we have established that RDI implemented throughout the fruit growing stage can save up to 25% of irrigation water and increase WP up to 35%. An option in future studies would be to consider selecting specific phenological stages, other *Vaccinium* cultivars and apply a greater number of water deficit treatments in order to establish the threshold for different cultivars in a greater number of productive areas.

Root growth is one aspect that has not been studied in these studies. There is a need to know how root growth and activity are affected throughout the season after applying water deficit treatments, not only in terms of growth but also its architecture. It has already been shown that depending on the irrigation method (Bryla and Linderman, 2007) and the amount of drip laterals used, irrigation practices have an effect on blueberry growth and development (Holzapfel et al., 2015) . Since it has been reported that there are two peaks in root growth during the season for northern highbush blueberries, and that root growth is inhibited by shoot and fruit growth (Abbott and Gough, 1986), timing of irrigation deficit should have a major impact on total root growth and on its rate of growth throughout the season.

Furthermore, a model-based approach needs to be developed to quantify the amounts of irrigation that can be applied to crops through deficit irrigation, as well as methods to follow up the degree of stress that plants are undergoing. The accuracy, efficiency, and effectiveness of these strategies should be validated using multiple year trials.

References

References

Abbott, J.D., Gough, R.E., 1986. Split-root water application to highbush blueberry plants. HortScience 21, 997–998.

Abdel-Razzak, H., Wahb-Allah, M., Ibrahim, A., Alenazi, M., Alsadon, A., 2016. Response of cherry tomato to irrigation levels and fruit pruning under greenhouse conditions. Journal of Agricultural Science and Technology 18, 1091–1103.

Acevedo-Opazo, C., Ortega-Farias, S., Fuentes, S., 2010. Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. Agricultural Water Management 97, 956–964. doi:10.1016/j.agwat.2010.01.025

Albacete, A.A., Martínez-Andújar, C., Pérez-Alfocea, F., 2014. Hormonal and metabolic regulation of source–sink relations under salinity and drought: From plant survival to crop yield stability. Biotechnology Advances 32, 12–30. doi:10.1016/j.biotechadv.2013.10.005

Alcobendas, R., Mirás-Avalos, J.M., Alarcón, J.J., Nicolás, E., 2013. Effects of irrigation and fruit position on size, colour, firmness and sugar contents of fruits in a mid-late maturing peach cultivar. Scientia Horticulturae 164, 340–347. doi:10.1016/j.scienta.2013.09.048

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration: guidelines for computing crop water requirements, FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations, Rome, Italy.

Améglio, T., Le Roux, X., Mingeau, M., Perrier, C., 2000. Water relations of highbush blueberry under drought conditions. Acta Horticulturae 537, 273–278. doi:10.17660/ActaHortic.2000.537.30

Araus, J.L., Slafer, G. a., Royo, C., Serret, M.D., 2008. Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Sciences 27, 377–412. doi:10.1080/07352680802467736

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Lopes, J.I., Gonçalves, B.C., Ferreira, T.C., Correia, C.M., 2007. Physiological behaviour, oxidative damage and antioxidative protection of olive trees grown under different irrigation regimes. Plant and Soil 292, 1–12. doi:10.1007/s11104-006-9088-1

Bai, T., Li, C., Ma, F., Feng, F., Shu, H., 2010. Responses of growth and antioxidant system to root-zone hypoxia stress in two Malus species. Plant and Soil 327, 95–105. doi:10.1007/s11104-009-0034-x

Ballinger, W.E., Kushman, L.J., Hamann, D.D., 1973. Factors affecting the firmness of highbush blueberries. Journal of the American Society for Horticultural Science 98, 583–587.

Behboudian, M., Mills, T., 1997. Deficit irrigation in deciduous orchards. Horticultural Reviews 21, 105–131.

Boland, A.M., Jerie, P.H., Mitchell, P.D., Goodwin, I., Connor, D.J., 2000. Long-term effects of restricted root volume and regulated deficit irrigation on peach: II. Productivity and water use. Journal of the American Society for Horticultural Science 125, 143–148.

Bordonaba, J.G., Terry, L.A., 2010. Manipulating the taste-related composition of strawberry fruits (*Fragaria×ananassa*) from different cultivars using deficit irrigation. Food Chemistry 122, 1020–1026. doi:10.1016/j.foodchem.2010.03.060

Bryla, D., Strik, B.C., 2006. Variation in plant and soil water relations among irrigated blueberry cultivars planted at two distinct in-row spacings. Acta Horticulturae 715, 295–300. doi:10.17660/ActaHortic.2006.715.43

Bryla, D.R., 2008. Water requirements of young blueberry plants irrigated by sprinklers, microsprays and drip. Acta Horticulturae 135–139. doi:10.17660/ActaHortic.2008.792.13

Bryla, D.R., Linderman, R.G., 2007. Implications of Irrigation Method and Amount of Water Application on Phytophthora and Pythium Infection and Severity of Root Rot in Highbush Blueberry. HortScience 42, 1463–1467.

