UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

ESTUDIO DEL EFECTO A CORTO Y MEDIO PLAZO DE LA TECNICA DEL “FORZADO DE YEMAS” SOBRE LA PRODUCTIVIDAD Y LA CALIDAD DE LA VENDIMIA DEL VIÑEDO ‘TEMPRANILLO’ EN EXTREMADURA

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PROGRAMA DE DOCTORADO EN MODELIZACIÓN Y EXPERIMENTACIÓN EN CIENCIA Y TECNOLOGÍA POR LA UNIVERSIDAD DE EXTREMADURA

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ÍNDICE DE CONTENIDOS

ÍNDICE DE FIGURAS

I. Introducción General

III. Materiales y Métodos Generales

IV. Capítulo 1

V. Capítulo 2

VI. Capítulo 3

VII. Capítulo 4

VIII. Capítulo 5

ÍNDICE DE TABLAS

III. Materiales y Métodos Generales

IV. Capítulo 1

V. Capítulo 2

VI. Capítulo 3

VII. Capítulo 4

VIII. Capítulo 5

LISTA DE ABREVIATURAS

∑A: Total acetyl- glucoside forms

Ant: Total Anthocyanin

BW: Berry weight

∑C: Total coumaroy- glucoside forms

Cat: Total Catechins Content

CC: Climate Change

CFT: Crop-Forcing Technique

∑Cy: Total cyanindin derivates

CyA: Cyanidin-3-glucoside acetate

CyC: Cyanidin-3-glucoside coumarate

CyG: Cyanidin-3-glucoside

DOY: Days of Year

∑Dp: Total delphinidin derivates

DpA: Delphidin-3-glucoside acetate

DpC: Delphidin-3-glucoside coumarate

DpG: Delphidin-3-glucoside

FiPAR: Fraction of intercepted par radition

∑G: Total monoglucoside forms

MAL: Malic Acid

MFA: Multiple Factor Analysis

∑Mv: Total malvidin derivates

MvA: Malvidin-3-glucoside acetate

MvC: Malvidin-3-glucoside coumarate

MvG: Malvidin-3-glucoside

PCA: Principal Components Analysis

PDO: Protected Designation of Origin

∑Pn: Total peonidin derivates

PnA: Peonidin-3-glucoside acetate

PnC: Peonidin-3-glucoside coumarate

PnG: Peonidin-3-glucoside

∑Pt: Total petunidin derivates

PtA: Petunidin-3-glucoside acetate

PtC: Petunidin-3-glucoside coumarate TSS: Total Soluble Solids

PtG: Petunidin-3-glucoside

SWP: Stem Water Potencial

TA: Titratable Acidity

Tan: Total Tannins Content

TAR: Tartaric Acid

TPP: Total Polyphenol Content

RESUMEN (ABSTRACT)

RESUMEN

Una de las limitaciones del viñedo en las zonas cálidas es la pérdida de calidad de los vinos debido a las incidencias de las altas temperaturas durante el periodo de maduración de la uva, que genera un aumento del grado alcohólico y una disminución de la acidez y el color en la uva y en el vino. Además, esta situación podría verse agravada en el futuro debido a que, según las predicciones de los modelos climáticos, se producirá un aumento de las temperaturas durante los próximos años, que ya empieza a ser evidente en las principales zonas de producción vitícola, incluida la extremeña.

Retrasar el proceso de maduración de la uva hacia periodos con temperaturas más suaves, podría ser una solución para promover una producción rentable y sostenible de uvas de calidad adaptadas al contexto del Cambio Climático. Mediante el "*forzado de yemas*", se produce el rebrote de las yemas francas y se reinicia del ciclo de desarrollo de la vid desplazando su fenología en el tiempo y, por tanto, la maduración de la uva hacia fechas donde las temperaturas son notablemente más bajas.

El objetivo de este trabajo es evaluar el efecto "forzado de yemas" o "crop-forcing" en un viñedo de 'Tempranillo' para las condiciones semiáridas de Extremadura. El estudio se realizó durante cuatro años (2017 – 2020), aplicando el "crop-forcing" en tres años consecutivos sobre las mismas cepas (2017 – 2019) en dos fechas diferentes y junto con dos estrategias de riego. Se establecieron tres tratamientos de poda: dos fechas diferentes de aplicación del "forzado de yemas", entre los estados fenológicos de floración y cuajado de la uva (F1), entre cuajado y tamaño guisante (F2) y un control con poda convencional en invierno (NF). Cada tratamiento de poda fue sometido a dos regímenes hídricos: riego para cubrir las necesidades hídricas de las plantas (C) y riego deficitario con estrés hídrico moderado durante pre-envero (RI).

Capítulo 1:

El "forzado de yemas" consiguió retrasar la vendimia una media de 32 y 56 días para F1 y F2 respectivamente en relación a NF, pero limitó el rendimiento del viñedo. La aplicación de RI también supuso un descenso en la produccion respecto a C. El "crop-forcing" incrementó la acidez titulable y los contenidos en ácido málico, polifenoles y antocianos totales en las uvas en comparación con NF. La combinación del "forzado de yemas" y riego RI, supuso una mejora sobre la composición de las uvas en comparación con la aplicación del "crop-forcing" con riego C, sin embargo, supuso una pérdida mayor de rendimiento.

Capítulo 2:

El “crop-forcing” demostró ser una técnica eficaz para mejorar el contenido fenólico, el contenido en antocianos y la acidez total de las bayas, con un menor contenido en sólidos solubles totales, por lo que es capaz de mejorar el acoplamiento entre la madurez fenólica y tecnológica. La aplicación conjunta del “forzado de yemas” y el déficil hídrico mantuvo este aumento en los parámetros de calidad a la vez que supuso una mejora en la eficiencia del consumo de agua.

Capítulo 3:

La aplicación del “crop-forcing” después de floración (F1) no supuso un desgaste en el nivel de reservas, sin embargo, cuando se aplicó después de cuajado (F2) se observó una leve disminución en el contenido de carbohidratos y una modificación de la distribución de biomasa con respecto a NF, incluso al dejar de aplicar el forzado (2020). La aplicación conjunta de “crop-forcing” con RI no modificó los resultados en relación con los correspondientes tratamientos con C.

Capítulo 4:

El “crop-forcing” aplicado después cuajado consiguió un aumento en el contenido de antocianos y catequinas en las bayas. En todos los años de aplicación (2017-2019), e independientemente del tratamiento de riego aplicado, esta técnica modificó el perfil de antocianos debido a que supuso un aumento en el contenido de las formas monoglucósidas así como en los derivados de delfinifina, cianidina, petunidina, peonidina y malvidina en dos de los tres años de ensayo.

Capítulo 5:

Los vinos procedentes de cepas forzadas también mostraron un mayor contenido en su composición ácida (acidez total y ácido málico) y fenólica (polifenoles totales, antocianos y catequinas). Las características cromáticas de estos vinos se vieron afectados, con un aumento en la intensidad de color y el contenido en antocianos copigmentados, aunque mostraron ser más sensibles a la oxidación que los vinos procedentes de cepas no forzadas. El “forzado de yemas” implicó también una modificación del perfil de antocianos de los vinos, con un aumento en el contenido de delfinidina, cianidina, petunidina y peonidina.

ABSTRACT

One of the limitations of vineyards in warm areas is the loss of wine quality due to the impact of high temperatures during the grape ripening period, which generates an increase in alcohol content and a decrease in acidity and color in grapes and wine. Moreover, this situation could be aggravated in the future due to the fact that, according to climate model predictions, there will be an increase in temperatures over the next few years, which is already beginning to be evident in the main wine production areas, including Extremadura.

Delaying the grape ripening process to periods with milder temperatures could be a solution to promote a profitable and sustainable production of quality grapes adapted to the context of climate change. By means of "*crop-forcing*", the regrowth of the frank buds is produced and the development cycle of the vine is restarted, shifting its phenology in time and, therefore, the ripening of the grape towards dates where temperatures are notably lower.

The objective of this work is to evaluate the effect of "crop-forcing" or "forzado de yemas" in a 'Tempranillo' vineyard for the semi-arid conditions of Extremadura. The study was conducted over four years (2017 - 2020), applying crop-forcing in three consecutive years on the same vines (2017 - 2019) on two different dates and together with two irrigation strategies. Three pruning treatments were established: two different dates of application of "crop-forcing", between the phenological stages of flowering and fruit set (F1), between fruit set and pea size (F2) and a control with conventional pruning in winter (NF). Each pruning treatment was subjected to two water regimes: irrigation to meet plant water requirements (C) and deficit irrigation with moderate water stress during pre-veraison (RI).

Chapter 1:

"Crop-forcing" succeeded in delaying harvest by an average of 32 and 56 days for F1 and F2 respectively relative to NF, but limited vineyard yield. The application of RI also resulted in a decrease in yield relative to C. “Crop-forcing” increased titratable acidity and malic acid, polyphenols and total anthocyanins contents in grapes compared to NF. The combination of “crop-forcing” and RI irrigation resulted in an improvement on grape composition compared to “crop-forcing” with C irrigation, however, it resulted in a higher yield loss.

Chapter 2:

“Crop-forcing” proved to be an effective technique to improve phenolic content, anthocyanin content and total berry acidity, with a lower total soluble solids content, thus being able to improve the coupling between phenolic and technological maturity. The joint application of "crop-forcing" and water deficit maintained this increase in quality parameters while leading to an improvement in water consumption efficiency.

Chapter 3:

The application of “crop-forcing” after flowering (F1) did not lead to a depletion in the level of reserves, however, when applied after fruit set (F2) a slight decrease in carbohydrate content and a modification of biomass distribution with respect to NF was observed, even when forcing was discontinued (2020). The joint application of “crop-forcing” with RI did not modify the results relative to the corresponding C treatments.

Chapter 4:

“Crop-forcing” applied after fruit set achieved an increase in anthocyanin and catechin content in berries. In all years of application (2017-2019), and regardless of the irrigation treatment applied, this technique modified the anthocyanin profile because it led to an increase in the monoglucoside forms content as well as delphinifin, cyanidin, petunidin, peonidin, and malvidin derivatives in two of the three trial years.

Chapter 5:

Wines from forced vines also showed higher contents in their acid (total acidity and malic acid) and phenolic (total polyphenols, anthocyanins and catechins) composition. The chromatic characteristics of these wines were affected, with an increase in color intensity and copigmented anthocyanin content, although they were shown to be more sensitive to oxidation than wines from unforced vines. “Crop-forcing" also implied a modification of the anthocyanin profile of the wines, with an increase in delphinidin, cyanidin, petunidin and peonidin content.

I. INTRODUCCIÓN GENERAL

I.1. EL CULTIVO DE LA VID EN EXTREMADURA

Según la Organización Internacional de la Vid y el Vino (OIV) en 2021 la superficie total de viñedo a nivel mundial superó los 7 millones de hectáreas, de las cuales el 13% está en España con más de 945000 hectáreas. A pesar de estar a la cabeza en superficie mundial de viñedos, España se sitúa por detrás de Italia y Francia en producción de vino.

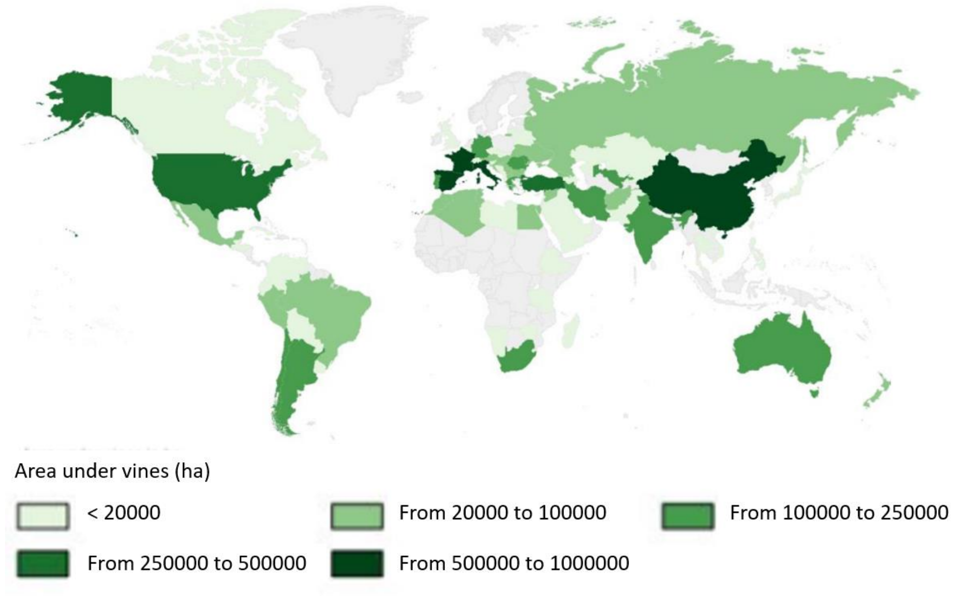

Figura I.1. Distribución mundial de viñedos en 2020. Fuente OIV

En Extremadura se localiza el 8.5% de la superficie nacional de viñedo, con más de 80000 hectáreas. Prácticamente toda la producción se destina a transformación. Las variedades dominantes en esta región son ‘Pardina’ en uva blanca con más de 19000 hectáreas y ‘Tempranillo’ en uva tinta con más de 18000 hectáreas.

España cuenta con 101 Denominaciones de Origen Protegidas (DOP) de Vino registradas en la Unión Europea. La Ribera del Guadiana es la DOP en Extremadura. Extremadura es la tercera comunidad española con mayor producción de vino, sólo por detrás de Castilla la Mancha y Cataluña, sin embargo, solo el 6% de esta producción se destina a vinos con DOP, situándose por detrás de otras 8 comunidades españolas en producción de este tipo de vinos, Andalucía, Aragón, Castilla y León, Murcia, Navarra, País Vasco, La Rioja y la Comunidad Valenciana.

El peso del sector agrícola y ganadero en Extremadura es tres veces mayor a la media nacional. El sector vitivinícola representa en torno al 2.2% del PIB regional, lo que supone un Valor Añadido Bruto (VAB) en torno a los 210 millones de euros por efecto directo de los cuales 60 millones corresponden a la viticultura, 35 millones a la elaboración y crianza de vinos y 115 millones a su comercialización.

I.2. EL CICLO DE LA VID

I.2.1 DESARROLLO VEGETATIVO Y REPRODUCTIVO

La vid pertenece a la familia *Vitaceae* y al género *Vitis el* cual se localiza principalmente en las zonas de clima templado y subtropical del hemisferio norte (Mullins et al., 1992; Wan et al., 2008). Todos los miembros de este género son vides o arbustos perennes con brotes con zarcillos. Este género comprende 60-70 especies repartidas principalmente en Asia (40 especies) y América del Norte (20 especies) (Alleweldt & Possingham, 1988; Wan et al., 2008). La especie euroasiática Vitis vinifera L. dio lugar a la inmensa mayoría de las variedades de uva cultivadas hoy en día.

Se trata de una planta de ciclo bianual, ya que para completar el ciclo reproductivo precisa de dos ciclos vegetativos. El inicio del ciclo vegetativo de la vid se sitúa a finales del invierno o principios de primavera, cuando la vid a menudo exuda savia del xilema de las superficies de poda y otras heridas que aún no han sido suberizadas. Este flujo de savia o "lloro" marca la transición de la latencia al crecimiento activo. El inicio del lloro está relacionado con el restablecimiento de la actividad metabólica en las raíces y está influenciado por la temperatura del suelo, que debe superar los 10 ºC (Keller, 2010). En este momento comienza la movilización de savia a lo largo de la vid después de la parada invernal. La presión de las raíces surge de la movilización de las reservas de nutrientes del almidón y las proteínas y el bombeo de azúcares (especialmente glucosa y fructosa) y aminoácidos (especialmente glutamina) hacia el xilema (Roubelakis-Angelakis & Kliewer, 1979). El aumento resultante de la presión osmótica de la savia del xilema proporciona la fuerza motriz para la captación osmótica de agua por las raíces desde el suelo, lo que genera una presión hidrostática positiva en todo el xilema de 0,2-0,4 MPa en la base del tronco y que disminuye a un ritmo de 0,01 MPa m^{-1} a medida que la savia asciende (Scholander et al., 1955; Sperry et al., 1987). Esta presión a su vez eleva el agua hacia las partes más altas de la vid y hacia las yemas, la cuales comienzan a hincharse. Comienza entonces en las yemas la división celular y la producción de auxina en el primordio de la hoja proximal de las yemas distales que marca el inicio de la brotación y del crecimiento vegetativo en primavera.

Cuanto más largo y más frío es el invierno, más se acelera la tasa de brotación al regresar las temperaturas cálidas (Dokoozlian, 1999; Lavee & May, 1997). En climas templados y fríos, temperaturas por encima de los 8 a 10 ºC inducen la brotación y el crecimiento de los brotes, pero esta temperatura base, dependerá de la especie y el cultivar. Los brotes en áreas con inviernos fríos, generalmente se rompen en unos pocos días (si no hay daños de frío en las yemas), sin embargo, en áreas con inviernos suaves esto puede tardar hasta 10 veces más (Keller, 2010). Como consecuencia, la brotación es a menudo errática en climas cálidos debido a que temperaturas más cálidas conducen a una mayor respiración y un mayor estrés oxidativo, pudiendo provocar la necrosis de la yema (Keller, 2010).

A la brotación le sigue un período de rápido crecimiento de los brotes, con la aparición consecutiva de hojas, y el crecimiento de las raíces. Este desarrollo es completamente dependiente las reservas acumuladas en órganos permanentes de la cepa (carbohidratos, proteínas y aminoácidos), hasta que la asimilación fotosintética de la cubierta vegetal formada por las hojas nuevas se incrementa hasta cubrir la demanda de la planta. Las reservas disminuyen, alcanzado un mínimo alrededor del momento de floración (Lebon et al., 2008; Weyand & Schultz, 2006; Williams, 1996; Zapata et al., 2004). En consecuencia, hay una pérdida de peso seco en las vides durante la primavera, hasta que cada brote consigue diferenciar de cinco a seis hojas aproximadamente, momento en el que se empieza a llevar a cabo la fotosíntesis (Eifert et al., 1960; Hale & Weaver, 1962). Temperaturas elevadas están asociadas con una movilización del almidón más rápida, y por lo tanto con un crecimiento más rápido de los brotes (Field et al., 2009; Skene & Kerridge, 1967; Woodham & Alexander, 1966; Zelleke & Kliewer, 1979). La tasa de crecimiento de los brotes se denomina vigor y depende tanto de la especie y el cultivar como de la temperatura, la disponibilidad de agua en del suelo, la edad de la cepa, el nivel de poda, la disponibilidad de nutrientes y la cantidad de reservas (Keller, 2010). El crecimiento de los brotes alcanza su estado óptimo cuando las temperaturas se sitúan en torno a los 25 – 30 ºC, disminuyendo a medida que aumenta la temperatura hasta que cesa cuando las temperaturas se sitúan entre 35 – 38 ºC (Buttrose, 1969b). Por otra parte, la producción de biomasa tiende a mantenerse constante, por lo que, al aparecer los frutos, el crecimiento vegetativo de la cepa tiende a pararse (Pallas et al., 2008; Petrie et al., 2000). Poco antes o durante el proceso de maduración de las uvas, los brotes comienzan a formar una peridermis, por lo que se convierten en "sarmientos" y pasan de ser verdes a un marrón amarillento-rojizo. Esta maduración del sarmiento, se conoce como "agostamiento" y se inicia desde la base hacia la punta y va acompañada de la reposición de las reservas de almacenamiento en preparación para la siguiente temporada de crecimiento (Eifert et al., 1960).

El ciclo de crecimiento de los sarmientos se completa con la senescencia de las hojas, asociada al reciclaje de nutrientes desde las hojas hacia los sarmientos y las partes permanentes de la vid (Conradie, 1986), la abscisión (desprendimiento) de hojas y, finalmente, la deshidratación y la aclimatación al frío de todas las partes leñosas en otoño. Este proceso se produce cuando las condiciones ambientales cambian, con una disminución de la duración del día y una caída de las temperaturas. La temperatura no tiene efecto en el inicio de la senescencia, pero si en la duración de la misma debido a que una temperatura más baja puede acelerar la tasa de senescencia una vez iniciada (Fracheboud et al., 2009).

El ciclo reproductivo de la vid se extiende a lo largo de dos años. En el primer año tiene lugar el inicio del ciclo con la formación de inflorescencias, y comprende tres procesos separados: la inducción, la iniciación y el desarrollo. En primer lugar, tiene lugar la inducción que consiste en la formación de un primordio reproductor (May, 2004). Una vez que se han formado los primordios, se lleva a cabo la diferenciación entre inflorescencias y zarcillos y empiezan a desarrollarse los tejidos meristemáticos (responsables del crecimiento vegetal) que se convertirán en flores en el segundo año. Durante el desarrollo, las yemas latentes reciben principalmente carbohidratos de las hojas (Hale & Weaver, 1962). Las temperaturas cálidas favorecen la inducción de inflorescencias, mientras que las temperaturas frescas (<20 ºC) promueven la formación de zarcillos(Buttrose, 1969a, 1970). Temperaturas por encima 35 ºC pueden causar problemas de fructificación en los brotes. Las inflorescencias crecen dentro de las yemas fructíferas durante el verano, entrando en periodo de latencia a finales de este, sin reanudarse hasta que los brotes comienzan a hincharse a finales del invierno o principios de primavera del año siguiente.

Cuando los brotes se reactivan cada inflorescencia da lugar a grupos de tres o cuatro meristemos florales y esto es lo que se conoce como *floración*. La floración suele comenzar en las yemas más bajas del pámpano, progresando hacia arriba y se produce durante aproximadamente 5 semanas después de la brotación. Para que la floración pueda llevarse a cabo es necesario la aportación de carbohidratos, que provienen tanto de las reservas de los órganos perennes como de la fotosíntesis (Lebon et al., 2005). El número de racimos por cepa queda determinado en la campaña previa a la floración, pero el número de uvas por racimos dependen de la propia campaña (Mullins et al., 1992).

El paso siguiente a la floración es la transformación de la flor en fruto, denominado cuajado. A partir de este momento se producen dos fases en el crecimiento del fruto: la formación y la maduración. Junto a la formación de la baya, la acumulación de ácidos se establece, con una pérdida en el contenido de los mismos con el paso del tiempo, pudiendo ser explicado por el efecto dilución. Las bayas ganan tamaño por el agua que absorben y la concentración de estos ácidos disminuye. La fase de formación de baya se extiende hasta el envero, momento en el cual comienza la maduración de la misma (Coombe & McCarthy, 2000). En esta fase, comienzan a acumularse carbohidratos (glucosa y fructosa) y antocianos, que son los responsables del cambio de color de verde a morado en uvas tintas. Además, la concentración de ácidos sigue disminuyendo. La fase de maduración es especialmente sensible a las condiciones climáticas, pues las concentraciones de los distintos metabolitos tienen comportamientos diferentes en función de las temperaturas.

I.2.2. FORMACIÓN Y REPARTO DE ASIMILADOS. BALANCE DE CARBONO

El balance de carbono de las plantas ha sido objeto de numerosos estudios debido a la relevancia que puede tener en la captación de CO_2 de la atmósfera. En el concepto de balance de carbono se incluye dos procesos fisiológicos de la cepa: la fotosíntesis y la respiración. Mediante la fotosíntesis, la cepa convierte el CO_2 que absorbe del aire y el H_2O que absorbe de las raíces en azúcares sencillos.

$$n\, CO_2 + 2n\, H_2O + energía \rightleftharpoons (CH_2\, O)_n + n\, O_2 + n\, H_2O$$

Estos azúcares sirven como recursos de la planta para dos procesos importantes. Por una parte, sirven como combustible en la respiración celular, dónde los carbohidratos y el O_2 forman CO_2 y H_2O. Por otra parte, los azúcares sencillos sirven de materia prima para la síntesis de sacarosa, almidón y celulosa. La glucosa y la fructosa sintetizan en primer lugar sacarosa en las hojas, que se transporta al resto de la cepa como fuente de energía para el crecimiento (Mullins et al., 1992). Cuando la síntesis de sacarosa excede la cantidad que la cepa puede transportar, comienza la síntesis de almidón y celulosa. La celulosa es el principal constituyente de la madera. En este proceso se lleva a cabo el crecimiento de la cepa, convirtiendo el carbono absorbido en biomasa. Se produce así un flujo de carbono a lo largo de la cepa. Sin embargo, no toda la biomasa producida permanece en la planta, una gran fracción de esta materia se elimina en la vendimia, en la poda de invierno o en la senescencia de las hojas (Poni et al., 2006; Tarara et al., 2011).

El Cambio Climático puede modificar el equilibrio de los ecosistemas en este balance de carbono debido a que tiene gran dependencia al aumento de CO_2, pero también al aumento de las temperaturas y a la sequía (IPCC, 2022). Aunque temperaturas más elevadas tienen a aumentar la capacidad fotosintética de la planta, cuando se excede unos límites, la tendencia es la contraria, y se inhibe la fotosíntesis. Además, para que la cepa sea capaz de capturar una cantidad más elevada de CO_2 necesita absorber una mayor cantidad de H_2O a través de las raíces, ya que la absorción de CO_2 tiene gran dependencia del agua disponible.

Durante el ciclo anual hay un flujo de carbohidratos entre los órganos anuales (hojas, inflorescencias y bayas) y los órganos permanentes (raíces y troncos). Las inflorescencias y las bayas actúan como “sumideros” de carbono, debido a que demandan carbohidratos como nutrientes para crecer. Las hojas, actúan como sumideros, demandando carbohidratos para crecer, y como “fuentes” ya que sintetizan estos carbohidratos mediante la fotosíntesis. Las raíces y el tronco actúan como fuentes en el inicio del ciclo, debido a que son capaces de liberar azúcares y almidón, sin embargo, cuando la fotosíntesis supera el consumo de carbohidratos, estos órganos empiezan a almacenar de nuevo reservas, actuando como sumideros. El equilibrio entre las interacciones fuente-sumidero es clave para el desarrollo de la planta.

En la parada invernal, el 90% de los recursos de carbono que tiene la cepa se acumulan en las raíces (Bates et al., 2002; Eifert et al., 1960). A principios de primavera, cuando la temperatura del suelo alcanza los 10 – 12 ºC se reactiva el metabolismo de las cepas, movilizando el almidón de las raíces y el tronco, para el crecimiento de los órganos vegetativos y reproductivos anuales (Scholefield et al., 1978; Zapata et al., 2004).

La movilización del almidón se produce desde principios de primavera hasta la floración, debido a que hasta entonces las hojas se comportan como órganos sumideros. Las hojas son órganos sumideros hasta que alcanzan entre un tercio y la mitad de su tamaño (Hale & Weaver, 1962; Petrie et al., 2000). La fotosíntesis aumenta desde la brotación hasta la floración, y a partir de este momento disminuye hasta la senescencia de la hoja (Stoev, 1952), alcanzando su máximo antes y durante la floración.

El flujo de CO_2 varía durante el año, pudiendo establecerse dos fases. Desde floración a envero está orientado al crecimiento de los órganos tanto anuales como permanentes. A partir de envero, el flujo de CO_2 se orienta a la restauración de las reservas de almidón (Stoev & Ivantchev, 1977) y a la maduración de las bayas.

I.2.3. ESTRUCTURA Y COMPOSICIÓN DE LA UVA Y DEL VINO

La uva se clasifica como una baya, porque presenta un pericarpio que encierra a las semillas. El pericarpio, está formado por tres tejidos anatómicamente distintos: el exocarpo (o epicarpio), mesocarpio y endocarpio (Galet, 2000). El exocarpio forma el sistema dérmico de la uva u "hollejo", que, dependiendo del grosor y del tamaño de la baya, constituye entre el 5 y el 18% del del peso fresco de las bayas maduras. El mesocarpio, que comúnmente se llama la "pulpa", se forma poco después del cuajado del fruto y consta de células de paredes finas, y altamente vacuoladas. Las vacuolas pueden constituir hasta el 99% del volumen celular en las bayas de uva maduras (Diakou & Carde, 2001) y sirven como depósito interno almacenando azúcares, ácidos orgánicos y nutrientes. El tejido más interno del pericarpio, el endocarpio, rodea las semillas y está formado por la hipodermis interna con células ricas en cristales de oxalato cristales de oxalato de calcio (drusas) y también la epidermis interna (Fougère-Rifot et al., 1995; Hardie et al., 1996). El endocarpo es a menudo es difícil de distinguir del mesocarpio en las uvas (Mullins et al., 1992). Las semillas tienen como papel principal proteger y nutrir al embrión en desarrollo (Roberts et al., 2002). Aunque la baya puede contar con 4-6 semillas, en la practica la mayoría de *Vitis vinifera* cultivadas suelen tener 1 ó 2 semillas por baya.

En cuanto a la composición química de la baya, está formada por diferentes compuestos entre los que destaca el agua, azúcares, ácidos orgánicos, compuestos nitrogenados, lípidos, compuestos aromáticos y compuestos fenólicos. El agua es el compuesto mayoritario de las uvas, representando entre el 75 y el 85% de su peso total, y sirviendo como disolvente de los demás compuestos (Keller et al., 2006; Robinson & Davies, 2000). El agua que contiene la uva se absorbe directamente de la raíz, por lo que tiene gran dependencia del estado hídrico del suelo (Chaves et al., 2010).

Los azúcares representan el 90% de los sólidos solubles totales de las uvas. Los azúcares mayoritarios en las uvas son la glucosa y la fructosa, que se transforman en la vinificación en alcohol etílico, por lo que el contenido de estos compuestos determinará el grado alcohólico del vino. La acumulación de azúcares tiene una tendencia ascendente durante el proceso de maduración y sirve como criterio de vendimia.

Los ácidos orgánicos mayoritarios en las uvas son el ácido málico y el tartárico. El ácido málico tiene una tendencia decreciente a medida que transcurre la maduración. El ácido tartárico también tiende a disminuir después de envero, pero con una evolución mucho más suave. La concentración de estos dos ácidos constituye el valor de acidez total de las uvas, que sigue una tendencia decreciente a medida que avanza la maduración.

Los compuestos nitrogenados se encuentran en forma inorgánica (NH_4^+, NO_2^- y NO_3^-) o en forma orgánica (aminoácidos libres, péptidos, proteínas, urea y derivados de ácidos nucleicos). Estos compuestos sirven de macronutrientes y son obtenidos directamente del suelo.

Los lípidos son compuestos con bajas concentración en las uvas, sin embargo, tienen gran importancia por su papel nutricional para las levaduras. La acumulación de estos compuestos se lleva a cabo desde envero a vendimia.

Los compuestos aromáticos son un conjunto de más de 500 compuestos que constituyen el aroma de los vinos. Entre ellos se encuentran algunos compuestos volátiles como son alcoholes, ésteres, aldehídos, ácidos, compuestos azufrados, lactonas, fenoles volátiles, derivados furfurales.

Los compuestos fenólicos son los responsables de algunas de sus propiedades más deseadas. Estos compuestos son los causantes de las propiedades antioxidantes que poseen los vinos, pero además también afectan a las propiedades organolépticas de los mismos. Según su estructura química, los compuestos fenólicos se clasifican en dos grandes grupos: no flavonoides (ácidos fenólicos y estilbenos) y flavonoides (flavanoles, antocianos y flavonoles) (Hidalgo Togores, 2003; Ribéreau-Gayon et al., 2006). En uvas tintas, las familias fenólicas que mayor importancia tienen en la calidad de los mostos son: los flavanoles y los antocianos. Los flavanoles están formados por taninos condensados y catequinas, que aportan el sabor amargo y la astringencia respectivamente (Zamora, 2003) mientras que los antocianos son los responsables del color. El color es uno de los aspectos más importantes en la calidad, ya que es la primera característica apreciable de un vino. Además, cada variedad de uva posee un perfil antocianico propio (Bakker & Timberlake, 1985; Mateus et al., 2002) que está compuesto por las cinco antocianidinas mayoritarias presentes en las uvas: delfidina, cianidina, petunidina, peonidina y malvidina y que a su vez pueden encontrarse en forma libre (como glucosidos) o en formas aciladas (acetatos y cumaratos). Los antocianos empiezan a formarse a partir del envero mediante dos vías diferentes. Por un lado, se forman los antocianos disustituidos: la cianidina se glucosida formando la cianidin-3-O-glucósido, y esta a su vez puede metilarse dando lugar a la peonidin-3-O-glucósido, estos compuestos son los responsables de los tonos rojizos. Por otra parte, se forman los antocianos trisustituos, que confieren a los mostos los tonos violetas, mediante la transformación de la delfindina, en delfidina 3-O-glucósido, que a su vez puede metilar un grupo hidróxido formando la petunidina-3-O-glucósido y/o metilar dos grupos hidróxidos dando lugar a la malvidin-3-O-glucósidos. Este último es el antociano mayoritario en las uvas ‘Tempranillo’. Una vez formados estos antocianos en forma libre, los grupos glucósidos pueden formar acetatos y cumaratos, dando lugar a las formas aciladas (Ford et al., 1998), que tienden a ser más estables.

I.3. FACTORES QUE AFECTAN A LA PRODUCTIVIDAD Y LA CALIDAD DE LA UVA Y DEL VINO

I.3.1 EL VIÑEDO Y SU ENTORNO

El clima es el mayor componente del “terroir” del viñedo (Hannah et al., 2013). La vid es una planta sensible al calor y a las heladas de invierno, tanto para su desarrollo vegetativo como para la maduración de las uvas. El cultivo *Vitis vinifera* no debe desarrollarse en zonas con temperaturas medias por debajo de los 9 ºC ni superiores a los 40 ºC. Es por esto que las grandes regiones vitivinícolas mundiales se distribuyen en dos zonas mundiales con climas templados (Figura I.2), comprendidas entre los paralelos de 30º y 50º de latitud Norte y los 30º y 40º de latitud Sur.

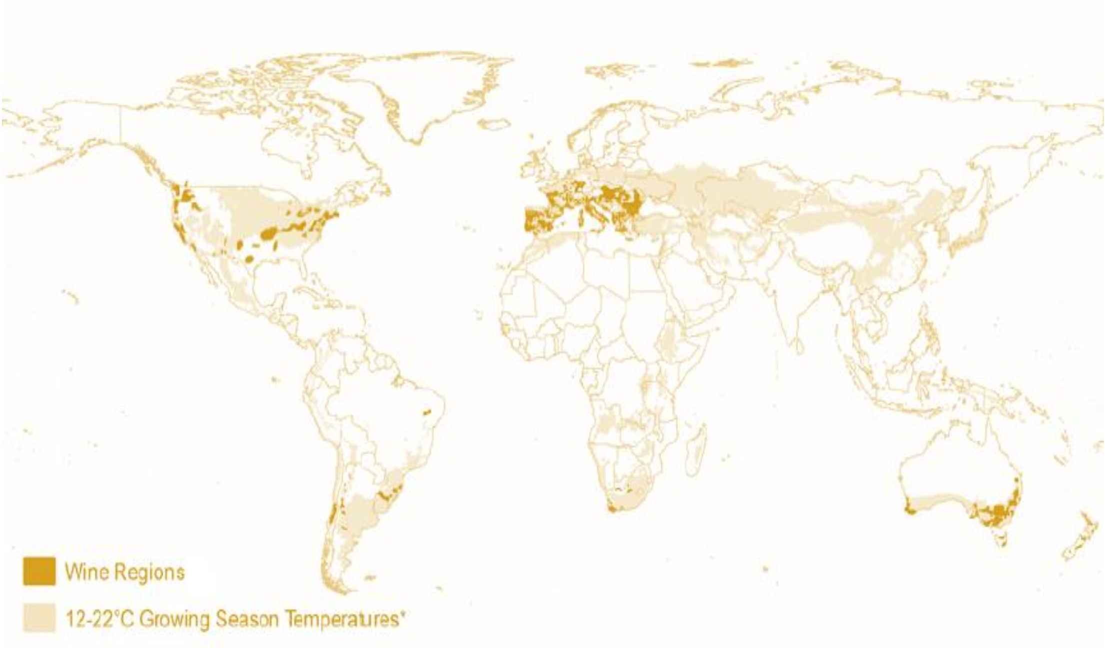

Figura I.2. Regiones vitivinícolas mundiales y zonas de temperatura de 12 a 22 °C en la temporada de crecimiento (abril a octubre en el Hemisferio Norte y octubre a abril en el Hemisferio Sur) (Jones, 2012)

Sin embargo, esta clasificación debe someterse a revisión, dado que, en los últimos años, el cultivo del viñedo comienza a estar presente en otras regiones como es el caso de Reino Unido, donde el aumento de las temperaturas está permitiendo su establecimiento en zonas donde antes las condiciones climáticas resultaban limitantes.

La influencia del suelo en la composición de la uva y el vino parece ser menos significativa que la del clima, o al menos no existen muchos trabajos en la literatura científica que correlacionen las características del suelo con la calidad del vino (Morlat et al., 1983). La influencia del suelo sobre la composición de la uva, tiende a expresarse indirectamente a través de características como la retención de calor, la capacidad de retención de agua y el estado nutricional. Por ejemplo, el color del suelo y la composición textural afectan a la absorción de calor por parte del suelo y, por tanto, a la maduración de la fruta y a la protección contra las heladas. Al hablar del suelo y sus efectos en el crecimiento de la vid, es importante distinguir entre las distintas propiedades fisicoquímicas del suelo: su textura, estructura de los agregados, disponibilidad de nutrientes, contenido orgánico, profundidad efectiva, pH, drenaje y disponibilidad de agua y es probable que la uniformidad de las condiciones del suelo resulte más importante que cualquiera de estas propiedades por sí sola. La variabilidad del suelo es una de las principales causas del desarrollo asincrónico de las bayas y de una menor calidad del vino (Jackson, 2008).

La disponibilidad hídrica, es también un factor determinante en el cultivo de la vid. En condiciones naturales, el agua suministrada por la nieve y/o la lluvia se almacena temporalmente en el suelo para ser extraída por las raíces de las cepas. Sin embargo, la cantidad de precipitaciones varía mucho de una región a otra y de un año a otro, lo que puede perjudicar drásticamente el rendimiento de la vid y la economía de la viticultura en algunas regiones y algunos años. La disponibilidad de agua no sólo depende de la cantidad de lluvia que recibe un viñedo sino también del momento en que cae la lluvia y de la rapidez con que se evapora. Además, la capacidad de retención de agua del suelo y, por tanto, la cantidad de agua disponible para las plantas, varía en función de la profundidad del suelo, la textura (es decir, las proporciones relativas de partículas de arcilla, limo y arena) y el contenido de materia orgánica. Por ejemplo, un suelo franco fino tiene hasta seis veces más agua disponible que la arena gruesa, aunque una mayor fracción del agua es retenida con demasiada fuerza para que sea accesible a las raíces (Keller, 2010).

Por otro lado, aunque la temperatura desempeña un papel importante en la regulación del ciclo anual de crecimiento de la vid, es la radiación solar la que genera el ciclo climático anual y proporciona la energía para la fotosíntesis. El grado en que el suelo y las vides reciben la radiación luminosa y térmica del Sol depende en gran medida del ángulo con el que los rayos solares inciden en la superficie de la Tierra. El ángulo solar varía con la hora del día, la estación del año, la latitud, la inclinación y la orientación del lugar. El cambio en la duración del día produce un ciclo fotoperiódico anual que es utilizado por muchas plantas para regular los ciclos anuales de crecimiento. Sin embargo, la mayoría de los cultivares de Vitis vinifera son relativamente insensibles al fotoperiodo, ya que el ciclo anual está influenciado principalmente por las fluctuaciones anuales de la temperatura (Keller, 2010). Además, la capacidad de la vid para captar la radiación solar se ve afectada por la dirección de las hileras, el espacio entre las cepas o el sistema de formación entre otros, pero también por el manejo del dosel vegetal, o la poda y todo ello, tiene su efecto sobre la composición de las uvas.

I.3.2. EL ESTADO HÍDRICO. EL RIEGO

El agua es el principal factor de producción en la viticultura de zonas cálidas, ya que la mayor parte del periodo vegetativo coincide con el de escasez de lluvias y altas temperaturas. En estas condiciones el riego incrementa considerablemente las producciones, contribuyendo a paliar algunos de los efectos negativos del cambio climático, manteniendo la actividad fotosintética en las hojas y por tanto la acumulación de azúcar y minorando la pérdida de acidez durante la maduración (Uriarte et al., 2016). A pesar de que existen numerosos trabajos en los que se ha evaluado el efecto del riego sobre la composición de la uva (Freeman & Kliewer, 1983; Ginestar et al., 1998; McCarthy, 1997; Petrie et al., 2004; Salón et al., 2004) es complicado generalizar y extrapolar los resultados obtenidos en dichos ensayos a otras condiciones edafoclimáticas, prácticas de cultivo y variedades. Aunque se acepta de forma general que la mayor producción puede ir acompañada de una pérdida de calidad en los mostos si el riego es aplicado en exceso. Respecto a los estudios publicados sobre respuestas del riego en variedades tintas, cabe mencionar que, el comportamiento depende en gran medida del cultivar en cuestión (Basile et al., 2011). En ‘Tempranillo’ los estudios indican que un cierto nivel de estrés hídrico puede ser favorable para la calidad del mosto, si éste es moderado y sucede durante el periodo de post-envero (Girona et al., 2009). La contrapartida es una cierta perdida en capacidad productiva (Girona et al., 2009). Cuando este estrés sucede en pre-envero el detrimento en producción puede incrementarse, y ademán puede llevar asociado un detrimento en calidad del mosto en cuanto a concentración de polifenoles y antocianos (Girona et al., 2009; Marsal et al., 2008). Estos resultados obtenidos mayoritariamente en maceta, se contradicen con los obtenidos por Intrigliolo et al. (2012) en esta misma variedad, ya que, en este trabajo, el periodo de pre-envero parece ser uno de los más idóneos para inducir estrés hídrico y obtener buenos resultados en cuanto a calidad de mosto se refiere. Experimentos realizados en Extremadura (Mancha et al., 2021; Uriarte et al., 2016) apoyan los obtenidos por Intrigliolo et al. (2012), en ‘Tempranillo’, En Extremadura de forma natural los niveles de estrés pre-envero fueron leves, lo que puede ser el origen de la discrepancia, ya que en algunas zonas de producción la disponibilidad de agua en el suelo debida a la lluvia de otoño y primavera resulta suficiente para limitan la capacidad de inducir cierto nivel de déficit hídrico antes del envero. A pesar de estas divergencias en calidad del mosto, existe una coherencia en cuanto a las citadas respuestas a nivel productivo. Otro aspecto al considerar estrategias deficitarias de riego es la mejora en la eficiencia en el uso del agua que ha pasado a ser una exigencia en el contexto de la escasez y perspectivas futura de competencia sobre los recursos hídricos en zonas áridas.

I.3.3. MANEJO DE LA VEGETACIÓN.

La productividad de la vid (crecimiento, cosecha y composición del fruto) depende en última instancia de la capacidad fotosintética del dosel vegetal, integrada a lo largo del periodo de crecimiento, y del equilibrio de la vid conocido en la literatura científica como “vine balance”. La elección de la localización del viñedo, el sistema de formación empleado, la disponibilidad hídrica y el propio manejo del viñedo están destinados a favorecer la capacidad fotosintética de la cepa, buscando el reparto equilibrado entres “fuente” y “sumidero” de fotoasimilados. En este sentido el manejo de la vegetación es determinante.

Para manejar correctamente la vegetación de la vid resulta importante atender al desarrollo del área foliar y su distribución en el espacio, la sombra que se genera dentro en la zona de fructificación/renovación y el equilibrio entre el crecimiento de los frutos y los brotes. Cuando el desarrollo de la vegetación durante el periodo de crecimiento exponencial (a principios de primavera) es rápida, se genera una gran superficie foliar expuesta que incrementa la interceptación de la luz solar y disminuye la densidad del dosel (Smart et al., 1990). En cuanto a la separación entre las hileras, no deben estar tan juntos como para causar una sombra excesiva en la zona de los racimos de la cepa adyacente y por tanto son preferibles posicionamientos verticales con alturas que no excedan la proporción 1:1 entre la altura y la anchura de la calle (Smart et al., 1990). Las hojas y los frutos deben tener un microclima lo más uniforme posible ya que una exposición adecuada de la uva favorece la calidad del vino. La sombra sobre los racimos, favorece las uvas con mayor contenido de K^+ y ácido málico y la incidencia de Uncinula necator y la podredumbre del racimo por Botrytis cinerea, y una reducción de los niveles de azúcar, ácido tartárico fenol y antocianina (Kliewer et al., 1988) e induce caracteres herbáceos en el vino (Pszczolkowski et al., 1985). La alta iluminación tiende a desviar el metabolismo de ácido málico hacia la síntesis de azúcares, es decir, hace aumentar el contenido de estos compuestos, mientras que provoca una reducción de la acidez total, modificando la relación sólidos solubles/acidez. Finalmente, el aumento de insololación del racimo hace disminuir el contenido en compuestos nitrogenados de los mostos (Pszczolkowski et al., 1985).

La distribución del fotosintato entre el crecimiento de los brotes y el de los frutos debe ser adecuada para evitar un exceso o un defecto de superficie foliar en relación con el peso de los frutos. Si los sarmientos son vigorosos, presentan un diámetro relativamente grande, entrenudos largos y hojas grandes, además de mostrar una marcada propensión al crecimiento activo de los anticipados. Estas características son indeseables e indican un desequilibrio entre el crecimiento vegetativo y el crecimiento del fruto (Smart, 1989). Por el contrario, los brotes demasiado cortos pueden tener una superficie foliar insuficiente para madurar adecuadamente la uva. El equilibrio entre la producción de brotes y de frutos puede evaluarse mediante ratio producción:peso de poda, conocido como Indice ravaz o "crop load", que de manera generalizada se asume un rango entre 5 a 10 necesario para los niveles ideales de acumulación de SST y la coloración de las bayas y el vino (Kliewer & Dokoozlian, 2005).

Los conceptos que han marcado la gestión de la cubierta vegetal en el viñedo, en las décadas pasadas, fomentaban en gran medida la máxima captación de la luz solar y una cierta iluminación de la zona de los racimos, para dar soluciones a las zonas del cultivo de las vertientes más frescas de clima mediterráneo. En la actualidad y debido al aumento generalizado de las temperaturas y la radiación solar y sobre todo la escasez de agua, estos planteamientos se encuentran bajo revisión como alternativa al cultivo del viñedo en zonas cálidas en donde una elevada radiación interceptada está asociada con un incremento en el consumo de agua en la cepa (Picón-Toro et al., 2012) y una sobre exposición de los racimos puede plantear problemas serios por golpes de calor y pérdida de calidad en la uva (Sadras & Moran, 2012). Todo ello, plantea prácticas de manejo que mantengan cierto sombreamiento sobre el entorno de los racimos y estrategias de riego deficitario que apoyen un desarrollo equilibrado en el viñedo (Sánchez-De-Miguel et al., 2010).

I.4. LA VITICULTURA DE ZONAS CÁLIDAS

Cada variedad de vid se desarrolla en un rango concreto de temperaturas (Jones & Webb, 2010). La Figura I.3 muestra el rango de temperaturas medias en el periodo vegetativo, adecuadas para el desarrollo y la maduración de las uvas de cada variedad.

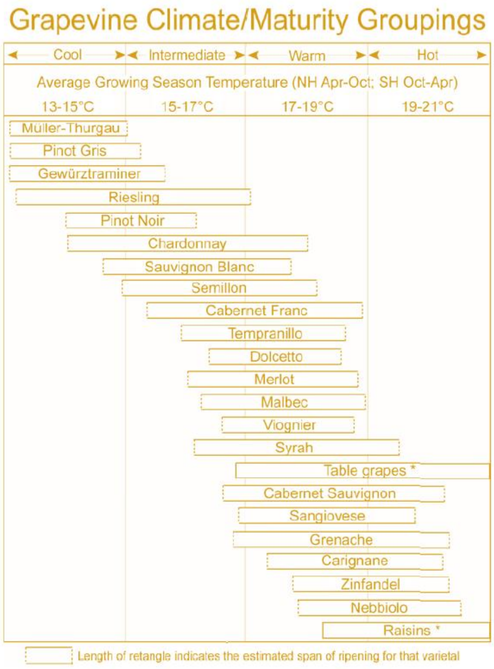

Figura I.3. Agrupaciones de madurez basadas en la media temperatura óptima durante la temporada de crecimiento para un conjunto de variedades de vid. (Jones, 2006)

El incremento de las temperaturas más allá de ciertos límites durante la maduración de las bayas tiene efectos negativos en su composición y en la calidad del vino (Duchêne & Schneider, 2005; Keller, 2010; Lacey et al., 1991; Mira de Orduña, 2010; Mori et al., 2007; Neumann & Matzarakis, 2011; Orlandini et al., 2008; Parra et al., 2010; Sadras & Moran, 2012). Se produce un acortamiento de los ciclos fenológicos (Duchêne & Schneider, 2005) que también pueden tener consecuencias negativas en el rendimiento (Mira de Orduña, 2010).

Temperaturas por encima de los límites climáticos de cada variedad aceleran la síntesis de SST (Petrie & Sadras, 2008) lo que aumenta el porcentaje de alcohol en vinos (Cook & Wolkovich, 2016; Jones et al., 2005; Koufos et al., 2014). Además, provocan pérdidas en la concentración de ácido málico, lo que conlleva una pérdida de acidez total y un aumento del pH de los mostos. Por otro lado, temperaturas por encima de los 30 ºC durante el periodo de maduración inhiben la acumulación de antocianos (Mori et al., 2007), provocando pérdida del color en los vinos. La ralentización en la síntesis de antocianos y la aceleración en la acumulación de sólidos solubles hace decrecer la relación antocianos/azúcares, provocando un desacople en los vinos (Sadras & Moran, 2012). Las altas temperaturas también están asociadas a un aumento de catequinas, lo que provoca un aumento de la astringencia de los vinos.

El desacople en los mostos provocado por las altas temperaturas hace difícil conservar el estilo de los vinos fuera de sus límites climáticos (Jones et al., 2005). En el caso de la ′Tempranillo′, los límites climáticos para la estación de crecimiento se encuentran entre 15 y 19ºC. ′Tempranillo′ es la variedad de uva tinta con mayor superficie cultivada en Extremadura. La Figura I.4 muestra los datos climáticos para la D.O. Ribera del Guadiana, desde 1997 a 2021 para el periodo vegetativo. Se observa que el promedio de temperaturas medias ha sido desde 1997, superior a los 19ºC, fuera o cerca del límite recomendado para las variedades cultivadas en esta región como se observa en la Figura I.3.

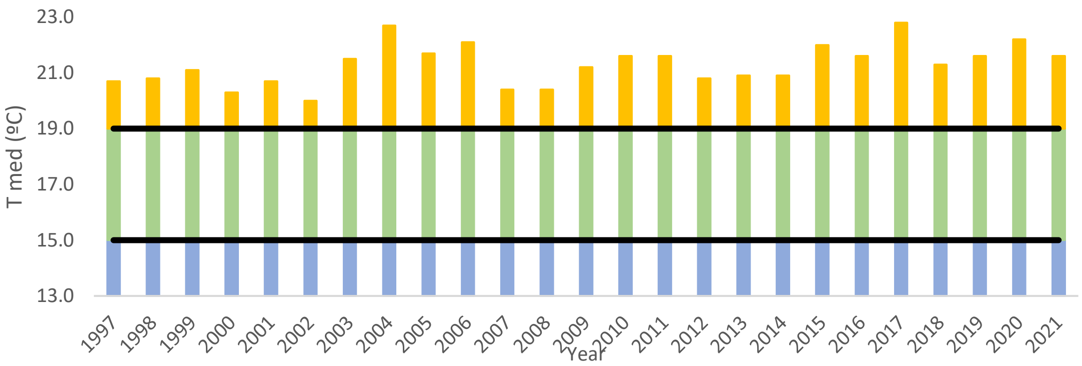

Figura I.4. Promedio de las temperaturas medias desde el 1 de abril al 30 de septiembre en Extremadura, España desde 1997 hasta 2021. En verde zona de temperaturas óptimas para el desarrollo de ′Tempranillo′ (15 - 19 ºC).

I.5. CONSECUENCIAS DEL CAMBIO CLIMÁTICO EN LA VITICULTURA

El Cambio Climático provoca un aumento de las temperaturas y un descenso en las precipitaciones o al menos un cambio en el patrón de distribución de las misma. Conlleva además un aumento de las condiciones extremas, con un mayor número de olas de calor, tormentas o periodos de sequía (UNFCCC, 2006).

El ICPP ha establecido cuatro nuevos escenarios de concentraciones de gases de efecto invernadero (IPCC, 2014) denominadas Trayectorias de Concentración Representativas (RCP). En estas trayectorias contemplan incluso las políticas orientadas a limitar el Cambio Climático del siglo XXI. Cada escenario analiza las concentraciones de CO_2 según su forzamiento radiactivo total para el año 2100, y varían desde 2.6 a 8.5 W m^{-2}, siendo el primero el que mejor pronóstico y el último el peor escenario posible. En España, según RCP 8.5 se prevé un aumento constante de temperaturas (Figura I.5), superando los 25ºC de medias anuales en gran parte del territorio. Las predicciones de temperaturas máximas durante el verano para este mismo escenario sitúan diferentes zonas de España por encima de los 38ºC de medias (Figura I.6). Esta situación se verá agravada por la disminución de las precipitaciones medias anuales (Figura I.7), donde la mayor parte del territorio español se sitúa por debajo de los 1.7 mm/día. Entre las zonas de España más afectadas por estas condiciones extremas se sitúa Extremadura siendo una de las zonas más afectadas la de la DOP Ribera del Guadiana.

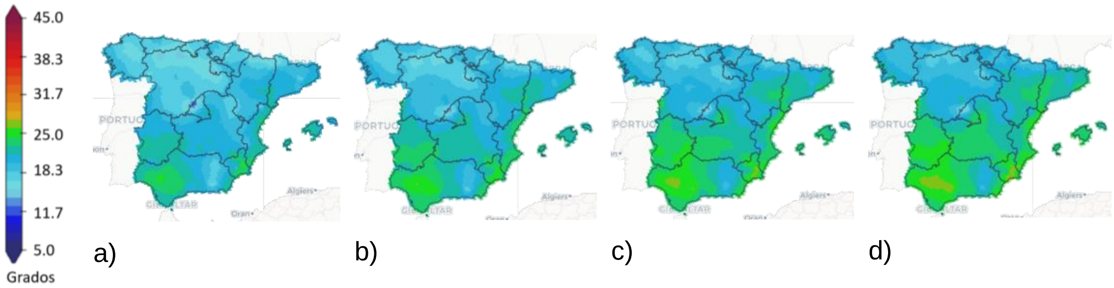

Figura I.5. Medias de temperaturas máximas durante todo el año según el escenario RCP 8.5: a) Histórico (1971-2000); b) Futuro cercano (2011-2040); c) Futuro medio (2041-2070); d) Futuro lejano (2071-2100). Fuente: Ministerio de Medio para la Transición Ecológica y el Reto Demográfico.

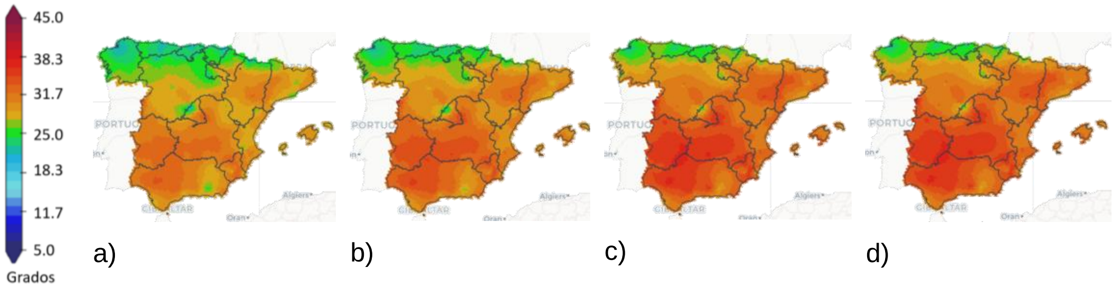

Figura I.6. Medias de temperaturas máximas durante el verano según el escenario RCP 8.5: a) Histórico (1971-2000); b) Futuro cercano (2011-2040); c) Futuro medio (2041-2070); d) Futuro lejano (2071-2100). Fuente: Ministerio de Medio para la Transición Ecológica y el Reto Demográfico.

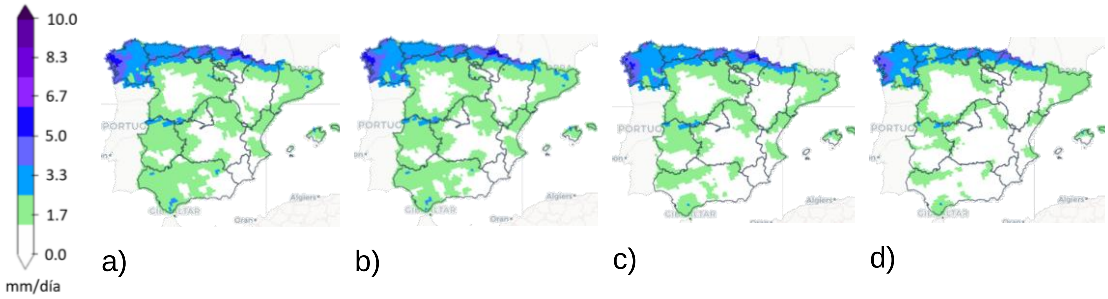

Figura I.7. Medias de precipitaciones diarias (mm/día) durante el año según el escenario RCP 8.5: a) Histórico (1971-2000); b) Futuro cercano (2011-2040); c) Futuro medio (2041-2070); d) Futuro lejano (2071-2100). Fuente: Ministerio de Medio para la Transición Ecológica y el Reto Demográfico.

Los posibles efectos de estas nuevas condiciones climáticas sobre la viticultura han sido estudiados en los últimos años (Keller, 2010; Sadras & Moran, 2012; Van Leeuwen et al., 2013). Las grandes regiones vitivinícolas han observado cambios de 1.3ºC en 50 años y se preveé que aumenten otros 2ºC en los próximos 50 (Jones, 2012). La estación de crecimiento ha aumentado 1.7ºC su temperatura desde 1950 a 2004 (Duchêne & Schneider, 2005; Fraga et al., 2012; Jones et al., 2005). Debido al adelanto de la fenología por el aumento de las temperaturas, se preveé un adelanto de 45 días en las vendimias en 2050 (Webb et al., 2008), lo que agravará aún más el problema, pues situará las vendimias en el periodo anual de temperaturas más elevadas.

