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LOS DÉFICIT HÍDRICOS TARDÍOS MEJORAN LA PRODUCTIVIDAD DEL AGUA Y LOS MECANISMOS FISIOLÓGICOS Y MICROBIOLÓGICOS DE TOLERANCIA A SEQUÍA EN CIRUELO EUROPEO INJERTADO SOBRE UN PORTAINJERTO ENANIZANTE Y EN SUPER ALTA DENSIDAD

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RESUMEN

Durante los últimos años, los huertos de ciruelo europeo se han establecido en super alta densidad, conducidos en seto e injertados sobre portainjertos enanos. El ciruelo europeo es ampliamente reconocido como un cultivo frutal tolerante al estrés hídrico. A pesar de esto, las principales regiones productoras de ciruelo europeo en Chile se han visto afectadas por reducciones considerables en la disponibilidad de agua para riego debido al cambio climático. El riego deficitario controlado se utiliza en ciruelo europeo no solo para inducir incrementos en el rendimiento seco, sino también para aumentar la productividad del agua. Al inicio de la maduración del fruto, se evaluaron dos regímenes de riego (convencional y deficitario) en plantas maduras de ciruelo europeo (*Prunus domestica L.* cv. French) establecidas en super alta densidad en Peralillo, Chile, durante dos temporadas consecutivas. Los resultados mostraron que las plantas bajo riego deficitario controlado tardío oscilaron entre un estrés hídrico moderado ($-1.4 \leq \Psi_{stem} \leq -1.2$ MPa) y severo (<-1.7 MPa). Las plantas bajo riego deficitario tardío tendieron a presentar mayores porcentajes de bacterias promotoras del crecimiento vegetal, particularmente del género *Azospirullum*. A pesar del corto periodo de tiempo en que las plantas estuvieron sometidas a estrés hídrico, este condujo a un ahorro considerable de agua y no causó reducciones ni en el rendimiento ni en la calidad de la fruta fresca y seca, lo que aumento la productividad del agua entre un 42 % y un 47 %. El presente estudio provee de evidencia que sostienen que el riego deficitario controlado tardío es una práctica sostenible y resiliente que no sólo permite ahorrar agua, sino también mejorar la microbiota de los suelos, lo cual es particularmente relevante en el escenario actual de sequía en muchas zonas productoras de fruta en el mundo.

SUMMARY

In recent years, European plum orchards have been established at super high density, hedge trained and grafted on dwarfing rootstocks. European plum is widely recognized as a water stress tolerant fruit crop. Despite this, the main European plum growing regions in Chile have been affected by considerable reductions in irrigation water availability due to climate change. Controlled deficit irrigation is used in European plum not only to induce increases in dry yield, but also to increase water productivity. At the onset of fruit ripening, two irrigation regimes (conventional and deficit) were evaluated on mature European plum (*Prunus domestica* L. cv. French) plants established at super high density in Peralillo, Chile, during two consecutive seasons. Results showed that plants under late controlled deficit irrigation ranged from moderate ($-1.4 \leq \Psi_{stem} \leq -1.2$ MPa) to severe (<-1.7 MPa) water stress. Plants under late deficit irrigation tended to have higher percentages of plant growth-promoting bacteria, particularly of the genus *Azospirullum*. Despite the short period of time that plants were subjected to water stress, it led to considerable water savings and did not cause reductions in either yield or quality of fresh and dry fruit, increasing water productivity by 42 % to 47 %. The present study provides evidence that late controlled deficit irrigation is a sustainable and resilient practice that not only saves water but also improves soil microbiota, which is particularly relevant in the current drought scenario in many fruit-growing areas of the world.

CAPÍTULO 1

INTRODUCCIÓN GENERAL

Chile es el primer exportador mundial de fruta deshidratada proveniente del cultivo ciruelo europeo (*Prunus dometica* L.) con una producción anual cercana a las 100.000 toneladas y 12.530 ha plantadas con este frutal (Sotomayor, 2023; ODEPA, 2022). Los modelos climáticos predictivos han proyectado que para la zona en que se cultiva ciruelo europeo y que corresponde a la región Centro-Sur de Chile se producirá un aumento de hasta 4°C en la temperatura del aire y una disminución de 39 % en la acumulación de nieve (Bambach et al., 2021). Estos cambios climáticos podrían generar una reducción considerable de la disponibilidad de agua para riego en las principales regiones productoras de fruta fresca de Chile. A pesar de esto, el ciruelo europeo es una especie frutal con una alta tolerancia al estrés hídrico severo (McCutchan and Shackel, 1992), por lo cual muchos huertos experimentales y comerciales han utilizado estrategias de riego conservacionistas para ahorrar agua en áreas afectadas por sequías frecuentes y prolongadas.

El riego deficitario controlado (LD) es una práctica cultural, en la cual la cantidad de agua de riego aplicada es inferior a la cantidad de agua evapotranspirada por el cultivo durante estados fenológicos específicos en que el crecimiento y desarrollo reproductivo son menos sensibles a la ocurrencia de estrés hídrico (Calderón-Orellana, 2020). En especies frutales, tales como damasco (*Prunus armeniaca* L.) (Girona et al., 2004), manzano (*Malus domestica* Borkh.) (Leib et al., 2006), ciruelo japonés (*Prunus salicina* L.) (Hajlaoui et al., 2022), y cerezo (*Prunus avium* L.) (Blanco et al., 2020), entre otros, se ha demostrado que el LD puede ahorrar hasta un 50 % de agua de riego, controlar el excesivo crecimiento vegetativo y mejorar la calidad de la fruta en cosecha o durante el almacenamiento (Pérez-Pastor et al., 2007).

En ciruelo europeo, la aplicación del LD ha logrado reducir la cantidad de agua aplicada en torno a un 40 %, mejorar el retorno floral en un 17 %, e inducir incrementos en el rendimiento seco en un 14 % (Goldhamer et al.,1994; Lampinen et al., 1995; Lampinen et al., 2001). Hasta la fecha, las investigaciones sobre LD en ciruelo europeo han sido

conducidas exclusivamente en huertos plantados a una baja a media densidad (400 a 800 plantas ha^{-1}) y principalmente injertados sobre patrones de vigor medio a alto, tales como Marianna 2624 o Myrobalan. (Goldhamer et al., 1994; Lampinen et al., 2001). Sin embargo, en Chile, desde hace algunos años han comenzado a establecerse huertos de ciruelo europeo en súper alta densidad (SHD) (>1.500 plantas ha^{-1}), conducidos en seto continuo e injertados sobre portainjertos enanizantes, tales como Rootpac-40, Rootpac-20, GF-677 (Lordan et al., 2019; Opazo et al., 2020). El establecimiento de huertos en SHD ha sido probado con éxito a nivel comercial en diversas especies frutales, tales como olivo (Diez et al., 2016), almendro (Casanova-Gascón et al., 2019), y ciruelo japonés (Buler and Mika, 2011). Los principales beneficios del SHD, en comparación con cultivos en densidades convencionales, incluyen la mayor mecanización de la cosecha y de las podas (Buler and Mika, 2011; Mika et al., 2015); la inducción de precocidad productiva (2° o 3° año) (Mika et al., 1998; Milosevic et al., 2008); y aumento del rendimiento por hectárea (Meland, 2005). Los huertos frutales en SHD se suelen establecer sobre portainjertos enanizantes, los cuales reducen fuertemente el crecimiento vegetativo de las plantas. Varios estudios han relacionado la disminución del vigor en naranjos, manzanos y ciruelos dulces con una mayor concentración de ácido absícico (ABA) en brotes de plantas injertadas sobre portainjertos enanizantes (Hayat et al., 2023). Debido a que el ABA es una hormona vegetal que inhibe el crecimiento vegetativo e induce el cierre estomático (Chen et al., 2020), el uso de portainjertos enanizantes con una mayor concentración de ABA podría alterar la respuesta estomática de las plantas a las prácticas de riego comercial. Además, debido a su menor desarrollo radical, los portainjertos enanizantes suelen asociarse a una menor capacidad de escape a la falta de agua u oxígeno en el suelo (Jiménez et al., 2013; Yahmed et al., 2016; Opazo et al., 2019). Estas características podrían limitar la aplicación de prácticas de riego conservacionistas en huertos con portainjertos enanizantes, en donde el suelo puede exhibir muy bajos contenidos de humedad a lo largo de la zona de enraizamiento efectivo, tales como el LD o el secado parcializado de raíces.

Las prácticas de riego inducen cambios transitorios en el balance agua-oxígeno del

suelo que modifican el pH de la rizósfera y estimulan la liberación de compuestos que fomentan la propagación de los microorganismos que conforman la microbiota (Gregory et al., 2013). Dentro de estos, las bacterias promotoras del crecimiento vegetal (PGPB) han despertado un gran interés en la industria hortícola, pues no sólo promueven el crecimiento de las plantas, sino que también mejoran la defensa frente al estrés abiótico y la adquisición de agua y nutrientes (Milošević et al., 2012). En condiciones de baja disponibilidad hídrica, los impactos de las PGPB sobre las plantas incluyen cambios en la morfología de la raíz y en la síntesis de osmolitos activos, antioxidantes, fitohormonas, polímeros extracelulares, compuestos orgánicos volátiles (COV), sideróforos y 1-aminociclopropano-1-carboxilato (ACC) deaminasa (Gouda et al., 2018). Sin embargo, la mayor parte de los estudios que abordan el impacto de las PGPB sobre la tolerancia a sequía en plantas cultivadas se han realizado en especies anuales, tales como cereales y hortalizas (Ahmad et al., 2022). En huertos frutales, las investigaciones sobre la interacción planta-bacteria en respuesta a las prácticas de riego ha sido escasa y poco concluyente. Por ejemplo, la inoculación de plantas de nogal (*Junglans regia* L.) con *Bacillus cereus* L90, una PGPR asociada a la síntesis de fitohormonas, aumentó la síntesis de ABA con riego abundante y deficitario, pero no mejoró la tolerancia de la planta al estrés hídrico severo (Liu et al., 2023).

