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Respuesta fisiológica del olivo a estrés biótico y abiótico: rendimiento, calidad del aceite y tolerancia a antracnosis

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RESUMEN

El olivo tiene alta tolerancia al déficit hídrico y en regiones de clima húmedo, como Uruguay, se cuestiona la necesidad del riego. Sin embargo, Uruguay presenta alta variabilidad climática, generándose períodos de déficit hídrico. Ante un déficit hídrico, las plantas desencadenan una serie de mecanismos de defensa para mantener la homeostasis celular, como ser la inducción de la actividad enzimática antioxidante y el engrosamiento de la cutícula para reducir la transpiración. Estas respuestas que ocurren ante un estrés abiótico también son comunes ante un estrés biótico ocasionado por la infección de algún patógeno. La antracnosis causada por el complejo *Colletotrichum* es la principal enfermedad en los olivos en nuestro país, ocasionando pérdidas de rendimiento y de calidad del aceite. Ha sido reportado que plantas expuestas a un estrés por sequía leve activarían la respuesta de defensa basal que permite a las plantas aumentar su tolerancia ante la infección de patógenos. Por lo tanto, la hipótesis planteada fue que plantas de olivo expuestas a un déficit hídrico moderado en la etapa de lipogénesis activarían la respuesta de defensa basal que conduce a una mayor tolerancia de los frutos a un estrés biótico causado por *Colletotrichum acutatum*. Se observó que el déficit hídrico moderado generó cambios anatómicos y bioquímicos en frutos de olivo que favorecieron la tolerancia a la antracnosis, y menor incidencia y severidad de los frutos inoculados con *C. acutatum in vitro* e *in vivo* en respuesta al déficit hídrico. El peso de los frutos y relación pulpa/hueso disminuyeron en respuesta al déficit hídrico, mientras que el grosor de la cutícula y el contenido de polifenoles en los frutos aumentó. Hubo inducción en las enzimas relativas a la eliminación del peróxido de hidrógeno (CAT y PER). Estas enzimas se indujeron ante la inoculación por *C. acutatum* y este efecto fue más acentuado ante el déficit hídrico. En condiciones de déficit hídrico severo también se observó una reducción significativa del peso de los frutos y del contenido graso, y los frutos mostraron una mayor tolerancia a la infección por antracnosis.

Palabras clave: *Olea europaea* L., espesor de cutícula, enzimas antioxidantes, crecimiento del fruto, severidad de enfermedad

Olive-Tree Physiological Response to Biotic and Abiotic Stress
Fruit Yield, Oil Quality and Tolerance to Anthracnose

SUMMARY

The olive tree is highly tolerant to water deficit and in humid climate regions, such as Uruguay, the need to irrigation is questioned. However, Uruguay's climate is characterized by high interannual variability, resulting in periods of water deficit. In the event of a water deficit, plants trigger a series of defense mechanisms to maintain cellular homeostasis, including increased antioxidant enzyme activity and cuticle thickening to prevent transpiration. These responses are common during abiotic stress and biotic stress caused by pathogen infections. Anthracnose, caused by the *Colletotrichum* complex, is a major disease in olive trees, leading to reduced fruit yield and oil quality. Mild drought stress has been found to activate the basal defense response, enhancing plant tolerance to pathogen infections. Therefore, the hypothesis was that exposing olive plants to moderate water deficit during the lipogenesis stage would trigger the basal defense response, increasing fruit tolerance to biotic stress caused by *Colletotrichum acutatum*. The study also examined whether this response was sustained under moderate water deficit. Results showed that water deficit induced anatomical and biochemical changes in olive fruits, promoting anthracnose tolerance with reduced incidence and severity of *C. acutatum* infection both *in vitro* and *in vivo*. Fruit weight and pulp/pit ratio decreased in response to water deficit, while cuticle thickness and fruit polyphenol content increased. An induction of enzymes related to hydrogen peroxide scavenging were observed, in particular PER and CAT. This enzymatic activity was induced by the pathogen inoculation treatment and was higher in the non-irrigated treatment. Severe water deficit resulted in significant reductions in fruit weight and oil content, but the fruits exhibited enhanced tolerance to anthracnose infection.

Keywords: *Olea europaea* L., cuticle thickness, antioxidant enzymes, fruit growth, disease severity

1. INTRODUCCIÓN

El olivo (*Olea europaea* L.) pertenece a la familia botánica Oleaceae, especie *Olea europaea*, dentro de la cual hay seis subespecies siendo el olivo, *Olea europaea* subesp. *europaea* var. *europaea*, la única especie con fruto comestible de esta familia (Rapoport y Moreno-Alías, 2017). Desde hace menos de 30 años, el consumo del aceite de oliva ha tenido un crecimiento en respuesta a los beneficios para la salud humana que se le atribuyen (Guasch-Ferré et al., 2014). Esto conllevó la expansión del cultivo a regiones climáticamente diferentes al mediterráneo, de donde es originario (Torres et al., 2017). Estos escenarios representan nuevos desafíos productivos para el olivo, lo cual suscita un gran interés por evaluar su adaptación y comportamiento.

1.1. OLIVICULTURA EN URUGUAY

Si bien existen en Uruguay plantaciones de aproximadamente 100 años (Pereira et al., 2018), es a partir del 2002 que comienza la nueva era de olivicultura, con plantaciones intensivas. Estas consisten, en su mayoría, en marcos de plantación de 7 m x 5 m, correspondiente a densidades de 285 plantas por hectárea. Además, se han instalado más de 25 almazaras que disponen de tecnología de avanzada con el principal objetivo de obtener aceite de oliva virgen extra (AOVE) (Ackermann et al., 2018).

En el año 2002, el Instituto Nacional de Investigación Agropecuaria (INIA) instala su primer ensayo comparativo de cultivares de olivo, donde se trabaja en generar conocimiento sobre los cultivares que mejor se adaptan a nuestras condiciones edafoclimáticas. Se caracterizó el comportamiento fenológico-productivo y se determinó el rendimiento potencial de los cultivares. A su vez, se identificaron las principales limitaciones que presenta el cultivo en nuestro país: la alternancia productiva y la sanidad del cultivo (Conde-Innamorato et al., 2019). Los estudios realizados fueron en sistemas bajo riego, ya que se buscaba darle óptimas condiciones al cultivo para que expresaran su máximo potencial. Sin embargo, el 87 % de las plantaciones se realizan en secano, por lo que es de interés conocer el rendimiento que se obtendría en esas condiciones (MGAP-DIEA, 2020).

1.2. RESPUESTA DEL OLIVO AL RIEGO

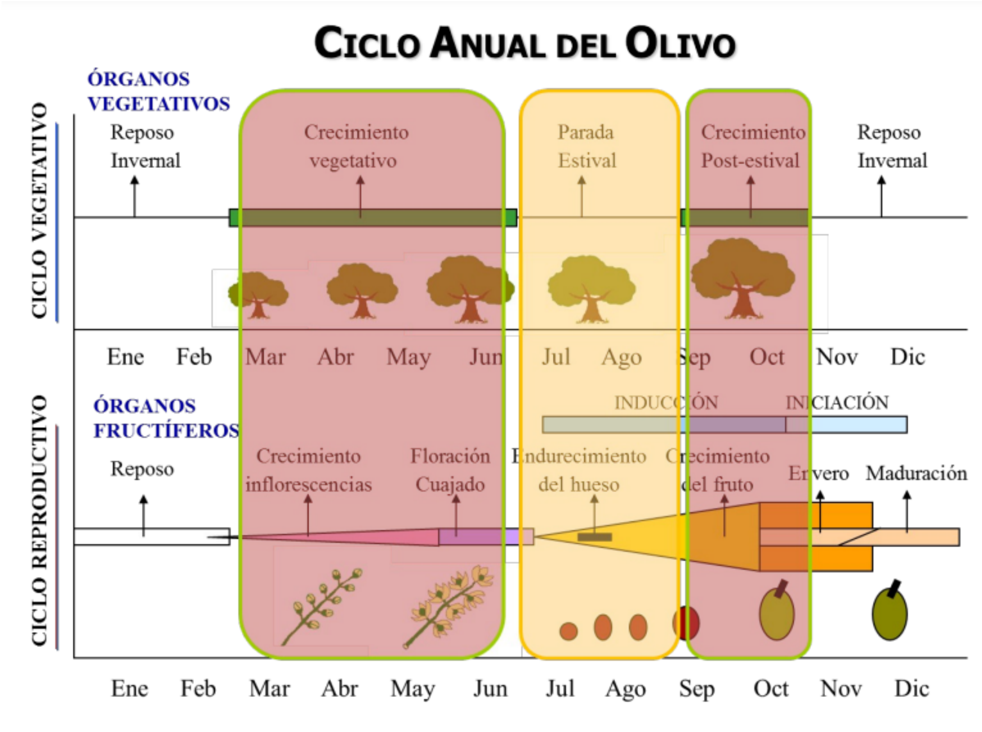
El olivo es un cultivo con alta tolerancia al déficit hídrico (Connor y Fereres, 2005), y en regiones de clima húmedo, con alta humedad relativa (70 %) y precipitaciones, como es el caso de Uruguay, se cuestiona la necesidad de la incorporación del riego. La precipitación media anual es de 1200 mm (Castaño et al., 2011), lo cual sería suficiente para cubrir los requerimientos hídricos del cultivo. Sumado a esto, nuestro déficit de presión de vapor (DPV) es menor que en la región mediterránea (Conde-Innamorato et al., 2022). El DPV se correlaciona directamente con las tasas de transpiración de la planta y, en consecuencia, del consumo de agua. La importancia de realizar evaluaciones locales ha sido destacada por varios autores que hacen énfasis en las condiciones de DPV de cada región (Mairech et al., 2020).

Las condiciones de alta pluviometría y baja demanda atmosférica de nuestras condiciones podrían ser aptas para un adecuado rendimiento de cultivo. Sin embargo, Uruguay presenta alta variabilidad climática interanual y una distribución irregular de las precipitaciones a lo largo del año (Tiscornia et al., 2016). Esto genera períodos de déficit hídrico, principalmente en verano. Se pronostica que cada vez será más frecuente la ocurrencia de eventos extremos (IPCC, 2021), incluyendo largos períodos de déficit hídrico que podrían afectar la productividad del olivo. La mayoría de las plantaciones se concentra en el este del país, donde los suelos predominantes son poco profundos (menor a 30 cm), con baja capacidad de almacenaje de agua, y de textura franco-arenosa con baja capacidad de retención de agua (MGAP-DIEA, 2020).

El olivo tiene una alta capacidad de crecer en condiciones de escasez de agua debido a sus características morfológicas y mecanismos fisiológicos de tolerancia al déficit hídrico (Fernández, 2014). Sin embargo, es un frutal que responde positivamente al riego, mejorando su productividad y estabilidad productiva (Lavee et al., 2007). El riego aumenta el crecimiento de los brotes, así como el tamaño final del fruto y el rendimiento (Moriana et al., 2003, Pierantozzi et al., 2020). Ajustar el manejo del riego permite maximizar el rendimiento y la calidad del aceite.

El crecimiento del fruto del olivo (expresado en peso fresco) sigue una curva doble sigmoidea (Hartmann, 1949). Reportes previos han identificado dos períodos durante el crecimiento del fruto que son particularmente sensibles a la restricción hídrica: una inicial durante la división celular y la fase de expansión, desde la floración hasta el final del cuajado, donde el riego deficitario puede reducir el número de frutos (Pierantozzi et al., 2020, Trentacoste et al., 2019), y una segunda durante la expansión celular y la fase de lipogénesis, después de endurecimiento del hueso hasta la cosecha, cuando el crecimiento de la fruta aumenta bruscamente a medida que se expanden las células del mesocarpio. Un déficit de riego en este período puede reducir el peso final del fruto y el contenido de aceite (Hueso et al., 2019) y puede afectar la composición del aceite, como el contenido de polifenoles (Ahumada-Orellana et al., 2018, Tovar et al., 2002) (figura 1).

Figura 1. Ciclo anual del olivo, donde se distinguen las dos fases más sensibles al déficit hídrico marcadas en color rosa (Leyva et al., 2017).



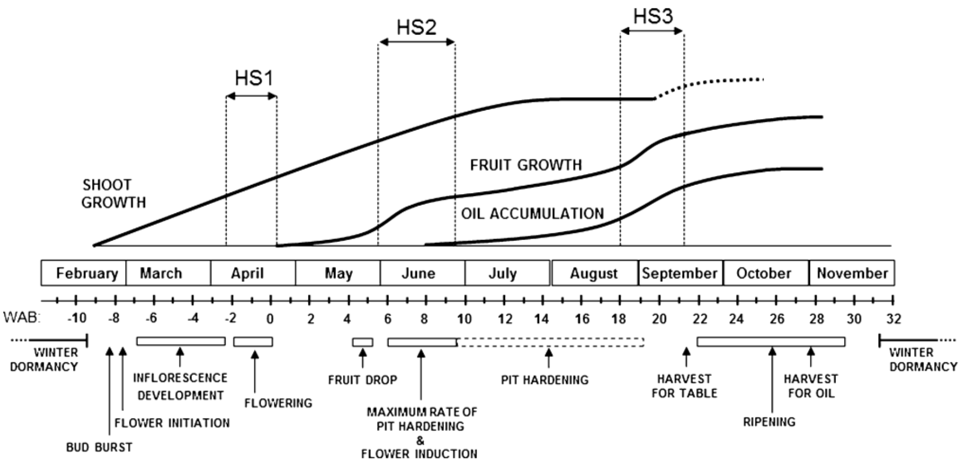


Fig. 1. Growing cycle of the olive tree in south Spain on a typical year. Shoot growth normally stops in July, although it may resume from late August. Fruit growth does not always show a double sigmoid curve as that depicted in the figure. Instead, a rather constant fruit growth rate is often observed, all throughout the summer, especially in fully irrigated trees. HS1–3 = periods of high sensitivity to water stress. WAB = weeks after bloom.

La mayoría de la información ha sido generada para climas áridos y es escasa para climas húmedos. En este contexto de variabilidad de disponibilidad de agua y características edafoclimáticas particulares, es necesario evaluar el comportamiento de los olivos en respuesta a diferentes condiciones de estado hídrico para realizar un manejo eficiente del agua identificando el mejor balance entre rendimiento, calidad del aceite y productividad del agua.

1.3. RESPUESTA DEL OLIVO AL ESTRÉS

Ante la ocurrencia de un estrés abiótico como el déficit hídrico, las plantas desencadenan una serie de mecanismos de defensa para sobrevivir y mantener la homeostasis celular (Miller et al., 2008). Tienen mecanismos que les permiten percibir distintas señales y, en consecuencia, modular una respuesta óptima. Hay respuestas enzimáticas y no enzimáticas. Dentro de las enzimáticas, una de las más comunes y generales es la respuesta al estrés oxidativo (Bartosz, 1997). Frente al estrés se generan especies reactivas de oxígeno (ROS), como H_2O_2 (peróxido de hidrógeno), $O^{2-\bullet}$ (anión superóxido), $^1O_2$ (oxígeno singulete) y $OH^\bullet$ (radical hidroxilo), y, por consiguiente, una inducción de la actividad enzimática antioxidante (De Gara et al., 2003). Estas especies son tóxicas para las plantas, pero, a la vez, son señalizadoras de estrés.

Dentro de las respuestas no enzimáticas se encuentran las relacionadas a la producción de ácido ascórbico, glutatión (Al-Ghamdi, 2009), vitamina E (Pérez et al., 2019) y polifenoles (Cirilli et al., 2017), que actúan como defensas. Bacelar et al.

(2006) reportaron que plantas de olivo expuestas a régimen hídrico deficitario desarrollaron ciertos mecanismos de defensa al estrés oxidativo con aumentos en concentración de fenoles. Además, puede haber modificaciones que no involucren cambios metabólicos, pero que también estén cumpliendo una protección como barrera física: cambios anatómicos en el grosor de la cutícula, engrosamiento de la pared celular, que hacen a una menor pérdida de agua por evapotranspiración (Gomes et al., 2009, Kunst y Samuels, 2003, Riederer y Schreiber, 2001).

Estas respuestas que ocurren ante un estrés abiótico también son comunes cuando ocurre un estrés biótico ocasionado por la infección de algún patógeno. Como se ha mencionado, otro de los desafíos de la olivicultura en el Uruguay es la sanidad del cultivo (Conde-Innamorato et al., 2019). Se ha identificado que la antracnosis es una de las principales enfermedades en los olivos en nuestro país (Conde et al., 2013), la cual ocasiona pérdidas directas de rendimiento de fruta y de calidad del aceite (Leoni et al., 2018, Romero et al., 2022) (figura 2). En Uruguay, muchas especies de *Colletotrichum* pertenecientes a los complejos *C. acutatum* y *C. gloeosporioides* causan antracnosis, siendo *C. acutatum* s.s. el más predominante (Moreira et al., 2021).

Figura 2. Escala de calificación de severidad de antracnosis en frutos de olivo del cultivar Arbequina, rango de 0 a 5, siendo 0: fruto sano, sin síntomas, 1: < 25 % de la superficie del fruto afectado, 2: 25-50 %, 3: 50-75 %, 4: 100 % y 5: fruto momificado).

La antracnosis es una enfermedad de rápido progreso, fulminante en condiciones de clima favorable (Moreira et al., 2022). Cada vez más el clima variable e impredecible hace complejo e ineficiente su control químico y, a su vez, las normativas internacionales respecto a la inocuidad de alimentos son cada vez más exigentes, por lo que es necesario realizar una estrategia de manejo cultural e integrada. En este sentido, se busca estudiar los mecanismos de defensa de las plantas que actúan frente a un ataque de *Colletotrichum* spp. para hallar aquellas estrategias de manejo que promuevan las defensas de la planta.

Las plantas se encuentran constantemente expuestas a diferentes factores de estrés, tanto bióticos como abióticos, e incluso a ambos simultáneamente. Sin embargo, son capaces de tolerar la ocurrencia independiente de uno o dos estreses, pero no necesariamente responden de igual forma cuando ocurren en simultáneo (Atkinson y Urwin, 2012).

Ha sido ampliamente reportado que plantas expuestas a un estrés por sequía leve activarían la respuesta de defensa basal que permite a las plantas aumentar su tolerancia ante la infección de patógenos. La interacción simultánea planta-estrés por sequía-patógeno es una de las combinaciones mayormente estudiadas (Ramegowda y Senthil-Kumarb, 2015). Sin embargo, la respuesta de las plantas a una combinación de estrés biótico y abiótico es compleja e implica la interacción de varias vías de señalización, lo que puede resultar en una mayor o menor susceptibilidad de las plantas dependiendo del estrés, la intensidad y del patógeno estudiado (Tippmann et al., 2006).

El engrosamiento de la cutícula de los frutos como consecuencia del déficit hídrico también cumple un rol en las interacciones planta-patógeno. La cutícula es el primer sitio de contacto entre el patógeno y el fruto (Diarte et al., 2019), actuando como barrera física contra la adhesión y penetración del patógeno (Gomes et al., 2009). Gomes et al. (2012) reportaron cambios significativos en el grosor de la cutícula en frutos de olivos de diferente susceptibilidad a *Colletotrichum acutatum* y encontraron mayor grosor en aquellos genotipos tolerantes.

1.4. HIPÓTESIS

Plantas de olivo expuestas a un déficit hídrico moderado en la etapa de lipogénesis activarían la respuesta de defensa basal que conducen a una mayor tolerancia de los frutos a un estrés biótico causado por *Colletotrichum acutatum*.