Pequeñas variaciones de las temperaturas en la estación de crecimiento pueden inducir cambios en la idoneidad varietal de muchas regiones (Jones et al., 2005). Ante esta situación, las posibles soluciones incluyen la adaptación de nuevas variedades y la migración de los viñedos a nuevas regiones más elevadas (Fraga et al., 2014; Hall & Jones, 2009; Hannah et al., 2013; Jones et al., 2005; Lobell et al., 2006; Moriondo et al., 2013; Salinger, 1987; White et al., 2006). Sin embargo, para regiones en zonas cálidas, resulta difícil encontrar una variedad que se adapte al aumento de temperatura que se está produciendo (Jones, 2006). A medio-largo plazo esto afectará a la distribución mundial de la viticultura (Caffarra & Eccel, 2011; Jones et al., 2005; Keller, 2010; Schultz, 2000), con una expansión de las regiones vitivinícolas al norte de Europa, puesto que las regiones del sur serán demasiado calurosas (Kenny & Harrison, 1992), lo que afectará a la “fotografía” de la distribución mundial de los viñedos (Schultz, 2000). Para los viñedos ya distribuidos en zonas con escenarios climáticos más severos, se están desarrollando estrategias destinadas a desplazar el periodo de maduración, como solución para mitigar los efectos de las altas temperaturas y poder mantener en la medida de los posible las características optimas de la uva destinadas a vinos de calidad.

I.6. ESTRATEGIAS PARA MODIFICAR EL PERIODO DE MADURACIÓN DE LA UVA

Las altas temperaturas en periodo de maduración inhiben la síntesis de antocianos, reducen la acidez y aumentan el grado alcohólico de vinos. En zonas cálidas, como es Extremadura, la maduración se lleva a cabo en el momento de más calor del año. En términos generales, la brotación se produce a finales de marzo o principios de abril, y el envero suele llegar a mediados de julio. El ciclo desde envero a vendimia se sitúa, por tanto, durante los meses más caluroso, desde julio-agosto. Este problema se verá aún más agravado por el aumento constante de temperaturas debido al Cambio Climático.

La viticultura debe adaptarse a esta difícil situación. Una opción es retrasar la fenología de la vid, para llegar a la cosecha en condiciones menos cálidas y los expertos han propuesto algunas técnicas, como la elección de variedades o clones de maduración tardía, el uso de portainjertos más vigorosos, el uso de variedades autóctonas, la adaptación de los sistemas de formación, el riego o la poda tardía (Gutiérrez-Gamboa, Liu, et al., 2020; Van Leeuwen et al., 2019), aunque estas soluciones no parecen resultar suficientes para evitar los efectos del calentamiento global.

Gutiérrez-Gamboa et al. (2020) realizó una revisión describiendo otras técnicas algo más agresivas dirigidas a retrasar la maduración de la uva para que esta se sitúe en un periodo con temperaturas moderadas. Así, limitar el ratio “fuente-sumidero” mediante el deshojado en envero, puede conducir a una menor capacidad de acumulación de sólidos en la uva y, por tanto, a una ralentización del proceso de maduración de la uva con ratios de 0.6 $m^2 kg^{-1}$ según Keller, (2020). En climas cálidos, las altas temperaturas se prolongan durante la temporada de crecimiento e incluso con ratios bajos, las uvas podrían alcanzar altos niveles de sólidos solubles en vendimia (Palliotti et al., 2014). Otra alternativa, es modificar este ratio aumentando el número de “sumideros”, en este sentido la poda mínima aumenta el número de racimos por cepa limitando la cantidad de fotoasimilados que nutren los racimos (Zheng, et al., 2017) lo que conduce a una ralentización en la madurez de la uva. El sombreamiento de la canopy mediante redes de sombreo que reducen el flujo de fotones fotosintéticos en la superficie de la hoja y disminuyen la actividad fotosintética o la aplicación de sprays antitranspirantes como el Kaolin (Gatti et al., 2016) puede también retrasar la maduración de la uva, además de disminuir la demanda hídrica e incrementar la eficiencia intrínseca del uso del agua.

Por último, otro tipo de técnicas están dirigidas, no a ralentizar el desarrollo de los procesos fisiológicos que conducen a la maduración, sino a desplazar en el tiempo la consecución de los diferentes estadios fenológicos incluida la madurez de la uva. Entre ellas, la poda tardía, que consiste en retrasar la poda de invierno hasta la primavera, permite retrasar la brotación de las yemas francas de menor rango hasta primeros de mayo, comenzando el ciclo de la vid desplazado en el tiempo. Sin embargo, el retraso de la vendimia observado mediante esta técnica, apenas alcanza las dos semanas en el mejor de los casos y por tanto, sin que ocurra un significativo cambio en las condiciones de temperatura durante la maduración de la uva (Gatti et al., 2016; Zheng, García, et al., 2017). Por ello, este trabajo de tesis, se centra en una técnica que permite desplazar la vendimia de uno a dos meses y sitúa la maduración de la uva en un entorno climático con temperaturas más bajas.

I.7. TÉCNICA DE “FORZADO DE YEMAS” (“CROP-FORCING”)

I.7.1. DESPLAZAMIENTO DEL CICLO FENOLÓGICO, USO DEL “FORZADO DE YEMAS” COMO TÉCNICA PARA RETRASAR LA FECHA DE VENDIMIA

Para adaptar los viñedos situados en climas semiáridos al aumento de temperaturas y conservar la calidad de los vinos, se necesitan estrategias capaces de retrasar la fecha de vendimia al menos hasta finales de septiembre lo que supone un retraso considerable respecto a la fecha de vendimia “convencional”. Forzar el rebrote de las yemas es una técnica innovadora que se ha propuesto para las regiones vitícolas cálidas para hacer frente al calentamiento global (Gu et al., 2012). Esta técnica consiste en cortar los sarmientos en crecimiento, dejando varios nudos con el objetivo de evitar la paradormancía y forzar así el desarrollo de nuevas yemas. El “forzado de yemas” o “crop-forcing” fue estudiado por Gu et al. (2012) en ‘Cabernet Sauvignon’ en California, USA, en un experimento durante 2009 y 2010. Desde entonces, otros autores en España han puesto en práctica esta técnica. En La Rioja, Martinez De Toda et al. (2019) estudiaron su efecto, en ‘Tempranillo’ durante 2015, 2016 y 2017 y en ‘Marutana Tinta’ en 2017. En Valencia, Martínez-Moreno et al. (2019) también estudiaron esta técnica en ′Tempranillo′ durante 2016 y 2017, y en Japón, Kishimoto et al. (2022) la aplicaron en los años 2017 y 2018 en ‘Merlot’.

La técnica del “forzado de yemas” consiste en aprovechar la capacidad de la viña de fructificar varias veces al año si se “fuerza” la brotación de las yemas después de la iniciación de la inflorescencia (Dry, 1987). Esta habilidad permite retrasar la vendimia y desplazar la maduración de la uva hacia un periodo con condiciones térmicas más favorables con menor temperatura media (Dry, 1987; Gu et al., 2012; Liu et al., 1998). Aunque las primeras investigaciones en esta técnica se sitúan en la década de los 80, es recientemente cuando en un estudio de Gu et al. (2012) se ha propuesto utilizarla para paliar los efectos de las altas temperaturas y el cambio climático en zonas cálidas. En este trabajo Gu y colaboradores consiguieron desplazar la fecha de vendimia desde principios de septiembre hasta mediados de noviembre. Martinez De Toda et al. (2019) y Martínez-Moreno et al. (2019) encontraron resultados muy similares, con un desplazamiento de la fecha de vendimia de más de dos meses al aplicar el forzado, mientras que Kishimoto et al. (2022) obtuvieron un retraso de un mes y medio con respecto a la fecha de vendimia con tratamientos convencionales.

I.7.2. INCIDENCIAS DEL “FORZADO DE YEMAS” SOBRE LA PRODUCCIÓN Y LA COMPOSICIÓN DE LAS UVAS Y DEL VINO

El “forzado de yemas” hace disminuir los niveles productivos. Las vides tratadas con técnicas de forzado producen bayas más pequeñas y con menor pH en los mostos, así como un mayor contenido de acidez total, antocianos, taninos y fenotipos totales que las vides no forzadas (Gu et al., 2012). En la mayoría de los estudios realizados en España, las bayas procedentes de cepas forzadas mostraron un pH más bajo y una acidez titulable más alta que las uvas de las vides no forzadas con sólidos solubles totales similares, y la relación antocianina/azúcar significativamente mayor que en las bayas procedentes de cepas no forzadas (Lavado et al., 2019; Martínez-Moreno et al., 2019; Martinez De Toda et al., 2019). Aunque forzado mejora la composición de la uva para la elaboración de vino de calidad, reduce notablemente el rendimiento, tanto en la campaña de aplicación como en la posterior según los resultados observados por Martínez-Moreno et al. (2019).

El conocimiento del momento más adecuado de la aplicación, el tipo de yema que se desarrolla una vez aplicada esta técnica, y el nivel de carga de yemas ajustado mediante el forzado, resultan fundamentales para determinar la sostenibilidad de esta técnica. Así, los forzados realizados de manera temprana, antes de flores separadas resultan menos productivos que los realizados después del cuajado (Martinez De Toda et al., 2019), por otro lado, los forzados realizados próximos a envero, muestran serios problemas en alcanzar una maduración completa de las uvas (Gu et al., 2012; Martinez De Toda et al., 2019). Por otro lado, la técnica puede realizarse con varios niveles de carga de yemas según se trate de forzado en pulgar (1-2 yemas dejadas por pámpano) o forzado en vara (+ de 3 yemas dejadas por pámpano), esto podría influir en el número final de racimos y por lo tanto en el nivel de rendimiento obtenido (Gu et al., 2012; Martínez-Moreno et al., 2019; Martinez De Toda et al., 2019). El origen de la yema que finalmente rebrota, también juega un importante papel en el rendimiento. Así, si rebrotan las yemas anticipadas, que dan lugar a los brotes de verano menos productivos, el rendimiento resultará menor que si son las yemas latentes las que se desarrollan según lo observado por Kishimoto et al. (2022).

Los resultados prometedores observados en la mejora de la calidad de la uva, invitan a continuar con estudios, que permitan definir tanto el momento más apropiado para la aplicación del forzado, como los efectos sobre los rendimientos a corto y medio plazo.

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II. OBJETIVOS GENERALES

En esta Tesis Doctoral se ha estudiado el efecto del “forzado de yemas” aplicado en dos fechas diferentes, después de floración y después de cuajado, comparando los resultados con la aplicación de una estrategia de riego sin déficit hídrico junto con otra de riego deficitario recomendada para ‘Tempranillo’ en las condiciones de Extremadura en viñas no forzadas, así como el efecto combinado de ambas técnicas.

El estudio ha abordado cuatro aspectos fundamentales: (i) Evaluar el efecto sobre el desarrollo vegetativo, el rendimiento y sus componentes y características fisicoquímicas de la baya. (ii) Analizar el efecto sobre la evolución de la maduración que determina aspectos críticos como la fecha optima de vendimia y las características de las bayas. (iii) Valorar el efecto a corto y medio plazo de esta técnica sobre el viñedo, a nivel de reservas, de forma que se pueda predecir un posible agotamiento prematuro de las cepas. (iv) Evaluar el efecto de los tratamientos sobre las características de las bayas y su potencial enológico.

Estos objetivos generales se desglosan en los siguientes objetivos parciales:

- Capítulo I: Conocer la influencia de la técnica del “forzado de yemas” y su combinación con una estrategia de riego deficitario sobre el desarrollo de las cepas la producción y características físico-químicas de las bayas en comparación con cepas tratadas según el sistema “convencional” de cultivo en la zona.
- Capítulo II: Comparar la evolución de la maduración de las bayas de cepas “forzadas” en relación con las correspondientes de cepas “convencionales” y evaluar el potencial impacto sobre las características de las bayas en vendimia.
- Capítulo III: Evaluar la evolución de las reservas de carbohidratos en las cepas a corto y medio plazo y determinar la producción estacional de biomasa del viñedo y la distribución de la misma entre órganos vegetativos y productivos y valorar el posible desgaste provocado por esta técnica año tras año.
- Capítulo IV: Analizar la influencia de esta técnica sobre las diferentes familias de polifenoles y antocianos.
- Capítulo V: Evaluar las características de los vinos procedentes de cepas “forzadas” en relación a las “convencionales”.

III. MATERIALES Y MÉTODOS GENERALES

III.1. LOCALIZACIÓN Y DESCRIPCIÓN DEL VIÑEDO

El estudio se llevó a cabo durante los años 2017, 2018, 2019 y 2020 en 1.8 hectáreas de un viñedo experimental de 'Tempranillo' (Vitis vinífera L.) localizado en Badajoz, Extremadura, España (38º 51´ N; 6º 40´ W; 198 m). Las vides tenían 17 años al inicio del experimento, y estaban dispuestas como cordones bilaterales en un sistema de espaldera vertical con un sistema de riego por goteo de 4 $L{\cdot}h^{-1}$ por vid. Todas las vides fueron podadas en invierno a seis espolones y dos yemas por espolón. Los surcos están orientados de E-O y el espacio entre hileras y bejucos fue de 2,5 m y 1,2 m (3333 bejucos ha^{-1}), respectivamente. Además de la poda de invierno, en primavera se ajustó manualmente el número de brotes por cepa (12 brotes por cepa).

III.2. TRATAMIENTOS

Se establecieron dos tratamientos de riego y en cada uno de ellos tres tratamientos de poda. El factor principal fue el forzado de yemas, y el segundo factor fue el régimen hídrico. El diseño experimental fue de parcela dividida, con cuatro repeticiones por tratamiento. La parcela experimental consta de 6 hileras para 18 vides. Se monitorean diez vides centrales de las cuatro filas centrales (dos filas como borde).

Figura III.1. Fotografía del ensayo

III.2.1. "FORZADO DE YEMAS"

El experimento se compone de dos tratamientos a los que se le ha aplicado el forzado de yemas en dos fechas diferentes, después de floración (F1) y después de cuajado (F2). Estos dos tratamientos se comparan con un tratamiento sin forzar (NF) sometido a prácticas convencionales. El "forzado de yemas" consistió en eliminar todos los brotes laterales, hojas e inflorescencias, así como todo el material vegetal por encima de los 6 nudos, para forzar el estallido de los brotes primarios desarrollados en la temporada actual. El forzado de yemas fue aplicado en 2017, 2018 y 2019 sobre las mismas cepas. En el año 2020 no se aplicó.

Tabla III.1. Fechas de aplicación del forzado de yemas.

	**Después de floración F1**		**Después de cuajado F2**
	18 mayo 2017		07 junio 2017
	29 mayo 2018		18 junio 2018
	20 mayo 2019		03 junio 2019

Figura III.2. Cepa forzada

III.2.2. RÉGIMEN HÍDRICO

Se establecieron dos tratamientos de riego. Un tratamiento sin limitaciones hídricas (C) aportando agua para mantener un potencial hídrico de tallo de mediodía (Ψsmd) cercano a -0,6 MPa y un riego deficitario (RI) preenvero aportando agua para alcanzar un SWP de -1,1 MPa, como máximo y -0,8MPa en postenvero.

Tabla III.2. Resumen de tratamientos aplicados en este estudio

	Sin forzar (NF)	**Forzado después de floración (F1)**	**Forzado después de cuajado (F2)**
Sin limitaciones hídricas (C)	C-NF	C-F1	C-F2
Riego Defictario (RI)	RI-NF	RI-F1	RI-F2

III.3. CONDICIONES CLIMÁTICAS

El suelo es aluvial, de textura franco a franco-arenosa, ligeramente ácido y carente de materia orgánica. La profundidad del suelo es mayor a 2.5m y con bajo contenido de piedra. La zona tiene un clima mediterráneo con suave influencia atlántica, veranos secos y calurosos, con alta demanda diaria de radiación y evaporación. Obtuvimos datos agrometeorológicos de una estación cercana al viñedo (100 m) con las características descritas en Martí et al. (2015).

Tabla III.3. Climatología anual durante los años 2017, 2018 y 2019.

Año	**Tª Max (ºC)**	**Tª Min (ºC)**	**Lluvias (mm)**
2017	25.3	9.5	282.2
2018	22.9	9.7	479.3
2019	24.3	9.4	292.9

III.4. VENDIMIAS

Las vendimias se realizaron manualmente, cuando la media de los mostos de las cuatro repeticiones de cada tratamiento alcanzó los 24±1 ºBrix (según el criterio de la zona). En 2018 y 2019 los mostos iniciales de los tratamientos F1 obtuvieron valores de Sólidos Solubles superiores a 26ºBrix, por lo que en el capítulo 5, se descartaron los tratamientos C-F1 y RI-F1.

Tabla III.4. Fechas de vendimias.

No forzado **NF**	**Después de floración** **F1**	**Después de cuajado** **F2**
22 agosto 2017	12 septiembre 2017	17 octubre 2017
27 agosto 2018	8 octubre 2018	29 octubre 2018
27 agosto 2019	30 septiembre 2019	15 octubre 2019
5 agosto 2020	5 agosto 2020	5 agosto 2020

III.5. ANÁLISIS ESTADÍSTICO

Para comprobar la normalidad de las muestras con un número de repeticiones menor a 30, se realizó el test de Shapiro-Wilks. Si la hipótesis de normalidad se rechazaba ($p<0.05$), las muestras se sometían a pruebas no paramétricas, usando el test de Kruskal Wallis como ANOVA de 1 vía y la comparación de medias a pares para establecer los grupos. Si la hipótesis de normalidad se confirmaba ($p>0.05$), o si el número de repeticiones por muestras era superior a 30, se comprobó la homogeneidad de las muestras usando el test de Barlett o test de Levene. Si la hipótesis de homogeneidad se rechazaba ($p<0.05$), los valores se sometieron a diferentes transformaciones, repitiendo el test de homogeneidad. Si la homogeneidad finalmente no se confirmaba ($p<0.05$), las muestras se sometían a muestras no paramétricas. Si la hipótesis de homogeneidad se confirmaba ($p>0.05$) las muestras se sometían a un análisis factorial múltiple (MANOVA), usando como factores el forzado y el riego, y usando como post-hoc el test de Tukey.

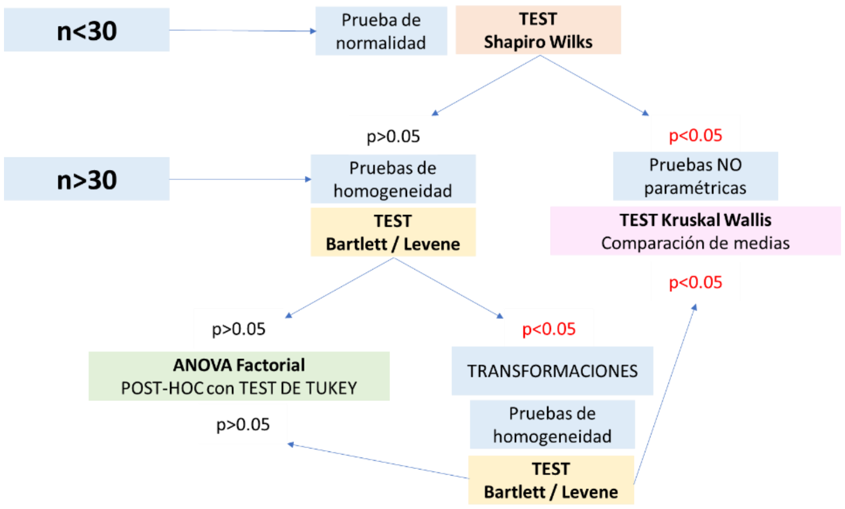

Figura III.3. Esquema de análisis estadístico.

III.4. REFERENCIAS

Martí, P., González-Altozano, P., López-Urrea, R., Mancha, L. A., & Shiri, J. (2015). Modeling reference evapotranspiration with calculated targets. Assessment and implications. *Agricultural Water Management*, *149*, 81–90. https://doi.org/10.1016/j.agwat.2014.10.028

IV. CAPÍTULO 1.

COMBINED EFFECT OF "CROP-FORCING" AND REDUCED IRRIGATION AS TECHNIQUES TO DELAY THE RIPENING AND IMPROVE THE QUALITY OF 'TEMPRANILLO' (VITIS VINIFERA L.) BERRIES IN SEMI-ARID CLIMATE CONDITIONS.

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IV. ABSTRACT

For vineyards in Mediterranean climates, subjected to high temperatures and scarce rainfall, new growing practices are increasingly required that minimize the adverse effects of global warming on grape yield and quality. The crop-forcing technique (CFT) consists of inducing the regrowth of buds developed during the season in course with the goal of modifying the phenology of the grapevine and shifting berry ripening to a time of moderate temperatures. The objective of this work is to evaluate the effect of CFT in a ′Tempranillo′ vineyard in the semi-arid conditions of Extremadura (Spain), together with different irrigation strategies. In the field trial which ran from 2017 to 2019 three pruning treatments were applied: on two different CFT application dates between the phenological stages of flowering and fruit set (F1) and between fruit set and pea size (F2); and in a control treatment with conventional winter pruning (NF)). In addition, each treatment received two different irrigation regimes: irrigation covering the water requirements of the plants (C); and a deficit irrigation with moderate water stress during pre-veraison (RI). For the C irrigation treatment, CFT application delayed harvest by an average of 32 and 56 days in F1 and F2, respectively, with a decrease in the mean temperature during ripening. In both cases, yield was lower than with the corresponding conventional pruning treatment, although it was more stable between years. The RI treatment also reduced yield compared to the corresponding C treatments, but NF-RI gave a higher yield than any of the CFT treatments. Titratable acidity, malic acid content, total polyphenol content and total anthocyanin content increased with CFT. In combination with RI, CFT had a synergic effect on grape composition with qualitative improvements, but yield was lower compared to the corresponding C treatments.

IV. RESUMEN

En viñedos de climas mediterráneos, sometidos a elevadas temperaturas y escasez de lluvia, es de interés la búsqueda de nuevas prácticas culturales que minimicen los efectos adversos del calentamiento global sobre la producción y calidad de la uva. La Técnica del Crop-Forcing (CFT) consiste en provocar el rebrote de las yemas desarrolladas en la temporada en curso con el objetivo de desplazar la fenología de la vid y que la maduración de la uva se realice con temperaturas moderadas. El objetivo de este trabajo es evaluar el efecto de la CFT en un viñedo de ‘Tempranillo’ para las condiciones semiáridas de Extremadura, junto con diferentes estrategias de riego. El ensayo de campo establecido durante los años 2017-2019 dispuso de tres tratamientos de poda: dos fechas diferentes de aplicación del CFT, entre los estados fenológicos de floración y cuajado de la uva (F1), entre cuajado y tamaño guisante (F2) y un control con poda convencional en invierno (NF). Cada tratamientos sometido a dos regímenes

hídricos: riego para cubrir las necesidades hídricas de las plantas (C) y riego deficitario con estrés hídrico moderado durante pre-veraison (RI). La CFT retrasó la vendimia una media de 32 y 56 días para F1 y F2 respectivamente en relación a C, descendiendo la temperatura media durante la maduración. En ambos casos la cosecha fue inferior a los correspondientes tratamientos con poda convencional, aunque más estable entre años. El RI también disminuyó la cosecha respecto de los correspondientes C, pero NF-RI fue más productivo que cualquiera de los tratamientos sometidos a CFT. La CFT incrementó la acidez titulable y los contenidos en ácido málico, polifenoles y antocianos totales en las uvas. El CFT junto con RI, tuvo un efecto sinérgico sobre la composición de las uvas con mejoras cualitativas, pero disminuyendo la cosecha en relación con los correspondientes C.

IV.1. INTRODUCTION

The practice of viticulture in semi-arid conditions is characterized by high temperatures and soil water deficit for a large part of the period of berry growth and development. Although grapevine (*Vitis vinifera L.*) is a species well adapted to the recurring droughts of the Mediterranean region, yield tends to be low and the grape has a high sugar concentration that goes against current market trends. This situation is aggravated during the summer months when rainfall is very scarce prior to budbreak, with severe stress levels being recached. Irrigation can counteract the negative effects of water scarcity, increasing yield and modifying berry composition (Williams & Matthews, 1990). Irrigation with doses that do not cover the water requirements of the plants at certain moments of their crop cycle, in addition to increasing yield compared to rainfed land, can also improve the final quality of the grape (Chapman et al., 2004; Intrigliolo & Castel, 2010; Mancha et al., 2020; Matthews & Anderson, 1988).

Another aspect that requires consideration in these climates is the effect of the high temperatures on the synthesis and accumulation of compounds in the berry during maturation, an effect that tends to lead to a loss of wine quality (Duchêne & Schneider, 2005; Keller, 2010; Lacey et al., 1991; Mira de Orduña, 2010; Mori, Goto-Yamamoto, Kitayama, et al., 2007; Sadras & Moran, 2012). The high temperatures usually accelerate the processes, reducing the time between budbreak and harvest (Duchêne et al., 2010; Duchêne & Schneider, 2005; Jones & Davis, 2000; Petrie & Sadras, 2008) as well as causing ripening to happen during the hottest summer months which negatively affects must composition (Fraga et al., 2016; García De Cortázar-Atauri et al., 2017; Van Leeuwen et al., 2019). Under these conditions of water limitations and high temperatures, grape composition is affected in terms of its sugars/acids ratio, with a reduction in malic acid content (Koundouras et al., 2006) which causes a loss of acidity and an increased pH (Keller, 2010). In addition, the accumulation of anthocyanins is inhibited (Buttrose et al., 1971; Kliewer & Antcliff, 1970; Mori et al., 2005; Mori, Goto-Yamamoto, Hashizume, et al., 2007; Spayd et al., 2002; Tarara et al., 2008; Winkler et al., 1974), with a consequent decoupling of phenological and technological maturity (Sadras and Moran, 2012). This effect can be observed even in the best adapted varieties to warm production areas where grape maturation tends to take place during the coolest months of the crop cycle (Jackson & Lombard, 1993). This situation is worsening as a result of global warming, as indicated in the latest reports of the International Panel on Climate Change (IPCC), making strategies to mitigate the effects of high temperatures during grape maturation a focus of attention of researchers and wine producers (Gutiérrez-Gamboa et al., 2021).

One such strategy is the crop-forcing technique (CFT), which aims to delay phenological stages so that grape maturation takes place during cooler periods. The technique consists of cutting growing shoots, leaving various nodes and removing from them all the leaves, water shoots and inflorescences. In this way, the paradormancy of the newly formed buds is broken, forcing their budbreak in the same season, with a new time-shifted harvesting being obtained. The extent to which CFT is able to shift grape harvest time depends on the phenological stage during which it is applied. In this way, it is also possible to modify the grape composition associated to its normal cycle, obtaining lower pH, and higher total acidity, anthocyanins and tannins in the berries compared to non-forced vines (Gu et al., 2012). Other authors who employed this technique in more recent works and in another winegrowing area obtained similar results (Martínez de Toda et al., 2019; Martínez Moreno et al., 2019). However, the main drawback associated to CFT application is the drastic reduction in yield that has been observed, with potential effects not only for the campaign in course but for subsequent ones as well as due to the decrease in vine reserves (Martínez-Moreno et al., 2019).

The most suitable moment for CFT application, the type of bud that develops when the technique is applied and the CFT-adjusted bud load level are factors that have been little studied but which are essential to know to deal with the decrease in grape yield. In this regard, Martínez de Toda et al. (2019) reported that early CFT application (before flower separation) resulted in lower fertility compared to later applications (after anthesis) which gave larger yields, but also that grape maturity was incomplete when CFT was applied close to veraison, which could indicate that full grape ripening in later CFT applications depends on climate conditions and grape variety (Martínez de Toda et al., 2019; Gu et al., 2012). The bud load left after CFT varies in the published studies depending on whether so-called short pruning (1-2 buds per shoot) or cane pruning (5-6 buds per shoot) is applied. Martinez-Moreno et al. (2019) found no statistically significant differences in number of clusters between the two types of pruning, and average decreases in yield of 82% compared to the non-forcing control treatment. However, less drastic yield reductions were reported by Gu et al. (2012) with cane pruning and by Martinez de Toda et al. (2019) with short pruning, with the latter study also reporting that increasing the number of buds per shoot could possibly increase yield in CFT treatments. The origin of the bud that regrows when the CFT is applied plays an important role in yield. The axillary buds comprise a lateral bud, which grows in the same campaign producing summer shoots that are not very fertile, and three latent buds whose development is inhibited by the growth of the summer shoots from the lateral bud (Keller, 2010). Kishimoto et al. (2022), comparing CFT when leaving and removing summer shoots, noted a significant positive difference in yield with the treatment that removed summer shoots, generating regrowth of the latent buds. This suggests that the most interesting buds to use in CFT are the latent as opposed to the lateral ones.

In addition to adjustments in form and time of CFT application, the effects of soil water availability and irrigation management in combination with CFT remain largely unexplored. In most published CFT trials which have included irrigation, it is either reported that irrigation was applied to satisfy the water requirements of the non-forcing control treatment or it is not specified if the irrigation schedule took into account the water requirements of the CFT treatment. Application of the CFT shifts in time the development of the phenological stages and in this way modifies the water requirements of the vines. Tian and Gu (2019), working with ′Cabernet Sauvignon′, applied three deficit irrigation regimes to CFT vines in post-veraison to improve grape composition. They reported no effect of RI on anthocyanin concentration compared to CFT vines irrigated without water limitations. For their part, Gu et al. (2012) applied to all the plants in their trial (Control and Forced) 65% ET_c in pre-veraison and 80% ET_c in post-veraison, observing lower pruning weights, fewer forced clusters, lower yields and faster ripening compared to the following year when irrigation was continuously applied to 80% ET_c. Martinez-Moreno et al. (2019), in a trial with ′Tempranillo′, reported lower water stress during the pea size and veraison phenological stages in the later CFT treatments, while the early treatments showed no water stress at harvest. In their study, the same irrigation regime was applied to all treatments.

The objective of the present study is to analyse the effect of two different CFT application dates and the interaction between CFT application and pre-veraison RI in ′Tempranillo′ on water status, vegetative growth, yield and its components, and grape composition under the semi-arid climate conditions of Extremadura.

IV.2. MATERIALS AND METHODS

IV.2.1. LOCATION, DESCRIPTION OF THE VINEYARD AND WEATHER CONDITIONS

The study area has a semi-arid Mediterranean climate, with annual rainfall of 450 mm and a mean temperature of 16.36 ºC (2004-2020 average). Durante the vegetative period (April-September), rainfall is just 131 mm and the mean temperature is 21.0 ºC. In this region, 'Tempranillo' maturation takes place during the months of July, August and September in mean temperatures of 24.3 ºC, 24.3 ºC and 21.5 ºC, respectively.

The study was carried out during the 2017, 2018 and 2019 agricultural seasons in a 1.8 ha experimental vineyard of the ‘Tempranillo’ (Vitis vinifera L.) variety grafted on Richter 110 rootstock, located at Badajoz, Extremadura, Spain (38º 51´ N; 6º 40´ W; 198 m). The vines were 17 years old at the beginning of the experiment, trained as bilateral cordons in a vertical trellis system with a drip irrigation system of $4L{\cdot}h^{-1}$ per vine. All vines were winter pruned to six spurs and two buds per spur. The rows are E-W oriented, and row and vine spacing are 2.5 m and 1.2 m (3333 $vine{\cdot}ha^{-1}$), respectively. In addition to winter pruning, the number of shoots per vine was adjusted manually in the spring (12 shoots per vine).

The soil is alluvial, with a loam to sandy texture, slightly acidic, and lacking in organic matter. Soil depth is greater than 2.5 m and with low stone content. The agrometeorological data were obtained from La Orden weather station (100 m from the vineyard) which forms part of the Agroclimatic Information System for Irrigation (SIAR by its initials in Spanish), with the characteristics described in Martí et al. (2015).

IV.2.2. TREATMENTS AND EXPERIMENTAL DESIGN

The trial had two treatments with CFT applications at two different times; F1, in which CFT application was between flowering and fruit set (May 18, 2017; May 29, 2018; May 20, 2019) and F2, in which it was applied between fruit set and pea size (June 6, 2017; June 18, 2018; June 3, 2019). F1 and F2 were compared with a non-forcing (NF) treatment grown under conventional practices (just winter pruning). The CFT consisted of hedging the growing shoots to six spurs and removing all the summer laterals, leaves and inflorescences with scissors to force the bursting of the primary buds developed in the current season. As a secondary factor, two irrigation treatments were established. A treatment with full irrigation (C), supplying water to maintain a midday stem water potential (SWP) close to -0.6 MPa (100% ET_c) and a pre-veraison regulated irrigation (RI) supplying water to reach an SWP of -1.1 MPa, as maximum, and -0.8 MPa in post-veraison. A total of 6 experimental treatments were therefore established, which are summarized in Table 1.

Table IV.1. Summary of the treatments applied in the study.

	NF. **Unforced**	**F1.** **Flowering to setting**	**F2.** **Setting to pea size**
C. Full irrigation	C-NF	C-F1	C-F2
RI. Regulated irrigation	RI-NF	RI-F1	RI-F2

Vine water requirements were calculated based on the crop evapotranspiration (ET_c), using the crop coefficient (K_c) recommended by the FAO for these latitudes for the NF treatments. For the F1 and F2 treatments, ET_c was calculated directly on a weighing lysimeter (Picón-Toro et al., 2012) integrated in the study plot with two F1 vines. Irrigation in F1 and F2 was started on the same day in each season and the same irrigation volume was applied in both treatments. F2 was irrigated based on the water needs obtained in the weighing lysimeter for F1.

Irrigation started when an SWP threshold value of -0.6 MPa was reached. Irrigation was applied five to six times per week,. Volumetric water meters were used to measure the amount of water applied to each subplot, and irrigation was maintained until the beginning or middle of October.

The experimental design was a split-plot, with four replicates per treatment. The experimental plot consists of 6 rows per 18 vines. The ten central vines of the four central rows were monitored (two rows as a border).

IV.2.3. VINE PHENOLOGY AND WATER STATUS

A phenological assessment was performed weekly according to the modified E-L system (Coombe, 1995). A visual inspection was made of ten plants per plot starting from mid-March (‘cotton bud’ stage), to determine the most representative growth stage (the stage shown by at least 50% of vines), as well as the most backward and the most advanced stages in the sample.

The SWP was measured with a pressure chamber (Soil Moisture Corp., Model 3500, Santa Barbara, CA, USA), following the procedure described by Martí et al. (2015), using leaves on the north side of the trellis (in the shade), close to trunk level and wrapped in aluminium foil at least 2 h before data recording. Measurements were taken weekly on one leaf per vine and in two plants per subplot.

IV.2.4. VEGETATIVE GROWTH

Vine growth was characterized by the fraction of intercepted photosynthetically active radiation (*fi*PAR), which was measured at solar noon with a linear ceptometer (80 cm probe length; Accupar Lineal PAR LP-80; Decagon Devices, Pulman, WA, USA) from two labelled vines per experimental plot. Measurements were taken every two weeks on clear days from flowering to the onset of senescence following the methodology described in (Mancha et al., 2021). The total leaf area of the vines was estimated using an LI-3100C laboratory leaf area meter (Li-Cor, Lincoln, NE, USA).

IV.2.5. YIELD AND YIELD COMPONENTS

The vines were manually harvested when the mean of the four replications of each treatment reached must total soluble solids (TSS) content of 23–24 °Brix (a common harvesting criterion for this variety in this area). NF vines were harvested on August 22, 2017, August 27, 2018, and August 27, 2019. F1 vines were harvested on September 12, 2017, October 8, 2018, and September 30, 2019. F2 vines were harvested on October 17, 2017, October 29, 2018, and October 15, 2019. Clusters were counted and weighed on a total of 10 vines per experimental plot. The number of berries per cluster was counted on 12 homogeneous clusters per treatment.

IV.2.6. GRAPE COMPOSITION

Samples of 500 g of berries per plot were collected randomly at harvest. In the laboratory, the grapes were destemmed and then crushed and homogenised in a Mycook blender (Taurus, Oliana, Spain) at speed setting 3 for 1 min. An aliquot of the obtained mash (pulp, juice, skins and seeds) was filtered and used to determine technological parameters. The TSS (ºBrix) was determined by refractometry (RE40D, Mettler Toledo, Greifensee, Switzerland), pH by pH-metro (Basic 20, Crison, Alella, Barcelona) and titratable acidity (TA, $g\cdot L^{-1}$) by titrator (T50, Mettler Toledo, Greifensee, Switzerland), according to ECC formal methods (ECC, 1990). Tartaric and malic acid content ($g\cdot L^{-1}$), were enzymatically analysed according to ECC formal methods (ECC 1990) using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

Phenolic compounds were extracted following the methodology previously described by Portu et al. (2016) with minor modifications. Samples of 100 g of berries per plot were collected randomly each week from veraison to harvest and frozen (-80°C). The grapes were crushed and homogenised in a Freshboost blender (Moulinex, Aleçon, France) for 30 seconds. One aliquot of homogenate per repetition (1.0 g) was incubated for 30 minutes in an ultrasound bath (USC-TH, VWR, Radnor, USA) in 10 mL of methanol/water/formic acid buffer (50:48.5:1.5). The incubated samples were centrifuged at 10 °C for 10 min (5810 R, Eppendorf, Hamburg, Germany). The extraction process was carried out in triplicate. Total polyphenol (TPP) content was determined according to Singleton and Rossi (1965) and total anthocyanin (Ant) content was quantified using the pH differential method (Lee et al., 2005). All determinations were carried out using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

IV.2.7. STATISTICAL DATA ANALYSIS

Normality and homogeneity of variances were checked using the Shapiro-Wilk's and Bartlett's test respectively. When the normality and homogeneity of variances were verified, data were subjected to multivariate analysis of variance (MANOVA) to investigate the effect of "crop-forcing", "irrigation" and their interaction on each parameter evaluated, selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons. The interaction between effects was evaluated by calculating the least-squares means (LS means), selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons and the Tukey test as post hoc test for parametric samples. When the normality and homogeneity of variances were not verified, non-parametric tests were carried out with the Kruskal-Wallis's test (alternative to one-way ANOVA) and multiple comparison p values (alternative to post-hoc pairwise comparisons). Differences between means were considered statistically significant when $p < 0.05$. These statistical tests were performed with XLSTAT-Pro 201610 (Addinsoft, 2009, Paris, France).

IV.3. RESULTS

IV.3.1. WEATHER CONDITIONS AND VINE PHENOLOGY

Compared to the 2004-2020 mean value for the area of 450 mm, 2017 and 2019 were relatively dry years, with respective annual precipitation values of 282.2 mm and 296.9 mm, In 2018, the corresponding value was 479.3 mm, with a notable contribution of 140 mm in March (Figure IV.1). From April to September (vegetative period), precipitation was 49.7 mm, 114.7 mm and 67.4 mm in 2017, 2018 and 2019, respectively, and the highest average temperature was recorded in 2017 (22.8 ºC). August was the hottest month, with maximum temperatures above 35ºC in all three study years, as well as minimum values higher than 16 ºC. In October, maximum temperatures were below 25 ºC in 2018 and 2019, but above 30 ºC in 2017.

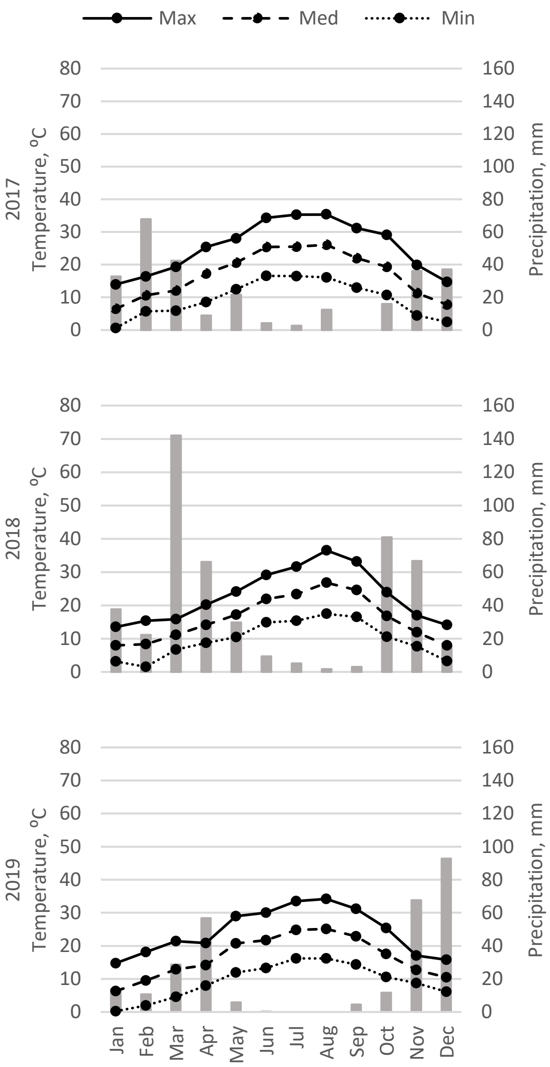

Figure IV.1. Temperatures (lines), and rainfall (bars) during 2017, 2018 and 2019.

The normal budbreak took place on April 3, day of the year (DOY) 93, in 2017 and 2018 and on March 26 (DOY 85) in 2019. Immediately after CFT application (Table IV.2), regrowth occurred in the F1 and F2 treatments, restarting the crop cycle. This resulted in the coexistence, in the same season, vineyard and variety, of three different phenological cycles: one 'normal' cycle for the NF treatments (C-NF and RI-NF), a different cycle for the F1 treatments (C-F1 and RI-F1), and another different cycle for F2 (C-F2 and RI-F2). After the second year of CFT application, a slight delay was observed in initial budbreak (March) of some 3-5 days in F2 compared to NF. In each pruning, the irrigation treatments did not modify vine phenology.

Table IV.2. Dates of the application of CFT and regrowth in F1 and F2 treatments.

Treatments	2017	2018	2019
NF (C-NF and RI-NF)	--	--	--
F1 (C-F1 and RI-F1)	18 May (138)*	29 May (149)	20 May (140)
F2 (C-F2 and RI-F2)	07 Jun (157)	18 Jun (168)	03 Jun (154)

**DOY in brackets*

In the NF treatments, the duration of the phenological cycle (budbreak-harvest) was 141 days in 2017, 148 days in 2018 and 154 days in 2019 (Table IV.3). The cycle was shorter in the F1 and F2 treatments (considered from the date of CFT application) with 133 days, while in 2017 it was 117 days. During the trial, we observed an average decrease in the duration of the period from budbreak to anthesis of 6.6 days in F1 and of 13.7 days in F2 with respect to NF. The mean duration of the period from anthesis to veraison was 8.6 days shorter in F1 and 5.3 days longer in F2 compared to NF., while the period from veraison to harvest was, on average, 5.3 days shorter in F1 and 4.3 days shorter in F2.

With modification of the crop cycle, the vines of the NF, F1 and F2 treatments were subjected to different meteorological conditions in each of the phenological stages during the season in course. The maximum temperatures recorded between budbreak and anthesis (mean values of the three years) were 7.4 ºC and 8.5 ºC higher in F1 and F2, respectively, compared to NF (Table IV.3), and around 3.5 ºC higher for both treatments in the anthesis to veraison period. However, during maturation (veraison to harvest) the mean maximum temperature was 2.4 ºC lower for F1 and 6.3 ºC lower for F2, with the largest difference for F2 of -8.7 ºC taking place in 2018 with harvest on DOY 302 (Table 3). Precipitations decreased in F1 and F2 in the budding to anthesis period, but increased in F2 from veraison to harvest in 2018 and 2019, while in 2017 there was no rainfall in this period. The 2019 season was notable for the low rainfall in the anthesis to veraison period in the three treatments. During the maturation period (veraison to harvest), rainfall was higher for F2, with the most notable value of 76.8 mm recorded in 2018 (Table IV.3).

Table IV.3. Duration of phenological periods in days, average maximum temperatures and rainfall in each period in the 2017, 2018 and 2019 growing seasons.

Year	Phenological stages	DOY			Days			T max (ºC)			Rainfall (mm)		
		NF	F1	F2	NF	F1	F2	NF	F1	F2	NF	F1	F2
2017	**budbreak - anthesis**	93-136	138-177	157-194	43	39	37	25.8	33.7	34.7	30.5	4.0	6.8
	anthesis - veraison	136-187	177-221	194-256	51	44	62	33.1	34.5	35.0	4.2	2.8	12.5
	veraison - harvest	187-234	221-255	256-290	47	34	34	35.9	34.4	30.7	2.6	12.5	0.0
2018	**budbreak - anthesis**	93-145	149-191	168-204	52	42	36	22.2	29.0	32.0	86.2	15.8	5.4
	anthesis - veraison	145-204	191-242	204-261	59	51	57	29.2	34.6	34.6	19.0	1.8	4.6
	veraison - harvest	204-239	242-281	261-302	37	39	41	35.8	32.7	27.1	1.6	3.0	76.8
2019	**budbreak - anthesis**	85-133	140-182	154-189	48	42	29	22.9	30.4	29.8	67.0	0.2	0.2
	anthesis - veraison	133-200	182-238	189-253	67	56	74	30.8	33.8	34.3	0.2	0.0	0.2
	veraison - harvest	200-239	238-273	253-288	39	35	35	34.2	31.6	29.1	0.0	4.6	9.9

DOY: Day of the year. Days: Duration of grape phenological stages; T max: average maximum temperatures during each of the phenological stages of the grapes; Rainfall: total rainfall during each of the phenological stages of the grapes.

IV.3.2. IRRIGATION, VINE WATER STATUS (SWP) AND FRACTION OF INTERCEPTED PAR RADIATION (FIPAR)

Irrigation was initiated in the C treatments (C-NF, C-F1 and C-F2) and at a later date in the RI treatments (RI-NF, RI-F1 and RI-F2) when, in each case, the SWP was reached that had been established for the corresponding proposed irrigation strategy. The RI irrigation amount was, on average for the three years of the study, 63% of that applied in C, which had a mean irrigation amount for the three years of the study of 416 mm. With respect to the effect of CFT application on total applied irrigation volumes, the C-F1 and C-F2 treatments received around 19% more water than C-NF, except in 2017 when the total applied water in the C treatments (C-NF, C-F1 and C-F2) was similar (Table 4). In the other campaigns, the C-F1 and C-F2 treatments received a similar total volume of water, but 143 mm and 83 mm more than applied in C-NF in 2018 and 2019, respectively. In the RI treatments, the total volume of applied water was higher in the RI-F1 and RI-F2 treatments in 2017 (+69%) and 2019 (+7%) compared to the RI-NF treatment (Table IV.4) and similar in 2018.

Table IV.4. Volumes of water applied (mm) in each treatment C (C-NF, C-F1 and C-F2) and RI (RI-NF, RI-F1 and RI-F2) in the different phenological periods in the 2017, 2018 and 2019 growing seasons.

Years	Phenological	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2
2017	**Pre-CFT**	0	0	17	0	0	20
	Pre-v	200	189	328	0	150	214
	Post-v	87	142	70	56	69	38
	Post-h	131	99	15	110	62	8
	Total	418	430	430	166	281	280
	Rainfall	37	19	19	37	19	19
2018	**Pre-CFT**	0	0	8	0	0	8
	Pre-v	156	339	420	0	145	187
	Post-v	119	175	99	144	112	69
	Post-h	109	12	0	119	8	0
	Total	384	526	527	263	265	264
	Rainfall	106	20	86	106	20	86
2019	**Pre-CFT**	0	4	25	0	10	33
	Pre-v	191	325	341	63	248	252
	Post-v	147	157	156	146	115	107
	Post-h	109	58	21	166	28	9
	Total	446	544	543	375	401	401
	Rainfall	67	5	10	67	5	10

Pre-CFT; pre-crop forcing technique; Pre-v: pre-veraison; Post-v: post-veraison; Post-h: post-harvest. Rainfall: total rainfall from budbreak to harvest.

The distribution of irrigation by periods was different in each of the treatments (Table IV.4). As the starting date of irrigation in F1 and F2 was the same, it coincided with the moment of CFT application in F1, and was 20 days before the CFT application date in F2. Therefore, the F2 treatments (C-F2 and RI-F2) were the only ones irrigated pre-CFT (between 8 mm and 33 mm, depending on treatment and year). For all treatments throughout the trial, with the exception of RI-NF, pre-veraison was the period which received the most irrigation: around 44% of the total for C-NF, 57% for C-F1 and 73% for C-F2. The RI-NF treatment did not require any irrigation as the SWP values remained above -1.1 MPa (value established for medium-high stress) during this period, except in 2019 when 63 mm were required (Table IV.4). In post-veraison, the C-NF treatment received an average of 118 mm, and C-F1 and C-F2 averages of 158 mm and 108 mm, respectively. After veraison, the RI treatments were irrigated to maintain light stress (-0.8 MPa), with RI-NF receiving an average of 115 mm, RI-F1 99 mm and RI-F2 71 mm. The irrigation campaign concluded after the harvest, when evaporative demand fell and the autumn rains commenced, with the average post-harvest irrigation volume amounts being 116 mm in C-NF, 56 mm in C-F1 and 12 mm in C-F2. In the RI treatments, the average post-harvest applied irrigation volume was 132 mm in RI-NF, 33 mm in RI-F1 and just 6 mm in RI-F2.

Figure IV.2 shows the evolution of SWP in the C treatments (C-NF, C-F1 and C-F2), with a similar trend in 2017 and 2019 when the lowest SWP values were recorded between DOY 200 and DOY 250, while in 2018 the values always remained around -0.6 MPa. The mean annual value for the three treatments remained between -0.6 MPa and -0.8 MPa, and were always less negative in C-F2 and more negative in C-NF. The RI-NF treatment had lower SWP values than C-NF, reaching severe stress levels with minimum values around DOY 200 (Figure 2B), before subsequently recovering to values close to those of C-NF throughout the trial. In 2017 and 2018, the RI-F1 and RI-F2 treatments also saw a larger decrease of SWP compared to C-F1 and C-F2 around DOY 225, with the biggest differences being reached in veraison. In RI-F1, the decrease was larger than in RI-F2. In 2019, there were only very slight differences between irrigation strategies in these treatments.

Vegetative growth, expressed as fiPAR, increased from budbreak to DOY 300 in C-NF (Figures IV.3A, IV.3B). In C-F1 and especially in C-F2, fiPAR decreased when removing vegetation at the moment of CFT application between DOY 150 and DOY 200. In C-F1, after regrowth, the rapid development of vegetation saw fiPAR reach similar or even higher values than C-NF in 2019 (Figure 3A). However, the fiPAR values of C-F2 were always lower than those of C-NF and C-F1 in the three study years (Figure IV.3A). With less irrigation, RI-NF had lower fiPAR than C-NF in 2017 and 2018 (Figure IV.3B). The RI-F1 treatment showed similar values to C-NF and higher values than RI-NF in 2017 and 2018, and were always higher than RI-F2. Although the application of RI decreased fiPAR, this was more affected by the CFT date than the applied water regime, with RI-F2 always having the lowest fiPAR values throughout the trial.

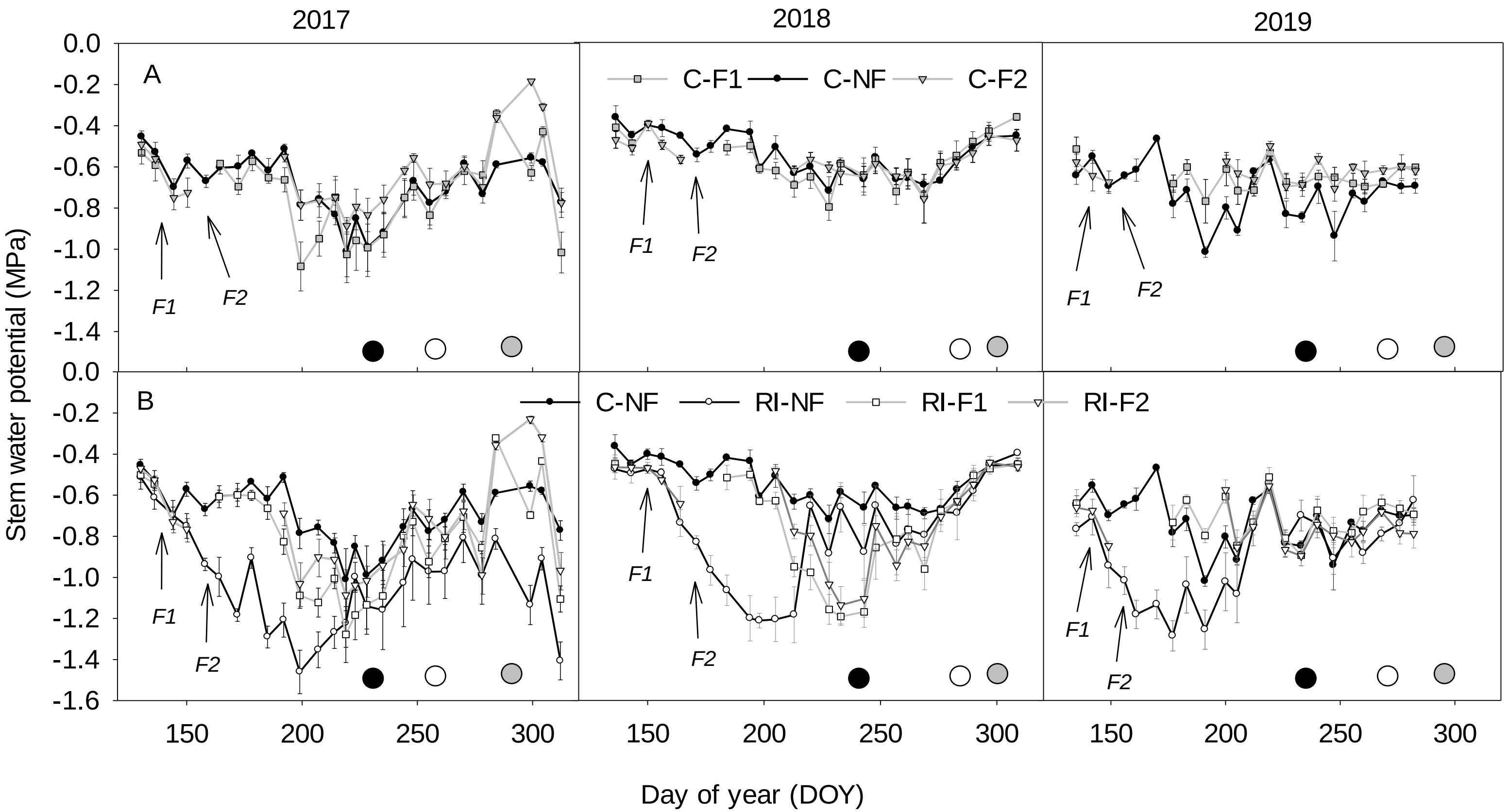

Figure IV.2. Seasonal evolution of SWP. A) Values for irrigation treatments C (C-NF, C-F1 and C-F2) in 2017, 2018 and 2019 growing season, and B) Values for treatment C-NF and treatments RI (RI-NF, RI-F1 and RI-F2) in 2017, 2018 and 2019 growing seasons. Black, white and grey circles represent the harvest date in NF, F1 and F2, respectively, in each year. Arrows represent the date of CFT in F1 and F2. Each point represents the mean of two leaves and four replicates of each treatment. Bars represent the standard error of the mean.

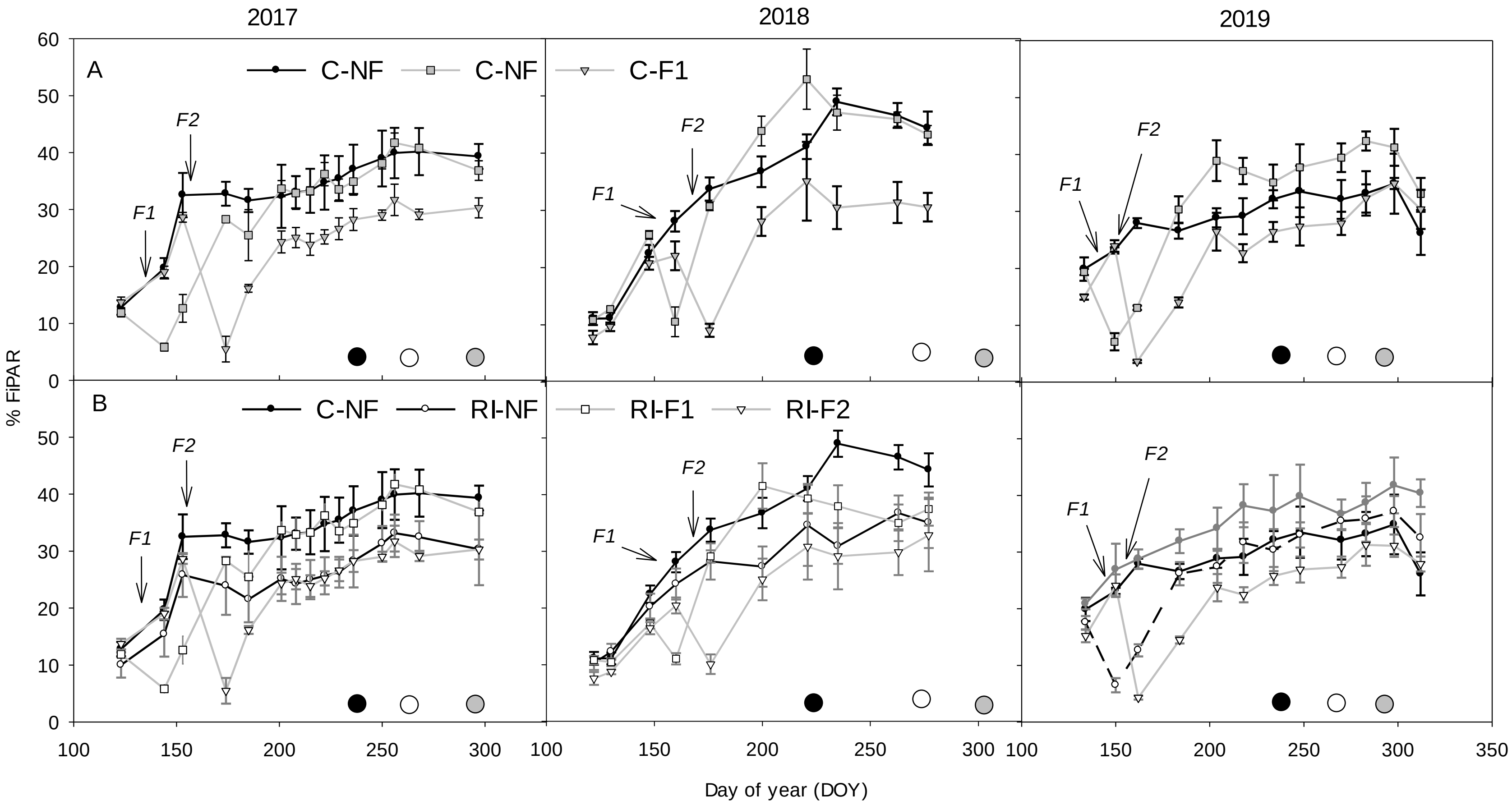

Figure IV.3. Seasonal evolution of the fraction of intercepted PAR radiation fiPAR. A) fiPAR values for irrigation treatments C (C-NF, C-F1 and C-F2) in 2017, 2018 and 2019 growing season, and B) fiPAR values for treatment C-NF and treatments RI (RI-NF, RI-F1 and RI-F2) in 2017, 2018 and 2019 growing seasons. Each point represents the mean of two vines and four replicates of each treatment. Black, white and grey circles represent the harvest date in NF, F1 and F2, respectively, in each year. Arrows represent the date of CFT in F1 and F2. Bars represent the standard error of the mean.