El “Rootpac-20” (*P. besseyi* x *P.cerasifera*) (Gasic and John, 2014) ha sido el portainjerto empleado en Chile para los nuevos huertos de ciruelo europeo en SHD debido a la inducción de un bajo crecimiento vegetativo, una alta precocidad de producción en el cv. French (Lordán et al., 2019), una baja transpiración por planta (Bellvert et al., 2021), y altos contenidos de materia seca en la fruta (Iglesias et al., 2019). Sin embargo, se desconoce cuál es el impacto que la falta de agua podría tener sobre huertos en SHD injertados sobre este portainjerto. Adicionalmente, debido a la necesidad de mejorar la resiliencia de los huertos frutales frente al estrés abiótico y a la relevancia que tienen las PGPB como herramientas biológicas complementarias para el manejo del estrés hídrico es muy importante evaluar el rol que las prácticas de riego pueden tener sobre la microbiota del suelo. Se realizó un estudio cuyo objetivo fue evaluar el impacto del LD sobre las relaciones hídricas, el crecimiento vegetativo y

reproductivo, la calidad de la fruta y la composición microbiológica del suelo en un huerto comercial de ciruelo europeo bajo un sistema de plantación en SHD injertado sobre Rootpac-20.

HIPÓTESIS

El riego deficitario controlado en ciruelo europeo en super alta densidad mejora la productividad del agua y la microbiota del suelo sin afectar la calidad de la fruta en fresco y seco.

OBJETIVO GENERAL

Evaluar el efecto del riego deficitario controlado sobre las relaciones hídricas, crecimiento, desarrollo y microbiota del suelo en un huerto comercial de Ciruelo europeo bajo super alta densidad.

OBJETIVOS ESPECÍFICOS

- Evaluar el efecto de las prácticas de riego sobre parámetros que caracterizan la respuesta al estrés hídrico de plantas de ciruelo europeo establecidas en super alta densidad.
- Evaluar el efecto de las prácticas de riego sobre la calidad de la ciruela fresca y deshidratada y los componentes del rendimiento de plantas de ciruelo europeo establecidas en super alta densidad.
- Evaluar el efecto de las prácticas de riego sobre el desarrollo de raíces y la abundancia relativa de bacterias anaeróbicas y aeróbicas estrictas en la zona de enraizamiento efectivo en plantas de ciruelo europeo establecidas en super alta densidad.

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CAPÍTULO 2

Regulated deficit irrigation improves water productivity and tolerance to severe water stress through physiological and microbiological mechanisms in a super high-density European plum orchard grafted on a dwarfing rootstock

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Keywords: Water stress, PGPR, Prunus domestica, Stomatic conductance, Stem water potential.

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Abstract

Recently, European plum (*Prunus domestica* L.) orchards have been planted in Chile at super high density using dwarfing rootstocks and hedge training. Late water deficits have increased water productivity in vigorous plants from low-density European plum orchards, which is highly relevant, as many prime-producing regions have experienced substantial reductions in irrigation water availability due to climate change. However, no study has addressed whether low-vigor plants grafted on a dwarfing rootstock can be successfully deficit-irrigated. Also, there is no clarity on how irrigation practices can affect soil microbiota in fruit trees with a shallow root system. Two irrigation treatments (WET: commercial irrigation and LD: late water deficit) were applied during fruit ripening in a European plum cv. French orchard at super high density, grafted on a dwarfing rootstock (Rootpac-20) and trained as a continuous hedge in Peralillo, Chile, for two consecutive seasons (2020-2021 and 2021-2022). Plants subjected to late deficit irrigation exhibited moderate (Ψ_{stem}~-1.2 MPa) to severe (Ψ_{stem}≤-1.4 MPa) water stress, increasing water productivity (>40%) and maintaining fresh and dry fruit quality attributes. Plants under late deficit irrigation maintained stomatal conductance and photochemical efficiency, even when the Ψ_{stem} was below -1.4 MPa. Transient water deficits caused abrupt drops in soil moisture, increasing the proportion of some plant growth-promoting bacteria (e.g., genus *Azospirillum*) the following season and raising the quantity of fine and thin roots. Consequently, late water deficit is a sustainable and resilient practice that saves water and improves drought tolerance in low-vigor plants through physiological and soil microbiological mechanisms.

Keywords: Water stress, PGPR, *Prunus domestica*, Stem water potential, soil microbiota.

1. Introduction

Chile is the world's leading exporter of dried prunes from the European plum (*Prunus domestica* L.), with an annual production of nearly 100,000 tons and 12,530 ha planted with this fruit crop (Sotomayor, 2023; ODEPA, 2022). In the worst case scenario, predictive climate models have projected an increase of 4°C in air temperature and a 39% decrease in snow accumulation for the area where European plum trees are grown in the south-central region of Chile (Bambach et al.2022). These climate changes could lead to a significant reduction in the availability of irrigation water in the major fresh fruit producing regions of Chile. However, European plum trees have a high tolerance to severe water stress (McCutchan and Shackel, 1992), which favors the adoption of conservation irrigation strategies to reduce water application in areas affected by frequent and prolonged droughts.

Regulated deficit irrigation (RDI) is a cultural practice in which the amount of irrigation water applied to the orchard is lower than the amount of water evapotranspired by the crop during certain phenological stages when the reproductive development is less sensitive to the occurrence of water stress (Chalmers et al.1981; Chai et al.2016; Calderón-Orellana, 2020). In fruit species such as apricot (*Prunus armeniaca* L.) (Girona et al.2004), apple (*Malus domestica* Borkh.) (Leib et al.2006), Japanese plum (*Prunus salicina* L.) (Hajlaoui et al.2022), and sweet cherry (*Prunus avium* L.) (Blanco et al.2020), among others, RDI has been shown to save up to 50% of irrigation

water, control excessive vegetative growth, and improve fruit quality at harvest or during storage (Pérez-Pastor et al.2007). The ability to maintain orchard productivity and fruit quality during a period of water stress makes RDI a highly resilient cultural practice, where water conservation through the use of RDI results in an internal and preventative regulatory mechanism to minimize the impact of a potential drought event on orchard profitability.

In European plum, the application of late water deficits (LD) has been successful in reducing the amount of irrigation water by about 40%, improving flowering by 17% and increasing dry yield by 14% (Goldhamer et al.1994; Lampinen et al.1995; Lampinen et al.2001). To date, LD research in European plum has been conducted exclusively in orchards planted at low to medium density (400 to 800 plants ha-1) and mainly grafted on medium to high vigor rootstocks, such as Marianna 2624 or Myrobalan (Goldhamer et al.1994; Lampinen et al.2001). Recently, several plantations of European plum in Chile have been established in super high density (SHD) (>1,500 plants ha-1), trained in continuous hedgerows and grafted on dwarfing rootstocks (Lordan et al.2019; Opazo et al.2020). The establishment of orchards in SHD has been successfully tested in several fruit species from the genus Prunus, such as almond (Casanova-Gascón et al.2019) and Japanese plum (Buler and Mika, 2011). The main advantages of SHD orchards compared to conventional orchards include increased mechanization of harvesting and pruning (Buler and Mika, 2011; Mika et al.2015), induction of productive earliness (second or

third year) (Mika et al.1998; Milosevic et al.2008), and increased yield per hectare (Meland, 2005).

Fruit orchards in SHD are typically established on dwarfing rootstocks, which severely reduce the vegetative growth of plants. Several studies have linked the low vigor of fruit trees to high levels of abscisic acid (ABA) in the shoots of plants grafted on dwarfing rootstocks (Hayat et al.2023). Because ABA is a plant hormone that inhibits vegetative growth and induces stomatal closure (Chen et al.2020), the use of dwarfing rootstocks with high concentrations of ABA may alter the stomatal response of plants to irrigation practices. In addition, dwarfing rootstocks are often associated with reduced escape capacity to water or oxygen deprivation due to their reduced root development (Jiménez et al.2013; Yahmed et al.2016; Opazo et al.2019). These characteristics may limit the use of conservative irrigation practices in orchards with dwarfing rootstocks where the soil may have very low moisture contents along the effective rooting zone.

Irrigation practices induce transient changes in soil water-oxygen balance that alter the pH of the rhizosphere and stimulate the release of compounds that promote the proliferation of microorganisms that constitute the microbiota (Gregory et al.2013). Among these, plant growth promoting bacteria (PGPB) have attracted great interest in the horticultural industry, as they not only promote plant growth, but also improve defense against abiotic stress and acquisition of water and nutrients (Milošević et al.2012). Therefore, PGPB are

considered as relevant inducers of drought resilience in agricultural crops (Bouremani et al.2023). Under conditions of low water availability, the effects of PGPB's on plants include changes in root morphology and the synthesis of active osmolytes, antioxidants, phytohormones, extracellular polymers, volatile organic compounds (VOCs), siderophores, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Gouda et al. 2018). However, most studies on the effects of PGPB on drought tolerance in cultivated plants have been conducted in annual species such as cereals and vegetables. In fruit trees, research on plant-bacteria interactions under contrasting irrigation practices has been scarce and inconclusive. For example, the inoculation of walnut plants (*Juglans regia* L.) with *Bacillus cereus* L90, a PGPB associated with phytohormone synthesis, increased ABA concentration under abundant and deficit irrigation, but did not improve plant tolerance to severe water stress (Liu et al.2023).

