



Universidad de Concepción
Dirección de Postgrado
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**INOCULACION CON BACTERIAS PGPR EN LA
RESPUESTA FISIOLÓGICA DE PLANTAS DE ARÁNDANO
(*Vaccinium corymbosum* L.) BAJO DIFERENTES
CONDICIONES DE RIEGO**

Tesis para optar al grado de Magíster en Ciencias Agronómicas

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TABLA DE CONTENIDOS

	Página
INDICE DE FIGURAS Y TABLAS	iv
RESUMEN	vii
SUMMARY	vii
CAPÍTULO 1. INTRODUCCIÓN GENERAL	1
HIPÓTESIS	3
OBJETIVO GENERAL	3
OBJETIVOS ESPECÍFICOS	4
REFERENCIAS	4
CAPÍTULO 2. Inoculation with PGPR bacteria in the physiological response of blueberry plants (<i>Vaccinium corymbosum</i> L.) under different irrigation conditions.	8
Abstract	8
Introduction	9
Materials and methods	12
Results	16
Discussion	20
Conclusions	25
References	26
Figures	31

INDICE DE FIGURAS

		Página
Figure 1	Valores diurnos del déficit de presión de vapor (DPV) del aire en el exterior e interior del invernadero en plantas jóvenes de arándano cv. Legacy sometidas a tres tratamientos de riego: control, riego deficitario controlado severo (RDC sv) y riego deficitario controlado extremo (RDC ex), con y sin inoculación de <i>Pseudomonas koreensis</i> . Cada punto representa el promedio diario del DPV estimado entre las 12:00 y 15:00 h.	31
Figure 2	Valores diurnos de Temperatura en el exterior e interior del invernadero en plantas jóvenes de arándano cv. Legacy sometidas a tres tratamientos de riego: control, riego deficitario controlado severo (RDC sv) y riego deficitario controlado extremo (RDC ex), con y sin inoculación de <i>Pseudomonas koreensis</i> .	32
Figure 3	Valores diurnos de la radiación solar fotosintéticamente activa (PAR) medidos en el exterior e interior del invernadero en plantas jóvenes de arándano cv. Legacy sometidas a tres tratamientos de riego: control, riego deficitario controlado severo (RDC sv) y riego deficitario controlado extremo (RDC ex), con y sin inoculación de <i>Pseudomonas koreensis</i> .	33
Figure 4	Contenido volumétrico del sustrato al mediodía (12:00–15:00 h) en plantas jóvenes de arándano sometidas a tres tratamientos de riego: control (riego comercial), riego deficitario controlado severo (RDC	34

sv) y riego deficitario controlado extremo (RDC ex), con y sin inoculación de *Pseudomonas koreensis*, en un huerto establecido en macetas.

- | | | |
|----------|---|----|
| Figure 5 | Potencial hídrico del tallo al mediodía (12:00–15:00 h) en plantas jóvenes de arándano sometidas a tres tratamientos de riego: control (riego comercial) (A), riego deficitario controlado severo (RDC sv) (B) y riego deficitario controlado extremo (RDC ex) (C), con y sin inoculación de <i>Pseudomonas koreensis</i> , en un huerto establecido en macetas. Barras de error representan 1 e.s. Letras distintas representan diferencias significativas entre tratamiento de inoculación al 95% de confianza (DMS). | 35 |
| Figure 6 | Conductancia estomática en hojas al mediodía (12:00–15:00 h) en plantas jóvenes de arándano sometidas a tres tratamientos de riego: control (riego comercial) (A), riego deficitario controlado severo (RDC sv) (B) y riego deficitario controlado extremo (RDC ex) (C), con y sin inoculación de <i>Pseudomonas koreensis</i> , en un huerto establecido en macetas. | 37 |
| Figure 7 | Índice de reflectancia fotoquímica (PRI) en hojas al mediodía (12:00–15:00 h) en plantas jóvenes de arándano sometidas a tres tratamientos de riego: control (riego comercial) (A), riego deficitario controlado severo (RDC sv) (B) y riego deficitario controlado extremo (RDC ex) (C), con y sin inoculación de <i>Pseudomonas koreensis</i> , en un huerto establecido en macetas. Los * representan diferencias significativas entre tratamiento de | 39 |

inoculación. Barras de error representan 1 e.s. Flecha roja indica las fechas de aplicación de tratamiento y flecha verde indica rehidratación de las plantas. Asteriscos indican diferencias significativas entre los tratamientos de riego al 95% de confianza (DMS).

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|----------|--|----|
| Figure 8 | Poblaciones totales de <i>Pseudomonas</i> (A) y bacterias totales (B) en sustrato de plantas jóvenes de arándano sometidas a dos tratamientos de riego: riego deficitario controlado severo (RDC sv) y riego deficitario controlado extremo (RDC ex), con y sin inoculación de <i>Pseudomonas koreensis</i> , en un huerto establecido en macetas. | 41 |
| Figure 9 | Peso seco de los órganos vegetativos de plantas jóvenes de arándano sometidas a tres tratamientos de riego: control (riego comercial) (A), riego deficitario controlado severo (RDC sv) (B) y riego deficitario controlado extremo (RDC ex) (C), con y sin inoculación de <i>Pseudomonas koreensis</i> , en un huerto establecido en macetas. | 42 |

RESUMEN

El estrés hídrico es uno de los principales factores que limita la productividad de cultivos frutales, especialmente en escenarios de creciente escasez de agua y altas temperaturas, por lo que resulta necesario evaluar estrategias que mitiguen sus efectos fisiológicos. El objetivo de este estudio fue evaluar la respuesta fisiológica de plantas jóvenes de arándano (*Vaccinium corymbosum* L. cv. *Legacy*) inoculadas con *Pseudomonas koreensis* sometidas a diferentes condiciones de riego durante una postcosecha bajo altas temperaturas. El ensayo se realizó en condiciones de invernadero bajo un diseño de parcelas divididas, considerando tratamientos de reposición hídrica equivalentes al 30% y 50% de la capacidad del contenedor entre eventos de riego, con y sin inoculación de *Pseudomonas koreensis* AG-97. Se registraron variables ambientales y fisiológicas, incluyendo temperatura del aire, humedad relativa, déficit de presión de vapor, potencial hídrico del tallo, conductancia estomática, eficiencia del fotosistema II, índice de reflectancia fotoquímica y contenido volumétrico de agua en el suelo. Los resultados mostraron diferencias significativas asociadas al tratamiento de riego, principalmente en el potencial hídrico del tallo y el contenido volumétrico de agua en el suelo. Asimismo, la inoculación con PGPR contribuyó a una mejor tolerancia al estrés hídrico, evidenciada por valores significativamente superiores de potencial hídrico del tallo en comparación con plantas no inoculadas. En conclusión, la inoculación con *Pseudomonas koreensis* AG-97 representa una alternativa prometedora para mitigar los efectos del déficit hídrico en el cultivo de arándano, aportando información relevante para optimizar su manejo bajo condiciones de disponibilidad limitada de agua.

SUMMARY

Water stress is one of the main factors limiting the productivity of fruit crops, particularly under scenarios of increasing water scarcity and high air temperature, highlighting the need to evaluate strategies that mitigate its physiological effects. The objective of this

study was to assess physiological responses of young blueberry plants to *Pseudomonas koreensis* inoculation under different irrigation regimes during postharvest under simulated warmer autumn conditions. The experiment was conducted under greenhouse conditions using a split-plot design, considering irrigation treatments equivalent to 30% and 50% of container capacity between irrigation events, with and without inoculation of *Pseudomonas koreensis* strain AG-97. Environmental and plant physiological variables were recorded, including air temperature, relative humidity, vapor pressure deficit, stem water potential, stomatal conductance, photosystem II efficiency, photochemical reflectance index, and volumetric soil water content. The results showed significant differences associated with irrigation treatments, mainly in stem water potential and volumetric soil water content. In addition, PGPR inoculation contributed to improved tolerance to water stress, evidenced by significantly higher stem water potential values compared to non-inoculated plants. In conclusion, inoculation with *Pseudomonas koreensis* AG-97 represents a promising alternative to mitigate the effects of water deficit in blueberry cultivation, providing relevant information for optimizing crop management under conditions of limited water availability.

CAPÍTULO 1. INTRODUCCIÓN GENERAL

El arándano alto (*Vaccinium corymbosum* L.), originario del hemisferio norte y perteneciente a la familia Ericácea, ha experimentado un notable crecimiento durante la última década (Yang et al., 2021). Actualmente, Chile ocupa el segundo lugar como exportador de arándanos en el hemisferio sur, con cerca de 72000 toneladas de fruta, y el cuarto puesto a nivel mundial de producción (ODEPA, 2025). El área productiva de este cultivo se ubica principalmente en la zona centro-sur del país, concentrándose más del 60% de la superficie plantada en las regiones del Maule y Ñuble (ODEPA, 2025).

Debido al cambio climático, el déficit hídrico se ha transformado en uno de los factores más limitantes para la seguridad alimentaria en zonas con clima Mediterráneo. En Chile, las proyecciones de modelos de cambio climático como el CMIP6, estiman que, en un escenario de altas emisiones de gases de efecto invernadero, las precipitaciones para fines de siglo disminuirían entre un 20% y un 40% (Salazar et al., 2024). El arándano es una especie particularmente sensible a la sequía debido a la ausencia de un sistema radical profundizador y a la falta de pelos radicales (Bryla & Strik, 2007), una alta sensibilidad estomática al estrés hídrico (Calderón-Orellana et al., 2023), y una mayor vulnerabilidad a cavitación (Améglio et al., 2000). Por ejemplo, la ocurrencia de un estrés hídrico moderado a severo por más de 21 días puede reducir la fotosíntesis de las plantas en más de un 30% (Rho et al., 2012). En plantas jóvenes de arándanos de los cultivares Blue Ribbon y Top Shelf, Calderón-Orellana et al., (2023), reportó que una caída de 0,5 MPa en el potencial hídrico del tallo en plantas hídricamente estresadas se asoció con una disminución de la conductancia estomática entre 38% y 58%, respectivamente.

Junto con la reducción en las precipitaciones, las proyecciones para la zona centro-sur de Chile anticipan un incremento sostenido de las temperaturas de hasta 4 °C (Bambach et al., 2022). Consecuentemente, los cultivos frutales no solo enfrentarían una exposición más prolongada al estrés hídrico, sino también a las altas temperaturas (Salazar et al., 2024), lo cual podría desplazar estas condiciones desfavorables hacia

etapas fenológicas en que usualmente las plantas están ambientalmente menos exigidas. En este contexto, resulta muy relevante estudiar en especies frutales caducifolias el efecto de los cambios climáticos proyectados no sólo para los meses de activo crecimiento vegetal, sino también para aquellos períodos de transición que preceden o suceden la endodormancia. Por ejemplo, en arándanos, las condiciones ambientales de postcosecha determinan la acumulación de reservas, el crecimiento de raíces, la diferenciación de yemas florales, el inicio de la senescencia foliar, y el desarrollo de mecanismos de aclimatación al frío invernal (Yang et al., 2021). La ocurrencia combinada de temperaturas elevadas y déficit hídrico durante esta etapa puede alterar sustancialmente dichos procesos, comprometiendo la entrada en dormancia y perjudicando la brotación, el crecimiento y el potencial productivo del cultivo (Luedeling et al., 2011). Debido a la variación ontogénica de la tolerancia al estrés hídrico (Cavender-Bares & Bazzaz, 2000), el impacto de condiciones climáticas adversas será mayor en plantas jóvenes de arándanos, pues su capacidad de resiliencia es menor debido: 1) una menor profundidad de su sistema radical que le permita buscar agua de reserva, 2) una menor capacidad de almacenamiento de carbohidratos que le permita soportar caídas en las tasas fotosintéticas debido al cierre estomático (Ru et al., 2024), 3) una menor altura y desarrollo de follaje que les permita disminuir su exposición a las altas temperaturas de un suelo expuesto a la radiación solar directa (Longstroth, 2012).