Bryla, D.R., Strik, B.C., 2007. Effects of cultivar and plant spacing on the seasonal water requirements of highbush blueberry. Journal of the American Society for Horticultural Science 132, 270–277.

Bryla, D.R., Yorgey, B., Shireman, A.D., 2009. Irrigation management effects on yield and fruit quality of highbush blueberry. Acta Horticulturae 649–656. doi:10.17660/ActaHortic.2009.810.86

Cameron, J.S., Brun, C.A., Hartley, C.A., 1989. The influence of soil moisture stress on the growth and gas exchange characteristics of young highbush blueberry plants (*Vaccinium corymbosum* L.). Acta Horticulturae 241, 254–259. doi:10.17660/ActaHortic.1989.241.41

Castrejón, A.D.R., Eichholz, I., Rohn, S., Kroh, L.W., Huyskens-Keil, S., 2008. Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. Food Chemistry 109, 564–572. doi:10.1016/j.foodchem.2008.01.007

Chai, Q., Gan, Y., Zhao, C., Xu, H.-L., Waskom, R.M., Niu, Y., Siddique, K.H.M., 2016. Regulated deficit irrigation for crop production under drought stress. A review. Agronomy for Sustainable Development 36, 3. doi:10.1007/s13593-015-0338-6

Chaitanya, K.V., Rasineni, G.K., Reddy, A.R., 2009. Biochemical responses to drought stress in mulberry (*Morus alba* L.): evaluation of proline, glycine betaine and abscisic acid accumulation in five cultivars. Acta Physiologiae Plantarum 31, 437–443. doi:10.1007/s11738-008-0251-6

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Annals of Botany 103, 551–560. doi:10.1093/aob/mcn125

Chaves, M.M., Oliveira, M.M., 2004. Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. Journal of Experimental Botany 55, 2365–2384. doi:10.1093/jxb/erh269

Choné, X., Van Leeuwen, C., Dubourdie, D., Gaudillères, J.P., 2001. Stem water potential is a sensitive indicator of grapevine water status. Annals of Botany 87, 477–483. doi:10.1006/anbo.2000.1361

Clothier, B.E., Green, S.R., 1994. Rootzone processes and the efficient use of irrigation water. Agricultural Water Management 25, 1–12. doi:10.1016/0378-3774(94)90048-5

Concha-Meyer, A., Eifert, J.D., Williams, R.C., Marcy, J.E., Welbaum, G.E., 2015. Shelf life determination of fresh blueberries (*Vaccinium corymbosum*) stored under controlled atmosphere and ozone. International Journal of Food Science 2015, 1–9. doi:10.1155/2015/164143

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2004. Breeding for high water-use efficiency. Journal of Experimental Botany 55, 2447–2460. doi:10.1093/jxb/erh277

Conesa, M.R., García-Salinas, M.D., de la Rosa, J.M., Fernández-Trujillo, J.P., Domingo, R., Pérez-Pastor, A., 2014. Effects of deficit irrigation applied during fruit growth period of late mandarin trees on harvest quality, cold storage and subsequent shelf-life. Scientia Horticulturae 165, 344–351. doi:10.1016/j.scienta.2013.11.023

Connor, A.M., Luby, J.J., Tong, C.B.S., Finn, C.E., Hancock, J.F., 2002. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. Journal of the American Society for Horticultural

Science 127, 89–97.

Costa, J.M., Ortuño, M.F., Chaves, M.M., 2007. Deficit irrigation as a strategy to save water: physiology and potential application to horticulture. Journal of Integrative Plant Biology 49, 1421–1434. doi:10.1111/j.1672-9072.2007.00556.x

Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species. Plant Signaling & Behavior 3, 156–165. doi:10.4161/psb.3.3.5536

Cui, N., Du, T., Kang, S., Li, F., Zhang, J., Wang, M., Li, Z., 2008. Regulated deficit irrigation improved fruit quality and water use efficiency of pear-jujube trees. Agricultural Water Management 95, 489–497. doi:10.1016/j.agwat.2007.11.007

Davies, F., Johnson, C., 1982. Water Stress, Growth, and Critical Water Potentials of Rabbiteye Blueberry (*Vaccinium ashei* Reade). Journal of American Society for Horticultural Science 107, 6–8.