1.5. OBJETIVO GENERAL

El propósito de este trabajo es contribuir al estudio de la respuesta del olivo a estrés biótico (*Colletotrichum acutatum*.), abiótico (déficit hídrico) y ambos en simultáneo. Para ello se plantea determinar cambios anatómicos, bioquímicos y fisiológicos en respuesta al déficit hídrico y que puedan estar interviniendo en los mecanismos de defensa de la planta frente al patógeno de interés.

1.6. OBJETIVOS ESPECÍFICOS

OE 1. Determinar el impacto en los parámetros de fruto del olivo (peso, relación pulpa/hueso, índice de madurez), en el rendimiento y en la calidad del aceite de oliva en respuesta al déficit hídrico en dos cultivares de olivo en una región de clima húmedo.

OE 2. Conocer los cambios anatómicos en frutos de olivo en respuesta al déficit hídrico.

OE 3. Estudiar los cambios en la actividad antioxidante en frutos de olivo en respuesta al déficit hídrico y al estrés biótico.

OE 4. Evaluar el progreso de la enfermedad causada por *Colletotrichum acutatum* en frutos de olivo *in vitro* e *in vivo* en respuesta al déficit hídrico.

2. THE IMPACT OF IRRIGATION ON OLIVE FRUIT YIELD AND OIL QUALITY IN A HUMID CLIMATE

2.1. RESUMEN

La expansión de la olivicultura a regiones no tradicionales y con climas húmedos, como Uruguay, con más de 1200 mm de precipitación anual, pone en cuestionamiento la necesidad de riego. Sin embargo, es frecuente que exista años con déficit hídrico principalmente durante el verano. El déficit de presión de vapor en Uruguay durante el verano es menor que en países con clima mediterráneo. La alta variabilidad interanual en las precipitaciones, acentuado en el actual contexto de cambio climático, con una tendencia creciente a la ocurrencia de eventos extremos, enfatiza la necesidad de evaluar la respuesta del olivo al riego. Para ello, se aplicaron tres tratamientos de riego en los cultivares Arbequina y Frantoio según el valor de la evapotranspiración máxima del cultivo: un primer tratamiento aplicando ETc al 100%, correspondiente a estar totalmente regado; un segundo tratamiento aplicando 50% ETc; y un tercer tratamiento en el que no se produjeron aportes de riego ni lluvia desde el final del período de endurecimiento del carozo hasta la cosecha. Los resultados mostraron un aumento del peso del fruto y la relación pulpa/hueso mediante riego en las condiciones edafoclimáticas de Uruguay. El contenido graso en la respuesta al riego fue diferente dentro de los cultivares. Las condiciones de restricción de agua no afectaron el contenido graso en Arbequina, mientras que en Frantoio lo aumentó. El contenido de polifenoles en fruto aumentó ante el déficit hídrico para ambos cultivares. La aplicabilidad tecnológica de los resultados obtenidos debería ir acompañada de un análisis económico. Los resultados obtenidos demuestran la necesidad de riego durante la fase de crecimiento y maduración del fruto del olivo en clima húmedo.

Palabras clave: *Olea europaea* L., estrés por sequía, potencial hídrico del xilema, crecimiento del fruto, contenido de aceite, polifenoles

2.2. SUMMARY

The expansion of olive orchards into regions with no tradition of olive production and humid climates, such as Uruguay, with more than 1200 mm of annual rainfall, calls into question the need for irrigation. In these regions, however, years with water deficit during summers are quite common. The vapor pressure deficit during summer is lower than in countries with a Mediterranean climate. The high variability in interannual water availability in the current context of climate change, with a growing tendency for extreme events to occur, emphasizes the need to evaluate the production response of olive trees to irrigation. To achieve this, three irrigation treatments were applied to Arbequina and Frantoio cultivars according to the value of the maximum crop evapotranspiration: a first treatment applying 100% ETc, corresponding to being fully irrigated; a second treatment applying 50% ETc; and a third treatment in which neither irrigation nor rain inputs occurred from the end of the pit hardening period until harvest. Results show the possibility of an increasing fruit weight and pulp/pit ratio through irrigation in the local environmental conditions. The oil content in response to irrigation was different within cultivars. Water restriction conditions did not affect the oil content of olives in Arbequina, while in Frantoio it increased it. Polyphenols in fruit increased under water stress for both cultivars. The technological applicability of the results obtained must be accompanied by an economic analysis. The results obtained highlight the need for better use of irrigation water during the growth and ripening phase of the olive fruit under a humid climate.

Keywords: *Olea europaea* L., drought stress, stem water potential, fruit growth, oil content, polyphenols agronomy

Article

The Impact of Irrigation on Olive Fruit Yield and Oil Quality in a Humid Climate

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Abstract: The expansion of olive orchards into regions with no tradition of olive production and humid climates, such as Uruguay, with more than 1200 mm of annual rainfall, calls into question the need for irrigation. In these regions, however, years with water deficit during summers are quite common. The vapor pressure deficit during summer is lower than in countries with a Mediterranean climate. The high variability in interannual water availability in the current context of climate change, with a growing tendency for extreme events to occur, emphasizes the need to evaluate the production response of olive trees to irrigation. To achieve this, three irrigation treatments were applied to Arbequina and Frantoio cultivars according to the value of the maximum crop evapotranspiration: a first treatment applying 100% ETc, corresponding to being fully irrigated; a second treatment applying 50% ETc; and a third treatment in which neither irrigation nor rain inputs occurred from the end of the pit hardening period until harvest. Results show the possibility of an increasing fruit weight and pulp/pit ratio through irrigation in the local environmental conditions. The oil content in response to irrigation was different within cultivars. Water restriction conditions did not affect the oil content of olives in Arbequina, while in Frantoio it increased it. Polyphenols in fruit increased under water stress for both cultivars. The technological applicability of the results obtained must be accompanied by an economic analysis. The results obtained highlight the need for better use of irrigation water during the growth and ripening phase of the olive fruit under a humid climate.

Keywords: *Olea europaea* L.; drought stress; stem water potential; fruit growth; oil content; polyphenols

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1. Introduction

The expansion of olive trees into new climate areas where temperature and precipitation regimes are different from those of the Mediterranean basin generates uncertainty regarding their ecophysiological response and represents challenges in crop management [1]. In temperate humid regions such as Uruguay, where annual precipitation is around 1200 mm, the need for irrigation is questioned. However, Uruguay presents high interannual climate variability and an irregular rainfall distribution throughout the year, which generates periods of water deficit [2,3]. In addition, these extreme events are expected to be more frequent [4], affecting productive behavior.

The importance of local evaluations has been highlighted by several authors who place emphasis on vapor pressure deficit (VPD) conditions [1,5,6], which is lower in Uruguay than in the Mediterranean region. Lower VPD values are associated with lower tree transpiration and, consequently, lower water consumption. In this context of variability of water supply and environmental characteristics, it is necessary to evaluate the productive response of olive trees under different water status conditions, identifying the best balance between yield, oil quality and water productivity.

Olive has a high capacity to grow under water scarcity conditions due to its morphological characteristics and physiological mechanisms, related with the escape, avoidance and tolerance components of stress resistance [7,8]. However, there is a lot of studies that confirm that this crop positively responds to irrigation. Irrigation increases vegetative shoot growth, as well as final fruit size and yield [9–15].

Olive fruit growth (expressed as fresh weight) follows a double sigmoid curve [16]. Previous reports have identified two periods during fruit growth that are particularly sensitive to water restriction: an initial one during cell division and the expansion phase, from flowering until the end of fruit set, where deficit irrigation can reduce the final fruit number [15,17]; and a second one, during cell expansion and the lipogenesis phase, after pit hardening until harvest, when fruit growth increases sharply as mesocarp cells expand. A deficit in irrigation during this period can reduce the final fruit weight and oil content, and it can affect the oil composition, such as the polyphenol content [18–20].

There are several studies on the effect of water restriction on olive trees in arid regions, but there is a knowledge gap on the response of olive trees to irrigation management in humid temperate climates. The aim of this work was to quantify the impact of different irrigation regimes on fruit growth development and oil quality in two olive cultivars in a humid climate region.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was conducted at the INIA "Las Brujas" Experimental Station in southern Uruguay (34°40′ S; 56°20′ W; 32 m above mean sea level) using the Arbequina and Frantoio cultivars. The olive trees were planted in 2006 at a density of 416 trees per hectare (4 m between trees and 6 m between rows) and were trained as single-trunk vase shapes, with three to four main branches. The orchard was managed as a commercial farm. Pest management was performed according to the Integrated Pest Management guidelines [21]. For each cultivar, a randomized complete design with three irrigation treatments and four replicates was used. The experimental unit is the tree and there are four trees per treatment, for each cultivar. Three irrigation treatments were applied according to the value of maximum crop evapotranspiration (ETc) (Penman–Monteith equation): a first treatment applying 100% ETc, corresponding to being fully irrigated; a second treatment applying 50% ETc; and a third treatment in which neither irrigation nor rain inputs occurred (non-irrigated) from the end of the pit hardening period until harvest. The experiment was repeated in two years, during the 2018/2019 and 2020/2021 seasons, with a different randomization of the experimental units. The assays were specifically made in years of high fruit load.

2.2. Soil, Irrigation and Tree Water Status

The soil at this site has a fine textured A horizon, with a maximum depth of 30 cm, 2.9% organic matter and pH 6.3, corresponding to a Typic-Vertic Argiudolls soil according to the USDA classification [22]. The soil water curve retention was characterized by measuring water content at tensions of 0.01 and 1.5 MPa (field capacity and permanent wilting point, respectively), using the Richards and Weaver methods [23]. Undisturbed soil sample were used for soil water extraction from different depths up to 0.50 m. Soil moisture was monitored throughout the experiment using three FDR sensors installed at three different depths (15, 30 and 45 cm deep), using an EM50 Digital/Analog Data Logger (Decagon Devices, Inc., Pullman, WA, USA). The total amount of applied water was 190 mm and 410 mm in the first season, and 240 mm and 540 mm in the second season, for 50% ETc and

100% ETc, respectively. Prior to the installation of the experiment, the crop was irrigated according to the value of maximum crop evapotranspiration, so that once the treatments were started, the soil was at field capacity. To avoid the effect of rainfall, after pit hardening the soil around the trees (24 m^2/tree) was covered with nylon (bilayer) in all treatments. The plastic was placed to prevent the entry of rain into the soil and it was not removed until harvest, so that the rain from January to May was not available for the plants and therefore did not affect the treatments. A complete drip irrigation system was used to supply the irrigation water. The system consisted of a 16-diameter (13.6 mm) lateral pipe PE (0.25 MPa) with 0.20 m of emitter spacing. The flow of the self-compensating emitters was 4 L h^{-1}. Therefore, the system was designed to apply 7 mm h^{-1} of water with an average pressure in the lateral pipe of 100 kPa.

The irrigation schedule for the 100% ETc treatment was accomplished daily using the simplified water balance method for the root zone of the crop [24,25], according to the following Equation (1):

$$Dri = ETci - Pei - Ii + DPi + Dri - 1 \quad (1)$$

where *Dr* stands for root zone depletion (mm); *ETc* for maximum crop evapotranspiration (mm), computed as ET_0**Kc*; *Pe* for effective precipitation (mm); *I* for irrigation depth (mm); *DP* for deep percolation outside the root zone (mm); *i* for the current day; and $i - 1$ for the day before. The value used for *Kc* was 0.65 at the beginning of the season and then 0.70 during the mid-season and end-season stages.

The potential crop evapotranspiration was determined as $ET_0 \times kc$ (*kc* values used to calculate the water balance, according to [26]). The irrigation schedule for the 50% ETc treatment was carried out using the same methodology as used for the 100% ETc treatment. The effective precipitation (Pe) used in the soil water balance equation was 0 during pit hardening until harvest. Irrigation depth was computed so that the depletion-water root zone was between the field capacity and readily available water [24]. The daily water balance was calculated for each irrigation treatment with the FAO 56 method [27], and it was used to apply the irrigation during both seasons.

Tree water status was assessed by measuring the stem water potential (SWP) using a Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) every 15 days from the end of pit hardening until harvest. Two hours before the measurement, the shoot was enclosed in a plastic bag, allowing the leaf water potential to balance with the stem water potential for a more stable value than that of the leaf water potential. Measurements on each tree were made between 12h00 and 14h00 on mature leaves exposed to the sun from the middle of the branches [28,29]. The measured SWP values were accumulated over the irrigation period and the cumulative leaf water potential (CLWP) was calculated to compare the level of water stress throughout the entire experiment [30].

2.3. Climate

The climate is temperate humid with an average annual rainfall of 1200 mm unequally distributed throughout the year. The rainfall, mean air temperature, relative air humidity, total day radiation and wind speed were obtained from a Campbell automatic weather station located near the experiment (approximately 0.5 km) (Figure 1). Considering the average historical data (1974–2020), the ET_0 values are as follows: 456.3 mm in summer (maximum values in December and January), 205.2 mm in autumn, 99 mm in winter (minimum values in June and July) and 310.5 mm in spring, with an annual total of 1071 mm.

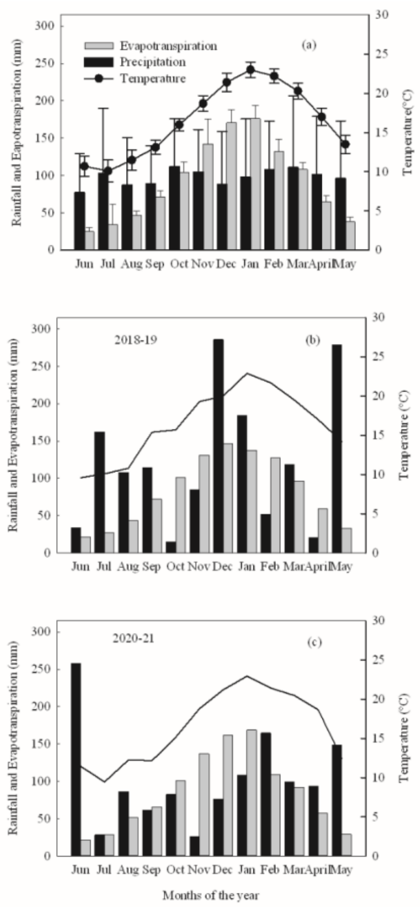

Figure 1. Average monthly values of mean air temperature (continuous line) (°C), evapotranspiration (gray bars) (ET_0, mm × 10) and precipitation (black bars) (mm) at the experimental site in INIA Las Brujas from 1973 to 2018 (**a**), for the 2018/2019 season (**b**), and for the 2020/2021 season (**c**). Vertical bars in (**a**) indicate the standard deviation. Data were recorded at an automatic weather station and is available at http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico 11 January 2022.

Historical data for precipitation and ET_0 show that during spring and summer evapotranspiration exceeds precipitation (Figure 1a), which causes a water deficit of approximately 250 mm during that period. The two seasons in which the experiments were carried out showed differences in rainfall and evapotranspiration. In the first season, high precipitations during December and January were recorded, which by far exceeded the evapotranspiration, and a water deficit occurred in October, November and February (Figure 1b). This generated differences in temperature and relative humidity and, consequently, a vapor pressure deficit that was lower compared to the second season of the experiment during January and higher during February (Figure 2). In the second season (Figure 1c), evapotranspiration exceeded rainfall by approximately 300 mm between the months of September and February, after which the values recorded were similar to what has occurred historically (Figure 1a). In order to characterize our climate region, we compared the VPD of Uruguay and Spain (Córdoba), a main traditional olive cultivation region, considering the average temperature (24 h) and average relative humidity for the 2009–2020 (Uruguay) and 2001–2020 (Spain) periods (Supplementary Figure S1).

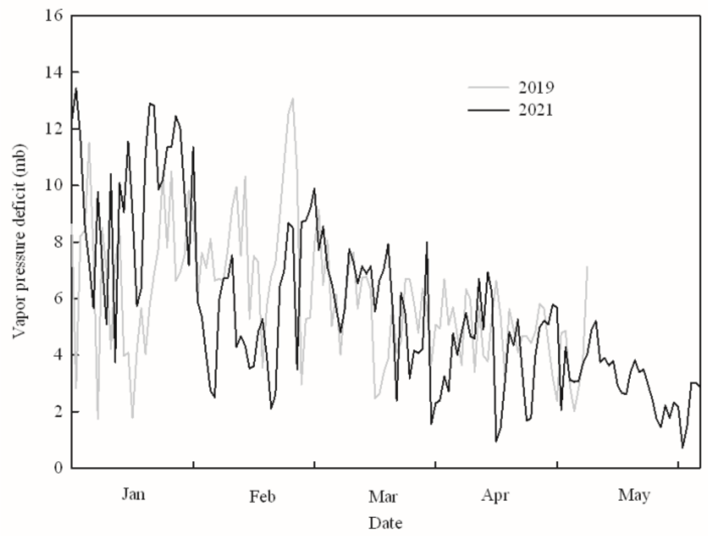

Figure 2. Daily vapor pressure deficit (mb) in Uruguay for the 2019 and 2021 seasons from 1 January to 30 April. Data were recorded at an automatic weather station at the experimental site in INIA Las Brujas (available at http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico 11 January 2022).

2.4. Productive Parameters

The fresh weight of the fruit (g), pit weight (g), and pulp/pit ratio were recorded monthly in 20 olive fruits per tree from the end of pit hardening to harvesting time. Each tree was harvested individually with a trunk vibrator machine at the end of April in both seasons, and fruit maturity index (MI) was recorded based on a 0–7 scale [31]. Fruit yield (kg/tree) was recorded and fruit number per tree was calculated from fruit yield and mean fresh weight of the fruit.

Oil content (%) was measured monthly from the end of pit hardening to harvest time on a sample of 200 g of olives per tree. To determine the fruit moisture content, each sample was ground with a hammer mill and dried at 105 °C for 48 h, after which the dried sample was grinded with a mortar and the oil content was determined following the Soxhlet method [32]. Olive oil from each tree was obtained in an Abencor mill (Mc2 Ingenieria y Sistemas, Sevilla, Spain) for oil composition analysis. Water productivity (WPf) was calculated as kg of fruit per unit of water applied (m^3) throughout the experiment [14].

2.5. Oil Chemical Composition

Acidity: the free fatty acid content was determined following the official analytical method described in [33] and expressed as acidity percentage.

Oil pigments: the content of total chlorophylls and total carotenoids was determined in a spectrophotometer by dissolving 7.5 g of each oil in cyclohexane and measured at 670 and 470 nm, respectively, according to Minguez-Mosquera et al. [34], and expressed as mg kg^{-1} EVOO.

Fatty acids profile: fatty acid methyl esters were prepared from trans-esterification reactions with a cold methanolic solution of potassium hydroxide and analyzed by gas chromatography as described by Feippe et al. [35], with some modifications. Briefly, 0.1 g of the olive oil sample were dissolved in 2 mL of heptane and vortexed for 1 min at 20 °C, then centrifuged at 12,000 rpm for 5 min. The supernatant was trans-esterified by adding 1 mL 4 N KOH in methanol and stirring manually for 1 min. The solution was dried with sodium sulfate powder, centrifugated at 12,000 rpm for 5 min, filtered and injected into a gas chromatograph with a flame ionization detector (GC-FID, Shimadzu model 2010-Plus, Kyoto, Japan). The column used was an Agilent DB-WAX (30 m × 0.25 mm ID, 0.25 µm). The injection temperature was 250 °C and the FID detector temperature was set at 300 °C. The oven temperature was set at 160 °C, increased to 200 °C after 13 min and maintained for 22 min, after which the temperature was increased to 240 °C for the final 25 min. The sample injection volume was 1 µL, and the mobile phase used was nitrogen at 30 mL/min. The hydrogen flow was set at 40 mL/min and the air flow at 400 mL/min. A standard certified Fatty Acids Methyl Esters (FAME) mix (Sigma-Aldrich, St Louis, MO, USA) was used to identify the peaks according to retention times and expressed as percentages.