Frente a estas serias limitaciones ambientales, el uso de bioestimulantes en base a microorganismos ha aparecido como una herramienta promisorio que busca mejorar la tolerancia de los cultivos al estrés abiótico y reducir el uso de agroquímicos (Beltrán & Berlan, 2022). En arándanos, la mayor parte de los estudios en esta área se ha enfocado en la acción de los hongos micorrícicos arbusculares. Sin embargo, existe comparativamente escasa información con relación al potencial de las bacterias promotoras del crecimiento vegetal (PGPB) como inoculantes para el género *Vaccinium*. Dentro de las múltiples especies y cepas de PGPB, el género *Pseudomonas* destaca por combinar simultáneamente una elevada capacidad de

colonización radicular y múltiples mecanismos directos e indirectos de promoción del crecimiento y tolerancia al estrés. En particular, la especie *Pseudomonas koreensis* ha demostrado efectos positivos sobre el crecimiento y la tolerancia al estrés, mejorando el comportamiento fisiológico de plantas bajo condiciones ambientales adversas (Mohanty *et al.*, 2021; Sati *et al.*, 2023). En Chile, la cepa AG-97 de *Pseudomonas koreensis* ha mostrado efectos positivos sobre la tolerancia a sequía en portainjertos de cerezo, logrando disminuir la deshidratación de estos y la caída en sus potenciales hídricos del tallo después de varios días sin agua (Cruzat-Hermosilla *et al.*, 2026). No obstante, actualmente se desconoce si estos efectos pueden manifestarse en arándano durante el período postcosecha, una etapa clave para la acumulación de reservas, el crecimiento radical y la preparación para la dormancia. Considerando esta brecha de conocimiento, se desarrolló un estudio destinado a evaluar el efecto de la inoculación con la cepa AG-97 de *Pseudomonas koreensis* sobre la tolerancia a la sequía de plantas jóvenes de arándano durante el período de postcosecha bajo condiciones ambientales que simulan los otoños más cálidos y secos proyectados por el cambio climático.

HIPÓTESIS

La inoculación con la cepa AG-97 de la rizobacteria promotora del crecimiento vegetal *Pseudomonas koreensis* mejora el estado hídrico y la respuesta fisiológica de plantas jóvenes de arándano (*Vaccinium corymbosum* L. cv. Legacy) sometidas a déficit hídrico y altas temperaturas durante el período de postcosecha.

OBJETIVO GENERAL

Evaluar el efecto de la inoculación con la cepa AG-97 de *Pseudomonas koreensis* sobre el estado hídrico, la respuesta fisiológica y el crecimiento de plantas jóvenes de arándano (*Vaccinium corymbosum* L. cv. Legacy) sometidas a diferentes niveles de disponibilidad hídrica y altas temperaturas durante el período de postcosecha.

OBJETIVOS ESPECÍFICOS

- Determinar el efecto de la inoculación sobre el estado hídrico de las plantas sometidas a distintos niveles de riego, mediante la evaluación del potencial hídrico del tallo y del contenido volumétrico de agua en el sustrato.
- Analizar el efecto de la inoculación sobre variables fisiológicas asociadas a la tolerancia al déficit hídrico, incluyendo la conductancia estomática, la eficiencia fotoquímica máxima del fotosistema II (Fv/Fm) y el contenido relativo de flavonoides en las hojas (PRI).
- Cuantificar la persistencia en la rizósfera de la cepa AG-97 de *Pseudomonas koreensis* en plantas jóvenes sometidas a distintos regímenes de riego.
- Evaluar el impacto de la inoculación en la acumulación de biomasa seca en hojas, raíces y tallos de plantas de arándano bajo distintos regímenes de riego.

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CAPÍTULO 2

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Physiological responses of young blueberry plants to *Pseudomonas koreensis* inoculation subjected to water deficit during postharvest under simulated warmer autumn conditions

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Keywords: Deficit irrigation; *Vaccinium corymbosum* L.; stem water potential; water stress; plant growth-promoting bacteria; PGPR; water use efficiency; ecological memory; drought resilience.

Abstract:

Climate change is increasing the frequency of combined drought and heat stress events in Mediterranean fruit-growing regions, highlighting the need for sustainable strategies to improve crop resilience. This study evaluated the effect of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas koreensis* AG-97 on the

physiological responses of one-year-old blueberry plants (*Vaccinium corymbosum* L. cv. Legacy) subjected to regulated deficit irrigation during postharvest under simulated warmer autumn conditions. Plants were exposed to three irrigation regimes corresponding to 50, 37.5, and $\leq 25\%$ of substrate container capacity, with or without bacterial inoculation. Under deficit irrigation, inoculated plants maintained stem water potentials approximately 0.2 MPa higher than those of non-inoculated plants and remained above the critical threshold of -1.2 MPa during periods of maximum stress. This improved water status was accompanied by greater substrate water content and significantly higher root biomass, which increased by 49% and 68% under severe and extreme deficit irrigation, respectively. In contrast, stomatal conductance did not differ significantly between inoculation treatments, indicating that improved plant water status was not associated with stronger stomatal regulation. Inoculated plants also tended to maintain higher PRI and Fv/Fm values under water deficit, suggesting improved maintenance of photosynthetic performance. These results indicate that *P. koreensis* AG-97 enhanced drought tolerance primarily through root-rhizosphere processes that improved water acquisition and retention. The use of microbial inoculation may therefore represent a promising strategy to improve the resilience of young blueberry plants to the warmer and drier autumn conditions projected for Mediterranean-climate production systems.

Introduction

The highbush blueberry (*Vaccinium corymbosum* L.), a fruit crop native to the Northern Hemisphere and belonging to the Ericaceae family, has experienced remarkable growth over the past decade (Retamales & Hancock, 2024). Currently, Chile ranks second among blueberry exporters in the Southern Hemisphere, with nearly 72,000 metric tons of fruit, and fourth globally in terms of production (ODEPA, 2025). The production area for this crop is located primarily in the south-central part of the country, with more than 60% of the planted area concentrated in the Maule and Ñuble regions (ODEPA, 2025).

Due to climate change, water scarcity has become one of the most limiting factors for food security in areas with a Mediterranean climate. In Chile, projections from climate change models such as CMIP6 estimate that, under a high greenhouse gas emissions scenario, precipitation by the end of the century would decrease by between 20% and 40% (Salazar et al., 2024). Blueberries are a species particularly sensitive to drought due to the absence of a deep-rooting system and the lack of root hairs (Bryla & Strik, 2007), high stomatal sensitivity to water stress (Calderón-Orellana et al., 2023), and greater vulnerability to cavitation (Améglio et al., 2000). For example, moderate to severe water stress, lasting more than 21 days, can reduce plant photosynthesis by more than 30% (Rho et al., 2012). In young blueberry plants of the Blue Ribbon and Top Shelf cultivars, Calderón-Orellana et al. (2023) reported that a 0.5 MPa drop in stem water potential in water-stressed plants was associated with a decrease in stomatal conductance of between 38% and 58%, respectively.

Along with reduced precipitation, projections for south-central Chile anticipate a sustained increase in temperatures of up to 4 °C (Bambach et al., 2022). Consequently, fruit crops would not only face prolonged exposure to water stress but also to high temperatures (Salazar et al., 2024), which could shift these unfavorable conditions to phenological stages when plants are typically under less environmental stress. In this context, it is highly relevant to study the effect of projected climate changes on deciduous fruit species, not only during months of active plant growth but also during the transitional periods preceding or following endodormancy. For example, in blueberries, environmental conditions before the onset of endodormancy determine the accumulation of carbohydrate reserves, root growth, floral bud differentiation, the onset of leaf senescence, and the development of mechanisms for acclimation to winter cold (Yang et al., 2021). The combination of high temperatures and severe water deficits during this stage can substantially alter these processes, compromising the onset of dormancy and impairing budbreak, growth, and the potential productivity of the crop (Luedeling et al., 2011). Given the ontogenetic variation in water stress tolerance (Cavender-Bares & Bazzaz, 2000), adverse weather conditions will have a greater

impact on young blueberry plants, as their resilience is lower due to: First, the shallower root system limits their ability to access water reserves. Second, the lower carbohydrate storage capacity limits their ability to withstand declines in photosynthetic rates due to stomatal closure (Ru et al., 2024). Third, the shorter height and less developed foliage limits their ability to reduce exposure to high soil temperatures caused by direct solar radiation (Longstroth, 2012).

Considering these significant environmental limitations, the use of microorganism-based biostimulants has emerged as a promising solution. These biostimulants can enhance crop resilience to abiotic stress and minimize the reliance on agrochemicals (Beltrán & Berlan, 2022). In blueberries, most studies in this area have focused on the action of mycorrhizal fungi (Yang et al., 2020; Gui et al., 2020; O'Neil et al., 2023). However, there is comparatively little information regarding the potential of plant growth-promoting bacteria (PGPB) as an inoculant for the genus *Vaccinium*. Because blueberry plants rely heavily on mycorrhizal associations for nutrient acquisition, the incorporation of PGPB may provide an additional advantage by acting synergistically with native or introduced mycorrhizal fungi, thereby improving root development, biomass accumulation, and tolerance to abiotic stress (Raklami et al., 2019; Fransgo et al., 2024). Among the many species and strains of PGPB, the genus *Pseudomonas* stands out for its ability to colonize roots and promote growth and stress tolerance through multiple mechanisms. In particular, *Pseudomonas koreensis* has demonstrated positive effects on growth and stress tolerance, improving the physiological performance of plants under adverse environmental conditions (Mohanty et al., 2021; Sati et al., 2023). In Chile, the AG-97 strain of *Pseudomonas koreensis* has demonstrated positive outcomes in enhancing drought tolerance in cherry rootstocks, reducing their dehydration and the decline in stem water potential after several days without water (Cruzat et al., 2026). However, the potential for these effects to occur in blueberry plants during the postharvest period remains to be elucidated. A study was conducted under greenhouse conditions to evaluate the effect of inoculation with the AG-97 strain of *Pseudomonas koreensis* on the drought tolerance of young

blueberry plants during the postharvest period. The study was conducted under environmental conditions that simulate the warmer and drier autumns projected by climate change.

Materials and methods

Study site

The experiment was conducted in a greenhouse at the Department of Plant Production, Faculty of Agronomy, University of Concepción, Chile (36°35'50" S, 72°05'02" W). The greenhouse covered 40 m² and had a transparent glass roof, north–south orientation, and lateral ventilation, without active climate control. Plants were placed on 1-m-high metal mesh benches to ensure adequate drainage.

Photosynthetically active radiation (PAR) was measured with a quantum sensor (LI-190R, LI-COR Biosciences, Lincoln, NE, USA). Air temperature and relative humidity were measured with a temperature and humidity probe (HMP60, Vaisala, Helsinki, Finland) installed 2 m above ground level. Measurements were collected every 5 min and stored as 15-min averages using a CR300 datalogger (Campbell Scientific Inc., Logan, UT, USA). Air vapor pressure deficit (VPD_{air}) was calculated from air temperature and relative humidity according to Allen et al. (1998).

The experiment was conducted from January 24th to May 3rd, 2024. One-year-old highbush blueberry plants (*Vaccinium corymbosum* L. cv. 'Legacy') were grown in 1.5-L pots filled with a substrate consisting of 70% coconut fiber and 30% sandy mineral soil (v/v). Prior to treatment imposition, plants were maintained in a nursery under a high-density polyethylene shade net providing 50% light reduction and irrigated with micro-sprinklers (30 L h⁻¹ emitters). This period allowed plant establishment and minimized transplant-related water and radiative stress.

On 6 February 2024, plants were inoculated with the AG-97 strain of *Pseudomonas koreensis*, by applying 10 mL of bacterial suspension at a concentration of 10⁶ colony-forming units (CFU) mL⁻¹. Before inoculation, all pots were irrigated to container capacity to facilitate bacterial movement and colonization of the rhizosphere. Following

inoculation, plants remained in the nursery until 19 March 2024 to allow bacterial establishment and plant acclimation.