Dichio, B., Xiloyannis, C., Sofo, A., Montanaro, G., 2007. Effects of post-harvest regulated deficit irrigation on carbohydrate and nitrogen partitioning, yield quality and vegetative growth of peach trees. Plant and Soil 290, 127–137. doi:10.1007/s11104-006-9144-x

Dodd, I.C., Theobald, J.C., Bacon, M.A., Davies, W.J., 2006. Alternation of wet and dry sides during partial rootzone drying irrigation alters root-to-shoot signalling of abscisic acid. Functional Plant Biology 33, 1081–1089. doi:10.1071/FP06203

Du, Z., Bramlage, W.J., 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. Journal of Agricultural and Food Chemistry 40, 1566–1570. doi:10.1021/jf00021a018

Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in highbush blueberry and species-introgressed blueberry cultivars. HortScience 37, 386–389.

Ehret, D.L., Frey, B., Forge, T., Helmer, T., Bryla, D.R., 2015. Age-related changes in response of highbush blueberry plants to drip irrigation. HortScience 50, 486–490.

Ehret, D.L., Frey, B., Forge, T., Helmer, T., Bryla, D.R., 2012. Effects of drip irrigation configuration and rate on yield and fruit quality of young Highbush blueberry plants. HortScience 47, 414–421.

Erb, W.A.A., Draper, A.D.D., Swartz, H.J., 1991. Combining ability for canopy growth and gas exchange of interspecific blueberries under moderate water deficit. Journal of American Society for Horticultural Science 116, 569–573.

Estrada, F., Escobar, A., Romero-Bravo, S., González-Talice, J., Poblete-Echeverría, C., Caligari, P.D.S., Lobos, G.A., 2015. Fluorescence phenotyping in blueberry breeding for genotype selection under drought conditions, with or without heat stress. Scientia Horticulturae 181, 147–161. doi:10.1016/j.scienta.2014.11.004

Fereres, E., Goldhamer, D.A., Parsons, L.R., 2003. Irrigation water management of horticultural crops. HortScience 38, 1036–1042.

Fereres, E., Soriano, M.A., 2007. Deficit irrigation for reducing agricultural water use. Journal of Experimental Botany 58, 147–159. doi:10.1093/jxb/erl165

Flexas, J., Barón, M., Bota, J., Ducruet, J.-M., Gallé, A., Galmés, J., Jiménez, M., Pou, A., Ribas-Carbó, M., Sajnani, C., Tomàs, M., Medrano, H., 2009. Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri*x*V. rupestris*). Journal of experimental botany 60, 2361–77. doi:10.1093/jxb/erp069

Forney, C.F., 2009. Postharvest issues in blueberry and cranberry and methods to improve market-life. Acta Horticulturae 785–798. doi:10.17660/ActaHortic.2009.810.105

García-Tejero, I., Jiménez-Bocanegra, J.A., Martínez, G., Romero, R., Durán-Zuazo, V.H., Muriel-Fernández, J.L., 2010. Positive impact of regulated deficit irrigation on yield and fruit quality in a commercial citrus orchard [*Citrus sinensis* (L.) Osbeck, cv. salustiano]. Agricultural Water Management 97, 614–622. doi:10.1016/j.agwat.2009.12.005

Gasque, M., Granero, B., Turegano, J. V., Gonzalez-Altozano, P., 2010. Regulated deficit irrigation effects on yield, fruit quality and vegetative growth of "Navelina" citrus trees. Spanish Journal of Agricultural Research 8, 40. doi:10.5424/sjar/201008S2-1347

Geerts, S., Raes, D., 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agricultural Water Management 96, 1275–1284. doi:10.1016/j.agwat.2009.04.009

Gelly, M., Recasens, I., Girona, J., Mata, M., Arbones, a, Rufat, J., Marsal, J., 2004. Effects of stage II and postharvest deficit irrigation on peach quality during maturation and after cold storage. Journal of the Science of Food and Agriculture 84, 561–568. doi:10.1002/jsfa.1686

Glass, V.M., Percival, D.C., Proctor, J.T.A., 2005. Tolerance of lowbush blueberries (*Vaccinium angustifolium* Ait.) to drought stress. I. Soil water and yield component analysis. Canadian Journal of Plant Science 85, 911–917. doi:10.4141/P03-027

Goldhamer, D.A., Viveros, M., 2000. Effects of preharvest irrigation cutoff durations and postharvest water deprivation on almond tree performance. Irrigation Science 19, 125–131. doi:10.1007/s002710000013

Goldhamer, D.A., Viveros, M., Salinas, M., 2006. Regulated deficit irrigation in almonds: Effects of variations in applied water and stress timing on yield and yield components. Irrigation Science 24, 101–114. doi:10.1007/s00271-005-0014-8

Grant, O.M., 2012. Understanding and Exploiting the Impact of Drought Stress on Plant Physiology, in: Ahmad, P., Prasad, M.N.V. (Eds.), Abiotic Stress Responses in Plants. Springer New York, New York, NY, pp. 89–104. doi:10.1007/978-1-4614-0634-1_5