IV.3.3. LEAF AREA AND DRY MATTER PRODUCTION

If we observe the C-NF treatment, leaf area was variable over the three study years, with values ranging between 7.1 $m^2 \cdot vine^{-1}$ in 2017 and 15.6 $m^2 \cdot vine^{-1}$ in 2018 (Table IV.5). From 2018 onwards, C-F1 developed a leaf area similar to C-NF, while the lowest leaf area values were recorded in C-F2. The RI-NF treatment developed a lower leaf area compared to the C-NF treatments. With CFT application leaf area was similar between the two irrigation regimes (C and RI). Independently of the water regime, the F2 treatments recorded the lowest leaf area per vine, with mean values of 4.2 $m^2 \cdot vine^{-1}$ (Table 5), which suggests a clear irrigation and CFT interaction (*CFTxI)* as the F1 treatments showed notable higher leaf area values. In addition, leaf area in F2 varied little between years if compared with the variability observed in NF and F1, reaching values of between 7 $m^2 \cdot vine^{-1}$ and 3.9 $m^2 \cdot vine^{-1}$ over the course of the study.

Likewise, in C-NF, pruning weight showed a clear year effect, with maximum values of 1177.8 $g \cdot vine^{-1}$ in 2018 and 814.0 $g \cdot vine^{-1}$ in 2017 (Table IV.5). Pruning weight was lower in the RI-NF treatment compared to C-NF in the three study years. In the case of the CFT treatments, the lowest pruning weight was observed in F2 independently of the irrigation applied, while the F1 treatment had a higher pruning weight than F2 with both the C and RI water regimes. The C-F1 treatment had a higher pruning weight than RI-NF in 2017, but in 2018 the difference decreased and in 2019 RI-NF had a higher value for this parameter than C-F1. The removal of shoot tips, inflorescences, leaves and secondary shoots, made in the CFT treatments (F1 and F2), saw a larger amount of dry matter removed in F2 than in F1 (Table IV.5), with weights of between 227.1 $g \cdot vine^{-1}$ and 335 g·vine-1 in F1 and between 569.8 $g \cdot vine^{-1}$ and 366.7 $g \cdot vine^{-1}$ in F2, due to the CFT application in F2 being 20 days later than in F1 and hence the shoots being more developed. Only in 2017 was the dry matter weight removed in CFT significantly lower in the RI treatments compared to C (Table IV.5).

Table IV.5. Values of leaf area after veraison, winter pruning weight and dry matter removed per vine at the application of CFT in each treatment.

Parameter	Year	*Statistical analysis*	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2	*CFTxI*	CFT			*CFT factor*	Irrigation		*Irrigation factor*
										NF	F1	F2		C	RI	
Leaf area ($m^{2}\cdot vine^{-1}$)	**2017**	*p.*	7.1 a	5.4 b	4.1 d	3.9 d	4.9 c	4.3 cd	***	5.5	5.1	4.2	***	5.5	4.4	***
	2018	*n.p.*	15.6 a	8.7 ab	4.1 d	5.3 cd	7.0 bc	3.9 d	***	10.5	7.8	4.0	***	9.5	5.4	***
	2019	*n.p.*	9.5 a	10.0 a	4.7 b	5.1 b	9.7 a	3.9 b	***	7.3	9.9	4.3	***	8.1	6.2	***
Pruning weight ($g\cdot vine^{-1}$)	**2017**	*n.p.*	814.0 a	741.0 a	502.8 b	504.8 b	713.3 a	406.5 b	***	659.4	727.1	454.6	***	685.9	541.5	***
	2018	*n.p.*	1177.8 a	763.8 b	528.0 c	803.8 b	636.8 bc	495.5 c	***	990.8	700.3	511.2	***	823.2	645.3	**
	2019	*p.*	995.0	686.5	433.5	917.5	649.0	465.8	*n.s.*	956.3 a	667.8 b	449.6 c	***	705.0	677.4	*n.s.*
Dry matter removed by CFT ($g\cdot vine^{-1}$)	**2017**	*p.*		236.9 c	687.4 a		217.2 c	452.3 b	**		227.1	569.8	***	462.2	334.8	***
	2018	*n.p.*		348.5 b	458.4 a		322.4 b	468.4 a	***		335.4	463.4	***	403.5	395.4	*n.s.*
	2019	*n.p.*		278.8 b	375.5 a		249.9 b	357.9 a	***		264.4	366.7	***	327.2	303.9	*n.s.*

Statistical analysis: p. indicates parametric statistics (MANOVA and Tukey test; $p < 0.05$); n.p., indicates nonparametric statistics (Kruskal-Wallis's test; $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level; and (***) significant at 0.1% level.

IV.3.4. YIELD, YIELD COMPONENTS AND MUST COMPOSITION

The interannual differences in yield ranged between 12428 kg·ha^{-1} and 23736 kg·ha^{-1} in C-NF for 2018 and 2019, respectively (Table IV.6). Application of CFT reduced yield compared to C-NF by an average of 54% for F1 and 43% for F2, with the lowest yield being for F1 with 5325.4 kg·ha^{-1} in 2018 and the highest being for F2 with 9793.8 kg·ha^{-1} in 2019. The RI also resulted in lower yields in 2017 and 2019 compared to C-NF, but the difference was not as marked as that observed for F1 and F2 compared to NF (Table IV.6). Only in 2019 was a *CFTxI* interaction observed, with the highest yield recorded in C-NF, followed by RI-NF, and with C-F1, C-F2 and RI-F2 presenting similar values and RI-F1 recording the lowest yield with 6685.8 kg·ha^{-1}.

The lower yield in the F1 and F2 treatments was mainly due to the lower number of berries per cluster (44% and 50% lower in F1 and F2, respectively, compared to NF) and the lower berry weight, with values of 1.6 g in NF and 1.3 g and 1.1 g in F1 and F2, respectively (Table IV.6). Mean cluster weight in the NF treatments was 212.3 g, while the corresponding values in F1 and F2 were 68.1 g and 77.7 g, respectively (Table 6). The RI treatments significantly decreased cluster weight in 2017 and 2019, whereas the effect of water restriction did not affect the number of berries per cluster, and only in 2018 was there a lower berry weight in the RI treatments (Table 6). Cluster weight only decreased with the RI-NF treatment against C-NF, but was unaffected in the RI-F1 and RI-F2 treatments against C-F1 and C-F2, which had similar cluster weights. Application of CFT had a greater impact on cluster weight than irrigation.

The number of clusters increased in the F1 and F2 treatments as this technique forces regrowth of a larger amount of buds (six per spur left), against the two buds per inch of the NF treatments. The differences in the number of clusters between F1 and F2 varied over the study years, with no clear trend observed. The number of clusters was higher in F2 in 2017 and 2019 and higher in F1 in 2018 (Table IV.6). Only in 2017 did RI decrease the number of clusters per vine in all treatments independently of CFT application, while in the other years, like berry weight, a strong *CFTxI* interaction was observed (Table IV.6).

Application of CFT increased the LA:Yield ratio, with the highest values obtained in F1 in the three study years (maximum values of 8.6 m^{2}·kg^{-1} in 2018), while no differences were observed between NF and F2 except between RI-NF and RI-F2 in 2017 (Table 6). For its part, the Ravaz index showed lower values for F1 in the three study years, and similar values between the NF and F2 treatments (Table IV.6).

Table IV.6. Yield and yield components values in the different treatments in the 2017, 2018 and 2019 growing seasons.

Parameter	Year	*Statistical analysis*	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2	*CFT xI*	CFT			*CFT factor*	Irrigation		*Irrigation factor*
										NF	F1	F2		C	RI	
Yield (kg·ha^{-1})	2017	*p.*	12952.5	6471.7	9216.7	7531.7	4290.0	4377.8	*n.s.*	10242.1 a	5380.8 c	6797.2 b	***	9546.9	5399.8	***
	2018	*p.*	12428.3	5515.8	6118.3	10177.5	5135.0	5935.0	*n.s.*	11302.9 a	5325.4 b	6026.7 b	***	8020.8	7082.5	*n.s.*
	2019	*p.*	23735.8 a	7488.9 cd	9986.7 c	14695.8 b	6685.8 d	9600.8 c	***	19215.8	7087.4	9793.8	***	13737.1	10327.5	***
Clusters per vine	2017	*n.p.*	16.3 bc	24.9 b	36.4 a	12.6 c	17.7 bc	21.6 b	***	14.4	21.3	29.0	***	25.8	17.3	***
	2018	*n.p.*	23.2 bc	37.6 a	21.8 c	19.8 c	32.9 ab	24.2 bc	***	21.5	35.2	23.0	***	27.5	25.6	*n.s.*
	2019	*n.p.*	22.2 bc	26.6 ab	32.7 a	20.0 c	22.6 bc	34.0 a	***	21.1	24.6	33.3	***	27.1	25.5	*n.s.*
Cluster weight (g)	2017	*p.*	240.6 a	81.1 c	77.1 c	168.6 b	71.1 c	58.2 d	*	204.6	76.1	67.7	***	132.9	99.3	***
	2018	*p.*	168.5	43.8	84.6	155.7	45.2	72.0	*n.s.*	162.1 a	44.5 c	78.3 b	***	99.0	91.0	*n.s.*
	2019	*p.*	325.1 a	82.0 c	91.6 c	215.5 b	85.7 c	82.4 c	***	270.3	83.9	87.0	***	166.2	127.9	***
Berries per cluster	2017	*p.*	163.6 ab	154.3 ab	100.2 c	178.0 a	128.5 bc	141.1 abc	*	170.8	141.4	120.7	***	139.3	149.2	*n.s.*
	2018	*n.p.*	193.1 ab	152.6 bc	126.0 bc	257.0 a	142.2 bc	122.4 c	***	225.1	147.4	124.2	***	157.2	173.9	*n.s.*
	2019	*p.*	412.0	146.6	122.6	290.0	115.6	127.2	*n.s.*	351.0 a	131.1 b	124.9 b	***	227.1	177.6	*n.s.*
Berry weight (g)	2017	*n.p.*	1.8 a	1.0 ab	1.0 ab	1.3 a	1.0 ab	0.8 b	**	1.5	1.0	0.9	***	1.2	1.0	*n.s.*
	2018	*n.p.*	1.9 a	1.4 ab	1.2 ab	1.3 ab	1.3 ab	1.0 b	**	1.6	1.3	1.1	**	1.5	1.2	*
	2019	*n.p.*	2.3 a	1.4 ab	1.4 ab	1.5 ab	1.5 ab	1.2 b	**	1.9	1.5	1.3	**	1.7	1.4	*n.s.*
LA:Y ratio (m2·kg^{-1})	2017	*p.*	2.1 b	4.4 a	1.9 b	2.4 b	5.9 a	5.2 a	***	2.3	5.2	3.6	***	2.8	4.5	***
	2018	*n.p.*	4.7 ab	10.0 a	3.4 bc	2.0 c	7.2 a	3.0 c	***	3.3	8.6	3.2	***	6.0	4.1	**
	2019	*n.p.*	1.4 b	7.1 a	2.0 b	1.4 b	6.6 a	2.0 b	***	1.4	6.9	2.0	***	3.5	3.3	*n.s.*
Ravaz Index	2017	*p.*	6.2	2.8	5.7	4.9	1.8	3.2	*n.s.*	5.6 a	2.3 b	4.5 a	***	4.9	3.3	***
	2018	*n.p.*	3.6 a	2.6 b	3.9 a	4.3 a	2.4 b	4.7 a	***	4.0	2.5	4.3	***	3.4	3.8	*n.s.*
	2019	*p.*	8.2 a	3.2 d	6.9 ab	5.1 c	3.0 d	6.4 bc	***	6.7	3.1	6.7	***	6.1	4.9	***

Statistical analysis: p., indicates parametric statistics (MANOVA and Tukey test; $p < 0.05$); n.p., indicates nonparametric statistics (Kruskal-Wallis's test; $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level; and (***) significant at 0.1% level.

Grape harvesting was carried out when the treatments reached TSS 23-24ºBrix (Table IV.7), and so no differences were observed between treatments. However, these values was not reached in the F2 treatments in 2018 (22.7º Brix) or NF in 2019 (22.5º Brix).

The musts with the lowest pH were from the F2 treatments, independently of irrigation, with a maximum value of 3.7 in 2017 and a minimum value of de 3.5 in 2018 and 2019, while the F1 values were more similar to those of NF (Table IV.7). The RI treatments increased pH in all years compared to the C treatments, which recorded mean values of 3.6. A *CFTxI* interaction was observed in all three years for this parameter, with pH higher in RI-NF against C-NF but maintaining similar values in C-F1 and RI-F1 as well as in C-F2 and RI-F2 (Table IV.7). The TA values increased in the F2 treatments (C-F2 and RI-F2) from 2018, while the F1 treatments (C-F1 and RI-F1) showed similar values to the NF treatments (C-NF and RI-NF). As for the effect of irrigation, all the RI treatments (RI-NF, RI-F1 and RI-F2) showed a lower TA value compared to the C treatments (C-NF, C-F1 and C-F2), which was statistically significant in 2018. Malic acid concentration was higher in the F1 and F2 treatments, though without any clear trend as it increased in F1 in 2017, in F2 in 2018, and in both treatments in 2019. The RI lowered malic acid concentration only in 2018, with higher values obtained for the C treatments. Tartaric acid was less sensitive to CFT application than malic acid and, especially, irrigation, with tartaric acid concentration decreasing with CFT application only in 2017 and no appreciable differences observed between any of the treatments in either 2018 or 2019 (Table IV.7).

The TPP and Ant values varied between years (Table IV.7). From 2018, F1, and especially F2, presented higher TPP and Ant, with maximum values in F2 of 7.5 $mg \cdot g^{-1}$ and 1.6 $mg \cdot g^{-1}$, respectively. However, the applied water regime only affected Ant values, with higher values in RI compared to C in 2017 and 2019, while no appreciable effect of irrigation was observed for TPP in any of the study years.

Table IV.7. Grape composition in the different treatments in the 2017, 2018 and 2019 growing seasons**.**

Parameter	Year	*Statistical analysis*	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2	*CFTxI*	CFT			*CFT factor*	Irrigation		*Irrigation factor*
										NF	F1	F2		C	RI	
TSS (º Brix)	**2017**	***p.***	24.8	23.6	23.0	24.2	24.1	23.7	*n.s.*	24.5 a	23.8 ab	23.3 b	*	23.8	24.0	*n.s.*
	2018	***p.***	23.1	23.9	22.7	23.9	23.9	22.7	*n.s.*	23.5 ab	23.9 a	22.7 b	**	23.2	23.5	*n.s.*
	2019	***p.***	21.9	24.9	24.1	23.2	25.3	24.4	*n.s.*	22.5 b	25.1 a	24.3 a	***	23.6	24.3	*
pH	**2017**	***n.p.***	3.9 a	3.7 ab	3.6 b	3.8 a	3.9 a	3.7 ab	**	3.9	3.8	3.7	*	3.7	3.8	*n.s.*
	2018	***p.***	3.6 b	3.8 a	3.5 c	3.9 a	3.8 a	3.6 bc	*	3.8	3.8	3.5	***	3.6	3.8	***
	2019	***p.***	3.6 bc	3.7 abc	3.5 c	3.8 a	3.8 ab	3.5 c	*	3.7	3.7	3.5	***	3.6	3.7	*
TA ($g \cdot L^{-1}$)	**2017**	***p.***	4.0	4.1	4.2	3.5	3.9	3.9	*n.s.*	3.8	4.0	4.1	*n.s.*	4.1	3.8	*n.s.*
	2018	***p.***	4.9	5.3	6.4	4.0	3.8	5.9	*n.s.*	4.4 b	4.5 b	6.2 a	***	5.5	4.6	**
	2019	***p.***	5.1	4.6	6.1	4.1	4.9	5.7	*n.s.*	4.6 b	4.7 b	5.9 a	**	5.3	4.9	*n.s.*
Ac. mal. ($g \cdot L^{-1}$)	**2017**	***p.***	2.0	2.5	2.3	1.3	2.3	2.3	*n.s.*	1.7 b	2.4 a	2.3 ab	*	2.3	2.0	*n.s.*
	2018	***p.***	3.0	2.9	3.5	2.6	2.0	3.1	*n.s.*	2.8 b	2.5 b	3.3 a	*	3.1	2.6	*
	2019	***p.***	1.8	2.6	2.6	2.3	2.7	2.5	*n.s.*	2.1 b	2.6 a	2.6 a	**	2.3	2.5	*n.s.*
Ac. tar. ($g \cdot L^{-1}$)	**2017**	***p.***	5.9	4.6	4.8	5.5	4.7	4.5	*n.s.*	5.7 a	4.6 b	4.6 b	***	5.1	4.9	*n.s.*
	2018	***p.***	5.1	5.2	5.2	5.2	4.8	4.7	*n.s.*	5.2	5.0	4.9	*n.s.*	5.1	4.9	*n.s.*
	2019	***p.***	5.1	5.3	5.0	5.4	5.2	5.1	*n.s.*	5.2	5.3	5.0	*n.s.*	5.1	5.2	*n.s.*
TPP ($mg \cdot g^{-1}$)	**2017**	***p.***	6.0	6.2	6.2	5.4	5.9	6.1	*n.s.*	5.7	6.1	6.1	*n.s.*	6.1	5.8	*n.s.*
	2018	***p.***	5.7	6.1	8.3	5.0	6.5	6.6	*n.s.*	5.3 b	6.3 ab	7.5 a	**	6.7	6.0	*n.s.*
	2019	***p.***	4.1	6.4	6.5	4.6	6.0	6.2	*n.s.*	4.3 b	6.2 a	6.3 a	***	5.7	5.6	*n.s.*
Ant ($mg \cdot g^{-1}$)	**2017**	***p.***	1.1	1.2	1.2	1.4	1.4	1.5	*n.s.*	1.3	1.3	1.4	*n.s.*	1.2	1.5	***
	2018	***p.***	1.1	1.3	1.5	1.1	1.4	1.8	*n.s.*	1.1 b	1.4 a	1.6 a	***	1.3	1.5	*n.s.*
	2019	***p.***	0.8	1.2	1.4	1.1	1.4	1.6	*n.i.*	1.0 b	1.3 a	1.5 a	***	1.1	1.4	**

TSS: Total soluble solids; TA Titratable acidity; Ac. mal.: Malic acid; Ac. tar.: Tartaric acid; TPP: Total polyphenols; Ant: Total anthocyanins. Statistical analysis: p., indicates parametric statistics (MANOVA and Tukey test; $p < 0.05$); n.p., indicates nonparametric statistics (Kruskal-Wallis's test; $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level; and (***) significant at 0.1% level

IV.4. DISCUSSION

The main objectives of this work were: to evaluate the capacity of the CFT to delay harvest and shift it in time to a period with cooler temperatures: to know the effect of this delay on ‘Tempranillo’ grape yield and quality in the crop conditions of Extremadura; and to evaluate irrigation strategies to complement CFT application. Unlike other works with a similar approach (Gu et al., 2012; Martínez-Moreno et al., 2019; Martinez De Toda et al., 2019), a key aspect of this study was to consider the carry-over effect, which can condition practical interest in this technique and its adoption form in commercial vineyards. For this latter purpose, the same treatments were maintained for the same plants and for all three years of the study.

The results of this work confirm that CFT application results in the growth of new shoots and clusters on the vine which develop in the same growing season. This concurs with results obtained by other authors (Gu et al., 2012; Martínez de Toda et al., 2019; Martínez-Moreno et al., 2019; Kisimoto et al. 2022). Of the two CFT applications studied, the F2 treatment (applied between fruit set and pea size) delayed the harvest by an average of 56 days and caused more consistent changes in temperature conditions during the ripening period. Martínez de Toda et al. (2019) in La Rioja and Martínez-Moreno et al. (2019) in Requena obtained respective harvest delays for the same variety of up to 70 and 72 days, with CFT application taking place during the berry pea size stage and 23 days after anthesis. Similar results have been obtained in other varieties, including 'Maturana Tinta' in Logroño, Spain (Martínez de Toda et al., 2019), 'Cabernet Sauvignon' in Fresno, USA (Gu et al., 2012; Tian & Gu, 2019), with a harvest delay of 83 days for CFT application 42 days after anthesis, and in 'Merlot' in Yamanashi, Japan (Kishimoto et al., 2022), with CFT application in anthesis and obtaining a harvest delay of 36 days compared to the control treatment. Excessively putting back CFT application can lead to difficulties to complete the ripening process. In an earlier trial we conducted in the same vineyard as the present study, a total of 5 different CFT application dates were analysed from pre-anthesis to veraison (data not shown). It was found that ripening was incomplete when the forcing technique was applied on dates later than in the F2 treatment of this study (after the pea size phenological stage). Gu et al. (2012) in ‘Cabernet Sauvignon’ and Martinez de Toda (2019) in ‘Tempranillo’ and ‘Maturana Tinta’ observed that, when CFT application is made close to veraison, the buds do not fully ripen and bud regrowth is irregular. This behaviour may be attributable to the combined action of low temperatures and the shortening of daylight hours which tends to lower leaf photosynthetic activity coinciding with the leaf fall period.

As well as shifting in time the harvest date, CFT application modifies the environmental conditions of each growth stage (Table IV.3). The F1 and F2 vines completed their budbreak to harvest cycle faster than the NF vines (by around 20 days in F1 and 13 days in F2). The results indicate that progression of the initial growth stages (budbreak to veraison) was achieved faster in the F1 and F2 treatments due to a higher daily accumulation of thermal time (Lebon et al., 2004), as the recorded temperatures were higher than for the NF treatments (Table IV.3). However, berry ripening (veraison to harvest) took place with lower temperatures compared to those of the same growth stages in NF and coinciding with reports in other studies (Gu et al., 2012; Kishimoto et al., 2022; Martínez-Moreno et al., 2019). Despite the lower temperatures, the veraison to harvest period was slightly shorter in comparison with the NF treatments. In other studies, the results have been variable, from shorter or similar ripening periods (Tian & Gu, 2019) to longer periods than for the vines conventionally treated. Other factors such as the sugar accumulation rate or vine capacity may be influencing the speed of berry ripening.

For both the NF and the F1 and F2 treatments, the application of RI resulted in a mean irrigation water saving of 37% compared to C (100% ET_c) in the three study years. Independently of the applied irrigation regime, the volume of consumed water was higher in the F1 and F2 treatments because the canopies were active for a longer time probably due their formation with younger leaves with a higher photosynthetic rate late in the seasons compared to the NF canopies, even though the treatments initiated leaf fall at the same time. In the case of C-F1, there was also a higher fiPAR (Figure 3A) in comparison with the NF treatments, with values that exceeded 50% of fiPAR in 2018, which, as reported by Picón-Toro et al. (2012), increases vine water requirements. This increase in water consumption with CFT can be a limitation for adoption of this technique in conditions of scarce water resources availability. In the case of F2, irrigated with the same volume of water as F1, the fiPAR values were always the lowest throughout the study, suggesting that the F2 water requirements were lower than those of F1. In addition, C-F2 had the lowest negative SWP value throughout the study (Figure IV.2A), which could have an associated water saving potential.

Irrigation is a factor that determines canopy development, as shown in the difference in leaf area between the C and RI treatments of this trial, independently of CFT application (Table IV.5). Application of RI in pre-veraison reduces total leaf area in comparison with non-stress conditions (Uriarte et al., 2015). However, leaf area is dependent on the date of CFT application. The F1 treatment regenerated a leaf area similar to the NF treatment from 2018, and higher than the F2 treatment. Similar results have been reported in a study by Martínez de Toda et al. (2019) in which the vines forced earlier in the season had a higher leaf area than the control and the vines forced later in the season. This effect may be related to the amount of dry matter removed as the later the CFT application date is delayed (F2) the greater the vegetative growth and, hence, the amount of dry matter to be removed (Table IV.5), In this way, F2 vine regrowth takes place with a lower amount of reserves. This effect is also noted in the pruning weight results which were lower for F2 throughout the study.

Although winegrowers tend to maintain the same crop practices in their vineyards, yield varies season-to season. The C-NF grape yield varied by 11300 kg·ha^{-1} between 2018 and 2019, with a coefficient of variation (CV) of 32%. For the study years, the CV decreased in C-F1 and C-F2 to 12% and 20%, respectively, indicating a decrease in interannual harvest variability that could contribute to facilitating logistical arrangements at harvest time. The lower yield variability on CFT applications may be due to the lower yield. Yield in the F1 and F2 vines was lower than in C-NF by, on average, 54% for F1 and 43% for F2, decreases which are greater than the 34% resulting from the pre-veraison RI action (RI-NF). This decrease in yield, more marked in the early CFT applications, has been reported for ‘Tempranillo’ by Martinez De Toda et al. (2019) and Martínez-Moreno et al. (2019), with decreases of between 11% and 98% and between 78% and 83%, respectively, compared to an unforced control treatment, depending on CFT application date.

In an attempt to address the loss in yield, a cane pruning CFT (6 buds per shoot) was applied to increase the number of clusters per vine. An increase in cluster numbers was achieved but did not compensate the loss in yield observed in the trial. Gu et al. (2012) in 'Cabernet Sauvignon' and with cane pruning, managed to increase the number of clusters compared to the unforced control after the second year of CFT application. Short pruning CFT (two buds per shoot) carried out in the works of Martinez De Toda et al. (2019) resulted in a lower number of clusters compared to the control from the second year of CFT application, while Martínez-Moreno et al. (2019) found no differences in the number of clusters per vine between short pruning and cane pruning. Kishimoto et al. (2022), with cane pruning CFT, found that also removing the water shoots in growth from the lateral buds in current season resulted in primary bud regrowth (but that would naturally become a dormant bud without sprouting). The development of this primary bud produces a larger number of inflorescences than water shoots. This could explain the difference in yield observed in this between different CFT application dates. The F1 treatment, in which the water shoots had not yet sprouted and were therefore not removed, thus favoured regrowth of the less productive water shoots. However, the F2 treatment, in which the already sprouted water sprouts were removed, favoured regrowth of the more productive primary buds (Table IV.6). In 2018, the number of clusters was higher in F1 than in F2, but this was the year in which CFT application was later in the season (Table IV.2), which facilitated water shoot removal in F1 and hence favoured primary bud regrowth in this treatment.

Both cluster weight and berry weight decreases with CFT. Keller et al. (2010) observed that temperature variations at the beginning of the season can substantially modify vine formation behaviour, reporting that high temperatures during the development of inflorescences produced clusters with fewer flowers. In our trial, the mean maximum temperatures in the budbreak-anthesis period were 7.4 ºC and 8.5 ºC higher in F1 and F2, respectively, compared to the control treatment, with fewer berried per cluster for both CFT application dates.

In this trial, no evidence was found that CFT has carry-over effects after three consecutive years of application on the same plants. The plants had a sufficient ‘a priori’ leaf area for adequate berry ripening and reserves accumulation for the start of the following season. The LA:Y values were above 5 $m^2 \cdot kg^{-1}$ and 2 $m^2 \cdot kg^{-1}$ in F1 and F2, respectively, and were in accordance with the values reported by Zheng et al. (2017). Similar LA:Y values have been reported by Martinez De Toda et al. (2019) and Gu et al. (2012). In their works, however, CFT application on the same vines was not repeated in various years. In contrast, Martínez-Moreno et al. (2019), who analysed the carry-over effects for two years, reported lower yield than the unforced control treatment in the following season when CFT was not applied, although it should also be noted that this trial was carried out in a vineyard with more than 25 years of age. In our trial, the highest yield was obtained in all treatments in 2019, with the F2 treatment producing yields of over 9000 $kg \cdot ha^{-}1$ after 3 consecutive years of CFT application. More studies would be required to analyse carbohydrate reserves in the different organs of the vine in order to discard a long-term depressive effect of CFT.

The decrease in yield due to RI was statistically significant in all three study years (Table IV.6), while for the other yield components (clusters per vine, cluster weight, berries per cluster and berry weight), the effect was year dependant. In most of these parameters, the distinction between C and RI was more evident in the NF treatments, as RI-NF had more negative SWP values throughout the season compared to C-NF than the differences observed between RI-F1 and RI-F2 against C-F1 and C-F2.

Despite using the same criterion of 23–24 ºBrix for the harvest, the F2 treatments showed a slightly lower TSS concentration than NF and F1, but higher TA, malic acid, TPP and

Ant values than the NF treatments, indicating a qualitative improvement in some of the most highly valued oenological parameters (Table IV.7). These results suggest that CFT application could avoid the decoupling of pulp and skin maturity described by Sadras and Moran, (2012). According to Mori et al. (2007), temperatures above 30ºC post-veraison can inhibit the synthesis of anthocyanins and modify the sugar/acid balance (Keller, 2010), as grape acid content is lowered (Downey et al., 2006; Haselgrove et al., 2000). In our trial, the distribution of temperatures (Figure IV.1) shows that from mid-September onwards the maximum temperatures were below 30ºC, and so the F2 grapes ripened when temperatures were more suited to improving the coupling between anthocyanins and sugars (Sadras and Moran, 2012) and allowed higher TA values and a lower pH (Table IV.7). The increase of TA observed in F2 was possible through the increase in malic acid concentration, which is especially sensitive to high temperatures (Koundouras et al., 2006). Similar results have been reported in different varieties and locations (Gu et al., 2012; Lavado et al., 2019; Martínez-Moreno et al., 2019; Martinez De Toda et al., 2019) in CFT vines.

With respect to the effect of RI on berry characteristics, an increase in pH was observed compared to the C treatments in 2018 and 2019 and a decrease in TA and malic acid concentration in 2018. In addition, Ant concentration was higher in the RI treatments, concurring with other observations made in the literature (Intrigliolo & Castel, 2010; Ojeda et al., 2002; Uriarte et al., 2016), but no differences were observed in TPP content between the different irrigation regimes (Table IV.7). Berry characteristics in the CFT treatments included increased TPP content independently of the irrigation regime applied, while Ant content showed the greatest increase with the combined application of CFT and RI, especially in F2.

IV. 5. CONCLUSIONS

“Crop forcing” delayed berry ripening to a period of lower temperatures, but decreased yield despite an increase in number of clusters. The phenological stage in which CFT application was performed affected the results. Application of “crop-forcing” after fruit set in the trial conditions was the most recommendable as it had a higher positive impact on berry composition and reduced yield loss compared to the earlier “crop-forcing” application. Forcing was found to be an effective technique to modify berry characteristics, giving rise to higher TA, PPT and Ant values and a lower pH. These results were constant over the three consecutive years of CFT application and no depressive effect on vine yield was noted. In both the CFT and non-CFT treatments, pre-veraison stress had a positive effect on berry quality. Pre-veraison stress caused a greater decrease in the different yield parameters in the non-forcing than in the forcing treatments.

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V. CAPÍTULO 2.

ASSESSMENT OF THE “CROP-FORCING” TECHNIQUE AND IRRIGATION STRATEGY ON THE RIPENING OF ‘TEMPRANILLO’ GRAPES IN A SEMI-ARID CLIMATE.

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V.ABSTRACT

Background and Aims: High temperatures during grape ripening have a negative effect on the winemaking characteristics of musts. The “crop-forcing” technique delays ripening to a period when temperatures are lower.

Methods and Results: This study of 3 growing seasons (2017-2019) analyzes the effect of this technique in a vineyard of ‘Tempranillo’ (Vitis vinifera L.) in Extremadura, together with two irrigation strategies. The grapevines were forced 4 and 22 days after anthesis (F1 and F2 respectively), compared to a treatment without “crop-forcing” techniques (NF). Each treatment was subjected to two irrigation strategies: to cover the water needs of the plants (C); and deficit irrigation during pre-veraison (RI). “Crop-forcing” delayed the harvest between 32 and 56 days on average in relation to NF. “Crop-forcing” and irrigation strategy modified berry composition at harvest: C-F1 and C-F2 had higher total polyphenol and anthocyanin concentrations, total acidity, malic acid content and lower pH relative to C-NF; RI-NF increased total anthocyanin concentration and pH, and decreased titratable acidity value.

Conclusions: “Crop-forcing” is able to delay grape ripening to lower temperature periods. This is a promising technique for restoring the coupling between phenolic and technological ripeness. The combination of both, “crop-forcing” and deficit irrigation strategy maintains the berry quality while improving water use efficiency.

Significance of the Study: The present work evaluates the effect of the “crop-forcing” technique, deficit irrigation strategy and the combined application of both on berry ripening process and final composition.

V. RESUMEN

Antecedentes y Objetivos: Altas temperaturas durante la maduración de las uvas tienen un efecto negativo sobre las características de los mostos para la vinificación. La técnica del “forzado de yemas”, provoca el retraso de la maduración hacia un periodo de temperaturas más bajas.

Métodos y Resultados: Este estudio de 3 campañas (2017 - 2019) analiza el efecto de esta técnica en un viñedo de ‘Tempranillo’ (Vitis vinifera L.) en Extremadura, junto con dos estrategias de riego. Las cepas se forzaron, 4 y 21 días después de la antesis (F1 y F2 respectivamente), comparando con un tratamiento no forzado (NF). Cada tratamiento se sometió a dos estrategias de riego: para cubrir las necesidades hídricas de las plantas (C); y un riego deficitario durante el preenvero (RI). El forzado retrasó la vendimia entre 32 días para y 56 días de media en relación con NF. El forzado de cultivo y la estrategia de riego modificaron la composición de las bayas en vendimia: C-F1 y C-

F2 tuvieron mayor concentración de polifenoles y antocianos totales, acidez total, contenido en ácido málico y menor pH en relación a C-NF; RI-NF incrementó la concentración de antocianos totales y el pH, y disminuyó el valor de acidez titulable.

Conclusiones: El “forzado de yemas” es capaz de retrasar la maduración de la uva a periodos de temperaturas más bajas. Se trata de una técnica prometedora para restaurar el acoplamiento entre la madurez fenólica y tecnológica. La combinación de ambas estrategias, “forzado de yemas” y riego deficitario, mantiene la calidad de la baya al tiempo que mejora la eficiencia en el uso del agua.

Importancia del estudio: El presente trabajo evalúa el efecto de la técnica del “forzado de yemas”, la estrategia de riego deficitario y la aplicación combinada de ambas sobre el proceso de maduración de las bayas y su composición final.

V.1. INTRODUCTION

Climate plays a fundamental role in the development of the vineyard. The temperature regime, the availability of water and the intensity of radiation determine plant growth, production and harvest quality (Dinis *et al.*, 2014; Mira de Orduña, 2010). Each grapevine variety has its optimum development in a specific range of temperatures. Small temperature variations can modify productivity, physical-chemical composition and, ultimately, the validity and adaptability of a given variety in a region (Jones, Duchêne & Schneider, 2005). In the ‘Tempranillo’, the average temperature range in the vegetative period (from April to October for the Northern Hemisphere) should be between 15 and 19 °C (Jones, 2006). Climate change (CC) is causing a progressive rise in temperatures, as well as increasingly frequent and prolonged episodes of heat stress, increased incident radiation, in addition to modifications in the seasonal pattern of rainfall, causing greater uncertainty in the availability of water resources (Van Leeuwen & Destrac-Irvine, 2017).

Over the past 50 years, major wine regions have recorded temperature increases of more than 1 °C and are expected to rise by a further 2 °C by 2050(Jones, 2012) Climate change is being identified as responsible for changes in grapevine phenology and physiology, such as longer lengthening of the growth cycle, faster phenological advancement in successive stages and earlier harvest dates. As a consequence, the composition of grapes and wines is modified and their quality altered (Jones et al., 2022; Van Leeuwen et al., 2019). Grape quality at harvest depends on the content of primary metabolites (sugars, organic acids and nitrogen compounds) and secondary metabolites (phenolic and aromatic compounds). As temperatures rise, the accumulation of sugars is accelerated by changing the synchrony between fruit development and the evolution of other metabolites such as acids, polyphenols and aromatic substances (Pons et al., 2017). While tartaric acid (TAR) is moderately stable against temperature changes, malic acid (MAL) is strongly affected by temperature and ripening stage (Buttrose & Hale, 1971; Mira de Orduña, 2010). On the other hand, temperatures above 30 °C can reduce anthocyanin synthesis and even inhibit it above 37 °C. Sadras & Moran (2012) observed a decoupling of anthocyanin and sugar contents in red varieties subjected to environmental stress conditions. Results vary depending on plant material and conditions but harvests with excessively high sugar concentrations, excessively low acidity and polyphenols, and an aromatic expression dominated by stewed fruit aromas and changes in wine style are cited (Duchêne & Schneider, 2005; Jackson & Lombard, 1993; Jones *et al.*, 2005; Keller, 2010; Petrie & Sadras, 2008; Sadras & Moran, 2012; Van Leeuwen & Seguin, 2006; Winkler *et al.*, 1974). The increase in potential alcohol contrasts with current consumer preferences, which are moving toward wines with moderate alcohol content (Palliotti *et al.*, 2014). Reducing sugar content is interesting to minimize the cost of desalcoholization of wines, which is limited to 2% vol (Commission Regulation (EC)No 606/2009, 2009).

Among the strategies proposed to reduce the effect of high temperatures and drought on grape production and quality are the selection of better adapted plant material (rootstock and variety) and agronomic techniques such as irrigation, modifications in plant architecture and different pruning strategies, among others (Van Leeuwen et al., 2019; Van Leeuwen & Destrac-Irvine, 2017). In 2012, Gu *et al.* published the results obtained from a green pruning technique they called “crop-forcing”, aimed at prompting the grapevine growth cycle to restart after flowering. These authors managed to shift the ripening period of ‘Cabernet Sauvignon’ in California from July-August to October-November, modifying grape production and characteristics at harvest. In recent years this technique has been studied in different parts of Spain in cv. Tempranillo in Valencia (Martínez-Moreno *et al.*, 2019) and the ‘Tempranillo’ and ‘Maturana Tinta’ in La Rioja (Martinez De Toda *et al.*, 2019) and recently in Japan, in the Yamanashi region, in ‘Merlot’, with similar results in all cases (Kishimoto et al., 2022). This technique decreased yield, but increased berry acidity and polyphenolic content. An alternative proposal to compensate for the crop loss caused by “crop-forcing” has been to keep the original clusters on the forced grapevines, which results in a double harvest (Martinez De Toda, 2021; Poni *et al.*, 2020).

The grapevine is considered a plant adapted to drought conditions (Chaves *et al.*, 2010; Grimplet *et al.*, 2007). Most of the vineyards are located in regions with strong seasonal droughts. Under these conditions, the plant depends on the water storage capacity of the soil to cover the strong evaporative demand that accompanies high temperatures and radiation levels and scarcity of rain during a good part of the growth cycle and usually leads to low yields and deterioration in quality. Although the highest yields are achieved in vineyards that do not suffer from water limitations, moderate stress can improve berry characteristics and sensory properties in wines (Chapman et al., 2004; Gamero et al., 2014; Intrigliolo & Castel, 2010; Valdés et al., 2009). Water deficit usually reduces the acidity (TA) of the wines, but increases the concentration of Total Polyphenols (TPP) and Total Anthocyanins (Ant) (Intrigliolo *et al.*, 2012) and tasting panels report that quality attributes improve with the application of a controlled water deficit (Gamero *et al.*, 2014). A number of deficit irrigation strategies have been proposed for vineyards that increase production compared to rainfed but enable the concentration of compounds of high oenological value. These include Regulated Deficit Irrigation (RDI) which consists of maintaining good water availability in the plant during production-sensitive periods and reducing irrigation water to cause controlled stress in periods that are not critical for production and/or important to stimulate or conserve compounds important for winemaking (Basile *et al.*, 2011, 2012; Girona *et al.*, 2009). Deficit irrigation during pre-veraison favours anthocyanin accumulation as a consequence of reduced berry size and increased expression of genes responsible for flavonoid synthesis (Basile *et al.*, 2011; Intrigliolo *et al.*, 2012; Romero *et al.*, 2013), bringing grape ripening forward (Castellarin et al, 2007), and has been used to minimize decoupling of phenolic and technological ripeness caused by high temperatures (Sadras & Moran, 2012). On the contrary, water stress applied post-veraison increases the proportion of seeds and skin in grapes without significantly affecting secondary metabolism (Roby & Matthews, 2004). In general, deficit irrigation increases total soluble solids (TSS) (Antolín et al., 2006; Koundouras et al., 2006; Ojeda et al., 2002) so that the wines obtained continue to show high alcoholic strength. The results of this technique may be different depending on the variety, timing, duration and intensity of the deficit periods, as well as the weather conditions and the previous history of the vineyard, so that inter-annual differences are observed in the response to the same strategy (Girona et al., 2009; Uriarte et al., 2016). Previous studies carried out in the region of Extremadura (Uriarte et al., 2016), with average annual rainfall around 450 mm, recommended applying a sustained deficit irrigation strategy of 25% of ETc, with which they achieved an average yield increase of 26% relative to rainfed with similar concentrations of TPP and Ant (Uriarte et al., 2016). Under the same conditions, regulated deficit irrigation with water deficit in the pre-veraison period increased TA by improving malic acid concentration compared to rainfed. (Mancha et al., 2021).

Spain's grape-growing surface area is in excess of one million hectares, of which more than 80% is devoted to wines with Protected Designation of Origin (PDO) (Ministry of Agriculture, Fisheries and Food). Most of this area is located in semi-arid climate zones, which include the main vineyard areas of Extremadura. Figure V.1 shows the average temperatures (Tmed) during the vegetative period of the grapevine from 1997 to 2021 in the trial plot, located in Vegas Bajas, Ribera del Guadiana PDO, southwestern Spain (Extremadura) from budburst to harvest in 1997-2021. In all cases in this area, the average mean air temperature was above 20 °C, outside or close to the recommended limit for the varieties grown in the region (Jones, 2006). Berry ripening coincides with the highest temperatures of the year, with averages of up to 35 °C for the months of June, July and August (Figure V.2), which then drop off in the fall. *V. vinifera* L. 'Tempranillo' is the main variety of half of the Spanish designations of origin, widely cultivated in the north, centre and south of the country (Cervera *et al.*, 2002).

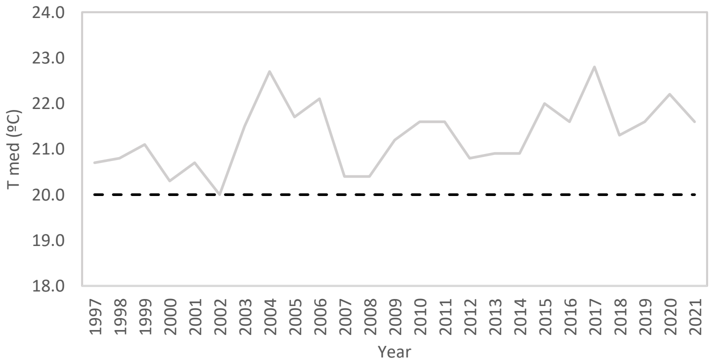

Figure V.1. Average mean temperatures from April 1 to September 30 in Extremadura, Spain from 1997 to 2019.

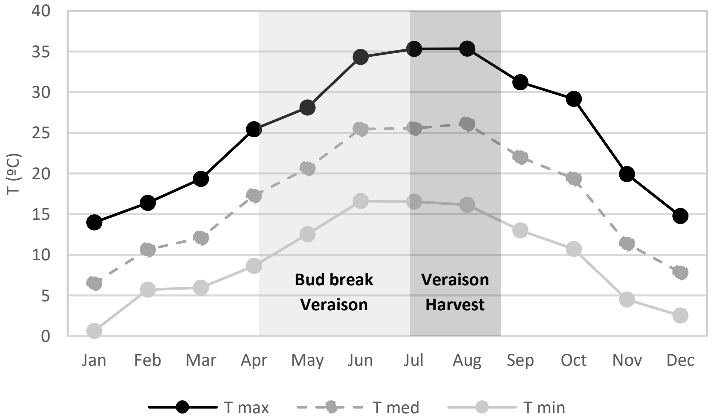

Figure V.2. Distribution of maximum (black), mean (grey dashed line) and minimum (grey solid line) temperatures throughout 2017 in Extremadura, Spain and average duration of the period from budburst to harvest of 'Tempranillo' in the same area.

This paper evaluates the effect of the “crop-forcing” technique on berry ripening process and final composition. The effects of this technique are compared to the application of a deficit irrigation strategy recommended for the ‘Tempranillo’ under Extremadura conditions in non-forced grapevines, as well as the combined effect of both techniques. The objective of this study is to provide winemakers with the most suitable raw ingredients for the desired winemaking processes; and to provide grape growers with tools to obtain higher value harvests that make their vineyards profitable and increase the resilience of vineyards in warm areas in the face of climate change.

V.2. MATERIALS AND METHODS

V.2.1. PLANT MATERIAL AND VINEYARD SITE

The study was carried out in an experimental vineyard located at Badajoz, Extremadura, Spain (38º 51´ N; 6º 40´ W; 198 m) planted with ‘Tempranillo' (*Vitis vinifera* L.) grafted on Richter 110 rootstock, trained as bilateral cordons in a vertical trellis system with a drip irrigation system of 8 L/h per grapevine. All the grapevines were winter pruned to six spurs and two buds per spur. The rows are laid out E-W and row and grapevine spacing is 2.5 m and 1.2 m, respectively.

V.2.2. TREATMENTS AND EXPERIMENTAL DESIGN

The experimental design was split-plot with four replications (Table V.1). The main factor consisted of two treatments with “crop-forcing” techniques on two different dates; F1, “crop-forcing” applied 4 days after anthesis (May 18, 2017; May 29, 2018; May 20, 2019) and F2, 22 days after anthesis, both treatments were compared with a treatment without “crop-forcing” techniques (NF) with grapevines grown following conventional practices (just winter pruning). “Crop-forcing” consisted of hedging the growing shoots to seven nodes and removing all the summer laterals, leaves and clusters with scissors to force the bursting of the primary buds developed in the current season. Two irrigation treatments were set up as a secondary factor. A treatment with no water stress (C), supplying water to maintain a midday stem water potential (Ψsmd) close to -0.6 MPa and a pre-veraison deficit irrigation (RI) supplying water to reach a SWP of -1.1 MPa, as maximum and -0.8 MPa at post-veraison. These treatments were maintained in the same vine during the three consecutive years.

Table V.1. Summary of the treatments applied in the study.

	No “Crop-forcing” (NF)	**Early “Crop-forcing” (F1)**	**Late “Crop-forcing” (F2)**
Fully irrigated (C)	C-NF	C-F1	C-F2
Deficit irrigation (RI)	RI-NF	RI-F1	RI-F2

The grapevine water requirements were calculated based on the crop evapotranspiration (ETc) using the crop coefficient (Kc) recommended by the FAO for these latitudes for the NF treatments. For the F1 and F2 treatments, ETc was calculated directly on a weighing lysimeter (Picón-Toro *et al.*, 2012) with two “crop-forcing” grapevines, integrated in the study plot. Irrigation started when a threshold value of Ψsmd of -0.6 MPa was reached. Irrigation was applied five to six times per week, measuring the amount of water applied to each subplot through volumetric water meters and maintaining irrigation until early and mid October. Meteorological data come from a weather station belonging to the Extremadura irrigation advisory network (REDAREX) located at a distance of 100 m from the plot. The experimental unit consists of 6 rows of 18 grapevines. Ten central grapevines of the four central rows are for sampling and harvest.

Table V.2. Irrigation applied and evapotranspiration (ETo), from budbreak to harvest on non-forced vines (NF) and from “crop-forcing” application date to harvest date in F1 and F2 during 2017, 2018 and 2019 years.

Treatment	**2017**		**2018**		**2019**	
	Irrigation (mm)	**ETo (mm)**	**Irrigation (mm)**	**ETo (mm)**	**Irrigation (mm)**	**ETo (mm)**
C-NF	286	881	285	763	347	909
C-F1	334	782	516	715	482	824
C-F2	405	788	524	671	499	781
RI-NF	56	848	155	763	220	876
RI-F1	228	782	256	715	373	824
RI-F2	212	788	261	671	373	781

V.2.3. MEASUREMENTS

The phenological observations were carried out in 10 grapevines in each experimental plot, every 7 days from budburst to veraison. For data analysis, only the two main phenological stages (budburst and veraison) were used. Veraison was determined by colour change in 50% of berries.

Three hundred berries per experimental unit were randomly collected, from different positions within clusters and plants, weekly from pea size to harvest. 100 berries were weighed fresh.

From veraison to harvest, 200 grapes were frozen in the laboratory until analyzed. The rest were destemmed, crushed and homogenized for 1 min at speed setting 3 (Mycook blender Taurus, Oliana, Spain). An aliquot of the resulting mash (pulp, juice, skins and seeds) was filtered and used to determine technological parameters. Total Soluble Solids (TSS) (°Brix) was determined by refractometry (RE40D, Mettler Toledo, Greifensee, Switzerland), pH with a pH-meter (Basic 20, Crison, Alella, Barcelona) and Titratable Acidity (TA, g tartaric acid /L) with a titrator (T50, Mettler Toledo, Greifensee, Switzerland) according to ECC formal methods (Commission Regulation No. 2676/90, 1990). Malic (MAL) and tartaric (TAR) acid content (g/L) was determined according to ECC formal methods using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

Extraction of phenolic substances from grapes was carried out employing a methodology based on previous works (Díaz-Fernández *et al.*, 2022; Portu *et al.*, 2016). 100 g samples of frozen berries (-80 °C) were crushed and homogenized for 30 seconds in a Freshboost blender (LM180110, Moulinex, Aleçon, France). 10 mL of the hydroalcoholic solution (methanol/water/formic acid 50:48.5:1.5, v/v/v) were added to an aliquot of the obtained mash (1.0 g) and macerated for 30 minutes at 4 °C in an ultrasonic bath (USC-TH, VWR, Radnor, USA) and centrifuged at 4 °C for 10 min (5810 R, Eppendorf, Hambug, Germany). The supernatant was separated, and the resulting pellet was extracted up to three times. The supernatants (phenolic extracts) were then combined and the final volume was annotated. Total Polyphenol Content (TPP) from the extracts was determined according to Singlenton & Rossi (1965), and Total Anthocyanin (Ant) content was quantified employing the pH differential method (Lee *et al.*, 2005). All determinations were carried out using an autoanalyzer (Y15, Biosystems, Barcelona, Spain). Two extractions were performed for each sample of a given plot and sampling date.

All treatments were harvested manually at 23–24 °Brix, a common criterion for picking red grape varieties in this area. The average TSS of the berries from the four elementary plots was considered for each treatment. All the clusters of 10 grapevines per experimental plot were weighed (40 grapevines per treatment). The numbers of clusters per shoot were counted and weighed on a total of ten vines per experimental plot.

Total pruning dry weight was determined in the different interventions carried out: green pruning, forcing pruning and winter pruning of the same ten grapevines per experimental plot.

V.2.4. STATISTICAL DATA ANALYSIS

Normality and homogeneity of variances was tested using Shapiro-Wilk's and Barlett's test respectively. When the normality and homogeneity of variances were verified, data were subjected to analysis of variance (MANOVA) to study the effect of “crop-forcing”, “irrigation” and their interaction on each parameter evaluated selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons. The interaction between effects was evaluated by calculating the least-squares means (LS means) selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons and the Tukey test as Post Hoc tests for parametric samples. When the normality and homogeneity of variances were not verified, non-parametric tests were carried out employing the Kruskal-Wallis test (alternative to one-way ANOVA) and multiple comparison *p* values (alternative to post-hoc pairwise comparisons). Differences between means were considered statistically significant when $p < 0.05$. The relationships between TSS values and the rest of investigated parameters were assessed by regression analysis and the comparison of slopes was tested by performing the corresponding analysis of variance (ANOVA). SPSS software package 12.0 for Windows (SPSS Inc., Chicago, IL) was used for processing the data. Also, principal component analysis (PCA) was performed to discriminate among treatments based on the values of acid and polyphenolic parameters, and the relationships between TSS values and the rest of parameters under study were assessed by regression analysis. This last statistical test was performed with XLSTAT-Pro 201610 (Addinsoft, 2009, Paris, France)

V.3. RESULTS

V.3.1. GRAPEVINE TIMELINE AND GROWING CONDITIONS

The irrigation strategies did not modify the phenological cycle of the grapevines (results not shown). However, the application of “crop-forcing” treatments displaced it, both in F1 and F2. Figure V.3 reflects the growth cycle in the different treatments: from natural budburst to harvest in C-NF and RI-NF and the first (C-F1 and RI-F1) and second post-forcing (C-F2 and RI-F2) budburst to respective dates of harvest. Figure V.3 also shows the maximum temperatures during the ripening period of the different treatments. Since the forcing treatments moved phenological stages to later dates in the year, they occurred with lower temperature than in NF treatments.

With respect to NF, the average decrease in maximum temperature for this period was 1.5 °C in 2017, 3 °C in 2018 and 2.7 °C in 2019 in F1 and 5.7, 8 and 4.6 °C for F2. Annual rainfall was 282, 479 and 293 mm in 2017, 2018 and 2019 respectively. 2018 was the wettest year in both spring and autumn. The length of the F1 and F2 ripening cycle was shortened in 2017 and 2019 relative to NF, while it was lengthened in 2018.

As shown in Table V.3, harvest dates varied according to year and “crop-forcing” treatment. With respect to NF, the “crop-forcing” technique shifted the harvest date, causing delays in the harvest, which were longer in F1 than in F2. In F1, grapes were harvested between September 12 and October 8, while in F2 the grapes were picked between October 15 and 29. In addition, when analyzing the different years, it is observed that, with respect to NF, the number of F1 delay days was 23, in 2017, 41 in 2018 and 34 in 2019. In F2, delays varied less depending on the year considered and were 58 days, 61 days and 43 days respectively in the same years.

Table V.3. Budburst and harvest dates, and duration of the cycle.

Treatment	Budburst date			Harvest date			Days (from budburst to harvest)		
	2017	2018	2019	2017	2018	2019	2017	2018	2019
NF	3 Apr	3 Apr	26-mar	22 Aug	27 Aug	27 Aug	141	148	154
F1	18-may	29-may	20-may	12-sep	08-oct	30-sep	117	132	133
F2	06-jun	17-jun	03-jun	17-oct	29-oct	15-oct	133	134	138

In F1 and F2 the budburst date is taken as the date of (forced) summer pruning.

V.3.2. BERRY WEIGHT EVOLUTION, YIELD COMPONENTS AND VINE VIGOR.

Figure V.4 shows the evolution of berry unit weight throughout the growth cycle (fruit set to harvest) and the veraison dates of the different treatments corresponding to the three years under study. In all cases the berry growth pattern follows a double sigmoid curve but, in the three years under study, the irrigation treatment and, particularly, the pruning technique, caused differences in the evolution of this parameter. On the one hand, in the “crop-forcing” treatments (C-F1, C-F2, RI-F1 and RI-F2), the onset of the curve was delayed. On the other, in the first two years, NF and F2 berry growth is evenly distributed between pre-veraison and post-veraison, but in F1 berries gain more weight after veraison (Figures V.4I and V.4II). In 2019, weight gain is higher during pre-veraison in all treatments (Figure V.4III). Also, it is noteworthy that a few days after fruit set, differences between C-NF and RI-NF begin to be identified, increasing as the cycle progresses, while in F1 and F2 there are hardly any differences in the evolution of the weight of the two irrigation treatments for the same forcing date in any year of the study.

The grapevines to which the forcing treatments were applied were less productive. In the three seasons analyzed, the mean value of the F2 treatments was higher than F1 although in 2018 the differences were not significant. In addition, 2019 should be highlighted as the most productive year, and also the year when there was the greatest difference in C-NF with the “crop-forcing” treatments. For the same pruning treatment, yield was higher in C than in RI. RI-NF had higher yield than any of the “crop-forcing” treatments, with significant differences only in 2019. Irrigation according to needs was more productive than RI although the differences were significant only in 2018. When comparing the two irrigation treatments with the same pruning, the control was always more productive, although differences were only significant in 2019.

All vines were pruned in winter leaving twelve buds per vine from which the corresponding shoots developed. In the forced treatments, each shoot was forced in the current season leaving six buds per shoot. Although the number of clusters per shoot varied across the study years, the forced treatments had a higher number of clusters per shoot during the study. The non-forced treatments (C-NF and RI-NF) had 1.3 average bunches per shoot, while F1 and F2 reached 1.6 and 1.8 bunches per shoot respectively (Table V.4). The effect of irrigation was significant only in 2017 where RI had lower number of bunches per shoot. This parameter showed a high irrigation-forcing interaction, but these interactions did not show a clear trend during the study, as they varied depending on the year. The effect of treatments on the total dry weight of the different pruning interventions (green pruning, forcing pruning and winter pruning) was different in each year (Table V.4). In 2017, the pruning was higher in F2 than NF. In 2018, C-NF and C-F1 had the highest wheight, and RI-NF the lowest. In these two years, the total dry weight of prunings was lower in the RI-NF treatment compared to C-NF, while in 2019 there was no difference.

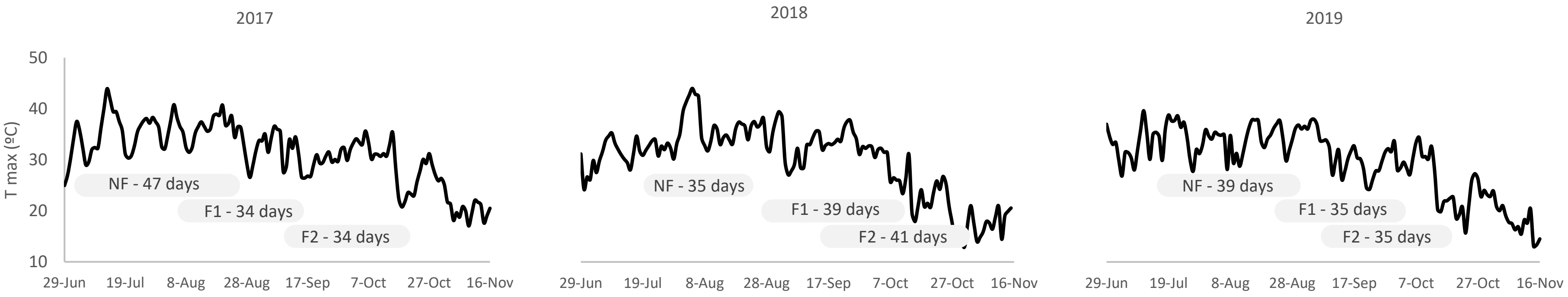

Figure V.3. Maximum daily temperatures from July 29 through November 16 for the three years under study. Horizontal bars represent the days between veraison and harvest for NF, F1 and F2.