On 19 March 2024, plants were transferred to the greenhouse, where they were exposed to air temperatures averaging approximately 6 °C above ambient outdoor conditions. This temperature regime was used to simulate the warmer autumn conditions projected for central-southern Chile under future climate scenarios. During this period, plants were irrigated with 0.15 L plant⁻¹ three times per week. This irrigation volume replenished approximately 30–50% of the water depleted from container capacity between irrigation events. The substrate's container capacity was previously determined in the laboratory as a volumetric water content (θ_v) of 0.4 m³ m⁻³.

Experimental Design and Treatments

Three irrigation treatments were evaluated. The Control treatment represented the commercial irrigation condition and maintained substrate volumetric water content (θ_v) at approximately 0.20 m³ m⁻³, equivalent to 50% of container capacity, throughout the experimental period. In the RDI-Severe treatment, substrate water content was maintained between 80 and 100% of container capacity before treatment imposition. From late March onwards, irrigation was adjusted to maintain θ_v at approximately 0.15 m³ m⁻³. In the RDI-Extreme treatment, substrate water content was also maintained between 80 and 100% of container capacity before treatment imposition. Thereafter, irrigation was restricted to maintain θ_v at ≤ 0.10 m³ m⁻³, a value close to the permanent wilting point of the substrate. Irrigation treatments were imposed from late March until the end of the experiment and were managed by adjusting irrigation frequency and volume according to substrate volumetric water content measurements. The split-plot factor corresponded to the inoculation treatment with the bacteria *Pseudomonas koreensis* AG-97, comparing plants with and without the application. The experimental unit consisted of two plants subjected to the same combination of irrigation and inoculation treatments; consequently, 12 plants were established in each of the three blocks, distributed according to the experimental design. Substrate volumetric water content (θ_v) was monitored throughout the experiment using a capacitance sensor

(GS1, Decagon Devices, Pullman, WA, USA). The sensor was inserted approximately 5 cm below the substrate surface and 15 cm from the plant stem in one pot per experimental unit.

Physiological Measurements

Physiological measurements were performed on non-fruiting plants during solar noon (12:00–15:30 h), when plant water stress was expected to be maximal. Stem water potential (Ψ_{stem}) was measured using a pressure chamber (Model 615, PMS Instrument Company, Albany, OR, USA) following the protocol described by Calderón-Orellana et al. (2023). Prior to measurement, selected leaves were enclosed in opaque, airtight plastic bags for at least 40 min to allow equilibration between the leaf and stem water potential.

Stomatal conductance (gs) was measured using a steady-state porometer (LI-600, LI-COR Biosciences, Lincoln, NE, USA). Measurements were taken on three fully expanded, sun-exposed leaves per plant, and values were subsequently averaged.

Maximum photochemical efficiency of photosystem II (Fv/Fm) was used as an indicator of photoinhibition and measured with a portable chlorophyll fluorometer (Pocket PEA, Hansatech Instruments, King's Lynn, UK). Measurements were performed on fully exposed leaves after a dark-adaptation period of at least 40 min using leaf clips.

Relative flavonoid content was estimated using the Photochemical Reflectance Index (PRI), which was used as an indicator of plant physiological response to abiotic stress. Measurements were obtained with a reflectance meter (PlantPen PRI 210, Photon Systems Instruments, Drásov, Czech Republic) on two basal leaves per plant at the beginning of the experiment and one day before harvest.

Bacterial inoculum preparation and population counts

The *Pseudomonas koreensis* AG-97 strain was obtained from the collection of the Agricultural Microbiology Laboratory at the Faculty of Agronomy. The bacteria were reactivated from cryopreservation at $-80\text{ }^{\circ}\text{C}$ and cultured in standard Nutrient Broth

(Merck) at 25 °C under constant orbital shaking at 150 rpm. After 30 hours of incubation, the optical density (OD) was measured at 600 nm (OD₆₀₀); the culture was then adjusted in a 1 % sucrose solution to reach an OD₆₀₀ of 0.1, equivalent to an approximate concentration of 1×10^7 CFU mL⁻¹.

Substrate samples (~1 g) were collected from the root zone of the potted plants. Samples were obtained under all treatment combinations of irrigation and inoculation following the application of the irrigation protocols; samples were placed in sterile plastic bags and maintained under refrigerated conditions until laboratory processing. Each 1 g substrate sample was suspended in 10 mL of sterile saline solution (0.89% NaCl) and subjected to decimal serial dilutions up to 10⁻³. Total bacteria populations were quantified using the surface plating method by spreading 100 µL aliquots of each dilution onto plate count agar media under aseptic conditions. For the quantification of *Pseudomonas* spp., dilutions were spread onto King B agar (2% protease-peptone, 1.5% agar, 8,614 mM K₂HPO₄·3H₂O, 6.08 mM MgSO₄·7H₂O and 1% glycerol, adjusted to pH 7.2). Plates were incubated at 25 ± 2 °C in darkness for 48 hours before colony counting. For the *Pseudomonas* spp. counts on King B agar, only green-fluorescent colonies visualized under a black light, were registered

Biomass Determination

At the end of the experimental period, each unit was harvested and separated into roots, stems, and aerial parts. Each fraction was weighed fresh and subsequently dried in a convection oven at 60 °C for at least 24 hours, or until a constant weight was achieved, to determine the total dry matter.

Statistical Analysis

The resulting data were subjected to an analysis of variance (ANOVA). Prior to the analysis, the assumptions of normality of residuals (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were verified. Mean differences were determined using the Least Significant Difference (LSD) test with a significance level of $p \leq 0.05$. All statistical procedures were conducted using SAS Studio software (SAS Institute Inc., Cary, NC, USA)

Results

Environmental Conditions and Characterization of Irrigation

During the period between March and April, the overall vapor pressure deficit (VPD) in the greenhouse (2.5–6.5 kPa) was 72 % higher than that of the outdoor environment (1.5–4.0 kPa) (Figure 1). Greenhouse VPD consistently exceeded outdoor values, exhibiting broader daily fluctuations and peaks near 6.5 kPa, while outdoor values remained mostly between 1.5 and 4.0 kPa. At the start of the evaluation period (mid-March), greenhouse VPD ranged from 3.5 to 4.5 kPa, increasing progressively toward early April. Specific episodes of elevated VPD were recorded in the greenhouse, particularly between April 8 and 18, where the period's maximum values occurred. In contrast, outdoor VPD showed only slight changes with moderate increases and lower variation. By late April, both environments showed a decreasing trend in VPD, though the gap between the greenhouse and the exterior remained constant at approximately 2 kPa.

Throughout the experimental period, maximum air temperature inside the greenhouse consistently exceeded that recorded under outdoor conditions (Figure 2). Mean maximum air temperature reached 34.9 °C inside the greenhouse compared with 28.3 °C outdoors, representing an average increase of 6.6 °C (23.2%) under greenhouse conditions. The highest temperatures were recorded during early April, when air temperature inside the greenhouse exceeded 40 °C, whereas outdoor maximum temperature remained below 35.5 °C. The temporal dynamics of both environments were similar; however, the greenhouse consistently amplified daytime temperature, resulting in a sustained increase in thermal load throughout the experimental period.

Photosynthetically active solar radiation (PPFD) exhibited similar temporal dynamics inside and outside the greenhouse throughout the experimental period (Figure 3). However, PPFD intensity was consistently lower under greenhouse conditions. Mean incident PPFD reached 374 $\mu\text{mol m}^{-2} \text{s}^{-1}$ inside the greenhouse and 607 $\mu\text{mol m}^{-2} \text{s}^{-1}$ outdoors, representing an average reduction of 233 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (38.4%) due to the

greenhouse glass cover. Peak PPFD values were recorded during late March and early April, exceeding $790 \mu\text{mol m}^{-2} \text{s}^{-1}$ outdoors, whereas maximum values inside the greenhouse remained below $670 \mu\text{mol m}^{-2} \text{s}^{-1}$. Although both environments showed a similar temporal pattern, PPFD values progressively declined toward the end of April while the greenhouse consistently attenuated incident radiation throughout the study period.

Substrate Water Content Dynamics

Substrate volumetric water content (θ_v) closely followed the irrigation regimes imposed during the experiment (Figure 4). Before the application of the irrigation treatment, θ_v remained near container capacity in all treatments, ranging from approximately 0.30 to $0.45 \text{ m}^3 \text{ m}^{-3}$. Following the onset of irrigation treatments on March 10th, clear differences among irrigation regimes were established and maintained throughout the experimental period. The Control treatment exhibited the highest θ_v values, whereas RDI-Severe and RDI-Extreme progressively declined to approximately 0.15 – 0.20 and $\leq 0.10 \text{ m}^3 \text{ m}^{-3}$, respectively. After inoculation, AG-97-inoculated plants generally maintained greater θ_v than non-inoculated plants within each irrigation regime. This trend became apparent shortly after water restriction began and persisted throughout most of the experiment. In April, all treatments were rehydrated to restore substrate water content close to container capacity, resulting in a rapid increase in θ_v across irrigation treatments.

Stem Water Potential (SWP)

Under the Control treatment (Figure 5A), Ψ_{stem} remained between -0.80 and -0.45 MPa throughout the experiment, whereas baseline plants ranged between -0.70 and -0.30 MPa. Significant differences between inoculated and non-inoculated plants were detected on 21 March and 3 May. On these dates, AG-97-inoculated plants exhibited Ψ_{stem} values that were approximately 0.20 and 0.15 MPa higher than those of non-inoculated plants, respectively. Throughout the experiment, both inoculation treatments

remained relatively close to the baseline value, although non-inoculated plants generally showed larger departures from the baseline.

Under the RDI-Severe treatment (Figure 5B), Ψ_{stem} progressively declined following water restriction, reaching values close to -1.0 MPa on 23 April. Differences between inoculated and non-inoculated plants ranged from approximately 0.10 to 0.25 MPa during the restriction period, with the largest separation occurring on 23 April. Following the rehydration event in late April, Ψ_{stem} increased in both treatments and differences between inoculated and non-inoculated plants were no significant. Relative to the baseline treatment, non-inoculated plants deviated by as much as 0.45 MPa, whereas inoculated plants generally remained within 0.20–0.30 MPa of the baseline.

The strongest inoculation effect was observed under RDI-Extreme (Figure 5C). In this treatment, significant differences between inoculation treatments were detected on 21 March, 23 April, 2 May, and 3 May. The magnitude of these differences ranged from approximately 0.18 to 0.30 MPa, with inoculated plants consistently maintaining higher Ψ_{stem} values than non-inoculated plants. Across all evaluation dates under severe and extreme water restriction, differences between inoculation treatments were generally close to 0.2 MPa. Relative to the baseline condition, non-inoculated plants frequently exhibited deviations of 0.40–0.60 MPa during the peak of water restriction, whereas AG-97-inoculated plants remained substantially closer to baseline values.

Physiological Stress Responses

Regardless of the irrigation strategy, g_s values ranged from approximately 15 to 105 $\text{mmol m}^{-2} \text{s}^{-1}$ throughout the study (Figures 6A, 6B, and 6C). Despite AG-97-inoculated plants frequently exhibiting slightly higher g_s values than non-inoculated plants (between 10 and 30 $\text{mmol m}^{-2} \text{s}^{-1}$) under the Control and Severe RDI treatments (Figures 6A and 6B), there were no significant differences in g_s between inoculation treatments. Under RDI-Extreme irrigation (Figure 6C), no clear trend emerged among the inoculation treatments.

The PRI estimates progressively decreased from values near zero to minimum values close to -0.2 across all irrigation strategies. Under control irrigation (Figure 7A), the PRI values of inoculated and non-inoculated plants tended to be similar until the end of the study. However, the inoculation induced changes in PRI a couple of times, but they were inconsistent. On one measurement date in February, the inoculated plants showed slightly lower PRI values, whereas in April, the inoculated plants showed higher PRI values than the control plants. Under deficit irrigation treatments (Figure 7B and 7C), the PRI tended to be higher in inoculated plants. The difference was significant in two and four instances in plants subjected to severe and extreme RDI, respectively.