Grant, O.M.O., Stoll, M., Jones, H.G.H., 2004. Partial rootzone drying does not affect fruit yield of raspberries, Journal of Horticultural Science & Biotechnology. doi:10.1080/14620316.2004.11511724

Guidi, L., Degl'Innocenti, E., Remorini, D., Massai, R., Tattini, M., 2008. Interactions of water stress and solar irradiance on the physiology and biochemistry of *Ligustrum vulgare*. Tree physiology 28, 873–83. doi:10.1093/treephys/28.6.873

Guo, Z., Ou, W., Lu, S., Zhong, Q., 2006. Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiology and Biochemistry 44, 828–836. doi:10.1016/j.plaphy.2006.10.024

Haman, D.Z., Smajstrla, A.G., Pritchard, R.T., Lyrene, P.M., 1997. Response of young blueberry plants to irrigation in Florida. HortScience 32, 1194–1196.

Hamdy, A., Ragab, R., Scarascia-Mugnozza, E., 2003. Coping with water scarcity: Water saving and increasing water productivity. Irrigation and Drainage 52, 3–20. doi:10.1002/ird.73

Hartz, T.K., 1996. Water management in drip-irrigated vegetable production. HortTechnology 6, 165–167.

Holzapfel, E.., Hepp, R.., Mariño, M.., 2004. Effect of irrigation on fruit production in blueberry. Agricultural Water Management 67, 173–184. doi:10.1016/j.agwat.2004.02.008

Holzapfel, E.A., Hepp, R.F., 1997. Effect of microjet and drip irrigation on fruit production of highbush blueberry. Acta Horticulturae 259–264. doi:10.17660/ActaHortic.1997.446.39

Holzapfel, E.A., Hepp, R.F., Riveros, C., Vera, P., Serri, H.E., Matta, R., 1994. Efecto del nivel de agua aplicado en la producción de frutos de arándano alto al segundo y tercer año de plantación. Agrociencia 10(1) 43–50.

Holzapfel, E.A., Pannunzio, A., Lorite, I., Silva de Oliveira, A.S., Farkas, I., 2009. Design and management of irrigation systems. Chilean Journal of Agricultural Research 69, 17–25. doi:10.4067/S0718-58392009000500003

Holzapfel, E., Jara, J., Coronata, A.M., 2015. Number of drip laterals and irrigation frequency on yield and exportable fruit size of highbush blueberry grown in a sandy soil. Agricultural Water Management 148, 207–212. doi:10.1016/j.agwat.2014.10.001

Houghton J. T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A., 2001. Projections of future climate change, in: Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York, NY, p. 881.

Howell, T.A., 2001. Enhancing water use efficiency in irrigated agriculture. Agronomy Journal 93, 281–289. doi:10.2134/agronj2001.932281x

Hu, T., Yuan, L., Wang, J., Kang, S., Li, F., 2010. Antioxidation responses of maize roots and leaves to partial root-zone irrigation. Agricultural Water Management 98, 164–171. doi:10.1016/j.agwat.2010.06.019

Intrigliolo, D.S., Castel, J.R., 2010. Response of grapevine cv. “Tempranillo” to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. Irrigation Science 28, 113–125. doi:10.1007/s00271-009-0164-1

Iturbe-Ormaetxe, I., 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiology 116, 173–181. doi:10.1104/pp.116.1.173

Jara-Rojas, F., Ortega-Farías, S., Valdés-Gómez, H., Acevedo-Opazo, C., 2015. Gas exchange relations of ungrafted grapevines (cv. Carménère) growing under irrigated field conditions. South African Journal of Enology and Viticulture 36, 231–242.

Johnson, H.E., Broadhurst, D., Goodacre, R., Smith, A.R., 2003. Metabolic fingerprinting of salt-stressed tomatoes. Phytochemistry 62, 919–928. doi:10.1016/S0031-9422(02)00722-7

Johnson, S.M., Doherty, S.J., Croy, R.R.D., 2003. Biphasic superoxide generation in potato tubers. A self-amplifying response to stress. Plant physiology 131, 1440–1449. doi:10.1104/pp.013300

Kalt, W., Forney, C.F., Martin, A., Prior, R.L., 1999. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. Journal of Agricultural and Food Chemistry 47, 4638–4644. doi:10.1021/jf990266t

Kays, S.J., 1999. Preharvest factors affecting appearance. Postharvest Biology and Technology 15, 233–247. doi:10.1016/S0925-5214(98)00088-X

Keen, B., Slavich, P., 2012. Comparison of irrigation scheduling strategies for achieving water use efficiency in highbush blueberry. New Zealand Journal of Crop and Horticultural Science 40, 3–20. doi:10.1080/01140671.2011.599398

