Universitat de Lleida

Evaluación de algunas tecnologías para la determinación de los efectos de la gestión del agua de riego y del nitrógeno en frutales

Miquel Pascual Roca

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EVALUACIÓN DE ALGUNAS TECNOLOGÍAS PARA LA DETERMINACIÓN DE LOS EFECTOS DE LA GESTIÓN DEL AGUA DE RIEGO Y DEL NITRÓGENO EN FRUTALES

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Lleida, 2015

El DR. JOSEP M. VILLAR MIR, catedrático de Universidad, y el

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DECLARAN:

Que la presente memoria titulada " EVALUACIÓN DE ALGUNAS TECNOLOGÍAS PARA LA DETERMINACIÓN DE LOS EFECTOS DE LA GESTIÓN DEL AGUA DE RIEGO Y DEL NITRÓGENO EN FRUTALES", presentada por Miquel Pascual Roca para optar al Título universitario oficial de DOCTOR por la Universidad de Lleida, dentro del programa de doctorado de esta Universidad, se ha realizado bajo nuestra dirección.

Y que revisada, autorizamos su presentación para ser juzgada por el tribunal asignado.

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RESUMEN

La optimización de la productividad del agua de los cultivos es un objetivo clave en la agricultura moderna. El riego deficitario es una estrategia ampliamente utilizada cuando los recursos de agua y energía son escasos y/o caros. En el cultivo de árboles frutales, la severidad y duración del estrés hídrico pueden afectar al rendimiento y al tamaño del fruto, imponiendo severas limitaciones en el resultado de los procesos de producción, la calidad del fruto y de sus transformados, además de comprometer la productividad del agua, tanto en términos agronómicos como económicos.

El capítulo II se dedica al estudio de la eficiencia de uso de agua (WUE) desde diferentes perspectivas y con diferentes métodos. La relación de isotopos de carbono y de nitrógeno son herramientas que se utilizan ampliamente en los estudios ecofisiológicos y de mejora vegetal. Sin embargo, el uso de técnicas de discriminación de isótopos en especies frutales ha sido muy limitado. Para conocer sus posibilidades a efectos de determinar su validez para la discriminación de períodos de estrés hídrico se utilizó un experimento en melocotonero en el que se cruzaron tres estrategias de riego (riego completo durante toda la temporada de crecimiento; restringido el riego durante la fase II de crecimiento del fruto (restricción del 70%); y restringido el riego durante la etapa-III (restricción 30%), con tres niveles de nitrógeno (0, 60 y 120 kg N ha^{-1}). La proporción de isótopos estables de carbono ($\delta^{13}C$) en frutos resultó ser un indicador fiable del estado hídrico de la planta y de la eficiencia del uso del agua. $\delta^{13}C$ en los tejidos del endocarpio del fruto exhibió una potente capacidad discriminatoria de estrés hídrico durante la fase II de crecimiento, mientras que $\delta^{13}C$ en el mesocarpio fue un trazador interesante para detectar el estrés de agua en la fase III de crecimiento. Se encontró una relación significativa entre el contenido de nitrógeno de la hoja N y la eficiencia fotosintética durante el periodo GSII, mejorando WUE, tanto si fue medida como WUE productiva (WUE_{WPy}) o a partir de las variables de intercambio gaseoso de la hoja (WUE intrínseca e instantánea). La proporción de isótopos de nitrógeno ($\delta^{15}N$) en el mesocarpio de la fruta se relacionó significativamente con la cantidad de N aplicado y el origen de éste. Sin embargo, la proporción de isótopos de nitrógeno no mostró una buena capacidad discriminativa de la influencia del agua y nitrógeno en la respuesta de la planta.