The F_v/F_m remained within a narrow range (0.70–0.85) throughout the experiment (data not shown). No significant effects of inoculation were detected under the Control treatment. Under deficit irrigation, inoculated plants tended to maintain slightly higher F_v/F_m values than non-inoculated plants, although significant differences were infrequent. In RDI-Extreme, inoculated plants exhibited lower F_v/F_m values shortly after rehydration, but both treatments showed similar values at the final evaluation (data not shown).

Bacterial populations

Total culturable bacterial counts in the substrate were not significantly affected by either irrigation treatments or inoculation with *Pseudomonas koreensis* AG-97, indicating that inoculation did not alter the overall culturable bacterial community (Figure 8A). Across all irrigation treatments, bacterial populations remained relatively stable, ranging from 6.7 to 7.3 log CFU g⁻¹ substrate. Regarding *Pseudomonas* spp. colony counts, populations were below the detection limit under control irrigation (CI) in both inoculated and uninoculated treatments. In contrast, colonies were successfully quantified in treatments subjected to deficit irrigation (RDI sv and RDI ext). Under both deficit irrigation conditions inoculation had a significant effect on *Pseudomonas* spp. populations ($P < 0.05$), reaching 6.6 log CFU g⁻¹ substrate under RDI sv and 5.7 log CFU g⁻¹ substrate under RDI ext, whereas uninoculated plants exhibited lower populations (approximately 1.9 and 1.1 log CFU g⁻¹ substrate, respectively (Figure 8

B). These results demonstrate that *P. koreensis* AG-97 successfully established and persisted in the substrate under both deficit irrigation regimes without affecting the total culturable bacterial population.

Biomass Accumulation

Dry weight partitioning varied among irrigation treatments and plant organs (Figures 9A, 9B, and 9C). Under the control irrigation treatment, roots accounted for the largest proportion of total biomass, followed by stems and leaves (Figure 9A). Significant differences were detected only in stem biomass between inoculation treatments, where AG-97-inoculated plants accumulated approximately 45% more dry matter than non-inoculated plants. No significant differences were observed in leaf or root biomass. A similar distribution pattern was observed under severe and extreme RDI (Figures 9B and 9C), with roots representing the largest biomass fraction. Compared to the control irrigation treatment, root biomass was reduced in non-inoculated plants under both deficit irrigation treatments, whereas AG-97-inoculated plants-maintained values that were similar to those observed under full irrigation. Within these irrigation regimes, root biomass was the only plant organ affected by inoculation. Thus, AG-97-inoculated plants accumulated approximately 49% and 68% more root dry matter than non-inoculated plants, respectively.

Discussion

The most consistent effect of AG-97 inoculation was the maintenance of higher plant water status under deficit irrigation. This response was clearer as the restriction became more severe as the season progressed. During much of the drought period, inoculated plants kept values of stem water potential that were approximately 0.2 MPa higher than those of non-inoculated plants. This effect was particularly evident under the Extreme RDI, where inoculated plants remained substantially closer to the well-watered baseline for blueberry plants (Calderón-Orellana et al., 2023). Maintaining blueberry plants above a stem water potential threshold of -1.2 MPa appears to be physiologically important for sustaining normal plant function under drought conditions

(Bryla and Strik, 2004). In young highbush blueberry plants, reductions in stem water potential below -1.0 MPa have been associated with substantial declines in stomatal conductance. A decrease of only 0.5 MPa has been shown to result in reductions of 38–58% in g_s , depending on cultivar (Calderón-Orellana et al., 2023). Such responses suggest that minor changes in plant water status can significantly impact gas exchange. Earlier studies in rabbiteye blueberry also demonstrated that moderate and severe water stress, characterized by reductions in stomatal conductance, significantly reduced leaf expansion and plant growth, highlighting the sensitivity of blueberry to declining water availability (Davies and Johnson, 1982). Because root elongation and vegetative growth depend on turgor-driven cell expansion, maintaining stem water potentials above values associated with severe stomatal limitation may be particularly important during postharvest, when young blueberry plants continue accumulating reserves, expanding roots, and preparing for dormancy. Under these conditions, the ability of inoculated plants to maintain a plant water status consistently above those recorded in non-inoculated controls suggests a potentially meaningful reduction in physiological stress.

In contrast, no significant differences in stomatal conductance were detected between inoculation treatments under any irrigation regime. Furthermore, inoculated plants generally exhibited similar or slightly greater stomatal conductance than non-inoculated plants. The lack of response in stomatal conductance, coupled with overall values ($<100 \text{ mmol m}^{-2}\text{s}^{-1}$), was likely driven by the extreme atmospheric demand ($\text{VPD} > 6 \text{ kPa}$). This severe evaporative demand imposed a physiological limit on the gas exchange capacity of leaves, which may have hidden statistical differences. These results suggest that maintaining higher stem water potential in AG-97-inoculated blueberry plants was not achieved through greater stomatal closure, disagreeing with previous studies, in which inoculating sweet cherry rootstocks with the same strain of *Pseudomonas koreensis* induced improvements in stomatal conductance (Cruzat et al., 2026).

Therefore, the improved water status of inoculated, deficit-irrigated plants appears to be associated with enhanced water acquisition and retention in the effective rooting zone. First, AG-97-inoculated plants consistently maintained a slightly higher substrate water content after irrigation restrictions were imposed. Several species of *Pseudomonas* can produce extracellular polysaccharides (EPS), which improve soil aggregation, biofilm formation, and water retention around roots (Carezzano et al., 2023; Zboralski & Fillion, 2023). Production of EPS has been proposed as an important mechanism by which PGPR improve plant performance under drought stress, creating a more favorable hydraulic environment in the rhizosphere (Carezzano et al., 2023). Second, inoculated plants exhibited significantly higher root biomass under both RDI-Severe and RDI-Extreme conditions. These responses are consistent with the known behavior of plant-growth-promoting *Pseudomonas*, which often promotes root development by synthesizing or stimulating auxin-related pathways (Uzma et al., 2022). Although osmotic adjustment was not directly evaluated in the present study, previous work has shown that *Pseudomonas koreensis* can increase the accumulation of stress-related metabolites, including proline and other amino acids involved in osmotic regulation (Guo et al., 2021). Because proline plays a central role in osmotic adjustment, maintenance of cell turgor, and protection of cellular structures under water deficit, these findings suggest that part of the improved plant water status observed in AG-97-inoculated plants may have been associated with enhanced osmotic regulation. However, further studies quantifying leaf osmotic potential and osmolyte accumulation are required to confirm this mechanism in blueberry. Enhanced root growth improves soil exploration and may induce greater water uptake when irrigation water becomes limited. This response is particularly relevant for avoiding drought in blueberries, which have shallow and relatively inefficient root systems (Bryla and Strik, 2004).

Interestingly, AG-97 altered biomass partitioning according to water availability. Under well-watered conditions, inoculated plants accumulated significantly more stem biomass, whereas under both deficit irrigation treatments root biomass was the only organ that increased significantly. These results suggest that AG-97 promoted growth

under all irrigation regimes, but drought shifted biomass allocation towards roots in inoculated plants. Such a response may have increased the capacity of AG-97 inoculated plants to acquire water and contributed to the maintenance of higher stem water potentials under deficit irrigation.

Although *Fv/Fm* and PRI responded differently, PRI and, to a lesser extent, *Fv/Fm* suggest that AG-97 contributed to maintaining photosynthetic function under water-limited conditions. Differences in PRI became more frequent as drought severity increased, particularly under RDI-Extreme, where inoculated plants generally exhibited higher values than non-inoculated controls. In contrast, changes in *Fv/Fm* were small and significant differences were uncommon, although inoculated plants typically maintained equal or slightly greater values throughout the drought period. While PRI is associated with photosynthetic radiation-use efficiency and light-energy partitioning, *Fv/Fm* reflects the maximum photochemical efficiency of photosystem II (Gamon et al., 1992; Maxwell & Johnson, 2000). Together, these responses indicate that AG-97 helped maintain photosynthetic performance during postharvest under restricted water availability and elevated autumn temperatures. Similar effects have been reported for *Pseudomonas* spp., where inoculation improved stress tolerance through the preservation of photosynthetic structures and enhanced antioxidant activity (Guo et al., 2021; Pravisya et al., 2019).

These physiological responses are of key importance for reasons that extend beyond drought tolerance itself. The analysis of the climatic conditions imposed during the study confirms that plants were exposed to an environment characterized by high atmospheric evaporative demand, consistent with the warmer and drier autumn conditions projected for Mediterranean-climate regions under future climate change scenarios. Vapor pressure deficit inside the greenhouse frequently exceeded 6 kPa and remained consistently higher than under outdoor conditions, indicating a substantial increase in atmospheric water demand. At the same time, the glass cover reduced incident photosynthetically active radiation (PAR), reducing the likelihood of severe photoinhibition. Previous studies in young blueberry plants have shown that

protected cultivation reduces radiation load, delays the onset of water stress, and helps maintain higher photosystem II efficiency under drought conditions (Calderón-Orellana et al., 2023). This explains why F_v/F_m values were between 0.7 and 0.8 for the whole experiment, despite the influence of high temperatures. Therefore, the environmental conditions generated during the experiment provided a suitable framework to evaluate the effects of combined thermal and water stress while minimizing the confounding influence of excessive solar radiation.

In this context, climate projections for Chile and other regions with a Mediterranean climate consistently indicate rising air temperatures, decreasing precipitation, and greater climatic aridity in the upcoming decades. In these scenarios, autumn conditions, like the environmental conditions established in this study, are expected to become progressively warmer and drier. This projection indicates that deciduous fruit species will face severe climatic constraints during developmental stages that have historically been considered transitional rather than stressful. Therefore, the ability of AG-97-inoculated blueberry plants to maintain higher stem water potential, greater root growth, and stabler physiological performance under drought conditions may be increasingly important as climate change progresses in Mediterranean production systems.

This aspect is particularly important because postharvest represents a critical developmental stage in blueberry. During this period, plants accumulate reserves, maintain root growth, initiate floral bud differentiation, and undergo the physiological adjustments associated with dormancy establishment (Yang et al., 2021). Previous evidence suggests that blueberry plants have a relatively limited capacity to accumulate carbohydrate reserves and depends strongly on current carbon assimilation to sustain growth and development (Petridis et al., 2018). In the present study, stomatal conductance remained relatively low throughout much of the experimental period ($<100 \text{ mmol m}^{-2}\text{s}^{-1}$), frequently remaining below values commonly reported for actively growing blueberry plants. These results suggest elevated temperatures may have imposed physiological constraints on carbon acquisition,

regardless of the irrigation strategy. Under such conditions, even modest improvements in photochemical performance may contribute to maintaining physiological activity during a developmental stage that ultimately determines subsequent vegetative development and orchard establishment.

The microbiological results further highlight the critical role of root-rhizosphere interactions in understanding plant responses. At the end of the experiment, *Pseudomonas* populations were not recovered from the well-watered treatment, suggesting that these environmental conditions did not favor the persistence of this bacterial group. In contrast, both deficit irrigation treatments supported detectable populations, and inoculated plants exhibited significantly greater colony-forming unit counts than non-inoculated controls. Similar responses have been observed in sweet cherry and plum production systems subjected to regulated deficit irrigation, where water limitation was associated with greater abundance of PGPR populations and bacterial communities adapted to drought conditions (Alvear et al., 2025; Calderón-Orellana et al., 2025). These results suggest that irrigation regime influenced bacterial persistence and establishment in association with the plant rhizosphere. The recovery of bacterial populations under deficit irrigation, together with the higher colony-forming unit counts observed in inoculated plants, suggests that water limitation may have favored the persistence of the AG-97 strain. However, further studies are needed to determine whether these responses are related to bacterial adaptation to water-limited conditions

Conclusions

Inoculation with the AG-97 strain of *Pseudomonas koreensis* improved the drought tolerance of young blueberry plants during postharvest under environmental conditions simulating warmer and drier autumns. The most consistent response was the maintenance of a better plant water status, with inoculated plants exhibiting stem water potentials approximately 0.2 MPa higher than non-inoculated plants under deficit irrigation. This response was accompanied by greater substrate water content and

significantly higher root biomass under both RDI-Severe and RDI-Extreme treatments. In contrast, no significant differences in stomatal conductance were detected, indicating that the improved water status was not associated with stronger stomatal regulation. Physiological responses related to photosynthetic performance were generally modest, although inoculated plants tended to maintain higher PRI values under severe water deficit and slightly greater Fv/Fm values throughout much of the experiment. The greater abundance of *Pseudomonas* populations under deficit irrigation, together with the enhancement of root growth and plant water status, suggests that AG-97 improved drought tolerance primarily through root-rhizosphere processes that enhanced water acquisition and retention. These findings indicate that *P. koreensis* AG-97 may represent a promising biological tool to improve the resilience of young blueberry plants to the warmer and drier autumn conditions projected for Mediterranean-climate production systems.