Total phenolics in olive fruits: total phenolics (TP) were determined according to the method adapted from Sánchez-Rangel et al. [36] with a Folin–Ciocalteu reagent. Two grams of olives were homogenized in an Ultraturrax for 2 min, extracted with 10 mL of 80% methanol, and centrifuged at 10,000 rpm for 4 min at 4 °C. The TP determination was carried out in 96-well microplates, with gallic acid (GA) as the calibration standard; 15 µL of diluted sample extract or GA dilutions were incubated for 15 min in the dark, after the addition of 240 µL of distilled water, 15 µL of Folin–Ciocalteu reagent and 30 µL of 1 N sodium carbonate. The absorbance was read at 760 nm in a Synergy H1 Hybrid Multi-Mode Reader with Gen 5 software (Bio-Tek Inc., Winooski, VT, USA). The results were expressed as mg of gallic acid equivalents (GAE) per kg of fresh olives.

Total phenolic analysis in Virgin Olive Oils (VOO): total phenol content was determined by the Folin–Ciocalteu method described by Gutfinger [37], with some modifications. Briefly, 5 g of olive oil samples were dissolved in 7 mL of MeOH:H_2O (80:20) and vortexed. The mixture was centrifuged for 10 min at 5800 rpm and the procedure was repeated twice. The supernatants were pooled and brought up to a volume of 25 mL with MeOH:H_2O (80:20). An aliquot of 1 mL was transferred to a 10-mL volumetric flask to which 8 mL of distilled water were added followed by 0.5 mL of Folin–Ciocalteu reagent and 0.5 mL of saturated Na_2CO_3. The samples were shaken and left for 15 min in the dark at room temperature. The absorbance was determined spectrophotometrically at 760 nm in a UV–VIS spectrophotometer (Shimadzu™ model UV-3000, Kyoto, Japan). The total amount of TP was calculated and expressed as mg of GAE equivalent per kg of oil by using a calibration curve prepared with pure gallic acid standard solution.

2.6. Statistical Analysis

Since fruit number is a yield component defined during fruit set [38–40] and irrigation treatments are installed after that phase, we analyzed whether there were significant differences between the treatments of fruit number per tree at harvest. As significant differences were detected, productive variables were analyzed with ANCOVA, with fruit number per tree as a covariate. The adjusted model for each cultivar included treatment effect, year effect, and their interaction. The Mixed Models procedure (SAS v.9.4 (SAS Institute, Cary, NC, USA 2013) was used, and the corrected means were contrasted using the Tukey–Kramer test, with a significance level of 5%. Linear functions were fitted to the relationships between the fresh weight of the fruit, fresh weight of the pulp, pulp/pit ratio, maturity index and the CLWP variables. We report those functions that provided the best fits with a significance level of 5%.

3. Results

3.1. Tree Water Status and Fruit Moisture

Plants water status was affected by the levels of irrigation applied during the experiment. The ranges of stem water potential (SWP) during the experiment in both seasons and for both cultivars are presented in Table 1. The values obtained for the non-irrigated treatment were lower than those for irrigated trees. The most negative water potential value reached throughout the experiment was −3.5 MPa, corresponding to the non-irrigated treatment. In the 2021 season, water potential values were more negative than in the 2019 season. During spring and summer of 2021 there were few rain events. The estimated evapotranspiration during those months was higher than the precipitation. The crop water demand exceeded the water supply from the rains. Despite the differences in the ranges between the two seasons, the differences between the treatments in each season were clearly defined and had the same response pattern.

Table 1. Midday stem water potentials (Ψstem) ranges obtained from Arbequina and Frantoio cultivars grown under different water irrigation levels (non-irrigated, 50% ETc and 100% ETc) in two seasons.

Cultivar	2019			2021		
	Non-Irrigated	50% ETc	100% ETc	Non-Irrigated	50% ETc	100% ETc
cv. Arbequina	−1.7 to −2.3	−1.3 to −1.6	−0.8 to −1.3	−2.5 to −3.4	−1.7 to −2.1	−1.3 to −1.8
cv. Frantoio	−1.9 to −2.7	−1.5 to −1.6	−1.1 to −1.5	−3.1 to −3.5	−2.0 to −2.8	−1.9 to −2.4

The values of cumulative leaf water potential (CLWP) of the non-irrigated treatment were lower than those of irrigated trees in both cultivars and seasons (Figure 3). The results show that the generated water deficit was progressive and constant throughout the experiment. The water deficit level reached in the 2021 season was more intense than in 2019 in both cultivars, 'Frantoio' being the cultivar that reached the most negative values. In the 2019 season, values of −248 and −295 MPa in Arbequina and Frantoio, respectively, were recorded in the non-irrigated treatments, while the 100% ETc treatment presented values between −137 and −149 MPa. In the 2021 season, values of −353 and −403 MPa in Arbequina and Frantoio, respectively, were recorded in non-irrigated treatments while the 100% ETc treatment presented values between −211 and −252 MPa.

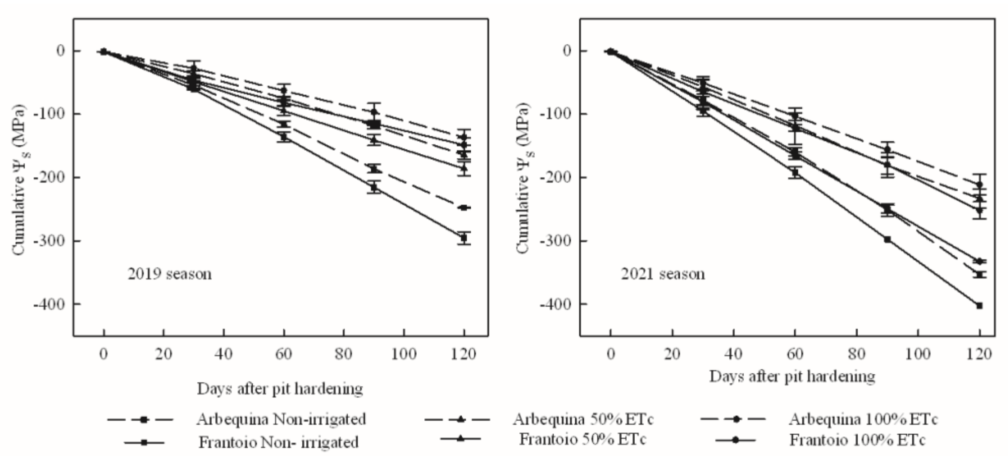

Figure 3. Seasonal evolution of cumulative leaf water potential (CLWP, MPa) of Arbequina (dotted line) and Frantoio (solid line) cultivars from the end of pit hardening to harvest time in the 2019 and 2021 seasons. Treatments included non-irrigated (■), 50% ETc (▲) and 100% ETc (●). Values are the means of four trees.

Fruit moisture content recorded during the experiment also presented differences between treatments, being lower in non-irrigated treatments than in irrigated ones, for both cultivars and both seasons (Figure 4). At the end of the experiment, the 100% ETc treatment presented at least 12% more moisture than the non-irrigated treatment in Arbequina and more than 8% in Frantoio. In 2019, fruit moisture in the non-irrigated treatments was 43 and 48% in Arbequina and Frantoio, respectively, compared to the respective 59 and 56% recorded in the 100% ETc treatments. In 2021, fruit moisture in the non-irrigated treatments was 54 and 49% in Arbequina and Frantoio, respectively, compared to the respective 66 and 63% recorded in the 100% ETc treatments.

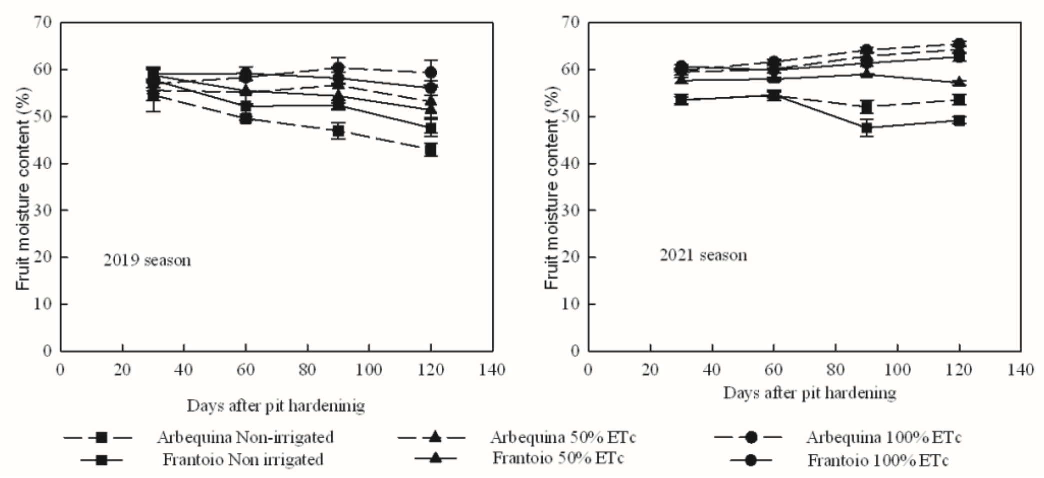

Figure 4. Fruit moisture content (%) in Arbequina (dotted line) and Frantoio (solid line) cultivars from the end of pit hardening to harvest time in the 2019 and 2021 seasons. Treatments included non-irrigated (■), 50% ETc (▲) and 100% ETc (•). Values are the means of four trees.

3.2. Productive Parameters

Significant differences between the treatments of fruit number per tree at harvest were detected, so productive variables were analyzed with ANCOVA, with fruit number per tree as a covariate. Fruit yield (kg/tree) did not show significant differences in Arbequina. In Frantoio, however, fruit yield was significantly higher in the irrigated treatments than in the non-irrigated treatment (Table 2). The maturity index for both cultivars was higher in the non-irrigated treatments than in the irrigated ones. Oil content (% DWB) did not show significant differences between treatments in Arbequina, whereas in Frantoio differences were only observed when comparing the 100% ETc and the non-irrigated treatment, with the oil content being higher in the latter. The fruit yield achieved in both irrigated treatments was similar. Therefore, regardless of the cultivar, WPf was higher in the treatment with 50% ETc than in the one with 100% ETc (Table 2). WPf was not calculated in the non-irrigated treatment, since there was no application of irrigation.

Correlations between the production parameters and CLWP were analyzed (Figure 5). CLWP better represents plant water status when it is closer to zero. Fresh weight of fruit increased with the best water status in both cultivars and in both seasons, as did the fresh weight of the pulp and pulp/pit ratio, presenting significant regressions in all cases. A positive relationship was observed between the fresh weight of fruit and CLWP, as irrigated trees of both cultivars presented a higher fresh weight of fruit than those of the non-irrigated treatment (Figure 5). In Arbequina, an adjustment greater than 0.4 was obtained, while in Frantoio this value was greater than 0.66. Similar results were obtained in the relationship between fresh weight of pulp and CLWP, with an adjustment greater than 0.51 in Arbequina and at 0.65 in Frantoio. A positive relationship was also observed between the pulp/pit ratio and CLWP, as Arbequina and Frantoio exhibited an adjustment greater than 0.68

and 0.71, respectively. The maturity index presented a negative relationship with CLWP, being significantly higher in non-irrigated treatments compared to those with irrigation (Figure 5). Arbequina presented an adjustment of 0.72, with MI ranges that varied between 1 and 3.7, while Frantoio presented an adjustment of 0.40, with MI ranges between 1.1 and 2.1 (Table 2).

Table 2. Fruit yield (kg/tree), maturity index, oil content (%) and water productivity (WPf, kg fruit/m^3 water applied) of Arbequina and Frantoio cultivars grown in the fully irrigated treatment with 100% ETc, in treatment with 50% ETc and in the non-irrigated treatment at INIA Las Brujas. Mean of the two seasons.

Evaluated Parameters	cv. Arbequina			cv. Frantoio		
	Irrigation Treatment					
	Non-Irrigated	50% ETc	100% ETc	Non-Irrigated	50% ETc	100% ETc
Fruit yield (kg/tree)	35.2 ^{a,*}	42.2 ^{a}	45.2 ^{a}	31.5 ^{b}	45.5 ^{a}	52.4 ^{a}
Maturity index	3.32 ^{a}	2.21 ^{b}	1.91 ^{b}	2.31 ^{a}	1.32 ^{b}	0.96 ^{b}
Oil content (% DWB)	39.6 ^{a}	37.7 ^{a}	37.6 ^{a}	39.2 ^{a}	36.9 ^{a,b}	34.5 ^{b}
WPf (kg fruit/m^3 water applied)	#	19.6	9.5	#	21.2	11.0

* Different letters within the row indicate significant differences for each cultivar separately (HSD Tukey–Kramer $p \leq 0.05$). # Since the non-irrigated treatment did not receive water applications, the WPf was not calculated.

3.3. Polyphenols Content in Fruits

Total phenols in fruit showed a negative relationship with the reduction in CLWP (Figure 6). Arbequina showed a reduction of 2730 and 1180 mg GAE/kg FW in total phenols in fruit, as CLWP was lower in 2019 and 2021, respectively. Frantoio showed a lesser reduction, being 470 and 610 mg GAE/kg FW in total phenols in fruit, as CLWP was lower in 2019 and 2021, respectively.

3.4. Oil Chemical Composition

The fatty acids profile was affected by irrigated treatments in both seasons, mainly for Arbequina. In the 2019 season, palmitoleic (C16:1) and linoleic (C18:2) acids increased in the non-irrigated treatment, whereas stearic (C18:0) and oleic (C18:1) decreased in Arbequina. In Frantoio, only stearic acid showed an increase without irrigation. In the 2021 season, Arbequina showed higher levels of linoleic acid in the non-irrigated treatment, similarly to the first season but in a much higher percentage in all treatments. Arachidic (C20:0) and eicosenoic (C20:1) acids showed lower levels under non-irrigated treatments in the 2021 season in Arbequina. As for Frantoio, linolenic (C18:3) and eicosenoic acids were lower and stearic acid was higher in the non-irrigated treatment. The MUFA/PUFA ratio was modified only in Arbequina in the first season, being significantly higher in the 100% ETc treatment. The oil polyphenol content was significantly higher in the non-irrigated treatment than in the 100% ETc treatment for Arbequina in the 2019 season and for Frantoio in 2021. Total carotenoids were significantly higher in the non-irrigated treatments in both cultivars, except for Arbequina in 2021, when no significant differences were recorded. Total chlorophylls in the non-irrigated treatment were lower in Arbequina in both seasons and higher in Frantoio, which only presented significant differences with the other treatments in the 2021 season. Free acidity was analyzed as the quality control of the extraction process, which in all cases was less than 0.15% (data not shown).

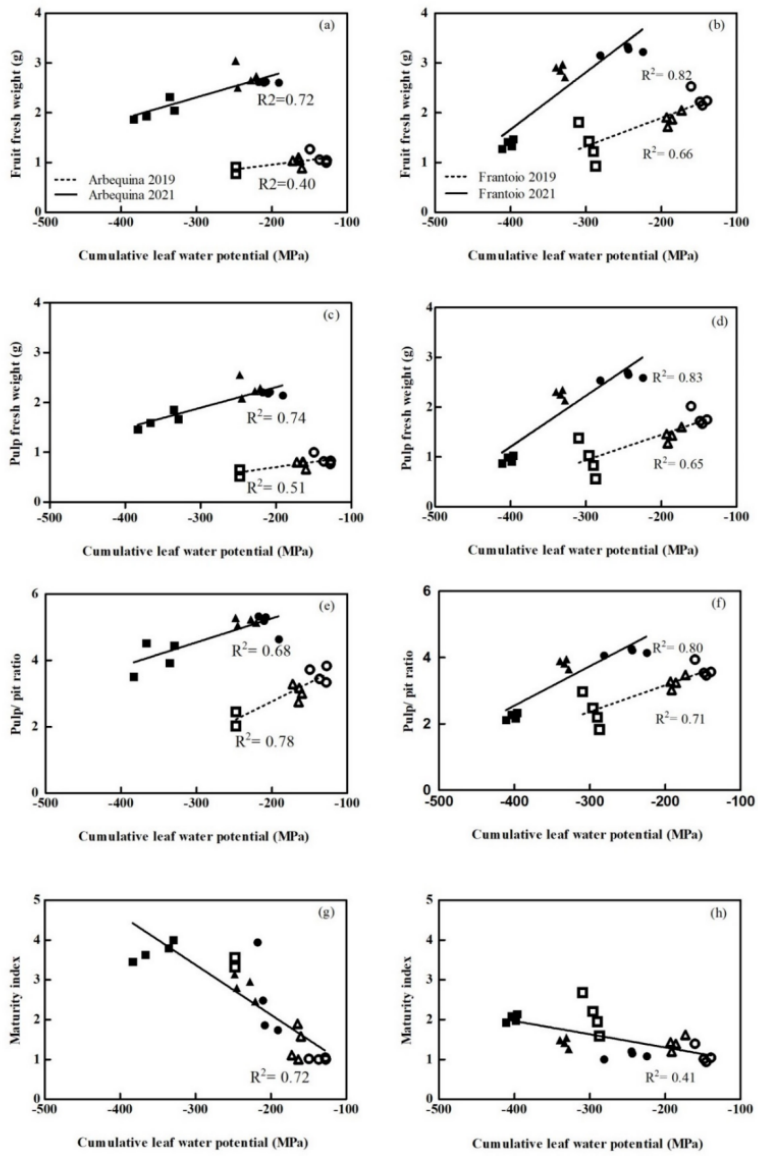

Figure 5. Fresh weight of fruit in grams (**a**,**b**), fresh weight of pulp in grams (**c**,**d**), pulp/pit ratio (in fresh weight basis) (**e**,**f**) and maturity index (**g**,**h**) at harvest time as a function of the cumulative leaf water potential (MPa) in Arbequina (**left**) and Frantoio (**right**) cultivars. Treatments included: non-irrigated (■), 50% ETc (▲) and 100% ETc (●). In panels (g) and (h), the regression was done for the two years together. The empty symbols correspond to the 2019 season and the full symbols to 2021.

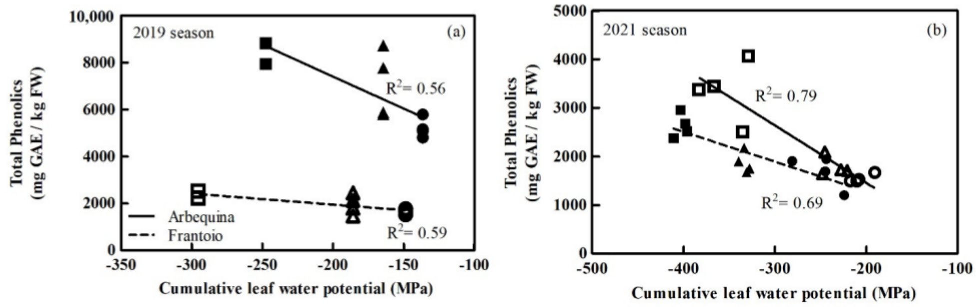

Figure 6. Total phenolics in fruit (mg GAE/kg FW) as a function of the cumulative leaf water potential (MPa) in the 2019 (**a**) and 2021 (**b**) seasons. Treatments included non-irrigated (■), 50% ETc (▲) and 100% ETc (•). Full symbols correspond to cv. Arbequina and empty symbols to cv. Frantoio.