Khadivi-Khub, A., 2014. Physiological and genetic factors influencing fruit cracking. Acta Physiologiae Plantarum 37. doi:10.1007/s11738-014-1718-2

Kim, J.G., Kim, H.L., Kim, S.J., Park, K.-S., 2013. Fruit quality, anthocyanin and total phenolic contents, and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea. Journal of Zhejiang University. Science. B 14, 793–9. doi:10.1631/jzus.B1300012

Laribi, A.I., Palou, L., Intrigliolo, D.S., Nortes, P.A., Rojas-Argudo, C., Taberner, V., Bartual, J., Pérez-Gago, M.B., 2013. Effect of sustained and regulated deficit irrigation on fruit quality of pomegranate cv. “Mollar de Elche” at harvest and during cold storage. Agricultural Water Management 125, 61–70. doi:10.1016/j.agwat.2013.04.009

Larkindale, J., Knight, M.R., 2002. Protection against heat stress-induced oxidative damage in

Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant physiology 128, 682–95. doi:10.1104/pp.010320

Lei, S., Yunzhou, Q., Fengchao, J., Changhai, S., Chao, Y., Yuxin, L., Mengyu, L., Baodi, D., 2009. Physiological mechanism contributing to efficient use of water in field tomato under different irrigation. Plant Spoil Environment 55, 128–133.

Liu, F., Savić, S., Jensen, C.R., Shahnazari, A., Jacobsen, S.E., Stikić, R., Andersen, M.N., 2007. Water relations and yield of lysimeter-grown strawberries under limited irrigation. Scientia Horticulturae 111, 128–132. doi:10.1016/j.scienta.2006.10.006

Liu, F., Shahnazari, A., Andersen, M.N., Jacobsen, S.E., Jensen, C.R., 2006. Effects of deficit irrigation (DI) and partial root drying (PRD) on gas exchange, biomass partitioning, and water use efficiency in potato. Scientia Horticulturae 109, 113–117. doi:10.1016/j.scienta.2006.04.004

Liu, M.C., Kojima, T., Tanaka, M., Chen, H., 2001. Effect of soil moisture on plant growth and fruit properties of strawberry. Acta Horticulturae Sinica 28, 307–311.

Loveys, B.R., Dry, P.R., Stoll, M., Mc Carthy, M.G., 2000. Using plant physiology to improve the water use efficiency of horticultural crops. Acta Horticulturae 187–197. doi:10.17660/ActaHortic.2000.537.19

Mansouri-Far, C., Modarres Sanavy, S.A.M., Saberali, S.F., 2010. Maize yield response to deficit irrigation during low-sensitive growth stages and nitrogen rate under semi-arid climatic conditions. Agricultural Water Management 97, 12–22. doi:10.1016/j.agwat.2009.08.003

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment 33, 453–467. doi:10.1111/j.1365-3040.2009.02041.x

Mingeau, M., Perrier, C., Améglio, T., 2001. Evidence of drought-sensitive periods from flowering to maturity on highbush blueberry. Scientia Horticulturae 89, 23–40. doi:10.1016/S0304-4238(00)00217-X

Mishra, A.K., Singh, V.P., 2010. A review of drought concepts. Journal of Hydrology 391, 202–216. doi:10.1016/j.jhydrol.2010.07.012

Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F., 2004. Reactive oxygen gene network of plants. Trends in Plant Science 9, 490–498. doi:10.1016/j.tplants.2004.08.009

Moggia, C., Retamales, J.B., Lobos, G.A., Caligari, P.D.S., 2009. Arándanos en Chile: ¿Un futuro azul o gris? Revista Frutícola 50–53.

Morales, C.G., Pino, M.T., del Pozo, a., 2013. Phenological and physiological responses to drought stress and subsequent rehydration cycles in two raspberry cultivars. Scientia Horticulturae 162, 234–241. doi:10.1016/j.scienta.2013.07.025

Morison, J.I.L., Baker, N.R., Mullineaux, P.M., Davies, W.J., 2008. Improving water use in crop production. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 363, 639–58. doi:10.1098/rstb.2007.2175

Mpelasoka, B., Behboudian, M., Mills, T., 2001. Effects of deficit irrigation on fruit maturity and quality of "Braeburn" apple. Scientia Horticulturae 90, 279–290. doi:10.1016/S0304-4238(01)00231-X

Mpelasoka, B.S., Behboudian, M.H., Dixon, J., Neal, S.M., Caspari, H.W., 2000. Improvement of fruit quality and storage potential of "Braeburn" apple through deficit irrigation. Journal of Horticultural Science and Biotechnology 75, 615–621. doi:10.1080/14620316.2000.11511296