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Figures

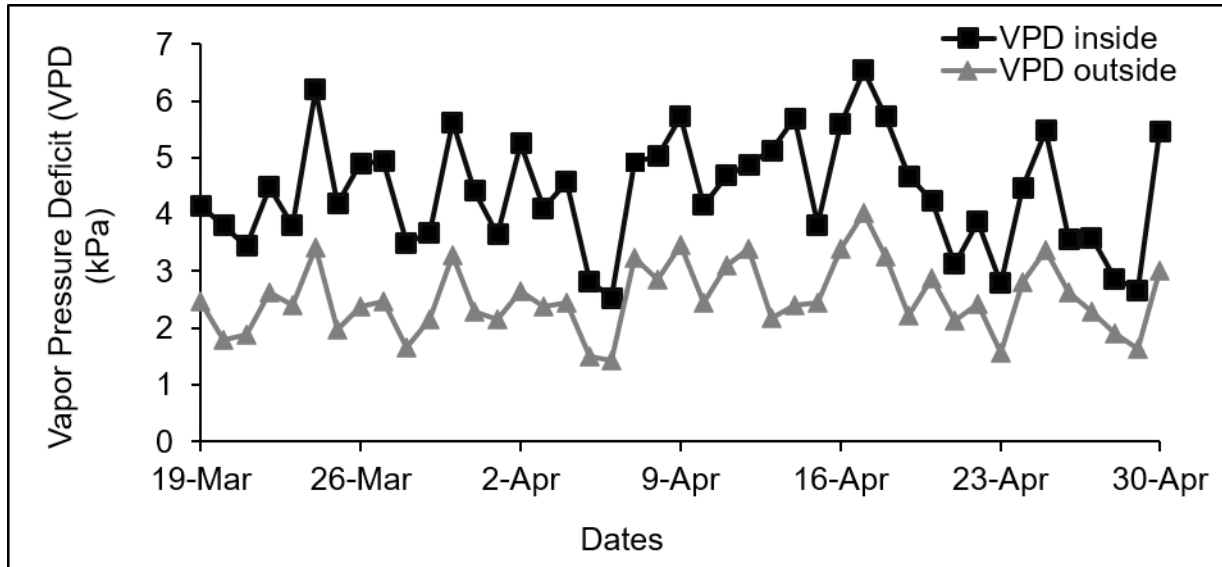


Figure 1. Diurnal averages of air vapor pressure deficit (VPD) outdoors and inside the greenhouse during an experiment with potted young blueberry plants (cv. Legacy) subjected to three irrigation treatments: Control (commercial irrigation) (A), Severe RDI (severe regulated deficit irrigation) (B), and Extreme RDI (extreme regulated deficit irrigation) (C). The experiment included two inoculation treatments (*Pseudomonas koreensis* AG-97 and a non-inoculated control). Each data point represents the daily average VPD estimated between 12:00 and 15:00 h.

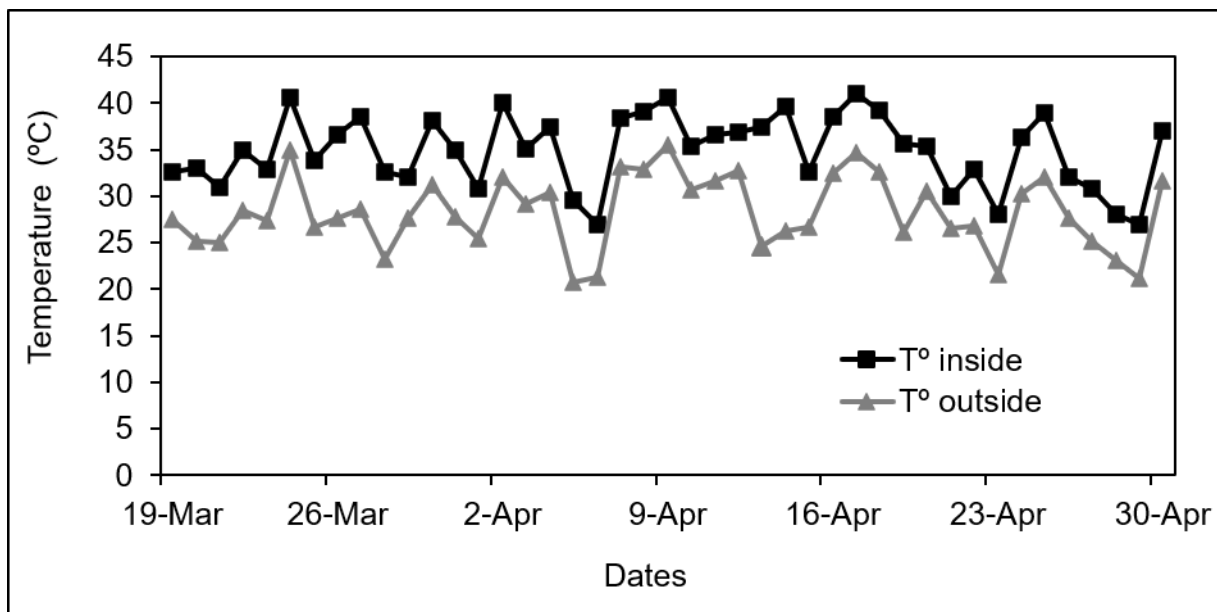


Figure 2. Diurnal values of temperature (T°) outdoors and inside the greenhouse during an experiment with potted young blueberry plants (cv. Legacy) subjected to three irrigation treatments: (Control: Commercial irrigation (A); Severe RDI: Severe regulated deficit irrigation (B); Extreme RDI: Extreme regulated deficit irrigation (C)) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control.

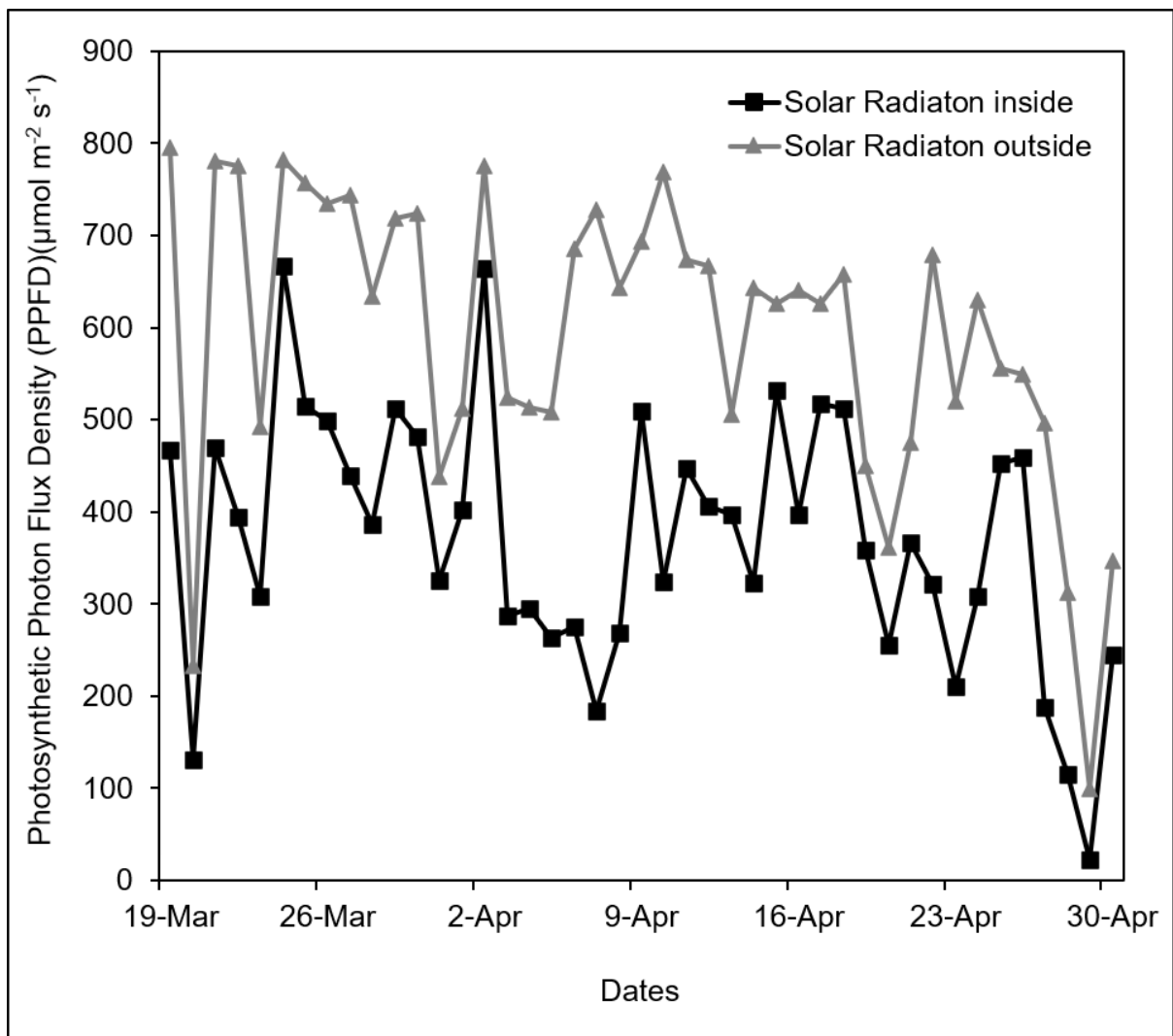


Figure 3. Diurnal values of photosynthetically active solar radiation outdoors and inside the greenhouse during an experiment with potted young blueberry plants (cv. Legacy) subjected to three irrigation treatments: (Control: Commercial irrigation (A); Severe RDI: Severe regulated deficit irrigation (B); Extreme RDI: Extreme regulated deficit irrigation (C)) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control.