The Rootpac-20 (*P. besseyi x P.cerasifera*) (Gasic and Preece, 2014) has been the dwarfing rootstock selected for the establishment of new European plum orchards in SHD in Chile. This rootstock has been reported to induce low vegetative growth and high yields per plant (Lordan et al.2019), low transpiration rates (Bellvert et al.2021), and high fruit dry matter content (Iglesias et al.2019). However, the degree of drought tolerance of European plum trees grafted on the Rootpac-20 rootstock is unknown. Furthermore, the need to improve the resilience of orchards to abiotic stress and the importance

of PGPBs as complementary biological tools for water stress management requires the evaluation of the role that irrigation practices can have on soil microbiota. The aim of this study was to evaluate the effect of LD on plant water relations, vegetative and reproductive growth, fruit quality and soil microbial composition in a commercial European plum orchard using a SHD planting system grafted on Rootpac-20.

2. Materials and method

2.1. Description of the study site and weather data.

This study was conducted for two consecutive seasons (2020-2021 and 2021-2022) in a commercial orchard of European plum (*Prunus domestica* L.) cultivar French, located in Peralillo (34°26'27.7"S 71°25'33.6"W), Libertador General Bernardo O'Higgins region, Chile. The trees were planted in 2014 and grafted on the Rootpack-20 rootstock (*P. besseyi x P. cerasifera*) at a spacing of 3.5 m x 1.5 m in a continuous hedge (canopy dimensions: width of 0.8 m and height of 2.5 m to 3 m). The orchard was drip irrigated with a double line, using two emitters per plant, 0.75 m apart, with an emission rate of 2 L h^{-1} per plant. The orchard was oriented north-south, showing an average fresh yield of 19.5 t ha^{-1} over the last 3 years. The soils belong to the Calleuque series, with a predominantly clayey texture (70% clay, 20% sand, 3% silt) and belong to the Vertisols family (Xererts). The A and B horizons are very clayey soils, with expansive clays and cracks associated with low moisture content. They are very

plastic and adhesive soils, with colors ranging from brown to reddish brown, shallow (0.6 m) with low permeability and slow infiltration. The C horizon is dark red, very clayey and overlies an impermeable layer rich in iron and manganese (hardpan). The plants were pruned twice during the season using a pruning machine (DE800, B.M.V., Sharp Innovators, Alba, CN, Italy). The first pruning was carried out in mid-November and consisted of cutting the hedge with blades, leaving a canopy width of 0.8 m to maintain the plant architecture. The second pruning was carried out at the end of April with saws, leaving a width of 0.6 m to renew the fruiting wood. Harvesting was carried out entirely with a mechanical grape harvester (New Holland VX 7090, CNH Global, Burr Ridge, IL, USA) with a frequency of 380-400 rpm, a working speed of 3 km h^{-1} and a harvesting efficiency of 0.4 ha h^{-1}. Pest, weed, and disease management was conducted according to commercial orchard practices.

2.2. Experimental design.

The experimental design was a completely randomized block design with 4 replications. Each experimental unit was a group of 12 plants distributed in 3 adjacent rows (4 plants per row), with measurements taken in the two middle plants of the central row. Two irrigation treatments were applied from the phenological stage of *veraison* (first week of January) (Table 1), with the aim of obtaining differences in plant water status during the fruit ripening period, when water availability is the minimum of the season and the relative growth rate (RGR) of the fruit is low. In the control treatment (WET), commercial irrigation practices

were maintained for the whole season, with the aim of satisfying at least 100% of the crop evapotranspiration (ETc) of orchards under conventional management (400-500 trees ha^{-1}), trying to maintain an optimum plant water status for European plum, stem water potential (Ψ_{stem}) between -1.0 and -0.8 MPa (McCutchan and Shackel, 1992). In the regulated deficit irrigation (LD) treatment, water was completely cutoff until Ψ_{stem} values reached a maximum water stress severity of -1.4 MPa (severe water stress) (McCutchan and Shackel, 1992).

Before *veraison* and after harvest, commercial irrigation practices were similarly applied in the whole orchard, regardless of the irrigation treatment. Irrigation requirements were calculated based on the estimation of the plum orchard evapotranspiration (ETc = ETo x kc). Crop coefficients (kc) used in this study from budbreak (September) to harvest (mid-February) were obtained from FAO 56 (Allen et al.1998) and considered conventional cultivation practices for this fruit crop (400-500 trees ha^{-1} and plant height between 4 to 6 m). In order to estimate ETc values for the SHD orchard, satellite image analysis was employed to facilitate a comparison between the maximum irrigation requirements and the cumulative irrigation applied. The SPIDERwebGIS® (System of Participatory Information, Decision Support and Expert Knowledge for Irrigation River Basin Water Management) platform, developed by the European PLEIADES project (D'Urso et al.2010) and currently operated by AgriSat Iberia (http://www.spiderwebgis.org/ and https://www.agrisat.es/en), was used to estimate kc values in the SHD orchard. This platform, in turn, integrates the

Plataforma Agrícola Satelital (PLAS) developed by the Instituto de Investigaciones Agropecuarias (INIA) (Balbontín, 2021; Jovanovic et al.2020). PLAS determines the crop coefficient (kc) as a function of the Normalized Difference Vegetation Index (NDVI) of the crop.

2.3. Environmental conditions.

Data on global solar radiation (Wm^{-2}), precipitation (mm), reference evapotranspiration ($m^3\ ha^{-1}$), relative humidity (%) and air temperature (°C) were obtained from an agrometeorological station installed in October 2020 at 200 m from the experimental orchard. Sensors were placed between 1.5 and 2 m above the soil surface and meteorological information was recorded and stored with a sampling frequency of 1 s and storage frequency of 15 min throughout both seasons. Air temperature and relative humidity were recorded with a sensor (HMP60, Vaisala, Helsinki, Finland), wind speed and direction with an anemometer (A100R, Vector Instruments Ltd., UK, SF), global radiation with a pyranometer (CM14, Kipp & Zonen Delft, The Netherlands), precipitation with a rain gauge (ARG100, Campbell Scientific Instrument, Logan, UT, USA). All data were stored in two data loggers (CR10X, Campbell Scientific Instrument, Logan, UT, USA). ETo values were calculated using the meteorological variables recorded with the FAO56 Penman-Monteith daily time step equation (FAO56 P-M) (Allen et al.1998).

Volumetric soil water content was measured across the effective rooting zone of the plants only in the first block of the orchard during both seasons from

one month before the onset of fruit maturity at *veraison* (mid-January) to the beginning of leaf senescence (end of March) (Table 1) during the first and second seasons. Evaluations were made with capacitance sensors (GS1, Decagon devices, Pullman, WA, USA) installed in the center row at 0.75 m from the plant, at two depths (-0.3 and -0.6 m). The effective rooting zone and the sampling depth were determined based on the root system development in four soil pits randomly distributed throughout the SHD orchard. Data were recorded and stored every 15 min in two dataloggers (Em5b and Em50, Decagon devices, Pullman, WA, USA). Four volumetric water meters (Dishnon, Arad Ltd, Dalia, Israel), one per block, were installed at the beginning of each irrigation line to estimate the amount of water irrigated on October 1st, 2020.

Photosynthetically active photon flux density (PPFD, µmol m^{-2} s^{-1}) and leaf area index (LAI) were determined weekly using a ceptometer (LP-80, Decagon Instruments, Washington, USA) with four measurements for each plant sampled at midday. Measurements were taken at 0.2 m below the plant canopy to estimate the internal PPFD, with each measurement taken at 0.05, 0.20, 0.30, and 0.40 m from the trunk. The outer PPFD was estimated in the inter-row at 1.75 m from the plant and at 1.6 m above the ground.

2.4. Plant water status, physiology, and growth

Severity of plant water stress was determined weekly from budbreak to harvest in both seasons by measuring midday stem water potential (Ψ_{stem}) in two leaves per sampled plant, selected from the shaded part of the canopy and

without visual symptoms of biotic or abiotic stress. Measurements were performed between 12:00 and 16:00 h using a pressure chamber (PMS-615, PMS Instruments, Portland, USA). Sampled leaves were previously covered with an opaque airtight bag for at least 40 min according to the method described by McCutchan and Shackel (1992). Stomatal conductance (mmol m^{-2} s^{-1}) was determined simultaneously with stem water potential measurements using a steady-state porometer (SC-1, Decagon devices, Washington, USA) on three mature sun-exposed leaves from the apical third of the shoots. Photosystem II (PSII) efficiency was determined as *Fv/Fm* using a chlorophyll fluorescence meter (Pocket PEA, Hansatech Instruments, Norfolk, UK). Both measurements were made at midday, once a week during both seasons. To determine the photochemical efficiency of the photosystem II (PSII), leaves were dark adapted for 30 min using leaf clips (Reyes-Díaz et al.2009) before measuring the minimum fluorescence (*F0*) and maximum fluorescence (*Fm*), from which the variable fluorescence (*Fv*) is determined as the difference between *Fm* and *F0*. Photosystem II efficiency (*Fv/Fm*) was calculated using the following relationship

$$\frac{Fv}{Fm} = \frac{(Fm - Fo)}{Fm}$$

2.5. Yield components and fruit quality.

The percentage of fruit drop in relation to the total yield at harvest was estimated by manually counting the fruit on the ground before harvest. This was done by considering a quadrant of the size of the planting frame (5.25 m^2) in the

two plants evaluated in each block treatment combination. At the time of harvest maturity for dehydrated fruit (24-25 Brix), the fruit of each sampled tree was manually harvested. At the time of harvest, the crop load of each sampled tree (kg plant^{-1}) was estimated, and the total number of fruits was counted. The total weight per tree was then determined using a platform scale. Subsequently, a random sample of 100 fruits was selected from each plant, stratified by block treatment. From this sample, a random subsample of 50 fruits was selected for analysis of fresh fruit quality, and an additional 50 fruits were selected for analysis of dried fruit quality. In the initial subsample for fresh fruit quality, the individual fresh weight of each fruit (g) was determined using a precision balance with an accuracy of ±0.1 g (APTP457A, Electronic Scale balance, Kuala Lumpur, Malaysia). Flesh firmness (lbf) was determined using a digital penetrometer with an 8 mm plunger (FM200, PCE Instruments, Southampton, UK). This measurement was taken at the midpoint of the longest side of the fruit after manual removal of a section of skin. The color of the pulp was then determined in a slice taken from each fruit using a portable colorimeter (CR-10, Konica Minolta, Tokyo, Japan). The color was determined within the CIELAB color space, expressed as the coordinates L*a*b*. L* represents lightness, a* represents red/green, and b* represents yellow/blue. The concentration of soluble solids (Brix) in the juice, extracted from each fruit by manual means, was determined using a digital refractometer (HI 96801, Hanna Instruments, Rhode Island, USA). Prior to each measurement, the refractometer was calibrated with distilled water.