Mpelasoka, B.S., Behboudian, M.H., Green, S.R., 2001. Water use, yield and fruit quality of lysimeter-grown apple trees: responses to deficit irrigation and to crop load. Irrigation

Science 20, 107–113. doi:10.1007/s002710100041

Nayyar, H., Gupta, D., 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environmental and Experimental Botany 58, 106–113. doi:10.1016/j.envexpbot.2005.06.021

NeSmith, D.S., Prussia, S., Tetteh, M., Krewer, G., 2002. Firmness losses of rabbiteye blueberries (*Vaccinium ashei* Reade) during harvesting and handling, in: Acta Horticulturae. pp. 287–293. doi:10.17660/ActaHortic.2002.574.43

Niculcea, M., Martinez-Lapuente, L., Guadalupe, Z., Sánchez-Díaz, M., Morales, F., Ayestarán, B., Antolín, M.C., 2013. Effects of water-deficit irrigation on hormonal content and nitrogen compounds in developing berries of *Vitis vinifer*a L. cv. Tempranillo. Journal of Plant Growth Regulation 32, 551–563. doi:10.1007/s00344-013-9322-z

Nora, L., Dalmazo, G.O., Nora, F.R., Rombaldi, C. V., 2012. Controlled water stress to improve fruit and vegetable postharvest quality, in: Mofizur, I., Hasegawa, H. (Eds.), Water Stress. InTech, pp. 59–72. doi:10.5772/30182

Nunes, M.C., Emond, J.P., Brecht, J.K., 2004. Quality curves for highbush blueberries as a function of the storage temperature. Small Fruits Review 3, 423–438. doi:10.1300/J301v03n03_18

Ortega-Farias, S., Fereres, E., Sadras, V.O., 2012. Special issue on water management in grapevines. Irrigation Science 30, 335–337. doi:10.1007/s00271-012-0356-y

Paniagua, A.C., East, a. R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biology and Technology 95, 50–59. doi:10.1016/j.postharvbio.2014.04.006

Paniagua, A.C.C., East, A.R.R., Hindmarsh, J.P.P., Heyes, J.A. a., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest

Biology and Technology 79, 13–19. doi:10.1016/j.postharvbio.2012.12.016

Peleg, Z., Reguera, M., Tumimbang, E., Walia, H., Blumwald, E., 2011. Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. Plant Biotechnology Journal 9, 747–758. doi:10.1111/j.1467-7652.2010.00584.x

Pérez-Pastor, A., Ruiz-Sánchez, M.C., Domingo, R., 2014. Effects of timing and intensity of deficit irrigation on vegetative and fruit growth of apricot trees. Agricultural Water Management 134, 110–118. doi:10.1016/j.agwat.2013.12.007

Perez-Sarmiento, F., Alcobendas, R., Mounzer, O., Alarcon, J., Nicolas, E., 2010. Effects of regulated deficit irrigation on physiology and fruit quality in apricot trees. Spanish Journal of Agricultural Research 8, 86–94. doi:10.5424/sjar/201008S2-1351

Prange, R.K., DeEll, J.R., 1997. Preharvest factors affecting postharvest quality of berry crops. HortScience 32, 824–830.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity [oxygen radical absorbance capacity (ORACFL)] of plasma and other biological and food samples. Journal of agricultural and food chemistry 51, 3273–3279.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC FL)) of plasma and other biological and food samples. Journal of Agricultural and Food Chemistry 51, 3273–3279. doi:10.1021/jf0262256

Retamales, J.B., Hancock, J.F., 2012. Blueberry field management and harvesting, in: Blueberries. CABI, Cambridge, USA, p. 323.

Retamales, J.B., Mena, C., Lobos, G., Morales, Y., 2015. A regression analysis on factors affecting yield of highbush blueberries. Scientia Horticulturae 186, 7–14. doi:10.1016/j.scienta.2015.02.003

Reyes-Díaz, M., Meriño-Gergichevich, C., Alarcón, E., Alberdi, M., Horst, W.., 2011. Calcium sulfate ameliorates the effect of aluminum toxicity differentially in genotypes of highbush blueberry (*Vaccinium corymbosum* L.). Journal of soil science and plant nutrition 11, 59–78. doi:10.4067/S0718-95162011000400005

Rho, H., Yu, D.J., Kim, S.J., Lee, H.J., 2012. Limitation factors for photosynthesis in "Bluecrop" highbush blueberry (*Vaccinium corymbosum*) leaves in response to moderate water stress. Journal of Plant Biology 55, 450–457. doi:10.1007/s12374-012-0261-1

Ribera, A.E., Reyes-Diaz, M., Alberdi, M., Zuñiga, G.E., Mora, M.L., 2010. Antioxidant compounds in skin and pulp of fruits change among genotypes and maturity stages in highbush blueberry (*Vaccinium corymbosum* L.) grown in southern Chile. Journal of soil science and plant nutrition 10, 509–536. doi:10.4067/S0718-95162010000200010

Robinson, J.E., Browne, K.M., Burton, W.G., 1975. Storage characteristics of some vegetables and soft fruits. The Annals of applied biology 81, 399–408.