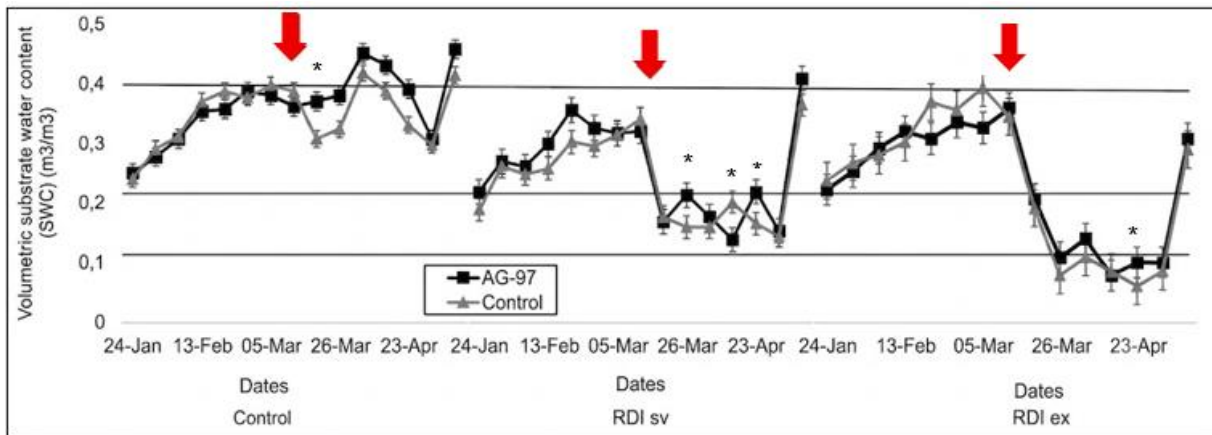
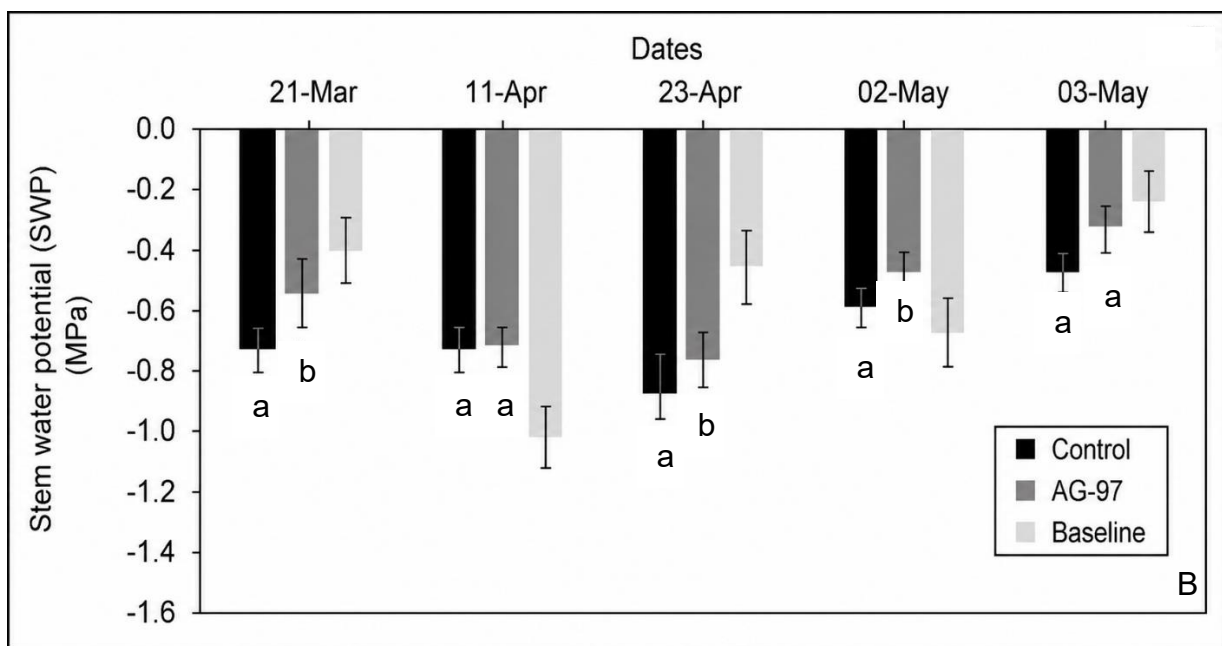
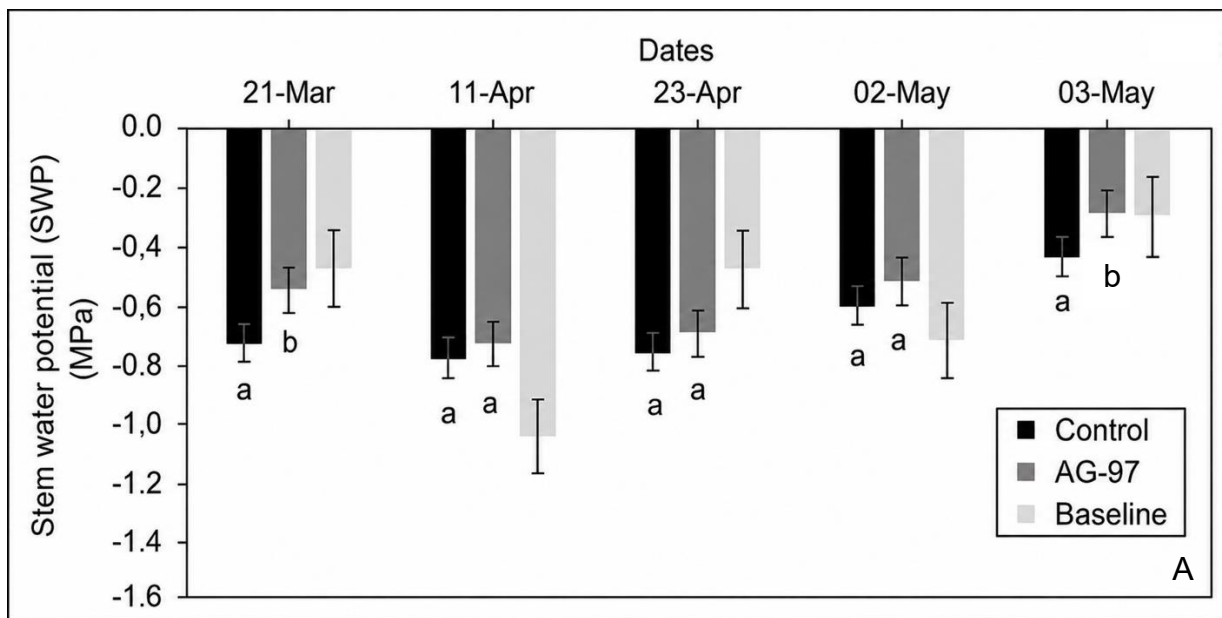


Figure 4. Average volumetric substrate water content at midday (12:00–15:00 h) in young blueberry plants (cv. Legacy) subjected to three irrigation treatments (Control: Commercial irrigation; RDI sv: Severe regulated deficit irrigation; RDI ex: Extreme regulated deficit irrigation) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control.



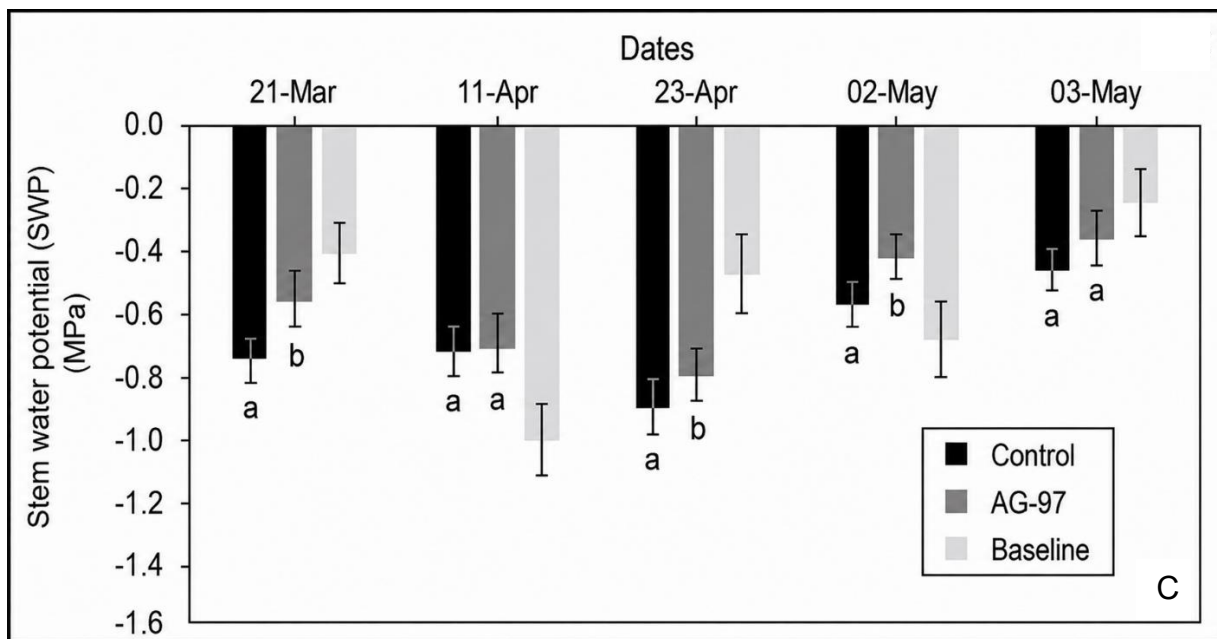
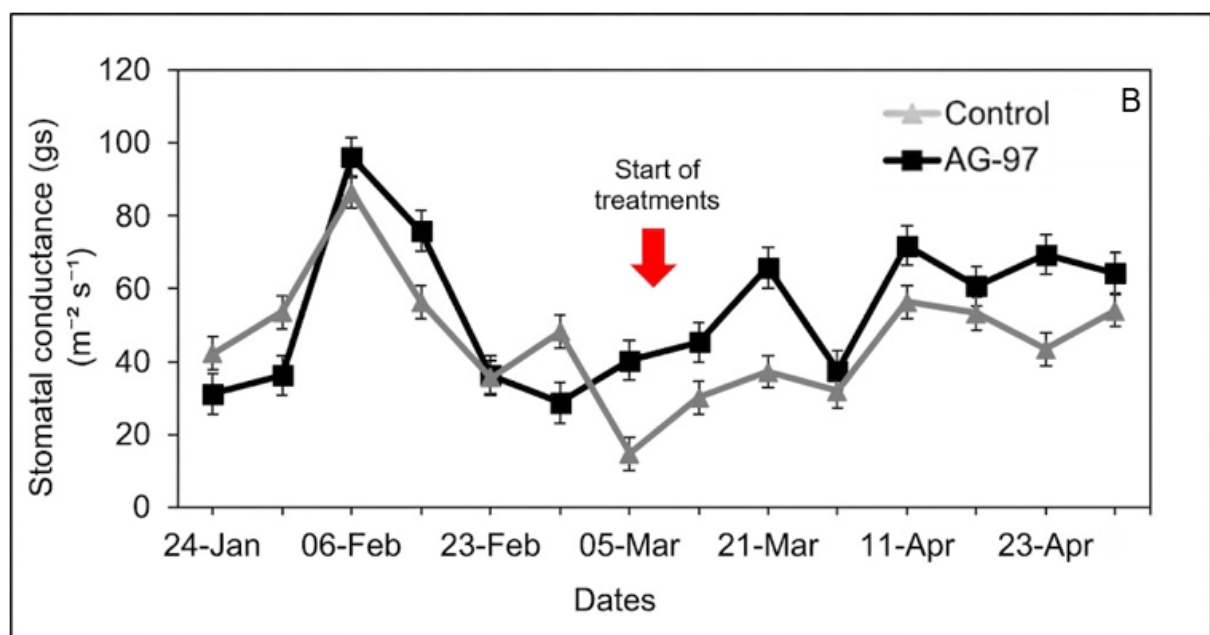
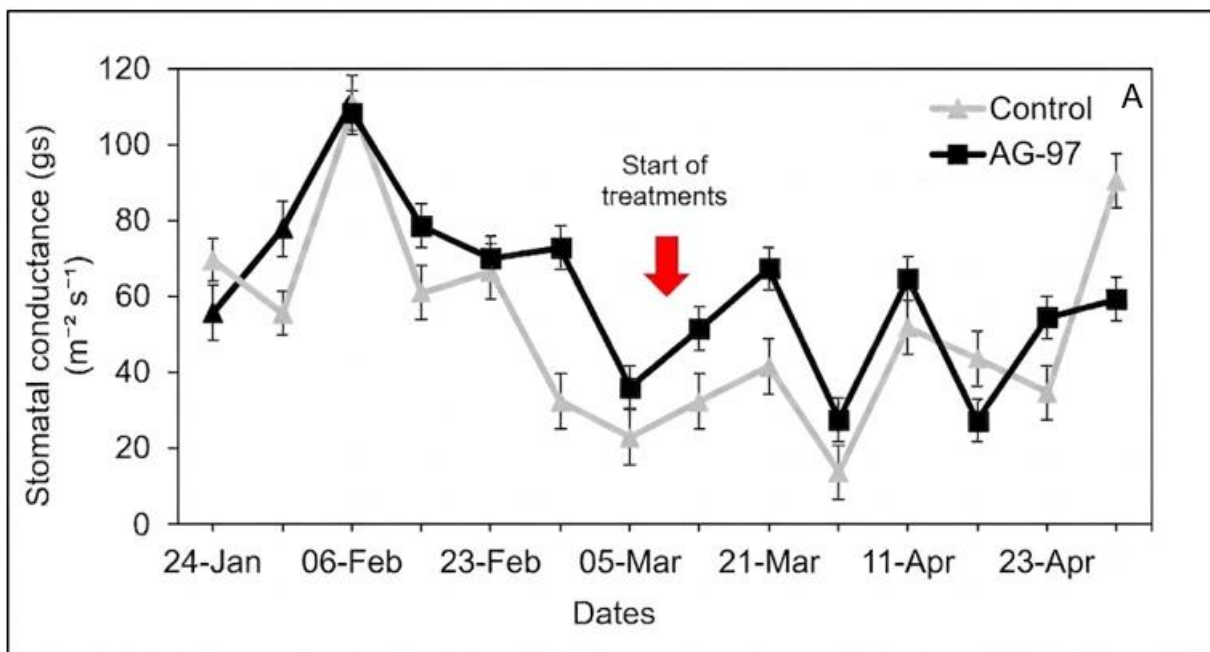


Figure 5. Midday stem water potential (12:00–15:00 h) in blueberry plants (cv. Legacy) subjected to three irrigation treatments (Control: Commercial irrigation (A); Severe RDI: Severe regulated deficit irrigation (B); Extreme RDI: Extreme regulated deficit irrigation (C)) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control. Error bars represent 1 S.E. Different letters represent significant differences between inoculation treatments at 95% confidence (LSD).