4. Discussion

Olive (*Olea europaea* L.) is a typical tree of the Mediterranean climate that has traditionally been cultivated in rainfed conditions and in regions with high vapor pressure deficit (VPD). VPD is the difference between the saturation vapor pressure and real vapor pressure during a given period [27]. Mairech et al. [5] express the need for calibrating irrigation at a local level given the high dependence of the water requirements with VPD. Many authors report the range of xylem potential reached during irrigation experiments, but few studies refer to VPD [15,41]. We compared the VPD of Uruguay and Spain (Córdoba) (Supplementary Figure S1) and it is observed that Uruguay presents notoriously lower values of VPD. During the summer, VPD values in Spain double those of Uruguay. VPD is directly associated with evapotranspiration (ET_0), as higher VPD would generate higher water consumption. Annual ET_0 values in our conditions are lower than those reported for other olive regions around the world [1,5]. During summer, the period in which the experiment was carried out, the most frequent ET_0 value in Uruguay was 456 mm (Figure 1a), while in the Mediterranean basin it is higher (for instance, 600 mm in Sevilla [1]). These climatic differences can influence olive tree physiology, affecting the productive variables.

Plant water status achieved by the different treatments in both seasons generated a moderate stress in the 50% ETc treatment and a severe one in the non-irrigated treatment according to Fernández et al. [42]. Stem water potential (SWP) in both seasons was in the range of −1.7 and −3.5 MPa in the non-irrigated treatment, −1.3 and −2.8 MPa in the 50% ETc treatment and between −0.8 and −2.4 MPa the in 100% ETc treatment, all within the range measured by other authors [14,30,43]. In Arbequina, the stress during the experiment reached cumulative leaf water potential gradients between treatments from −137 to −248 in 2019 and from −211 to −353 in 2021. In Frantoio, the CLWP gradient was −149 to −295 in 2019 and −251 to −403 in 2021 (Figure 3). These values were similar to those reported by Gucci et al. [30]. This allows us to classify the stress level of treatments and therefore to compare our results with reports made in other sites and cultivars.

Final yield is determined by fruit number, fruit weight and oil content [44]. Fruit number is defined in the flowering–fruit setting phase, prior to establishing the experiment [38–40]. As significant differences in fruit number per tree were detected at harvest between treatments, this parameter was used as a covariate. Therefore, we focused on fruit weight and oil content responses. It was observed that, under the experimental conditions, both cultivars responded positively to irrigation, increasing the fruit weight and the pulp/pit ratio in comparison to the non-irrigated treatment. A similar response was also observed during the same water deficit period by Lavee et al. [45] in cv. Muhasan, where fruits were significantly smaller in the non-irrigated trees than in the irrigated ones. The magnitude of the irrigation effects was different depending on the cultivar. Fruit weight in

Arbequina in the irrigated treatment increased by 40% in comparison to the non-irrigated treatment, while in Frantoio it increased by 68%, which represents a 66% increase in yield per tree. Moreover, a positive linear relationship between the pulp/pit ratio and water plant status was observed in both cultivars (Figure 5b). This is in accordance with previous studies by Gómez-Rico et al. [46] and Lavee et al. [45] who observed that irrigation increases the pulp/pit ratio in comparison to rain-fed trees. However, other authors did not find effects in this ratio under mild water stress [12,45]. In the framework of this experiment, the impact of irrigation on absolute fruit weight was lower in Arbequina, a cultivar with small fruits. In addition, it did not translate into a yield increase (kg/plant). In Frantoio, the increase in fruit weight doubled with irrigation, impacting the yield (Table 2 and Figure 5). This could be due to the genetic characteristic of the fruit size limiting the response to irrigation.

Fruit oil content does not yet show a consensual pattern of response to water restriction. This parameter is highly variable depending on the moment and level of water restriction applied [14,46]. In Arbequina, we found no effect of irrigation on the oil content on a dry basis (Table 2). Similar results were obtained by Hueso et al. [14] in Arbequina under similar water-stress conditions (up to −2.6 MPa) and by Ahumada-Orellana et al. [47] even under severe water stress (up to −6 MPa). However, Iniesta et al. [40] found a higher oil content in water deficit treatments in comparison to irrigated ones, under similar stress conditions as those of our work (−2.9 and −3.6 MPa), also in Arbequina. Oil content is conditioned by the genotype–environment interaction [48]. In this study, a different response in oil content according to cultivars was observed, in agreement with Iniesta et al. [40]. In particular, the oil content in Frantoio was higher in the non-irrigated treatment than in the 100% ETc treatment (Table 2), while in other works a reduction in oil content has been recorded when stem water potential was near −4 MPa [46,49].

Regarding MI, the negative effect of the fruit load on the advancement of maturity has been widely reported. In this sense, it is expected that the treatments with more load have a delayed maturity. Despite having corrected the maturity mean values for fruit number, a negative linear relationship was maintained between MI and water status in both cultivars, with a greater slope in Arbequina (Figure 5). The same pattern was found by Inglese et al. [50] in cv. Carolea when the irrigation in the final phase of fruit development delayed the MI, and by Motilva et al. [51] in Arbequina. However, Iniesta et al. [40] did not find that a deficit in irrigation leads to an earlier ripeness.

Polyphenols have been associated with defense mechanisms against water stress [52]. In our study, total phenols in fruit increased in the non-irrigated treatment in both cultivars. According to Talhaoui et al. [53], the transfer of phenolic compounds from fruits to oil did not surpass 2% in a study with six cultivars that explains qualitative and quantitative changes in phenolic compounds of olive oil during oil extraction in relation to fruits. Our results show that the content of polyphenols in Arbequina and Frantoio oil increased for the non-irrigated treatment compared with the 100% ETc treatment, as also reported by several authors [51,54,55]. During the 2021 season, Arbequina showed the same tendency but the differences were not statistically significant. In the 2019 season, the content of polyphenols in Frantoio increased with irrigation restriction at 50% ETc, reaching the highest level. A similar effect was observed by Tognetti et al. [56], who reported that total phenolics at 66% ETc where higher than in the non-irrigated and 100% ETc treatments. Other works with Frantoio found inconsistent results between years in the phenol content in response to the level of irrigation [57]. Despite the fact the levels found in both seasons are different, they are in concordance with previously reported oils from similar experiments [18,46,51]. In humid climate conditions, the differences between two growing seasons could affect not only the oil content [58] but also the minor oil components, such as phenolic compounds and the fatty acid profile.

The carotenoids content in oil was affected by plant water status, increasing with water restriction between the non-irrigated and 100% ETc treatments in Arbequina and Frantoio, respectively. Only Arbequina showed no differences in season 2021 (Table 3). However, previous works on the effects of irrigation on the carotenoid content found different responses. Sena-Moreno et al. [59] reported an increase in carotenoids with water restriction, consistent with our results, while Tovar et al. [18] did not find differences. The chlorophyll content in Arbequina was reduced by 65% (2019) and 81% (2021) in the non-irrigated treatment compared to the irrigated ones. This response was probably due to the advanced maturity index in the non-irrigated treatment at harvest. For Frantoio, the only difference was found in the 2021 season, where the chlorophyll content increased by 76%. The pigments content (chlorophylls and carotenoids) varies depending on the cultivar, the fruit ripening stage, the weather conditions and the oil-extraction processes [60]. The lipophilic characteristics of these compounds determine the affinity of the oily phase and the migration ratio into the EVOO, playing a role in oxidative stability [61].

Table 3. Physicochemical composition of olive oil in the non-irrigated, 50% ETc and 100% ETc treatments: total phenolics, chlorophylls, total carotenoids, free acidity and fatty acids composition. Olives from the Arbequina and Frantoio cultivars harvested in 2019 and 2021 obtained the corresponding virgin olive oil (VOO).

	2019						2021					
	cv. Arbequina			cv. Frantoio			cv. Arbequina			cv. Frantoio		
	Non-Irrigated	50% ETc	100% ETc	Non-Irrigated	50% ETc	100% ETc	Non-Irrigated	50% ETc	100% ETc	Non-Irrigated	50% ETc	100% ETc
Total Phenolics (mg GAE/kg EVOO)	147.5 ^{a,*}	138.3 ^{a,b}	121.7 ^{b}	343.5 ^{a,b}	372.4 ^{a}	306.4 ^{b}	86.4 ^{a}	72.6 ^{a}	80.2 ^{a}	133.5 ^{a}	105.9 ^{b}	104.0 ^{b}
Totals Carotenoids (mg Car/kg EVOO)	3.54 ^{a}	3.36 ^{b}	2.88 ^{b}	7.29 ^{a}	5.81 ^{b}	5.59 ^{b}	0.68 ^{a}	0.77 ^{a}	0.79 ^{a}	5.19 ^{a}	3.08 ^{b}	3.41 ^{b}
Total Chlorophylls (mg Ch/kg EVOO)	0.69 ^{b}	1.94 ^{a}	1.99 ^{a}	6.81 ^{a}	5.16 ^{a}	5.79 ^{a}	0.14 ^{b}	0.75 ^{a}	0.75 ^{a}	7.47 ^{a}	3.85 ^{b}	4.04 ^{b}
Fatty acid composition:												
Palmitic Acid (%)	14. 82 ^{a}	15.07 ^{a}	15.25 ^{a}	13.54 ^{a}	14.19 ^{a}	13.67 ^{a}	16.69 ^{b}	17.41 ^{a}	17.18 ^{a,b}	14.00 ^{a}	13.81 ^{a}	14.64 ^{a}
Palmitoleic Acid (%)	2.19 ^{a}	1.99 ^{a,b}	1.93 ^{b}	1. 23 ^{a}	1.45 ^{a}	1.55 ^{a}	1.62 ^{b}	2.16 ^{a,b}	2.28 ^{a}	1.07 ^{a}	0.85 ^{a}	1.09 ^{a}
Stearic Acid (%)	1.51 ^{c}	1.74 ^{a}	1.65 ^{b}	2.25 ^{a}	1.89 ^{b}	1.58 ^{b}	1.71 ^{a}	1.75 ^{a}	1.69 ^{a}	2.26 ^{a}	1.90 ^{b}	1.70 ^{c}
Oleic Acid (%)	67.56 ^{b}	68.73 ^{a,b}	69. 57 ^{a}	73.45 ^{a}	73.05 ^{a}	74.07 ^{a}	60.23 ^{a}	60.52 ^{a}	61.24 ^{a}	71.10 ^{a}	70.28 ^{a}	71.01 ^{a}
Linoleic Acid (%)	12.84 ^{a}	11.41 ^{b}	10.42 ^{c}	8.17 ^{a}	8.14 ^{a}	7.78 ^{a}	18.42 ^{a}	16.66 ^{a,b}	16.14 ^{b}	10.11 ^{b}	11.59 ^{a}	9.808 ^{b}
Linolenic Acid (%)	0.50 ^{a}	0.51 ^{a}	0.56 ^{a}	0.75 ^{a}	0.68 ^{b}	0.68 ^{b}	0.60 ^{a}	0.65 ^{a}	0.66 ^{a}	0.63 ^{c}	0.70 ^{b}	0.82 ^{a}
Arachidic Acid (%)	0.30 ^{a}	0.29 ^{a}	0.31 ^{a}	0.31 ^{a}	0.31 ^{a}	0.31 ^{a}	0.34 ^{b}	0.40 ^{a}	0.40 ^{a}	0.41 ^{a}	0.40 ^{a}	0.40 ^{a}
Eicosenoic Acid (%)	0.28 ^{a}	0.27 ^{a}	0.31 ^{a}	0. 30 ^{a}	0.29 ^{a}	0.37 ^{a}	0.27 ^{b}	0.31 ^{a}	0.30 ^{a}	0.31 ^{c}	0.36 ^{b}	0.40 ^{a}
MUFA/PUFA	5.23 ^{c}	5.94 ^{b}	6.55 ^{a}	8.39 ^{a}	8.49 ^{a}	9.00 ^{a}	3.28 ^{a}	3.66 ^{a}	3.81 ^{a}	6.76 ^{a}	5.84 ^{b}	6.83 ^{a}

* Means (n = 4) followed by the same letter within a row for each cultivar and each season are not significantly different at $p < 0.05$ (Tukey's test). GAE: Gallic acid equivalent; Ch: total chlorophylls; Car: total carotenoids. MUFA/PUFA: ∑ monounsaturated fatty acids (MUFA)/∑ polyunsaturated fatty acids (PUFA) ratios.

The effect of different irrigation strategies on the olive oil fatty acid composition remains unclear [4]. In our study, oleic acid, the main olive oil fatty acid, was significantly lower for Arbequina in the 2019 non-irrigated treatment (Table 3). Severe and prolonged water stress during fruit growth increases fruit temperature [62] and consequently the oleic acid proportion fell [63]. In the same regard, we found a reduction in oleic acid as linoleic acid increases with severe water restriction in Arbequina. The MUFA/PUFA ratio decreased concomitantly for Arbequina in the 2019 season in the non-irrigated treatment, with no changes in Frantoio in either season. Despite slight differences in the percentages, the obtained quality complied with the COI specifications for EVOOs regarding the fatty acid composition of both cultivars.

It is important to find the best balance between yield, oil quality and water-saving issues [30]. The relationship between fruit production and ETc was shown to be curvilinear by Moriana et al. [10], which means that high production could be reached at lower values than those of the maximum potential ETc. It was demonstrated that under full irrigation olives can achieve high yields (8 t/ha/year) in humid template regions [58]. In our study, if we compare WP (estimated according to kg/tree based on the applied water) between both irrigation treatments, we observe that it was always higher in the 50% ETc treatment than in the 100% ETc one, since yields in kg/tree were similar between both and the water applied was half in the 50% ETc one. Moreover, oil polyphenols content was similar in both irrigated treatments, which may affect oil stability [64]. Fruit moisture at harvest ranged between 43% and 65.5% in Arbequina in the non-irrigated and 100% ETc treatments, respectively. In Frantoio, the range was between 47.6% and 62.7% in the non-irrigated and 100% ETc treatments, respectively. High fruit moisture may have negative effects on the oil extraction process [65,66]. A reduction in irrigation translates into a lower percentage of fruit moisture, which facilitates oil extraction [64–66] and reduces costs at the olive oil mill. The economic valuation of the investment in irrigation must take into account other benefits, such as avoiding severe droughts in the spring or in the years of installation of the crop. Our results provide information on how to manage the irrigation already installed during the fruit growth phase, a phase in which the published results are highly variable and for which there is no evidence in our agroecological conditions.

In summary, this work provided experimental evidence about the productive behavior of the Arbequina and Frantoio cultivars in a temperate humid climate under different water deficit conditions. We demonstrated that irrigation in a low VPD environment increases fruit weight and the pulp/pit ratio, and that it resulted in a significant increase in yield (kg/tree) in Frantoio. The oil content in response to irrigation was different between cultivars. Water restriction conditions did not affect the oil content of olives in Arbequina, while in Frantoio it increased by water restriction in the evaluated range of stem water potential (from −0.8 to −3.5 MPa). The content of polyphenols in fruits and in oil increased under water restriction, with lesser changes in other oil quality parameters. A moderate water restriction (50% ETc) produced the most balanced result between yield, oil quality and WP. Irrigation during the growth and ripening of the fruit also affects the vegetative development and therefore will affect the flowering potential for the next season, in this way it is also intervening in the expression of alternate bearing. For this reason, future studies should address aspects of partition and the relationship of vegetative–reproductive growth to carry out a comprehensive analysis of the benefits of irrigation in our agroclimatic conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12020313/s1, Figure S1: Annual vapor pressure deficit (mb) in Uruguay and in Spain. Data recorded by an automatic weather station at the experimental site in INIA Las Brujas as an average of the 2009–2020 period (available at http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico 11 January 2022) and by the weather station at Córdoba as an average of the 2001–2020 period (available at https://www.juntadeandalucia.es/agriculturaypesca/ifapa/riaweb/web/estacion/14/6 11 January 2022).

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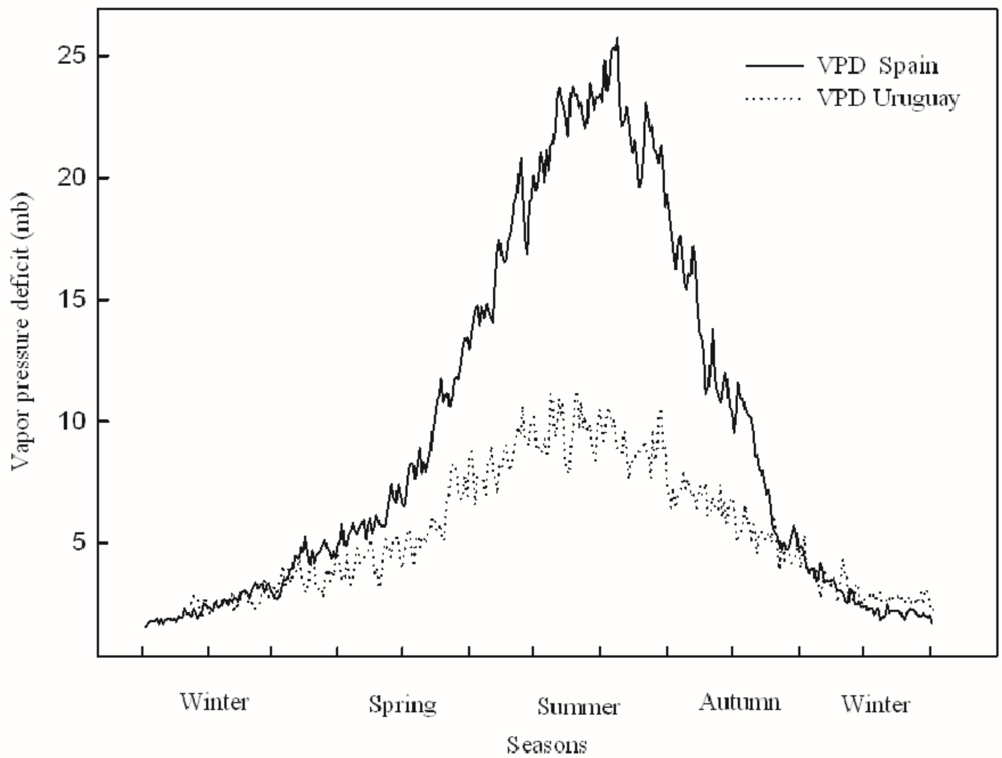

Figure S1: Annual vapor pressure deficit (mb) in Uruguay and in Spain. Data recorded by an automatic weather station at the experimental site in INIA Las Brujas as an average of the 2009–2020 period (available at http://www.inia.uy/gras/

Clima/Banco-datos-agroclimatico 11 January 2022) and by the weather station at Córdoba as an average of the 2001–2020 period (available at https://www.juntadeandalucia.es/agriculturaypesca/ ifapa/riaweb/web/estacion/14/6 11 January 2022).