Romero, P., Gil-Muñoz, R., del Amor, F.M., Valdés, E., Fernández, J.I., Martinez-Cutillas, A., 2013. Regulated Deficit Irrigation based upon optimum water status improves phenolic composition in Monastrell grapes and wines. Agricultural Water Management 121, 85–101. doi:10.1016/j.agwat.2013.01.007

Ruiz Sánchez, M.C., Domingo Miguel, R., Castel Sánchez, J.R., Ruiz-Sanchez, M.C., Domingo, R., Castel, J.R., 2010. Deficit irrigation in fruit trees and vines in Spain. Spanish Journal of Agricultural Research 8, 5–20. doi:10.5424/sjar/201008S2-1343

Sajedi, N., Madani, H., Naderi, A., 2011. Effect of microelements and selenium on superoxide dismutase enzyme, malondialdehyde activity and grain yield maize (*Zea mays* L.) under water deficit stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 39, 153–159. doi:10.15835/nbha3925500

Sams, C.E., 1999. Preharvest factors affecting postharvest texture. Postharvest Biology and Technology 15, 249–254. doi:10.1016/S0925-5214(98)00098-2

Sánchez-Blanco, M.J., Alvarez, S., Navarro, A., Bañón, S., 2009. Changes in leaf water relations, gas exchange, growth and flowering quality in potted geranium plants irrigated with different water regimes. Journal of plant physiology 166, 467–76. doi:10.1016/j.jplph.2008.06.015

Santesteban, L.G., Miranda, C., Royo, J.B., 2011. Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv. "Tempranillo." Agricultural Water Management 98, 1171–1179. doi:10.1016/j.agwat.2011.02.011

Sauter, a, Davies, W.J., Hartung, W., 2001. The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. Journal of experimental botany 52, 1991–1997. doi:10.1093/jexbot/52.363.1991

Savić, S., Stikić, R., Radović, B.V., Bogičević, B., Jovanović, Z., Šukalović, V.H.T., 2008. Comparative effects of regulated deficit irrigation (RDI) and partial root-zone drying (PRD) on growth and cell wall peroxidase activity in tomato fruits. Scientia Horticulturae 117, 15–20. doi:10.1016/j.scienta.2008.03.009

Schroeder, J.I., Kwak, J.M., Allen, G.J., 2001. Guard cell abscisic acid signalling and engineering drought hardiness in plants. Nature 410, 327–330. doi:10.1038/35066500

Shackel, K.A., Greve, C., Labavitch, J.M., Ahmadi, H., 1991. Cell turgor changes associated with ripening in tomato pericarp tissue. Plant physiology 97, 814–6.

Shahnazari, A., Liu, F., Andersen, M.N., Jacobsen, S.E., Jensen, C.R., 2007. Effects of partial root-zone drying on yield, tuber size and water use efficiency in potato under field conditions. Field Crops Research 100, 117–124. doi:10.1016/j.fcr.2006.05.010

Shibairo, S.I., Upadhyaya, M.K., Toivonen, P.M.A., 1997. Postharvest moisture loss characteristics of carrot (*Daucus carota* L.) cultivars during short-term storage. Scientia Horticulturae 71, 1–12. doi:10.1016/S0304-4238(97)00077-0

Singh, R., Van Dam, J.C., Feddes, R.A., 2006. Water productivity analysis of irrigated crops in Sirsa district, India. Agricultural Water Management 82, 253–278. doi:10.1016/j.agwat.2005.07.027

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2004. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Science 166, 293–302. doi:10.1016/j.plantsci.2003.09.018

Spreer, W., Ongprasert, S., Hegele, M., Wünsche, J.N., Müller, J., 2009. Yield and fruit development in mango (*Mangifera indica* L. cv. Chok Anan) under different irrigation regimes. Agricultural Water Management 96, 574–584. doi:10.1016/j.agwat.2008.09.020

Suzuki, A., Kikuchi, T., Aoba, K., 1997. Effects of Ethylene on Fruit Set and Maturation of Highbush Blueberry (*Vaccinium corymbosum* L.). Engei Gakkai zasshi 66, 267–272. doi:10.2503/jjshs.66.267

Szőllősi, R., 2014. Superoxide dismutase (SOD) and abiotic stress tolerance in plants, in: Ahmad, P. (Ed.), Oxidative Damage to Plants. Elsevier, pp. 89–129. doi:10.1016/B978-0-12-799963-0.00003-4