The dry subsample was transferred to dehydration ovens, where the fruit was placed on trays and exposed to 85°C for 19 hours, resulting in a reduction of the fruit's moisture content from 85% to 19%. Subsequently, the dried fruits were individually weighed, and the drying rate per treatment was calculated using the ratio of the fresh weight to the dry weight. Flower return was conducted at full bloom (early September) (Table 1), and flowers were counted on four branches per tree in the two trees monitored per treatment block combination. Flower return was expressed as the number of flowers per meter squared.

2.6. Root system characterization.

During the period of maximal floral development (September), the number and size of roots in one block were determined for both irrigation treatments. A soil pit measuring 1 m in depth and width was excavated in the center of the center row. A grid of the same dimensions as the soil pit (1 m^2) was divided into 100 grids of 0.1 m^2. Prior to counting, the soil pit was meticulously cleaned with an agrological knife in order to expose the root system. Each visible root was classified according to diameter, with roots measuring less than 0.5 mm classified as fine, those between 0.5 and 2.0 mm classified as thin, those between 2.0 and 5.0 mm classified as medium, those between 5.0 and 7.0 mm classified as medium to coarse, and those greater than 7.0 mm classified as coarse. The proportion of roots of varying diameters to the total number of roots sampled in each test soil pit was determined.

2.7. Evaluations of the cultivable soil microbiota.

During the first week of September, the populations of four groups of bacteria (*Azotobacter* spp., *Azospirillum* sp., Actinobacteria and anaerobic bacteria) were evaluated to characterize the effect of irrigation on the cultivable soil microbiota. The sampling time was chosen to evaluate whether irrigation practices applied at the end of the previous season were able to alter the soil microbiota that will interact with plant growth at the beginning of the following season. For this purpose, soil samples were collected with an auger from the effective rooting zone of the plants in three of the four blocks evaluated, at the same locations where the soil moisture probes were installed. Composite samples of 250 g of soil, including free and rhizospheric soil, were collected. Prior to each sampling, the materials used were disinfected in 70% ethanol. Each composite sample was placed in a polyethylene bag, previously identified according to the number of samples, cultivar and density. Finally, the bags containing the samples were sealed and placed in a refrigerated box (5°C) until their analysis in the laboratory. Each soil sample was homogenized to select a 10 g subsample, which was dissolved in 100 mL of sterile saline solution (0.89% NaCl) in an Enlenmeyer flask. The flasks containing the dilution were kept on an orbital shaker at 150 rpm with constant agitation for two hours. Dilutions of 10 were made from the suspension until the 10-5 dilution was reached. Dilutions ranging from 10-2 to 10-5 were used for inoculation into the different culture media, depending on the microbial group to be quantified. In addition, for the determination of the dry weight of the soil, a subsample of 50 g of each experimental unit was placed on metal

plates and placed in a dry air oven at 60°C until the weight was constant. All culture media were prepared in deionized water and autoclaved at 120°C / 1 atm for 20 minutes. After sterilization, they were distributed in Petri dishes with 10 ml of medium in each one, this procedure was performed under aseptic conditions in a laminar flow chamber. The methodology used to count all the populations is based on the distribution of an aliquot of 100 µL of each dilution on the surface of the agar using a sterile glass rod. The plates were incubated at 25 ± 2 °C in the dark for 3 days, except for the Congo Red medium, which was incubated for 7 days before counting the colony forming units (CFU). For counting, dilutions were chosen in which the number of colonies was between 30 and 300. For strict anaerobic bacteria: Standard nutrient agar medium (MERCK) was used and incubated under anaerobic conditions in the GasPak™ EZ Anaerobe Container System Sachets chamber. Actinobacteria were enumerated on Jensen agar (2 g/L dextrose, 0.2 g/L casein; 0.5 g/L K_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$, trace $FeCl_3.6H_2O$, 2.5% (w/v) agar). Bacteria of the genus *Azotobacter* were isolated on LG medium (Dobereiner et al.1995) and those of the genus *Azospirillum* on RC selective medium (Rodriguez, 1982). The relative amount of bacteria was determined as the ratio between the CFU of each group and the sum of the CFU of the four groups evaluated.

2.8. Statistical analysis

The data were subjected to an analysis of variance (ANOVA) after testing for normality distribution (Shapiro-Wilk), homogeneity of error variances (Levene's

test), and additivity (Tukey). Differences between means were determined using the LSD test (alpha = 0.05). The relationship between physiological variables and root development measures was analyzed using linear and quadratic regression analysis. All statistical analyses were performed using the statistical software SAS 9.4 (SAS Studio, University Edition, SAS Institute, NC, USA).

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During the first week of September, the populations of four groups of bacteria (*Azotobacter* spp., *Azospirillum* sp., Actinobacteria and anaerobic

bacteria) were evaluated to characterize the effect of irrigation on the cultivable soil microbiota. The sampling time was chosen to evaluate whether irrigation practices applied at the end of the previous season were able to alter the soil microbiota that will interact with plant growth at the beginning of the following season. For this purpose, soil samples were collected with an auger from the effective rooting zone of the plants in three of the four blocks evaluated, at the same locations where the soil moisture probes were installed. Composite samples of 250 g of soil, including free and rhizospheric soil, were collected. Prior to each sampling, the materials used were disinfected in 70% ethanol. Each composite sample was placed in a polyethylene bag, previously identified according to the number of samples, cultivar and density. Finally, the bags containing the samples were sealed and placed in a refrigerated box (5°C) until their analysis in the laboratory. Each soil sample was homogenized to select a 10 g subsample, which was dissolved in 100 mL of sterile saline solution (0.89% NaCl) in an Enlenmeyer flask. The flasks containing the dilution were kept on an orbital shaker at 150 rpm with constant agitation for two hours. Dilutions of 10 were made from the suspension until the 10-5 dilution was reached. Dilutions ranging from 10-2 to 10-5 were used for inoculation into the different culture media, depending on the microbial group to be quantified. In addition, for the determination of the dry weight of the soil, a subsample of 50 g of each experimental unit was placed on metal plates and placed in a dry air oven at 60°C until the weight was constant. All culture media were prepared in deionized water and autoclaved at 120°C / 1 atm

for 20 minutes. After sterilization, they were distributed in Petri dishes with 10 ml of medium in each one, this procedure was performed under aseptic conditions in a laminar flow chamber. The methodology used to count all the populations is based on the distribution of an aliquot of 100 µL of each dilution on the surface of the agar using a sterile glass rod. The plates were incubated at 25 ± 2 °C in the dark for 3 days, except for the Congo Red medium, which was incubated for 7 days before counting the colony forming units (CFU). For counting, dilutions were chosen in which the number of colonies was between 30 and 300. For strict anaerobic bacteria: Standard nutrient agar medium (MERCK) was used and incubated under anaerobic conditions in the GasPak™ EZ Anaerobe Container System Sachets chamber. Actinobacteria were enumerated on Jensen agar (2 g/L dextrose, 0.2 g/L casein; 0.5 g/L K_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$, trace $FeCl_3.6H_2O$, 2.5% (w/v) agar). Bacteria of the genus *Azotobacter* were isolated on LG medium (Dobereiner et al.1995) and those of the genus *Azospirillum* on RC selective medium (Rodriguez, 1982). The relative amount of bacteria was determined as the ratio between the CFU of each group and the sum of the CFU of the four groups evaluated.

2.9. Statistical analysis

The data were subjected to an analysis of variance (ANOVA) after testing for normality distribution (Shapiro-Wilk), homogeneity of error variances (Levene's test), and additivity (Tukey). Differences between means were determined using the LSD test (alpha = 0.05). The relationship between physiological variables and

root development measures was analyzed using linear and quadratic regression analysis. All statistical analyses were performed using the statistical software SAS 9.4 (SAS Studio, University Edition, SAS Institute, NC, USA).

3. Results

3.1. Environmental conditions and characterization of irrigation

The amount of irrigation for the WET plants was about 8500 m^3ha^{-1} in the first season, while the irrigation of the same treatment increased by about 40% in the second season (Table 2). The estimated cumulative evapotranspiration of the SHD orchard for the first and second seasons was 5204 and 5288 m^3 ha^{-1}, respectively. The amount of water applied in the first and second seasons was 9219 and 12423 m^3 ha^{-1}, respectively, with water savings of about 23% in plants under LD in both seasons. In general, plants under LD had a clear effect on water productivity, increasing values between 40% and 43%. Seasonal differences in monthly ETc (close to 0.5 mm) were observed in December and February (Figure 1A). In the first season, the maximum evaporation rate of 3.5 mm was recorded in December, followed by an abrupt 40% decrease in monthly ETc in March. The maximum crop coefficients (kc) were reached in November in both seasons and were very close to 0.7 (Figure 1B). In December, the month in which mechanized training pruning was carried out, kc was reduced by 15%. In February, kc returned to values relatively similar to those before pruning. However, the kc of the first season in February was slightly lower (5%) than that of the second season.