3. <u>RESPONSE OF OLIVE FRUITS TO DROUGHT STRESS DETERMINES *COLLETOTRICHUM ACUTATUM* INFECTION PROGRESS</u>

3.1. RESUMEN

La antracnosis del olivo causada por *Colletotrichum* spp. es la enfermedad del olivo más extendida y económicamente importante a nivel mundial. Nuestra hipótesis es que el déficit hídrico moderado en olivo genera cambios anatómicos y bioquímicos que pueden conducir a una mayor tolerancia de los frutos a antracnosis. Se llevó a cabo un experimento de tres años en Arbequina bajo dos tratamientos de riego: con riego total (sin estrés hídrico) y sin riego (estrés hídrico moderado), desde fin del endurecimiento de hueso hasta la cosecha. A cosecha, los frutos fueron inoculados *in planta* e *in vitro* con un aislado de *C. acutatum*. Nuestros resultados mostraron que los frutos con estrés hídrico moderado presentaron una significativamente menor incidencia y severidad que los frutos sin estrés hídrico. Además, el estrés hídrico moderado aumentó la actividad de las enzimas relacionadas con la eliminación del peróxido de hidrógeno (catalasa y peroxidasa) y el grosor de la cutícula del fruto. En conjunto, estos factores contribuyeron a una mayor tolerancia a la infección por *C. acutatum*, tanto *in vitro* como *in planta*, lo que se refleja en diferentes áreas bajo la curva de progreso de la enfermedad (en promedio, un 45% y un 30% menos de incidencia y severidad, respectivamente). Estos hallazgos podrían explicar las diferencias observadas en la expresión de la enfermedad en olivares entre temporadas y manejos.

Palabras clave: Arbequina, espesor de cutícula, severidad de enfermedad, *Olea europaea* L., ROS

3.2. SUMMARY

Olive anthracnose caused by Colletotrichum spp. is the most spread and economically important olive fruit disease worldwide. We hypothesize that induced water deficit in olive trees generates anatomical and biochemical changes which contributes to anthracnose fruit rot tolerance. A three-year experiment was conducted in Arbequina under two irrigation treatments: fully irrigated (no water stress) and non-irrigated (moderate water stress), from pit hardening until harvest period. At harvest, fruits were inoculated in planta and in vitro with an isolate of C. acutatum. Our results showed that fruits with moderate water stress presented significantly lower disease incidence and severity than fruits without water stress. Additionally, moderate water stress increased the activity of the enzymes related to hydrogen peroxide scavenging (Catalase and Peroxidase) and cuticle fruit thickness. Altogether, these factors contributed to a greater tolerance to C. acutatum infection, both in vitro and in planta, reflected by different area under the disease progress curve (in average 45% and 30% lower incidence and severity, respectively). These findings could explain differences observed in disease expression in olive orchards among seasons and managements.

Keywords: Arbequina, cuticle thickness, disease severity, *Olea europaea* L., ROS

Response of olive fruits to drought stress determines *Colletotrichum acutatum* infection progress

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Abstract

Olive anthracnose caused by *Colletotrichum* spp. is the most spread and economically important olive fruit disease worldwide. We hypothesize that induced water deficit in olive trees generates anatomical and biochemical changes which contributes to anthracnose fruit rot tolerance. A three-year experiment was conducted in Arbequina under two irrigation treatments: fully irrigated (no water stress) and non-irrigated (moderate water stress), from pit hardening until harvest period. At harvest, fruits were inoculated *in planta* and *in vitro* with an isolate of *C. acutatum*. Our results showed that fruits with moderate water stress presented significantly lower disease incidence and severity than fruits without water stress. Additionally, moderate water stress increased the activity of the enzymes related to hydrogen peroxide scavenging (Catalase and Peroxidase) and cuticle fruit thickness. Altogether, these factors contributed to a greater tolerance to *C. acutatum* infection, both *in vitro* and *in planta,* reflected by different area under the disease progress curve (in average 45% and 30% lower incidence and severity, respectively). These findings could explain differences observed in disease expression in olive orchards among seasons and managements.

Key words: Arbequina, cuticle thickness, disease severity, *Olea europaea* L., ROS

1. Introduction

Olive (*Olea europaea* L.) cultivation has expanded to regions outside the Mediterranean Basin (Torres et al., 2017) such as those with humid climates like Uruguay. The highly conducive weather conditions for the development of fungal diseases, with annual relative humidity between 70% and 78%, annual rainfall above 1100 mm per year and moderate temperatures pose a challenge for disease management (Conde-Innamorato et al., 2019). Olive anthracnose caused by *Colletotrichum* spp. was reported for the first time in 1899 in Portugal by Almeida (Almeida, 1899) and it is the most spread and economically significant olive fruit disease worldwide (Moral et al., 2012; Talhinhas et al., 2018). In Uruguay *Colletotrichum acutatum* species complex is the prevalent one causing olive anthracnose (Moreira et al., 2021), producing direct damage to fruits and oil quality (Leoni et al., 2018; Romero et al., 2022). *Colletotrichum* can survive in different organs over the tree with mummified fruit as the most important site followed by leaves and branches (Ferronato et al., 2023). Chemical control is difficult, in part due to its rapid progress in the orchards. Furthermore, it is necessary to transform food systems towards the ultimate goal of sustainability by minimizing the use of chemical pesticides to reduce their negative impacts (Gliessman, 2016; Lechenet et al., 2017). Therefore, enhancing olive tree natural defenses is proposed as a strategy to reduce the disease progress and improve the effect exerted by chemical control.

In nature, plants are simultaneously exposed to a combination of biotic and abiotic stresses that limit crop yields (Atkinson & Urwin, 2012; Ramegowda & Senthil-Kumar, 2015). Survival under these stressful conditions depends on the plant's ability to perceive the stimulus and generate anatomical (Gomes et al., 2009), physiological and chemical changes (Al-Ghamdi, 2009; HongBo et al., 2005). At the anatomical level, water deficit generates responses meant to reduce water loss, for example reduction of epidermal and mesophyll cells size in olive leaves (Boughalleb & Hajlaoui, 2011) and increasements of the cuticle thickness (Bacelar et al., 2004). The cuticle in olive fruit is more developed than those of other commercial drupes with larger fruits, suggesting the critical role of the cuticle in olive fruit biology (Hammami & Rapoport, 2012). The cuticle of the fruits plays an essential role as a barrier to water loss, controlling temperature fluctuations, and protecting the cells against pathogens (Gomes et al., 2009; Kunst & Samuels, 2003; Riederer & Schreiber, 2001). The cuticle is also the first site of contact between the fungus and the plant (Diarte et al., 2019). Therefore it plays an important role in forming a physical barrier against the adhesion and penetration of the pathogen (Gomes et al., 2009).

The infection process of *C. acutatum* includes spore adhesion to the fruit cuticle, germination, and production of adhesive appressoria that are crucial for cuticle penetration, growth and fruit colonization. Inside the fruit, the pathogen grows through the mesocarp until it colonizes all host cells. Hyphae grows extensively inter- and intracellularly and across cell walls from one cell to the next, killing host cells and dissolving cell walls ahead of the infection (Gomes et al., 2012). Significant differences in the thickness of the cuticle have been observed in olive fruits of cultivars that exhibit different degrees of tolerance to *Colletotrichum*. For instance, the susceptible cultivar Galega shows a thinner cuticle than the cultivar Picual which has a moderate tolerance to this fungal disease (Gomes et al., 2009).

At the biochemical level, one of the most common and general defense mechanism to stress is the enzymatic response related to oxidative stress (Bartosz, 1997). During biotic and abiotic stress, reactive oxygen species (ROS) are generated that could damage plant metabolism (De Gara et al., 2003). ROS include mainly the superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), perhydroxyl radical ($HO_2^{\cdot}$), and singlet oxygen ($^1O_2$) (Bartosz, 1997). Hydrogen peroxide is one of the major and the most stable ROS that regulates basic acclimation, defense and developmental processes in plants (Ślesak et al., 2007). In order to overcome ROS toxic effects, plants respond to stress using different enzymatic and non-enzymatic antioxidative mechanisms to scavenge ROS (Ben Ahmed et al., 2009; Signorelli et al., 2013). Major ROS-scavenging mechanisms of plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) (Mittler, 2002) and peroxidase (POD) (HongBo et al., 2005), which are considered the main enzymatic systems for protecting cells against oxidative damage (De Gara et al., 2003). The balance between the formation and detoxification of activated oxygen species is critical to cell survival during periods of water stress (Zhang & Kirkham, 1994). Proline accumulation is a well-known adaptive mechanism in the olive tree against water stress conditions (Munns & Tester, 2008; Sofo et al., 2004). This osmolyte promotes water retention in the cytoplasm (Ashraf & Foolad, 2007; Parida & Das, 2005), prevents membrane damage and protein denaturation during severe drought stress (Ain-Lhout et al., 2001).

Plants exposed to mild drought stress activate basal defense responses that allow plants to respond to pathogen infection (Ramegowda & Senthil-Kumar, 2015). However, the simultaneous occurrence of drought and pathogen infection during plant growth generates complex pathways controlled by different signaling events resulting in a positive or negative impact of one stress over the other (Tippmann et al., 2006). We hypothesize that induced water deficit in olive trees generates anatomical and/or biochemical changes that can lead to greater tolerance of the fruits to disease caused by *C. acutatum*. The aim of this study was to describe changes in olive fruits subjected to the interaction of abiotic stress (water deficit) and biotic stress (challenged by *C. acutatum*) in Arbequina, the most planted cultivar in Uruguay. This study aims to comprehend the mechanisms behind differences in the disease expression observed in olive orchards among seasons and managements.

2. Materials and Methods

2.1. Experimental site

The experiment was established in mature olive trees of cultivar Arbequina in the orchard located at the National Agriculture Research Institute, INIA Las Brujas Experimental Station in Southern Uruguay (34°40'S; 56°20'W). The olive trees were planted in 2006 at a density of 416 trees per hectare. The soil has a fine textured A horizon, with a maximum depth of 30 cm, 2.9% soil organic matter and pH 6.3, corresponding to a Typic-Vertic Argiudolls soil according to the USDA classification (Durán et al., 2006). From December to April, no phytosanitary interventions were carried out for anthracnose fruit rot control. Detailed specifications of the experimental orchard management were previously reported (Conde-Innamorato et al., 2022). Meteorological data was retrieved from a Campbell automatic weather station located at INIA Las Brujas (data available at http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico) (Table 1).

Table 1 Average mean daily temperature (C°), effective precipitation (Rain, mm), monthly evapotranspiration (Evap., Class A pan evaporation, mm) and average mean daily relative humidity (RH, %) from December to April 2018 to 2022 and average historical data (1997-2017) at INIA Las Brujas, Southern Uruguay (S 34° 67', W 56° 37'). Data was recorded from an automatic weather station and is available at http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico

Year	Month	Mean temperature (°C)	Rain (mm)	Evap. (mm)	RH (%)
1997-2017	December	21.2	84.7	221.1	69.9
	January	22.9	85.4	223.7	71.6
	February	22.2	94.3	170.1	75.8

	March	20.2	95.6	146.4	77.9
	April	16.9	81.0	101.0	79.8
	December	20.0	150.1	225.0	70.0
	January	22.9	163.9	201.3	75.8
2018-2019	February	21.7	51.7	192.5	70.7
	March	19.5	92.9	160.1	75.8
	April	17.0	20.3	140.5	76.6
	December	21.2	69.4	248.8	63.5
	January	23.0	101.7	254.2	67.0
2020-2021	February	21.4	214.1	160.3	77.5
	March	20.5	85.4	164.0	78.2
	April	18.7	71.9	108.9	80.9
	December	22.1	7.3	287.1	68.1
	January	24.0	140.0	265.4	71.3
2021-2022	February	21.2	116.9	154.1	76.8
	March	18.8	50.5	151.2	76.3
	April	16.5	61.8	129.6	77.1

2.2. Experimental design

A completely randomized design with two water stress treatments and four replicates was used, where the experimental unit was one tree. The experiment was repeated for three seasons (2018-19, 2020-21, 2021-22). Two water stress treatments were imposed from the end of the pit hardening (ending December) until harvest (April): fully irrigated (no water stress) and non-irrigated (moderate water stress). The fully irrigated criterion was to apply water according to the maximum crop evapotranspiration (ETc) (Penman–Monteith equation) with a drip irrigation system with 4 L/h flow. In the non-irrigated treatment neither irrigation nor rain inputs occurred, so to prevent rainwater filtration the soil around the trees was covered with a plastic film. Tree water status was assessed by measuring the stem water potential (SWP, MPa) using a Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) every 15 days. In non-irrigated treatment, SWP values varied between -2 and -3 MPa depending on the year, considered as moderated drought stress in olives trees (Moriana et al., 2002).

Olive fruits from the irrigation experiment (4 trees per irrigation treatment) were inoculated with *Colletotrichum acutatum* isolate N°81 from the collection of the Plant Protection Department, Faculty of Agronomy, Universidad de la República, Uruguay. The isolate was obtained from diseased olives and was characterized (Montelongo et al., 2013). The inoculum for the *in vitro* and *in planta* assays was prepared by dislodging conidia from one week-old colony growing on PDA at 24 °C. The conidial water suspension was adjusted to 1 x 10^5 conidia ml^{-1} using a Neubauer chamber. A two-factorial experiment with water irrigation and inoculation treatment as experimental factors with four replications was conducted. The *in vitro* and *in planta* assays were established along the three seasons (2018-19, 2020-21, 2021-22).

2.3. Assessment of olive fruits

2.3.1. Disease incidence and severity

For *in vitro* assays, one hundred fruits per tree were collected at harvest and surface disinfected. Fifty fruits were inoculated by immersion for 30 minutes in the conidial suspension and other fifty were placed in sterile distilled water as control (Moral et al., 2008). Inoculated and control fruits were placed in separate trays and kept in a moist chamber at 24 °C to allow disease development. Disease incidence and severity were evaluated along one week. Disease severity was assessed with a 0-5 rating scale, from 0 = no symptoms to 5 = mummified fruit (Leoni et al., 2018) (Fig. 1). Disease severity index (DSI) was estimated as DSI=Σ (ni*i)/N, where ni=number of olives, with i level of disease, i=disease level with i going from 0 to 5, N=total number of olives evaluated. Disease incidence (DI) was estimated as a percentage of affected fruits. The area under the disease progress curve (AUDPC) was calculated and used for the statistical analysis.

Fig. 1 Anthracnose disease severity 0-5 rating scale of Arbequina olives (0: no symptoms, 1: <25% affected surface fruit, 2: 25–50%, 3: 50–75%, 4: 100%, 5: mummified fruit)

In the *planta* assays, two branches per tree (4 trees per treatment) were selected, containing approximately 100 fruits each. One branch was inoculated one month before harvest, by sprinkling a suspension of *C. acutatum* conidia prepared as mentioned previously, and the other branch was sprinkled with sterile water as a control. The branches were bagged to maintain the necessary humidity for 48 hours. Disease severity was evaluated 25 days after inoculation and the severity index was calculated. The area under the disease progress curve (AUDPC) was estimated and used for the statistical analysis.

2.3.2. Fruit characterization

Three hundred olives per tree were manually harvested. The maturity index, using a 0–7 ripening scale was determined in 100 fruits (Uceda & Frias, 1975). Fresh fruit weight and pulp/pit ratio were recorded in 20 olives. Fruit moisture content was determined in a sub-sample of 100 fruits, after grounding with a hammer mill and dried at 105 °C for 48 h.

2.3.3. Fruit anatomical study

The cuticle thickness of the fruit and the surface area of the epidermal cells were measured on pericarp anatomical cross-sections, in 2018-19 season (harvest: April 2019). From each tree, six fruits were collected and the outer portion of the fruit exposed to the sun was sliced without touching the pit. Immediately the slices were fixed in formalin: acetic acid: ethanol 70% = (FAA, 5:5:90) solution (Johansen, 1940). After 48hs, the slices were transferred to ethanol 70% and kept until further processing. Cubic pieces from the slices were infiltrated in paraffin and the blocks were sectioned in a manual rotary microtome (Trademarck SLEE, Model CUT 4062) to obtain 7 μm thick sections. Then, the cells were stained with Safranine-Fast Green (Johansen, 1940) and mounted with Entellan medium. Cuticle thickness was measured on six different and consecutive sections and the area of epidermic cell was quantified for ten epidermic cells per section. The images were obtained with DinoCapture 2.0 and processed with Image J software (https://imagej.es.download.it/download) (Fig. 4 a, c).

2.3.4. Fruit biochemical study

Ten sampled fruits per treatment were selected from the trays of the *in vitro* experiment at 48 hours after inoculation with *C. acutatum* and were frozen in liquid N2. Frozen fruits were peeled with a scalpel and the skin was ground in an ice-cold mortar. Crude enzyme extracts were obtained (Signorelli et al., 2013). Enzyme assays were performed from crude extracts after filtration through a Sephadex 25 column. The supernatant was used for the determination of Superoxide dismutase (EC 1.15.1.1) (SOD, U SOD.min^{-1}.mg protein^{-1}) (Sainz et al., 2010), Catalase (EC 1.11.1.6) (CAT, μmol H_2O_2.min^{-1}.mg protein^{-1}) (Beers & Sizer, 1952), Peroxidases (EC 1.11.1) (POD, μmol H_2O_2.min^{-1}.mg protein^{-1}) (Maehly & Chance, 1954), and Ascorbic peroxidases (EC 1.11.1.11) (APX, μmol ascorbic acid.min^{-1}.mg protein^{-1}) (Chen & Asada, 1989). One unit of SOD activity was the amount of enzyme activity that caused 50% inhibition of the initial reaction rate in the absence of enzyme. CAT activity was assayed by monitoring the decomposition of H_2O_2 spectrophotometrically at 240 nm (Aebi, 1984) and was calculated using the molar extinction coefficient of H_2O_2 (ε= 39.4 mM^{-1}.cm^{-1}); POD activity was determined spectrophotometrically at 460 nm and calculated using the molar extinction coefficient of 4-Aminoantipyrine (ε= 1.13 x 10^4 mM^{-1}.cm^{-1}); APX activity was determined by monitoring the oxidation of ascorbate by H_2O_2 at 290 nm and was calculated using the molar extinction coefficient of ascorbic acid (ε= 2.8 mM^{-1}.cm^{-1}). Other osmotic/oxidative stress indexes such as proline accumulation and membrane damage through measurement of TBARS was determined. Proline (nmoles/g DW) was extracted from 100 mg of fruits skin tissue with methanol–chloroform–water (12:5:1) and quantified (Borsani et al., 1999). Lipid peroxides were detected as TBARS (nmoles/g DW) by measurement of malonaldehyde absorbance at 532 nm and concentration was determined using an extinction coefficient of 156 mM^{-1}.cm^{-1} (Rustérucci et al., 1996). The fruit biochemical study was established along two seasons (2018-19 and 2020-21).

2.4. Statistical analysis

An analysis of variance (ANOVA) was performed for stem water potential, fruit parameters, fruit anatomical measurement considering irrigation treatment for each season. Tukey's test at $p \leq 0.05$ were calculated to separate means. DI and DSI *in vitro* and *in planta* values were used for estimating the area under the disease progress curve (AUDPC), and an analysis of variance (ANOVA) was performed for each season considering irrigation treatment, inoculation treatment and its interaction. As well, biochemical measurements were analyzed by ANOVA. In all cases the statistical software used was InfoStat version 2020 (INFOSTAT, 2020).

3. Results

3.1. Effect of water deficit on disease progress

Fruits from the non-irrigated treatment presented lower DI and DSI than fully irrigated ones in the *in vitro* and *in planta* assays, as well as a slower infection progress in the first days after inoculation resulting in lower AUDPC. Moreover, control fruits without inoculation remained healthy, without signs of latent infections or lower values depending on the season. Contrastingly, fully irrigated treatments showed *Colletotrichum* spp. latent infection (Table 2, Fig. 2 and 3).