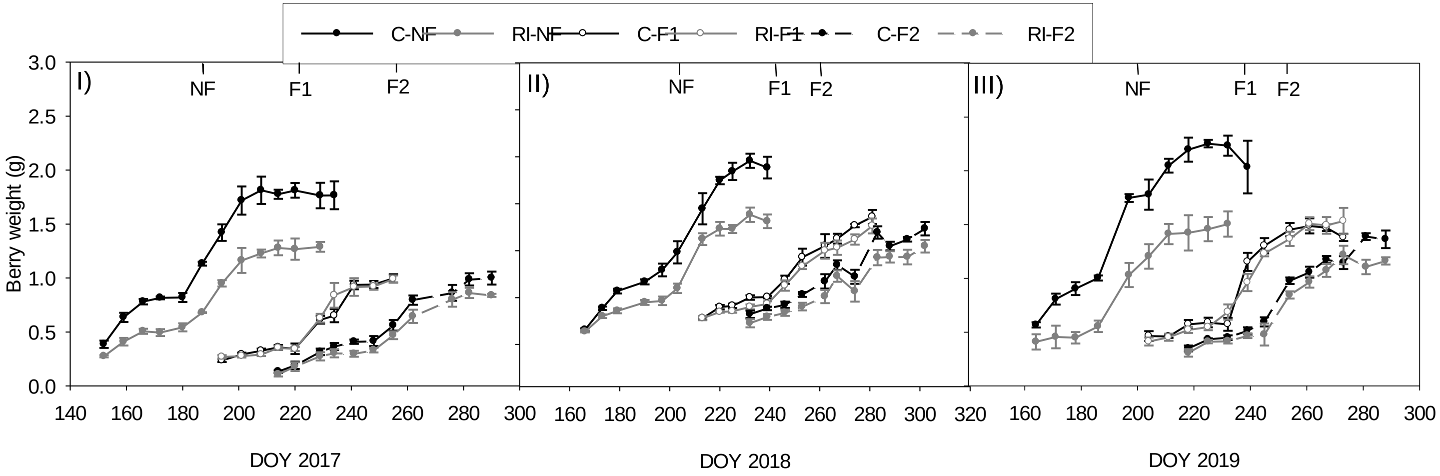

Figure V.4. Evolution of berry fresh weight in: (I) 2017; (II) 2018; (III) 2019. Each point represents the mean of 8 samples (4 blocks, 2 replicates). The bars represent the standard error of the mean. In the upper part, the time of veraison for the different forcing treatments is shown. DOY: Days of Year.

Table V.4. Yield, number of clusters per vine and total pruning weight (green pruning, forcing pruning and winter pruning) in pruning (NF, F1 and F2) and irrigation (C and RI) treatments.

Parameter	**Year**	***Statistical analysis***	**C**			**RI**			***FxI***	**Forcing**			***FF***	**Irrigation**		***IF***
			NF	**F1**	**F2**	**NF**	**F1**	**F2**		**NF**	**F1**	**F2**		**C**	**RI**	
Yield	**2017**	*p.*	3.89	1.94	2.77	2.26	1.29	1.31	n.s.	3.07 a	1.61 c	2.04 b	***	2.86	1.62	***
(kg/vine)	**2018**	*p.*	3.73	1.65	1.84	3.05	1.54	1.78	n.s.	3.39 a	1.60 b	1.81 b	***	2.41	2.12	n.s.
	2019	*p.*	7.12 a	2.25 d	3.00 c	4.41 b	2.01 d	2.88 c	***	5.77	2.13	2.94	***	4.12	3.10	***
Clusters	**2017**	*n.p.*	1.1 bc	1.4 b	2.1 a	0.9 c	0.9 c	1.0 bc	***	1.0	1.2	1.6	***	1.6	0.9	***
per shoot	**2018**	*n.p.*	1.3 b	2.4 a	1.6 b	1.5 b	2.3 a	1.8 ab	***	1.4	2.3	1.7	***	1.8	1.8	n.s.
	2019	*n.p.*	1.5 b	1.3 bc	2.2 a	1.4 bc	1.1 c	2.3 a	***	1.5	1.2	2.2	***	1.7	1.6	n.s.
Total	**2017**	*n.p.*	0.9 b	1.1 ab	1.2 a	0.6 c	1.0 ab	0.9 b	***	0.7	1.0	1.0	***	1.0	0.8	***
pruning weight	**2018**	*n.p.*	1.2 a	1.2 a	1.0 ab	0.8 b	1.0 ab	1.0 ab	**	1.0	1.1	1.0	n.s.	1.1	0.9	**
(kg/vine)	**2019**	*n.p.*	1.1	1.0	0.9	1.0	1.0	0.9	n.s.	1.0	1.0	0.9	n.s.	1.0	1.0	n.s.

Statistical analysis: MANOVA and Tukey test as a parametric test and Kruskal-Wallis test as a nonparametric test (both p < 0.05). FxI: Interaction between forcing and irrigation effects; FF: Forcing factor; IF: Irrigation factor. Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; n.i., no interaction; (*), significant at 5 percent level; (**), significant at 1 percent level and (***), significant at 0.1 percent level.

V.3.3. GRAPE RIPENING

Technological Ripeness (Total Soluble Solids and Acids)

As reflected in Figure V.5, “crop-forcing” treatments delayed the grape ripening period but did not affect the metabolite increase and decrease patterns in the berries. Typical decreases in MAL and TA values and increases in berry °Brix and pH were observed in these treatments. The delay in ripening dates did not prevent reaching the °Brix value set for harvesting in F1 and F2. Of note is the evolution of these parameters in the F2 treatments in 2018 and 2019. Figure V.5 shows that, in F2, the drops in MAL and AT were less pronounced and a stabilization of the values of these parameters was observed and also the pH values were lower than in the rest of the treatments during the whole period analyzed. In 2017, there was a greater chronological separation between the three prunings, while in 2018 and 2019, F1 was further away from C and closer to F2 (Figure 5).

It is noteworthy that, for the same pruning treatment, the ripening processes took place on identical dates under both irrigation strategies. With regard to C, the effect of RI was higher in NF berries than in F1 and F2 berries. In the three years, the evolution of TSS values was practically identical in the C and RI treatments except for the 2019 NF treatments. The MAL evolution curves show lower values in RI-NF than in C-NF throughout the process, while no differences were observed in F1 and F2 curves, except for F1 in 2018 when RI-F1 falls below C-F1. In TAR, RI maintains higher values than C in NF, with these differences being clearer in 2017. In the forced grapevines the values are similar in the two irrigation treatments, except for 2018, when C has higher RI values throughout ripening in F1 and F2. As a result, in all three seasons, the TA values of the C-NF treatments remained below those of RI-NF treatments, while in F1 and F2, they were very similar throughout the cycle. Finally, pH values of RI-NF berries remained above those of C-NF in all samples of the 2018 and 2019 vintages, while C-F1 and RI-F1 were very similar. In F2, pH stabilized at lower values in C in the first two harvests under study and no differences were observed in the third harvest.

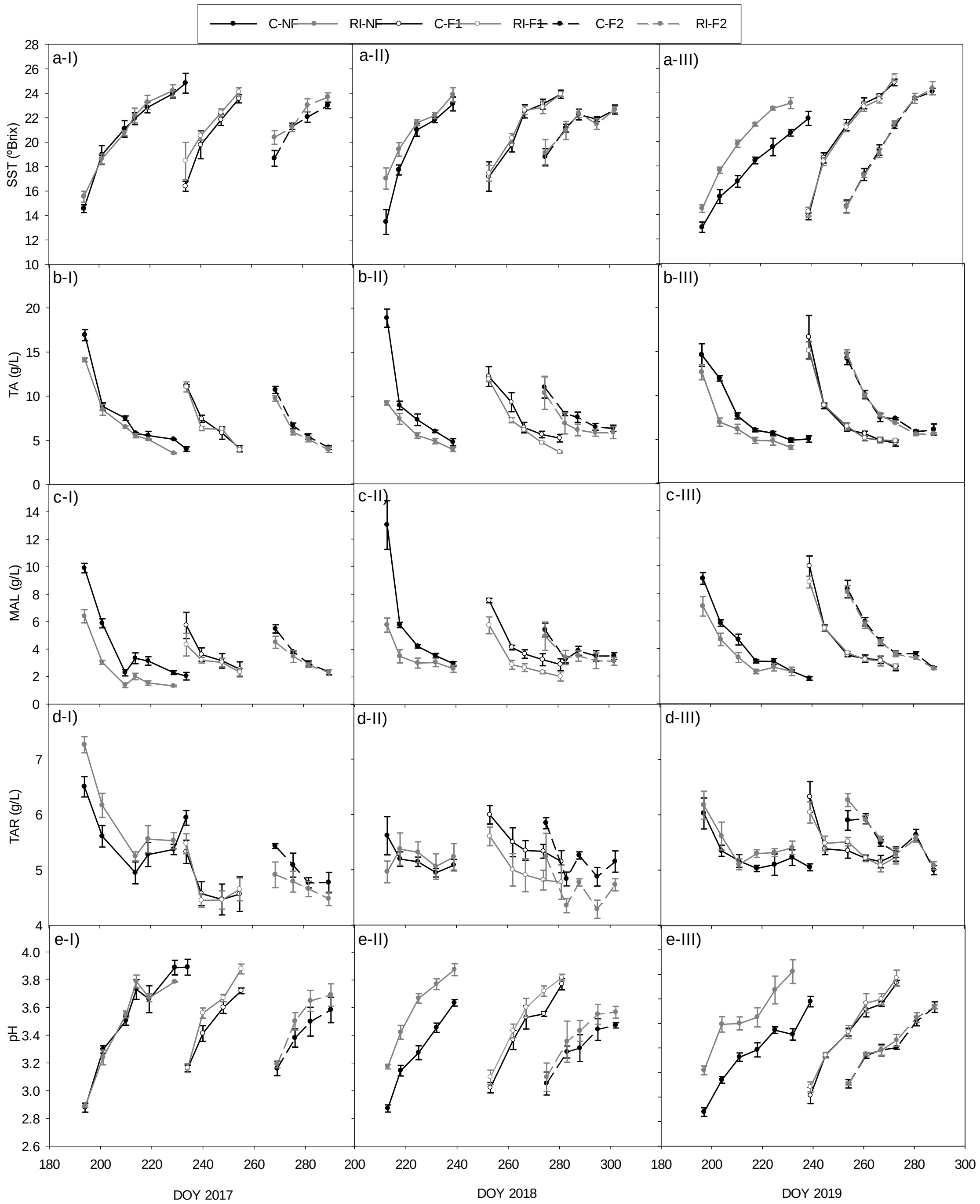

Figure V.5. Evolution of: (a-I) Total Soluble Solids (TSS) in 2017, (a-II) Total Soluble Solids (TSS) in 2018 and (a-III) Total Soluble Solids (TSS) in 2019; (b-I) Titratable Acidity (TA) in 2017, (b-II) Titratable Acidity (TA) in 2018 and (b-III) Titratable Acidity (TA) in 2019; c-I) Malic Acid (MAL) in 2017, c-II) Malic Acid (MAL) in 2018 and c-III) Malic Acid (MAL) in 2019; d-I) Tartaric Acid (TAR) in 2017, d-II) Tartaric Acid (TAR) in 2018 and d-III) Tartaric Acid (TAR) in 2019; e-I) pH in 2017, e-II) pH in 2018 and e-III) pH in 2019. Each point represents the mean of 8 samples (4 blocks, 2 replicates). The bars represent the standard error of the mean. DOY: Days of Year.

Phenolic Ripeness

Figure V.6 shows the evolution of Ant and TPP values. The treatments subjected to forcing had a similar evolution dynamic to the corresponding NF; increase and tendency to stabilization in TPP and decrease and tendency to stabilization in Ant. However, the initial values of F1 and F2 are higher and at the time of harvest they still maintain higher values than NF. Deficit irrigation acted as a stimulus for anthocyanin synthesis in all three pruning treatments in 2017 and in NF also in 2018 and 2019. The effect of forcing treatments on these substances was more evident in the last two harvests. In F1 and F2 the accumulation of these compounds was higher. In 2017 and 2818, in the forced grapevines, Ant dropped faster and final stabilization was shorter as we came closer to the harvest date.

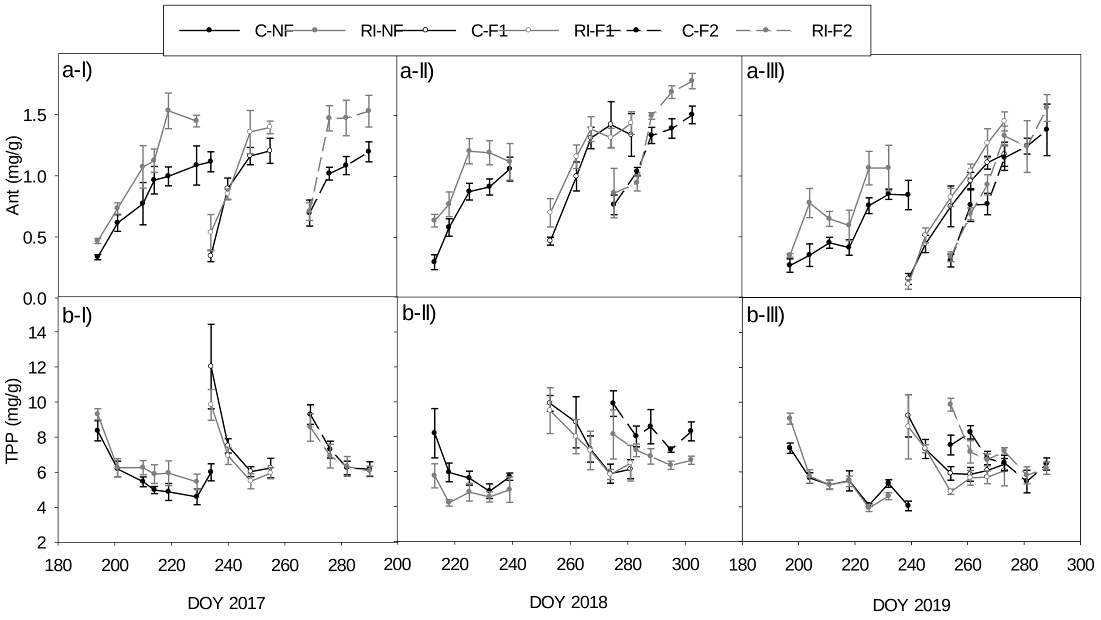

Figure V.6. Evolution of the content in: a-I) Total Anthocyanins (TAN) in 2017, a-II) Total Anthocyanins (TAN) in 2018 and a-III) Total Anthocyanins (TAN) in 2019; b-I) Total Polyphenols (TPP) in 2017, b-II) Total Polyphenols (TPP) in 2018 and b-III) Total Polyphenols (TPP) in 2019. Each point represents the mean of 8 samples (4 blocks, 2 replicates). The bars represent the standard error of the mean. DOY: Days of Year.

V.3.4. BALANCE OF BERRY TRAITS

To analyze the effect of treatments on the balance between different berry compounds, regressions were fitted between TAN, TPP, TA, pH, MAL and TAR with TSS throughout ripening (Figure V.7). To simplify the presentation of the data, treatments C-F1 and C-F2 have been excluded from the figures, since the developments presented in the previous section (Figure V.5) indicate that with the same green pruning date ripeness development was similar in the two irrigation treatments, with greater differences with the F treatments in the case of RI. Table V.5 shows Pearson's coefficients for the correlations between these compounds and the TSS and the significance. The Table S1 of the *supplementary information* include the equations, the coefficient of determination and the comparisons of the slopes of the correlation lines. In all cases, the comparisons of the slopes were statistically significant, al least two groups were established between the four treatments analyzed (Table V.6).

The correlation between pH and TSS of the different treatments was different in the three years under study (Figure V.7a). In 2017, all treatments maintained a similar balance between both compounds, except RI-F, which started from a significantly lower pH for the same TSS, and the rise in pH as ripening progressed was significantly higher. In the two subsequent years, more differentiated relationships between treatments were identified. The pH of RI-NF was higher with 22 TSS. In these same years, RI-F2 reaches this TSS concentration at a similar or lower pH than C-NF.

The correlation between TA and TSS showed that as maturation progressed, TSS increased and TA decreased. (Figure V.7b). In 2017, the 4 treatments maintain a similar correlation. In 2018, with increasing TSS the 4 treatments tend to equal TA with 24 °Brix, while in 2019 RI-F1 and RI-F2 have higher TA with similar TSS throughout.

The correlation between malic acid and TSS also decreases linearly (Figure V.7c). In 2017, at approximately 22 °Brix, RI-F1 and RI-F2 match the C-NF line, while RI-NF shows lower malic acid values. In 2018, the trend is very similar to the previous year but, in this case, RI-F1 shows similar values to RI-NF. In 2019, C-NF is the treatment showing the lowest values across the line, followed by RI-NF. RI-F1 and RI-F2 are the treatments that show the highest values and are very similar to each other.

The correlation between TPP and TSS showed a decreasing trend (Figure V.7d). The differences between treatments are appreciable throughout the line. C-NF shows the lowest values in all three years, followed by RI-NF (with the exception of 2018 which does not show RI-NF data). The treatment with the highest values is RI-F2 in 2017 and 2019, while in 2018 it is RI-F1, although the differences between these two treatments are small in all three years.

The correlation between Ant and TSS increases linearly (Figure V.7e). RI-F2 is the treatment with the best results over the three years, followed by RI-F1. In 2017, RI-F1 is no different from RI-NF in 2017. In all three years, C-NF shows the lowest values in all cases, although in 2019 the RI-NF and C-NF values are very similar.

Table V.5. Pearson correlation between total soluble solids content (TSS) and: pH, total acidity (TA), malic acid (MAL), tartaric acid (TAR), total polyphenols (TPP) and total anthocyanins (TAN).

		C-NF	RI-NF	RI-F1	RI-F2
2017	**pH**	0.908 (***)	0.918 (***)	0.836 (***)	0.845 (***)
	TA	-0.944 (***)	-0.930 (***)	-0.823 (***)	-0.841 (***)
	MAL	-0.934 (***)	-0.888 (***)	-0.842 (***)	-0.844 (***)
	TAR	-0.540 (**)	-0.834 (***)	-0.435 (n.s.)	-0.557 (*)
	TPP	-0.715 (***)	-0.734 (***)	-0.854 (***)	-0.739 (**)
	TAN	0.841 (***)	0.889 (***)	0.862 (***)	0.717 (**)
2018	**pH**	0.931 (***)	0.910 (***)	0.970 (***)	0.909 (***)
	TA	-0.935 (***)	-0.848 (***)	-0.929 (***)	-0.908 (***)
	MAL	-0.944 (***)	-0.686 (**)	-0.802 (***)	-0.784 (***)
	TAR	-0.691 (**)	0.349 (n.s.)	-0.485 (*)	-0.653 (**)
	TPP	-0.698 (**)	-0.025 (n.s.)	-0.501 (*)	-0.750 (***)
	TAN	0.949 (***)	0.776 (***)	0.852 (***)	0.770 (***)
2019	**pH**	0.943 (***)	0.852 (***)	0.972 (***)	0.951 (***)
	TA	-0.897 (***)	-0.907 (***)	-0.950 (***)	-0.928 (***)
	MAL	0.945 (***)	-0.906 (***)	-0.958 (***)	-0.950 (***)
	TAR	-0.695 (***)	-0.672 (***)	-0.818 (***)	-0.851 (***)
	TPP	-0.711 (***)	-0.882 (***)	-0.568 (***)	-0.735 (***)
	TAN	0.815 (***)	0.698 (***)	0.969 (***)	0.918 (***)

Pearson correlation: n.s. indicates not significant; (*), significant at 5 percent level; (**), significant at 1 percent level and (***), significant at 0.1 percent level. Comparison of slopes was tested by performing the corresponding analysis of variance (ANOVA), in all cases p<0.001.

Table V.6. Coefficient of determination (R^2) and equation between x=Total Soluble Solids (TSS) and y= pH; Titratable Acidity (TA); Malic Acid (MAL); Tartaric Acid (TAR); Total Polyphenols (TPP) and Total Anthocyanins (TAN), in year 2017, 2018 and 2019.

	Treatment	**2017**			**2018**			**2019**		
		Equation	***Slope***[a]	**R^2**	**Equation**	***Slope***[a]	**R^2**	**Equation**	***Slope***[a]	**R^2**
pH	**C-NF**	$y = 0.0944x + 1.552$	*bc*	$R^2 = 0.8252$	$y = 0.0692x + 1.9309$	*d*	$R^2 = 0.8674$	$y = 0.0875x + 1.5930$	*a*	$R^2 = 0.8885$
	RI-NF	$y = 0.1001x + 1.4088$	*b*	$R^2 = 0.8432$	$y = 0.0939x + 1.6260$	*c*	$R^2 = 0.8284$	$y = 0.0771x + 1.9269$	*b*	$R^2 = 0.7257$
	RI-F1	$y = 0.0909x + 1.6236$	*c*	$R^2 = 0.6995$	$y = 0.1061x + 1.2605$	*b*	$R^2 = 0.9409$	$y = 0.0816x + 1.6751$	*ab*	$R^2 = 0.9439$
	RI-F2	$y = 0.1237x + 0.7748$	*a*	$R^2 = 0.7143$	$y = 0.1262x + 0.7096$	*a*	$R^2 = 0.8258$	$y = 0.0571x + 2.1008$	*c*	$R^2 = 0.904$
TA	**C-NF**	$y = -1.1546x + 32.107$	*b*	$R^2 = 0.8917$	$y = -1.3285x + 35.061$	*a*	$R^2 = 0.8745$	$y = -1.0817x + 27.444$	*a*	$R^2 = 0.8048$
	RI-NF	$y = -1.0622x + 29.257$	*c*	$R^2 = 0.8643$	$y = -0.6638x + 20.107$	*c*	$R^2 = 0.7199$	$y = -0.8767x + 24.042$	*c*	$R^2 = 0.8233$
	RI-F1	$y = -0.8937x + 25.989$	*d*	$R^2 = 0.6768$	$y = -1.1065x + 30.534$	*b*	$R^2 = 0.8639$	$y = -0.9534x + 27.496$	*b*	$R^2 = 0.9024$
	RI-F2	$y = -1.2539x + 33.881$	*a*	$R^2 = 0.7079$	$y = -1.3285x + 35.432$	*a*	$R^2 = 0.8245$	$y = -0.8464x + 25.411$	*c*	$R^2 = 0.8613$
MAL	**C-NF**	$y = -0.7525x + 20.043$	*a*	$R^2 = 0.8729$	$y = -1.0193x + 25.71$	*a*	$R^2 = 0.8909$	$y = -0.7485x + 17.72$	*a*	$R^2 = 0.8927$
	RI-NF	$y = -0.5372x + 13.757$	*b*	$R^2 = 0.7877$	$y = -0.3475x + 10.818$	*c*	$R^2 = 0.4712$	$y = -0.5324x + 14.308$	*bc*	$R^2 = 0.8204$
	RI-F1	$y = -0.3503x + 10.702$	*c*	$R^2 = 0.7091$	$y = -0.4826x + 13.482$	*b*	$R^2 = 0.6438$	$y = -0.5604x + 16.196$	*b*	$R^2 = 0.9186$
	RI-F2	$y = -0.5406x + 15.206$	*b*	$R^2 = 0.7122$	$y = -0.5579x + 15.536$	*b*	$R^2 = 0.6153$	$y = -0.5107x + 14.857$	*c*	$R^2 = 0.9033$
TAR	**C-NF**	$y = -0.0853x + 7.4104$	*b*	$R^2 = 0.2917$	$y = -0.0675x + 6.5076$	*c*	$R^2 = 0.4773$	$y = -0.0919x + 6.9316$	*a*	$R^2 = 0.4835$
	RI-NF	$y = -0.1933x + 9.9613$	*a*	$R^2 = 0.696$	$y = 0.053x + 4.0839$	-	$R^2 = 0.1219$	$y = -0.0925x + 7.3254$	*a*	$R^2 = 0.452$
	RI-F1	$y = -0.0880x + 6.6360$	-	$R^2 = 0.1888$	$y = -0.0992x + 7.1437$	*b*	$R^2 = 0.2355$	$y = -0.0804x + 7.1108$	*b*	$R^2 = 0.6687$
	RI-F2	$y = -0.1146x + 7.2372$	*b*	$R^2 = 0.3102$	$y = -0.193x + 8.7879$	*a*	$R^2 = 0.426$	$y = -0.0993x + 7.6049$	*a*	$R^2 = 0.7237$
TPP	**C-NF**	$y = -0.2889x + 11.891$	*c*	$R^2 = 0.5109$	$y = -0.3049x + 12.000$	*b*	$R^2 = 0.4868$	$y = -0.2697x + 10.172$	*b*	$R^2 = 0.5058$
	RI-NF	$y = -0.3593x + 13.975$	*b*	$R^2 = 0.5387$	$y = -0.0095x + 5.0595$	-	$R^2 = 0.0006$	$y = -0.4824x + 15.273$	*a*	$R^2 = 0.7771$
	RI-F1	$y = -0.6655x + 21.276$	*a*	$R^2 = 0.729$	$y = -0.4101x + 16.196$	*b*	$R^2 = 0.2508$	$y = -0.2761x + 12.141$	*b*	$R^2 = 0.3228$
	RI-F2	$y = -0.6569x + 21.474$	*a*	$R^2 = 0.5457$	$y = -0.565x + 19.097$	*a*	$R^2 = 0.5623$	$y = -0.3041x + 13.257$	*b*	$R^2 = 0.5401$
TAN	**C-NF**	$y = 0.0783x - 0.8179$	*c*	$R^2 = 0.7069$	$y = 0.078x - 0.7698$	*c*	$R^2 = 0.9009$	$y = 0.0695x - 0.6885$	*b*	$R^2 = 0.6641$
	RI-NF	$y = 0.1190x - 1.4075$	*b*	$R^2 = 0.7901$	$y = 0.0877x - 0.8398$	*c*	$R^2 = 0.6019$	$y = 0.0717x - 0.6764$	*b*	$R^2 = 0.4872$
	RI-F1	$y = 0.1426x - 2.0128$	*ab*	$R^2 = 0.7432$	$y = 0.1077x - 1.1079$	*b*	$R^2 = 0.7257$	$y = 0.1224x - 1.6916$	*a*	$R^2 = 0.9389$
	RI-F2	$y = 0.1831x - 2.7493$	*a*	$R^2 = 0.5142$	$y = 0.1971x - 2.8476$	*a*	$R^2 = 0.5936$	$y = 0.1168x - 1.3280$	*b*	$R^2 = 0.8426$

[a] *Comparison of slopes was tested by performing the corresponding analysis of variance (ANOVA), in all cases $p<0.001$. Different letters indicate the existence of statistically significant differences between treatments.*

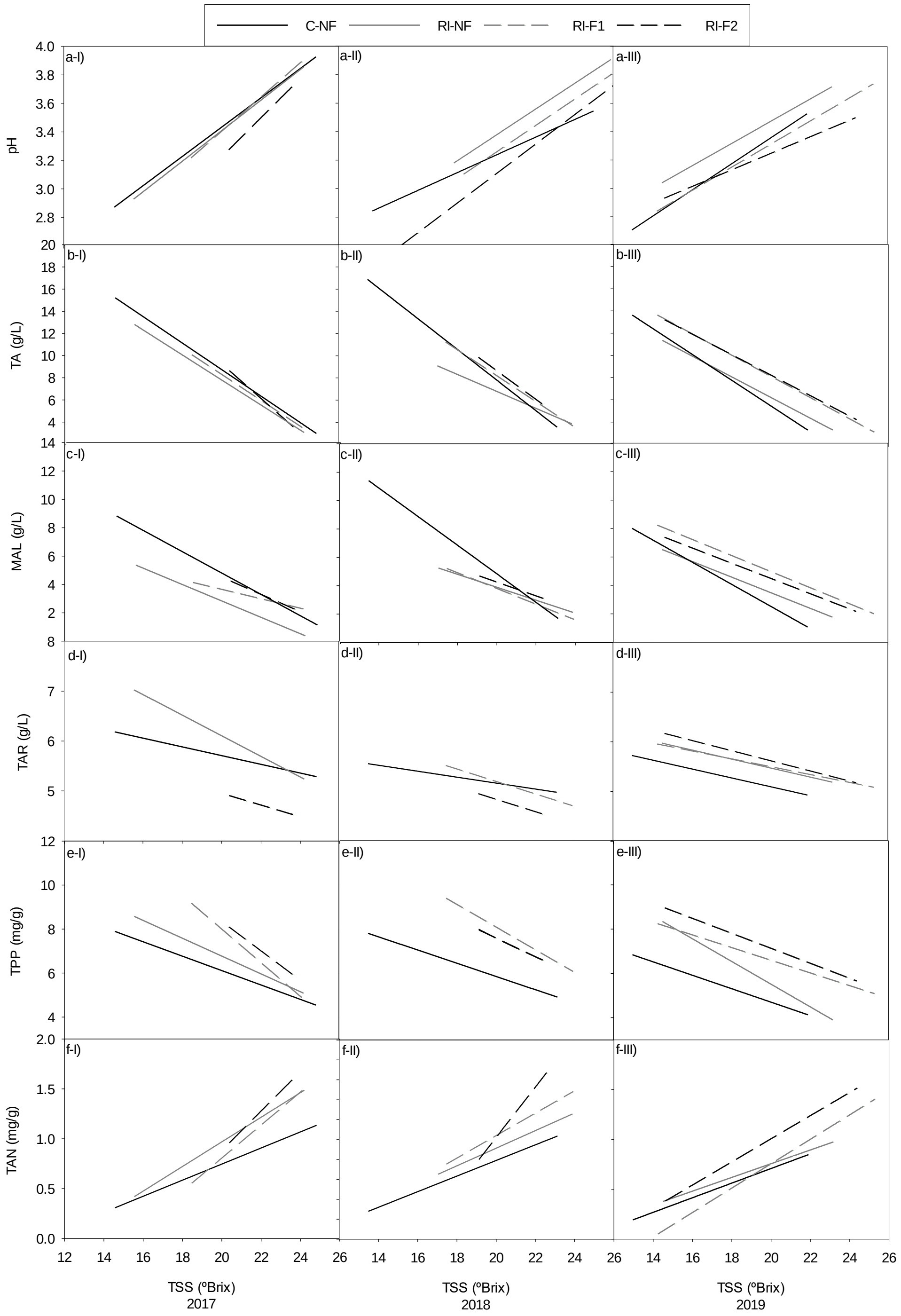

Figure V.7. Regression between Total Soluble Solids (TSS) and a-I) pH in 2017, a-II) pH in 2018 and a-III) pH in 2019; b-I) Titratable Acidity (TA) in 2017, b-II) Titratable Acidity (TA) in 2018 and b-III) Titratable Acidity (TA) in 2019; c-I) Malic Acid (MAL) in 2017, c-II) Malic Acid (MAL) in 2018 and c-III) Malic Acid (MAL) in 2019; d-I) Tartaric Acid (TAR) in 2017, d-II) Tartaric Acid (TAR) in 2018 and d-III) Tartaric Acid (TAR) in 2019; e-I) Total Polyphenols (TPP) in 2017, e-II) Total Polyphenols (TPP) in 2018 and e-III) Total Polyphenols (TPP) in 2019; f-I) Total Anthocyanins (TAN) in 2017, f-II) Total Anthocyanins (TAN) in 2018 and f-III) Total Anthocyanins (TAN) in 2019.

V.3.5. BERRY COMPOSITION. EFFECTS OF TREATMENTS

Table V.7 shows the composition of the berries for a concentration of 22 °Brix, considering this value as a reference for making wines that are less alcoholic. It is noteworthy that a significant interaction of the forcing and irrigation effect was only observed in MAL in 2017 and in TPP in 2018 and 2019. In all three cases, minimum values were recorded in RI-NF. For the remaining parameters and years, the F*I effect was not significant. This allows the effect of forcing and irrigation strategy to be analyzed separately.

The significance of the effect of forcing on berry acidity parameters depended on the parameter and season considered. In 2018 and 2019 the values of TA show an increase for F1 and F2 with respect to NF. However, differences between F1 and F2 only occur in 2019, the latter having a higher value. Overall, the effect of F1 and F2 relative to NF on TAR values was not clear. In 2017, the differences are found between NF and F2, while F1 results are intermediate. In 2018, NF and F1 achieve similar values and higher than F2. In 2019, it is F2 that shows a higher value, with differences with respect to NF, while F1 achieves intermediate values. For malic acid content in 2017, there is interaction between the two effects, with differences between C-NF and RI-NF, while all “crop-forcing” treatments achieve intermediate values. In 2018 there is no difference in this parameter. The effect on malic acid is seen only in 2019, with higher results for F1 and F2 with respect to NF. All years showed the same tendency to higher berry pH values in forced grapevines with respect to the NF grapevines, and the effect was always greater in F2 than in F1. The effect on pH was significant in 2017 and 2019.

Regarding the effect of treatments on phenolic ripeness indicator parameters, a significant interaction was observed in TPP in the last two seasons, so that in both years the minimum values were recorded in C-NF. The values found in this treatment in 2019 were significantly lower than those corresponding to C-F1, C-F2 and RI-F2. In 2017, there is no interaction between the two effects, but there is an effect from forcing. The mean values of this parameter in F2 were significantly higher than those of the control grapevines. The results of forcing are clear and evident in the Ant values since in all years the contents of these F2 substances were significantly higher than those of NF.

Table V.7. Berry composition of pruning (NF, F1 and F2) and irrigation (C and RI) treatments: pH, Titratable Acidity (TA), malic acid, tartaric acid, Total Polyphenols (TPP) and Total Anthocyanins (TAN) at 22 °Brix.

Parameter	**Year**	***Statistical analysis***	**Treatment**						***FxI***	**Forcing**			***FF***	**Irrigation**		***IF***
			C-NF	**C-F1**	**C-F2**	**RI-NF**	**RI-F1**	**RI-F2**		**NF**	**F1**	**F2**		**C**	**RI**	
pH	**2017**	*p.*	3.72	3.61	3.49	3.76	3.64	3.56	*n.s.*	3.74 a	3.63 ab	3.53 b	**	3.61	3.65	*n.s.*
	2018	*p.*	3.48	3.50	3.45	3.74	3.56	3.56	*n.s.*	3.61	3.53	3.50	*n.s.*	3.48	3.62	**
	2019	*p.*	3.58	3.39	3.27	3.55	3.45	3.31	*n.s.*	3.56 a	3.42 b	3.29 c	***	3.42	3.44	*n.s.*
TA (g/L)	**2017**	*n.p.*	5.77	5.65	5.53	5.65	6.28	5.60	*n.s.*	5.71	5.97	5.56	*n.s.*	5.65	5.84	*n.s.*
	2018	*p.*	5.90	7.03	6.57	5.17	6.54	5.93	*n.s.*	5.53 b	6.78 a	6.25 a	***	6.50	5.88	**
	2019	*p.*	5.06	5.98	6.88	4.88	5.82	6.54	*n.s.*	4.97 c	5.90 b	6.71 a	***	5.98	5.75	*n.s.*
Malic Acid (g/L)	**2017**	*p.*	3.31 a	3.08 ab	3.01 ab	1.93 b	3.06 ab	3.16 ab	*	2.62	3.07	3.08	*n.s.*	3.13	2.73	*n.s.*
	2018	*p.*	3.46	3.75	3.52	3.04	2.73	3.16	*n.s.*	3.25	3.24	3.34	*n.s.*	3.57	2.98	*
	2019	*p.*	1.82	3.42	3.86	2.46	3.43	3.50	*n.s.*	2.14 b	3.43 a	3.68 a	***	3.03	3.13	*n.s.*
Tartaric Acid (g/L)	**2017**	*p.*	4.98	4.47	4.79	5.24	4.45	4.73	*n.s.*	5.04 a	4.46 b	4.76 ab	**	4.75	4.76	*n.s.*
	2018	*p.*	4.97	5.37	4.91	5.13	4.93	4.47	*n.s.*	5.05 a	5.15 a	4.69 b	*	5.08	4.84	*n.s.*
	2019	*p.*	5.05	5.30	5.42	5.31	5.37	5.36	*n.s.*	5.18 b	5.34 ab	5.39 a	*	5.26	5.35	*n.s.*
TPP (mg/g)	**2017**	*p.*	4.98	6.05	6.32	5.94	5.82	6.67	*n.s.*	5.46 b	5.93 ab	6.49 a	*	5.78	6.14	*n.s.*
	2018	*n.p.*	5.00 ab	7.57 a	7.42 a	4.63 b	7.43 ab	6.48 ab	**	4.82 b	7.50 a	6.95 a	***	6.66	6.18	*n.s.*
	2019	*p.*	4.06 d	5.90 abc	6.17 ab	4.78 cd	5.26 bcd	6.85 a	*	4.42 c	5.58 b	6.51 a	***	5.37	5.63	*n.s.*
TAN (mg/g)	**2017**	*p.*	0.97	1.17	1.08	1.12	1.24	1.47	*n.s.*	1.04 b	1.20 ab	1.28 a	*	1.07	1.28	**
	2018	*p.*	0.94	1.26	1.41	1.20	1.33	1.73	*n.s.*	1.07 c	1.29 b	1.57 a	***	1.20	1.42	***
	2019	*p.*	0.84	0.82	1.18	0.81	0.93	1.31	*n.s.*	0.83 b	0.88 b	1.24 a	***	0.95	1.02	*n.s.*

Statistical analysis: p. indicates parametric statistics ($p < 0.05$); n.p. indicates nonparametric statistics ($p < 0.05$). FxI: Interaction between forcing and irrigation effects; FF: Forcing factor; IF: Irrigation factor. Different letters indicate the existence of statistically significant differences between treatments; n.s., not significant; (*), significant at 5 percent level; (**), significant at 1 percent level and (***), significant at 0.1 percent level.

The effect of irrigation strategy was less than that of forcing. In fact, most of the differences were found in 2018, when the lower volume of water applied in RI caused significant decreases in TA and MAL and increases in pH in berries with this treatment compared to those of treatment C. In addition, RI also caused increases in Ant in 2017 and 2018. In all years, RI-F1 and RI-F2 treatments resulted in the highest Ant values, therefore, a synergistic effect of forcing and deficit irrigation should be considered.

V.3.6. PRINCIPAL COMPONENT ANALYSIS (PCA). CLASSIFICATION OF TREATMENTS

Principal component analysis (PCA) was used to classify the different treatments in terms of values of acid (pH, TA, MAL and TAR) and phenolic parameter values (TPP and TAN). The first PCA (Figure V.8a) was performed with the data from the 2017 season. The figure reflects that PC1, 45.06 % of the total variance, is strongly correlated whit pH and Ant (positive and negative side respectively). According to the Figure V.8a, three groups can be distinguished: two of them located at the negative side of PC1 includes F1 and F2 treatments. RI-F1 and C-F1 were associated with the highest concentrations of MAL and F2 treatments with the highest of Ant and TPP values.

The second PCA was performed with the values obtained in 2018. In this year, PC1 correlated whit pH and TA, in positive side discriminated the NF treatments (C-NF and RI-NF) from the rest. It is of note that C treatments were characterized by their values of TA.

Finally, Figure 8c shows that the first two main components (PC1 and PC2) explained 94.13 % of the total variance (84.41 and 9.72 %, respectively) of samples from 2019. RI-F1 and C- F1 and RI-F2 and C-F2 are located on the negative side of PC1 while NF treatments are on the positive side of the same axis. The F2 treatments strongly correlated with TA and TPP.

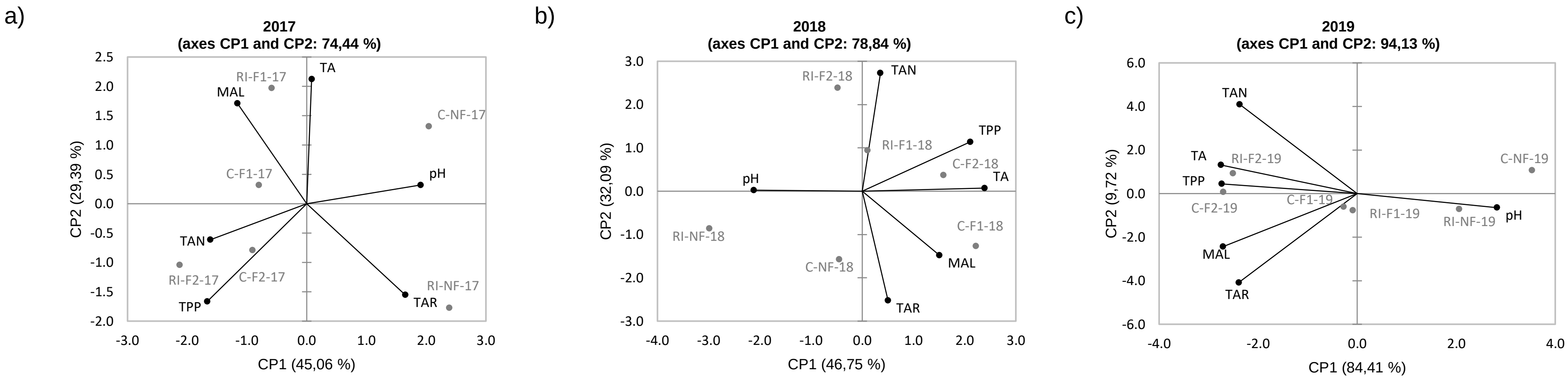

Figure V.8. Principal components analysis on ‘Tempranillo’ grapes composition in basis to forcing (NF, F1 and F2) and irrigarion (C and RI) treatments: a) 2017; b) 2018; c) 2019.

V.4. DISCUSSION

High temperatures during ripening are typical of warm zone viticulture and often have negative effects on some of the desirable characteristics of red wine grapes. Severe water stress causes yield losses and, although it can improve some berry characteristics, it can also lead to an imbalance in grape ripening. With proper irrigation management, it is possible to improve both production and some of these characteristics, although it can also prejudice other traits. Numerous studies have aimed at designing irrigation strategies that make both aspects compatible. Such strategies are usually not universally valid, and need to be adapted to the environment and the variety, often leading to varying results depending on the year. As a result of the work carried out in Extremadura, a strategy of regulated deficit irrigation with a reduction in water supply during pre-veraison is recommended to improve the content of polyphenols and total anthocyanins, yet with low titratable acidity values (Uriarte et al., 2016). Another important aspect is that the application of RI causes faster ripening, which leads to an increase in TSS accumulation and alcoholic strength (Duchêne & Schneider, 2005; Jones, 2005). This study aims to go a step further in the modification of berry characteristics, seeking to offer new oenological possibilities or to alleviate some of the negative consequences of climate change on quality in hot areas.

The starting hypothesis of this work is that shifting grape ripening to a period with more suitable temperatures (lower and with a wider diurnal range) has a positive effect on berry characteristics. The “crop-forcing” technique has been effective in achieving this displacement by lowering daily maximum temperatures during ripening (post-veraison) by 5 to 8 °C, particularly when applied after fruit set (F2). This change has modified berry composition by increasing phenolic compounds, like RI-NF, but reducing pH and increasing titratable acidity relative to this irrigation strategy (Gu *et al.*, 2012; Kishimoto *et al.*, 2022; Martinez-Moreno *et al.*, 2019; Martinez De Toda *et al.*, 2019; Tian & Gu, 2019). The effect of this technique was clearer in the F2 treatment because it caused a greater delay in the period of berry development.

Forcing displaced and modified the conditions of cluster development throughout the berry formation period, both pre-veraison and post-veraison. Their composition at harvest depends on the whole process of berry formation: in the first phase after fruit set, organic acids, tannins and some precursors of phenolic compounds accumulate (Zoccatelli *et al.*, 2013). At this stage, the treatments with “crop-forcing” ripen at higher temperatures, between 5 °C and 6 °C, and with a shortening of the duration of this period. Figure 7 shows that the “crop-forcing” treatments initiate veraison with a higher

TSS content than the non "crop-forcing" treatments. In the post-veraison stage, anthocyanins, compounds that are very sensitive to high temperatures, accumulate. At this time, the treatments with "crop-forcing" are located in a period with significantly lower temperatures. On the other hand, summer pruning causes a shift of the clusters to a higher area of the trellis with greater exposure to solar radiation (*photographs are shown in Figure V.9*). Bergqvist *et al.* (2001) observed that, at high temperatures, excess sun exposure decreases colour in grapes. However, other studies (Poni *et al.*, 2009) point to improvements in colour with increasing exposure as was the case in this study, possibly due to the combination of lower temperatures and higher lighting in post-veraison.

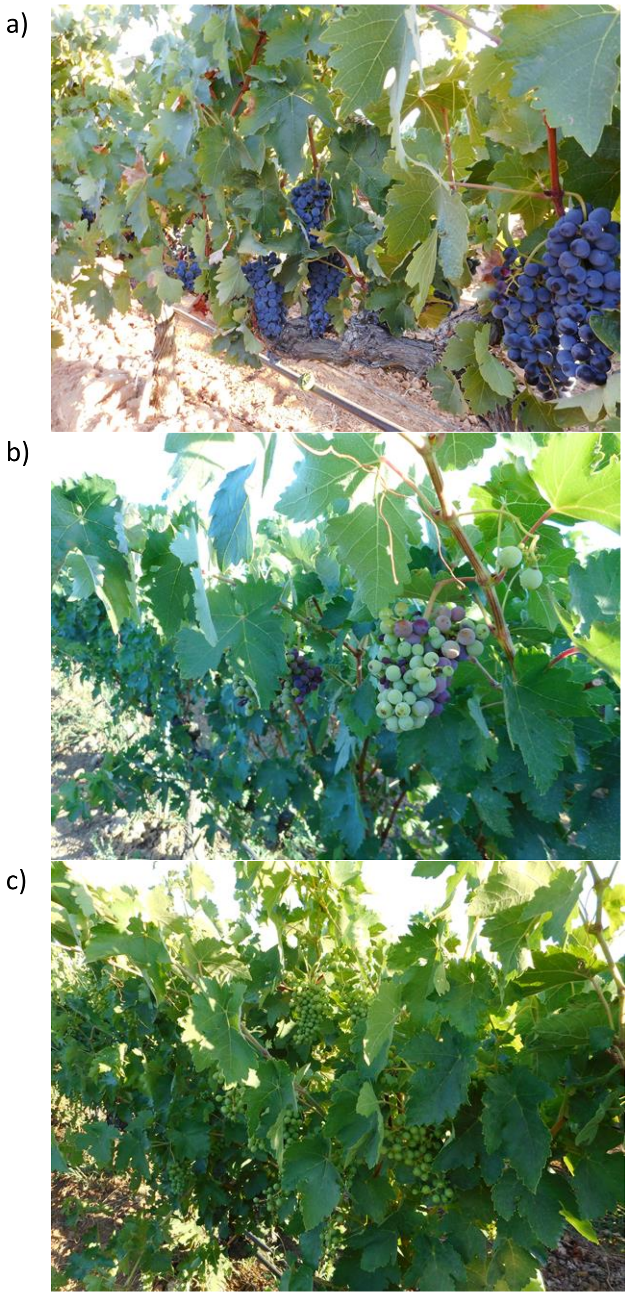

Figure V.9. Photographs of strains of treatments (a) C, (b) F1 and (c) F2 on 11th August 2017.

The increase in phenolic compounds in grapes from deficit irrigation treatments has been attributed in part to the smaller size of the berries, as the proportion of skin and pips (where these compounds are located) increases in relation to the pulp (Ojeda *et al.*, 2002). As we have seen, the "crop-forcing" treatments experienced a clear tendency to reduce berry size, reaching sizes similar to the RI-NF treatment (Figure V.4). However, the dynamics of the different berry compounds throughout ripening were different between RI-NF and "crop-forcing" treatments (Figure V.7), so the smaller berry size was not solely responsible for the increase in phenolic compounds.

According to Sadras & Moran (2012), high temperatures during ripening accelerate the accumulation of sugars, causing a decoupling of phenolic and technological ripeness. The "crop-forcing" technique, although it effectively shifted ripening to a cooler period, did not prolong the duration of ripening and even shortened it in the 2017 season (Figure V.3). The best coupling occurred when changing the dynamics of increasing or decreasing qualitative parameters (Figure V.6). Figure V.7 shows that the ripening process of the forced grapevines manage to improve the coupling between phenolic and technological ripeness, reaching a higher Ant and TPP content (in the three years of application) and TA (in 2018 and 2019) with a lower accumulation of TSS. The improvement of these parameters is greater with forcing (F1 and F2) than with deficit irrigation alone (RI-NF), when compared to the C-NF treatment. The combination of forced and deficit irrigation (RI-F1 and RI-F2) also improves the total anthocyanin content compared to the "crop-forcing" treatments with control irrigation (C-F1 and C-F2), but decreases titratable acidity. Among the different "crop-forcing" dates, the one applied after fruit set, F2, achieves the best results in terms of final fruit quality with a greater displacement of berry formation and ripening.

Although in this work we do not present results for wines made with grapes from forced vines, it is to be expected that, starting with better quality grapes, the vinification process will result in higher quality wines. At the very least, with this technique the enologists are provided with different grapes that increase the possibilities for enological elaborations. In view of the figures shown in Table V.7, F1 and F2 could be harvested at 22 °Brix, in order to aim the harvest toward wines with lower alcoholic strength, obtaining improvements in TAN, TPP and TA content, even when compared to the values obtained in NF at 24 °Brix. These results are reaffirmed with those obtained in the PCA (Figure V.8), where F2 stands out with better results in the content of these compounds. The composition is even more balanced if forced irrigation is combined with deficit irrigation during pre-veraison. These results demonstrate that it is possible to provide winemakers in this area with grapes of the 'Tempranillo' for the production of wines with lower alcoholic strength and higher acidity and total anthocyanin and polyphenol content than with conventional practices. Martínez De Toda *et al.* (2014) managed to delay harvest by two weeks in the 'Garnacha' maintaining the TSS of the unpruned control but with increases in anthocyanin content, while (Santesteban *et al.*, 2017) applied this same technique on the 'Tempranillo' but did not achieve notable increases in anthocyanin concentration, which highlights the importance of adapting this type of technique to the particular grape-growing area.

Although there are few published papers on this technique, in all cases it implies lower production than treatments used as control with conventional pruning practices (Gu *et al.*, 2012; Kishimoto *et al.*, 2022; Martinez-Moreno *et al.*, 2019; Martinez De Toda *et al.*, 2019). This reduction in production also occurs with other viticultural practices aimed at improving berry quality, such as deficit irrigation, cluster thinning or cluster tip removal. As with these other practices, for "crop-forcing" to be of interest, it must demonstrate a significant and interesting effect for winemaking. When compared to an RI, these production differences are smaller, since between C-NF and RI-NF there is a drop in production of between 20 and 40 percent depending on the year. The forced treatments had a more stable production over the three years compared to C-NF. The Table V.4 shows that F2 can reach 3 kg/grapevine, which with a planting layout of 2.5 x 1.2 m, means a production close to the maximum limit authorized by Spanish designations of origin (8000 kg/ha). In an attempt to reduce yield loss, the vines were forced by increasing the crop load (6 buds per shoot). This was intended to increase the number of bunches per vine. The number of bunches per shoot varied throughout the year, and although it was higher in F1 and F2, yields were not similar to those of the non-forced treatments, as the number of berries per bunch and consequently the average weight of the bunches was drastically reduced from 212.3 g in the C-NF treatments compared to 68.1 g and 77.7 g observed in F1 and F2 ($p>0.001$). The basal inflorescences usually forms the most flowers, and numbers decrease for higher inserted inflorescences (Vasconcelos et al., 2009) and elevated temperatures either before or after budburst reduces the flower number (Petrie & Clingeleffer, 2005) what happens when budburst occurs after forcing pruning. Likewise, fruit set can be negatively affected by temperatures above 32°C (Kliewer, 1977), but also under conditions of high light intensity (due to its impact on the current supply of photoassimilates) as reported by Friend (2005). Both high temperature and high light intensity conditions occur during the flowering and fruit set process with the phenology shift in forced vines and could be responsible for the lower number of berries per bunch observed in this study.

In order to minimize the loss of production caused by green pruning, several authors have proposed a double harvest (Martinez De Toda, 2021; Poni *et al.*, 2020). With this technique, it is possible to obtain a higher production with the quality benefits of green pruning. However, the feasibility of this proposal will depend on the short- and mid-term on the vineyard, as well as cost overruns and harvest difficulties.

Unlike previous studies with this same technique (Gu *et al.*, 2012; Martinez-Moreno *et al.*, 2019; Martinez De Toda *et al.*, 2019; Tian & Gu, 2019), in this study, the “crop-forcing” treatments were maintained on the same grapevines throughout the three years. In 2019, the third consecutive year of application of the treatments on the same grapevines, is when the greatest differences in grape composition between the “crop-forcing” and “non-crop-forcing” treatments were observed. These results agree with those of (Kishimoto et al., 2022), who studied the effect of two types of “crop-forcing” application in two consecutive years on the same grapevines of ‘Merlot’, in Japan. The greater differences obtained in 2019 could either be a cumulative effect or coincide with the year in which there are greater differences in production between NF and F treatments, since the greatest differences in temperatures during ripening correspond to 2018, which is when the harvests in F1 and F2 were most delayed in relation to NF. Higher yields can decrease berry quality due to a dilution effect (Ojeda *et al.*, 2002). Gu *et al.* (2012) and Tian & Gu (2019) achieved an increase in TPP, Ant and TA content in the first year of application of this technique for ‘Cabernet Sauvignon’ for the same degree of TSS accumulation (24 °Brix). In 2019, Martinez De Toda *et al.*, got similar results for ‘Garnacha’, ‘Tempranillo’ and ‘Maturana Tinta’, with a significant improvement also in malic and tartaric acid content, and with a difference in TSS between unforced (22 °Brix) and forced (20 °Brix) grapes. In a preliminary study in 2017, an increase in TPP, Ant and TA content in ‘Tempranillo’ grapes from forced vines at harvest for the same TSS value, involved an increase in TPP, Ant and malic acid content in wines from those same vines (Lavado et al., 2019). In this study, an improvement in TPP and Ant content has been achieved from the first year of application for ‘Tempranillo’ at 22 °Brix. However, the TA content did not improve until the second year of application on the same grapevines. Malic acid content improved in 2019, as did tartaric acid and only in the case of “crop-forcing” applied after fruit set.

Although the results obtained with the forced pruning technique may be interesting from the point of view of the characteristics of the berries, it should be taken into account that its application would only be recommended for areas that allow the development cycles of the vine to be extended, those where, after the harvest date, there are sufficient climatic conditions for the vine to continue its activity for a few more months. On the other hand, the varieties should not be long-cycle varieties, which naturally ripen during the coolest periods of the season. It is also necessary to previously evaluate the optimum moment for forced pruning in each variety, so that the fruiting buds are already developed, but with enough time to complete ripening before the leaves start to fall. The application of RI together with "crop-forcing" maintained or improved berry characteristics and production compared to the respective irrigated treatment, so the combined use of both techniques is recommended to improve water use efficiency while maintaining compositional benefits. An important aspect to take into account for the application of this technique is the higher water consumption observed. Although ETo was lower in treatments F1 and F2 during the period analysed, the volume of water consumed was higher than in NF (Table V.2). This effect was due to the fact that the canopies were more active during the summer period of higher evapotranspirative demand (July to September) with young leaves with a higher photosynthetic rate compared to NF canopies (Oliver-Manera *et al.*, 2022; Poni *et al.*, 1994). In addition, the intercepted fraction of photosynthetically active radiation (PAR) was equal or higher in the case of C-F1 compared to C-NF (data not shown), which increases the water requirements of the vine (Picón-Toro *et al.*, 2012). All this indicates that in areas with a lack of rainfall during the summer months, this technique is probably not recommended in rainfed conditions.

V.5. CONCLUSIONS

Under the conditions in which the "crop-forcing" experiment was carried out, it was an effective technique for delaying grape ripening to lower temperature periods, particularly when applied after fruit set. This technique limits vineyard yields but manages to improve phenolic content, anthocyanin content and total berry acidity, with a lower total soluble solids content, thus restoring the coupling between phenolic and technological ripeness. When "crop-forcing" is carried out with water limitation during pre-veraison increases the anthocyanins content of the grapes of 'Tempranillo' while improving water use efficiency with respect to the application of "crop-forcing" without water limitations.

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VI. CAPÍTULO 3.

EVALUATION OF THE CARRY-OVER EFFECT OF THE "CROP-FORCING" TECHNIQUE AND WATER DEFICIT IN GRAPEVINE 'TEMPRANILLO'

Este capítulo está enviado a la revista: *Agronomy*

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VI. ABSTRACT

“Crop-forcing” is an effective technique to delay grape maturation to a period of lower temperatures and in this way improve grape quality. Because of the aggressiveness of this technique (removal of leaves and fruit to reinitiate a second vegetative cycle), it may affect the level of reserves and could provoke progressive vine exhaustion. The aim of the present work is to evaluate the short- and medium-term evolution of carbohydrate reserves in different plant organs and the effect of “crop-forcing” under different irrigation regimes on seasonal biomass production and its distribution. The study was carried out over a four years period (2017-2020), applying “crop-forcing” in three consecutive years (2017-2019) to the same vines on two different dates and using two irrigation strategies. The application of “crop-forcing” did not decrease root reserve levels in either the year of application or the following year, but did modify starch and soluble sugar levels in shoots and leaves in some moments of the vegetative cycle during the years of “crop-forcing” application. Total biomass production in terms of grams per vine was lower in the “crop-forcing” treatments and continued to be so when “crop-forcing” was no longer applied. The percentage of biomass in vegetative organs increased at the expense of productive organs.

VI. RESUMEN

El “forzado de yemas” es una técnica efectiva para retrasar la maduración de las uvas hasta un periodo con temperaturas más bajas y mejorar así su calidad. Debido a la “agresividad” de esta técnica (eliminación de hojas y frutos para reiniciar un segundo ciclo vegetativo), puede afectar al nivel de reservas y podría provocar un agotamiento progresivo de las cepas. El objetivo de este trabajo es evaluar la evolución de las reservas de carbohidratos en distintos órganos a corto y medio plazo, así como el efecto sobre la producción estacional de biomasa y su distribución en cepas sometidas al “forzado de yemas” bajo diferentes regímenes hídricos. El estudio se realizó durante cuatro años (2017 – 2020), aplicando el “crop-forcing” en tres años consecutivos sobre las mismas cepas (2017 – 2019) en dos fechas diferentes y junto a dos estrategias de riego. La aplicación del “forzado de yemas” no disminuyó los niveles de reservas en las raíces, ni durante su aplicación ni en el año siguiente, aunque sí modificó los niveles de almidón y azúcares solubles en sarmientos y hojas en algunos momentos del ciclo vegetativo durante los años de aplicación del forzado. La distribución de biomasa total también fue menor con, incluso al dejar de aplicar el forzado. El porcentaje de biomasa en órganos vegetativos se incrementó a costa de los productivos.

VI.1. INTRODUCTION

The “crop-forcing” technique involves the application of a severe pruning when the vegetative period has begun and the fruit-bearing buds of the following year are already formed. In this way, the cycle of the vines from budbreak is reinitiated with a consequent delay of the ripening cycle and transformation of the biannual reproductive cycle into an annual one. This technique was proposed by Gu et al. (2012) and gave interesting results, delaying harvest to a period of more favourable temperatures. It has been proposed as an interesting option for vineyards in warm regions and as a strategy to combat the loss of grape quality which the extreme temperatures of global warming will cause (Gu et al., 2012; Kishimoto et al., 2022; Martínez-Moreno et al., 2019; Martinez De Toda et al., 2019).

In Extremadura (Spain), the harvesting of ‘Tempranillo’ takes place from the end of August to the beginning of September. The application of “crop-forcing” at the pea size phenological stage has enabled grape maturation to be delayed to a time of milder temperatures and harvesting to mid-October, increasing total acidity and total polyphenol and anthocyanin content but with a fall in yield (Lavado et al., 2019; Martinez De Toda et al., 2019).