In WET and LD plants, soil volumetric water content (SWC) was close to 0.5 m^3 m^{-3} before the first irrigation cutoff in January (Figures 2A and 2B). For the first irrigation cutoff, early January 2020 and late December 2021, SWC values decreased to 0.4 m^3 m^{-3} in LD plants. For the second cutoff in late February, SWC reached 0.3 m^3 m^{-3} in LD plants. A third irrigation cutoff in the second season reduced SWC in LD plants from 0.4 to 0.3 m^3 m^{-3} in just one week. At the end of the second season, both irrigation treatments showed a decrease in SWC, reaching values close to 0.25 m^3 m^{-3}.

3.2 Physiological responses

Measured Ψ_{stem} values for both irrigation treatments were between 2.0 and 0.5 MPa near the SWP baseline until the first irrigation cutoff (Figures 3A and 3B). After treatment application, LD plants exhibited reductions in Ψ_{stem} in both seasons, but measured Ψ_{stem} values for both irrigation treatments were consistently lower in the second season. In the first season, LD plants reduced their Ψ_{stem} to -0.8 MPa two weeks after the first irrigation cutoff, but there were no significant differences between irrigation treatments. During the second irrigation cutoff, plants reached a Ψ_{stem} of -1.2 MPa after 12 days, with a difference of -0.5 MPa from the WET treatment (Figure 3A). In the second season, LD plants showed a Ψ_{stem} of -1.5 MPa one week after the first irrigation cutoff. After the second irrigation cutoff, the maximum water stress severity reached -2.0 MPa, while for the third cutoff, the Ψ_{stem} reached -1.7 MPa (Figure 3B). The regression analyses showed that Ψ_{stem} values near -1.5 MPa were associated with the

maximum seasonal values of gs (Figure 4A) and *Fv/Fm* (Figure 4B). Once plants reached a -2.0 MPa Ψ_{stem}, g_s decreased by 20% (Fig. 4A), while *Fv/Fm* values remained close to the maximum of 0.8 (Fig. 4B). Plants with Ψ_{stem} values above -0.5 MPa experienced a 30% decrease in g_s and 20% decrease in *Fv/Fm*.

3.3 Yield and fruit quality estimates

The yield of fresh fruit per plant was not affected by LD. The average yield for the first and second seasons were 9.3 and 32.3 tons per hectare, respectively (Table 3). The number of fruits per tree was not significantly affected by irrigation treatment in either season. The mean number of fruits per tree was 260 and 1,065 in the first and second seasons, respectively. There were no treatment differences in the number of flowers (Table 3). The fresh fruit quality parameters were similar between the irrigation treatments in both seasons (Table 4). The soluble solids concentration and the fresh weight were 5 Brix and 2 g higher in the first season. There was a linear relationship between crop load in fresh weight per fruit and yield per plant (Figure 5).

3.4 Root system response and cultivable soil microbiota

The plants showed a poorly developed root system at a maximum depth of 60 cm. Of the total roots, 90% were fine and thin (data not shown). LD plants had almost 20% more fine and thin roots than WET plants (Figure 6A). Regression analysis revealed a clear quadratic relationship between the average seasonal stem of plants and the number of fine and thin roots per m^2 of soil (Figure 6B). The application of LD had no statistical effect on the total UFC, but it did affect the

relative abundance of some groups of cultivable microorganisms in free soil (Figures 7A and 7B). The relative abundance of *Azospirillum* spp bacteria in soils under LD was ten times higher than that under the WET treatment (Figure 7B). While there were no significant differences overall, the relative contents of the genus Azotobacter and the phylum Actinobacteria were three and eight times higher, respectively, under LD. Conversely, soils under the WET treatment exhibited a relative abundance of anaerobic bacteria close to 90%, which was three times higher than that observed in soils under the LD treatment.

4. Discussion

The results of this study prove that European plum orchards established in SHD and grafted on the dwarfing rootstock Rootpac-20 can be deficit irrigated without reducing fresh and dry yields. Applying a late water deficit resulted in significant water savings, with an improvement in water productivity of approximately 40% in both seasons. The period of the late water deficit application coincides with the time of the year when (1) the atmospheric evaporative demand is at its maximum and (2) the availability of irrigation water is at its lowest in Central Chile. The water savings generated by LD during this period are of great significance, as they increase the water availability to irrigate fruit species that are more sensitive to water stress than the European plum. These results contradict those reported by McCutchan and Shackel (1992). Their findings indicated that severe late water deficits (Ψ_{stem} <-1.5 MPa) had no effect on dry yield, but resulted in a 4 t ha^{-1} reduction in fresh yield. The lack of irrigation effects on fresh and dry

yield was the result of a combination of factors, including: (A) The application of LD at the onset of *veraison* in January did not coincide with any phenological event that determines the number and weight of fruits. For example, the number of fruits per tree is largely determined by the number of flowers in the current season, which is defined during bud induction and initiation in late November of the previous season (Wells and Bukovac, 1978). Furthermore, drupes from reach their maximum relative growth rate (RGR) during the first few weeks after fruit set (Basile et al.2002). Any significant alteration of the carbohydrate supply rate during fruit set will inevitably result in a discrepancy between the actual and potential RGR, which in turn will lead to a reduction in final fruit size and yield at harvest. Since the severe water stress in LD plants occurred between one and two months away from bud induction and fruit set, both phenological stages occurred under optimal water conditions, regardless of the irrigation treatment. (B) Even though previous studies in vigorous orchards have shown a reduction near 60% in the photosynthesis rate of severely water-stressed European plum trees (Lampinen et al.2004), the transient occurrence of moderate (Ψ_{stem} ~-1.2 MPa) to severe water stress (Ψ_{stem} ~-2.0 MPa) in low-vigor trees grafted on the Rootpac-20 rootstock had no significant impact on parameters that alter net assimilation rates, such as g_s and *Fv/Fm*. When plants reached a Ψ_{stem} of -2.0 MPa, g_s was reduced by 20%, but *Fv/Fm* remained close to the maximum values recorded for European plum trees (*Fv/Fm*~0.8) (Buwalda and Noga, 1994). These findings are inconsistent with those reported by Lampinen et al. (2004), which

indicated about 50% reduction in g_s and photosynthesis in plants of cv. French when Ψ_{stem} reached -1.7 MPa. In almond trees (*Prunus dulcis* L.) grafted on Rootpac-20, only the application of a very severe level of water stress ($\Psi_{leaf} \leq$ -2.0 MPa) reduced g_s to values that compromised the photosynthesis rates of leaves (Álvarez-Maldini et al.2021). Since dwarfing rootstocks have been reported to exhibit higher concentrations of ABA in shoots (Hayat et al.2023), it is possible that the grafting of *Prunus* spp plants onto a dwarfing rootstock, such as Rootpac-20, may induce hormonal changes associated with modifications in the stomatal response to water stress in comparison to that reported in previous studies with plants grafted on rootstocks of higher vigor. (C) The crop loads of the plants in SHD were at least four times lower than those reported in previous irrigation studies for conventional European plum orchards. In the present study, the application of LD did not affect yield estimates or any fruit quality parameter, suggesting that moderate, severe, and very severe water stress showed little impact on the carbohydrate supply to fruits during stage III of fruit growth and development. This finding highlights the importance of the competition for carbohydrates among fruits as a key determinant of the impact of water stress on yield and fruit quality. The fruit load in the orchard in SHD did not exceed 1200 fruits, whereas in a conventional production system with lower planting densities, fruit loads are usually higher than 5000 fruits. From a commercial perspective, the lack of differences in fruit quality and yield between irrigation treatments is of

greater consequence nowadays, given the increasing attractiveness of exporting fresh fruits from European plum trees to the Asian market in Chile.

Despite the lack of irrigation effects on fruit size parameters, the equatorial diameter and fruit weight did not reach the commercial optimum values required by the fresh and dry markets (33 mm and 20-23 g, respectively). Although the crop load of the first season was reduced to 259 fruits per tree-1 by a spring frost close to flowering, both irrigation treatments exhibited the lowest fruit sizes in the first season. Previous studies found that the relationship between crop load and fruit size was quadratic in European plum trees, with the highest slope value for this relationship observed at low crop loads (Lampinen et al.2001). In this study, the relationship between crop load and fresh fruit weight was linear for the range between 0 and 1200 fruits per plant, suggesting that competition for assimilates among fruits was not a limiting factor for fruit growth in plants with a low crop load. A failure in the irrigation system that lasted two weeks and induced severe levels of water stress (Ψ_{stem}~-1.7 MPa) near the fruit set may have been the main factor that explains the low fruit size in the first season. In the second season, the 312% increase in crop load was probably the primary cause of the small fruit size at harvest. A substantial increase in crop load in the second season was expected for European plum trees with unusually low crop loads the preceding season, as this species is considered a plant with a pronounced alternate bearing behavior (Lampinen et al.1995).