Table 2 Area under the disease progress curve (AUDPC) for disease incidence (DI, %) and disease severity index (DSI) in fruits as a function of days after inoculation with *Colletotrichum acutatum* in Arbequina cultivar grown under non-irrigated and fully irrigated treatments, in 2019, 2021 and 2022 seasons for the *in vitro* assays, and in 2021 and 2022 seasons for the *in planta* assays

	Season	Disease incidence (DI)		Disease severity index (DSI)	
		Non-irrigated	Fully irrigated	Non-irrigated	Fully irrigated
AUDPC *in vitro*	2019	76.6 ^{b*}	141.8 ^{a}	7.0 ^{b}	11.1 ^{a}
	2021	96.8 ^{b}	143.8 ^{a}	9.5 ^{b}	10.9 ^{a}
	2022	234.2 ^{b}	417.2 ^{a}	7.6 ^{b}	13.9 ^{a}
AUDPC *in planta*	2021	227.5 ^{b}	412.3 ^{a}	9.2 ^{b}	17.1 ^{a}
	2022	37.4 ^{b}	368.1 ^{a}	1.2 ^{b}	14.0 ^{a}

* Different letters within the row indicate significant differences for each season separately at $p < 0.05$ (Tukey's test)

In the *in vitro* assays in 2019, DI 4 days after inoculation was 50% in the irrigated treatment while less than 20% in the non-irrigated one (Fig. 2 a). Regarding DSI, the infection progressed more slowly in the non-irrigated treatment and DSI values at the 4th day after inoculation were around one for non-irrigated and two for fully irrigated treatments (Fig. 2 b). As mentioned previously, in fruits without inoculation, those from fully irrigated showed some disease symptoms, due to latent infections by *Colletotrichum* spp., with an impact on the DSI. In 2021 the trend was similar (Fig. 2 c, d), DI was significantly lower in non-irrigated and on the 2nd day after inoculation represented the half of the fully irrigated treatment. The control fruits from fully irrigated presented 50% DI from natural infection while those from the non-irrigated treatment presented DI below 30%. Similar results were observed in 2022 where DI and DSI in the non-irrigated treatment were significantly lower than in the fully irrigated one (Fig. 2 e, f). Values were higher than in the previous seasons and the infection progressed faster.

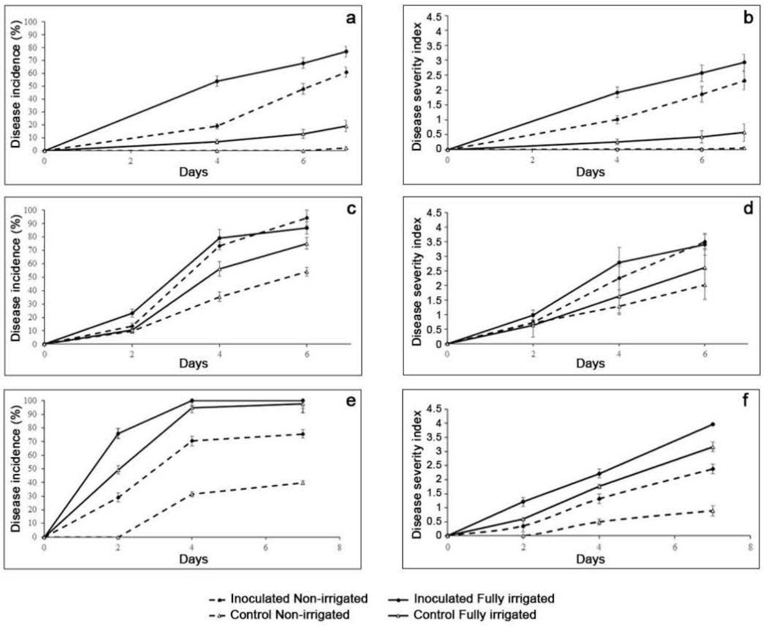

Fig. 2 Disease progress in fruits *in vitro* as a function of days after inoculation with *Colletotrichum acutatum* in Arbequina cultivar grown under non-irrigated and fully irrigated treatments. Disease incidence in 2019 (a), 2021 (c) and 2022 (e) seasons. Disease severity index in 2019 (b), 2021 (d) and 2022 (f) seasons. Bars represent the standard error

Regarding the *in planta* assays, in the 2019 season the infection did not occur because the temperature in the days following the inoculation was very low and avoid the progression of the infection. However, in 2021 and 2022 seasons the climatic conditions were favorable to infection and Arbequina showed a visible disease progress. In both seasons, fruits from the non-irrigated treatment presented lower DI (Fig. 3 a, c) and DSI (Fig. 3 b, d) than fully irrigated ones. Moreover, fruits from fully irrigated without inoculation also presented natural infection, while those from non-irrigated treatment not. In 2022 the effect was more evident with significantly higher DI and DSI in the fully irrigated treatment than in the non-irrigated one, whose fruits were barely affected (Fig. 3 c, d).

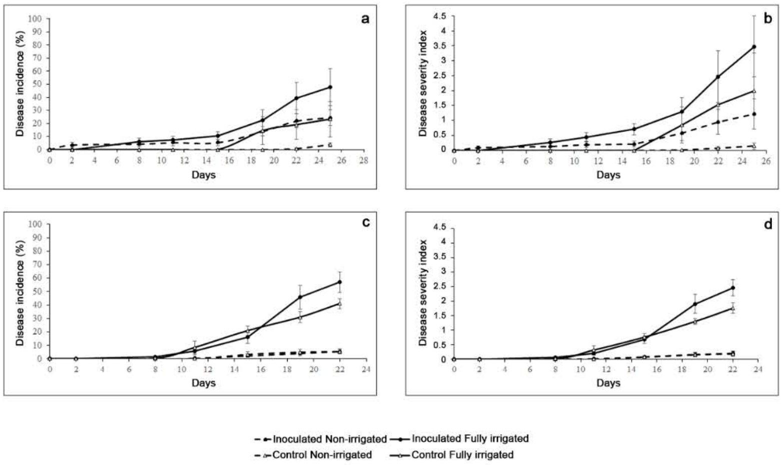

Fig. 3 Disease progress in fruits *in planta* as a function of days after inoculation with *Colletotrichum acutatum* in Arbequina cultivar grown under non-irrigated and fully irrigated treatments. Disease incidence in 2021 (a) and 2022 (c) seasons. Disease severity index in 2021 (b) and 2022 (d) seasons. Bars represent the standard error

3.2. Effect of water deficit on plant physiological and fruit parameters

The treatments applied during the experiment generated differences in the plants' water status, which was quantified by measuring the SWP. The SWP (MPa) values obtained by the non-irrigated treatment were more negative than those for the fully irrigated treatment in the three seasons (Table 3). The irrigation deficit achieved in plants also translated into a significantly lower fruit moisture content, at the end of the water deficit experiment, in the non-irrigated treatment respect to the fully irrigated, which presented at least 10% more water content than the non-irrigated treatment (Table 3). Fresh fruit weight and pulp/pit ratio were significantly higher in the fully irrigated treatment than in the non-irrigated one. The evolution of the maturity index was faster in non-irrigated treatment than in fully irrigated treatment in the first two seasons (Table 3).

Table 3 Average midday stem water potential (Ψstem), fruit moisture (%), fruit fresh weight (g), pulp/pit ratio, and maturity index of Arbequina cultivar at harvest, grown under fully irrigated and non-irrigated treatment at INIA Las Brujas, in 2019, 2021 and 2022 seasons

Evaluated parameters	2019		2021		2022	
	Non-irrigated	Fully irrigated	Non-irrigated	Fully irrigated	Non-irrigated	Fully irrigated
Midday stem water potential (Ψstem, MPa)	-2.0 ^{a*}	-1.1 ^{b}	-3.0 ^{a}	-1.6 ^{b}	-2.6 ^{a}	-1.8 ^{b}
Fruit moisture (%)	43.0 ^{b}	59.4 ^{a}	53.6 ^{b}	65.5 ^{a}	51.6 ^{b}	62.1 ^{a}

Fruit fresh weight (g)	0.73 ᵇ	1.13 ᵃ	2.03 ᵇ	2.64 ᵃ	0.82 ᵇ	2.80 ᵃ
Pulp/pit ratio	2.47 ᵇ	3.51 ᵃ	4.08 ᵇ	5.14 ᵃ	2.85 ᵇ	5.70 ᵃ
Maturity index	3.17 ᵃ	1.11 ᵇ	3.66 ᵃ	2.61 ᵇ	3.83 ᵃ	3.65 ᵃ

* Different letters within the row indicate significant differences for each season separately at $p < 0.05$ (Tukey's test)

3.3. Effect of water deficit on fruit anatomy

After four months of different irrigation treatments, fruit cuticle thickness was increased in plants under water restriction. Cuticle thickness (Fig. 4 a) of the fruits increased by 57% comparing the non-irrigated and the fully irrigated treatment (Fig. 4 b). Average values were 24 µm and 16 µm, in non-irrigated and fully irrigated treatment, respectively. The area of epidermal cells (Fig. 4 c) was significantly lower in the non-irrigated treatment (Fig. 4 d), with average values of 237 μm^2 and 291 μm^2, in non-irrigated and fully irrigated treatment, respectively.

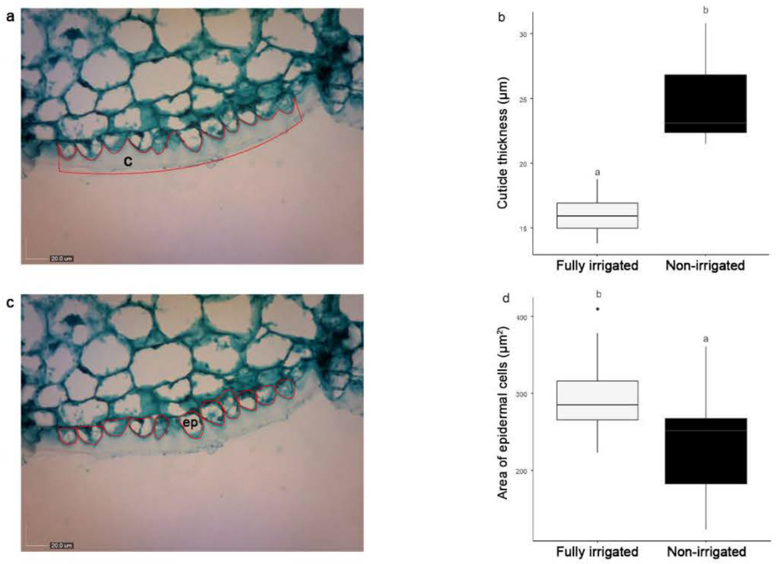

Fig. 4 Analysis of Arbequina fruit cuticle, grown under fully irrigation and non-irrigated treatments in the 2019 season. (a) Cross section of the fruit cuticle, red line indicates the measured area, (b) cuticle thickness values estimated with Image J program, (c) cross section of the epidermal cells, red line indicates the measured area, (d) area of epidermal cells values estimated with Image J program. Each value represents the mean ±SE of four replicates. Different letters indicate significant differences at $p < 0.05$ (Tukey's test). Abbreviations: cuticle (c); epidermal cells (ep)

3.4. Effect of water deficit on fruits oxidative stress and antioxidant enzymes

CAT activity in not inoculated plants was similar in fully irrigated and non-irrigated treatments. However, significant differences were observed when *C. acutatum* was inoculated. CAT activity was induced by the inoculation and this response was more evident under moderate water stress (Fig. 5 a). POD activity was also induced by the inoculation treatment, which was significantly higher in non-irrigated treatments (Fig. 5 b). Approximately 20% of induction in non-irrigated treatment could be assigned to the combination of biotic and abiotic stress. APX and SOD activities showed a non-consistent response pattern with a high variability between seasons or treatments (Supplementary Fig. 1).

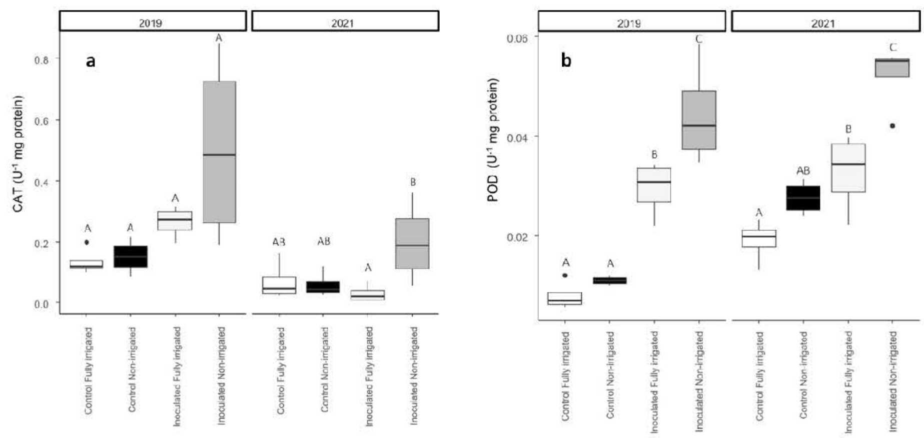

Fig. 5 Catalase (a) and Peroxidase (b) activity in olive fruits from Arbequina cultivar grown under non-irrigated and fully irrigated treatments, and with and without (control) inoculation with *Colletotrichum acutatum*, in two seasons. Different letters indicate significant differences between treatments in each season separately at $p < 0.05$ (Tukey's test). Abbreviations: Catalase (CAT), Peroxidase (POD)

In the first and in the second season, proline level was not significantly affected by treatments, with average values between 1.44 and 1.66 (µmoles/g DW) and 0.34 and 0.61 (µmoles/g DW) respectively (Fig. 6 a). Also average values of Oxidative membrane damage estimated through TBARS, did not present significant differences between treatments with average values between 2.6 and 5.9 (nmoles/g DW) in the first season and between 11.0 and 20.4 (nmoles/g DW) in the second one (Fig. 6 b). These results would indicate that water deficit treatments were not severe enough to induce a common osmolyte accumulation or severe damage in fruit cell membranes.

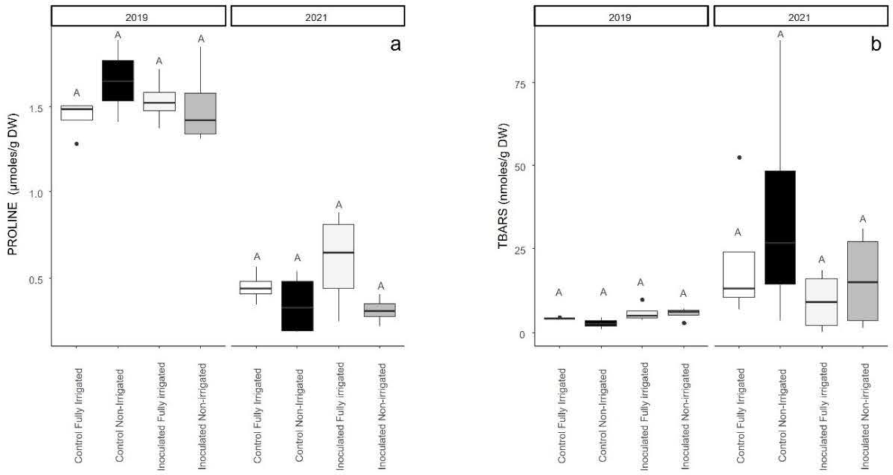

Fig. 6 Proline (a) and lipid peroxidation, detected as TBARS (b) in olive fruits from Arbequina cultivar grown under non-irrigated and fully irrigated treatments, with and without (control) *Colletotrichum acutatum* inoculation, in two seasons. Different letters indicate significant differences between treatments in each season separately at $p < 0.05$ (Tukey's test)

4. Discussion

The highly conducive weather conditions of Uruguay for development of olive anthracnose caused by *Colletotrichum* spp. threatens olive production while the use of chemical products is increasingly restricted (Gliessman, 2016). Therefore, enhancing olive tree natural defense by anatomical and/or biochemical mechanisms could play a role for mitigating disease outbreaks. This is particularly relevant for Arbequina, the most planted cultivar in Uruguay (Conde-Innamorato et al., 2019) and currently one of the most grown cultivars worldwide (Rubio-Valdés et al., 2022), under variable climatic conditions. In this regard, mild drought stress can be used to activate basal plant defense responses against pathogens (Ramegowda & Senthil-Kumar, 2015). The moderate water stress generated in our experiment caused structural changes in the olives such as a decrease in moisture content, in fruit weight and in pulp/pit ratio under moderate water stress (Table 3), in agreement with previous reports (Gucci et al., 2009; Hueso et al., 2019). It was hypothesized that these changes, as well as anatomic and biochemical changes, could lead to differences in disease tolerance when fruits were challenged by *C. acutatum*.

At the anatomical level, cuticle thickness was affected by water deficit, being 57% higher in stressed plants. Cuticle thickness is known to influence not only water loss but also reduce penetration by fungal pathogens. In this regard, significant differences in cuticle thickness from *C. acutatum*-susceptible and resistant cultivars were found (Gomes et al., 2012), and the authors concluded that a thicker cuticle of olive fruits could provide a mechanical obstacle to pathogens attack. This could help explain the lowered incidence and severity showed by fruits of Arbequina cultivar when grown under water deficit. Interestingly, deficit irrigation speed up the maturity, which was then associated with healthier (Motilva et al., 2000). Therefore, although susceptibility to *Colletotrichum* spp. has been observed to increase linearly with fruit maturity (Moral et al., 2008), this was not verified in our study (Fig. 2 and 3, Table 3). Under our experimental conditions, higher maturity was accompanied by a thicker cuticle, which could explain the higher tolerance to disease development.

Interestingly, the non-irrigated treatment with thicker cuticle also showed higher enzymatic activity. This supports that drought was not too intense to reduce biological activity and metabolism. Besides, this suggests that the association of healthier fruits already described with thicker cuticle should be extended to include the enzymatic response. We found that Arbequina´s fruits were able to induce CAT and POD activity when pathogen inoculation treatment was imposed, and this induction was higher in non-irrigated treatments (Fig. 5). Therefore, an interaction was seen in the response of peroxide-related enzymes to the combination of the stresses. Hydrogen peroxide is one of the major and the most stable ROS that regulates basic acclimation, defense and developmental processes in plants (Ślesak et al., 2007). Although other enzymes such as APX and SOD have shown in olive leaves an important role in the antioxidative defense mechanism against water stress effects (Ben Ahmed et al., 2009), in fruits, no clear response was observed (Supplementary Fig. 1). Drought stress triggered the antioxidant defense mechanism of olive trees, increasing antioxidative enzymes activities (Denaxa et al., 2020). Thus, the enzymatic response was modified according to the simultaneous occurrence of the biological stress posed by *C. acutaum* infection and water stress.

Regarding metabolites involved with cellular osmotic homeostasis, proline accumulation in olive leaves has been related with drought tolerance mechanisms (Ben Ahmed et al., 2009), but this observation was conditioned by the water stress level, with lower proline associated with low to moderate stress (Bacelar et al., 2009; Sofo et al., 2004). However, in our work there was no significant change in proline content in fruits that may be associated with a moderate drought stress (Fig. 6). This suggests that, in the case of olive fruits, proline metabolism may not be responsive to drought. A well-known target of ROS at cellular level is the membranes which are affected by oxidative damage. Irrigation reduced the oxidative damage on cell membranes by lipid peroxidation evaluated in leaves of cultivar Cobrançosa (Bacelar et al., 2007). In our work an induction of membrane damage through measurement of TBARS in response to drought stress was observed, although differences were not significant (Fig. 6).