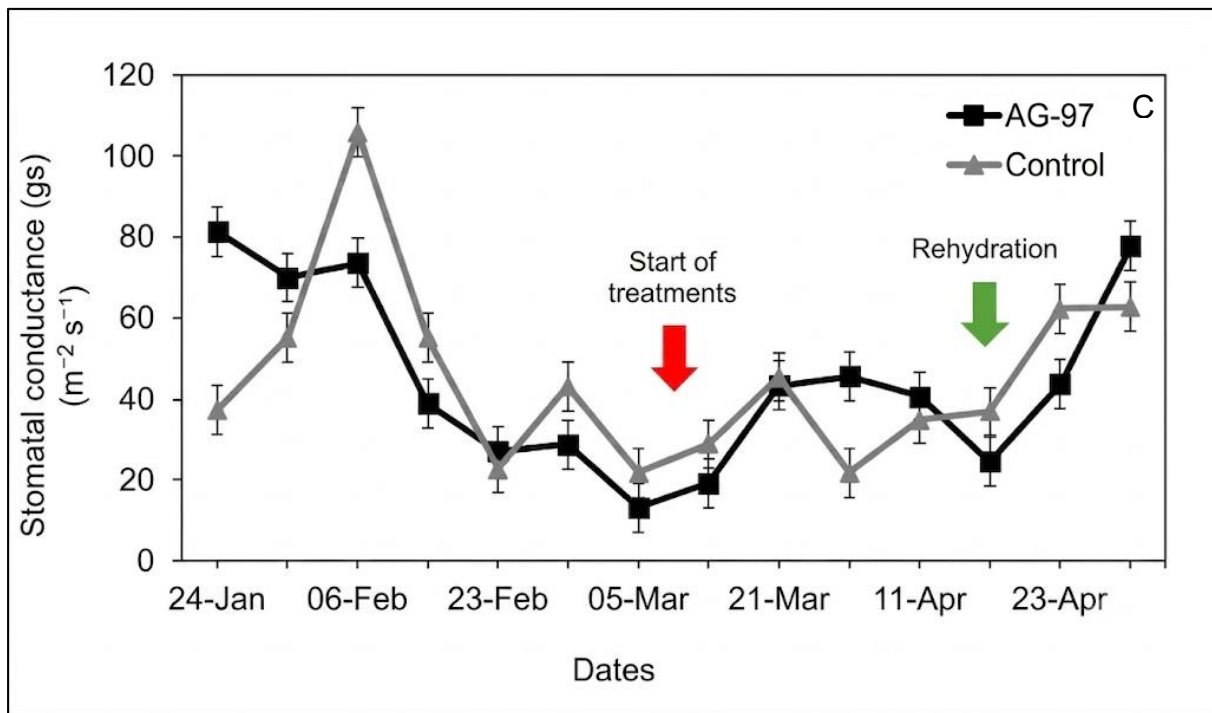
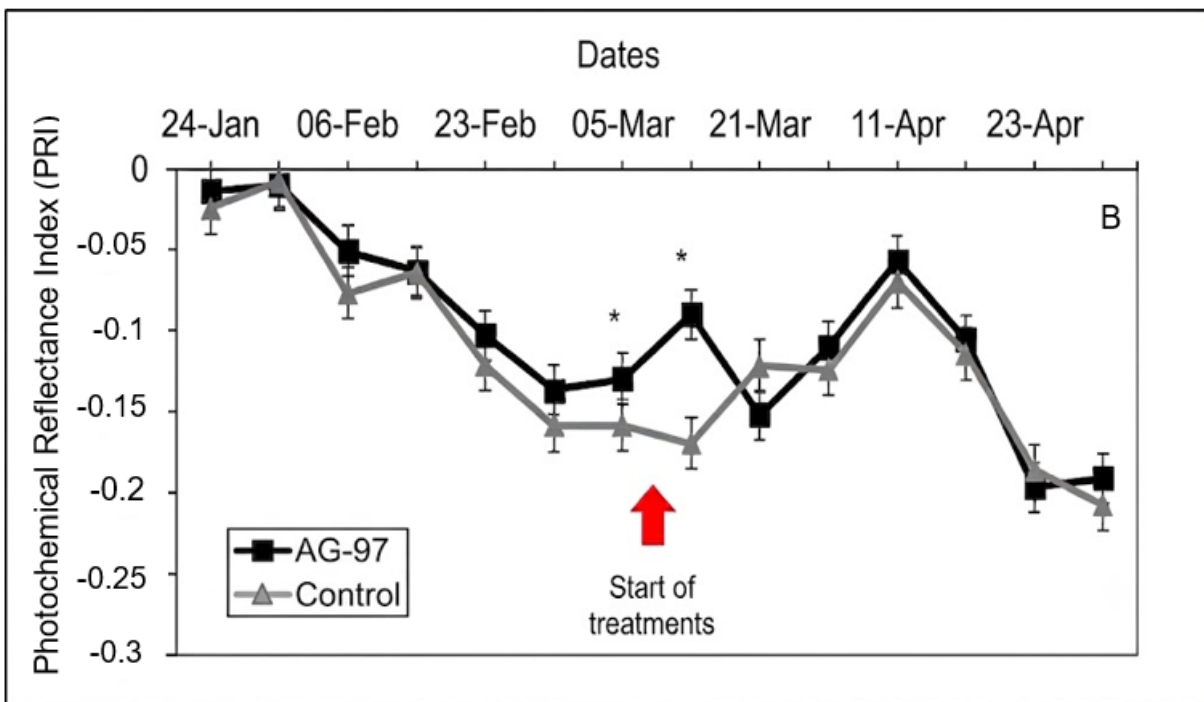
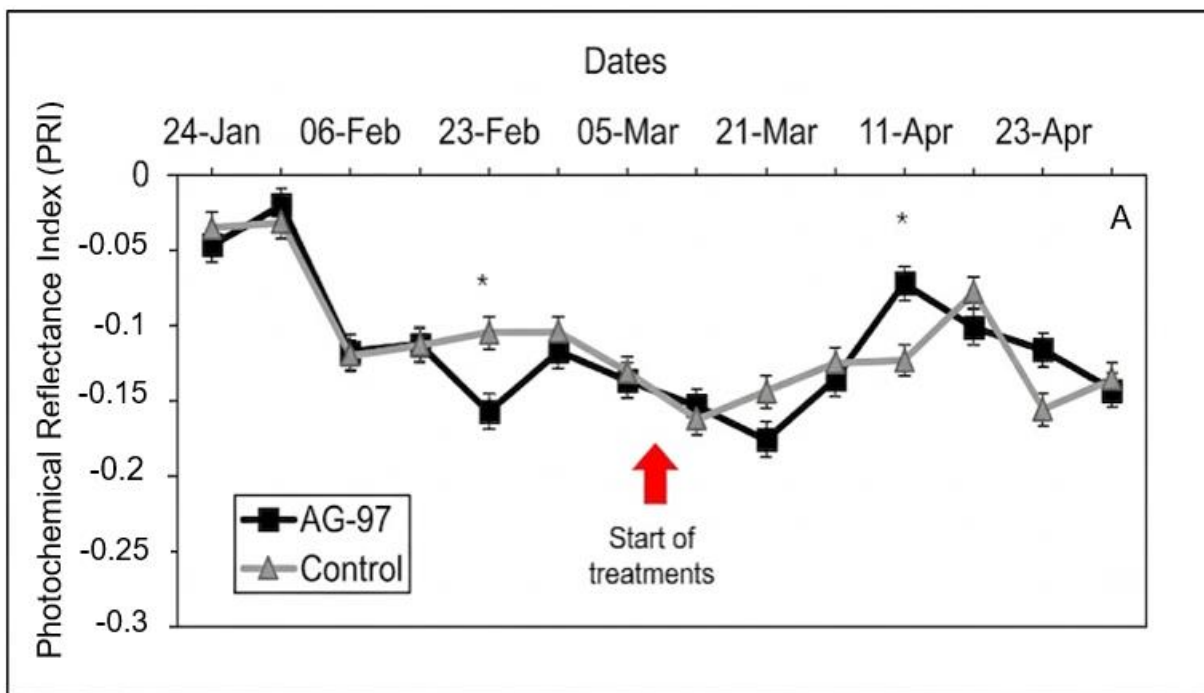


Figure 6. Leaf stomatal conductance at midday (12:00–15:00 h) in young blueberry plants (cv. Legacy) subjected to three irrigation treatments (Control: Commercial irrigation (A); Severe RDI: Severe regulated deficit irrigation (B); Extreme RDI: Extreme regulated deficit irrigation (C) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control. Error bars represent 1 S.E. The red arrow indicates the treatment application dates, and the green arrow indicates plant rehydration.



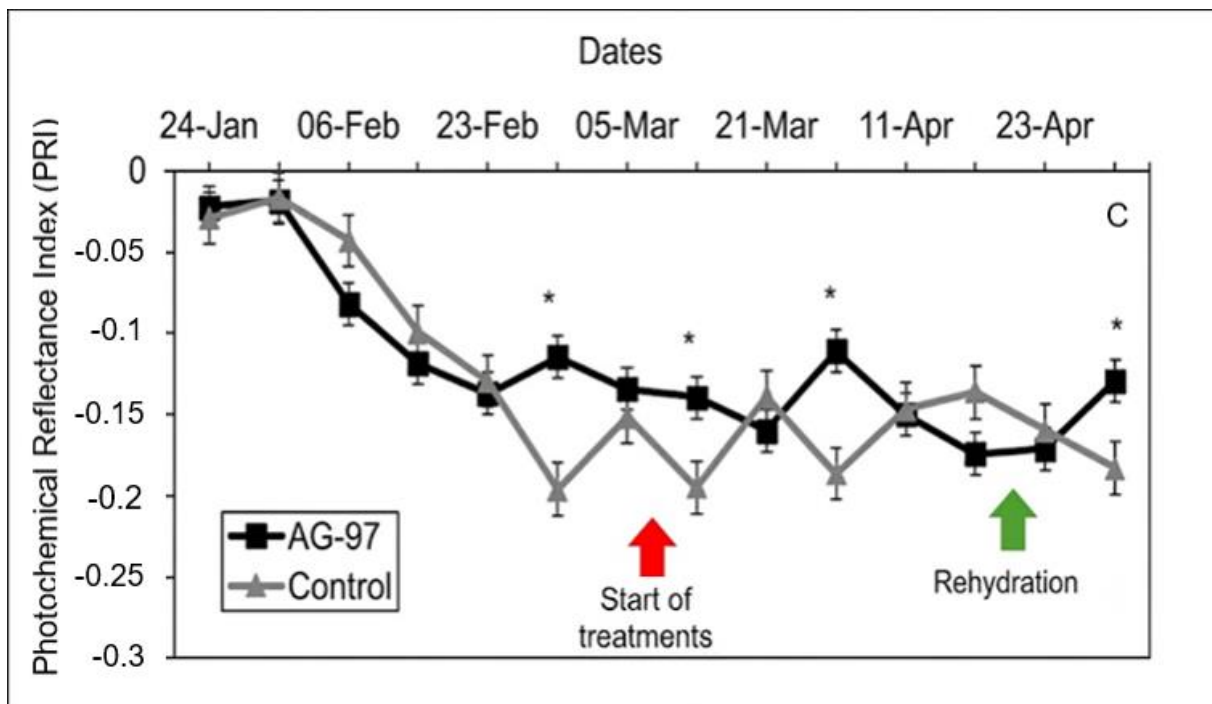


Figure 7. Photochemical Reflectance Index (PRI) measured at midday (12:00–15:00 h) in young blueberry plants (cv. Legacy) subjected to three irrigation treatments (Control: Commercial irrigation (A); Severe RDI: Severe regulated deficit irrigation (B); Extreme RDI: Extreme regulated deficit irrigation (C)) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control. Asterisks (*) represent significant differences between inoculation treatments at 95% confidence (LSD). Error bars represent 1 S.E. Red arrows indicate treatment application dates, and green arrows indicate plant rehydration.