European plum trees grafted on Rootpac-20 would have a higher photosynthetic risk under over-watered conditions than under moderate or severe water stress. Plants that exhibited Ψ_{stem}~-0.5 MPa were near or above the SWP baseline for well-irrigated conditions, inducing decreases of 30% in g_s and 20% in *Fv/Fm*. These reductions may be related to poor oxygenation of roots subjected to abundant irrigation (Parent et al.2008; Ashraf, 2012). The regulation of stomatal opening and closing under conditions of excess moisture has been attributed to both an increase in abscisic acid (ABA) concentration and a decrease in cytokinins and gibberellins in roots (Domingo et al. 2002; Habibi et al. 2023). Reductions of physiological importance in g_s and *Fv/Fm* were observed in apple plants grafted on the M9 dwarfing rootstock after 30 days under hypoxic conditions (Bhusal et al.2020). In the present study, the amount of irrigation water applied throughout the season to plants in the SHD of the WET treatment was calculated to satisfy the irrigation needs of conventionally managed European plum orchards (between 9,000 and 12,000 m^3 ha^{-1}) (Goldhamer et al.1994). However, the analysis of satellite images and NDVI estimates for the SHD orchard showed that the total evaporative demand for the first and second seasons was 5204 and 5288 m^3 ha^{-1}, respectively. Therefore, the quantity of irrigation water applied in the SHD orchard would have exceeded the amount necessary to meet at least twice the water demand in both seasons, which clearly showed that plants were consistently under overirrigation conditions. The objective of irrigation in conventionally managed European plum orchards is to meet the evaporative

demand of tall trees (between 5 and 6 m in height) with canopies that cover the ground almost entirely. This results in maximum crop coefficients close to 1.0 (Shackel et al.2012). In this study, plant canopies in SHD did not completely cover the 3.5 m interrow, as the canopy width was between 0.8 and 0.9 m using trimming machines. As a result, approximately 75% of the orchard area was without vegetation during the growing season.

The impact of overirrigation in the SHD orchard was more evident in the WET treatment, as its average soil volumetric water content was closer to saturation (0.5 m^3m^{-3}) than field capacity (FC) (0.45 m^3m^{-3}) during the experimental period in both years. Despite an increase of 40% in irrigation water applied, water stress severity was higher in WET and LD plants during the second season. In the case of WET plants, Ψ_{stem} values indicated optimal plant water conditions during the first season. Conversely, in the subsequent season, Ψ_{stem} values of WET plants represented mild to moderate water stress levels for European plum, ranging from -1.2 to -1.0 MPa (Lampinen et al.2001). Plants under LD in the first season reached moderate water stress levels for European plum for approximately one week, with Ψ_{stem} values ranging from -1.4 to -1.2 MPa (Lampinen et al.2001). In contrast, water stress severity in the second season was moderate for the first irrigation cutoff but very severe for the second cutoff (<-1.7 MPa) (Lampinen et al.2001). Consequently, applying 40% more irrigation water in the second season caused no increase in water stored in the effective rooting zone, highlighting how unnecessary overwatering is as an irrigation

practice to avoid water stress. The higher severity of water stress registered by both treatments during the second season mostly reflected the influence of higher evaporative water demand, as the monthly average ETc during February of the second season, when the most severe Ψ_{stem} drops occurred, was approximately 30% higher than that observed during the same month of the previous year. The observed increase in ETc during February of the second season was likely due to two primary factors: (1) higher VPD and (2) slightly higher kc values resulting from the stimulation of sprouting following the spring pruning in November. Yet, previous research has indicated that the decline in Ψ_{stem} in LD treatments may be less pronounced and less severe when irrigation is partially restricted rather than fully withheld (Lampinen et al.2001).

The application of LD with a complete irrigation cutoff, as opposed to a proportional decrease in irrigation amount or frequency, reduced SWC to values close to PMP (0.25 $m^3\ m^{-3}$). However, once plants reached moderate to severe levels of water stress, LD plants were irrigated as WET plants. The transient and abrupt processes of severe drying and rehydration of soils caused changes in microbiological dynamics, as previously stated by Meisner et al. (2018). Soils subjected to LD tended to show higher relative abundances of bacterial genera and phyla adapted to aerobic conditions and regarded as beneficial microorganisms for plants (Pallo, 2011; Cassán et al.2020). For instance, the relative abundance of *Azospirillum* spp. was approximately tenfold higher in the LD treatment. Despite the lack of significant differences between irrigation

treatments, the relative abundance of the genus *Azotobacter* and the phylum Actinobacteria was three and eight times higher in the LD treatment. *Azospirillum* is a bacterial genus that, like *Azotobacter*, is characterized by nitrogen fixation and the production of phytohormones (Cassán et al.2020) that are often associated with nitrogen and water scarcity (de-Bashan et al.2007). On the other hand, Actinobacteria, known for their role as plant symbionts and biological control agents, have the potential to increase their population under conditions of low soil moisture content (Xie et al.2021; Barnard et al.2013). The reduction in soil moisture in plots under the LD treatment can kill a substantial number of bacteria, releasing high quantities of organic matter within the effective rooting zone of plants (Manzanera, 2021). After irrigation restarted in LD plants, soil water content increased to values near field capacity, solving organic matter and increasing bacterial populations to a greater extent than in the soils maintained at high moisture levels in the WET treatment (Denef et al.2001). Soils subjected to the WET treatment exhibited a relative abundance of anaerobic bacteria near 90%, approximately threefold higher than that observed in soils under the LD treatment. Soil conditions in the WET treatment may have stimulated the proliferation of anaerobic bacteria, outcompeting the populations of obligate aerobic bacteria. The shallow root system and the low leaf area limited the water uptake capacity of dwarfing rootstocks, which probably modulated the magnitude of the irrigation effects on *Azospirillum* and anaerobic bacteria population changes.

The higher relative abundance of some groups of beneficial soil bacteria in the LD treatment may explain the higher number of fine and thin roots in plants subjected to transient periods under water stress. These kind of bacteria stimulate root growth and phytohormone synthesis, such as indole acetic acid and gibberellins (González, 2010; Cassán et al.2020). The stimulation of root growth improves the adaption to drought by increasing the quantity of fine and thin roots, which are the ones that exhibit the highest rates of water and nutrient uptake and represent nearly 90% of all roots sampled. Furthermore, the highly significant correlation between season-average stem water potential and the total number of fine and thin roots confirms that transient moderate water stress stimulates root development, while very high humidity conditions may reduce it. The findings of the present study suggest that European plum trees grafted on a dwarfing rootstock in an SHD orchard can be effectively deficit-irrigated to enhance tolerance to a wide range of water stress severities not only through stomatal regulation and root development stimulation, but also through the maintenance and preferential reproduction of specific beneficial soil bacteria. Surprisingly, the microbiological response to regulated deficit irrigation was statistically significant even nine months after its application, following the winter rainy season (>400 mm $year^{-1}$) and three weeks before the beginning of the following season in spring.

5. Conclusions

The findings of the present study demonstrated that plants of the cv. French grafted on a dwarfing rootstock like Rootpac-20 with a shallow root system

exhibited no decline in yield and fresh and dry fruit quality even when water stress severity was transiently very high (Ψ_{stem} < -2.0 MPa). Moderate to severe water stress levels were associated with mild stomatal closure and high photosystem II efficiency despite soil water content approaching, more than once, the permanent wilting point each season. Regulated deficit irrigation induced changes in the soil microbiota towards the prevalence of potentially growth-promoting bacteria (PGPB), including members of the genus *Azospirillum*, that increased the quantity of fine and thin roots. In conclusion, the present study proved that a late water deficit is a sustainable and resilient practice that improves water productivity and soil microbiota. High resilience to water stress is highly relevant, as the lack of irrigation water represents a significant challenge to the profitability of European plum cultivation in Mediterranean climates under the current climate change scenario.

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Figure 1. Monthly average of (A) crop evapotranspiration and (B) crop coefficient in a European plum orchard in super high density (SHD) in Peralillo, O'Higgins Region, during the 2020-2021 and 2021-2022 seasons. Error bars represent ±1 se.

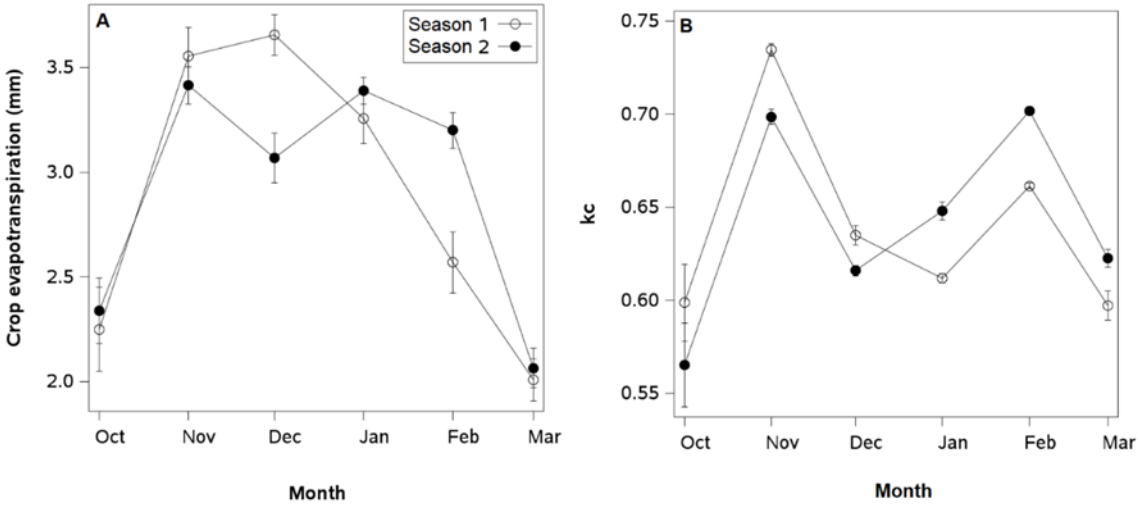

Figure 2. Weekly values of volumetric soil water content over two depths (-30 and -60 cm) from fruit ripening to the onset of leaf senescence (December to March) in a European plum orchard cv. French in super high density (SHD) under two irrigation treatments (LD: late water deficit and WET: commercial irrigation) in Peralillo, O'Higgins Region, during the (A) 2020-2021 and (B) 2021-2022 seasons.