In addition, although not mentioned here, we have already shown that polyphenols in Arbequina fruits were higher under moderate water deficit when compared with no water stress (Conde-Innamorato et al., 2022). Regarding non-enzymatic antioxidant systems, polyphenols play a crucial role in plant–environmental interactions and plant defense against pathogens (Šamec et al., 2021), increasing in plants in response to abiotic stresses (Cirilli et al., 2017). This finding could also explain the higher tolerance to *C. acutatum* infection registered in the non-irrigated treatment.

In conclusion, we have investigated anatomical and biochemical changes in fruits of Arbequina olive cultivar grown under simultaneous abiotic stress (moderate drought stress) and biotic stress caused by *C. acutatum*. Our results showed that the imposed drought stress generated an increase in the cuticle thickness and an induction of enzymes related to hydrogen peroxide scavenging, in particular POD and CAT. This enzymatic activity was also induced by the pathogen inoculation treatment and was higher in the non-irrigated treatment. Altogether, these factors contributed to a greater tolerance of fruits to infection by *C. acutatum* evaluated in *in vitro* and *in planta* assays, obtained from trees under moderate drought. These findings could explain differences in disease expression observed in olive orchards among seasons and managements (irrigation strategy, permanent or temporary cover crops under the trees). A moderate water stress could lead to a slower progress of fungal infection and disease development, enabling a wider harvest window for growers, since for obtaining Arbequina extra virgin olive oil, anthracnose fruit rot incidence should be lower than 13% (Leoni et al., 2018). Future research will focus on studying the expression of defense related genes in susceptible and resistant cultivars to elucidate the molecular basis of defense mechanisms involved in this plant-pathogen interaction.

Supplementary Materials: Supplementary Fig. 1 Ascorbate peroxidase (a) and Superoxide dismutase (b) activity in olive fruits from Arbequina cultivar grown under non-irrigated and fully irrigated treatments, and with inoculation with Colletotrichum acutatum and without inoculated (control), in two seasons. Different letters indicate significant differences between treatments in each season separately at $p < 0.05$ (Tukey's test). Abbreviations: Ascorbate peroxidase (APX), Superoxide dismutase (SOD)

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4. *COLLETOTRICHUM ACUTATUM* INFECTION IN ARBEQUINA OLIVE FRUITS UNDER SEVERE DROUGHT STRESS

4.1. RESUMEN

La antracnosis del olivo causada por *Colletotrichum* spp. es la enfermedad del olivo más extendida y de importancia económica en todo el mundo. El olivo es un cultivo con alta tolerancia al déficit hídrico. Sin embargo, la duración e intensidad del déficit hídrico condiciona la respuesta productiva y fitosanitaria del olivo. Las plantas expuestas a un estrés por sequía moderado activan respuestas de defensa basales que les permiten reaccionar contra la infección por patógenos. Particularmente en frutos de olivo, la sequía moderada contribuyó a aumentar la tolerancia a la infección por *Colletotrichum acutatum*. Sin embargo, se desconoce el efecto del déficit hídrico severo sobre los parámetros de la fruta y el progreso de la enfermedad. Para dilucidar estos efectos, se estableció un experimento en seis lisímetros de drenaje en condiciones de riego controlado (protegidos de la lluvia) con olivos del cultivar Arbequina a lo largo de dos temporadas consecutivas. Se impusieron dos tratamientos: totalmente irrigado y no irrigado, desde el final del endurecimiento del hueso hasta la cosecha. En la cosecha, se evaluó la calidad de las aceitunas y se inoculó una submuestra con un aislado de *C. acutatum* para evaluar el progreso de la enfermedad in vitro. La humedad de la fruta fue significativamente diferente entre tratamientos, en no irrigado fue $<43\%$ y en totalmente irrigado $>64\%$. El rendimiento de frutos (kg/árbol) se duplicó y el peso de frutos frescos (g) se triplicó en el tratamiento totalmente irrigado en comparación con el no irrigado. Además, el contenido de rendimiento de aceite en base seca mostró valores más altos en el tratamiento totalmente irrigado. El índice de severidad de la enfermedad al sexto día después de la inoculación fue la mitad en el tratamiento no irrigado en comparación con el totalmente irrigado. Se encontró que el estrés por déficit hídrico severo reduce significativamente el contenido graso, pero los frutos mostraron una mayor tolerancia a la infección por antracnosis. Estos resultados subrayan la importancia de estudiar cómo el estrés abiótico, como la sequía, puede promover mecanismos de defensa en las plantas.

Palabras clave: Antracnosis, Severidad de enfermedad, Lisímetro, *Olea europaea* L.

4.2. SUMMARY

Olive anthracnose caused by Colletotrichum spp. is the most spread and economically significant olive fruit disease worldwide. The olive tree is a crop with high tolerance to water deficit. However, the length and intensity of water deficit determine olive productive and phytosanitary responses. Plants exposed to moderate drought stress activate basal defense responses allowing plants to react against pathogen infection. Particularly in olive fruits, moderate drought contributed to increase tolerance to Colletotrichum acutatum infection. However, the effect of severe drought on fruit parameters and disease progress is unknown. To unravel these effects, an experiment in six drainage lysimeters under controlled irrigation conditions (rain-out shelter) with Arbequina olive trees was established along two consecutive seasons. Two treatments were imposed: fully irrigated and non-irrigated from the end of pit hardening until harvest. At harvest olives were assessed for fruit quality and a subsample was inoculated with an isolate of C. acutatum to evaluate disease progress in vitro. Fruit moisture was significantly different among treatments, in non-irrigated was <43% and in fully irrigated >64%. Fruit yield (kg/tree) doubled and fresh fruit weight (g) tripled in the fully irrigated treatment compared to the non-irrigated one. Also, oil yield content in dry bases showed higher values in fully irrigated treatment. Disease severity progress at 6th day after inoculation was half in non-irrigated treatment compared with fully irrigated one. Severe drought stress was found to significantly reduce olive oil yield, but the fruits showed increased tolerance to anthracnose fruit rot. These results underscore the importance of studying how abiotic stress, such as drought, can elicit defense mechanisms in plants.

Keywords: Anthracnose, Disease severity, Lysimeter, Oil content, *Olea europaea* L.

Colletotrichum acutatum infection in Arbequina olive fruits under severe drought stress

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Abstract

Olive anthracnose caused by *Colletotrichum* spp. is the most spread and economically significant olive fruit disease worldwide. The olive tree is a crop with high tolerance to water deficit. However, the length and intensity of water deficit determine olive productive and phytosanitary responses. Plants exposed to moderate drought stress activate basal defense responses allowing plants to react against pathogen infection. Particularly in olive fruits, moderate drought contributed to increase tolerance to *Colletotrichum acutatum* infection. However, the effect of severe drought on fruit parameters and disease progress is unknown. To unravel these effects, an experiment in six drainage lysimeters under controlled irrigation conditions (rain-out shelter) with Arbequina olive trees was established along two consecutive seasons. Two treatments were imposed: fully irrigated and non-irrigated from the end of pit hardening until harvest. At harvest olives were assessed for fruit quality and a subsample was inoculated with an isolate of *C. acutatum* to evaluate disease progress *in vitro*. Fruit moisture was significantly different among treatments, in non-irrigated was <43% and in fully irrigated >64%. Fruit yield (kg/tree) doubled and fresh fruit weight (g) tripled in the fully irrigated treatment compared to the non-irrigated one. Also, oil yield content in dry bases showed higher values in fully irrigated treatment. Disease severity progress at 6th day after inoculation was half in non-irrigated treatment compared with fully irrigated one. Severe drought stress was found to significantly reduce olive oil yield, but the fruits showed increased tolerance to anthracnose fruit rot. These results underscore the importance of studying how abiotic stress, such as drought, can elicit defense mechanisms in plants.

Keywords: Anthracnose, Disease severity, Lysimeter, Oil content, *Olea europaea* L.

INTRODUCTION

Olive trees are highly tolerant to water deficit (Connor and Fereres, 2010). The effect of water deficit was found to be strongly related to phenological stage and environmental conditions (El Yamani et al., 2019). Moreover, the water deficit degree and the duration conditionate the tree productive response, affecting fruit weight (Lavee et al., 2007) and oil yield (Pierantozzi et al., 2020). In addition, plants exposed to moderate drought stress activate basal defense responses, allowing plants to respond against pathogen infection (Ramegowda and Senthil-Kumar, 2015). Some of the mechanisms that may be involved are cuticle reinforcement to reduce water loss (Bacelar et al., 2004) and increasing activity of antioxidative enzymes (Denaxa et al., 2020).

Olive anthracnose caused by *Colletotrichum* spp. it is the most spread and economically significant olive fruit disease worldwide (Talhinhas et al., 2018). This pathogen causes direct losses in fruit yield and oil quality (Leoni et al., 2018; Romero et al., 2022). In Uruguay, many *Colletotrichum* species belonging to *C. acutatum* and *C. gloeosporioides* complexes cause anthracnose, being *C. acutatum* s.s. the prevalent one (Moreira et al., 2021). The expression of this disease requires environmental conditions such as high relative humidity and frequent rainfall, as well as temperatures above 10°C (Moral et al., 2012; Moreira et al., 2022). Most studies on drought stress have focused on arid environments, where the olive anthracnose incidence is low. But there is a lack of information on humid and low VPD (vapor pressure deficit) climates, such as Uruguayan one. While water deficits are more common in arid zones, our country has a highly variable climate where drought events can occur prior to periods of high humidity, suitable for anthracnose development. We want to evaluate whether drought stress prior to conducive conditions for anthracnose development can activate the defenses of the olive tree, increasing its tolerance towards the disease.

A study conducted under a humid-temperate climate showed that moderate drought stress increased the tolerance to *C. acutatum* in fruits of the Arbequina cultivar (Conde-Innamorato et al., submitted). However, the effect of severe drought on infection progress remained unknown. To investigate the impact of severe drought stress on fruit productive parameters and anthracnose infection response, we conducted an experiment under controlled conditions using lysimeters with rainout shelters to impose severe and prolonged deficit irrigation conditions on four-year-old olive trees.

MATERIALS AND METHODS

Plant material and experimental site

The experiment was established in six drainage lysimeters (1.9 × 0.9 × 1.35 m depth) located at the National Agriculture Research Institute, INIA-Las Brujas Experimental Station in Southern Uruguay (34°40'S; 56°20'W). These devices were protected from rain by a rain-out shelter, which would automatically close every time rain exceeded 3 mm (Puppo et al., 2014). Lysimeters were surrounded by irrigated olives to avoid an "oasis" effect as a consequence of advective processes. The separation between lysimeters with olive trees simulated a plantation framework of 2.5 × 5.5 m (Puppo et al., 2014). The soil is silty clay loam texture.

Two irrigation treatments were imposed in four-year-old olive trees Arbequina cultivar, fully and non-irrigated, from the end of the pit hardening until harvest. In the fully irrigated, the criterion was to apply water according to the maximum crop evapotranspiration (ETc) (Allen et al., 1998). A randomized complete design with two irrigation treatments and three replicates was used, where the experimental unit was one tree. The experiment was repeated for two consecutive seasons (2020 and 2021). Drip irrigation with 2 drippers per tree with a flow of 4 l/h was installed in treatment that received 100% of the crop water requirement (CWR) according to the FAO methodology (Allen et al., 1998). This treatment (100%) provided all crop water requirements throughout the crop cycle, allowing maximum crop production. A treatment without irrigation was used, where irrigation was applied only to avoid reaching permanent wilting point according water content in the soil profile.

The climate of the study area, according to Köppen-Geiger climate classification system, classifies as Warm temperate - fully humid (Cfa). In the first evaluated season, from January to April 2020 the relative humidity was lower, the evapotranspiration and the deficit pressure value was higher than in the second season (Table 1).

Table 1. Average mean daily temperature (C°), monthly evapotranspiration (Evap., Penman, mm), average mean daily relative humidity (RH, %) and average vapor pressure deficit (VPD, mb) from January to April 2020, 2021 and average historical data (1997-2019) at INIA Las Brujas, Southern Uruguay (S 34° 67', W 56° 37'). Meteorological data was retrieved from a Campbell automatic weather station located at INIA Las Brujas (data available at http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico).

Year	Month	Mean daily temperature (°C)	Monthly Evap. (mm)	Mean daily RH (%)	VPD (mb)
2020	January	22.7	172.5	64.1	9.9
	February	22.7	152.0	64.5	10.0
	March	22.2	110.3	72.7	7.5
	April	17.2	59.3	77.0	4.5
2021	January	23.0	163.3	67.0	9.4
	February	21.4	109.0	77.5	5.8
	March	20.5	91.9	78.2	5.2
	April	18.7	57.7	80.9	4.2
1997-2019	January	23.1	173.6	70.8	Nd[a]
	February	22.1	132.4	70.5	
	March	19.9	111.6	73.4	
	April	18.1	60.0	78.4	

[a]Nd= no data

Water status

Soil water content variation in backfilled soil of each lysimeter was measured with a neutron probe (model 503 DR Hydroprobe). A 1-meter aluminium access tube was installed in each lysimeter to monitor soil moisture through the whole depth. This tube was located 0.20 m from the surface drip line (orthogonally), and at an equal distance from the two nearest emitters. Measurements at the depths 0.20; 0.40; 0.60 and 0.80 m, were conducted twice a week, before irrigation, following published methodology (García Petillo and Castel, 2007; Morales et al., 2010). The neutron probe was calibrated with the gravimetric method for each measured depth and then multiplied by bulk density to obtain volumetric moisture content (Haverkamp et al., 1984). Irrigation and drainage water volumes in each lysimeter were recorded on a daily basis. There were no rain entries due to the rain-out shelter.

Tree water status was assessed by measuring the stem water potential (SWP) using a Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) every 15 days in each tree between noon to 2:00 pm (local time) in mature leaves, from the middle of the branch that were exposed to the sun, from the end of pit hardening until harvest (Scholander et al., 1965). The water potential was measured up to -4 MPa, a limit determined for users' safety. The measured SWP values were accumulated over the irrigation period and the cumulative leaf water potential (CLWP) was calculated to compare the level of drought stress throughout the entire experiment (Gucci et al., 2019). Along the whole day SWP and stomatal conductance measured with a METER Group SC-1 Porometer were determined during solar time in each season, every two hours.

Fruit and physiological parameters

Three hundred fruits per tree were manually harvested (end of March 2020 and beginning of April 2021) and fruit yield (kg/tree) was registered. The maturity index, using a 0–7 ripening scale was determined in 100 fruits (Uceda and Frias, 1975). Fresh fruit weight was recorded in 20 olives. Fruit moisture content was determined in a sub-sample of 100 fruits, after grounding with a hammer mill and dried at 105 °C for 48 h. Oil content in dry basis (%) was measured per tree on a sample of 200 g of olives at harvest following the Soxhlet method.

Fungal inoculation

Olives collected for pathogenicity tests were inoculated with a *Colletotrichum acutatum* isolate N°81 from the collection of the Plant Protection Department, Faculty of Agronomy, Universidad de la República, Uruguay, by immersion for 30 minutes in the conidial suspension. The inoculum was prepared by dislodging in sterile water conidia from one week-old colony growing on PDA at 24 °C. The conidial water suspension was adjusted to 1 x 10^5 conidia ml^{-1} using a Neubauer chamber. One hundred fruits per tree were collected at harvest and the surface disinfected. Fifty fruits were inoculated and the other fifty fruits were placed in sterile distilled water as control (Moral et al., 2008). A two-factorial experiment with water irrigation and inoculation treatment as experimental factors with three replications was established in two seasons (2020 and 2021).

Inoculated and control fruits were placed in separate trays and kept in a moist chamber at 24 °C to allow disease development. Disease severity index was assessed along one week using a 0-5 rating scale, with 0 = no symptoms and 5 = mummified fruit (Leoni et al., 2018). Disease severity index was estimated as DSI=Σ (ni*i)/N, where ni=number of olives with i level of disease, i=disease level with i going from 0 to 5, N=total number of olives evaluated. Finally, the area under the disease progress curve (AUDPC) was estimated for each treatment and used for the statistical analysis.

Statistical analysis

An analysis of variance (ANOVA) was performed for stem water potential and productive parameters considering irrigation treatment for both seasons. Disease incidence and disease severity index values were used for estimating the area under the disease progress curve (AUDPC), and an analysis of variance (ANOVA) was performed. Tukey's test at $p \leq 0.05$ was calculated to separate means. In all cases the statistical software used was InfoStat version 2020 (INFOSTAT, 2020).

RESULTS

Water status

The irrigation treatments established in the experiment generate differences in the water status of the trees. The SWP values obtained by the non-irrigated treatment were more negative than those for the fully irrigated treatment along the experiment in both seasons, lower than -3.4 MPa in non-irrigated treatment and more than -1.8 MPa in the fully irrigated treatment (Table 2). The CLWP was −408 MPa in the non-irrigated treatment while the fully irrigated treatment −174 MPa. The soil water content readings taken by neutron probe at soil profile in the two treatments in the two studied season are shown in Figure 1. Treatment of fully irrigation kept the soil moisture content near upper limit, while in the other treatments the soil moisture decreased as the deficit conditions occurred. It can be observed from January 12 to April 20 that soil water content kept values near 160 mm of water. In the non-irrigated treatment, soil moisture kept around 110 mm along all growing season, causing severe stress in the trees.

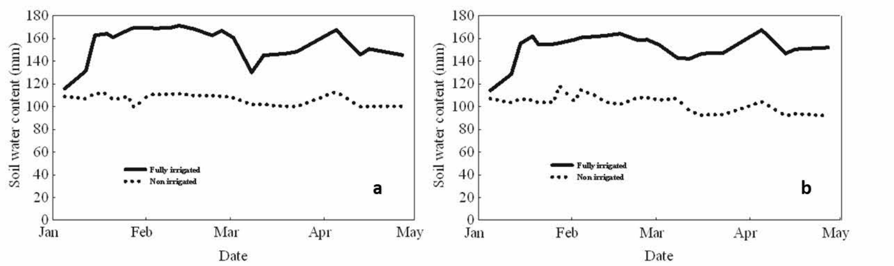

Figure 1. Soil water content in the soil profile in 2020 (a) and in 2021 (b), in lysimeters located at INIA Las Brujas, Southern Uruguay.

Daily measurement of SWP dynamics were significantly lower in the non-irrigated treatment during the day than in fully irrigated one (-3.5 MPa and -1.0, respectively). The lower value presented in fully irrigated treatment was achieved at 15 h (Figure 2 a). The stomatal conductance was significantly lower also in the non-irrigated treatment with values between 150 and 200 mmol.m^2.seg, while in the fully irrigated treatment remained up to 250, presenting a natural decrease at 17 h (late afternoon) (Figure 2 b). The trend was similar in the two years, that is why the two years are presented together.

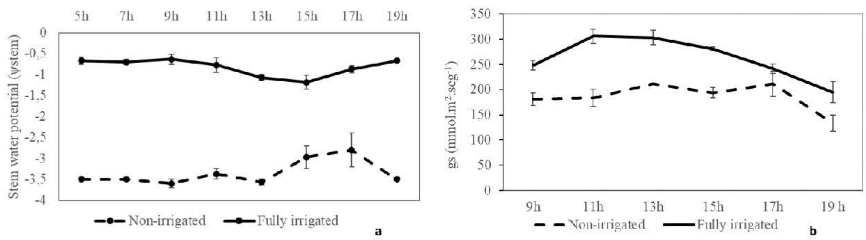

Figure 2. Daily evolution of stem water potential (ψstem, MPa) (a) and stomatal conductance (gs) (mmol m^{-2}s^{-1}) (b) of Arbequina cultivar in February. Treatments included non-irrigated (dotted line) and fully irrigated (solid line). Values are the means of 2020 and 2021 seasons. Bars represent the standard error.