Although good results have been observed in grape quality using this technique, the modification to the vegetative cycle caused by the “crop-forcing” technique may have an impact on the seasonal carbon balance and hence on grapevine reserve levels. Each year the vines have to initiate two reproductive cycles, an unfinished one that is interrupted with the forcing and one that takes place after the forcing. Non-structural carbohydrates (NSCs) are the energy source of the vine, composed of photassimilates synthesised in leaves (soluble carbohydrates) and reserves accumulated in the perennial woody organs of the previous year (starch) (Lebon et al., 2008; Loescher et al., 1990; Mullins et al., 1992; Zapata et al., 2001). Thanks to the exchange of gases that takes place at leaf level throughout the canopy, these compounds are formed by photosynthesis through a reversible reaction, and are the source of energy required for vine growth in the moments when photosynthesis is not carried out.

$$n\,CO_2 + 2n\,H_2O + energy \rightleftharpoons (CH_2\,O)_n + n\,O_2 + n\,H_2O$$

Glucose and fructose are the main products of photosynthesis and serve as raw material for the synthesis of sucrose and starch. Glucose and fructose synthesise sucrose in the leaves, which is transported to the rest of the plant as an energy source for growth (Mullins et al., 1992). The synthesis of starch commences when the synthesis of sucrose exceeds the amount that the vine can transport. Regulation of the formation of sucrose and starch depends on the ambient conditions and changes in the source-sink ratio (Mullins et al., 1992).

Starch is the main carbohydrate stored in the vine (Mullins et al., 1992), and is found in the roots and trunk (Köse & Ateş, 2017; Pellegrino et al., 2014; Zapata et al., 2004b). This carbohydrate reserve is restored in the perennial organs through photosynthesis which extends from flowering to leaf senescence (Candolfi-Vasconcelos et al., 1994; Chaumont et al., 1994). Starch accumulation in the previous campaign is essential for the mobilization of reserves that act as 'source' until photosynthesis commences (Smith & Holzapfel, 2009; Zapata et al., 2004a). After budbreak, the vine grows rapidly thanks to the energy provided by carbohydrates mobilised from the perennial organs during the beginning of spring (Conradie, 1980; Hale & Weaver, 1962; Kriedemann & Kuewer, 1970; Scholefield et al., 1978; Weyand & Schultz, 2006), with the accumulation of carbohydrates therefore slow in the weeks following budbreak. When the leaves are sufficiently mature the growth energy of the vine is based on photosynthesis, coinciding with the end of spring and beginning of summer, which is the period when the "crop-forcing" technique is applied. With this pruning, all leaves, inflorescences and part of the shoots are removed, as therefore are also the reserves mobilised up to that moment from the roots and trunk to these organs. The vine has to restart the 'forced' budbreak under low carbohydrate availability, which explains the decrease in vigour of the campaign in course that could have a cumulative effect in following campaigns (Poni et al., 2020). However, it has been shown in warm climates that the greatest accumulation of carbohydrates takes place in the post-harvest period (Bennett et al., 2005; Vaillant-Gaveau et al., 2014). Application of the "crop-forcing" technique might therefore modify grapevine carbohydrate accumulation as harvest is delayed in time to senescence, although very little has been studied about this effect.

Agricultural practices can also modify the accumulation of reserves. Water stress reduces the rate of photosynthesis and can modify the distribution of assimilates in the vine. Differences between cultivars in their response to stress have been reported in the literature. Whereas water stress did not induce a diminution of carbohydrate reserves in 'Garnacha' and 'Semillon' (Rogiers et al., 2011), this was not the case in 'Shiraz' (Holzapfel et al., 2010). It can be assumed that aspects such as meteorological conditions and the moment and intensity of the water stress can also influence its impact on grapevine reserves.

Use of the “crop-forcing” technique has been proposed in previous studies in combination with the application of a pre-veraison water deficit with the aim of achieving better results in terms of grape quality (Lavado, Prieto, et al., 2023) (unpublished manuscript) and reducing the amount of irrigation water used. The aim of the present study is to analyse the short- and medium-term impact of “crop-forcing” with and without the adoption of a controlled deficit irrigation strategy on the concentration of carbohydrates in the same vines over the course of the annual cycle and for three consecutive years.

VI.2. MATERIALS AND METHODS

VI.2.1. LOCATION, DESCRIPTION OF THE VINEYARD AND WEATHER CONDITIONS

The study was carried out in an experimental vineyard located at Badajoz, Extremadura, Spain (38º 51´ N; 6º 40´ W; 198 m) in a ‘Tempranillo’ vineyard (Vitis vinifera L.) grafted on Richter 110 rootstock, trained as bilateral cordons in a vertical trellis system with a drip irrigation system of 8 L/h per vine. The vines were 16 years old at the beginning of the study and the cultivation practices were the usual ones in the area (besides the experimental treatments). All the vines were winter pruned to six spurs and two buds per spur. The rows are E-W oriented and row and vine spacing were 2.5 m and 1.2 m, respectively. In addition to winter pruning, the number of shoots per vine was adjusted manually in the spring (12 shoots per vine). The study was initiated in 2017, which was the year in which the first “crop-forcing” was applied, which was repeated in 2018 and 2019. In 2020, only conventional pruning was carried out to evaluate the cumulative effect of this practice on the plants.

The agrometeorological data were obtained from La Orden weather station (100 m from the vineyard) which forms part of the Agroclimatic Information System for Irrigation (SIAR by its initials in Spanish), with the characteristics described in Martí et al. (2015).

VI.2.2. TREATMENTS AND EXPERIMENTAL DESIGN

The experimental design was a split plot with four replications (Table VI.1). The main factor was pruning with three treatments. Two of the treatments involved “crop-forcing” on different dates. In the F1 treatment, “crop-forcing” was applied three days after anthesis (May 18, 2017; May 29, 2018; May 20, 2019) and in the F2 treatment 22 days after anthesis. Both treatments were compared with a treatment without forcing techniques (NF) with vines grown under conventional practices (just winter pruning). “Crop-forcing” consisted of hedging the growing shoots to seven nodes and removing all the summer laterals, leaves and clusters with scissors to force the bursting of the primary buds developed in the current season. The secondary factor was the irrigation strategy. Two treatments were assigned to the subplots: good water status (C), supplying water to maintain a midday stem water potential (SWP) close to -0.6 MPa, and a pre-veraison deficit irrigation treatment (RI) supplying water to reach a maximum SWP of -1.1 MPa and -0.8 MPa in post-veraison. The experimental field design was kept intact for the first three years and in 2020 only the irrigation treatments were applied.

Table VI.1. Summary of the treatments applied in the study

	No "crop-forcing" (NF)	**Early "crop-forcing" (F1)**	**Late "crop-forcing" (F2)**
Full irrigation (C)	C-NF	C-F1	C-F2
Deficit irrigation (RI)	RI-NF	RI-F1	RI-F2

Vine water requirements were calculated based on the crop evapotranspiration (ETc) using the crop coefficient (Kc) recommended by FAO for these latitudes for the NF treatments. For the F1 and F2 treatments, ETc was calculated directly on a weighing lysimeter (Picón-Toro et al., 2012) integrated in the study plot, using two F1 vines. Irrigation started when a threshold SWP value of -0.6 MPa was reached. Irrigation was applied five to six times per week, measuring the amount of water applied to each subplot with volumetric water meters and maintaining irrigation until the beginning or middle of October. The meteorological data were obtained from a weather station belonging to the Extremadura irrigation advisory network (REDAREX) which was located 100 m from the plot. The experimental unit comprised 6 rows per 18 vines. The ten central vines of the four central rows were used for sampling and harvested.

VI.2.3. VINE PHENOLOGY AND WATER STATUS

A phenological assessment was performed weekly according to the modified E-L system (Coombe, 1995). A visual inspection was made of ten plants per plot starting from mid-March ('cotton bud' stage), to determine the most representative growth stage (the stage shown by at least 50% of vines), as well as the most backward and the most advanced stages in the sample.

The SWP was measured at noon with a pressure chamber (Soil Moisture Corp., Model 3500, Santa Barbara, CA, USA), following the procedure described by Martí et al. (2015) using leaves on the north side of the trellis (in the shade) close to trunk level and wrapped in aluminium foil at least 2 h before data recording. Measurements were taken weekly on one leaf per vine and in two plants per subplot.

VI.2.4. PRODUCTION OF BIOMASS

Yield dry weight - Vines were harvested by hand when the mean of the four replicates of each treatment reached total soluble solids (TSS) content of 23-24 °Brix (a common harvest criterion for this variety in this area). NF vines were harvested on 22 August 2017, 27 August 2018, 27 August 2019 and 5 August 2020. F1 vines were harvested on 12 September 2017, 8 October 2018, 30 September 2019 and 5 August 2020. F2 vines were harvested on 17 October 2017, 29 October 2018, 15 October 2019 and 5 August 2020. Clusters were cut, counted and weighed from a total of 10 vines per experimental plot.

Leaf dry weight - Leaf area per vine was determined at harvest by multiplying the number of shoots per vine by the average leaf area of the shoot (LAS). LAS was estimated by measuring the area of all leaves of 4 shoots per experimental plot with a leaf area meter (LI-3100C, LI-COR Bioscience, Lincoln, NE). Leaf dry weight per vine was then estimated from the leaf area to dry weight ratio (dry weight (g) = 0.0089 leaf area (cm^2)-1.651; r^2=0.95) calculated for this variety under the same trial conditions. Leaf dry weight was estimated for 10 vines per experimental plot.

Dry weight of the pruning interventions - Ten vines per experimental plot were selected and dry weight was determined in the different interventions carried out: winter pruning, green pruning and forcing pruning only in the case of forced vines.

For all dry weight determinations, a forced ventilation stove was used to dry the samples at 65 ºC until they reached a constant weight. A precision balance (Sartorius Mechatronics BP61S, Göttinger, GER) with a sensitivity of 0.01 g was used to determine the dry weight.

VI.2.5. SOLUBLE SUGAR EXTRACTION AND STARCH DIGESTION

Root, shoot and leaf samples were collected from ten plants per subplot at different moments of the annual vine cycle. Root samples were collected in the 2017/2018, 2018/2019, 2019/2020 and 2020/2021 winters, and were washed with paper soaked in cold distilled water. Shoot samples were collected in the periods of winter pruning (2017, 2018 and 2019), bleeding sap (2019 and 2020), application of “crop-forcing” (2018 and 2019 only in the F1 and F2 treatments) and harvest (2018, 2019 and 2020). Leaf samples were collected in the periods of application of “crop-forcing” (2018 and 2019 only in F1 and F2 treatments), harvest (2018, 2019 and 2020) and leaf fall (2019 and 2020). A forced ventilation stove was used to dry the samples at 65 ºC, and the samples were kept there until they reached a constant weight. Once dry, they were crushed to a fine powder (MF 10, Ika, Staufen, Germany). A sample of 50 mg was added to 10 mL of ethanol 80% and macerated for 10 minutes at 80 ºC in a water bath (WNE, Memmert, Schwabach, Germany) and centrifuged for 5 minutes at 3000 rpm (5810, Eppendorf, Hamburg, Germany). The supernatant was separated and the resulting pellet was extracted up to three times. The supernatants (soluble sugar extracts) were then combined and stored at -20 ºC.

The extraction of soluble sugars was carried out using a methodology based on a previous work (Landhäusser et al., 2018). A 5 mL aliquot of soluble sugar extract was dried and redissolved in 2 mL of H_2Od for 5 minutes in an ultrasonic bath (USC-TH, VWR, Radnor, USA). The sample was macerated for 10 minutes at 80 ºC in a water bath (WNE, Memmert, Schwabach, Germany) and centrifuged for 5 minutes at 3000 rpm (5810, Eppendorf, Hamburg, Germany). Determination of soluble sugar content from extracts (%Glu-Fru) was carried out using an autoanalyzer (Y15, Biosystems, Barcelona, Spain). Two extractions were performed for each sample of a given plot and sampling date.

The extraction of starch was carried out using a methodology based on a previous work (Quentin et al., 2015). Extracted sugar pellets were dried for 24 h (65 ºC). 4 mL of sodium acetate (pH 4.6) was added and the mixture was macerated for 60 minutes at 100 ºC (WNE, Memmert, Schwabach, Germany). The sample was cooled in a water-ice bath for 10 minutes. 1 mL of α-amylase was added and homogenized (ZX3, VELP, Monza and Brianza, Italy), macerated for 30 minutes at 85 ºC and centrifugated for 5 minutes at 3000 rpm (5810, Eppendorf, Hambug, Germany). An aliquoted of 100 µL was added 500 µL of α- amyloglucosidase and homogenized (ZX3, VELP, Monza and Brianza, Italy) and macerated for 30 minutes at 60ºC. Determination of starch content from the extracts (%Starch) was carried out using an autoanalyzer (Y15, Biosystems, Barcelona, Spain). Two extractions were performed for each sample of a given plot and sampling date.

VI.2.6. STATISTICAL DATA ANALYSIS

Normality and homogeneity of variances were checked using the Shapiro-Wilk and Bartlett tests respectively. When normality and homogeneity of variances were verified, the data were subjected to a multivariate analysis of variance (MANOVA) to investigate the effect of “crop-forcing”, “irrigation” and their interaction on each parameter evaluated, selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons. The interaction between effects was evaluated by calculating the least-squares means (LS means), selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons and the Tukey test as post hoc test for parametric samples. When normality and homogeneity of variance were not verified, non-parametric tests were carried out using the Kruskal-Wallis test (alternative to one-way ANOVA) and multiple comparison p values (alternative to post-hoc pairwise comparisons). Differences between means were considered statistically significant when $p < 0.05$. These statistical tests were performed with XLSTAT-Pro 201610 (Addinsoft, 2009, Paris, France).

The evolution data were statistically analysed (SPSS Inc., Chicago, IL) using the Shapiro-Wilks test as the test of normality, Levene’s test as the test of homogeneity of variances, with the one-factor ANOVA and the Tukey-B test and the t-Student test as parametric test, and Kruskal-Wallis’s test and multiple comparison p values and the Mann–Whitney U Test as nonparametric test. Differences between means were considered statistically significant when $p < 0.05$.

VI.3. RESULTS

VI.3.1. CLIMATOLOGY, PHENOLOGY AND WATER STATUS

Figure VI.1 shows the seasonal evolution of maximum, minimum and mean temperature and monthly rainfall over the course of the 4 years of the study. Although the temperature pattern was similar in all years, 2018 saw the most spring and autumn rainfall of the three years in which “crop-forcing” was applied as well as the highest temperatures in August. The year of highest rainfall was 2020.

While irrigation strategy did not modify vine phenology, “crop-forcing” did (Table VI.2). The duration of the vegetative cycle reinitiated with the pruning in F1 and F2 was shortened in the three years of “crop-forcing” application, with all stages of the vegetative cycle reduced except for the veraison-to-harvest period in 2018. The shift in the growth cycle caused an increase in temperature in the initial stages of the new crop cycle, but the mean values were lower during ripening (veraison to harvest). Overall, mean, maximum and minimum temperatures from budbreak to harvest were higher in the treatments subjected to “crop-forcing” compared to those with conventional pruning (Figure VI.1).

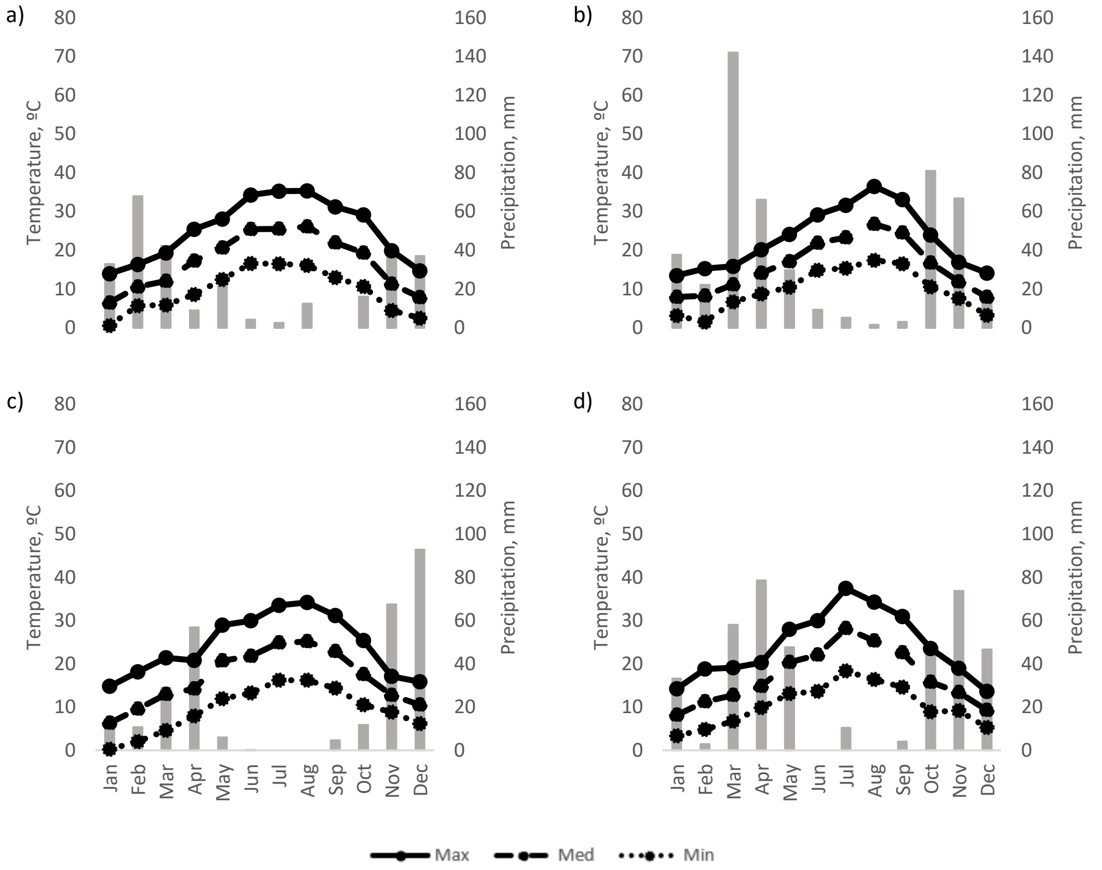


Figure VI.1. Temperatures (lines), and rainfall (bars) during: a) 2017; b) 2018; c) 2019 and d) 2020.

Table VI.2. Day of year of the different phenological states from budbreak to leaf fall in the different pruning treatments. Each pruning treatment represents the two irrigation treatments as no differences were found between them.

	2017			**2018**			**2019**			**2020**		
	NF	**F1**	**F2**	**NF**	**F1**	**F2**	**NF**	**F1**	**F2**	**NF**	**F1**	**F2**
Budbreak	93	93	93	93	93	99	85	90	91	78	78	78
“Crop-forcing”		138	157		149	168		140	154			
Flowering	136	177	194	145	191	204	133	182	189	133	133	133
Fruit set	150	187	201	156	204	214	140	189	200	141	141	141
Veraison	187	221	256	204	242	261	200	238	253	195	195	195
Harvest	234	257	292	241	282	302	240	274	283	218	218	218
Leaf fall	322	322	322	295	310	310	295	308	308	287	287	287

Table VI.3 shows average SWP in each period of the growth season. In the first three years, all the “crop-forcing” treatments maintained SWP values between -0.45 and -0.73 MPa, indicating an absence of water stress before application of “crop-forcing”. Considering the entire vegetative cycle, the mean SWP values of the C treatments ranged between -0.41 and -0.82 MPa, with values for the C-F1 treatment between -0.45 and -0.89 MPa and for the C-F2 treatment between -0.42 and -0.79 MPa. The RI treatments supported a moderate water deficit in pre-veraison. The mean SWP of RI-NF was -1.00 MPa in this period, while for RI-F1 and RI-F2 the values were above -0.90 MPa in 2017 and 2018 and in 2019 did not exceed -0.80 MPa. In 2020, only very slight differences were found between the water status of the different treatments, despite maintaining the two irrigation strategies (C and RI), due to the abundant rains.

Table VI.3. Average midday stem water potential (MPa) throughout the different phenological stages in 2017, 2018, 2019 and 2020. BB= Budbreak; CFP=“Crop-forcing” Pruning; F= Flowering; FS= Fruit Set; V= Veraison; H= Harvest; PostH= Postharvest.

		C-NF	RI-NF	C-F1	RI-F1	C-F2	RI-F2
2017	**BB-CFP**	-	-	-0.56	-0.52	-0.63	-0.62
	CFP-F	-0.49	-0.56	-0.64	-0.6	-0.55	-0.69
	F-FS	-0.64	-0.72	-0.61	-0.63	-0.79	-1.03
	FS-V	-0.61	-1.12	-0.89	-1.06	-0.74	-0.90
	V-H	-0.82	-1.24	-0.86	-0.98	-0.58	-0.71
	PostH	-0.7	-1.01	-0.63	-0.7	-0.42	-0.50
2018	**BB-CFP**	-	-	-0.45	-0.45	-0.49	-0.51
	CFP-F	-0.41	-0.48	-0.51	-0.52	-	-
	F-FS	-0.41	-0.48	-0.56	-0.57	-0.62	-0.78
	FS-V	-0.49	-1.00	-0.67	-0.98	-0.61	-0.96
	V-H	-0.61	-0.92	-0.65	-0.87	-0.59	-0.67
	PostH	-0.59	-0.67	-0.45	-0.51	-0.47	-0.46
2019	**BB-CFP**	-	-	-0.52	-0.64	-0.64	-0.73
	CFP-F	-	-	-0.69	-0.73	-	-
	F-FS	-0.65	-0.77	-0.61	-0.62	-0.58	-0.58
	FS-V	-0.70	-1.06	-0.68	-0.74	-0.64	-0.78
	V-H	-0.75	-0.78	-0.68	-0.71	-0.62	-0.77
	PostH	-0.74	-0.77	-0.60	-0.66	-0.54	-0.67
2020	**BB-CFP**	-	-	-	-	-	-
	CFP-F	-	-	-	-	-	-
	F-FS	-0.55	-0.45	-0.48	-0.5	-0.47	-0.45
	FS-V	-0.63	-0.68	-0.61	-0.71	-0.61	-0.71
	V-H	-0.65	-0.75	-0.66	-0.62	-0.69	-0.73
	PostH	-0.67	-0.69	-0.66	-0.65	-0.66	-0.68

VI.3.2. STARCH CONTENT IN VEGETATIVE ORGANS

VI.3.2.1. Roots

Figure VI.2 shows root starch concentration during winter dormancy in the 4 years from 2017/2018. There were significant differences between years, with the lowest concentrations in 2020/2021 and the highest in 2019/2020. However, in each year all the treatments had similar values, with no differences between pruning treatments for the same irrigation treatment (Figure VI.2a and VI.2b) or between irrigation treatments for the same pruning treatment (data not shown).

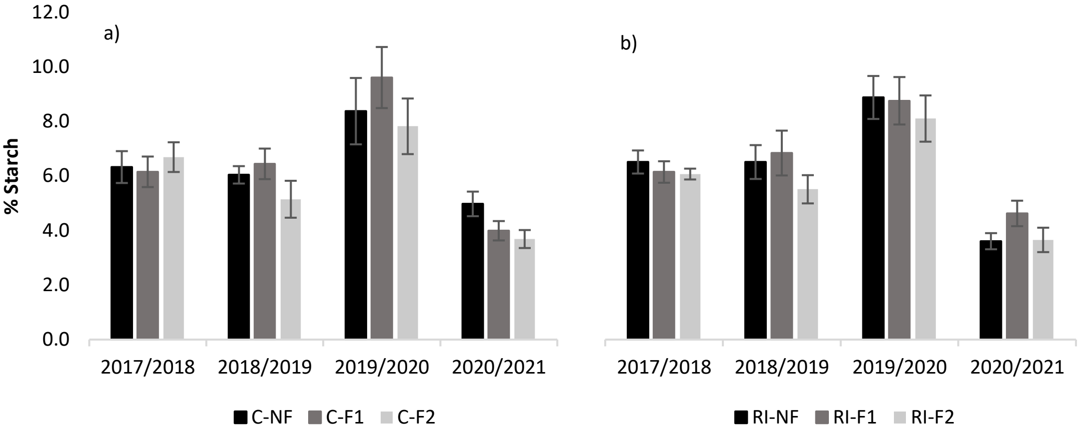


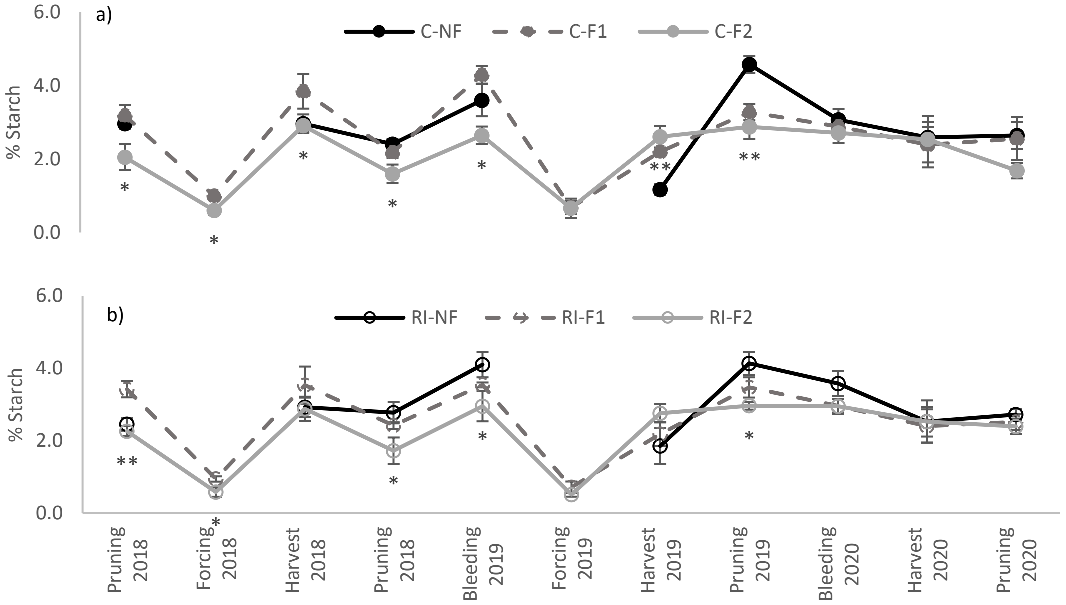
Figure VI.2. Percentage of starch in relation to dry weight (%Starch) in roots during the winter dormancy period: a) in C treatments C; b) in RI treatments. Bars represent the standard error of the mean. Statistical analysis: ANOVA and Tukey test as parametric test and Kruskal-Wallis test as nonparametric test (both p < 0.05).

VI.3.2.2. Shoots (winter pruning)

Starch content in shoots pruned in winter was higher in 2019 than in the other years, as was also the case for roots (Table VI.4). No significant interaction was observed between forcing effect and irrigation. As can be seen in Table VI.4, the lowest winter pruning starch levels were found in F2. The highest shoot starch content in 2017 was in F1, and in 2018 and 2019 in NF. In 2020, the results were the same for all three treatments. At harvest, the results were different in each year, with the highest content in 2018 corresponding to F1. In 2019, the most noteworthy result was the low starch content of NF, while when no “crop-forcing” was applied in 2020 there were no differences between treatments. No effect was observed of irrigation strategy on shoot starch content in winter or at harvest (Table VI.4).

Figure VI.3 shows the evolution of shoot starch content in different moments of the vegetative cycle from the winter pruning of 2018 to that of 2020. Starch content decreased from its highest levels in the winter pruning and after budbreak (moment of bleeding sap), with the lowest values corresponding to the samples collected in the “crop-forcing” pruning and with a subsequent recovery such that concentrations at harvest were similar to those of the winter pruning. The general pattern was similar in all treatments, although the F2 treatments had lower starch levels from the 2018 pruning to the 2019 “crop-forcing”. The NF and F1 treatments had similar concentrations throughout the samplings, except in the 2019 pruning when NF had a higher concentration. In 2020, all treatments showed similar starch concentrations (Table VI.4).

Differences between the different irrigation treatments for the same pruning treatment were only observed in NF in the 2018 winter pruning, in F1 in the 2019 bleeding sap (both with higher values in C) and in F2 in the 2020 pruning (with higher values in RI).



NF	*		n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.
F1	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
F2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*

Figure VI.3. Evolution of shoot starch percentage (%Starch) in: a) C treatments; b) RI treatments. Bars represent the standard error of the mean. The table indicates statistically significant differences between irrigation treatments for the same pruning treatment. Statistical analysis: ANOVA and the Tukey-B test and t-Student test as parametric test; Kruskal-Wallis's test and multiple comparison p values and Mann–Whitney U test as nonparametric test. Differences between means were considered statistically significant when $p < 0.05$.

Table VI.4. Shoot starch percentage during winter pruning and at harvest.

	Year	*Statistical analysis*	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2	F x I	Forcing			Forcing factor	Irrigation		Irrigation factor
										NF	F1	F2		C	RI	
Pruning Shoots	**2017**	***p.***	3.0	3.2	2.0	2.5	3.4	2.3	n.s.	2.7 b	3.3 a	2.2 c	***	2.7	2.7	n.s.
	2018	***p.***	2.4	2.2	1.6	2.8	2.4	1.7	n.s.	2.6 a	2.3 a	1.7 b	**	2.1	2.3	n.s.
	2019	***p.***	4.6	3.3	2.9	4.1	3.5	3.0	n.s.	4.4 a	3.4 b	2.9 b	***	3.6	3.5	n.s.
	2020	***p.***	2.6	2.5	1.7	2.7	2.5	2.4	n.s.	2.7	2.5	2.0	n.s.	2.3	2.5	n.s.
Harvest Shoots	**2018**	***n.p.***	3.0	3.8	2.9	2.9	3.5	2.9	n.s.	3.0 ab	3.7 a	2.9 b	*	3.2	3.1	n.s.
	2019	***p.***	1.2	2.2	2.6	1.9	2.2	2.8	n.s.	1.5 b	2.2 a	2.7 a	**	2.0	2.3	n.s.
	2020	***p.***	2.6	2.4	2.5	2.5	2.4	2.5	n.s.	2.6	2.4	2.5	n.s.	2.5	2.5	n.s.

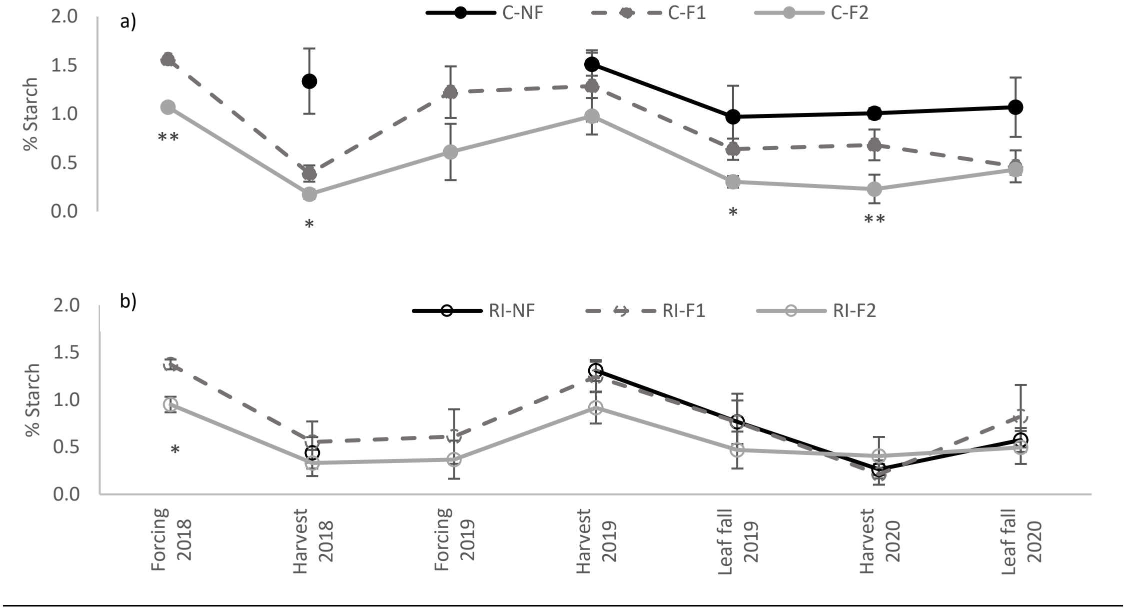
Treatments: C-NF (no forcing and full irrigation); C-F1 (forcing applied between flowering and fruit set and full irrigation); C-F2 (forcing applied between fruit set and pea size and full irrigation); RI-NF (no forcing and deficit irrigation); RI-F1 (forcing applied between flowering and fruit set and deficit irrigation) and RI-F2 (forcing applied between fruit set and pea size and deficit irrigation).

Statistical analysis: p. indicates parametric statistics ($p < 0.05$); n.p. indicates nonparametric statistics ($p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VI.3.2.3. Leaves

As can be seen in Figure VI.4, evolution of leaf starch content was similar in all treatments although it was more stable in the case of C-NF along the different samplings. While certain seasonal variations were observed in the rest of the treatments, the same pattern was not repeated in the three years the samples were taken. In 2018 and 2020 the levels decreased at harvest, but not in 2019. In the absence of water stress, C-NF maintained high levels at all times and C-F2 the lowest levels, with intermediate values in F1. In RI, the three treatments maintained the same values, although there was a slight tendency to lower values in RI-F2. This tendency was not observed in 2020, when no “crop-forcing” was applied.

Differences between irrigation treatments for the same pruning treatment were observed in the 2018 harvest in NF, in the 2019 leaf fall in F1 and in the 2020 harvest in NF and F1. In all cases, the highest values were in C.



NF		*		n.s.	n.s.	***	n.s.
F1	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
F2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Figure VI.4. Evolution of leaf starch percentage (%Starch) in: a) C treatments; b) RI treatments. Bars represent the standard error of the mean. The table indicates statistically significant differences between irrigation treatments for the same pruning treatment. Statistical analysis: ANOVA and the Tukey-B test and t-Student test as parametric test; Kruskal-Wallis’s test and multiple comparison p values and Mann–Whitney U test as nonparametric test. Differences between means were considered statistically significant when $p < 0.05$.

VI.3.3. SOLUBLE SUGAR CONTENT IN VEGETATIVE ORGANS

VI.3.3.1. Roots

Figure VI.5 shows the percentage of soluble sugars in roots during the winter dormancy period for the four study years. As in the case of starch, no differences between forcing treatments were observed in any year, either in the C or RI treatments. As for the effect of irrigation for the same forcing treatment, statistically significant differences were only observed in the 2019/2020 winter for the NF treatments, with lower results in RI than in C (data not shown).

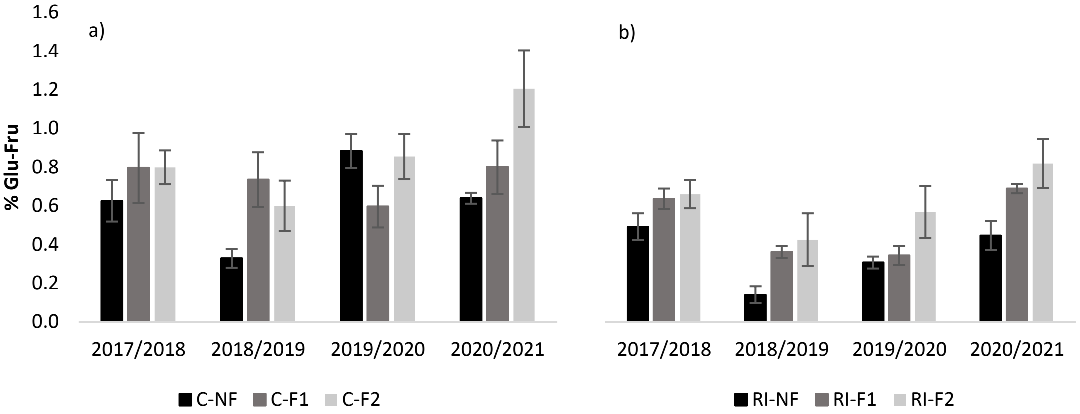


Figure VI.5. Percentage of root soluble sugar content during the winter growth pause: a) Percentage of soluble sugars (%Glu-Fru) in C treatments; b) Percentage of soluble sugars (%Glu-Fru) in RI treatments. Bars represent the standard error of the mean. Statistical analysis: ANOVA and Tukey test as a parametric test and Kruskal-Wallis test as a nonparametric test (both $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments.

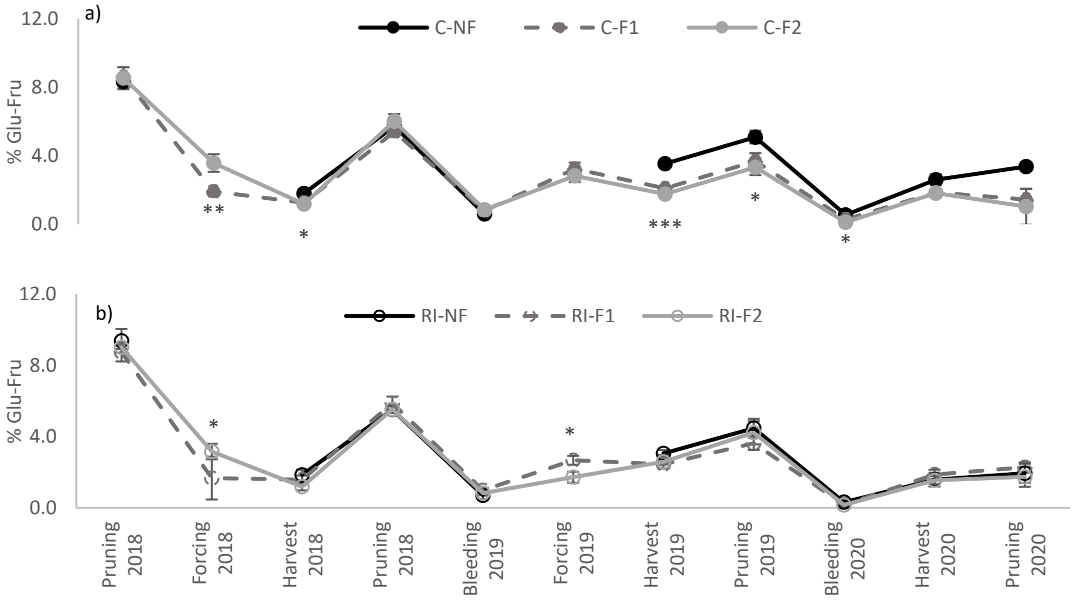
VI.3.3.2. Shoots

Table VI.5 shows shoot soluble sugar content at the time of winter pruning from 2017 to 2020 and at the time of harvest from 2018 to 2020. Significant interaction between irrigation and pruning was only observed in the 2019 harvest shoots. Shoot soluble sugar content decreased year-on-year in all treatments.

At the moment of pruning, differences were only observed in 2017 with higher values in RI than in C and in 2019 with higher concentrations in NF than in F1 and with intermediate values in F2. In the 2019 harvest there was interaction between the two effects, with differences in the C treatments (higher values in C-NF than in C-F1 and C-F2) but no differences in the RI treatments between RI-NF and RI-F1 and RI-F2. In 2020, the C treatments had higher soluble sugar content than the RI treatments. There were no differences in the concentration of soluble sugars between irrigation treatments with the same pruning treatment and, in general, the effect of irrigation did not show any clear trend over the course of the study years.

The evolution of shoot glucose-fructose content is shown in Figure VI.6. The highest concentrations correspond to the winter dormancy period (winter pruning), which decreased to their minimum value in the bleeding sap stage, followed by a subsequent recovery in 2019 and 2020, but not in 2018. These evolutions in C showed no differences between pruning treatments until the 2019 harvest, when higher values were recorded for NF (Figure VI.6a). The trend was maintained until the 2020 bleeding sap stage, without differences between treatments from that moment onwards. Similar differences were not observed in RI. The only moment when differences between these treatments were observed was when applying “crop-forcing” in 2018 and 2019, with differences between F1 and F2, although not always in the same direction.

The only differences between irrigation treatments for the same pruning treatment were observed in the 2019 forcing in both F1 and F2, with higher values in C.



	Pruning 2018	Forcing 2018	Harvest 2018	Pruning 2018	Bleeding 2019	Forcing 2019	Harvest 2019	Pruning 2019	Bleeding 2020	Harvest 2020	Pruning 2020
NF	n.s.		n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.
F1	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
F2	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.

Figure VI.6. Percentage of shoot soluble sugar content during the winter dormancy period: a) Percentage of soluble sugars (%Glu-Fru) in C treatments; b) Percentage of soluble sugars (%Glu-Fru) in RI treatments. Bars represent the standard error of the mean. The table indicates statistically significant differences between irrigation treatments for the same pruning treatment. Statistical analysis: ANOVA and the Tukey-B test and t-Student test as parametric test; Kruskal-Wallis’s test and multiple comparison p values and Mann–Whitney U test as nonparametric test. Differences between means were considered statistically significant when $p < 0.05$.

Table VI.5. Percentage of shoot soluble sugar content during winter pruning and at harvest.

	Year	*Statistical analysis*	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2	F x I	Forcing			Forcing factor	Irrigation		Irrigation factor
										NF	F1	F2		C	RI	
Pruning Shoots	**2017**	***p.***	8.3	8.6	8.5	9.4	8.8	9	n.s.	8.9	8.7	8.8	n.s.	8.5	9.1	*
	2018	***p.***	5.7	5.4	6	5.5	5.9	5.5	n.s.	5.6	5.6	5.8	n.s.	5.7	5.6	n.s.
	2019	***p.***	5.1	3.7	3.3	4.5	3.6	4.2	n.s.	4.8 a	3.7 b	3.8 ab	*	4	4.1	n.s.
	2020	***p.***	3.4	1.4	1	1.9	2.3	1.8	n.s.	2.7	1.9	1.4	n.s.	1.9	2	n.s.
Harvest Shoots	**2018**	***p.***	1.8	1.3	1.2	1.8	1.6	1.2	n.s.	1.8	1.4	1.2	n.s.	1.4	1.5	n.s.
	2019	***p.***	3.5 a	2.1 c	1.8 c	3.0 ab	2.4 bc	2.6 bc	*	3.3	2.3	2.2	***	2.5	2.7	n.s.
	2020	***p.***	2.6 a	1.8 b	1.8 b	1.6 b	1.9 b	1.6 b	n.s.	2.1	1.9	1.7	n.s.	2.1	1.7	*

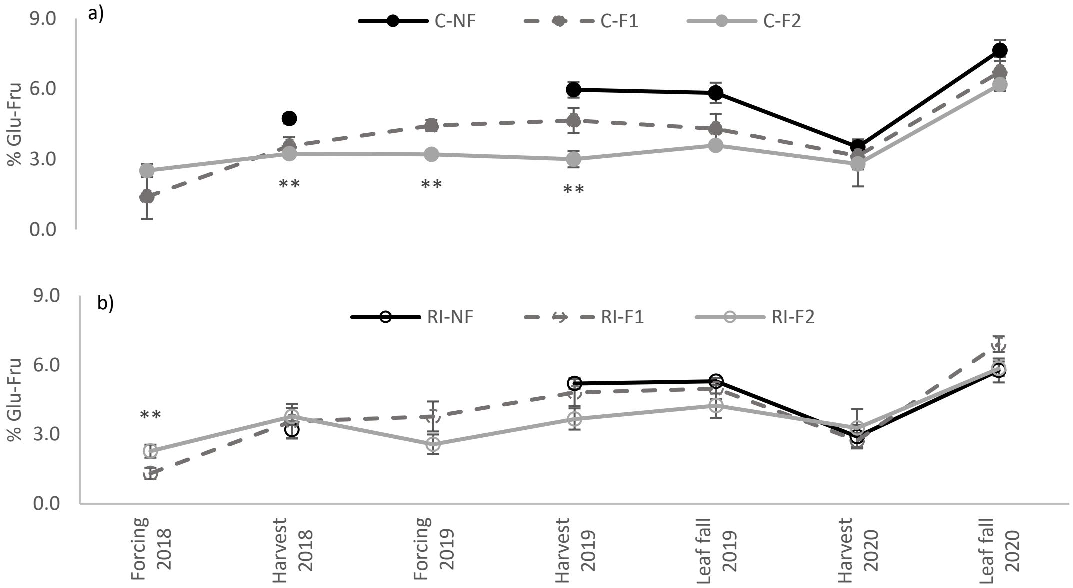
Treatments: C-NF (no forcing and full irrigation); C-F1 (forcing applied between flowering and fruit set and full irrigation); C-F2 (forcing applied between fruit set and pea size and full irrigation); RI-NF (no forcing and deficit irrigation); RI-F1 (forcing applied between flowering and fruit set and deficit irrigation) and RI-F2 (forcing applied between fruit set and pea size and deficit irrigation).

Statistical analysis: p. indicates parametric statistics (p < 0.05); n.p. indicates nonparametric statistics (p < 0.05). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VI.3.3.3. Leaves

Figure VI.7 shows the evolution of leaf glucose-fructose content. Levels were similar along the different samplings except for 2020. The evolution showed stable values, with higher accumulation found at 2020 leaf fall. In the C treatments, F2 maintained lower values of these sugars from the 2018 harvest to the 2019 harvest, followed by F1., while in 2020 the three treatments showed similar values. Differences between treatments in RI were only observed in the 2018 forcing.

Differences between irrigation treatments for the same pruning treatment were only observed in the 2018 harvest in NF, in the 2019 forcing in F2, and in the 2020 leaf fall in NF. Again, the highest values were in C.



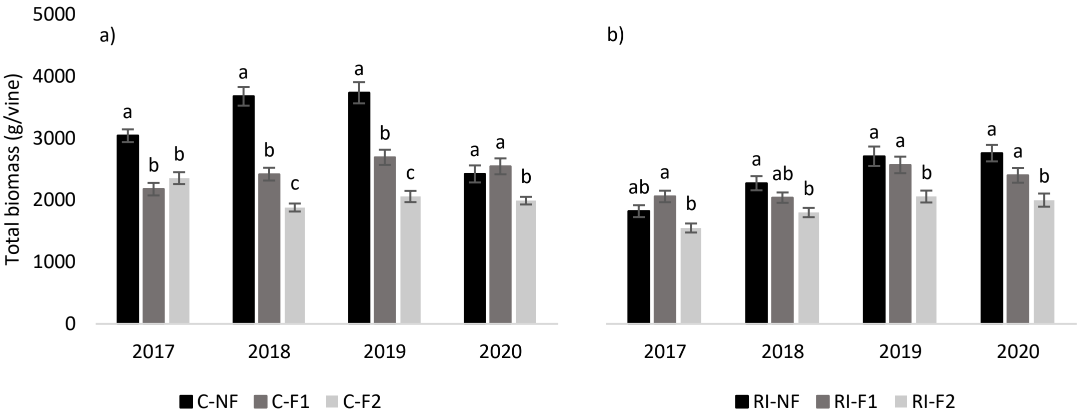
NF		**		n.s.	n.s.	n.s.	*
F1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
F2	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.

Figure VI.7. Evolution of leaf soluble sugar content percentage (%Glu-Fru) in: a) C treatments; b) RI treatments. Bars represent the standard error of the mean. The table indicates statistically significant differences between irrigation treatments for the same pruning treatment. Statistical analysis: ANOVA and the Tukey-B test and t-Student test as parametric test; Kruskal-Wallis's test and multiple comparison p values and Mann–Whitney U test as nonparametric test. Differences between means were considered statistically significant when $p < 0.05$.

VI.3.4. BIOMASS

Figure VI.8 shows total dry biomass produced by the vines of each treatment obtained as the sum of dry matter of yield, leaves and pruning interventions (winter pruning, green pruning and forcing pruning). During the forcing years (2017 to 2019) in the treatments with no water limitations C-NF obtained the highest biomass of the study, while in C-F2 the biomass was lower than in C-F1 in 2018 and 2019. In 2020, no differences were observed between C-NF and C-F1, but C-F2 had lower values. Also in 2020, C-NF obtained its lowest biomass, with weights lower than 2500 g/vine showing a notable interannual variability that was not observed in C-F1 and C-F2. In all the analysed years, RI had lower total biomass production than C. Differences between pruning treatments were also lower than those observed in C.

Among the non-forcing treatments, C-NF obtained higher values than RI-NF in 2017, 2018 and 2019, while in the forcing treatments C-F2 had higher values than RI-F2 only in 2017.



	2017	2018	2019	2020
NF	***	***	***	n.s.
F1	n.s.	n.s.	n.s.	n.s.
F2	***	n.s.	n.s.	n.s.

Figure VI.8. Total biomass (g/vine) in: a) C treatments; b) RI treatments. Bars represent the standard error of the mean. The table indicates statistically significant differences between irrigation treatments for the same pruning treatment. Statistical analysis: ANOVA and the Tukey-B test and t-Student test as parametric test; Kruskal-Wallis's test and multiple comparison p values and Mann–Whitney U test as nonparametric test. Differences between means were considered statistically significant when $p < 0.05$.

Figure VI.9 shows the percentage distribution of biomass (leaves, yield and pruning interventions). Biomass distribution for the same treatment was similar over the first three years. Generally, "crop-forcing" in both C and RI increased the proportion of pruning biomass at the expense of a reduction in biomass percentage (%) in yield and leaves in the case of F2.

F2 had a lower leaf biomass % in 2017, 2018 and 2019, while NF and F1 had similar leaf biomass % values in 2017 and 2018, with F1 increasing leaf biomass % compared to NF in 2019. In 2020, when no "crop-forcing" was applied, the deficit irrigation treatments of RI-F1 and RI-F2 saw a higher leaf biomass % than RI-NF. However, this effect was not observed between treatments with no water limitations (C-NF, C-F1 and C-F2). During 2017, 2018 and 2019 the lowest biomass % in yield was observed in C-F1, and in RI-F1 in 2020. Irrespective of irrigation treatment, F2 showed a lower yield biomass % than NF, except for C-F2 in 2018 and RI-F2 in 2019. In 2020, C-F2 had the highest yield biomass %, while no differences between treatments were observed in RI.

In 2020, the percentage distribution of biomass showed a similar trend in all treatments, with a significant decrease in yield biomass %, although with slight differences in C with the C-F2 treatment and in RI with the RI-NF treatment which do no not seem to be related to the pattern of the previous years.

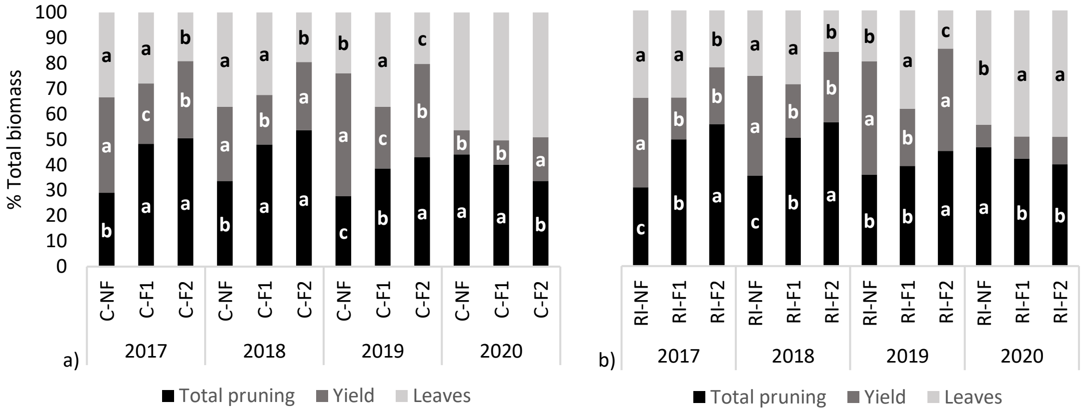


Figure VI.9. Total biomass content (%) in leaves, yield and pruning interventions in: a) C treatments; b) RI treatments. Statistical analysis: ANOVA and Tukey test as a parametric test and Kruskal-Wallis test as a nonparametric test (both $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments.

Table VI.6 shows the biomass extracted in the different interventions.

In the first three years, the highest yield biomass productions were observed in the NF treatments, especially in the C-NF treatment. The lowest were observed in F1 and F2 in 2017, 2018 and 2019. Application of RI decreased yield biomass significantly, except in 2018. Of the two forcing dates, F1 had lower yield biomass, although the differences were only statistically significant in RI in 2019. In 2018, 2019 and 2020, the amount of biomass produced as winter pruning in C-NF was higher than in the other treatments, followed by C-F1, RI-NF and RI-F1, while C-F2 and RI-F2 had the lowest values. In 2017, there was higher winter pruning biomass in C-NF, C-F1 and RI-F1 than in the other treatments. The biomass from the forcing pruning was higher in F2 than in F1 and only in 2017 were there differences between irrigation treatments, with more biomass extracted in C-F2 than in RI-F2. In the first three years, C-NF had the highest leaf biomass and F2 the lowest, with the differences being statistically significant. F1 had intermediate values compared to the other two pruning treatments (NF and F2), in both C and RI. The RI-NF and RI-F1 treatments had the same leaf biomass weights in 2017 and 2018.

The green pruning data are shown in Table VI.6. This pruning was not carried out in 2017 in the F2 treatments. Interaction between the two effects was observed in 2017 and in 2018, while in 2019 differences were observed due to the effect of forcing and irrigation without interaction between the two. In 2017, a higher amount of matter removed in green pruning was observed in RI-F1 than in RI-NF, but these differences were not observed in C. In 2018, C-F2 had the lowest extracted material, with significant differences with respect to C-F1, RI-NF and RI-F1. In 2019, in the NF treatments the amount of removed material in the green pruning was lower than in F1 and F2. This value was also affected by the irrigation regime, with higher values in RI than in C.

Table VI.7 shows the ratio between dry weight and leaf area at harvest and between leaf area and leaf dry weight. Yield per leaf area unit of C-F2 was similar to that of C-NF in 2017 and 2018, and in 2018 and 2019 the RI-F2 values were similar to those of RI-NF. In the first three years, the F1 treatments had the lowest canopy yield in both C and RI. All treatments had similar values in 2020, although some differences were detected but with generally very low values due to the low grape yield as a result of damage caused by cryptogamic diseases. The most notable values observed in the leaf area to dry weight ratio (Table VI.7) were the high F2 values, principally for RI, with RI-F2 obtaining a better ratio than C-NF, RI-NF and RI-F1 in 2017 and than RI-NF in 2018. In 2019, C-F2 and RI-F2 had higher values than the rest of the treatments. In 2020, no differences were found between treatments.

Table VI.6. Biomass in different organs during the years of "crop-forcing" application (2017, 2018, 2019) and the year of recovery (2020).

	Year	***Statistical analysis***	**C-NF**	**C-F1**	**C-F2**	**RI-NF**	**RI-F1**	**RI-F2**	***FxI***	**Forcing**			***Forcing factor***	**Irrigation**		***Irrigation factor***
										NF	**F1**	**F2**		**C**	**RI**	
Yield (g dry/vine)	**2017**	*p.*	1137.0	548.1	743.0	671.8	354.4	356.0	*n.s.*	904.4 a	451.3 b	549.5 b	***	809.4	460.7	***
	2018	*p.*	1066.5	506.0	523.4	885.7	465.0	508.1	*n.s.*	976.1 a	485.5 b	515.7 b	***	698.6	619.6	*n.s.*
	2019	*p.*	1784.8 a	670.1 cd	801.8 cd	1225.6 b	609.7 d	857.0 c	**	1505.2	639.9	829.4	***	1085.5	897.4	**
	2020	*p.*	232.4	250.9	359.8	259.1	228.5	247.4	*n.s.*	245.8	239.7	303.6	*n.s.*	281.1	245.0	*
Winter Pruning (g dry/vine)	**2017**	*n.p.*	814.0 a	741.0 a	502.8 b	504.8 b	713.3 a	406.5 b	***	659.4	727.1	454.6	***	685.9	541.5	***
	2018	*n.p.*	1178.1 a	763.8 b	528.0 c	803.8 b	636.8 bc	495.5 c	***	990.9	700.3	511.8	**	823.3	645.3	***
	2019	*n.p.*	995.0 a	686.5 ab	433.5 c	917.5 ab	649.0 bc	465.8 c	***	956.3	667.8	449.6	*n.s.*	705.0	677.4	***
	2020	*n.p.*	1114.5 a	1023.3 ab	672.5 c	1277.0 a	1016.3 a	773.2 bc	***	1195.8	1019.8	722.8	*	936.8	1022.1	***
Green Pruning (g dry/vine)	**2017**	*p.*	87.6 a	81.9 ab		60.7 b	88.6 a		**	74.1	85.2		*n.s.*	84.7	74.6	*n.s.*
	2018	*n.p.*	35.3 ab	44.1 a	27.2 b	38.0 a	41.4 a	34.3 ab	***	36.7	42.7	30.7	***	35.5	37.9	*n.s.*
	2019	*p.*	62.7	79.9	73.6	66.4	82.7	85.7	*n.s.*	64.6 b	81.3 a	79.6 a	***	72.1	78.3	*
Forcing Pruning (g dry/vine)	**2017**	*p.*		239.8 c	687.4 a		217.2 c	452.3 b	**		228.5	569.8	***	463.6	334.7	***
	2018	*n.p.*		348.5 b	458.4 a		322.4 b	468.4 a	***		335.4	463.4	***	403.5	395.4	*n.s.*
	2019	*n.p.*		278.8 b	375.5 a		249.9 b	357.9 a	***		264.4	366.7	***	327.2	303.9	*n.s.*
Leaves (g dry/vine)	**2017**	*n.p.*	1001.7 a	578.0 b	422.3 c	584.3 b	685.7 b	335.3 c	***	793.0	631.8	378.8	***	667.3	535.1	**
	2018	*n.p.*	1396.6 a	757.0 b	343.6 d	545.7 c	574.4 bc	293.0 d	***	971.1	665.7	318.3	***	832.4	471.0	***
	2019	*n.p.*	892.2 a	975.5 a	373.9 bc	498.9 b	977.0 a	291.4 c	***	695.6	976.2	332.6	***	747.2	589.1	***
	2020	*p.*	1076.4	1271.7	958.7	1222.5	1155.0	979.2	*n.s.*	1149.5 a	1213.3 a	968.9 b	***	1102.3	1118.9	*n.s.*

Treatments: C-NF (no forcing and full irrigation); C-F1 (forcing applied between flowering and fruit set and full irrigation); C-F2 (forcing applied between fruit set and pea size and full irrigation); RI-NF (no forcing and deficit irrigation); RI-F1 (forcing applied between flowering and fruit set and deficit irrigation) and RI-F2 (forcing applied between fruit set and pea size and deficit irrigation).

Statistical analysis: p. indicates parametric statistics ($p < 0.05$); n.p. indicates nonparametric statistics ($p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

Table VI.7. Ratio between vine dry weight (Yield) and leaf area (LA) and ratio between leaf area and leaf dry weight (Leaves) during the years of "crop-forcing" application (2017, 2018, 2019) and during the year of recovery (2020).