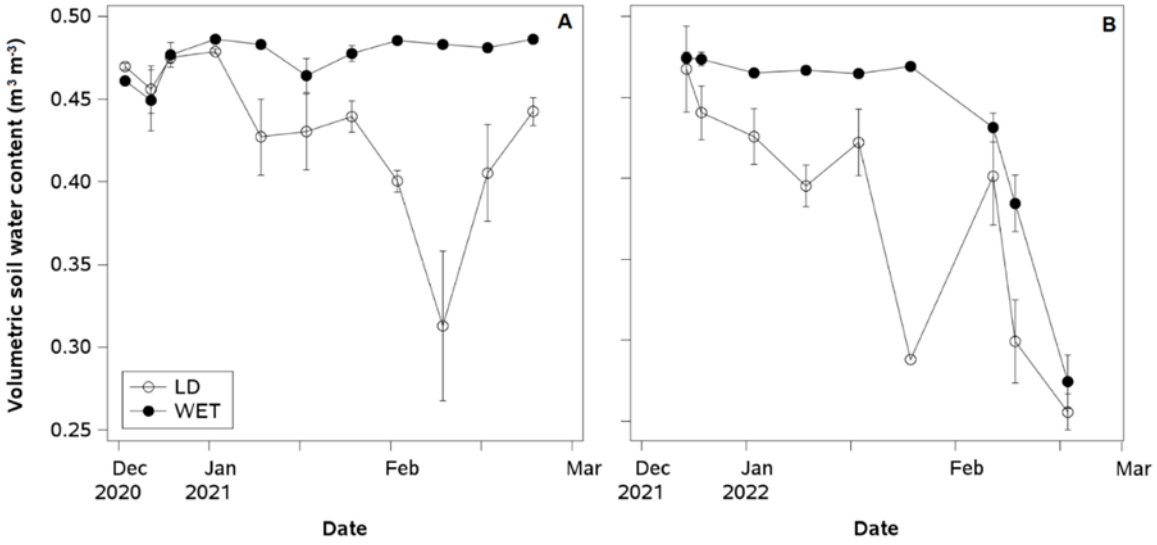

Figure 3. Stem water potential at midday (12:00-15:00 h) in a European plum orchard in super high density (SHD) under two irrigation treatments (LD: late deficit and WET: commercial irrigation) in Peralillo, O'Higgins Region, during the (A) 2020-2021 and (B) 2021-2022 seasons. Black arrows indicate the date of irrigation treatment application, white arrows indicate the date of irrigation resumption. The gray line without markers indicates the baseline optimum water status for European plum (McCutchan and Shackel, 1992). Asterisks indicate significant differences ($P \leq 0.05$, $n = 4$). Error bars represent ±1 se.

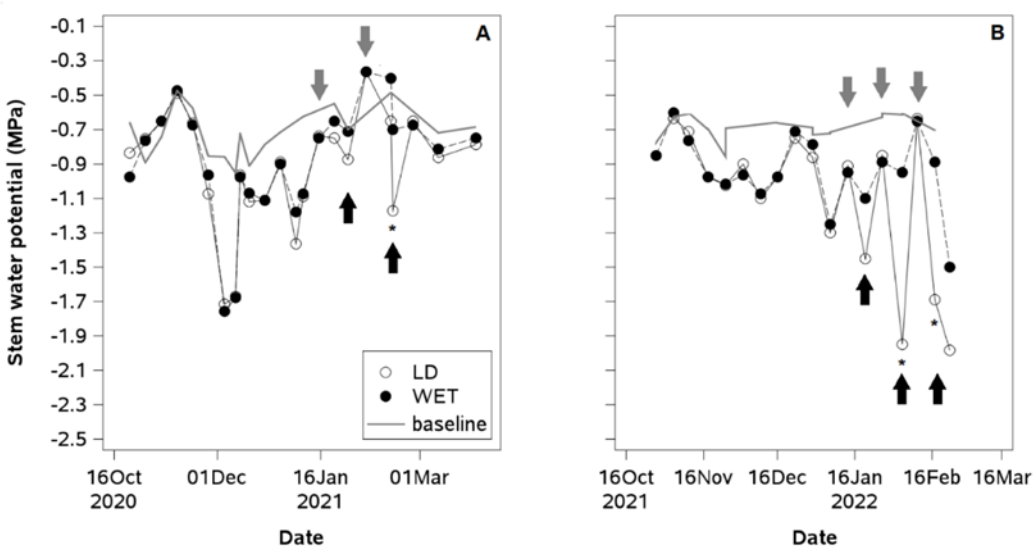

Figure 4. Relationship between midday stem water potential (Ψ stem) (12:00-15:30 h) and (A) leaf stomatal conductance (gs) (R2:0.74; p-value<0.05; y=-198.6x2-550.6x+183.7) and (B) Fv/Fm (R2:0.66; p-value<0.05; y=-0.66x2-0.219x+0.62) in a European plum orchard in super high density (SHD) under two irrigation treatments (LD: late water deficit and WET: commercial irrigation) in Peralillo, O'Higgins Region, during the 2020-2021 and 2021-2022 seasons.

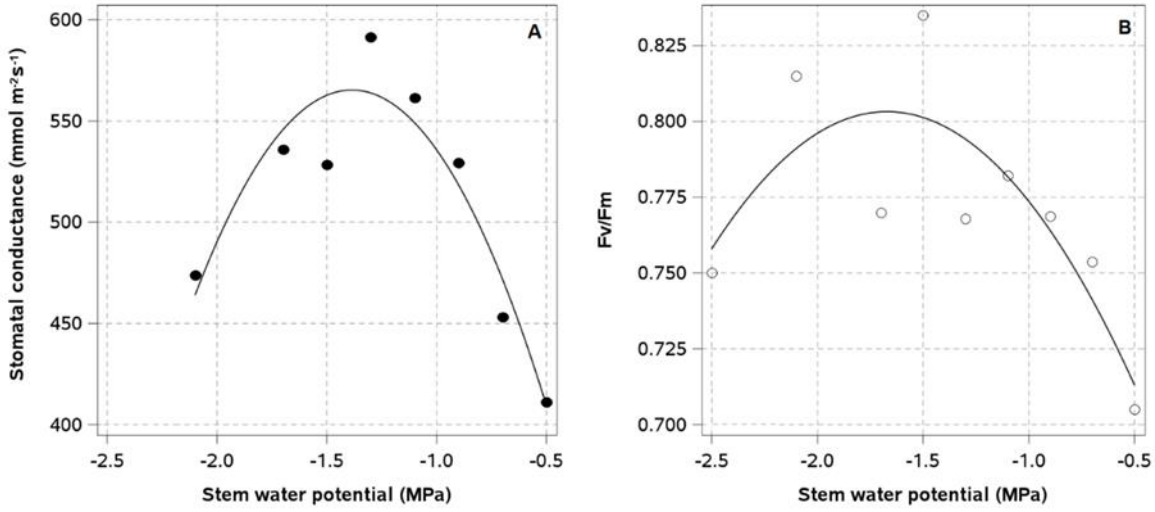

Figure 5. Linear regressions between fruit load with yield per plant (y= 1.27+0.015x (p-value<0.0001; R2:0.97; n=32) and with fresh weight of an individual fruit (y= 19.4-0.0026x (p-value<0. 0001; R2:0.67; n=32) in a European plum orchard in super high density (SHD) under two irrigation treatments (LD: late water deficit and WET: commercial irrigation) in Peralillo, O'Higgins Region, during the 2020-2021 and 2021-2022 seasons.

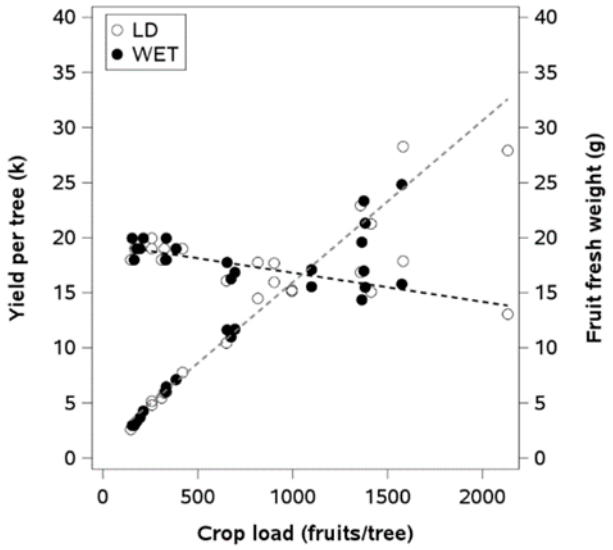

Figure 6. (A) Number of fine and thin roots per m^2 of soil and (B) quadratic regression between the seasonal average of stem water potential and the number of fine and thin roots per m^2 of soil in two blocks of a European plum orchard in super high density (SHD) under two irrigation treatments (LD: late deficit irrigation and WET: commercial irrigation) in Peralillo, O'Higgins Region during the 2021-2022 season). Error bars represent ±1 se.