Fruit and physiological parameters

Fruit parameters presented significant differences between treatments in both seasons after four months of different irrigation treatments (Table 2). Fruit yield (kg/tree) was doubled in fully irrigated treatment in comparison with non-irrigated one in both seasons (Table 2). Yield increases between seasons were due to tree growth, but the differences between treatments remained constant. The maturity index at harvest was significantly higher in non-irrigated treatment than in fully irrigated in both seasons. Non-irrigated treatment showed significantly less fruit moisture than fully irrigated (less than 43% and more than 64%, respectively). The fully irrigated treatment resulted in a significantly higher fresh fruit weight compared to the non-irrigated. In terms of oil yield content in dry bases, the non-irrigated treatment had lower values, with a 13.5 percentage point difference in 2020 and 9.2 percentage points lower in 2021. However, there were no differences between treatments in fresh bases. These results suggest that irrigation significantly improves fruit weight, but non-irrigated trees have a higher oil concentration.

Table 2. Average midday stem water potential (Ψstem), fruit yield (kg/tree), fruit moisture (%), fruit fresh weight (g), maturity index, oil content (%, dry and fresh weight basis) and fruit oil yield (kg/tree) of Arbequina cultivar at harvest, grown under fully irrigated and non-irrigated treatment at INIA Las Brujas, in 2020 and 2021 seasons.

Evaluated parameters	2020		2021	
	Non-irrigated	Fully irrigated	Non-irrigated	Fully irrigated
Midday stem water potential (Ψstem)	-3.6 a*	-1.0 b	-3.4 a	-1.8 b
Fruit yield (kg/tree)	3.2 c	6.7 b	6.7 b	13.0 a
Fruit moisture (%)	42.8 b	65.7 a	41.0 b	64.3 a
Fruit fresh weight (g)	0.6 c	1.9 a	0.6 c	1.4 b
Maturity index	3.8 a	2.1 b	4.0 a	1.0 c
Oil content (% DWB)	23.0 b	36.5 a	22.6 b	31.8 a
Oil content (% FWB)	13.2 a	12.5 a	13.1 a	11.3 a
Fruit oil yield (kg/tree)	0.4 c	0.8 b	0.9 b	1.5 a

* Different letters within the row indicate significant differences for both seasons at $p < 0.05$ (Tukey's test).

Disease progress

Analyzing the AUDPC it was observed significantly lower values for the non-irrigated treatment than in the fully irrigated one, for both DI and DSI (Table 3). Fruits from the non-irrigated treatment presented significantly lower disease incidence (DI) and disease severity index (DSI) than fruits from the fully irrigated one in both seasons (Figure 3). In the first season, DI eight days after inoculation in the fully irrigated treatment reached values around 100% and DSI reached values around four, while in the non-irrigated treatment fruits remained healthy, as well as the control without inoculation (Figure 3 a, b). In the second season the DI and DSI were higher than in the first one. Six days after inoculation there was a 100% DI and almost DSI=4 in irrigated treatment. While in the non-irrigated treatment disease development represented half of the fully irrigated treatment, around 60% DI and DSI=2 (Figure 3 c, d). In the control treatment (without inoculation), the fruits showed natural *Colletotrichum* spp. infection. The control fruits from fully irrigated treatment presented DI values around 40% and DSI values around two, while in the non-irrigated control represented half of the fully irrigated treatment (Figure 3 c, d).

Table 3. Area under the disease progress curve (AUDPC) of disease incidence (DI) and disease severity index (DSI) in fruits as a function of days after inoculation with *Colletotrichum acutatum* in Arbequina cultivar grown under non-irrigated and fully irrigated treatments, in 2020 and 2021 seasons.

	Season	Disease incidence		Disease severity index	
		Non-irrigated	Fully irrigated	Non-irrigated	Fully irrigated
AUDPC	2020	15.48 b*	372.96 a	0.45 b	13.28 a
	2021	301.84 b	555.13 a	10.46 b	19.91 a

* Different letters within the row indicate significant differences for each season separately at $p < 0.05$ (Tukey's test).

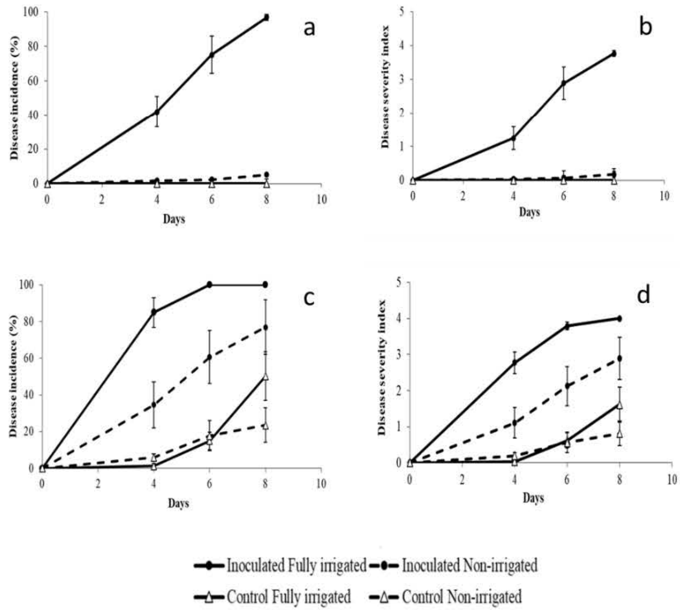

Figure 3. Disease progress in fruits *in vitro* as a function of days after inoculation with *Colletotrichum acutatum* in Arbequina cultivar grown under non-irrigated and fully irrigated treatments. Disease incidence in 2020 (a) and 2021 (c) seasons. Disease severity index in 2020 (b) and 2021 (d) seasons. Bars represent the standard error.

DISCUSSION

Olive trees have a high capacity to grow under water scarcity conditions. However, differences in stress intensity determine the productive response of trees (Pierantozzi et al., 2020). It has been reported that a moderate drought stress (Ψstem levels > −2.21 MPa) during lipogenesis do not reduced oil content (Hueso et al., 2019; Conde-Innamorato et al., 2022). This is because stomatal conductance and therefore photosynthesis are processes affected at lower water potentials (El Yamani et al., 2019). Our results show that the trees without irrigation presented severe drought stress since they reached steam water potentials below -4 MPa, accompanied by a reduction in stomatal conductance (Figure 2). A decrease in oil content in dry basis was observed in non-irrigated treatment (Table 2), same as reported in other works when stem water potential was near −4 MPa (Gómez-Rico et al., 2007; Gómez-del-Campo, 2013). Severe stress reduced oil yield per tree, probably because this condition limits the generation of carbon skeletons available for lipogenesis. Fruit maturity was anticipated under stress conditions as has been reported for several cultivars (Inglese et al., 1996; Motilva et al., 2000; Lavee and Wodner, 2004; Conde-Innamorato et al., 2022). This response is dependent on the fruit load of the trees (Trentacoste et al., 2010), which was lowest in trees without irrigation (Trentacoste et al., 2010) (Table 2).

The intensity of water deficit also determines fruit health response (Ramegowda and Senthil-Kumar, 2015). Moderate drought stress during the lipogenesis period promoted tolerance to anthracnose fruit rot, explained by increased fruit cuticle thickness, higher fruit phenols content and increased in antioxidant activity in Arbequina olive fruits (Conde-Innamorato et al., submitted). In the present study the severe drought stress magnified the tolerance to anthracnose rot, compared to the response under a moderate stress, probably because the defense mechanisms increased with the more intense stress. Climatic conditions of the year also affected disease development. In the first season (2020) the relative humidity was lower than the historical average and there were no conducive conditions for disease development (Table 1). This was evidenced by the fact that the fruits of the control treatment were not infected. Also, no disease symptoms were observed in fruits from non-irrigated treatment, while in fully irrigated treatment were (Figure 3 a,b). The second season (2021) was more conducive for the disease development, with higher relative humidity than the historical average (Table 1). The fruits from the control treatment showed natural *Colletotrichum* spp. infection, and in the inoculated treatment, fruits from the non-irrigated treatment presented lower severity index than those from the fully irrigated treatment (Figure 3 c,d).

Olive trees with moderate stress have been seen in clayey soils in Uruguay, with a moderate to high water storage capacity (Conde-Innamorato et al., 2022). However, the degree of stress generated in the present work is frequently observed in Eastern Uruguay, where most of the olive groves are established. In this region soils are sandy loam, with low water retention capacity, leading to severe drought stress evidenced by wrinkled fruits. Moreover, in Eastern Uruguay, agroclimatic conditions are more conducive to anthracnose (Moreira et al., 2022). The great climatic variability of Uruguay during the fruit growth development period, with frequent water deficit periods but also environmental conditions conducive to anthracnose, highlights the relevance of appropriate water management in olive orchards. This management could be achieved by combining irrigation and soil mulching (either hay/straw or permanent cover crop), enhancing soil biology and overall soil health to promote plant productivity and healthy fruits (Bernaschina et al., 2023).

In summary, severe drought stress, from the end of pit hardening until harvest in Arbequina fruits, reduces significantly olive oil yield, but increases tolerance to anthracnose fruit rot. Future research that evaluates the commitment between economic loss and health performance will be important. In addition, deepen the knowledge of the defense mechanisms in response to abiotic stress, as a tool of disease tolerance, is needed.

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5. DISCUSIÓN

Se generó información sobre el efecto del déficit hídrico en clima húmedo, donde la precipitación media anual es de 1200 mm y la humedad relativa mayor a 70 % (Castaño et al., 2011), y donde el DPV es menor que en la zona originaria del cultivo del olivo, la cuenca mediterránea (Conde-Innamorato et al., 2022). El experimento a campo fue realizado en suelos poco profundos de baja capacidad de almacenaje de agua, pero franco-arcillosos, con moderada a alta capacidad de retención de agua (Durán et al., 2006). Es importante considerar el nivel del déficit hídrico, el tiempo de duración y el momento del ciclo en el que se produce el déficit, ya que condicionan la respuesta vegetativa y productiva de la planta (Pierantozzi et al., 2020). A campo se logró generar un déficit hídrico moderado durante la fase de lipogénesis luego de cuatro meses sin aporte de agua, cuando los árboles alcanzaron potenciales xilemáticos de -2,5 MPa en el cultivar Arbequina y de -2,8 MPa en el cultivar Frantoio. El contenido de humedad de los frutos de Arbequina en el tratamiento con déficit hídrico fue un 12 % menor que en el tratamiento regado y en Frantoio un 8 % menor. Esta disminución favorece la extractabilidad del aceite en almazara (Ellis y Gámbaro, 2018, Fernández et al., 2018).

El peso fresco de los frutos aumentó con el mejor estado hídrico en ambos cultivares y en ambas temporadas, al igual que el peso fresco de la pulpa y la relación pulpa/hueso, presentando regresiones significativas en todos los casos. Esta respuesta coincide con reportes previos sobre el efecto del riego en los parámetros del fruto (Gómez-Rico et al., 2007, Lavee et al., 2007). Por otra parte, el déficit hídrico generó un engrosamiento de la cutícula; esta respuesta se asocia a la reducción de pérdidas de agua por evapotranspiración (Bacelar et al., 2004). También se observó un aumento en el contenido de polifenoles en los frutos, siendo similar en ambos cultivares, a pesar de que Frantoio tiene mayor contenido de fenoles en el aceite que Arbequina (Conde-Innamorato et al., 2022). La madurez de los frutos se adelantó en respuesta al déficit hídrico, en coincidencia con lo reportado por Inglese et al. (1996) y Motilva et al. (2000), si bien el principal factor que afecta la madurez de los frutos es la carga del árbol (Trentacoste et al., 2010). La acumulación de aceite comienza inmediatamente después del endurecimiento del hueso (Beltran et al., 2017), momento en el cual se llevó a cabo el experimento. Sin embargo, no se observó una disminución en el rendimiento graso de los frutos (en base seca) en respuesta al déficit hídrico, al igual que lo reportado por Ahumada-Orellana et al. (2017) y Hueso et al. (2019). El déficit hídrico no afectó el rendimiento (kg/pl) de Arbequina, aunque sí disminuyó en el cultivar Frantoio.

Hubo diferencias en las respuestas al déficit hídrico según su magnitud, moderada o severa. En condiciones de lisímetro, en el cultivar Arbequina, el potencial xilemático alcanzado durante los cuatro meses sin aporte de riego luego de endurecimiento de hueso fue menor a -3,5 MPa durante todo el período. Este estrés hídrico considerado severo provocó una disminución en el rendimiento (kg/pl), en el peso de los frutos, en la relación pulpa/hueso y en la humedad de los frutos, y alcanzó valores demasiado bajos (40 % aproximadamente), lo cual dificulta la extracción del aceite. Además, se observó una disminución en el contenido graso de los frutos (en base seca). Un déficit moderado parecería no tener una repercusión importante en el rendimiento final. Sin embargo, un déficit hídrico severo condicionaría mucho la productividad del cultivo. En el país, gran parte de la producción olivícola se desarrolla sobre suelos franco-arenosos superficiales (MGAP-DIEA, 2020), con menor capacidad de retención de agua que los suelos donde se realizó el experimento a campo, por lo cual el impacto del déficit podría ser más acentuado, similar al experimento en lisímetro.

Con respecto a la calidad del aceite, el perfil de ácidos graso no mostró verse afectado por los tratamientos, así como tampoco el porcentaje de ácidos grasos libres, en tanto se observó un leve aumento en el contenido de polifenoles y de carotenoides en aceite en el tratamiento con déficit hídrico, en concordancia con reportes previos (Dag et al., 2008, Gómez-Rico et al., 2006, Sena-Moreno et al., 2017).

Se ha demostrado que las plantas estresadas por la sequía resisten ciertos patógenos que requieren constante ambiente húmedo (Ramegowda y Senthil-Kumar, 2015). Frutos del cultivar Arbequina inoculados con *Colletotrichum acutatum* mostraron una menor incidencia y severidad en el tratamiento en déficit hídrico, donde el progreso de la enfermedad fue al menos dos días más lento en condiciones *in vitro*, óptimas para el desarrollo del hongo. Este enlentecimiento se traduce en una amplitud de ventana del período de cosecha en condiciones a campo para los productores. En condiciones de déficit hídrico severo en lisímetros, se vio más acentuada la tolerancia al patógeno. A su vez, este menor progreso de la enfermedad también fue observado en campo con la inoculación *in vivo* en el tratamiento con déficit hídrico. En todos los casos el tratamiento regado presentó mayor incidencia y severidad en el tratamiento control sin inoculación, lo que evidencia la presencia de posibles infecciones latentes por su mayor susceptibilidad a campo. Moreira et al. (2022) han reportado que la incidencia de infecciones latentes de *Colletotricum* fue mayor en la región sureste del Uruguay en comparación con el centro-sur, debido a que las condiciones ambientales eran más propicias para el desarrollo de la enfermedad.

En cuanto a los metabolitos implicados en la homeostasis osmótica celular, se describe que la acumulación de prolina en hojas de olivo está involucrada en el mecanismo de tolerancia al déficit hídrico (Ahmed et al., 2009). Sin embargo, en nuestro trabajo no hubo cambios significativos en el contenido de prolina en los frutos: quizá el metabolismo de la prolina no contribuye a la respuesta de los frutos a la interacción de los estreses estudiados. Se ha reportado que el riego redujo el daño oxidativo en las membranas celulares por peroxidación lipídica en hojas del cultivar Cobrançosa (Bacelar et al., 2007). En nuestro trabajo se observó una inducción del daño de membrana a través de la medición de TBARS en respuesta al déficit hídrico, aunque sin diferencias significativas.

El déficit hídrico desencadena el mecanismo de defensa antioxidante (Denaxa et al., 2020). Se observó una inducción en las enzimas relativas a la eliminación del peróxido de hidrógeno, como ser las catalasas y las peroxidasas en frutos del cultivar Arbequina en respuesta al déficit hídrico. Estas enzimas se indujeron ante la inoculación por *C. acutatum* y este efecto fue más acentuado en condiciones de déficit hídrico; por lo tanto, hubo una interacción en la respuesta a ambos estreses en simultáneo.

Con base en los resultados, se podría pensar que controlar el estatus hídrico de la planta y generarle un déficit moderado en el período de lipogénesis (verano) ayudará a reducir las infecciones por *C. acutatum*. En un futuro habría que buscar alternativas que logren engrosar la cutícula de los frutos y/o aquellas que promuevan la inducción de enzimas antioxidantes.

En resumen, el peso de los frutos, la relación pulpa/hueso y el contenido de humedad disminuyó en respuesta el déficit hídrico. En déficit severo se observó además una disminución en el rendimiento y en el contenido graso. Por lo tanto, la respuesta en los parámetros de rendimiento está condicionada por el grado de déficit hídrico. Por otra parte, se observó un aumento en el contenido de polifenoles en fruto y en el grosor de la cutícula en respuesta al déficit hídrico. Los frutos inoculados con *C. acutatum* presentaron menor progreso de la enfermedad cuando provenían de plantas con déficit hídrico en comparación con las regadas. Se observó una inducción en la actividad enzimática antioxidante en respuesta a la inoculación y este efecto fue más acentuado en condiciones de déficit hídrico. Consideramos que el aumento del grosor de la cutícula junto con el aumento en el contenido de polifenoles y la inducción de enzimas antioxidantes favorecieron la mayor tolerancia de los frutos de olivo a antracnosis.

6. CONCLUSIONES Y PERSPECTIVAS

Se generó información sobre el comportamiento productivo ante el déficit hídrico en olivos en un clima húmedo y con bajo DPV y se determinó qué parámetros se afectaron. El déficit hídrico moderado (hasta valores de -3,5 MPa) no causó una disminución en el rendimiento ni en la calidad del aceite. En tanto el déficit hídrico severo (valores más negativos que -3,5 MPa) causó una disminución significativa en el rendimiento. El déficit hídrico moderado en la etapa de lipogénesis aumentó la tolerancia de frutos de olivo a *Colletotrichum acutatum*. Esta tolerancia se mantuvo en condiciones de déficit hídrico más severo.

El déficit hídrico generó cambios anatómicos y bioquímicos en frutos de olivo del cultivar Arbequina que favorecieron la tolerancia a la antracnosis, presentando una menor incidencia y severidad de los frutos inoculados con *C. acutatum* tanto *in vitro* como *in vivo*. Estos cambios fueron: un aumento en el contenido de fenoles en fruto, un engrosamiento de la cutícula y una inducción en las enzimas relativas a la eliminación del peróxido de hidrógeno, como ser las catalasas y las peroxidasas. Estas enzimas se indujeron ante la inoculación por *C. acutatum* y este efecto fue más acentuado en condiciones de déficit hídrico.

En un futuro se espera continuar evaluando el manejo del riego, maximizando la eficiencia en el uso del agua. También, estudiar los mecanismos de defensa en cultivares tolerantes a antracnosis, profundizando en el estudio sobre la composición y estructura de la cutícula de los frutos, así como en el perfil de polifenoles en fruto. A su vez, se pretende estudiar la expresión de genes relacionados con la defensa en cultivares susceptibles y resistentes para dilucidar la base molecular de los mecanismos de defensa involucrados en esta interacción planta-patógeno.

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