Tahi, H., Wahbi, S., Wakrim, R., Aganchich, B., Serraj, R., Centritto, M., 2007. Water relations, photosynthesis, growth and water-use efficiency in tomato plants subjected to partial rootzone drying and regulated deficit irrigation. Plant Biosystems 141, 265–274.

doi:10.1080/11263500701401927

Terry, L.A., Chope, G.A., Bordonaba, J.G., 2007. Effect of water deficit irrigation and inoculation with botrytis cinerea on strawberry (*Fragaria* x *ananassa*) fruit quality. Journal of Agricultural and Food Chemistry 55, 10812–10819. doi:10.1021/jf072101n

Tognetti, R., D'Andria, R., Lavini, A., Morelli, G., 2006. The effect of deficit irrigation on crop yield and vegetative development of *Olea europaea* L. (cvs. Frantoio and Leccino). European Journal of Agronomy 25, 356–364. doi:10.1016/j.eja.2006.07.003

Toivonen, P.M.A., Hodges, D.M., 2011. Abiotic Stress in Harvested Fruits and Vegetables, in: Shanker, A. (Ed.), Abiotic Stress in Plants - Mechanisms and Adaptations. InTech, p. 428. doi:10.5772/22524

Verreynne, J.., Rabe, E., Theron, K.., 2001. The effect of combined deficit irrigation and summer trunk girdling on the internal fruit quality of "Marisol" Clementines. Scientia Horticulturae 91, 25–37. doi:10.1016/S0304-4238(01)00233-3

Wang, C.Y., Chen, C.T., Wang, S.Y., 2009. Changes of flavonoid content and antioxidant capacity in blueberries after illumination with UV-C. Food Chemistry 117, 426–431. doi:10.1016/j.foodchem.2009.04.037

Xiong, L., Zhu, J.-K., 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant, cell & environment 25, 131–139. doi:10.1046/j.1365-3040.2002.00782.x

Yordanov, I., Velikova, V., Tsonev, T., 2003. Plant Responses To Drought and Stress Tolerance. Bulg. J. Plant Physiol 187–206. doi:10.1023/A:1007201411474

Zarrouk, O., Francisco, R., Pinto-Marijuan, M., Brossa, R., Santos, R.R., Pinheiro, C., Costa, J.M., Lopes, C., Chaves, M.M., 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. Agricultural Water Management 114, 18–29. doi:10.1016/j.agwat.2012.06.018

Zheng, Y., Wang, C.Y., Wang, S.Y., Zheng, W., 2003. Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. Journal of Agricultural and Food Chemistry 51, 7162–7169. doi:10.1021/jf030440k

Zwart, S.J., Bastiaanssen, W.G.M., 2004. Review of measured crop water productivity values for irrigated wheat, rice, cotton and maize. Agricultural Water Management 69, 115–133. doi:10.1016/j.agwat.2004.04.007

Appendices

Appendix A

Table A1. Effect of irrigation level (% of actual evapotranspiration: ETa) on the proportion of sound, dehydrated or decayed fruit of blueberry cv. Brigitta after 30 d of cold storage (0-2 ºC) plus 3 d at ambient temperature.

Season (year)	Treatment (% ETa)	Sound	Dehydrated	Decay
Colbún 2013/14	100	60.1	36.0	3.9
	75	65.0	30.2	4.8
	50	45.2	52.0	2.8
Colbún 2014/15	100	81.0	9.1	9.9
	75	82.9	8.5	8.6
	50	78.4	12.1	9.5
South Haven 2014	100	62.7	33.6	3.7
	75	63.8	33.3	2.9
	50	60.9	35.8	3.3

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between DI treatments. Mean values ± standard error (n=4). n.s. = no significance

Table A2. Effect of irrigation level (% of actual evapotranspiration: ETa) on the proportion of sound, dehydrated or decayed fruit of blueberry cv. Brigitta after 60 d of cold storage (0-2 ºC) plus 3 d at ambient temperature.

Season (year)	Treatment (% ETa)	Sound	Dehydrated	Decay
Colbún 2013/14	100	42.3	52.1	5.6
	75	37.9	56.0	6.1
	50	34.1	62.1	3.8
Colbún 2014/15	100	68.8	13.9	17.3
	75	67.6	13.6	18.8
	50	65.5	15.8	18.7
South Haven 2014	100	46.5	47.8	5.7
	75	44.0	51.8	4.2
	50	44.4	51.1	4.5

Averages within a column followed by different lower case letters indicate statistically significant differences (Tukey's HSD at P≤0.05) between DI treatments. Mean values ± standard error (n=4). n.s. = no significance