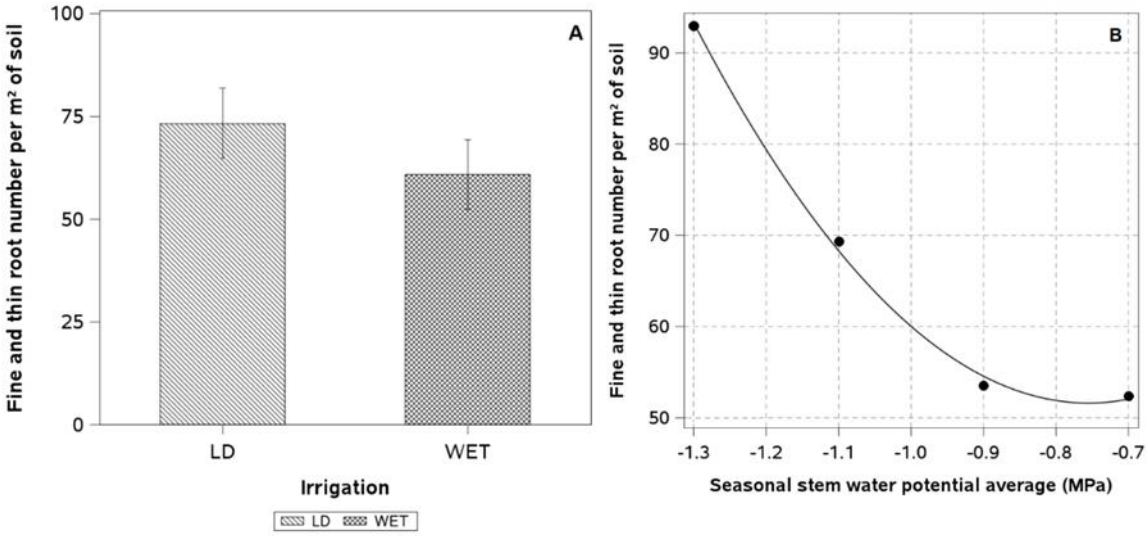

Figure 7. (A) Absolute content (logarithm of colony forming units, CFU) and (B) relative abundance of bacteria in the soil in a European plum orchard in super high density (SHD) under two irrigation treatments (LD: late deficit irrigation and WET: commercial irrigation) in Peralillo, O'Higgins Region during the 2020-2021 season. ACT= Actinobacteria; ANE= Strict anaerobic bacteria; AZO= Bacteria of the genus *Azospirillum*; AZOT= Bacteria of the genus *Azotobacter*. Asterisk indicates significant differences (P ≤ 0.05, n = 3).). Error bars represent ±1 se.

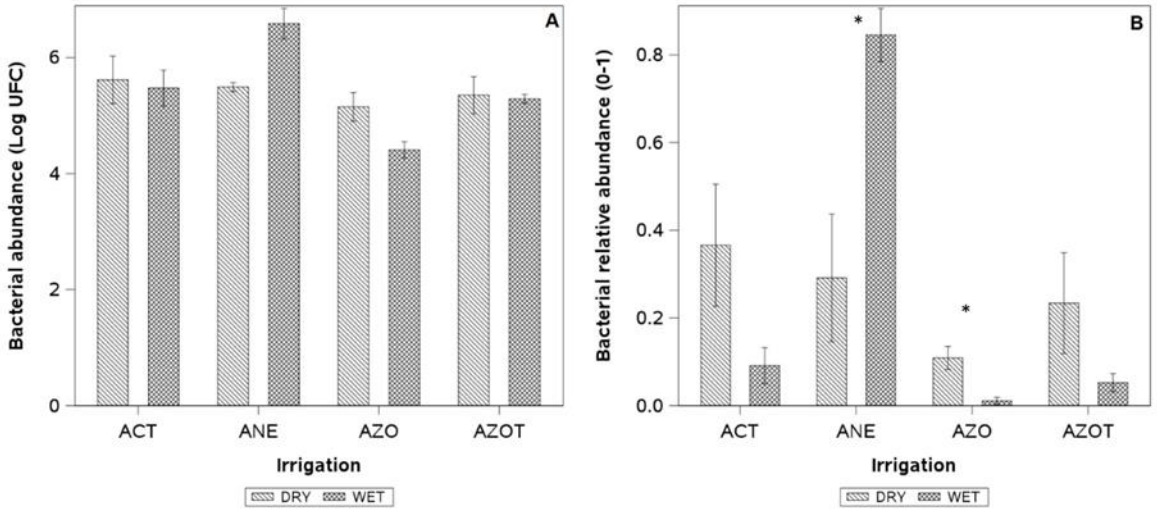

Table 1. Date of occurrences of several phenological stages of a SHD European plum orchard in Peralillo, O'Higgins Region, Chile during the 2020-2021 and 2021-2022 seasons

Phenological stages*	Seasons	
	2020	2021
Bloom	September 25th	September 30st
Budbreak	September 29th	October 4th
Fruit set	October 5th	October 7th
Pit hardening	November 7th	November 11th
Veraison	January 17th	January 20th
Harvest	February 18th	February 23rd

*****The date of occurrence of each phenological stage was recorded when the typical visual characteristics of that stage were observed at least in 50% of the evaluated plants.

Table 2. Cumulative values of irrigation, precipitation, applied water (irrigation + precipitation) and water productivity in a European plum orchard in super high density (SHD) under two irrigation treatments (LD: Late water deficit and WET: Commercial irrigation) in Peralillo, O'Higgins Region, during the 2020-2021 and 2021-2022 seasons.

	Season 2020-2021			Season 2021-2022		
Acumulative values	WET	LD	Diff	WET	LD	Diff
Crop evapotranspiration (m^3 ha^{-1})	5204			5288		
Irrigation (m^3 ha^{-1})	8544	6591	23%	11991	9097	24%
Precipitation (m^3 ha^{-1})	672			432		
Applied water (m^3 ha^{-1})	9216	7263	23%	12423	9529	23%
Water productivity (kg fruits m^{-3})	1.1	1.6	45%	3.0	4.2	40%

Table 3. Orchard and tree yield, number of fruits per tree, floral return, fallen and split fruit at harvest in a super high density (SHD) European plum orchard subjected to two irrigation treatments (LD: late deficit irrigation and WET: commercial irrigation) during the 2020-2021 and 2021-2022 seasons. NS means not significantly different, n=8.

Production	Irrigation treatment	
	WET	LD
2020-2021		
Orchard yield (ton ha^{-1})	8,8	9,8
Plant yield (kg $treel^{-1}$)	4,6	5,1
Fruit load (nº $tree^{-1}$ fruits)	243	275
Floral reversion (flowers m^{-1})	101	115
Fruit drop (%)	25,3	33,9
2021-2022		
Orchard yield (ton ha^{-1})	30,4	34,1
Plant yield (kg $tree^{-1}$)	15,9	17,9
Fruit load (n° $tree^{-1}$ fruits)	1001	1130
Floral reversion (flowers m^{-1})	24	22
Fruit drop (%)	10,8	11,3

Table 4. Fruit quality parameters at harvest in a super high-density (SHD) orchard subjected to two irrigation treatments (LD: Late deficit irrigation and WET: Commercial irrigation) during the 2020-2021 and 2021-2022 seasons.

Harvest quality	Irrigation Treatment	
	WET	LD
2020-2021		
Split fruit (%)	4,3	4,8
Fresh weight per fruit (g)	18,8	18,2
Equatoril diameter (mm)	26,8	26,6
Polar diameter (mm)	33	32
Pulp firmness (lbf)	2	2,4
Soluble solids concentration (Brix)	25	25,1
Color		
L	31,9	34,6
a	11	11,8
b	28,7	30,7
Conversion ratio	2,8	3,4
2021-2022		
Split fruit (%)	4	4,3
Fresh weight per fruit (g)	16,1	16,2
Equatoril diameter (mm)	28	27,5
Polar diameter (mm)	34,8	34,5
Pulp firmness (lbf)	1,4	1,4
Soluble solids concentration (Brix)	20,1	21,3
Color		
L	29,2	29
a	10,5	10,2
b	28,2	28,4
Conversion ratio	3,6	3,7

CAPÍTULO 3

CONCLUSIONES GENERALES

La falta de agua de riego es una de las más grandes amenazas para la rentabilidad del ciruelo europeo en climas Mediterráneos. Los resultados del presente estudio mostraron que las plantas del cv. French sobre Rootpac-20 no vieron afectados el rendimiento, ni la calidad de la fruta en fresco y seco, incluso cuando la severidad del estrés hídrico fue transitoriamente muy alta (SWP<-2.0 –MPa). Las severidades de estrés hídrico entre moderada y muy severa se asociaron a un leve cierre estomático y a una alta eficiencia del fotosistema II, a pesar de que el SWC llegase casi a PMP en ambas temporadas. Las modificaciones de la relación agua-oxígeno asociadas a la aplicación de los tratamientos de riego en los meses de verano indujeron cambios en la microbiota del suelo que fueron detectados al inicio de la siguiente temporada. Mientras los suelos de plantas bajo riego comercial abundante tuvieron un mayor porcentaje de bacterias anaeróbicas (~90 %), los suelos de plantas bajo LD tendieron a presentar mayores porcentajes de bacterias potencialmente promotoras de crecimiento (PGPB), como las del género *Azospirillum.* Estos resultados muestran que el LD en la combinación French sobre Rootpac-20 puede aumentar la resiliencia a sequía, pues el corte transitorio y absoluto del riego en la etapa III de crecimiento y desarrollo del fruto se asoció a un cierre estomático que no disminuyó la capacidad fotosintética de las hojas, ni el rendimiento del huerto o la calidad de la fruta. Adicionalmente, la aplicación del LD mejoró la microbiota del suelo, induciendo aumentos en el número de raíces finas y delgadas. Finalmente, el presente estudio provee de evidencia que sostiene que el LD es una práctica sostenible y resiliente que no sólo permite ahorrar agua, sino también mejorar la microbiota de los suelos, lo cual es particularmente relevante en el escenario actual de sequía en muchas zonas productoras de fruta en el mundo.