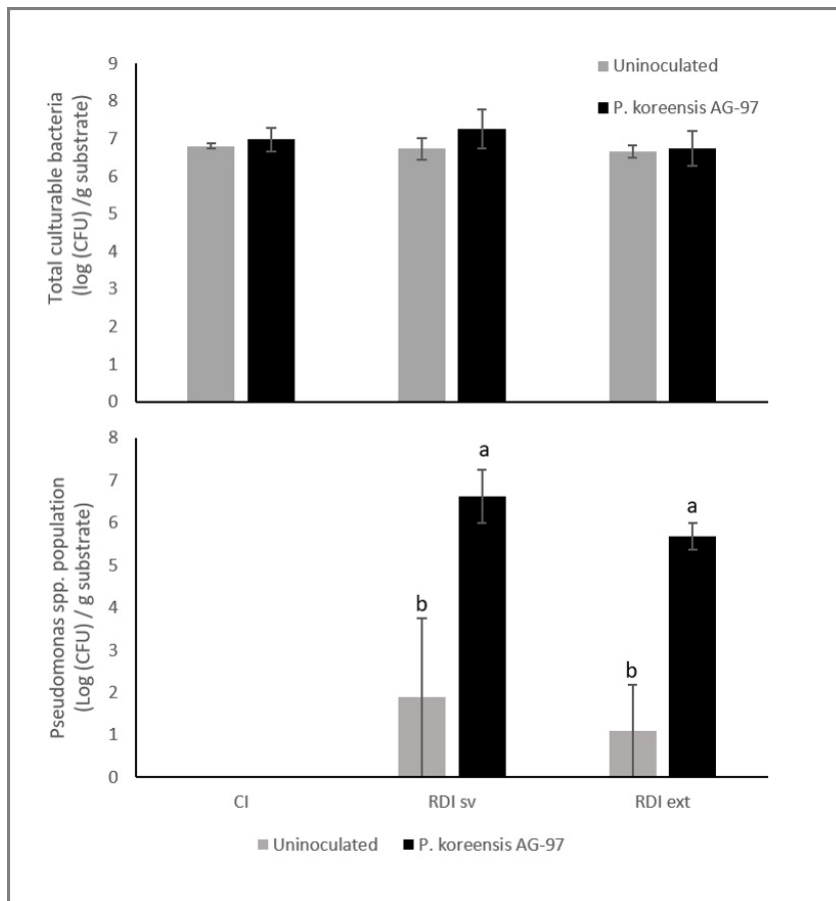
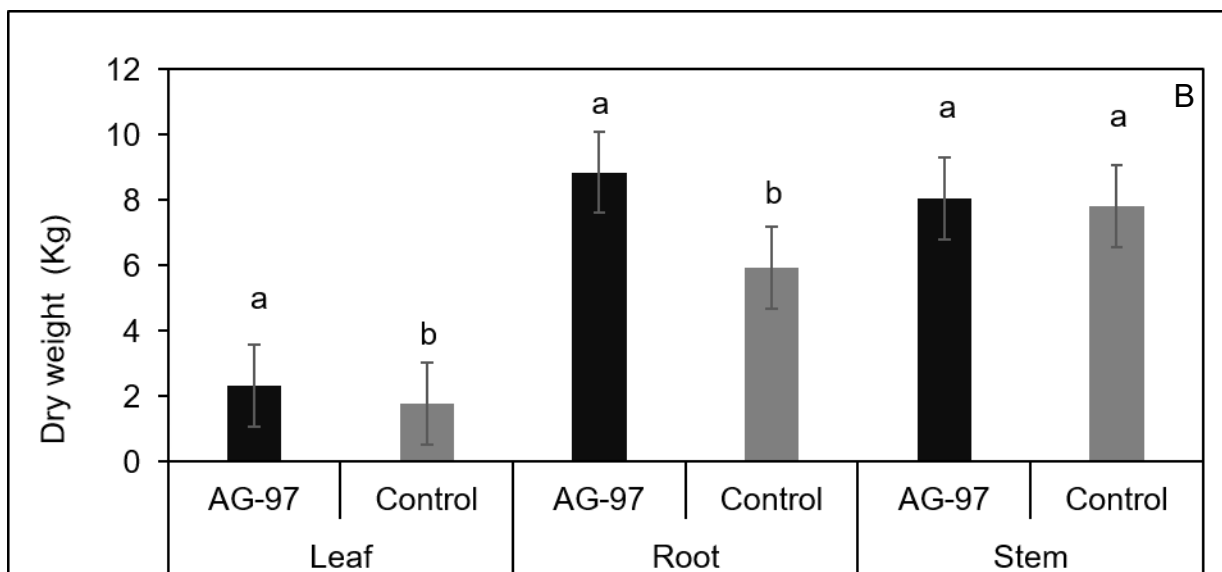
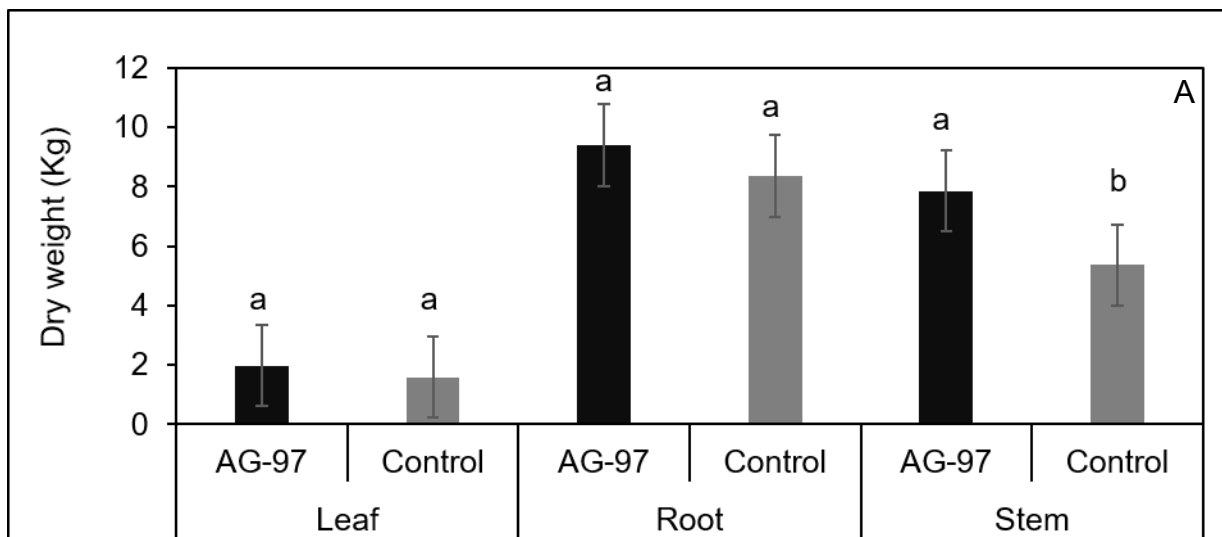


Figure 8. Total bacteria counts (A) and *Pseudomonas* spp. populations (B) in the substrate under three irrigation treatments (Control irrigation, Severe RDI and Extreme RDI) in a pot-established orchard of young blueberry plants (cv. Legacy) with two inoculation treatments: application of *Pseudomonas koreensis* and an uninoculated control. Error bars represent ± 1 S.E. Different letters represent significant differences between inoculation treatments at 95% confidence (LSD).



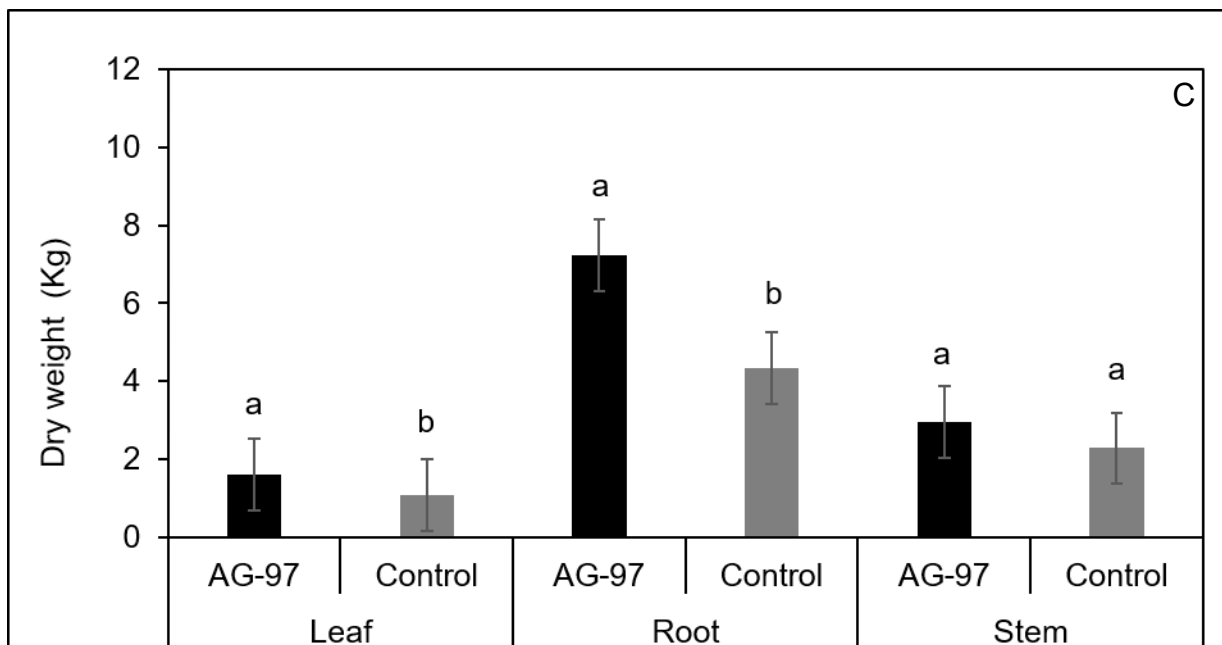


Figure 9. Vegetative organ dry weight of young blueberry plants (cv. Legacy) subjected to three irrigation treatments (Control: Commercial irrigation (A); Severe RDI: Severe regulated deficit irrigation (B); Extreme RDI: Extreme regulated deficit irrigation (C)) in a pot-established orchard with two inoculation treatments: application of the bacterium *Pseudomonas koreensis* and a non-inoculated control. Error bars represent 1 S.E. Different letters represent significant differences between inoculation treatments at 95% confidence (LSD).