	Year	***Statistical analysis***	**C-NF**	**C-F1**	**C-F2**	**RI-NF**	**RI-F1**	**RI-F2**	***FxI***	**Forcing**			***Forcing factor***	**Irrigation**		***Irrigation factor***
										NF	**F1**	**F2**		**C**	**RI**	
Yield / LA (g/m^2)	**2017**	*n.p.*	180.0 a	108.8 b	183.8 a	178.2 a	77.0 b	86.7 b	***	179.1	92.9	135.2	***	157.5	113.9	**
	2018	*n.p.*	81.4 bc	59.8 c	151.6 ab	172.6 a	70.1 c	160.6 a	***	127.0	64.9	156.1	***	97.6	134.4	***
	2019	*n.p.*	210.3 a	70.5 b	180.7 a	248.5 a	70.0 b	231.6 a	***	229.4	70.2	206.2	***	153.8	183.4	*n.s.*
	2020	*n.p.*	17.8 ab	20.4 ab	38.1 a	17.0 b	17.7 b	19.6 ab	***	17.4	19.0	28.9	**	25.4	18.1	**
LA / Leaves (cm^2/g)	**2017**	*n.p.*	71.1 b	92.5 ab	98.5 ab	67.0 b	71.6 b	131.5 a	**	69.1	82.1	115.0	**	87.4	90.1	*n.s.*
	2018	*n.p.*	112.2 ab	114.6 ab	121.6 ab	99.4 b	121.3 ab	138.0 a	*	105.8	118.0	129.8	**	116.1	119.6	*n.s.*
	2019	*p.*	105.5	102.6	126.0	101.5	96.8	134.0	*n.s.*	103.5 b	99.7 b	130.0 a	***	111.3	110.8	*n.s.*
	2020	*p.*	142.9	101.5	113.0	130.5	129.8	125.4	*n.s.*	136.7	115.7	119.2	*n.s.*	119.1	128.6	*n.s.*

Yield / LA: Relation between vine dry weight and its leaf area; LA / Leaves: Ratio between leaf area per vine and leaf dry weight.

Treatments: C-NF (no forcing and full irrigation); C-F1 (forcing applied between flowering and fruit set and full irrigation); C-F2 (forcing applied between fruit set and pea size and full irrigation); RI-NF (no forcing and deficit irrigation); RI-F1 (forcing applied between flowering and fruit set and deficit irrigation) and RI-F2 (forcing applied between fruit set and pea size and deficit irrigation).

Statistical analysis: p. indicates parametric statistics ($p < 0.05$); n.p. indicates nonparametric statistics ($p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments; n.s. indicates not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VI.4. DISCUSSION

“Crop-forcing” is a severe pruning technique that modifies not only the phenology of the grapevine but also the distribution of biomass among the vegetative and productive organs and hence the source:sink ratios. This work aims to provide information about how use of this technique can affect biomass distribution and vine reserve levels and, in this way, determine whether the technique may lead to premature vine exhaustion. This information is of particular importance for the establishment of systematic criteria for application of this technique should a commercial vineyard consider its benefits to be of interest in the achievement of its goals.

Carbohydrate reserves in roots, trunks and shoots play a vital role in the annual cycle of the grapevine and are determining factors for the vegetative and productive growth of the campaign in course as well as those following given the biannual nature of the reproductive development of the grapevine. Low levels of reserves during the induction phase of the fruit-bearing buds that will bear fruit in the following campaign can reduce the potential number of inflorescences (Jackson, 2008). Budbreak and growth of shoots in their initial phase depend on the reserves accumulated at the end of the growth season of the previous year (May, 1987) and additionally coincide with the phases of initiation and differentiation of inflorescences when flower fertility of the campaign in course is determined and hence the number of berries per cluster. Through application of “crop-forcing”, with the removal of recently formed photosynthetic cover (part of the shoots and all the leaves), the new budbreak takes place at the expense of some reserves partially consumed by the ‘natural’ budbreak. As can be seen in Figure VI.3, at the moment when the forcing pruning is performed the shoots have lower starch, glucose and fructose content than at the moment of ‘natural’ budbreak, indicating a lower availability of carbohydrates to tackle bud regrowth. Similar results were found by Oliver-Manera et al. (2022), who observed a lower total NSC concentration in the trunk at forcing date. However, the meteorological conditions of late spring, with its high temperatures, favoured rapid shoot development and the development and expansion of photosynthetically active new leaves as well as canopy recovery, especially in F1 (data not shown). This allowed recovery of carbohydrates accumulated at harvest to levels similar to those observed in the previous winter pruning, as also observed by Oliver-Manera et al. (2022) in a similar study in ‘Tempranillo’. Under our study conditions, the application of RI did not entail differences to those observed between F1 and F2 with respect to NF in the C treatments at any moment during the cycle, indicating that the application of RI together with forcing did not result in a greater exhaustion of reserves (Figures VI.2-VI.7).

“Crop-forcing” shifts the vegetative cycle of the vine to a period of milder temperatures during grape maturation (Kishimoto et al., 2022; Martínez-Moreno et al., 2019; Martinez De Toda, 2021; Martinez De Toda et al., 2019). In warm climates, the greatest accumulation of carbohydrates in reserve organs takes place from post-harvest to leaf fall (Bennett et al., 2005; Vaillant-Gaveau et al., 2014), and so the duration of this period can affect the synthesis of these compounds. Application of “crop-forcing” delayed harvest by up to two months, while the initiation of leaf fall took place on the same date for all treatments, reducing the post-harvest period in the “crop-forcing” treatments. In F1 and F2, leaf retention time on the vine was lower than in NF (Table VI.2), which was reflected in lower shoot starch concentrations during the winter dormancy period (Table VI.4) in both C and RI. However, this decrease was not observed in the roots (Figure VI.2), the main reserve organ of the grapevine (Köse & Ateş, 2017; Mullins et al., 1992; Pellegrino et al., 2014; Zapata et al., 2004b).

With respect to root reserve levels, the characteristics of the year were more important than the pruning treatments applied. In view of the results of this work, it seems that importance should be given to the role of shoot reserve levels with respect to budbreak, as in F2, with similar root reserve levels, delays to budbreak were produced in 2018 and 2019, and in F1 in 2019, when shoot starch content was lower than in the NF treatments. In 2020, budbreak took place on the same date in all treatments, coinciding with the highest reserve levels being obtained in 2019. The break from dormancy is a complex and poorly understood process which, in addition to the influence of the carbohydrate reserves, it is believed may be associated to an increase in the production of hydrogen peroxide and the development of oxidative stress associated to low levels of catalase (Jackson, 2008), but it is not unreasonable to acknowledge the possibility that budbreak delay was caused by lower accessibility to reserves.

The concentration of carbohydrates in leaves was more sensitive to application of the “crop-forcing” technique than the other organs which were analysed, with the lower levels in F2 and the higher levels in NF being the most noteworthy results. It is probable that dominance of other sinks decreased leaf carbohydrate accumulation as they rapidly mobilized after photosynthetic assimilation. It should be noted that the leaves are perennial organs that cease to constitute a reserve for the vine with autumn leaf fall.

Water stress can accentuate the fall in vine reserve levels. Starch is the main reserve carbohydrate accumulated in grapevine (Köse & Ateş, 2017; Mullins et al., 1992; Pellegrino et al., 2014). Different studies have been conducted on the effect of the application of deficit irrigation strategies on starch accumulation. In ‘Garnacha’ and

‘Semillon’, water deficit resulted in decreased starch accumulation (Rogiers et al., 2011), but not in ‘Shiraz’ (Holzapfel et al., 2010). The results of the present work show that the application of water limitations in ‘Tempranillo’ did not, in general terms, result in a decrease in starch or soluble sugars content in shoots or roots (Figures VI.2-VI.7 and Tables VI.4-VI.5), including when water limitations were applied to vines subjected to forcing.

As with application of deficit irrigation, the forcing technique decreased total biomass production of biomass. The biomass data show that delaying the forcing date resulted, in F2, in a higher proportion of biomass in vegetative organs that were removed with the pruning applications (Table VI.6), modifying the annual distribution of biomass (Figure VI.8). Due to this larger amount of removed dry matter, the F2 treatments always obtained the lowest leaf and pruned wood percentages (Figure VI.9), without exceeding NF either in yield percentage. This was not the case in F1 which, although it obtained lower yield and pruned wood percentages, had, in general lines, an increase in leaf percentage compared to the other two treatments (NF and F2).

In F2, leaf proportion was generally lower than in NF, but with leaves of lower density that could capture more solar radiation per gram of leaf, with the result that yield per leaf area unit was similar in F2 and NF. In F1, yield per leaf area unit was lower as there was a greater proportional decrease in yield biomass than in leaf area. The F2 treatment was able to form clusters with mature berries that improved total acidity and anthocyanin content compared to the NF and F1 berries (articles in press).

It should be noted that, despite the decrease in total biomass as a result of “crop-forcing”, the values remained more or less stable over the course of the study in both the F1 and F2 treatments (Figure VI.8a and VI.8b), whereas in 2020 (when “crop-forcing” was not applied) a drastic reduction was observed in the NF treatments. This effect was due to the dramatic decrease in yield observed in 2020 caused by environmental conditions which favoured cryptogamic diseases. Thus, the biomass distribution in 2020 was concentrated among pruned organs and leaves (Figure VI.9a and VI.9b) and differences were only observed with F2 in total biomass production. This result suggests that it is yield biomass that gives rise to the differences between the different pruning types, and that the rest of the organs are not as affected by “crop-forcing”, especially with F1. The source:sink balance favours biomass recovery and allows restoration of carbohydrate levels at the end of the season in the forcing treatments. Similar conclusions have been reported by Oliver-Manera et al., (2022), with net carbon exchange rates per harvest unit around 2.5 times higher than in the non-forcing control treatment.

The results obtained in the present work show that the “crop-forcing” technique modifies NSC concentrations, principally in shoots and leaves, and that this is more evident in the case of the later forcing date. The differences established between F2 and the treatments with conventional winter pruning were maintained over the different years of the study, with no increase in the differences as the study progressed. In F1, as yield was reduced, photosynthetic assimilation was sufficient to maintain similar levels of reserves in the vine to NF. In F2, the lower canopy size and the short post-harvest period lowered reserves.

It does not therefore seem to be the case that “crop-forcing” results in progressive exhaustion, or that the deficit irrigation strategy causes cumulative exhaustion with “crop-forcing”. However, in 2020, when all the treatments were subjected to the same pruning, F2 had a lower number of clusters (17.4 clusters/vine in F2 against 22.3 and 22.5 in F1 and NF, respectively). This may be related to the lower carbohydrate content between budbreak and flowering, when induction of the following year’s flower buds takes place and the number of clusters is determined. As 2020 progressed the carbohydrate levels in all treatments tended towards similar values, and therefore a rapid recovery is expected in subsequent campaigns.

VI.5. CONCLUSIONS

In the three years in which the pruning treatments and irrigation strategies were applied no clear symptoms were observed of progressive grapevine decline caused by the application of “crop-forcing”, water stress, or a combination of the two.

The date on which “crop-forcing” was applied was important in terms of shoot and leaf carbohydrate levels, which were lower throughout the season in the forcing treatment applied at a later date compared to the treatment with conventional pruning and the earlier applied forcing treatment. With the forcing treatment applied at a later date, slight phenological deviations were observed which could be related to the availability of carbohydrates over the course of the vegetative cycle, and in the recovery year the lower number of clusters per vine may be a first symptom of vine exhaustion.

Application of the “crop-forcing” technique decreased the seasonal production of biomass and modified its distribution among the different aerial organs. The deficit irrigation strategy, which induced water stress during pre-veraison, decreased biomass production but did not result in modification of either biomass distribution among the different aerial parts of the vine or reserve levels of carbohydrates.

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VII. CAPÍTULO 4.

“CROP-FORCING” TECHNIQUE AND IRRIGATION STRATEGY MODIFIED THE CONTENT AND PHENOLIC PROFILE OF ‘TEMPRANILLO’ GRAPE GROWN IN SEMI-ARID CLIMATE.

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VII. ABSTRACT

Backgroung: Climate Change modifies the content and the phenolic profiles of grapes and wines. It is known that high temperatures, related to Climate Change, reduce the anthocyanins and procianidin (catechin and tannin) compounds accumulated in the berries. In last years, in order to improve phenolic composition of the berries, “crop-forcing” technique has been proposed to delay grape ripening to a more favourable period of temperatures. In this study, “crop-forcing” was applied to ‘Tempranillo’ vines on two different dates after flowering (F1) and after fruit set (F2), and compared to a treatment control (NF, without forcing). Besides, as a secondary factor, two irrigation strategies were established in each treatment. A irrigation with no water stress (C), and a pre-veraison deficit irrigation (RDI). The study was carried out in three consecutive years (2017-2019)

Results: Regardless irrigation strategy, berries F2 achieved higher contents of catechins and anthocyanins than NF berries. Every year and regardless irrigation strategy, “crop-forcing” increased the content of monoglucoside forms, and had a positive effect on the total content of malvidin, petunidin, delphinidin, peonidin and malvidin derivatives, but only affected acetyl and coumaryl forms in 2017. However, since it depended of metereology annual, the effect of irrigation strategy was less significant and less consistent.

Conclusion: “Crop-forcing” technique applied after fruit set could become a technique employed by the vine growers to delay the ripening of the grapes and achieve an increase in the anthocyanin characteristics of the grapes.

VII. RESUMEN

Antecedentes: El Cambio Climático modifica el contenido y los perfiles fenólicos de las uvas y los vinos. Se sabe que las altas temperaturas, relacionadas con el Cambio Climático, reducen los compuestos antocianos y procianidinas (catequinas y taninos) acumulados en las bayas. En los últimos años, con el fin de mejorar la composición fenólica de las bayas, se ha propuesto la técnica del forzado del cultivo para retrasar la maduración de la uva a un periodo de temperaturas más favorables. En este estudio, el “forzado de yemas” se aplicó a vides ‘Tempranillo’ en dos fechas diferentes después de la floración (F1) y después del cuajado (F2), y se comparó con un tratamiento control (NF, sin forzado). Además, como factor secundario, se establecieron dos estrategias de riego en cada tratamiento. Un riego sin estrés hídrico (C), y un riego deficitario pre-envero (RDI). El estudio se llevó a cabo en tres años consecutivos (2017-2019)

Resultados: Independientemente de la estrategia de riego, las bayas F2 alcanzaron mayores contenidos de catequinas y antocianinas que las bayas NF. Cada año e independientemente de la estrategia de riego, el "forzado de yemas" aumentó el contenido de formas monoglucósidas, y tuvo un efecto positivo en el contenido total de derivados de malvidina, petunidina, delfinidina, peonidina y malvidina, pero solo afectó a las formas acetil y cumaril en 2017. Sin embargo, al depender de la metereología anual, el efecto de la estrategia de riego fue menos significativo y menos consistente.

Conclusiones: La técnica del "forzado de yemas", aplicada después del cuajado del fruto, podría convertirse en una técnica empleada por los viticultores para retrasar la maduración de la uva y conseguir un aumento de las características antociánicas de la uva.

VII.1. INTRODUCTION

The color of red wine is one of its most important quality parameters and significantly determines consumer acceptance. Since the phenolic substances are responsible for significant and desirable characteristics of red wines as color, astringency and antioxidant properties, the amount and profile of these compound are important factors when the quality of berries for red winemaking is considered. According to their chemical structure, phenolic compounds can be classified into two main groups: non-flavonoids (phenolic acids and stilbenes) and flavonoids (anthocyanins, flavanols, and flavonols) (Ribéreau-Gayon et al., 2006). In red grapes, the most important phenolic families are: flavanols and anthocyanins. Flavanols are classified in tannins and catechins, which provide the bitter taste and astringency respectively while anthocyanins are responsible for the color (Kennedy et al., 2006).

It is know that the anthocyanin, profiles depend mainly on the variety, and are therefore useful elements for varietal classification (Bakker & Timberlake, 1985; Díaz-Fernández et al., 2022; Mateus et al., 2002). Generally, the five major anthocyanidins present in grapes are: delphidin, cyanidin, petunidin, peonidin and malvidin, which in turn can be found in free form (as 3-O-glucoside) or in acylated forms (acetyl and coumaril 3-O-glucoside). Anthocyanins begin to be sintetized from veraison by the following routes. On the one hand, disubstituted anthocyanins are formed: cyanidin is glycosidised to form cyanidin-3-O-glucoside, which in turn can be methylated to form peonidin-3-O-glucoside, these compounds are responsible for the red color. On the other hand, trisubstituted anthocyanins are formed, which give the berries their violet tones, through the transformation of delphinidin into delphinidin-3-O-glucoside, which in turn can methylate a hydroxide group to form petunidin-3-O-glucoside and/or methylate two hydroxide groups to form malvidin-3-O-glucosides. The latter is the main anthocyanin in ‘Tempranillo’ grapes (Matus et al., 2009). Once these anthocyanins are formed in free form, the glycoside groups can form acetates and coumarates, giving rise to the acylated forms which tend to be more stable (Ford et al., 1998).

To achieve the phenolic potential of grapes, it is necessary that all these transformations are completed. However, high temperatures, especially in the final ripening cycle, inhibit anthocyanin synthesis (Mori et al., 2007; Tarara et al., 2008). The accumulation of each anthocyanidin derivative is individually temperature sensitive. Thus, high temperatures decrease delphinidin, cyanidin, petunidin and peonidin content, but do not affect malvidin content as much (Mori et al., 2007; Spayd et al., 2002; Tarara et al., 2008). On other hand, the non-acylated forms are more unstable at increasing temperatures than the acylated forms. Wine color is linked to the accumulation of anthocyanins in the grape berries and particularly in the skin. However, it is not only the anthocyanin concentration and profile that is responsible for wine color: copigmentation can account for 30 to 50% of color in young wines (Boulton, 2001). For copigmentation in young wines to increase, there must be sufficient quantities of substances that can act as copigments. The most common cofactors are compounds such as phenolic acids, flavonoids, and in particular, flavonol and flavone derivatives (Rustioni et al., 2012). Flavanol content is also sensitive to climatic conditions. High temperatures lead to increases in catechins, and decreases in tannin content (Rienth et al., 2016). On the other hand, the increase in CO_2 associated with climate change leads to an increase in tannin concentration (Tate, 2001).

Nowadays, the high temperatures associated with climate change, during the final phase of ripening, cause changes in the chemical composition of the grapes. “Crop-forcing”, a technique proposed by Gu et al. (Gu et al., 2012), is investigated as a possible solution to these high temperatures in the final ripening cycle of the berry. The technique consists of "winter" pruning once the vegetative period has advanced, taking advantage of the vine's biannual reproductive cycle. Forcing thus leads to two crop cycles in the same year, one unfinished before forcing and the real one after forcing. With the application of this technique, ripening is shifted to a period with lower temperatures and the harvest is delayed by up to two months. Previous works indicated increases in total acidity and anthocyanin values (Gu et al., 2012; Lavado et al., 2019a; Martinez De Toda et al., 2019) in the berries from the crop forced vines. These same works reported that the results of “crop-forcing” depended on the phenological moment of application of this technique.

Irrigation management is a fundamental tool in order to improve the grape quality especially when the vines are cultivated in semiarid environment. However, irrigation to ensure 100 % potential vine evapotranspiration (ETc) normally reduces wine quality and regulated deficit irrigation (RDI) in vines has been used to improve it (Salón et al., 2005; Valdés et al., 2009). RDI consist of applying water in quantities smaller than those required to fully satisfy ETc needs during certain periods of the growth cycle. Besides, it is very important the phenological timing on vine and berry response to water stress. In this sense, the post-veraison water application was necessary to increase grape sugar level and wine alcohol content, however, water restrictions during the pre-veraison period left to more concentrated berries in terms of total phenolic and anthocyanins (Basile et al., 2011; Intrigliolo & Castel, 2010). Take account this, it is necessary determinate the impact of “crop-forcing” under different irrigations strategies.

The objective is to evaluate the effect of “crop-forcing”, applied in two different dates, on the phenolic content and anthocyanin profile of ‘Tempranillo’ grapes and its interaction with the application of a pre-veraison RDI, across three years.

VII.2. MATERIALS AND METHODS

VII.2.1. PLANT MATERIAL AND VINEYARD SITE

The study was carried out in an experimental vineyard located at Badajoz, Extremadura, Spain (38º 51´ N; 6º 40´ W; 198 m) in a ‘Tempranillo’ vineyard (Vitis vinifera L.) grafted on Richter 110 rootstock, trained as bilateral cordons in a vertical trellis system with a drip irrigation system of 8 L/h per vine. All the vines were winter pruned to six spurs and two buds per spur. The rows are E-W oriented and row and vine spacing were 2.5 m and 1.2 m, respectively. The soil is alluvial, with a loam to Sandy loam textura, slightly acidic, and lacking in organic matter. Soil depth is greater than 2.5 m and with low stone content. The area has a Mediterranean climate with a mild Atlantic influence, dry and hot summers, with high daily radiation and evaporative demand. The meteorological data come from a weather station belonging to the Extremadura irrigation advisory network (REDAREX) located 100 m from the plot with the characteristics described in Martí et al., (Martí et al., 2015) (See Supplementary Information, Table S1).

VII.2.2. TREATMENTS AND EXPERIMENTAL DESIGN

The experiment design was a split-plot with four replicacions (Table VII.1).

Table VII.1. Summary of the treatments applied in the study

	No “Crop-forcing” (NF)	**Early “Crop-forcing” (F1)**	**Late “Crop-forcing” (F2)**
Full-irrigated (C)	C-NF	C-F1	C-F2
Deficit irrigation (RI)	RI-NF	RI-F1	RI-F2

The experimental unit consists of 6 rows per 18 vines. Ten central vines of the four central rows are for sampling and harvest. The principal factor consisted of two treatments with “crop-forcing” techniques on two different dates; F1, “crop-forcing” applied three days after anthesis (May 18, 2017; May 29, 2018; May 20, 2019) and F2, 22 days after anthesis, both treatments were compared with a treatment without forcing techniques (NF) with vines grown under conventional practices (just winter pruning). “Crop-forcing” consisted of hedging the growing shoots to seven nodes and removing all the summer laterals, leaves and clusters with scissors to force the bursting of the primary buds developed in the current season. As a secondary factor, two irrigation treatments were established. A treatment with no water stress (C), supplying water to maintain a midday stem water potential (Ψsmd) close to -0.6 MPa and a pre-veraison deficit irrigation (RI) supplying water to reach a SWP of -1.1 MPa, as máximum and -0.8MPa in post-veraison. The vines water requirements were calculated based on the crop evapotranspiration (ETc) using the crop coefficient (Kc) recommended by FAO for these latitudes for the NF treatments. Water status of vines was given in Table VII.2. Yield and berry weight are indicated in TableVII.3.

Table VII.2. Weather conditions on budburst to harvest cycle and anual, stem water potencial (SWP) and forcing and harvest dates.

Year	Treatment	Tª Max (ºC)	Tª Min (ºC)	Thermal oscillation	Days Tª > 35ºC	Irrigation (mm)	SWP (MPa)		Forcing Date	Harvest Date
							C	RI		
2017	**NF**	35.9	16.4	19.5	32	2.6	-0.7	-1.0		22 Aug
	F1	34.5	15.6	18.9	19	12.5	-0.8	-0.9	18 May	12 Sep
	F2	31.0	12.0	19.0	2	0.0	-0.7	-0.9	07 Jun	17 Oct
	ANUAL	*25.3*	*9.5*	*15.9*	*63*	*282.2*				
2018	**NF**	35.7	17.0	18.7	17	1.6	-0.5	-0.8		27 Aug
	F1	32.9	15.3	17.6	10	3.0	-0.6	-0.8	29 May	08 Oct
	F2	27.9	12.7	15.2	4	76.8	-0.6	-0.8	18 Jun	29 Oct
	ANUAL	*22.9*	*9.7*	*13.2*	*36*	*479.3*				
2019	**NF**	34.3	16.3	18.0	15	0.0	-0.7	-0.9		27 Aug
	F1	31.6	14.9	16.7	8	4.6	-0.7	-0.7	20 May	30 Sep
	F2	29.6	13.1	16.5	0	9.9	-0.6	-0.7	03 Jun	15 Oct
	ANUAL	*24.3*	*9.4*	*14.9*	*34*	*292.9*				

Treatments: NF: no forcing; F1: forcing applied between flowering and fruit set; F2: forcing applied between fruit set and pea size; C: full irrigation treatments; RI: deficit irrigation treatment.

The agrometeorological data were obtained from a station close to the vineyard (100 m) with the characteristics described in Martí et al. (Martí et al., 2015). We calculated the vapor pressure déficit (VPD) with the maximum and minimum temperaturas and relative humidity (Allen et al., 1998).

The stem water potencial (SWP) was measured with a pressure chamber (Soil Moisture Corp., Model 3500, Santa Barbara, CA, USA), following the procedure described by Martí et al. (2015), using leaves on the north side of the trellis (in the shade), close to trunk level and wrapped in aluminium foil at least 2 h before data recording. Measurements were taken weekly on one leaf per vine and in two plants per subplot

Table VII.3. Effect of forcing and irrigation on yield and berry weight.

	Year	C-NF	% Regarding C-NF (100%)					
			C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2
Yield (kg/ha)	**2017**	12952.5 a	100 a	50 cd	71 b	58 bc	33 d	34 d
	2018	12428.3 a	100 a	44 b	49 b	82 a	41 b	48 b
	2019	23735.8 a	100 a	32 cd	42 c	62 b	28 d	40 c
Berry weight (g)	**2017**	1.8 a	100 a	56 ab	56 ab	72 a	56 ab	44 b
	2018	1.9 a	100 a	74 ab	63 ab	68 ab	68 ab	53 b
	2019	2.3 a	100 a	61 ab	61 ab	65 ab	65 ab	52 b

Treatments: C-NF (no forcing and full irrigation); C-F1 (forcing applied between flowering and fruit set and full irrigation); C-F2 (forcing applied between fruit set and pea size and full irrigation); RI-NF (no forcing and deficit irrigation); RI-F1 (forcing applied between flowering and fruit set and deficit irrigation) and RI-F2 (applied between fruit set and pea size and deficit irrigation).

Each value represents the mean of 8 samples (4 blocks, 2 replicates).

All treatments were harvested manually at 23–24 °Brix, a common criterion for picking red grape varieties in this area. The average TSS of the berries from the four elementary plots was considered for each treatment. All the clusters of 10 grapevines per experimental plot were weighed (40 grapevines per treatment). Samples of 100 g of berries per plot were weighed fresh.

Statistical analysis: Analysis of variance (ANOVA). Different letters indicate the existence of statistically significant differences between treatments.

VII.2.3. EXTRACTION AND DETERMINATION OF PHENOLIC COMPOUNDS

All treatments were hand harvested at 23 to 24 °Brix, a common harvesting criterion for red grape varieties in this area. The average of TSS of the berries from the four elementary plots by treatment was considered.

The extraction of phenolic substances from grapes was carried out according a methodology based in previous works (Díaz-Fernández et al., 2022; Portu et al., 2016). Briefly, samples of 100 g of berries frozen (-80°C), were crushed and homogenised for 30 seconds in a freshboost blender (LM180110, Moulinex, Aleçon, France). 10 mL of the hydroalcoholic solution (methanol/water/formic acid 50:48.5:1.5, v/v/v) were added to an aliquot of the obtained mash (1.0 g) and was macerated for 30 minutes in a at 4 ºC in an ultrasonic bath (USC-TH, VWR, Radnor, USA) and centrifuged at 4°C for 10 min (5810 R, Eppendorf, Hambug, Germany). The supernatant was separated, and the resulting pellet was extracted up to three times. The supernatants (phenolic extracts) were then combined and the final volume was annotated. Separation, identification, and quantification of phenolic substances were performed by HPLC analysis (Díaz-Fernández et al., 2022; Portu et al., 2016). For quantification and calibration of each compound, calibration curves of their respective standards ($R^2 > 0.999$) were used. Anthocyans present in extracts were identified and quantified as simple glucosides-nonacylated (G), acetyl derivatives (A) and coumaroyl (C) forms of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv). Total amount of anthocyans (Ant, µg malvidine-3-glucoside / g fresh berry) was calculated from the values of individual compounds.

Total polyphenol (TPP, µg gallic acid / g fresh berry) content was determined according to Singleton and Rossi (Singlenton & Rossi, 1965). Catechins (Cat, µg catechin / g fresh berry) content was determinated according to McMurrough & Dowell (McMurrough & McDowell, 1978). These two determinations were carried out using an autoanalyzer (Y15, Biosystems, Barcelona, Spain). Tannins (Tan, µg/g) was determinated following the methods described by Sarneckis et al. (Sarneckis et al., 2006) using (+)-catechin as standard.

VII.2.7. STATISTICAL DATA ANALYSIS

Normality and homogeneity of variances was tested using Shapiro-Wilk's and Barlett's test respectively. Differences between means were considered statistically significant when $p < 0.05$. Phenolics data were subjected to analysis of variance (MANOVA) to investigate the effect of “crop-forcing”, “irrigation” and their interaction on each parameter evaluated selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons. The interaction between effects was evaluated by calculating the least-squares means (LS means) selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons and the Tukey test as Post Hoc tests for parametric samples. On the other hand, when the normality and homogeneity of variances were not verified, non-parametric tests were carried out and the Kruskal-Wallis test (alternative to one-way ANOVA) and multiple comparasion *p* values (alternative to post-hoc pairwise comparisons). Differences between means were considered statistically significant when $p< 0.05$. Principal component analysis (PCA) was performed with the values of anthocyanins parameters. Statistical tests were performed with XLSTAT-Pro 201610 (Addinsoft, 2009, Paris, France).

VII.3. RESULTS

VII.3.1. BERRIES PHENOLIC COMPOSITION

Figures VII.1 (a-d) show the effect of “crop-forcing” (F) and irrigation strategy (RI) on total polyphenols (TPP), total anthocyanins (Ant), catechins (Cat) and tannins (Tan) respectively on ‘Tempranillo’ berries. Because a not significant effect was found for F*I in all these families, the impact of F and I were analysed separately.

The effect of treatments on TPP values varied between years (Figure VII.1a). In 2018 and 2019 F2 improved TPP values, with increases of 40% respect to NF. However, F1 only increased it in 2019 (40% respect to NF). No appreciable effect of irrigation strategy was observed for TPP in any of the study years.

The Ant values depended on the year (Figure VII.1b). In every treatment the highest values of these compounds were registered in 2018, with exception of RI-NF and RI-F1, where the maximum values corresponded to 2017 and 2019 respectively. Respect to effect of treatments applied, as general trend, F increased the Ant accumulated in the berries and every year, F berries (C and RI) showed higher values or these compounds than NF ones. However, the significance and extent of the effect depended on the date when “crop-forcing” was applied, and every year, F2 > F1 > NF but a significant increase of F1 compared with NF was observed in 2018 only. On other hand, respect to C berries, regardless pruning applied, RI increased the content of Ant also, and significatively in 2017 and 2019. As results, since additive effects (i.e. lack of interaction) between F and RDI were found, the maximum and the minimum values were registered in RI-F2 and C-NF respectively every year of the trial. When the variation in percentage was calculated, the highest percentages were registered in C-F2 vs C-NF berries (about 60% in 2018 and 2019) and an average increase of 20% were found in RI respect C berries in 2017 and 2019, but they did not reach the 6% (2.2 and 5.8% respectively in NF and F1) in 2018.

Figure VII.1c reflects that, like in Ant, the maximum Cat values were found in 2018 for most of treatments with exception C-F1. Besides, F improved the amounts of Cat (p<0,001 in 2017 and 2019 and p< 0,01 in 2018) too; however, unlike Ant, the general trend observed was F1 > F2 > NF in 2017 and 2019 and in 2018, RI-F1 > RI-NF, while C-F1 < C-NF. On the other hand, when the contents of RI berries were compared to the C ones, a trend to lower values in RI, only significant in 2017, was registered. Thus, in 2017 and 2019 the highest values corresponded to C-F1 (1348.27 and 1617.55 respectively) while in 2018 to C-F2 (2086.83). Finally, unlike Ant and Cat, by general, the maximum values of Tan were reached in 2019 (Figure VII.1d) only C-F2 registered the maximum value in 2018. On other hand, a scarce effect of F and RI was found in the content of Tan, and no clear trend found either. Significant differences caused by forcing crops were registered in 2019 only, with increases of 19% and 23% in F1 and F2 respectively while RDI decreased in 2018.

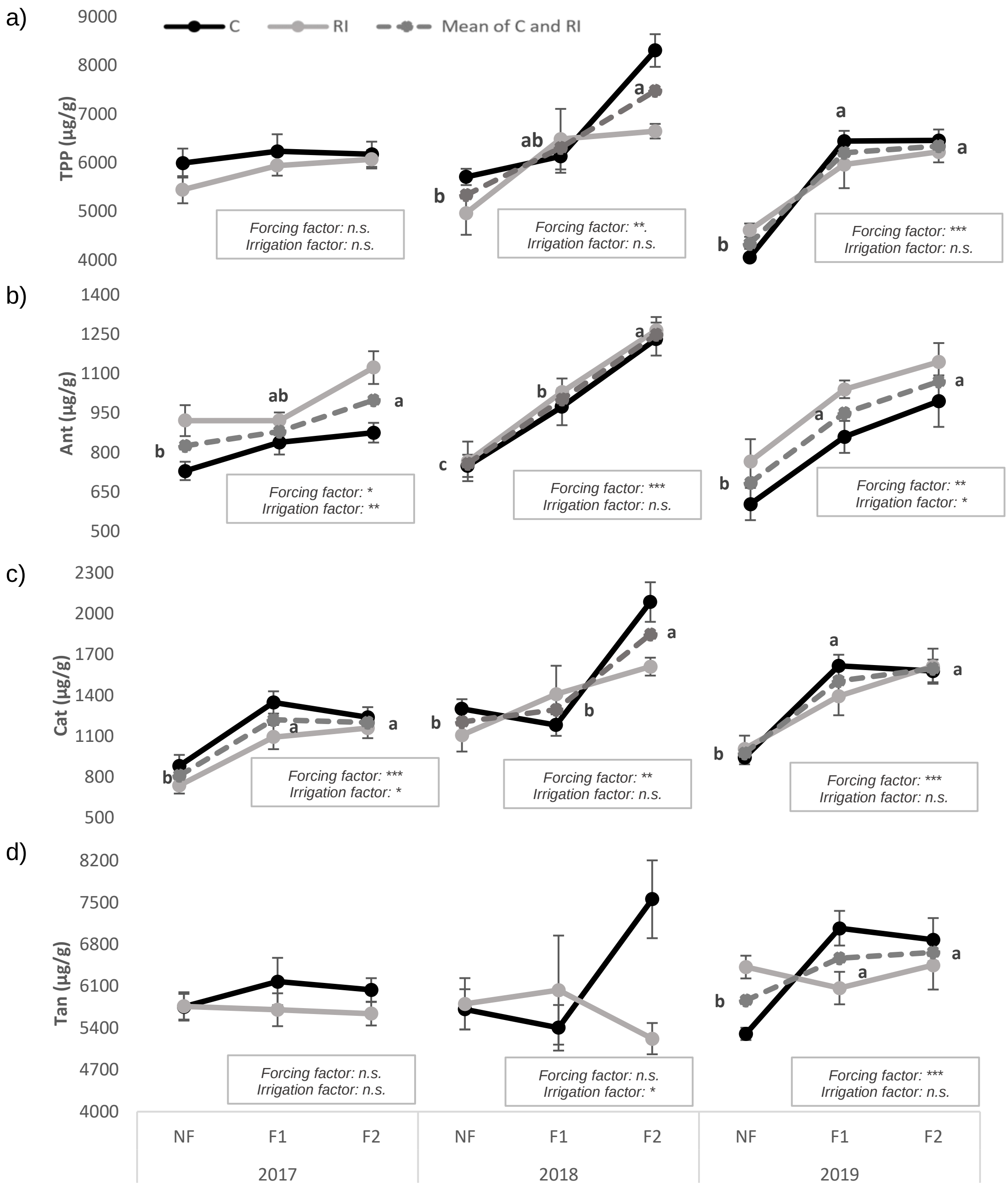

Figure VII.1. Effect of “crop-forcing” technique (forcing factor) and irrigation strategy (irrigation factor) on phenolic composition (µg substance /g fresh berry) of ‘Tempranillo’ grapes: a) Total polyphenols (TPP); b) Anthocyanins (Ant); c) Catechins (Cat); d) Tannins (Tan). Each point represents the mean of 8 samples (4 blocks, 2 replicates). The bars represent the standard error of the mean. Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VII.3.2. ANTHOCYANIN PROFILE

Table VII.4 indicate the anthocyanin profile of ‘Tempranillo’ berries. “Crop-forcing” had a consistent effect across the year, since 11, 10 and 11 compounds showed significant increases in 2017, 2018 and 2019 respectively. However, the effect of irrigation strategy depended on the year considered. Thus, while in the 2017 and 2019 years, RI had a significant effect on 11 and 9 compounds respectively, in the 2018 only on 3.

When the profiles of different treatments across 2017-2019 year were analysed, it is noteworthy that i) regardless treatment, MvG reached the highest values while the lowest were found in CyC and CyA. ii) after MvG, MvC was the second most abundant compound in NF berries, however, in F berries, the general behaviour was MvG > DpG > MvC. iii) In 2017 the highest values of all individual monoglucosides compounds were registered in RI-F2, while the highest of acetyl and coumaryl monoglucosides in RI-NF. In 2018, no trend between treatments and substances could be established and finally, in 2019, the maximum of every monoglucosides compounds were again recorded in RI-F2 while those of the DpC, PnC, CyC and all individual acetyl monoglucosides forms in RI-F1.

The effects of the techniques applied on the total content of monoglucoside (∑G), acetyl (∑A) and coumaryl (∑C) monoglucoside forms on ‘Tempranillo’ berries at harvest are show in Figure VII.2 (a-c) and Figure VII.3 (a-e) shows the effects of “Crop-forcing” (F) and irrigation values of malvidin, (∑Mv) petunidin (∑Pt), delphinidin (∑Dp), peonidin (∑Pn) and cyanidin (∑Cy) derivates respectively. As in the previous results, no interactions were found between “crop-forcing” (F) and irrigation strategies (I).

According Figures VII.2, respect to NF, “crop-forcing”, and more specifically F2, caused significant increases on the content of ∑G every year, however, ∑A and ∑C were less reactive. As Figure VII.2a reflects, the order F2 > F1 > NF was observed every year in the values of ∑G and with exception of 2018, RI compared to C, increased the values of these compounds in the berries. As consequence, all years the highest values of ∑G corresponded to RI-F2 and specifically the maximum was found in RI-F2 of 2018 year while the minimum in C-NF of 2019 year. In 2017 an opposite trend to ∑G was observed in ∑A and ∑C (Figures VII.2b and VII.2c); therefore F2 berries had lower values of these last compounds than NF ones in that year. In the following years, the application of forcing factor did not affect the values of ∑A and ∑C. Besides, regardless pruning treatment, by general RI berries had higher values than C of these compounds by only significant differences were found in ∑A in 2019.

Table VII.4. Effect of forcing and irrigation on anthocyanin profile of 'Tempranillo' grapes (µg substance /g fresh berry).

	Year	C-NF	C-F1	C-F2	RI-NF	RI-F1	RI-F2	F*I	NF	F1	F2	F	C	RI	I
MvG	**2017**	263.7	309.2	332.6	338.3	331.8	409.3	n.s.	301.0 b	320.5 ab	371.0 a	*	301.9	359.8	**
	2018	275.2	350.5	451.6	271.1	377.6	445.3	n.s.	273.1 c	364.0 b	448.4 a	***	359.1	364.7	n.s.
	2019	233.7	323.2	395.8	276.5	393.0	446.5	n.s.	255.1 b	358.1 a	421.2 a	**	317.6	372.0	n.s.
PtG	**2017**	90.6	108.5	121.7	108.5	123.9	168.3	n.s.	99.6 b	116.2 b	145.0 a	**	106.9	133.6	*
	2018	87.9	128.1	169.9	85.9	137.6	189.6	n.s.	86.9 c	132.9 b	179.8 a	***	128.6	137.7	n.s.
	2019	69.6	114.8	141.5	96.1	141.2	168.5	n.s.	82.9 b	128.0 a	155.0 a	***	108.7	135.3	*
DpG	**2017**	106.0	131.5	152.9	125.6	153.7	224.5	n.s.	115.8 b	142.6 b	188.7 a	***	130.1	167.9	**
	2018	101.7	157.2	216.8	100.7	171.1	263.0	n.s.	101.2 c	164.2 b	239.9 a	***	158.6	178.3	n.s.
	2019	78.6	143.7	180.4	117.8	176.7	222.6	n.s.	98.2 b	160.2 a	201.5 a	***	134.2	172.4	*
PnG	**2017**	34.0	46.1	50.9	34.1	50.4	64.9	n.s.	34.1 b	48.2 ab	57.9 a	*	43.7	49.8	n.s.
	2018	64.5	84.0	125.6	61.1	84.7	98.3	n.s.	62.8 b	84.4 ab	112.0 a	*	91.4	81.4	n.s.
	2019	24.0	66.0	67.9	39.9	75.1	76.8	n.s.	31.9 b	70.5 a	72.4 a	***	52.6	64.0	n.s.
CyG	**2017**	16.4	27.0	30.8	15.0	29.5	43.4	n.s.	15.7 b	28.3 ab	37.1 a	**	24.7	29.3	n.s.
	2018	33.7	49.1	74.0	33.2	48.4	63.6	n.s.	33.5 b	48.8 ab	68.8 a	*	52.3	48.4	n.s.
	2019	11.5	38.3	39.4	23.0	42.6	46.0	n.s.	17.3 b	40.5 a	42.7 a	***	29.7	37.2	n.s.
MvA	**2017**	26.3	26.0	20.5	38.1	28.4	23.8	n.s.	32.2 a	27.2 ab	22.2 b	**	24.2	30.1	**
	2018	20.8	22.5	19.0	25.4	24.2	21.0	n.s.	23.1	23.4	20.0	n.s.	20.8	23.5	n.s.
	2019	18.7	20.4	19.1	25.5	26.1	21.7	n.s.	22.1	23.2	20.4	n.s.	19.4	24.4	*
PtA	**2017**	6.3	7.3	5.9	8.5	8.4	7.6	n.s.	7.4	7.8	6.8	n.s.	6.5	8.2	**
	2018	5.0	6.2	6.3	6.1	6.6	7.4	n.s.	5.5 b	6.4 ab	6.8 a	*	5.8	6.7	*
	2019	4.1	6.0	5.8	6.4	7.6	6.8	n.s.	5.2	6.8	6.3	n.s.	5.3	6.9	**
DpA	**2017**	5.8	6.7	5.9	7.8	8.1	8.0	n.s.	6.8	7.4	7.0	n.s.	6.2	8.0	**
	2018	4.7	5.9	6.8	5.7	6.8	8.7	n.s.	5.2 c	6.4 b	7.7 a	***	5.8	7.1	**
	2019	3.6	6.1	6.3	6.2	7.8	7.7	n.s.	4.9 b	7.0 a	7.0 a	*	5.3	7.2	**

Table VII.4 cont. Effect of forcing and irrigation on anthocyanin profile of 'Tempranillo' grapes (µg substance /g fresh berry).

	Year	**C-NF**	**C-F1**	**C-F2**	**RI-NF**	**RI-F1**	**RI-F2**	**F*I**	**NF**	**F1**	**F2**	**F**	**C**	**RI**	**I**
PnA	**2017**	2.3	2.3	1.9	3.0	2.6	2.3	n.s.	2.6 a	2.4 ab	2.1 b	**	2.2	2.6	**
	2018	3.0	3.4	3.8	3.1	3.6	3.4	n.s.	3.0	3.5	3.6	n.s.	3.4	3.4	n.s.
	2019	1.8	3.1	2.5	2.7	3.7	3.0	n.s.	2.3 b	3.4 a	2.7 ab	**	2.5	3.1	*
CyA	**2017**	1.5	1.6	1.2	2.2	1.8	1.7	n.s.	1.8	1.7	1.5	n.s.	1.4	1.9	*
	2018	1.4	1.9	1.9	1.6	2.3	1.9	n.s.	1.5	2.1	1.9	n.s.	1.7	1.9	n.s.
	2019	0.7	1.6	1.3	1.5	2.0	1.7	n.s.	1.1 b	1.8 a	1.5 ab	**	1.2	1.7	**
MvC	**2017**	124.2 b	112.9 b	99.7 b	169.5 a	116.3 b	110.9 b	*	146.8	114.6	105.3	***	112.3	132.2	**
	2018	103.3	108.6	96.6	119.2	104.7	94.5	n.s.	111.2	106.7	95.6	n.s.	102.8	106.1	n.s.
	2019	114.7	83.8	81.6	117.6	100.2	84.6	n.s.	116.2 a	92.0 b	83.1 b	**	93.4	100.8	n.s.
PtC	**2017**	9.8	8.2	7.4	11.2	9.2	7.4	n.s.	10.5 a	8.7 b	7.4 b	***	8.5	9.3	n.s.
	2018	9.3 bc	8.6 bc	6.3 c	8.6 bc	15.4 a	12.6 ab	**	9.0	12.0	9.5	*	8.1	12.2	***
	2019	10.4	12.4	16.9	9.9	15.9	17.7	n.s.	10.1 c	14.1 b	17.3 a	***	13.2	14.5	n.s.
DpC	**2017**	28.7	33.7	27.8	42.2	37.6	34.3	n.s.	35.5	35.6	31.0	n.s.	30.1	38.0	***
	2018	21.8	26.4	27.0	27.5	26.9	32.9	n.s.	24.6	26.7	30.0	n.s.	25.1	29.1	n.s.
	2019	21.2	23.6	22.7	29.0	28.9	26.1	n.s.	25.1	26.3	24.4	n.s.	22.5	28.0	*
PnC	**2017**	13.3	17.9	13.9	16.2	19.4	15.8	n.s.	14.7 b	18.7 a	14.9 b	**	15.0	17.1	*
	2018	16.6	20.4	25.3	16.5	19.4	21.9	n.s.	16.5 b	19.9 ab	23.6 a	**	20.8	19.3	n.s.
	2019	10.5	15.6	13.7	13.7	18.6	14.4	n.s.	12.1 b	17.1 a	14.1 ab	**	13.3	15.6	n.s.
CyC	**2017**	1.0	0.4	3.8	1.3	0.2	0.8	n.s.	1.2	0.3	2.3	n.s.	1.7	0.8	n.s.
	2018	0.4	1.0	1.1	0.6	1.0	1.3	n.s.	0.5 b	1.0 a	1.2 a	**	0.9	1.0	n.s.
	2019	0.7	0.9	0.7	0.9	1.3	1.0	n.s.	0.8	1.1	0.8	n.s.	0.7	1.1	*

Each value represents the mean of 8 samples (4 blocks, 2 replicates). Parameters: Malvidin-3-glucoside (MvG); Petunidin-3-glucoside (PtG); Delphidin-3-glucoside (DpG); Peonidin-3-glucoside (PnG); Cyanidin-3-glucoside (CyG); Malvidin-3-glucoside acetate (MvA); Petunidin-3-glucoside acetate (PtA); Delphidin-3-glucoside acetate (DpA); Peonidin-3-glucoside acetate (PnA); Cyanidin-3-glucoside acetate (CyA); Malvidin-3-glucoside coumarate (MvC); Petunidin-3-glucoside coumarate (PtC); Peonidin-3-glucoside coumarate (PnC); Cyanidin-3-glucoside coumarate (CyC). Statistical analisis: Multiple analysis of variance (MANOVA) to investigate the effect of "crop-forcing", "irrigation" and their interaction on each parameter. Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

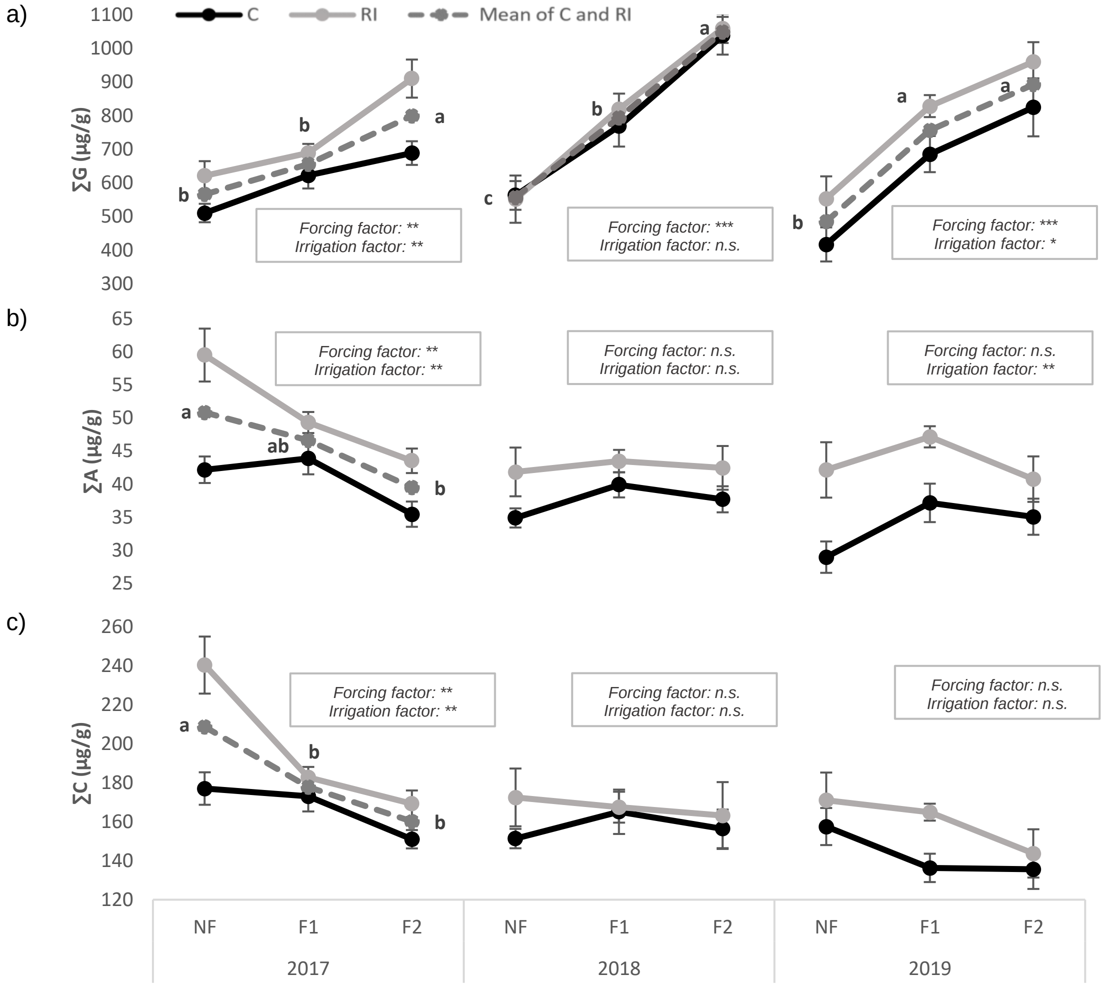

Figure VII.2. Effect of “crop-forcing” technique (forcing factor) and irrigation strategy (irrigation factor) on anthocyanin profile of ‘Tempranillo’ grapes (µg substance /g fresh berry): a) Total monoglucoside forms (∑G); b) Total acetyl- glucoside forms (∑A); c) Total coumaroy- glucoside forms (∑C). Each point represents the mean of 8 samples (4 blocks, 2 replicates). The bars represent the standard error of the mean. Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

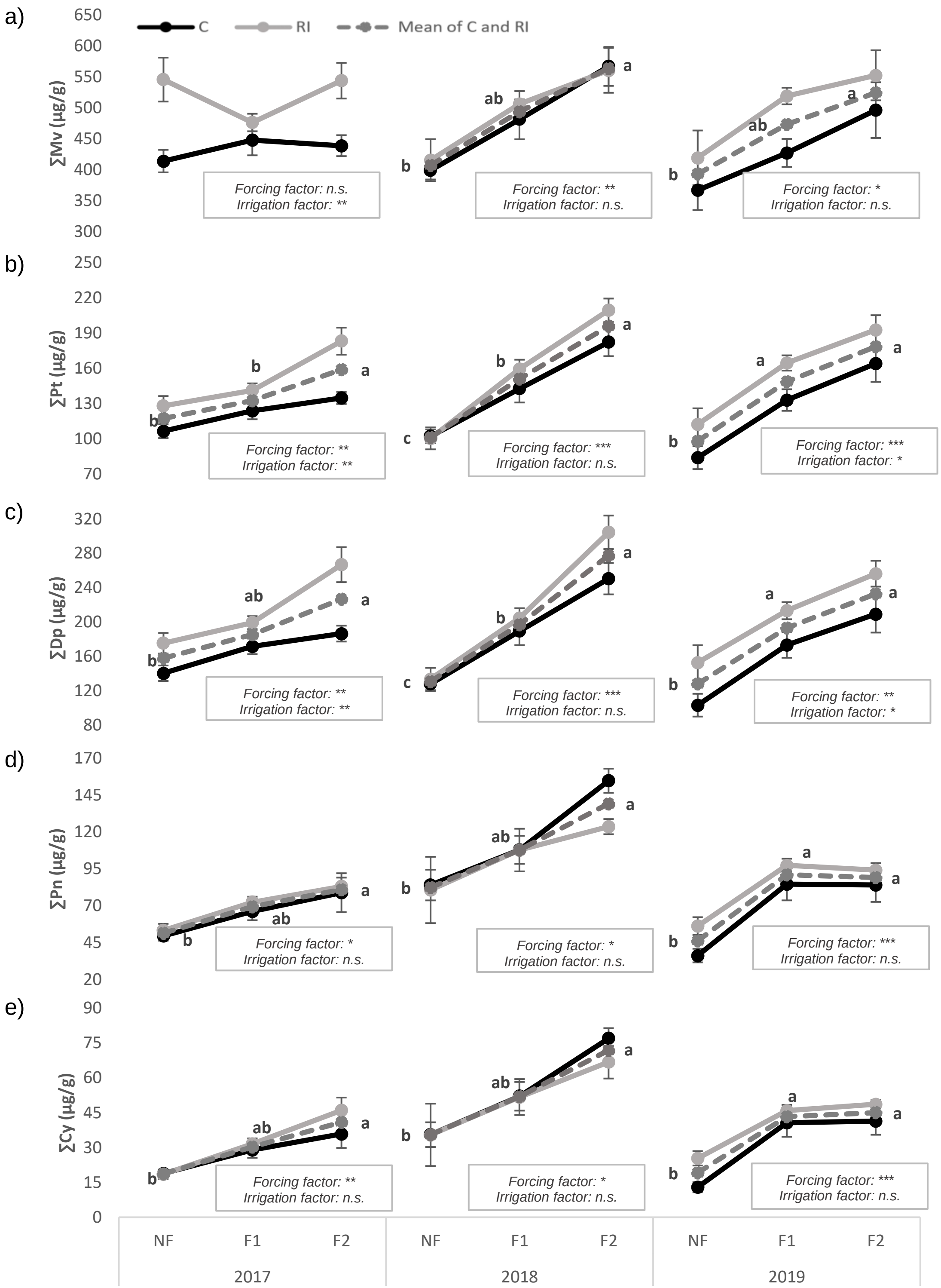

Figure VII.3. Effect of “crop-forcing” technique (forcing factor) and irrigation strategy (irrigation factor) on anthocyanin profile of ‘Tempranillo’ grapes (µg substance /g fresh berry): a) Total malvidin derivates (∑Mv); b) Total petunidin derivates (∑Pt); c) Total delphinidin derivates (∑Dp); d) Total peonidin derivates (∑Pn); e) Total cyanindin derivates (∑Cy). Each point represents the mean of 8 samples (4 blocks, 2 replicates). The bars represent the standard error of the mean. Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

When the impact of “crop-forcing” and irrigation strategies were investigated on total values of anthocyanidin derivates (Figure VII.3, a-e) the following results were obtained: i) higher amounts (p<0.05) of ∑Mv, ∑Pt, ∑Dp, ∑Pn and ∑Cy derivates were registered in F2 respect NF berries every year with exception of ∑Mv in 2017.ii) the effect of F1 was lower (in extent and significance): compared to NF, in 2017 significant differences were reported in ∑Pt only, in 2018 in ∑Pt and ∑Dp, and in 2019 for every derivate with exception of Mv. Therefore, it could be conclude that Dp derivates are highly sensitive to “crop-forcing”, while Mv derivates had a scarce response to this technique. Respect to irrigation strategy, the values of almost ∑derivates were in RI berries similar o higher than those C ones. More specifically, RDI increased in 2017 ∑Mv, ∑Pt, ∑Dp and in 2019 ∑Pt and ∑Pt

VII.3.3. CLASSIFICATION OF TREATMENTS

Three principal component analysis (PCA) were used to classify the different treatments in terms of values of total anthocynin forms and total anthocynin derivates (Figure VII.4). The first PCA performed with the data from the 2017 season (Figure VII.4a) accounted 97.27% of the total variance. According this Figure, PC1 (63.63% of the total variance) associated with ∑Dp, ∑G, ∑Pn and ∑Pt, separated the treatments according pruning, while PC2 (33.64% of total variance), correlated with ∑A and ∑C, separated them according irrigation strategy. The second (Figure VII.4b) was performed with the values obtained in 2018, and PC1 and PC2 explained 95.22% of the total variance (72.50 and 22.71%, respectively). PC1 strongly correlated with ∑G, ∑Dp, ∑Pt, and ∑Mv allows to differentiate three groups consisted of NF, F1 and F2 samples according pruning treatment, while PC2 associated a ∑A and ∑C according irrigation strategy. It is noticed that RI-F2-18 and C-F2-18 were placed more closely than RI-F-18 and C-F1-18 and C-NF-18 and RI-NF-8 respectively. Figure VII.4c shows the results of PCA performed with the results from 2019 year allows to found a similar distribution of parameters and treatments observed in 2017 season.

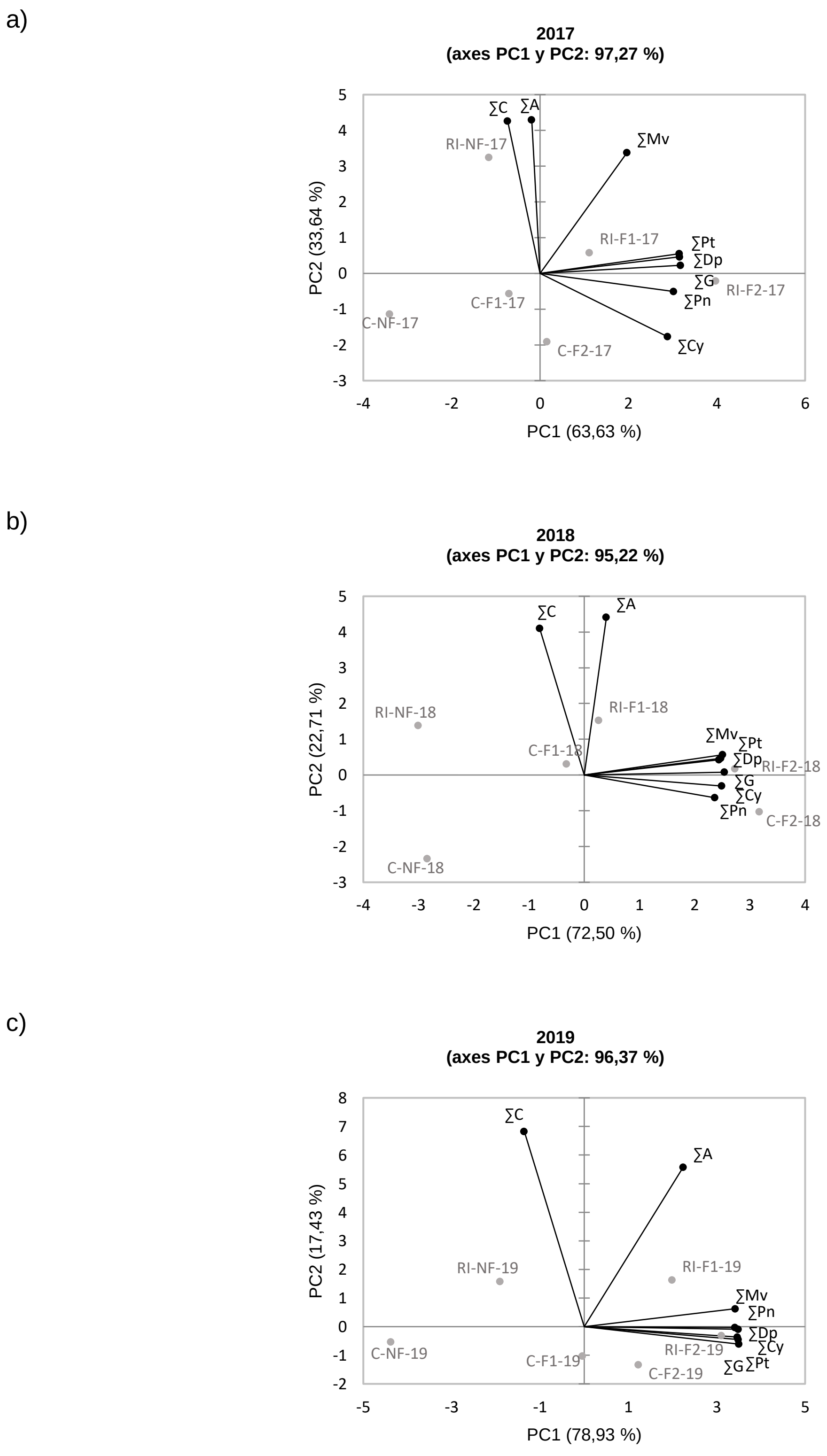

Figure VII.4. Principal components analysis on anthocyanin profile of ‘Tempranillo’ grapes: a) 2017; b) 2018; c) 2019. Total monoglucoside forms (∑G); Total acetyl- glucoside forms (∑A); Total coumaroy- glucoside forms (∑C). Glucoside (G), Acetyl-glucoside (A); Coumaril-glucoside (C); Total malvidin derivates (∑Mv); Total petunidin derivates (∑Pt); Total delphinidin derivates (∑Dp); Total peonidin derivates (∑Pn); Total cyanindin derivates (∑Cy).

VII.4. DISCUSSION

It is known that the values of polyphenols in berries from a specific cultivar and vineyard and similar TSS are highly dependent on season (Díaz-Fernández et al., 2022; Downey et al., 2006). In this work, the highest values of polyphenols compounds were reached in 2018 (the rainiest year) (Table VII.2). These results disagree with those of previous studies carried out in wetter areas where the highest amounts of polyphenols have been detected in the driest years (Rienth et al., 2016; Tarara et al., 2008). It is possible that the spring rainfall during this season contributed to a higher synthesis and accumulation of these compounds in the conditions of our trial.

At present, as in the area where this work was performed, the average of maximum and minimum temperature during the ripening are over 35ºC and 16ºC respectively. It is widely stated that temperatures above 30ºC after veraison can inhibit the synthesis and accumulation of phenolic compounds (Mori et al., 2007; Rienth et al., 2016; Tarara et al., 2008). Thus, since lower temperatures improves these events, “crop-forcing” could be as a possible solution to shift the phenological cycle to a period of more favorable temperatures as it was reported in previous researches (Gu et al., 2012; Kishimoto et al., 2022; Lavado et al., 2019; Martínez-Moreno et al., 2019; Martínez de Toda et al., 2019). In this work, when the “crop-forcing” technique was applied after flowering (F1), the ripening cycle was conducted with maximum temperatures between 32 and 35ºC and minimum around 15ºC. and a little variation respect to the current typical temperatures of control cycle of that area was reached. Therefore, a scarce impact in the content and profile of phenolic compounds were obtained. However, when the forcing crop was applied after fruit set (F2), the temperatures were between 28-31ºC, and 12-13ºC and the anthocyanins and catechins content was improved.

On other hand, Girona et al. (Girona et al., 2009) demonstrated that ‘Tempranillo’ berries had great phenological sensitivity to water stress. In this sense, according previous works carried out in ‘Tempranillo’, in this and in other areas the RDI applying water in quantities blow than those required to fully satisfy ETc needs during some periods of the vegetative cycle, increased the content of anthocyanins and catechins families in the berries (Intrigliolo et al., 2012; Uriarte et al., 2016). However, it is noticed that the significance and extent of the results obtained depended on the meteorological conditions of the season (Valdés et al., 2022). Thus, increases of anthocyanins in RI-NF respect to C-NF berries were registered in 2017 and 2019, but not in 2018, the rainiest year wettest. In 2018, the abundant rainfall recorded during the preveraison period reduced the water stress of the RI vines. Therefore, a certain level of difference in stress levels during pre and post veraison periods is necessary to find differences in the anthocyanin values of berries from the different treatments in this area of study.

When the anthocyanin profile of ‘Tempranillo’ berries was analyzed, regardless of the season and the treatment, ∑G and ∑Mv were the most abundant forms and derivates respectively in grape of ‘Tempranillo’ berries, while ∑A and ∑Cy exhibited the lowest values. Similar profile was found in ‘Tempranillo’ in different geographic areas from Spain (Díaz-Fernández et al., 2022; Moreno et al., 2021; Revilla et al., 2009). Since higher values of ∑G were found in F respect to NF berries, these compounds, were mainly responsible of the increase of total anthocyanin; however, ∑A and ∑C showed decreases in 2017, and any significant effect in the following years, our results confirm the researches of Tian & Gu (Tian & Gu, 2019), who did not report increases of ∑A and ∑C also. Acylated and coumaryl forms are sinthetyzed from the non-acylated ones (Ford et al., 1998). Therefore, it can may occurred that these last forms were not yet synthetized and accumulated in the berries at harvest of F1 and F2. Whit exception of ∑Mv in 2017 season, “crop-forcing” increased all derivates compounds, whit a higher increase in F2 than in F1. Moreover, it is noticed that the extent of variation respect to values found in C-NF berries depended of derivate considered. Thus, when the percentage of variation respect to these values were calculated, the maximum values were reached in ∑Cy (135.17% in RI-F2 in 2017), ∑Dp (120.83 % in RI-F2 in 2018) and ∑Cy (219.94% in C-F2 in 2019). However, the lowest percentages of variation were always found in ∑Mv. Specifically, decreases of 10.32% were found in F1-RI in 2017, and increases of 20.62% in 2018 and 16.43% in 2019, both in C-F1. As summer temperature rises to high temperatures values the anthocyanin biosynthetic genes are downregulated, reducing berry skin anthocyanin biosynthesis (Conde et al., 2016; Tarara et al., 2008). For instance, Tarara et al. showed that lower temperatures are associated with increases in grapevine Dp, Cy, Pt, and Pn derivates contents, but found no influence on Mv derivates concentration. These results can be explained on basis that Mv is the final product on the biosintetic route of anthocyanin compound sinthetyzed (Matus et al., 2009).

It is reported that, respect full irrigation RDI increases anthocyanin concentration in grapes by the enhanced accumulation of anthocyanins, through the stimulation of anthocyanin hydroxylation, probably by upregulating the gene encoding the enzyme F3050H (Mattivi et al., 2006). F3050H transforms Cy and Dp into Pn, Pt and Mv (Castellarin et al., 2007). According Castellarin et al. (Castellarin et al., 2007), the principal anthocyanins synthesized in the berries under water deficits are PnG and MvG, because methoxylation of Dp to produce its derivate Pt rarely occurs. However, in this work no significant changes were found in ∑Pn any year. Currently, to the best of our knowledge, this is the first work analyzing the effect of preveraison RDI in crop forced. Tian & Gu (Tian & Gu, 2019) compared the effect of different postveraison RDI and 100% ETc treatments on forced cv. Sauvignon vines on the values of some anthocyanidin derivates and total anthocyanin grown in California in 2013 and 2014. Every year, forced treatment increased the contents of these compounds respect to Control. However, in 2013, few differences were found between RDI treatments (40-80% ETc), and all of them had higher values than 100% ETc treatment, and in 2014 they did not obtain differences in any of them. This result confirms the interannual variability of the RDI strategy in forced vines.

The Figure VII.4 (a-c) shows a lighter and stronger separation of NF and F samples than C and RI ones. These results indicate that the effect of the temperature was higher the water status. According to Torres (Torres et al., 2017), the samples can be separated according to the temperature during the vegetative period of the vines. In the same way, when Arrizabalaga et al. (Arrizabalaga et al., 2018) analyzed by principal component analysis (PCA) 'Tempranillo' samples from plants grown at 24 °C / 14 °C and 28 °C / 18 °C, the first two principal components explained about 75% of the total variability and clearly separated samples according to the temperature regime.

Since Gamero et al. (Gamero et al., 2018) correlated the intensity of color of 'Tempranillo' wines whit the content of monoglucosides forms and Dp and Pt derivates and the "crop-forcing" increased the total content of these derivates, it can be expected that F1 and F2 wines to have a higher CI than those made from NF berries. These good oenological results would compensate for the loss of yield implied by the the application of Crops Forcing.

VII.5. CONCLUSIONS

This research provides evidence that "crop-forcing" causes changes in the phenolic content and profile of 'Tempranillo'. grapes in response to the decrease in temperature during the vegetative period. Thus, the timing of "crop-forcing" was applied a high impact on these results. Under this study conditions, forcing after fruit set has a greater impact on the phenological characteristics of the grapes than forcing after flowering. Berries from forced vines showed, in general, higher values of polyphenols, catechins and anthocyanins. The increase in anthocyanin content is caused by the increase in the monoglucoside forms, while the acetylated forms did not change their content. All the anthocyanidin derivates increased their value in berries from forced vines. The impact of water stress was less consistent than the impact of "crop-forcing", with a large interannual variability.

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VIII. CAPÍTULO 5.

FORCING VINE REGROWTH UNDER DIFFERENT IRRIGATION STRATEGIES: EFFECT ON POLYPHENOLIC COMPOSITION AND CHROMATIC CHARACTERISTICS OF ‘TEMPRANILLO’ WINES GROWN IN SEMI-ARID CLIMATE.

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VIII.ABSTRACT

High temperatures during grape ripening have negative effects on the phenolic composition of the grapes. The effects of climate change in warm areas aggravate this problem. One of the immediate effects is the asynchrony between the dates of technological and maturity of grapes. This is important because the quality and colour stability of red wines are directly related to content and distribution of phenolic compounds. A novel alternative proposed to delay grape ripening, and make it coincide with a seasonal period more favourable for the formation of phenolic compounds, is the forcing bud. This consists of severe green pruning after flowering, when the buds of the following year have already differentiated. In this way, the buds formed during the same season are forced to sprout, initiating a new delayed cycle. The aim of the present work is to study the effect on the phenolic composition and colour of wines elaborated from control (grown under conventional practices) and forced vines irrigated with different irrigation strategies. Thus, four types of wines were analysed: wines from vines full irrigated (C), grown under conventional (NF) and forced (F) vines (C-NF and C-F), and wines from vines subjected to regulated deficit irrigation (RI), grown under NF and F techniques (RI-NF and RI-F). The trial was carried out in an experimental vineyard of the ‘Tempranillo’, located in a semi-arid area (Badajoz, Spain) in the 2017-2019 season. The wines (four by treatment) were elaborated and stabilised according to the classic methodologies for red wine. All wines had the same alcohol content and malolactic fermentation was not carried out in any of them. Anthocyanin profiles were analysed by HPLC and total Polyphenolic content, anthocyanins, cathechins, the contribution to colour due to copigmented anthocyanins and chromatic parameters by spectrophotometric techniques. Although a significant effect of year was found for almost all parameters analysed, a general trend to increase in F wines was found for most of them. It is noticed that the anthocyanin profile of F wines was different from the C wines, especially in delphinidin, cyanidin, petunidin and peonidin content, thus the anthocyanin profile was modified. These results indicate that by using the forcing technique, it was possible to increase the polyphenolic content by ensuring that the synthesis and accumulation of these substances occurred at more suitable temperatures.

VIII.RESUMEN

Las altas temperaturas durante la maduración de la uva tienen efectos negativos sobre la composición fenólica de las uvas. Los efectos del Cambio Climático en las zonas cálidas agravan este problema. Uno de los efectos inmediatos es la asincronía entre las fechas de maduración tecnológica y madurez de las uvas. Esto es importante porque la calidad y la estabilidad del color de los vinos tintos están directamente relacionadas con

el contenido y la distribución de los compuestos fenólicos. Una alternativa novedosa propuesta para retrasar la maduración de la uva, y hacerla coincidir con un periodo estacional más favorable para la formación de compuestos fenólicos, es el forzado de la yema. Consiste en una poda en verde severa tras la floración, cuando las yemas del año siguiente ya se han diferenciado. De esta forma, las yemas formadas durante la misma temporada son forzadas a brotar, iniciándose un nuevo ciclo retardado. El objetivo del presente trabajo es estudiar el efecto sobre la composición fenólica y el color de vinos elaborados a partir de cepas testigo (cultivadas bajo prácticas convencionales) y forzadas, regadas con diferentes estrategias de riego. Así, se analizaron cuatro tipos de vinos: vinos procedentes de viñas regadas completamente (C), cultivadas bajo técnicas convencionales (NF) y forzadas (F) (C-NF y C-F), y vinos procedentes de viñas sometidas a riego deficitario regulado (RI), cultivadas bajo técnicas NF y F (RI-NF y RI-F). El ensayo se realizó en un viñedo experimental de ‘Tempranillo’, situado en una zona semiárida (Badajoz, España) en la campaña 2017-2019. Los vinos (cuatro por tratamiento) se elaboraron y estabilizaron siguiendo las metodologías clásicas para vino tinto. Todos los vinos tenían la misma graduación alcohólica y no se realizó fermentación maloláctica en ninguno de ellos. Se analizaron los perfiles antociánicos mediante HPLC y el contenido polifenólico total, los antocianos, las catequinas, la contribución al color debida a los antocianos copigmentados y los parámetros cromáticos mediante técnicas espectrofotométricas. Aunque se encontró un efecto significativo del año para casi todos los parámetros analizados, se observó una tendencia general al aumento en los vinos F para la mayoría de ellos. Se observa que el perfil antociánico de los vinos F era diferente del de los vinos C, especialmente en el contenido de delfinidina, cianidina, petunidina y peonidina, por lo que el perfil antociánico se modificó. Estos resultados indican que, utilizando la técnica del forzado, fue posible aumentar el contenido polifenólico haciendo que la síntesis y la acumulación de estas sustancias se produjeran a temperaturas más adecuadas.

VIII.1. INTRODUCTION

Grape quality for winemaking is a concept linked to a good balance of primary metabolites such as sugars and organic acids (e.g., tartaric and malic acid) and secondary metabolites such as aromas and phenolic compounds (e.g., tannins, hydroxycinnamic acids, stilbens, flavanols, flavonols and anthocyanins) that control the organoleptic properties of the wine (Jackson & Lombard, 1993). The climate is a determining factor of the physico-chemical characteristics of the grapes and, consequently, of the wines obtained (Mira de Orduña, 2010). Air temperature, thermal oscillation, CO_2, content in the air and solar radiation play an essential role, as they condition the processes of synthesis, transport and accumulation of primary and secondary metabolites during the grape ripening (Jones et al., 2022; Van Leeuwen et al., 2019; Van Leeuwen & Destrac-Irvine, 2017). Since the last decades of the last century, that balance has been altered by climate change characterised by Global Warming associated with temperature increases over more and more long periods, increasing periods of drought, and increases in CO_2 and ultraviolet radiation (Moriondo et al., 2013; Sadras & Moran, 2012). High temperatures display various direct and indirect effects on the physiology of the grapevine fruit depending on the developmental stage. It is known that high temperatures accelerate the accumulation of Total Soluble Solids (Petrie & Sadras, 2008) and also affect the content of acids present in the berries. Tartaric acid, is generated early in the ripening period and is less sensitive to high temperatures. However, malic acid, decreases drastically with increasing temperatures because disappears by combustion throughout this period (Poni et al., 2018). These facts lead to a decrease in the total acidity of grapes (Buttrose et al., 1971; Kliewer, 1971). In addition, increasing temperatures raise potassium content (Tarara et al., 2008). This decrease in acidity coupled to the increase in potassium lead to values lead to values close to or even higher than 4 increases in the pH of wines. These values cause unbalanced and unstable wines in microbiological, physical-chemical and sensory aspects (Sigler, 2008)

Phenolic compounds are important determinants of sensory characteristics of wines (colour, astringency and bitterness, among others). High temperatures modify the total contents and the distribution of these compounds in grapes (Arrizabalaga-Arriazu et al., 2021), with the consequent modification of the sensory characteristics of the wines made from them. It is reported that daytime temperatures above 35 °C inhibit the regular biosynthesis of anthocyanins, the main compounds responsible for the colour of red wines (Spayd et al., 2002). Anthocyanins compounds are composed of a glycoside, which can be esterified by a phenolic acid, generically caffeic or p-coumaric acid (p-coumaric anthocyanins), or acetic acid (acetylated anthocyanins). Cyanidin, peonidin, petunidin, delphinidin and malvidin are the main anthocyanidins identified in Vitis vinifera L. Their synthesis and accumulation start at veraison. It is known that these subtances are also temperature sensitive (Downey et al., 2006; Monagas et al., 2005). Previous works reported that high temperatures decreased delphinidin, cyanidin, petunidin and peonidin content and do not affect malvidin content (Mori et al., 2007; Spayd et al., 2002; Tarara et al., 2008). Moreover, it is not only the anthocyanin content that is responsible for wine colour; co-pigmentation can account for 30-50% of the colour in young wines (Boulton, 2001). Co-pigmentation in wine results from molecular interactions between anthocyanins and other organic molecules called cofactors, which form molecular associations or complexes. The most common cofactors include a variety of compounds, such as phenolic acids, flavonoids and, in particular, derivatives of the flavonol and flavone subgroups (Rustioni et al., 2012). The amount and distribution of flavanols are also sensitive to climatic conditions. According to Tate (2001), high temperatures are associated with an increase in catechins, and the increase in CO_2 associated with Climate Change leads to an increase in tannin concentration. Rienth et al. (2016) suggested that high temperatures during the first phase of berry growth could be at the detriment of the synthesis of these compounds while cool day temperature stimulates their synthesis.

On the other hand, soil water availability is also a critical factor for vine performance and wine composition (Intrigliolo & Castel, 2010b). The water status is factor affects the composition of the grapes at harvest and, consequently, modify the sensory characteristics of the wines (Gamero et al., 2014; Moreno et al., 2022). In arid and semi-arid environments, irrigation is a major tool to regulate soil water availability to vines. Due to the increase in periods of drought and the decreasing availability of water above mentioned, Regulated Deficit Irrigation (RDI) is a widely used strategy to reduce the possible negative impact of irrigation on grapes, improving grape composition and resulting in water savings (Pérez-Alvarez et al., 2021). Regulated deficit irrigation (RDI) in vines has been used to improve berry and wine quality (Romero et al., 2013). Deficit irrigation consists in applying water rates to replace only part of the potential vine evapotranspiration either during the whole season or only during some phenological periods previously established. to control growth and reproductive development and/or improve water use efficiency (Intrigliolo & Castel, 2010b). Previous research reported that respect to conventional irrigation, the RDI techniques modified the anthocyanin profile, increased the anthocyanin content of the grapes and the corresponding wines and improved the sensory characteristics of 'Tempranillo' from vines grown in different semi-arid climate regions of the Iberian Peninsula (Gamero et al., 2014; Intrigliolo & Castel, 2010a, 2011; Santesteban et al., 2011; Valdés et al., 2009). In addition, it has been found that the effect of RDI is highly dependent on the timing of the water deficit (Girona et al., 2009; Intrigliolo et al., 2012; Valdés et al., 2009). Torres et al., (2017) even proposed that the adaptation to climate change in south Mediterranean Europe might be plausible with the optimization of timing of water deficit and the appropriate selection of clones.

The ripeness of grapes at the harvest time is one of the most important parameters for obtaining high quality red wines. The coupled accumulation of primary and secondary metabolites during maturation aids in defining fruit quality in red Vitis vinifera cultivars (Vanderweide et al., 2018). In this sense, the technological (adequate concentrations of sugars and acids) and the corresponding phenolic maturity (determined by the optimal concentration and profile of phenolic compounds) are not simultaneously reached, on the contrary they tend to separate depending on some factors as cultivar, terroir, adverse weather conditions, water availability and cultural practices. Besides, this divergence is increasing as a consequence of the Climate Change (larger quantities of CO_2, less rain, and higher temperatures) (Lopez et al., 2007; Meléndez et al., 2013). When Parra et al. (2010) investigated the impact of predicted Climate Change (elevate CO_2 and temperature, and moderate drought) on grapevine ripening, concluded that Climate Change shortened the time between grape veraison and full maturity, and at harvest time, many of the grape quality parameters were affected. Under current climatic conditions, the grapes reach high total soluble solids and very low total acidity before achieving phenolic maturity. the warmer conditions over ripening, lead to the production of unbalanced wines with high alcohol levels, low acidities, a modified varietal aroma and a lack of color (Martinez De Toda et al., 2019). These changes affect microbiology, winemaking progress, physico-chemical composition and organoleptic characteristics of the wine (Jones et al., 2005; Mira de Orduña, 2010; Palliotti et al., 2014).

Take account the above mentioned, to maintain and/or improve the characteristics of the wines typical of each area, and in order to preserve the viability of the vineyards is necessary to adapt them to these new climatic conditions. Some viticultural long-term strategies may offer a natural solution to mitigate the negative effects of global warming in viticulture. Decisions concerning vineyard topography variables, as well as rootstock, variety, clone, training system, and row orientation and slope selection may be combined (Díaz-Fernández et al., 2022; Morales-Henríquez et al., 2022; Muñoz-Organero et al., 2022; Torres et al., 2017).

Besides, vinegrowers can implement some strategies in the short-term. They consist of establishing techniques capable of delaying grape ripening and, therefore, to move the harvest date to periods of more favourable temperatures. Several techniques have been investigated for this purpose: reducing leaf area (Martínez De Toda & Balda, 2013; Petrie & Clingeleffer, 2006; Poni et al., 2009); bunch thinning or increasing vine load (Keller, 2010; Parker et al., 2014). These techniques allow harvest delays of a maximum of two weeks, with little temperature difference. The late pruning, which consists of delaying the time of pruning usually carried out in winter and carrying it out in spring, proved to be an effective technique in counteracting the asynchrony between the time of technological and phenological maturity due to the high summer temperatures. In addition, the plant is protected from possible spring frosts (Friend & Trought, 2007; Frioni et al., 2016; Moran et al., 2017).

A recently proposed alternative technique is “crop-forcing”. This technique consists of severe green pruning after flowering when buds have already differentiated the following year. In this way, the flowering of the buds formed that same season is forced, and the whole phenological cycle is delayed, the beginning of the ripening cycle and the harvest date. This technique was studied by Gu et al. (2012), who managed to shift the harvest date significantly. Other authors have achieved similar shifts (Kishimoto et al., 2022; Lavado et al., 2019; Martínez-Moreno et al., 2019; Martínez de Toda et al., 2019). These studies show that these techniques increased the acidity values and those corresponding to phenolic compounds although it caused decreases in yield. However, the effects of “crop-forcing” and their interaction with irrigation strategy on the acid, phenolic and chromatic characteristics on the wines are scarcely known. Another question is to investigate the effect of the technique over the years and how the effect changes according to the meteorology of the year in question.

This work aims to study for three consecutive years the effect of “crop-forcing” and the interaction with a pre-veraison RDI strategy on the physical-chemical characteristics, and in greater depth on the anthocyanin profile, of wines produced. In addition, the aim is to observe the influence of different meteorological circumstances on the effect of the techniques applied.

VIII.2. MATERIALS AND METHODS

VIII.2.1. LOCATION AND DESCRIPTION OF THE VINEYARD

The study was carried out in an experimental vineyard located at Badajoz, Extremadura, Spain (38º 51´ N; 6º 40´ W; 198 m) in a ‘Tempranillo’ vineyard (Vitis vinifera L.) grafted on Richter 110 rootstock, trained as bilateral cordons in a vertical trellis system with a drip irrigation system of 8 L/h per vine. All the vines were winter pruned to six spurs and two buds per spur. The rows are E-W oriented and row and vine spacing were 2.5 m and 1.2 m, respectively. The soil is alluvial, with a loam to sandy texture, slightly acidic, and lacking in organic matter. Soil depth is greater than 2.5 m and with low stone content. The

VIII.2.2. TREATMENTS AND EXPERIMENTAL DESIGN

The experiment design was a split-plot with four replicacions (Table VIII.1). The principal factor consisted of a treatment with “crop-forcing” techniques (F). “Crop-forcing” applied 22 days after anthesis (May 18, 2017; May 29, 2018; May 20, 2019). This treatment was compared with a treatment without forcing techniques (NF) with vines grown under conventional practices (just winter pruning). "Crop-forcing" consisted of hedging the growing shoots to seven nodes and removing all the summer laterals, leaves and clusters with scissors to force the bursting of the primary buds developed in the current season. As a secondary factor, two irrigation treatments were established. A treatment with no water stress (C), supplying water to maintain a midday stem water potential and a pre-veraison deficit irrigation (RI).

Table VIII.1. Summary of the treatments applied in the study.

	No "Crop-forcing" (NF)	**"Crop-forcing" (F)**
Full-irrigated (C)	C-NF	C-F
Deficit irrigation (RI)	RI-NF	RI-F

The vines water requirements were calculated based on the crop evapotranspiration (ETc) using the crop coefficient (Kc) recommended by FAO for these latitudes for the NF treatments. For the F1 and F2 treatments, ETc was calculated directly on a weighing lysimeter (Picón-Toro et al., 2012) with two crop forzing vines, integrated in the study plot. Irrigation started when a threshold value of Ψsmd of -0.6 MPa. Irrigation was applied five to six times per week, measuring the amount of water applied to each subplot through volumetric water meters and maintaining irrigation until the beginning or middle of October. The meteorological data come from a weather station belonging to the Extremadura irrigation advisory network (REDAREX) located 100 m from the plot. The experimental unit consists of 6 rows per 18 vines. Ten central vines of the four central rows are for sampling and harvest.

All treatments were hand-harvested with 23 to 24 °Brix, a common harvesting criterion for red grape varieties in this area. The average of TSS of the berries from the four elementary plots by treatment was considered. All the clusters of 10 vines per experimental plot were counted and weighed (40 vines per treatment).

VIII.2.3. WATER STATUS

The stem water potencial (SWP) was measured with a pressure chamber (Soil Moisture Corp., Model 3500, Santa Barbara, CA, USA), following the procedure described by Martí et al., (2015), using leaves on the north side of the trellis (in the shade), close to trunk level and wrapped in aluminium foil at least 2 h before data recording. Measurements were taken weekly on one leaf per vine and in two plants per subplot.

In C-NF treatment, SWP values remained -0.7, -0.5 and -0.7 MPa during the fruit set - harvest period in 2017, 2018 and 2019 respectively. In RI-NF treatment, values remained

-1.0, -0.8 and-0.9 MPa, in C-F treatment, values remained -0.7, -0.6 and -0.6 MPa and in RI-F treatment, values remained -0.9, -0.8 and -0.7 MPa.

VIII.2.4. WEATHER CONDITIONS

The area has a Mediterranean climate with a mild Atlantic influence, dry and hot summers, with high daily radiation and evaporative demand. We obtained agrometeorological data from a station close to the vineyard (100 m) with the characteristics described in Martí et al., (2015).

Table VIII.2. Weather conditions on budburst to harvest cycle and anual and forcing and harvest dates.

Año	Tratamiento	Tª Max (ºC)	Tª Min (ºC)	Oscilación térmica	Días Tª > 35ºC	Lluvias (mm)	Forcing date	Harvest date
2017	**NF**	35.9	16.4	19.5	32	2.6		22 Aug
	F	31.0	12.0	19.0	2	0.0	07 Jun	17 Oct
2018	**NF**	35.7	17.0	18.7	17	1.6		27 Aug
	F	27.9	12.7	15.2	4	76.8	18 Jun	29 Oct
2019	**NF**	34.3	16.3	18.0	15	0.0		27 Aug
	F	29.6	13.1	16.5	0	9.9	03 Jun	15 Oct

NF: No “crop-forcing” treatment; F: “Crop-forcing” treatment

VIII.2.5. YIELD PARAMETERS

All treatments were harvested manually at 23–24 °Brix, a common criterion for picking red grape varieties in this area. The average TSS of the berries from the four elementary plots was considered for each treatment. All the clusters of 10 grapevines per experimental plot were weighed (40 grapevines per treatment). Samples of 100 g of berries per plot were weighed fresh.

VIII.2.6. MICROVINIFICATIONS

Samples of about 60 kg from each plot, thus four wines per treatment were obtained each year. ‘Tempranillo’ grapes from the different experimental blocks were mechanically crushed and destemmed (Modelo Micra/15, Agrovin, España). Must were undertaken immediately after crushing and analyzed. The mash from each experimental block was fermented in 50-L steel tanks at 22 – 24 °C. Initially sulfur dioxide (SO_2) was added at 50 mg/kg and Saccharomyces cerevisiae (Viniferm 3D, Agrovin, Spain) was added at 25 g/hL. Fermentation was monitored daily, measuring density and total phenolic index (TPI) by spectrophotometric absorbance at 280 nm (UV/visible UV-1700 spectrophotometer, Shimadzu, Shimadzu Corporation, Kyoto, Japan). During vatting, fermenting must vines were punched twice per day. The musts were racked when the increase in TPI levelled off. Once fermentation was completed, the wines were settled at 4 °C and sulphur content was then added to the wine to achieve 30 mg·L-1 of free SO2. Finally, the wines were bottled and stored at 15 °C until analysis, without initiating malolactic fermentation.

VIII.2.7. ANALYTICAL METHODS

VIII.2.7.1. General Oenological Parameters of must and wines

The Total Soluble Solids (TSS, ºBrix) of must was determined by refractometry (RE40D, Mettler Toledo, Greifensee, Switzerland). Wine analysis was carried out four months after bottling. In must and wines, analysis for pH and total acidity (TA, as g tartaric acid/L), were made according to OIV (Organisation Internationale de la Vigne et du Vin) methods (1990) in an authomatic titrator (T50, Mettler Toledo, Greifensee, Switzerland) according to ECC formal methods ((Commission Regulation No. 2676/90, 1990). Tartaric acid (TAR, g/L) and malic acid (MAL, g/L) were analyzed following the Rebelein and the enzymatic reaction methods, respectively, in an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

VIII.2.7.2. Nitrogen Parameters

Free Amino Nitrogen (FAN, mg/L) and Amonium (NH_4^+, mg/L) content were enzymatically analyzed according to ECC formal methods (ECC, 1990) using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

VIII.2.7.3. Wine Phenolic Compounds

Wine Total polyphenol content (TPP, mg/L) was determined according to Singlenton & Rossi (1965). Catechins concentration (Cat, mg/L) was determinated according to Broadhurst & Jones (1978) and Tannins (Tan, mg/L) following the methods described by Sarneckis et al. (2006) using (+)-catechin as standard in these last two determinations. Total anthocyan was determinated from the amount of all individual compounds as determined by HPLC Furthermore, the copigmented anthocyanins contents (%C-Ant) were determined using the colorimetric effects of acetaldehyde and SO2 on different forms of anthocyanins (Boulton, 2001).

VIII.2.7.4. Wine Chromatic Characteristics

Samples were filtered through Millipore-AP20 filters (Bedford, MA, USA) prior to the color determination. Color intensity (CI) was calculated as the sum of absorbance at 420, 520, 620 nm, and Hue as the ratio of the absorbance at 420 nm and 520 nm. From absorbance values at 420, 520 and 620 nm, percentage of yellow (Yellow %), percentage of red (Red %) and percentage of blue (Blue %) were determined according to the Glories method (Glories, 1984): Yellow % = 100% [Abs 420 / (Abs 420 + Abs 520 + Abs 620)]; Red % = 100% [Abs 520 / (Abs 420 + Abs 520 + Abs 620)]; Blue % = 100% [Abs 620 / (Abs 420 + Abs 520 + Abs 620)]. Absorbance measurements were taken using a Shimadzu spectrophotometer with data system control software (Shimadzu Corporation, Kyoto, Japan).

VIII.2.7.5. Anthocyanin Compounds by HPLC

HPLC separation, identification and quantification of anthocyanins were performed on an Agilent 1200 LC system (Agilent Technologies, Palo Alto, CA) equipped with a degasser, quaternary pump, column oven, 1290 infinity autosampler, UV-Vis diode-array detector (DAD) and the Chemstation software package for LC 3D systems (Agilent Technologies) to control the instrument and for data acquisition and analysis. Separation was performed in a Kromasil® 100-5-C18 (250 x 4.6 mm) column (AkzoNobel, Bohus, Sweden). The analysis was carried out as described in Natividade et al. (2013) with slight modifications to improve peak resolution. For the analysis of anthocyanins, a 10 mL extract was injected directly into the HPLC and the column was maintained at 40 °C. The mobile phase consisted of a gradient mixture of a solvent A (0.85% phosphoric acid solution) and solvent B (acetonitrile), with a flow rate of 1 mL·min-1. The gradient was started with 100% of solvent A and adjusted for 90% of solvent A and 10 % of solvent B at 10 min; 85% of solvent A and 15% of solvent B at 20 min; 80% of solvent A and 20 % of solvent B at 30 min; 67 % of solvent A and 33 % of solvent B at 40 min; 65% of solvent A and 35% of solvent B at 45 min; and 100% of solvent B at 55 min. Absorbance at 520 nm was measured by the DAD detector for identification of anthocyanins by their elution order and by comparison to the retention times of commercially available standards (malvidin-3-glucoside, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside (Extrasynthese, Genay, France)). The anthocyanins present in wines were identified as the monoglucoside forms (G) of malvidin (MvG), petunidin (PtG), delphinidin (DpG), peonidin (PnG) and cyanidin (CyG); the acetylglucoside forms (MvA, PtA, DpA, PnA and CyA), and the p-coumaroyl-glucoside forms (MvC, PtC, PnC and CyC). All measures were expressed in mg malvidin glucoside /L.

VIII2.8. STATISTICAL DATA ANALYSIS

Normality and homogeneity of variances was tested using Shapiro-Wilk’s and Barlett’s test respectively. When the normality and homogeneity of variances were verified, yield and berry weight data were analysed by one-factor ANOVA and the Tukey test. Differences between means were considered statistically significant when $p < 0.05$. Must and wine data were subjected to analysis of variance (MANOVA) to investigate the effect of “crop-forcing”, “irrigation” and their interaction on each parameter evaluated selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons. The interaction between effects was evaluated by calculating the least-squares means (LS means) selecting $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for significance of comparisons and the Tukey test as Post Hoc tests for parametric samples. On the other hand, when the normality and homogeneity of variances were not verified, non-parametric tests were carried out and the Kruskal-Wallis test (alternative to one-way ANOVA) and multiple comparasion p values (alternative to post-hoc pairwise comparisons). Differences between means were considered statistically significant when $p < 0.05$. Multiple factor analysis (MFA) was applied. The weather conditions were used as suplementary variable, while yield parameter, and acid, phenolics and chromatics of wine as active variables. Futhermore, principal component analysis (PCA) was performed to discriminate among treatments in basis to the values of Mv, Pt, Dp, Pn and Cy. These lasts statistical test was performed with XLSTAT-Pro 201610 (Addinsoft, 2009, Paris, France).

VIII.3. RESULTS

The effect of the year on agronomical parameters and all the must and wine composition parameters studied was highly significant (data not shown), suggesting that the effect of treatments, specially the caused by the irrigation regime, on these parameters was different among seasons For this reason, the results of each harvest are analysed separately in this work. To minimise compositional differences between treatments associated with TSS level, a common TSS between treatments and year was considered.

VIII.3.1. YIELD PARAMETERS

Table VIII.3 shows the yield (Kg/ha) and berry weight (BW, g) of the control treatment (C-NF) in 2017-2019 period. Yields were above 12000 on 2017 and 2018 and close to 24000 in 2019 in the control treatment (C-NF) and respect to this, decreases were found on the rest of treatments all years. Table 3 indicates the corresponding decreases (in percentage) respect to C-NF. Significant decreases always were found in F treatments. In 2017, the decreases were higher in RI (66%) than in C (29%) while in 2018 were similar (51 and 52% respectively), and finally, in 2019 slightly higher in RI-F (48%) than in C-F (39%). The loss of yield provoked by RDI reached 42%, 18% and 36% in 2017, 2018 and 2019 respectively with significant effect in 2017 and 2019. Thus, respect to C-NF, the highest decreases of yield corresponded to RI-F all years of the trial, while the lowest to C-F, in 2017 and in RI-NF the following years. On other hand, it is noticed that in C-F the reduction of yield was greater as the number of “crop-forcing” applications increased.

As regarding BW, C-F, RI-NF and RI-F berries were smaller than C-NF with values of 1.8 in 2017, 1.9 in 2018 and 2.3 g in 2019. The decreases were slight lower in C-F than in RI-F: 44 and 56% in 2017, 37 and 47% in 2018 and 39 and 48% in 2019 respectively while the lowest decreases were registered in RI-NF berries (28%, 37% and 35%).

Table VIII.3. Effect of “crop-forcing” and water status on yield (kg/ha) and berry weight (g).

	Year			**%Decrease respect to C-NF**		
			C-NF	**C-F**	**RI-NF**	**RI-F**
Yield (kg/ha)	**2017**	12952.5	0a	29 b	42 c	66 d
	2018	12428.3	0a	51 b	18 a	52 b
	2019	23735.8	0a	58 c	38 b	60 c
Berry weight (g)	**2017**	1.8	0a	44 ab	28 a	56 b
	2018	1.9	0a	37 ab	32 ab	47 b
	2019	2.3	0a	39 ab	35 ab	48 b

Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and deficit irrigation) and RI-F (forcing and deficit irrigation). Each value represents the mean of 8 samples (4 blocks, 2 replicates)

Statistical analysis (with yield and berry weight values): ANOVA. Different letters indicate the existence of statistically significant differences between treatments.

VIII.3.2. MUST COMPOSITION

Table VIII.4 shows the acid, nitrogen, and phenolic composition of the initial musts from the different treatments applied to the vines and the results of the MANOVAs performed to analyse the statistical significance of the effect of forcing (F), irrigation strategy (I), and their interaction on these parameters in 2017, 2018 and 2019 years. In general, no statistical significance was observed in the F*I interaction (only on 3 occasions). Although the statistical significance of F and I, depended on the parameter analysed and the year considered it should be noted that forcing had a more significant number of times statistical significance (13) than the irrigation strategy (8).

As it was cited above and since the aim of this work, was to observe the effect of the treatments on wines of similar alcoholic strength, the musts showed similar values of total soluble solids (TSS) in all years and all treatments

The results obtained do not allow to conclude a general behaviour about the effect of F on the tartaric acid (TAR) values in the musts because a high variability among years studied was observed. In 2017, an interaction F*I was detected because F caused a significant increase (12.0%, $p<0.05$) in the contents of the C musts (CF vs C-NF) while no effect was detected in RI. The following year, all musts presented similar values and, finally, in 2019, when F were compared with NF must, similar decreases were found in C and RI strategies.

On the other hand, regardless pruning treatment, irrigation strategy did not have any effect any year and RI, showed similar TAR values to C musts in all years.

As regarding malic values, increases were found in C-F and RI-F vs C-NF and RI-NF respectively all years. It is noticed that the increases were higher in RI strategy as presented in Table 4: Thus, in RI-F-2017 respect to RI-NF musts an increase of 267% was registered while in CF respect to C-NF the increases reached 30%. The same trend was observed in 2018, with increases of 53% and 6% respectively for these treatments and 105% and 71% and in 2019. In 2017 a significant interaction F*I was found because the value of C-NF was higher than the RI-NF ones (233% increase), but similar values were found in C-F and RI-F. In 2018 C-NF and C-F were higher than RI-NF and RI-F respectively, and finally in 2019 similar values were found in C and RI must. As a consequence of these results, the RI-NF musts had the lowest malic acid values in the three years of the trial. It is clear that the values found in F mainly depended of the changes caused by RI treatment in the year considered.

The variations in tartaric acid and especially malic acid values modified total acidity (TA) values. Thus, in 2017 and 2019, higher TA values were observed in the F musts than in the NF musts ($p<0.001$ in 2017 and $p<0.01$ in 2019). In 2018, and as a logical consequence of the values of both MAL and TAR, the C-NF and C-F musts presented similar values of TA (7.0 and 7.3, respectively), however, the RI-NF values (4.8) was very far of the RI-F (6.0) apart. Moreover, TA of the RI musts was significantly lower than those of the corresponding C musts. As a consequence of the above results, the C-F and RI-NF musts had the highest and the lowest TA values respectively in the three years of the study. Finally, in the 2017 and 2018 vintages, the pH of the F musts was significantly lower than that of the corresponding NF musts. Moreover, RI musts, especially NF, showed higher pH values than the corresponding C. Consequently, in those vintages, the highest pH values corresponded to RI-NF musts (3.9 and 3.7 in 2017 and 2018) and the lowest to C-F musts (3.6 and 3.4 in those same years). However, in 2019, all musts presented similar pH values.

Regarding the nitrogen compounds, regardless of the treatment considered, it is noteworthy that as the study progressed, a gradual decrease in nitrogen compounds (both inorganic and organic) values was observed. In 2017 and 2018, the NH_4^+ values of the F musts were lower than those of NF. In both irrigation strategies, the decreases were around 50%. In 2019 no differences were found. In none of the years did the use of RDI cause significant changes in the values of this parameter. Finally, the free amino nitrogen (FAN) contents of the musts were modified in a very similar way to those of NH4 and thus, the mean values of this parameter of the F musts in 2017 and 2018 (144.6 and 85.4) were significantly lower than those recorded in the NF (65.8 and 53.1) and no significant differences were observed in the PAN values recorded in the musts of the different treatments in 2019.

Table VIII.4. Effect of "crop-forcing" and water status on composition of cv. 'Tempranillo' grape juice

		C-NF	**C-F**	**RI-NF**	**RI-F**	***FxI***	**NF**	**F**	***F***	**C**	**RI**	***I***
TSS	**2017**	24.8	23.7	24.0	23.2	*n.s.*	24.4	23.5	*n.s.*	24.3	23.6	*n.s.*
(ºBrix)	**2018**	25.2	24.1	23.8	22.8	*n.s.*	24.5	23.5	*n.s.*	24.7	23.3	*n.s.*
	2019	22.5	23.2	23.6	23.9	*n.s.*	23.0	23.5	*n.s.*	22.8	23.7	*n.s.*
Acid composition												
TAR	**2017**	3.9 b	4.4 a	4.1 ab	4.2 ab	*	4.0	4.3	**	4.2	4.2	*n.s.*
(g/L)	**2018**	4.6	4.8	4.5	4.4	*n.s.*	4.5	4.6	*n.s.*	4.7	4.5	*n.s.*
	2019	5.0	4.6	5.2	4.7	*n.s.*	5.1	4.7	**	4.8	4.9	*n.s.*
MAL	**2017**	2.0 b	2.6 a	0.6 c	2.2 ab	*	1.3	2.4	***	2.3	1.4	***
(g/L)	**2018**	3.1	3.3	1.9	2.9	*n.s.*	2.5	3.1	*	3.2	2.4	**
	2019	2.1	3.6	1.8	3.7	*n.s.*	2.0	3.6	***	2.8	2.8	*n.s.*
TA	**2017**	4.4	5.6	3.7	4.9	*n.s.*	4.1	5.3	***	5.0	4.3	**
(g/L)	**2018**	7.0	7.3	4.8	6.0	*n.s.*	5.9	6.7	*n.s.*	7.1	5.4	**
	2019	6.8	7.2	5.3	7.0	*n.s.*	6.1	7.1	**	7.0	6.2	*
pH	**2017**	3.9	3.6	3.9	3.6	*n.s.*	3.9	3.6	***	3.7	3.7	*n.s.*
	2018	3.5	3.4	3.7	3.4	*n.s.*	3.6	3.4	**	3.4	3.5	*
	2019	3.4	3.5	3.6	3.5	*n.s.*	3.5	3.5	*n.s.*	3.4	3.5	*n.s.*
Nitrogen composition												
NH_4^+	**2017**	125.0	71.8	121.8	68.0	*n.s.*	123.4	69.9	***	98.4	94.9	*n.s.*
(mg/L)	**2018**	81.8	40.9	93.8	47.7	*n.s.*	87.8	44.3	**	61.3	70.7	*n.s.*
	2019	67.7	58.2	82.0	54.5	*n.s.*	74.8	56.3	*n.s.*	62.9	68.3	*n.s.*
FAN	**2017**	148.3	68.8	141.0	62.8	*n.s.*	144.6	65.8	***	108.5	101.9	*n.s.*
(mg/L)	**2018**	70.3 b	52.8 b	100.5 a	53.3 b	*	85.4	53.1	***	61.5	76.9	*
	2019	57.7	57.0	91.0	56.4	*n.s.*	74.3	56.7	*n.s.*	57.3	73.7	*n.s.*

Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and decit irrigation) and RI-F (forcing and deficit irrigation). Each value represents the mean of 8 samples (4 blocks, 2 replicates).

Parameters: Total Soluble Solids (TSS), Tartaric acid (TAR), Malic acid (MAL), Titrable acid (TA), pH, Free Amino Nitrogen (FAN) and Amonium (NH_4^+).

Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VIII.3.3. WINE COMPOSITION

VIII.3.3.1. Acid composition

Table VIII.5 shows the values of the alcohol content (AD, % v/v), TAR, MAL, TA and pH at the end of fermentation and the results of the MANOVAs carried out.

The statistical significance of the F*I interaction was only observed 5 times and the effect of F and I also depended on the parameter analysed and on the year in question. As in the case of musts, forcing was statistically significant more times (7) than the irrigation strategy (2).

In 2017 and 2018, all wines showed similar values of (AD), while in 2019, contrary to expected, since the initial musts had similar TSS values, the AD of the C-F (12.8) and RI-F (13.2) wines was lower than that of the C-NF (14.0) and RI-NF (14.2) wines.

As Table VIII.5 reflects, a general trend to increase was found in the values of TAR and MAL in F respect NF wines. The significance of the effect depended on the year considered and, in any case, for a given year, of the irrigation strategy employed in the vineyard. Thus, in 2018 higher values of MAL were registered when RIF were compared to RI-NF only, and the following year when C-F was compared to C-NF. As consequence of those results, by general TA values were higher in F than in NF wines with the most noticeable effect on the RI wines. In this way, it has been noted that a significant interaction F*I was found in 2018 and 2019, because only the value of RI-F wines (7.5 and 7.8 in 2018 and 2019) was higher than the corresponding RI-NF (6.2 and 7.5, respectively). Finally, forcing decreased the values of pH in 2018 only.

On the other hand, in agreement with the trend registered in the musts, no differences were observed on TAR values of C and RI (F and NF) wines in any year. Besides, the differences in MAL values found between musts C and RI were hardly reflected in the wines, and only the C-NF value was significantly higher than the RI-NF one in 2018. Finally, irrigation did not modify eh values of TA in 2017, next year caused decreases in C wines, NF and F wines, and in 2019 in NF wines only. As consequence, in 2018 pH of RI wines was lower than C wines (regardless pruning) but in 2019 only significant decreases were registered in RI-NF vs C-NF only.

Table 5. Effect of "crop-forcing" and water status on composition of 'Tempranillo' wines.

		C-NF	C-F	RI-NF	RI-F	*FxI*	NF	F	*F*	C	RI	*I*
AD (%)	**2017**	14.8	13.8	14.0	13.5	*n.s.*	14.4	13.6	*n.s.*	14.3	13.7	*n.s.*
	2018	14.9	14.1	14.3	13.3	*n.s.*	14.6	13.7	*n.s.*	14.5	13.8	*n.s.*
	2019	14.0	12.8	14.2	13.2	*n.s.*	14.1	13.0	*	13.4	13.7	*n.s.*
TAR (g/L)	**2017**	1.8	2.4	2.2	2.6	*n.s.*	2.0	2.5	**	2.1	2.4	*n.s.*
	2018	2.2	2.2	2.3	2.5	*n.s.*	2.3	2.3	*n.s.*	2.2	2.4	*n.s.*
	2019	2.4	2.5	2.3	2.5	*n.s.*	2.3	2.5	*n.s.*	2.4	2.4	*n.s.*
MAL (g/L)	**2017**	2.0	2.8	1.6	2.7	*n.s.*	1.8	2.8	***	2.4	2.2	*n.s.*
	2018	3.2 a	3.1 a	2.5 b	3.1 a	*	2.9	3.1	*n.s.*	3.2	2.8	*
	2019	2.1 c	2.9 a	2.4 bc	2.7 ab	*	2.2	2.8	***	2.5	2.6	*n.s.*
TA	**2017**	5.5	6.8	5.0	6.9	*n.s.*	5.2	6.9	***	6.1	6.0	*n.s.*
(g/L)	**2018**	7.8 ab	8.2 a	6.2 c	7.5 b	*	7.0	7.8	***	8.0	6.8	***
	2019	7.5 a	7.6 a	6.0 b	7.8 a	**	6.8	7.7	**	7.6	6.9	*
pH	**2017**	4.1	4.0	3.9	3.9	*n.s.*	4.0	3.9	*n.s.*	4.0	3.9	*n.s.*
	2018	3.7	3.7	3.8	3.7	*n.s.*	3.8	3.7	**	3.7	3.8	*
	2019	3.5 b	3.6 ab	3.7 a	3.6 ab	*	3.6	3.6	*n.s.*	3.5	3.6	*

Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and decit irrigation) and RI-F (forcing and deficit irrigation). Each value represents the mean of 8 samples (4 blocks, 2 replicates).

Parameters: Alcoholic Degree (AD), Tartaric acid (TAR), Malic acid (MAL), Titrable acid (TA) and pH.

Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VIII.3.3.2. Phenolic composition and chromatic characteristics

As the general trend no interactions were found in the phenolic composition of wines either chromatic characteristics and anthocyanin profile between the two techniques applied to the vines (Tables VIII.6, VIII.7 and VIII.8) therefore the effect of "crop-forcing" and irrigation can be examined, in most cases, separately.

As Table VIII.6 shows, regardless the irrigation strategy, the "crop-forcing" significatively increased the values of the polyphenolic parameters of wines with exception of anthocyanins (Ant) in 2017 and tannins (Tan) in 2018. Compared to "crop-forcing", the effect of irrigation was less significant (significant differences were found 5 times only). Regarding the effect of this technique on the anthocyanin values, the behaviour observed depended on year and pruning applied: In 2017, respect to C-NF and C-F increases of 53% and 9% were registered in RI-NF and RI-F wines respectively. However, in 2018 decreases of 14.4% and 15,3% for these comparisons. Finally, in 2019 a significant interaction (F*I) was registered and increases of 19% in RI-NF compared with C-NF and decreases of 11.4 in C-NF vs RI-NF were recorded. On other hand, a general trend to lower catechins (p<0.01 in 2018, p<0.05 in 2019) and tannin (p>0,05 in 2017) was recorded in RI respect to C wines. As consequence TPP were always higher in F wines than in NF ones, however it is not clear the effect of RI in TPP because not significant changes were found in 2017 and 2019, however a significant decrease in RI wines compared with C wines was registered in 2018.

Table VIII.6. Effect of "crop-forcing" and water status on phenolics composition (mg/L) of 'Tempranillo' wines.

		C-NF	**C-F**	**RI-NF**	**RI-F**	***FxI***	**NF**	**F**	***F***	**C**	**RI**	***I***
Ant	**2017**	148.2	199.2	227.3	217.5	*n.s.*	187.8	208.4	*n.s.*	173.7	222.4	*
	2018	280.0	441.6	239.7	373.9	*n.s.*	259.9	407.7	**	360.8	306.8	*n.s.*
	2019	187.5 c	321.3 a	232.0 bc	288.4 ab	*.	209.8	304.9	***	254.4	260.2	*n.s.*
Cat	**2017**	995.7	1752.1	715.8	1655.2	*n.s.*	855.7	1703.6	***	1373.9	1185.5	*n.s.*
	2018	1528.3	2837.3	1052.5	1888.9	*n.s.*	1290.4	2363.1	***	2182.8	1470.7	**
	2019	1810.8 b	3641.1 a	1149.5 c	3633.6 a	**	1480.2	3637.3	***	2726.0	2391.5	*
Tan	**2017**	1330.7	1995.9	951.7	1634.7	*n.s.*	1141.2	1815.3	***	1663.3	1293.2	*
	2018	2005.7	1535.6	1526.3	1624.5	*n.s.*	1766.0	1580.0	*n.s.*	1770.7	1575.4	*n.s.*
	2019	1871.3 b	2707.0 ab	990.5 c	2983.8 a	*	1430.9	2845.4	***	2289.2	1987.1	*n.s.*
TPP	**2017**	1581.9	2212.0	1546.4	2101.3	*n.s.*	1564.1	2156.6	***	1896.9	1823.8	*n.s.*
	2018	2228.6	2570.1	1783.4	2233.1	*n.s.*	2006.0	2401.6	***	2399.4	2008.2	***
	2019	1855.8	2594.5	1762.0	2581.7	*n.s.*	1808.9	2588.1	***	2225.1	2171.9	*n.s.*

Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and deficit irrigation) and RI-F (forcing and deficit irrigation). Each value represents the mean of 8 samples (4 blocks, 2 replicates)

Parameters: Anthocyanins (Ant); Catechins (Cat), Tannins (Tan) and Total Polyphenolics (TPP).

Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

These changes in the values of phenolic compounds of the wines had an impact on their chromatic characteristics. According to the results shown in Table VIII.7, on the three years of the study, F had higher C-Ant values (%) than NF wines, and this trend was observed when RI were compared with the C wines. Moreover, the IC values registered in the F and RI wines were higher than in the NF and C wines. It should be noticed that only F had a significant effect. Therefore, the C- Ant (%) and CI values followed the order RIF>C-F>RI-NF>C-NF. No significant changes were observed in the colour composition (calculated as %420, %520 and %620). Differences were found in the values of these percentages between C-NF and RI-NF wines in the 2019 vintage only. Finally, the Hue values of the RI wines tended to be lower than those found in the C wines, with significantly different values in the 2017 and 2019 vintage.

Table VIII.7. Effect of "crop-forcing" and water status on chromatics characteristics of 'Tempranillo' wines.

		C-NF	**C-F**	**RI-NF**	**RI-F**	***FxI***	**NF**	**F**	***F***	**C**	**RI**	***I***
C-Ant (%)	**2017**	17.8	26.1	22.6	31.9	*n.s.*	20.2	29.0	*	22.0	27.3	*n.s.*
	2018	32.3	37.1	33.8	44.1	*n.s.*	33.1	40.6	**	34.7	38.9	*
	2019	25.9	40.2	32.3	40.5	*n.s.*	29.1	40.3	***	33.0	36.4	*n.s.*
CI	**2017**	3.7	5.8	4.1	6.5	*n.s.*	3.9	6.1	**	4.7	5.3	*n.s.*
	2018	13.6	14.8	13.8	15.7	*n.s.*	13.7	15.3	*	14.2	14.8	*n.s.*
	2019	8.7	12.9	9.7	14.1	*n.s.*	9.2	13.5	**	10.8	11.9	*n.s.*
Yellow (%)	**2017**	33.8	31.8	31.3	30.1	*n.s.*	32.5	31.0	*n.s.*	32.8	30.7	*
	2018	31.0	29.4	31.9	28.2	*n.s.*	31.4	28.8	**	30.2	30.1	*n.s.*
	2019	28.6 b	29.6 ab	30.3 a	29.4 ab	*	29.5	29.5	*n.s.*	29.1	29.9	*n.s.*
Red (%)	**2017**	53.8	57.0	57.4	59.2	*n.s.*	55.6	58.1	*n.s.*	55.4	58.3	*
	2018	56.8	61.2	54.4	62.3	*n.s.*	55.6	61.8	***	59.0	58.3	*n.s.*
	2019	62.2 a	59.9 ab	58.3 b	60.0 ab	**	60.2	60.0	*n.s.*	61.0	59.2	**
Blue (%)	**2017**	12.4	11.2	11.3	10.7	*n.s.*	11.9	10.9	*n.s.*	11.8	11.0	*n.s.*
	2018	12.2	9.4	13.7	9.5	*n.s.*	13.0	9.4	***	10.8	11.6	*n.s.*
	2019	9.2 b	10.5 ab	11.3 a	10.5 a	**	10.3	10.5	*n.s.*	9.9	10.9	**
Hue	**2017**	3.1	2.8	2.7	2.6	*n.s.*	2.9	2.7	*n.s.*	3.0	2.6	*
	2018	5.5	4.8	5.9	4.5	*n.s.*	5.7	4.7	**	5.1	5.2	*n.s.*
	2019	4.6 b	4.9 ab	5.2 a	4.9 ab	*	4.9	4.9	*n.s.*	4.8	5.1	*

Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and deficit irrigation) and RI-F (forcing and deficit irrigation). Each value represents the mean of 8 samples (4 blocks, 2 replicates)

Parameters: Percentage of color due to anthocyanins (C-Ant); Color Intensity (CI); % Absorbance 420 nm (Yellow%); % Absorbance 520 nm (Red%); % Absorbance 620 nm (Blue%) and Color Tonality (Hue).

Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level.

VIII.3.3.3. Anthocyanin profile of 'Tempranillo' wines

The amounts of the different anthocyanin substances (g/L) found in the wines from the different treatments in 2017-2019 years are indicated in Table VIII.8. The anthocyanins present in wines were identified as the monoglucoside forms (G) of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv); the acetylglucoside forms (A) (DpA, CyA, PtA, PnA, and MvA), and the p-coumaroylglucoside forms (C) (CyC, PtC, PnC, and MvC).

Regardless of year, Mv and its derivatives were the most abundant in all samples. In NF the content of anthocyanidin derivates in the samples was: Mv>Pt>Dp >Pn>Cy every year and in NF in 2017; however in F wines, in 2018 and 2019 wines the values were Mv > Dp >Pt >Pn>Cy was found. In terms of forms, monoglucosides were always more abundant than acetyl glycosides, and acetyl glycosides more than acetates. Thus, the major compound was MvG in all samples analyzed. The amounts (mg/L) of this compound ranged from 88.9, found in the C-NF-2019, to 202.5 found in C-F-2018. The lowest values of individual anthocyanin compounds were found in NF wines and they corresponded to PtA (0.3 in C-NF-2017), and CyA (0.3 in C-NF-2018 and 1.3 in RI-NF 2019).

The effect F was significant for 7 individual anthocyanin compounds in 2017, 11 in 2018 and 9 in 2019. Table 8 reflects as a general trend, higher values of anthocyanin compounds in F wines than in NF wines. Therefore, the values of Mv, Pt, Dp, Pn and Cy were higher in F than in NF wines every year with exception of Pt and Mv in 2017 and, in the same way, the wines F showed higher contents of monoglucoside forms (∑G) than the respective NF wines with exception of MvG in 2017. However, this effect was significant in a lower number of cases in acetyl (∑A) and coumaryl glucosides forms (∑C). These results indicate that the extent and significance of effect of “crop-forcing” in anthocyanin profile depended of the compound, irrigation treatment and year considered. By general, the increases were higher in C than in the respective RI wines in 2017 and 2019. In these years, the highest increases (in percentage) were found in Dp with increases of 123% and 153% in CF wines compared to C-NF and 67.3% and 109.1 in the RIF wines respect to RI-NF. However, in 2018 the increases for these derivates were higher when in RIF vs RI-NF (237.8%) than C-F vs C-NF (112.8%).

The effect I was significant for 7 individual anthocyanin compounds in 2017, 3 in 2018 and none of them in 2019. The sense of variation (higher values in C or in RI wines), the significance and extent depended on the compound, year and pruning treatment considered. In 2017, as general behaviour higher values were found in RI than in C wines and in percentage higher increases were found in NF than in F wines. The highest increases on RI-NF values respect to those C-NF ones were found in PtA (633.3%) MvA (100%), PtC (91.9%) and PtG (52.9%). In 2018, the irrigation had a scarce effect; although as general trend, decreases were found in RI respect to C, only in CyG, PnG and PnC the effect was significant. Besides, the decreases were (in percentage) slightly higher in NF wines. Finally, in the last year, 2019, the irrigation treatment did not cause changes in the values of any anthocyanin substances present in the wines both in NF and F wines. The effect of irrigation depended on the pruning treatment: A trend to increase values of Mv, Pt and Dp was observed in RI-NF respect to C-NF. However, the opposite trend was detected when CF and RI-F were compared.

As consequence, in 2017, the highest values of Mv were found in RI-NF (170.7), those of Pt (33.5) and Dp (26.6) in RI-F, and those of Pn and Cy in C-F. The following year, 2018, the maximum values of all anthocyanidin groups were registered in C-F, Finally, the last year, the maximum values of Mv, Pt, Dp, Pn, Cy corresponded to C-F treatment and very close values were registered in RI-F. On the other hand, the minimum values of all anthocianidin groups were registered in NF treatments, more specifically: in 2017 in C-NF, in 2018 Mv in C-NF, and of the rest in RI-NF, and in 2019 Mv, Pt and Dp in C-NF and Pn and Cy in RI-NF.

Table VIII.8. Effect of “crop-forcing” and water status on on anthocyanin profile of ‘Tempranillo’ wines.

	Year	**C-NF**	**C-F**	**RI-NF**	**RI-F**	***F*I***	**NF**	**F2**	***F***	**C**	**RI**	***I***
MvG	**2017**	88.3	115.9	129.9	124.4	n.s.	109.1	120.2	n.s.	102.1	122.9	*
	2018	141	202.5	144.1	178.8	n.s.	142.6	190.7	**	171.7	173.3	n.s.
	2019	88.9 b	150.9 a	118.0 ab	137.8 a	*	103.5	144.3	***	119.9	134.4	n.s.
PtG	**2017**	13.8	24.3	21.1	27.8	n.s.	17.5	26.1	**	19	22.7	n.s.
	2018	32.9	61.9	24	53.4	n.s.	28.5	57.6	***	47.4	42.9	n.s.
	2019	16.0 b	40.6 a	23.2 b	37.3 a	*	19.6	39	***	28.3	31.9	n.s.
DpG	**2017**	9.1	21.7	14	24.9	n.s.	11.5	23.3	**	15.4	17.8	n.s.
	2018	31.6	77.2	17	65.9	n.s.	24.3	71.6	***	54.4	47.1	n.s.
	2019	18	52.7	18.7	46.7	n.s.	18.4	49.7	***	35.4	35.7	n.s.
PnG	**2017**	3.4	8	3.4	6.9	n.s.	3.4	7.4	***	5.7	5.7	n.s.
	2018	23.1	36.6	8.4	22	n.s.	15.7	29.3	***	29.8	22.5	***
	2019	4.2	13.1	3.4	10.8	n.s.	3.8	12	***	8.7	8.3	n.s.
CyG	**2017**	0.6 c	1.8 a	0.8 c	1.5 b	**	0.7	1.7	**	1.2	1.3	n.s.
	2018	5.1	11.5	1.5	5.7	n.s.	3.3	8.6	***	8.3	6.5	**
	2019	2.3	6.5	2.2	5.5	n.s.	2.2	6	***	4.4	4.4	n.s.
∑G	**2017**	115.2	171.7	169.2	185.6	n.s.	142.2	178.7	*	143.5	170.5	*
	2018	233.7	389.6	195	325.8	n.s.	214.3	357.7	***	311.7	292.3	n.s.
	2019	129.4 b	263.8 a	165.5 b	238.0 a	*	147.4	250.9	***	196.6	214.7	n.s.

Table VIII.8 cont. Effect of “crop-forcing” and water status on on anthocyanin profile of ‘Tempranillo’ wines.

	Year	**C-NF**	**C-F**	**RI-NF**	**RI-F**	**F*I**	**NF**	**F2**	***F***	**C**	**RI**	***I***
MvA	**2017**	2.9	2.9	5.8	4.1	n.s.	4.3	3.5	n.s.	2.9	4.3	**
	2018	3.4	5.5	3	4.8	n.s.	3.2	5.1	**	4.4	4.2	n.s.
	2019	4.8	6.3	5.9	4.6	n.s.	5.3	5.5	n.s.	5.6	6.1	n.s.
PtA	**2017**	0.3 c	1.3 b	2.2 a	1.6 b	***	1.3	1.5	n.s.	0.8	1.8	***
	2018	2.4	3.2	2.3	3	n.s.	2.4	3.1	**	2.8	2.8	n.s.
	2019	2.4	3.8	3.4	3.3	n.s.	2.9	3.5	n.s.	3.1	3.6	n.s.
DpA	**2017**	1.2	1.3	2	1.7	n.s.	1.6	1.5	n.s.	1.3	1.6	**
	2018	1.9	3.2	1.5	3.2	n.s.	1.7	3.2	***	2.5	2.3	n.s.
	2019	2.3	4.8	3.3	5.1	n.s.	2.8	4.9	*	3.5	4	n.s.
PnA	**2017**	0.4 b	0.5 b	0.7 a	0.6 ab	**	0.5	0.5	n.s.	0.4	0.6	**
	2018	1.4	1.5	1.4	1.1	n.s.	1.4	1.3	n.s.	1.4	1.4	n.s.
	2019	2.3	1.6	2.8	1.6	n.s.	2.5	1.6	n.s.	1.9	2.2	n.s.
CyA	**2017**	0.8	0.8	0.9	0.8	n.s.	0.8	0.8	n.s.	0.8	0.9	n.s.
	2018	0.3	0.6	0.3	0.6	n.s.	0.3	0.6	*	0.5	0.5	n.s.
	2019	1.3	2.9	1.2	3.3	n.s.	1.2	3.1	***	2.1	2	n.s.
∑A	**2017**	5.5 b	6.8 b	11.7 a	8.8 ab	*	8.6	7.8	n.s.	6.1	9.2	**
	2018	9.5	14	8.5	12.8	n.s.	9	13.4	**	11.7	11.2	n.s.
	2019	13	19.4	16.6	17.9	n.s.	14.8	18.6	n.s.	16.2	18	n.s.
MvC	**2017**	20.8 b	14.3 b	34.9 a	16.1 b	*	27.9	15.2	**	17.6	24.6	*
	2018	25	23.5	26.2	21.9	n.s.	25.6	22.7	n.s.	24.3	24.9	n.s.
	2019	25.2	19.6	29.3	17.7	n.s.	27.3	18.7	**	22.4	24.5	n.s.
PtC	**2017**	3.7 b	3.5 b	7.1 a	4.0 b	*	5.4	3.7	*	3.6	5.3	**
	2018	5	6.2	5	6.1	n.s.	5	6.1	n.s.	5.6	5.6	n.s.
	2019	9.2	7.0	10.3	5.4	n.s.	9.7	6.2	*	8.1	8.7	n.s.
PnC	**2017**	1.8	1.7	2.5	1.8	n.s.	2.1	1.8	n.s.	1.7	2.1	n.s.
	2018	3.9	4.8	2.6	3.7	n.s.	3.2	4.2	*	4.3	3.7	*
	2019	4.5	3.8	4.5	3.3	n.s.	4.5	3.5	*	4.1	4.1	n.s.
CyC	**2017**	1.4	1.2	1.8	1.2	n.s.	1.6	1.2	*	1.3	1.5	n.s.
	2018	2.9	3.5	2.5	3.6	n.s.	2.7	3.5	*	3.2	3	n.s.
	2019	6.2	7.7	5.8	6.1	n.s.	6	6.9	n.s.	6.9	6.7	n.s.
∑C	**2017**	27.6 b	20.7 b	46.4 a	23.1 b	*	37	21.9	**	24.1	33.5	*
	2018	36.8	38	36.3	35.2	n.s.	36.6	36.6	n.s.	37.4	37.1	n.s.
	2019	45.1	38	49.9	32.5	n.s.	47.5	35.3	*	41.6	44	n.s.

Table VIII.8 cont. Effect of “crop-forcing” and water status on on anthocyanin profile of ‘Tempranillo’ wines.

	Year	C-NF	C-F	RI-NF	RI-F	*F*I*	NF	F2	*F*	C	RI	*I*
∑Mv	**2017**	112	133.1	170.7	144.7	n.s.	141.4	138.9	n.s.	122.6	151.9	*
	2018	169.4	231.4	173.3	205.6	n.s.	171.4	218.5	*	200.4	202.4	n.s.
	2019	118.9 b	176.8 a	153.2 ab	160.1 a	*	136.1	168.5	**	147.9	165	n.s.
∑Pt	**2017**	17.7	29.1	30.5	33.5	n.s.	24.1	31.3	n.s.	23.4	29.8	*
	2018	40.3	71.3	31.3	62.5	n.s.	35.8	66.9	***	55.8	51.3	n.s.
	2019	27.6 c	51.4 a	36.8 bc	46.0 a	*	32.2	48.7	***	39.5	44.1	n.s.
∑Dp	**2017**	10.3	23	15.9	26.6	n.s.	13.1	24.8	**	16.7	19.5	n.s.
	2018	33.5	80.4	18.5	69.1	n.s.	26	74.8	***	57	49.4	n.s.
	2019	20.3	57.5	22	51.8	n.s.	21.2	54.7	***	38.9	39.7	n.s.
∑Pn	**2017**	5.5	10.2	6.6	9.3	n.s.	6.1	9.7	***	7.8	8.4	n.s.
	2018	28.3	42.8	12.4	26.8	n.s.	20.3	34.8	**	35.6	27.6	***
	2019	11	18.5	10.7	15.7	n.s.	10.8	17.1	**	14.7	14.6	n.s.
∑Cy	**2017**	2.7 b	3.8 a	3.5 ab	3.5 ab	*	3.1	3.6	*	3.2	3.7	n.s.
	2018	8.4	15.6	4.3	9.9	n.s.	6.3	12.8	***	12	10	**
	2019	9.7	17.1	9.3	14.9	n.s.	9.5	16	**	13.4	13.2	n.s.

Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and decit irrigation) and RI-F (forcing and deficit irrigation). Each value represents the mean of 8 samples (4 blocks, 2 replicates)

Parameters: Malvidin-3-glucoside (MvG); Petunidin-3-glucoside (PtG); Delphidin-3-glucoside (DpG); Peonidin-3-glucoside (PnG); Cyanidin-3-glucoside (CyG); Total monoglucoside forms (∑G); Malvidin-3-glucoside acetate (MvA); Petunidin-3-glucoside acetate (PtA); Delphidin-3-glucoside acetate (DpA); Peonidin-3-glucoside acetate (PnA); Cyanidin-3-glucoside acetate (CyA); Total acetyl- glucoside forms (∑A); Malvidin-3-glucoside coumarate (MvC); Petunidin-3-glucoside coumarate (PtC); Peonidin-3-glucoside coumarate (PnC); Cyanidin-3-glucoside coumarate (CyC); Total coumaroy- glucoside forms (∑C); Total malvidin derivates (∑Mv); Total petunidin derivates (∑Pt); Total delphinidin derivates (∑Dp); Total peonidin derivates (∑Pn); Total cyanindin derivates (∑Cy).

Statistical analysis: Different letters indicate the existence of statistically significant differences between treatments; n.s. indicate not significant; (*) significant at 5% level; (**) significant at 1% level and (***) significant at 0.1% level

VIII.3.4. CLASSIFICATION OF WINES. CLASSIFICATION PARAMETERS

Multiple factor analysis (MFA) is a statistical helpful method to analyse the similarities and discrepancies between a set of observations explained by data tables of different parameter sets and can also be used to explore the correlation between these parameter sets (Escofier et al., 1994; Salkind, (Ed.) 2007). In this study, MFA was performed on the data matrices of the yield (Yield and BW), acid (TAR, MAL, TA and pH), phenolic (TPP, Ant, Cat and Tan) and chromatic (%C-Ant, CI and Hue) group parameters. In addition, weather conditions (maximum temperature and steam water potential, SWP) were used as supplementary (non-active) variables to further explore the influence of both temperature and SWP on the composition of grapes and wines. The MFA allowed, on the one hand, to explore the differences and similarities between the different wines due to the treatment applied to the vines and, on the other hand, to know which set of variables can be used as markers of the wines from the different treatments.

Figures VIII.1a, VIII.1b and VIII.1c show the results of the MFA carried out on the results of the wines from the 2017, 2018 and 2019 vintages, respectively. In all years, a good variance explanation was achieved (98.48, 89.32 and 98.53% in 2017, 2018 and 2019, respectively). All years, the wines were distributed along the horizontal axis F1 (which explained 71.71, 57.22 and 66.16% of the total variance) and were grouped according wines from forced (F) or non-forced (NF) vines. On the other hand, the vertical axis F2, which explained 28.77, 32.09 and 32.37%, separated the samples according to irrigation treatment.

In 2017, NF and F samples were located on positive and negative side of F1 respectively. This axe was defined by the yield (25.01%), acid (27.15%), phenolic (20.79%) and chromatics (27.05%) group parameters. Thus, all groups of variables contributed with a similar extent to differentiate the F from the NF samples. Almost of the individual variables were related to higher values on F wines, with exception of Hue, pH, yield and berry weight, on the corresponding NF wines. However, F2 was mainly associated with phenolic (43.48%) and acid (35.57%) groups of variables. Thus, F2 axe allows to distinguish the samples C from RI. These last located on the negative side of F2, were defined mainly by higher Ant values.

In 2018, the distribution of samples on the plane defined by F1 and F2 was similar from the previous year. F1 grouped the samples according the pruning and F2 by irrigation strategy. In the similar way of last year, the yield (24.68%), phenolic (27.76%) and chromatic (31.61%) parameters group contributed with similar values to F1. However, acid parameters with 15.96% only. Thus, F samples were located on negative side of F1 and NF on positive side. These last were associated to higher BW, Tan, yield, Hue and pH. Like in 2017 harvest, C samples and RI were located in positive and negative side respectively of F2 axe. RI samples were mainly defined by higher TAR and pH values.

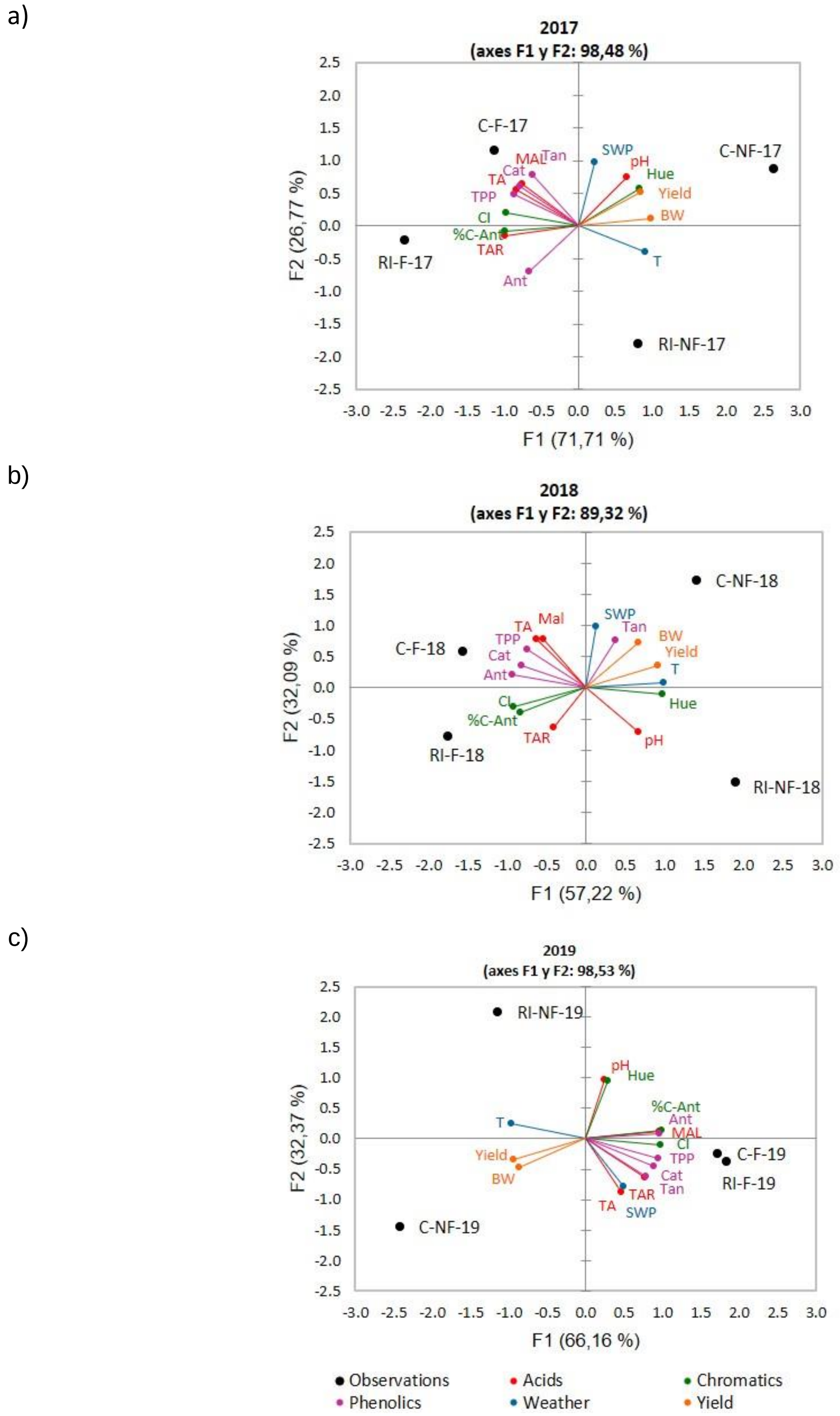

Figure VIII.1. Multiple Factorial Analysis (MFA) and of acid, phenolic, chromatic, weather and yield parameters data: a) 2017; b) 2018; c) 2019. Acids parameters: Total Acidity (TA), Malic acid (MAL), Tartaric acid (TAR) and pH. Phenolics parameters: Total Polyphenolic content (TPP), Anthocyanins content (Ant), Tannins content (Tan) and Catechins contents (Cat). Chromatics parameters: Color Intensity (CI), Hue and Color-Anthocyanins (C-Ant). Weather paratemeters: Temperature (T) and Stem Water Potencial (SWP). Yield parameters: Yield and Berry Weight (BW).

Finally, as Table VIII.9 shows, in 2019, all group of variables contributed in the same extent to F1. This axe grouped NF (C and RI) wines on the negative side. These samples were defined by higher yield and BW. It is noticed that F2 only differentiate RI-NF and C-NF by pH and Hue values (higher on RI-NF) and on this year, RI-F and C-F were F2 closely located. Therefore, in all years of the trial, F (C and RI) wines, elaborated from F vines with lower production and smaller berries than NF, were distinguished by higher amounts of MAL, TAR, TA, TPP, Ant, Cat, CI and % C-Ant than NF wines. It is noticed that the RI-F wines were characterized by the maximum values of TAR and TA and C-F by the maximum of polyphenolic substances. However, the impact of irrigation strategy varied with the year considered. Thus, in 2017 and 2019, RI (F and NF) wines, from RI with lower production and smaller berries than C vines, were characterized by lower pH and Hue values than C wines.

PCA were performed with the values of Mv, Dp, Pt, Pn and Cy of each year to determine general trends in the different wines. Figure VIII.2 (a, b and c) shows the distribution of samples in 2017, 2018 and 2019 years. PC-a, PC-b and PC-c accounted higher than 95% of the total variance. F1 covered 75% in 2017 and about 90% in the following years. From these figures, it is clear that, with exception of RI-NF in 2017, the wines can be grouped in NF and F. These last samples were related with higher values of all derivate compounds with exception of Mv in 2017.

Table VIII.9. Variable contributions (%) to Multiple Factorial Analysis (MFA).

	2017		**2018**		**2019**	
	F1	**F2**	**F1**	**F2**	**F1**	**F2**
TAR	9.9	0.6	2.1	8.8	6.9	9.7
MAL	5.9	11.4	3.6	13.4	10.8	0.4
TA	6.9	8.3	4.9	13.1	2.5	18.7
pH	4.4	15.3	5.4	10.8	0.7	22.4
Acids parameters	**27.1**	**35.6**	**16.0**	**46.2**	**20.9**	**51.1**
TPP	7.1	6.1	6.9	8.6	7.2	1.8
Ant	4.2	12.4	10.8	0.9	7.6	0.1
Cat	6.0	9.5	8.3	3.0	6.5	3.3
Tan	3.5	15.4	1.7	13.1	5.0	6.3
Phenolics parameters	**20.8**	**43.5**	**27.8**	**25.6**	**26.2**	**11.4**
%C-Ant	10.0	0.2	8.8	3.5	13.7	0.5
CI	9.9	1.1	10.7	2.2	13.5	0.3
Hue	7.1	8.8	12.1	0.2	1.2	26.2
Chromatics parameters	**27.0**	**10.1**	**31.6**	**5.9**	**28.4**	**27.0**
Yield	10.5	10.3	16.1	4.2	13.3	3.5
BW	14.5	0.6	8.6	18.1	11.2	6.9
Yield parameters	**25.0**	**10.8**	**24.7**	**22.4**	**24.5**	**10.5**

Acids parameters: Total Acidity (TA), Malic acid (MAL), Tartaric acid (TAR) and pH. Phenolics parameters: Total Polyphenolic content (TPP), Anthocyanins content (Ant), Tannins content (Tan) and Catechins contents (Cat). Chromatics parameters: Color Intensity (CI), Hue and Color-Anthocyanins (C-Ant). Weather paratemeters: Temperature (T) and Stem Water Potencial (SWP). Yield parameters: Yield and Berry Weight (BW).

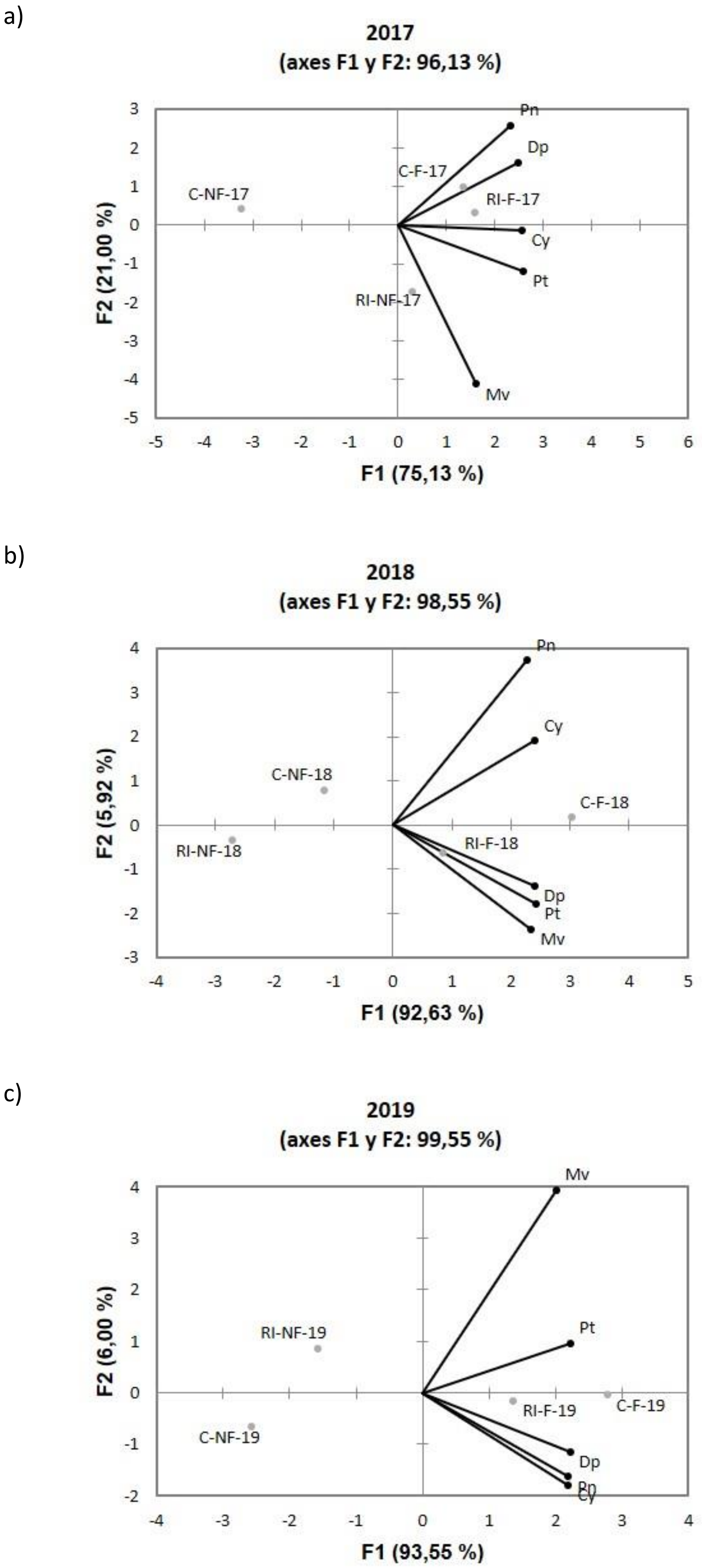

Figure VIII.2. Principal components analysis on anthocyanin profile of ‘Tempranillo’ wines: a) 2017; b) 2018; c) 2019. Malvidin (Mv), petunidin (Pt), delphinidin (Dp), peonidin (Pn) and cyanidin (Cy). Treatments: C-NF (no forcing and full irrigation); C-F (forcing and full irrigation); RI-NF (no forcing and decit irrigation) and RI-F (forcing and deficit irrigation).

VIII.4. DISCUSSION

The application of “crop-forcing” on ‘Tempranillo’ grapevines growing under C and RI irrigation strategies, altered phenology dates, berry weight, yield and in consequence the acidic and phenolic composition of must and the corresponding wines. The effect was different among the years under study, and the parameter and treatment considered and sometimes the significance and extent of effect of “crop-forcing” depended of irrigation strategy applied.

When Gu et al. (2012) in ‘Cabernet Sauvignon’ and Martinez De Toda et al. (2019) in ‘Tempranillo’ investigated the effect of “crop-forcing”, decreases on the berry size and yield at harvest were reported. Arrizabalaga et al. (2018) reported that elevated temperature hastened berry development, with a greater influence before the onset of ripening in different clones of ‘Tempranillo’. Changes in berry weight have been attributed by several authors to changes in temperature and the light regime within the cluster that probably influence the berry sink capacity (i.e. the ability of attracting photo-assimilates) (Kliewer & Antcliff, 1970).

In this work, regardless prunning applied, decreases on RI berry weight were registered respect to C ones. It has been reported that water deficits reduce berry size and yield, and some studies showed that the decreases are linearly related to decreases in stem water potential (Grimes & Williams, 1990; Santesteban et al., 2011). In a recent meta-analysis, Mirás-Avalos & Intrigliolo (2017) found that this relationship was variety dependent. In this sense, our results obtained agree with the studies of Girona et al. (2009) who published hat the ‘Tempranillo’ has a high phenological sensitivity to pre-veraison water stress. Besides, previous works have also shown that the effect of irrigation on final berry size at harvest in ‘Tempranillo’ is linked with the timing of water stress (Intrigliolo et al., 2012; Intrigliolo & Castel, 2010b; Uriarte et al., 2015; Valdés et al., 2009). In general, the pre-veraison water stress, as the used in this work, tends to more strongly restrict berry size, and have a more negative effect on yield compared to post-veraison water stress (Intrigliolo et al., 2012). At this regard, it must be considered that the degree of pre-veraison stress that can be induced naturally depends on the soil water available at flowering, which in turn depends on winter and early spring rainfall levels and on the water used during spring (Lopes et al., 2011). In 2018, the wettest year, the lowest differences in water status were recorded and in consequence, the lowest differences on yield recorded in that year.

VIII.4.1. ACID COMPONENT

Acidity is not only important for flavour balance and organoleptic properties of wine, but also contribute to wine stability (van Leeuwen & Darriet, 2016). Malic and tartaric acids are the most common organic acids in grapevine fruit and they are the determinants of TA of the berries and wines. Normally, both acids reach their highest concentrations near veraison. In the second phase of growth, termed ripening, metabolite concentrations increase or decrease, depending on net biosynthesis or metabolization and growth dilution, both mechanisms being genotype-dependent (Dai et al., 2011; Keller et al., 2016). It is believed that, once synthesised, tartaric acid remains stable and that their content does not varies in terms of quantity per fruit during ripening (Rösti et al., 2018; Terrier et al., 2001); however malic acid is metabolized and used as an energy source during the process (Rienth et al., 2016). Therefore, tartaric acid concentration decreased in the samples of must and wines from more irrigated treatments and the highest berry weight (C-NF). On other hand, it is known that temperature is the main environmental factor affecting the evolution of because this acid is less affected than malic acid by environmental conditions (Ruffner, 1982), and thus, its concentration was probably more determined by the dilution effect in must as well as by increased precipitation of bitartrate potassium salts (Iland & Coombe, 1988). In our case, the lower temperatures during the ripening period of the F berries impeded the combustion of this acid in NF samples. Torres et al. (2017) reported that in ‘Tempranillo’ the extent of alteration in primary metabolism due to temperature was higher than in secondary metabolism, which was mainly affected by deficit irrigation. The effect of water stress during the herbaceous period of berry development on acidity has been earlier reported for ‘Tempranillo’ and other varieties (Girona et al., 2009; Intrigliolo & Castel, 2010b; Salón et al., 2005). Must and wines from F were more acid mainly because of the much larger concentration of malic acid than NF samples specially in RI treatments. This organic acid was the main one contributing to changes of acidity (García Romero et al., 1993). According Jackson and Lombard moderate temperatures result in good TA, and pH, values assuming the growing season is warm and long enough to ripen the fruit. The cultivar best suited to a particular region usually ripens its fruit during the cooler portion of the season (Jackson & Lombard, 1993).

In this work, by general, the highest values of TA were found in in C-F samples (must and corresponding wines). These results are consequence of an increase on the synthesis of malic and tartaric acid resulting from higher assimilation rates (De Souza et al., 2005; Esteban et al., 1999; Salón et al., 2005) and above allow higher malic acid respiration rate decreased by the lower temperatures reached at the clusters of crop forced vines and less exposed to sunlight as a result of the increase in leaf area involved in C irrigation practice as a result of higher vegetative growth (De Souza et al., 2005; Spayd et al., 2002). Our findings are in agreement with previous work that analyzed the effect of forcing (Martínez de Toda et al., 2019) and RDI (Uriarte et al., 2016) independently of each other on 'Tempranillo'. At this regard, it is noticed that for first time is reported that the better climatic conditions and temperatures during the ripening of RI-F samples, mitigated the decrease caused by RI strategy in NF wines, thus the TA values of RIF wines were always close to found in C-F wines. Since the malic is a weaker acid than tartaric (i.e. malic acid has higher pKa and it dissociates incompletely), Unfortunately, the effect of "crop-forcing" on wines pH was scarce. These results reduce the success of the "crop-forcing" technique because one of the main problems, particularly pronounced in 'Tempranillo' wines, of current oenology is the high pH of wines.

VIII.4.2. PHENOLIC COMPOSITION AND CHROMATICS CHARACTERISTICS

Temperature, water status drought and light intensity are factor of influence in the synthesis, accumulation and concentration at harvest of phenolic substances of grapes, and in consequence of the wines elaborated. Since in this work, "crop-forcing" and RDI modified the temperature and water status during vegetative period and the berry weight and yield were different, the C-NF, C-F, RI-F and RI-NF wines elaborated had different phenolic composition and in consequence different chromatic characteristics. Additionally, many works describe the relationship between berry weight and the values at harvest of components which determine must quality. In the present work, the values of polyphenol families at harvest should be related to the effect on berry weight.

As general trend, no interactions were found between water and temperature on the phenolic composition of wines, therefore, the effect of water and temperature can be examined separately.

The increase in the content of anthocyanin in F wines is associated with the lower temperatures during the vegetative cycle of F grapes. It is known that as summer temperature rises to atypical values, the anthocyanin biosynthetic genes are downregulated, reducing berry skin anthocyanin biosynthesis (Conde et al., 2016). On the work above cited Arrizabalaga et al. (2018) shown that elevated temperature reduced anthocyanin concentration in different 'Tempranillo' clones. At the same concentration of total soluble solids, the anthocyanin concentration was lower at 28 °C/ 18 °C than 24 °C/14 °C, indicating a decoupling effect of elevated temperature during berry ripening.

This decoupling was explained by changes in the relative rate of response of anthocyanin and sugar build-up, rather than delayed onset of anthocyanin accumulation. These authors also refer the inhibition of mRNA transcription of the anthocyanin biosynthetic genes, as well as chemical and/or enzymatic degradation reported in previous works (Mori et al., 2007). Furthermore, temperature may also reduce the anthocyanin content, affecting its subcellular transport through the down-regulation of several transmembrane transporter-encoding genes involved in the import of anthocyanins in the vacuole (Carbonell Bejerano & Martinez Zapater, 2013). On other hand, although the studies reported an increase in skin anthocyanins under water deficit either by a berry-size concentrating effect or by up-regulation of the biosynthetic pathway (Castellarin et al., 2007). However, only in 2017, the driest year, the value of anthocyanin in RI wines was higher than in C, and in the wines from vines no forced (RI-NF vs C-NF) in 2019. There is not a general trend in the response of anthocyanins of wines to water stress and the results are influenced by the cultivar, terroir and year. In our previous work performed in 'Tempranillo' grown in similar edaphic and climatic conditions, the different irrigation strategy had almost no effect on wine anthocyanin concentration values and only slightly lower concentrations of these substances were found in the wine from less irrigated vines but the differences were non-significant (Gamero et al., 2018).

Anthocyanin profile determinate the colour and its stability of the wines. Acylated and coumarates derivatives are considered to be among the most stable compounds (Ortega-Regules et al., 2006). On the other hand, Cy, Dp and Pt are more sensitive to enzymatic oxidation (except for laccase) and non-enzymatic oxidation (catalysed by copper or iron ions) to produce o-diquinones, or even o-diphenol dimmers than Mv and Pn (Jackson, 2008).

The forcing crop applied to the vines, modified the anthocyanin profile of the wines elaborated. Certainly, as general a trend to increase the anthocyanin compounds was found in F, respect to NF samples. However, the extent of increase varied in function of the derivate considered. Thus, regardless irrigation strategy, the highest increases were registered in Dp anthocyanidin. Tarara et al. (2008) reported that the lower temperatures were associated with increases in Dp, Cy, Pt, and Pn derivates but found no influence on Mv derivatives concentrations. This behaviour was only found in 2017. Otherwise, Mv and Pn compounds are more resistant to oxidation, than Cyan, Del and Pet. When HS/LS (highly sensitive/low sensitive) relation as $\sum(Cy + Dp + Pt) / \sum (Mv + Pn)$ was calculated, the mean values reached 0.68 in C-NF; 0.85 in C-F; 0.55 in RI-NF and 0.83 in RI-F. The order RI-NF < C-NF < RI-F < C-F was observed all year. Therefore, the F wines were more sensitive to oxidation than NF and for a given pruning treatment RI more than respective C.

Together anthocyanins, catechins and tannins (also known as proanthocyanidins), can strongly impact the quality of red wines via their contributions to wine bitterness and astringency (Cheynier et al., 1997; Kennedy et al., 2006; Vidal et al., 2003). Our results are in agreement with studies that show a reduced response of tannins to irrigation treatments (Ollé et al., 2011). Respect to increase of these compounds in F wines (C and RI) can be explained on the basis to the high temperatures impaired tannin synthesis of the berries and also and degree of galloylation at the transcriptomic levels exposed by Rienth et al., (2016). On other hand, as Bonada et al. (2015) exposed that temperature could affects tannins extractability from seed or skin indirectly by uncoupling berry seed and skin development and modifying the number of seeds or skin mass per berry. In this last sense, grapes F had higher percentage of seeds in the fresh berry weight than NF ones (4.6 and 4.0 respectively as global interanual mean).

Many studies have used statistical techniques to find correlations between phenolic compounds and colour parameters during the maturation and ageing processes of red wine (Gamero et al., 2018). In one interesting work, (Monagas et al., 2006) showed that chromatic attributes of red wines could be predicted by their phenolic profile using polynomial regression techniques. The substances which provided the best fitting model in that study were the anthocyanin compounds. Besides, when Gamero et al. (2018), investigated the correlations between the phenolic composition and the chromatic charcteristics of ‘Tempranillo’ wines found that CI was high and positively correlated with the presence of G, C, Dp, Mv and Pt. In consequence CI was higher in F than in NF wines.

VIII.4.3. CLASSIFICATION OF WINES. CLASSIFICATION PARAMETERS

The “crop-forcing” modified the maximum temperature during vegetative period. This parameter considered supplementary variable in MFA, was significant and strongly correlated to F1 (0.91, 0.99 and 0.97 in 2017, 2018 and 2019 years). The irrigation strategies modified SWP, parameter correlated with F2 (0.97, 0.98 y 0.79 in those years)

Since F1 explained 71.71%, 57.22% and 66.16% of the variation, and the location of samples was similar all years, it implies that the temperature during the vegetative period had a strong effect and a consistent response. Our results are in agreement with previous researchs: Torres et al. (2017) reported a good separation of grape samples grown at different temperature mainly based on differences in TAR. Arrizabalaga et al.

(2018) analyzed by principal component analysis (PCA) plants grown at 24 °C/14 °C and 28 °C/18 °C. The first two principal components explained about 75% of the total variability and clearly separated samples according to the temperature regime. The loading plot reflected that that distinction was associated with an increased Hue, as well as lower Ant, TPP, CI and TA. F2 explained a lower percentage of the variance of F1 and two aspects should be taken account the distribution of the samples along this axe first, the contribution of acid parameter group to F2 was similar on the three years under study (35.57, 46.15 and 35.00 in 2017, 2018 and 2019), while those of the rest of parameters depended on the year considered and NF samples were more affected by strategy of irrigation of F wines. When Bonada et al. (2015) subjected to PCA the chemical and sensory profiles of Barossa Shiraz grapes wines produced from vines exposed and no exposed to hydric and thermal stress, found that F1 explained ~53% of the variation and was a function of the temperature treatment; the remaining 37% was explained by F2 and F3, which were related to the water treatment. According they, those differences suggest a comparatively higher impact of temperature over water on grape and wine composition. Thus, it can be concluded that in in line with that reported by Arrizabalaga et al. (2018) in the work above cited, the extent of alteration in primary metabolism due to temperature was higher than in secondary metabolism, which was mainly affected by deficit irrigation. Finally, it has been noted that the berry weight did not was a determining factor in wine composition. In this sense, these results should would support the findings of Walker et al. (2005) and Matthews & Nuzzo (2007) who stated that the the viticultural practices used to control yield in a vineyard are more important than the yield values per se in determining the quality of the resulting grapes and wines; and the environmental conditions determine berry size are more important the size per se in determining the quality of the grapes and resulting wines.

VIII.5. CONCLUSIONS

The impact of climate change factors, requires the use of direct short-term methods that involve changing environmental factors. This research provides evidence of changes in the composition of ‘Tempranillo’ wines in response to temperature of berries during vegetative period and water status. The significance and extent of impact by temperature was higher and more consistent than the of water status one. Wines from forced vines (elaborated with berries grown at lower temperature) had, in general, highest values of total acidity, malic acid, anthocyanins, catechins, total polyphenols. In additions, color intensity and copigmented anthocyanins contents were higher in these wines. However, wines from forced vines were more sensitive to oxidation than wines from non forced vines.

Thus, these results indicate that the adaptation to climate change in south Mediterranean Europe might be plausible with the application of “crop-forcing” should be appropriate. Due to the limitations of yield, wines from “crop-forcing” can be used as “good modifiers” of wines from vines grown with conventional techniques and improved their chromatic characteristics.

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IX. DISCUSIÓN GENERAL

En el año 2012 Gu et al. publicaron un artículo en el que presentaban una técnica novedosa para el viñedo, que tenía como principal objetivo mejorar la calidad de la uva retrasando la fecha de vendimia al desplazar la fenología de la vid hacia un periodo con temperaturas más frescas, para lo proponían transformar el ciclo reproductivo bianual de la viña en un ciclo anual. A pesar de los positivos resultados obtenidos con esta propuesta, tuvieron que pasar siete años hasta que se publica un nuevo trabajo sobre esta técnica. A partir de entonces la producción científica ha sido algo mayor. El efecto del cambio climático ha despertado un interés creciente por las prácticas agronómicas que puedan mitigar las consecuencias negativas del aumento de las temperaturas en algunas de las principales zonas productoras de cultivo del viñedo, entre las que se encuentra el "crop-forcing". A día de hoy es difícil pensar, a tenor del descenso en el rendimiento que se observa con esta técnica, que los viñedos comerciales estén dispuestos a adoptarla entre sus prácticas habituales. El aspecto más interesante de esta técnica es que es efectiva para mejorar algunos de los parámetros que definen la calidad de las bayas para vinificación en uva tinta, principalmente aquellos que se ven más perjudicados por elevadas temperaturas o exceso de radiación durante la maduración. Como contraposición incrementa los costes de producción, al introducir una poda adicional compleja y reduce significativamente la producción de uva. Sin embargo, disponer de una mayor información sobre el efecto de esta técnica puede convertirla en una opción interesante a tener en cuenta desde el punto de vista de la gestión de los viñedos con unos objetivos productivos claros, ofreciendo a los enólogos una gama más amplia de materias primas. Cuando se habla de esta técnica se suele hacer en el contexto del Cambio Climático, pero puede tener también interés en viñedos de zonas cálidas, para modificar algunas características de las bayas, dirigidas a elaboraciones enológicas más exigentes.

En trabajos previos se ha abordado algunos aspectos de la aplicación del "crop-forcing", pero en este caso se plantea un estudio bajo un enfoque amplio analizando tanto el comportamiento de las cepas en campo a corto y medio plazo, considerando aspectos ecofisiológicos y de desarrollo vegetativo y productivo en el viñedo (Capítulo 1), como la evolución de la maduración de la uva en condiciones ambientales diferentes (Capítulo 2) y las posibles consecuencias interanuales que supone esta modificación del ciclo biológico y el posible agotamiento de las cepas (Capítulo 3). El estudio se ha prolongado fuera del viñedo para estudiar las características y composición de las bayas (Capítulo 4) y el impacto sobre los vinos (Capítulo 5). Se trata de un trabajo amplio que ha generado unos resultados de interés que, aunque en algunos puntos no ha resultado concluyente, supone una aportación relevante en el conocimiento sobre esta técnica.

Hemos podido demostrar que esta técnica resulta efectiva para desplazar la vendimia una media de 32 días cuando el "crop-forcing" se realiza después de floración (F1) y 56 días cuando se lleva a cabo después de cuajado. Además de desplazar la fecha de vendimia, el "crop-forcing" modifica la duración y condiciones ambientales de cada etapa del ciclo fenológico (Tabla IV.3). El periodo desde brotación a vendimia se acortó en torno a 20 días cuando el "crop-forcing" se realizó después de floración, y 13 días cuando se llevó a cabo después de cuajado. El retraso provocó una aceleración en los procesos al elevarse la temperatura incrementándose la acumulación diaria de tiempo térmico en las cepas sometidas al "crop-forcing" (Lebon et al., 2004). Por el contrario, las temperaturas durante el periodo de envero a vendimia fueron más bajas en las cepas "crop-forcing" coincidiendo con los resultados obtenidos en otros estudios (Gu et al., 2012; Kishimoto et al., 2022; Martínez-Moreno et al., 2019; Martínez de Toda et al., 2019).

A pesar del aumento significativo en el número de racimos por cepa observado al aumentar la carga de yemas, El "crop-forcing" provoca una pérdida de producción que es más notoria cuando se realiza después de floración (F1) (Tabla IV.6). Además, disminuye el peso de baya, así como el número de bayas por racimos, pero en este caso la pérdida es mayor cuando el "crop-forcing" se realiza después de cuajado (F2). Esta menor producción, se debe principalmente a un menor número de bayas por racimo y en segundo lugar al menor peso de bayas. Las diferencias en estos componentes del rendimiento, en relación con el tratamiento C pudo ser debida tanto a la posición de las yemas fructíferas (las que dieron lugar a racimos) tras aplicar la poda de "crop-forcing", como al cambio en las condiciones meteorológicas bajo las que se produjo el desarrollo de las flores, floración y cuajado (Khanduja & Balasubrahmanyam, 1972). Aunque algunos autores han propuesto la doble vendimia (Martinez de Toda, 2021; Poni et al., 2020) como una opción para compensar esta pérdida de cosecha, supone que la "ventaja" en cuanto al cambio en la composición de las bayas solo afectaría a parte de la producción, y la valoración quedaría en manos de los enólogos. En 2020, Poni et al. (2020), estudiaron el efecto de la doble poda en 'Pinot Noir' con el fin de producir un retraso en la maduración tanto de la cosecha principal, como de la segunda cosecha, sin las pérdidas de producción que conlleva la aplicación del "crop-forcing". En su estudio, consiguieron un rendimiento adicional del 40-50% de la producción. Además, la segunda vendimia supuso un aumento en el contenido en sólidos solubles totales, acidez total, ácido málico y antocianos. TA y ácido málico. Resultados similares fueron obtenidos por Martinez De Toda (2021) en 'Garnacha', 'Tempranillo' y 'Maturana Tinta'. La doble poda puede ser una buena técnica que consiga combinar los efectos positivos del “crop-forcing” en la calidad de las uvas y el vinos, pero mitigando la pérdida o incluso aumentando la producción que esta técnica conlleva.

Aunque es cierto que el descenso en la cosecha ha sido una constante en los trabajos publicados hasta el momento (Gu et al., 2012; Kishimoto et al., 2022; Martínez-Moreno et al., 2019; Martinez De Toda et al., 2019), sería interesante ampliar la información disponible con un mayor número de variedades y condiciones agroclimáticas, incluyendo viñedos vigorosos y puede tener interés para enfrentar situaciones que de forma natural implicarían la pérdida de la cosecha, como sería el caso del granizo o problema fitosanitario grave en etapas iniciales post-brotación.

Otra cuestión práctica que plantea la aplicación de esta técnica es la fecha de entrada en bodega. En las condiciones en que se ha realizado este ensayo la vendimia de los tratamientos “crop-forcing” (principalmente el segundo) se realizó con las bodegas cerradas para la recepción de uvas, retrasó la vendimia una media de 56 días con respecto a la fecha del tratamiento sin forzar y estando coincidiendo con los resultados obtenidos por otros autores (Gu et al., 2012; Kishimoto et al., 2022b; Martínez-Moreno et al., 2019; Martínez de Toda et al., 2019), planteándose como una opción interesante para escalonar la recepción en las bodegas.

El menor tamaño de baya y las temperaturas más favorables modifican la composición de las bayas. Este es el aspecto clave de esta técnica y en la valoración de la importancia de este cambio reside el interés y por tanto el futuro de la misma. Las uvas procedentes de cepas “crop-forcing” muestran un mayor contenido en polifenoles y antocianos totales (Tabla IV.7). Cuando el “crop-forcing” se realiza después de cuajado, se consigue aumentar el contenido en ácido málico y disminuir el valor de pH. Estos resultados se mantienen en los vinos resultantes, en los que se ha obtenido un mayor contenido en acidez total y ácido málico que los vinos procedentes de cepas sin “crop-forcing” (Tabla VIII.5).

La aplicación del “crop-forcing” también modifica el perfil antociánico de las uvas y del vino. El aumento en el contenido en antocianos (Tabla IV.7) se produce por un aumento de los derivados de glucósidos (Figura VII.2). Sin embargo, el “crop-forcing” solo consigue mejorar el contenido en acetilados y cumaratos en 2017 en uvas (Figura VII.3). En los vinos, se mantiene la tendencia en el contenido en derivados glucósidos en los vinos procedentes de cepas “crop-forcing” después de cuajado (Tabla VIII.8), pero los datos de acetilados y cumaratos muestran un comportamiento diferente. Con la aplicación del “crop-forcing” el contenido en acetilados aumenta en 2018, sin embargo, el contenido en cumaratos disminuye en 2017 con respecto a los vinos procedentes de cepas sin “crop-forcing” (Tabla VIII.8). Debido a que las formas aciladas se forman a partir de las no aciladas (Ford et al., 1998), cabe pensar que las uvas de F1 y F2 en 2018 y 2019 no han tenido tiempo suficiente para dar lugar a sus formas aciladas, lo que puede provocar una disminución en la estabilidad de los vinos resultantes.

El contenido de los derivados de antocianos en función de su grupo fenólico también muestra una misma tendencia en uvas y vinos (Figura VII.3 y Tabla VIII.8). Excepto los derivados de malvidina en 2017 (tanto en uvas como en vinos), la aplicación del “crop-forcing” supone un aumento en el contenido de todas las antocianidinas analizadas. Estos resultados están de acuerdo con los obtenidos por Tian and Gu, (2019), cuyos resultados en uvas sostienen que el aumento de antocianos producido por el “crop-forcing” se debe al aumento de la concentración de la forma libre, mientras que no apreciaban este aumento en las formas acetiladas.

La hipótesis de partida de esta tesis era desplazar la maduración de la uva hacia un periodo de temperaturas más favorables. La técnica del “crop-forcing” ha sido efectiva para conseguir este desplazamiento, provocando un descenso en las temperaturas máximas diarias durante la maduración (desde envero a vendimia) entre 5 y 8°C más bajas, sobre todo cuando el “crop-forcing” se aplica después del cuajado. Según Sadras and Moran (2012), las altas temperaturas durante la maduración aceleran la acumulación de azúcares, provocando un desacople entre la madurez fenólica y tecnológica. La técnica del “crop-forcing”, consiguen mejorar el acoplamiento entre la madurez fenólica y tecnológica, alcanzando un mayor contenido en polifenoles y antocianos totales (en los tres años de aplicación) y acidez total (en 2018 y 2019) con una menor acumulación de sólidos solubles totales (Figura V.7). Las uvas procedentes de cepas “crop-forcing”, tanto después de floración como después de cuajado podrían vendimiarse a 22ºBrix, para dirigir la vendimia hacia vinos de menor grado alcohólico, obteniéndose mejoras tanto en el contenido en acidez total, polifenoles y antocianos totales, incluso comparando con los valores obtenidos en las cepas no “crop-forcing” a 24ºBrix (Tabla V.5).

Un aspecto de indudable interés práctico que también se ha tratado en este trabajo es el efecto acumulado del “crop-forcing” sobre las cepas. El “crop-forcing” es una técnica “agresiva” que llega a modificar no solo la temporización de la fenología de la vid, sino también las relaciones entre órganos vegetativos y productivos y por tanto las relaciones fuente-sumidero. El “crop-forcing” disminuyó la producción de biomasa total, sobre todo cuando se aplica después de cuajado (F2). Además, supuso una modificación en la distribución de biomasa, con una mayor proporción de material retirado en las podas en

F2, mientras que disminuye su proporción de hojas con respecto a las cepas NF o “crop-forcing” después de floración (F1) (Figura VI.8 y VI.9 y Tabla VI.6). Sin embargo, la aplicación del “crop-forcing” no supone un desgaste en el nivel de reservas en F1 y leve en F2. La cantidad de almidón y azúcares solubles no se ha reducido de manera clara en los años en el que el “crop-forcing” se ha llevado a cabo, ni en el año siguiente tras dejar de aplicarlo, ni en raíces, ni en sarmientos ni en hojas (VI.Capítulo 3).

En este trabajo se ha tratado de forma conjunta la aplicación del “crop-forcing”, riego deficitario con estrategias de RI por varios motivos: el primero por la importancia que tiene en los viñedos de zonas semi-ardidas como Extremadura el uso eficiente del agua; en segundo lugar para contrastar con una de las técnica más utilizadas para mejorar las características de las uvas tintas para vinificación; y finalmente para aprovechar la oportunidad que ofrece el desplazamiento del ciclo del viñedo para inducir déficit hídrico en el periodo pre-envero, que en estas condiciones es difícil conseguir con la meteorología habitual. La aplicación del riego deficitario (RI) no modificó el ciclo fenológico de las cepas. Someter las cepas a la estrategia de RI supuso una pérdida de rendimiento de en torno al 20-40% (Tabla IV.6). Esta disminución de la producción apenas se vio compensada en la composición de la baya (Tabla IV.7), ni en la de los vinos (Tablas VIII.5, VIII.6 y VIII.7). Tampoco se aprecia un acoplamiento mayor entre la madurez fenólica y tecnológica de las uvas procedentes de cepas con limitaciones hídricas (Figura V.7).

En el perfil de antocianos, las diferencias entre los dos tratamientos de riego en uvas se aprecian en 2017 y 2019 para los compuestos trisustituidos (malvidina, petunidina y delfinifina), con un aumento de estos compuestos para RI con respecto a C, mientras que en los derivados disustituidos (peonidina y cianidina) no se aprecian estas diferencias. En los vinos, los resultados son diferentes, la malvidina y la petunidina muestran un valor mayor en RI que en C solo en 2017, mientras que la peonidina y la cianidina muestran en 2018 un mayor valor en C que en RI. En 2018, debido a las altas lluvias, la aplicación de RI no consigue aumentar significativamente ninguno de estos compuestos, demostrando la variabilidad interanual de esta estrategia en función de las condiciones meteorológicas (Girona et al., 2009; Tian and Gu, 2019; Uriarte et al., 2016).

El efecto combinado del “crop-forcing” y un RI pre-envero supone, en términos generales, un aumento del contenido en antocianos de las uvas y los vinos (Tabla IV.6 y Figuras VIII.1-VIII.3), aunque con gran dependencia de las condiciones climáticas del año estudiado. Sin embargo, si supone un uso más eficiente del agua. Cuando el “crop-forcing” se aplica sin limitaciones hídricas, puede llegar a suponer en torno a un 20%

más de agua que el tratamiento sin forzar y sin limitaciones hídricas (C-NF), mientras que al aplicar el "crop-forcing" con un RI pre-envero se ahorra en torno a un 25% del agua con respecto a C-NF, mientras que apenas supone un cambio con respecto al tratamiento sin forzar y con déficit hídrico (RI-NF) (Tabla IV.4), aunque estos resultados dependen de la climatología anual.

Sin embargo, estos resultados se han obtenido con una variedad y condiciones agroclimáticas concretas por lo que da pié a otros trabajos en los que se verifique e incluso se ofrezcan opciones para superar algunas de las desventajas de esta técnica.

A la vista de los resultados obtenidos entendemos que la técnica del "crop-forcing" puede tener interés en determinados contextos, pero que para su puesta a punto es necesario ampliar la información disponible ampliando los escenarios e incidir en aspectos que no han sido objeto de este trabajo, como la valoración económica o la mecanización de las labores de cultivo. Otro aspecto inexplorado es el efecto que puede tener el cambio en las condiciones meteorológicas en los diferentes estados fenológicos sobre la incidencia de plagas y enfermedades. Como se mencionó en el inicio de esta discusión, esta ha sido una aportación más para explorar una nueva técnica y muy probablemente no la última.

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X. CONCLUSIONES GENERALES

En este trabajo se ha demostrado que el "forzado de yemas" es una técnica efectiva para retrasar el periodo de maduración de las uvas del 'Tempranillo' en las condiciones agroclimáticas de Extremadura. Del análisis de los resultados obtenidos se pueden extraer las siguientes conclusiones:

Capítulo 1:

- ✓ El "forzado de yemas" es una técnica útil para retrasar la maduración a periodos con temperaturas más favorables.
- ✓ Esta técnica provoca una pérdida de producción, aunque se acerca a las limitaciones establecidas por las DOP.
- ✓ Al aplicar el "forzado de yemas" durante tres años consecutivos sobre las mismas cepas no se aprecia un efecto depresor sobre el rendimiento.
- ✓ La aplicación del "forzado de yemas" después de cuajado consigue aumentar el contenido en polifenoles y antocianos totales y la acidez titulable, así como disminuir el pH.
- ✓ La combinación del "forzado de yemas" y la estrategia de riego deficitario controlado preenvero mejora la calidad de las uvas sometidas al "forzado de yemas" sin limitaciones hídricas, aunque provoca una mayor pérdida en los parámetros productivos.

Capítulo 2:

- ✓ El "forzado de yemas" es una técnica capaz de restablecer el desacoplamiento entre madurez fenólica y tecnológica.
- ✓ La aplicación del "forzado de yemas" permite mejorar el contenido en acidez total, polifenoles y antocianos totales de las bayas con valores más bajos de sólidos solubles totales.
- ✓ Cuando el "forzado de yemas" se lleva a cabo con una limitación hídrica en el periodo de preenvero se produce un incremento en el contenido en antocianos y además una mejora en la eficiencia del uso del agua.

Capítulo 3:

- ✓ El "forzado de yemas", el estrés hídrico o la combinación de ambos no suponen un desgaste progresivo de las cepas. Estas técnicas no provocaron una disminución en los niveles de reservas de las cepas, ni durante su aplicación ni en el año siguiente.
- ✓ El "forzado de yemas" aplicado después de cuajado si supone una disminución y una reorganización de la biomasa acumulada.

Capítulo 4:

- ✓ El “forzado de yemas”, sobre todo aplicado después de cuajado, consigue valores más elevados de polifenoles, catequinas y antocianos.
- ✓ El aumento del contenido de antocianinas se reflejó en un incremento de la forma monoglucósida y en todas las antocianidinas analizadas.
- ✓ El impacto del estrés hídrico tiene una gran variabilidad interanual.

Capítulo 5:

- ✓ La mejora en la composición ácida y fenólica de las uvas sometidas al “forzado de yemas” se corresponde con una mejora de estos mismos parámetros en los vinos, en respuesta a una menor temperatura durante la maduración de las uvas.
- ✓ En general, los vinos procedentes de cepas forzadas obtienen valores más elevados de acidez total, ácido málico, antocianos, catequinas y polifenoles totales, así como una mayor intensidad de color y un porcentaje de antocianos copigmentados más elevado.
- ✓ Los vinos procedentes de cepas forzadas son más sensibles a la oxidación que los correspondientes procedentes de cepas no forzadas.