En el mismo experimento, WUE también fue estudiado con el propósito de realizar un estudio global de la eficiencia de uso de agua integrando el efecto año (el cual no solo es atribuible al clima). Se utilizaron los resultados obtenidos durante 4 años de la medida de la asimilación neta de carbono y el intercambio gaseoso de hoja, del estado hídrico de las plantas, la biomasa producida (podas y rendimiento de fruta) y la calidad de los frutos. En términos agronómicos WUE se midió como la productividad del agua de frutos y biomasa (WUE_{WPy}, b), y en términos fisiológicos, como la eficiencia de uso de agua intrínseca e instantánea de hoja (WUEint y WUEins respectivamente). Como índice integrado de WUE se utilizó las relaciones isotópicas de carbono y oxígeno ($\delta^{13}C$ y $\delta^{18}O$) en la madera del tronco, una vez identificados los anillos de crecimiento de cada año. Los resultados sugieren que la variación de la productividad agronómica del agua (WUE_{WPy}) fue causado por una compleja regulación entre el crecimiento de las plantas y la fructificación, en relación con el estado hídrico de las plantas y la disponibilidad de nitrógeno, mostrando ser dos factores clave de WUE en términos de productividad (relación peso de fruta por fracción de agua transpirada respecto ETo). Las relaciones isotópicas de carbono y oxígeno en madera se relacionaron con la conductancia estomática y la transpiración, y también con los efectos del riego y nitrógeno. No se observó una relación clara entre estos indicadores y WUE_{WPy}. El estado hídrico de la planta fue dependiente del riego aportado, de la carga de frutos y del crecimiento vegetativo, relacionándose estrechamente con WUE_{WPy} (masa de frutos/agua transpirada respecto ETo) y WUE_{WPb} (masa de vegetación/agua transpirada respecto ETo).

En conjunto, los resultados muestran que el riego completo y la aplicación de nitrógeno con dosis suficientes tienen efectos positivos y aditivos en WUE_{WPy}, tanto en términos agronómicos como económicos. Los resultados también muestran que cuando el régimen de lluvia es muy variable en primavera y pueden producirse restricciones de agua en verano, el riego deficitario con restricciones moderadas en la fase de crecimiento del fruto III, posibilita un ahorro de agua similar o superior al riego deficitario de fase II, constituyendo una estrategia más eficiente que éste. En este sentido, el riego deficitario en GSIII, utilizado en una forma flexible, es un método a considerar en las recomendaciones destinadas a mejorar la eficiencia de agua y del nitrógeno en cultivos frutales en zonas donde las restricciones de agua se producen en pleno verano y de manera variable.

El capítulo III trata la eficiencia desde el punto de vista de la optimización de la calidad de la fruta como medio para incrementar la productividad económica del agua. Es conocido que la alta actividad enzimática de los frutos se asocia a condiciones indeseables para mantener la calidad necesaria de estos para su manipulación, conservación y distribución,

además de alcanzar la calidad de consumo más adecuada a los estándares de cada mercado. Una de las enzimas que ha sido reconocida como causante de pardeamientos y de la pérdida de calidad de los frutos es la *o*-polifenol-oxidasa (PPO), que también ha sido relacionada con la estabilidad de los frutos en post-cosecha y, por tanto, afectando las posibilidades de mercadeo de los mismos. La actividad de esta enzima depende de varias variables de distinta naturaleza, siendo poco estudiadas algunas de las agronómicas con mayor peso en la fruticultura mediterránea (agua y nutrición). El conocimiento de su influencia en la actividad PPO y la relación entre ellas puede llegar a ser interesante para administrar los insumos de los cultivos a fin de lograr la calidad deseada de la fruta.

Para ello se planteó un experimento en melocotonero tipo platicarpa, en el que fueron aplicados diferentes dosis de nitrógeno (4) durante dos años. La PPO se midió en diferentes momentos de la maduración de los frutos y se relacionó con las variables productivas (rendimiento), la calidad de los frutos y el contenido de nutrientes de las hojas y frutos. Los frutos se recolectaron escalonadamente conforme los tratamientos alcanzaban iguales condiciones de madurez, medidas como los sólidos solubles, la firmeza y el color de la epidermis. Utilizando técnicas de análisis multivariante se identificaron las relaciones entre las diferentes variables, indicando que una dosis relativamente baja de fertilización fue suficiente para evitar cualquier deficiencia de nutrientes, además de mostrar una actividad baja de la PPO frente a las dosis de N elevadas. La relación N/Ca en los frutos resultó ser un buen indicador de actividad de la PPO, más que el contenido de N ó Ca condiderados independientemente. El contenido de nutrientes en las hojas presentó una débil correlación con PPO de los frutos y con la calidad en general. Las variables más útiles para predecir la actividad PPO fueron la relación N/Ca, la firmeza de la fruta y el color de la piel. Los resultados mostraron que las dosis altas de fertilización promovieron mayores rendimientos de frutos pero también una mayor actividad de PPO, lo que indica un riesgo potencial alto para las posibilidades comerciales de la fruta.

En el capítulo IV se aborda la eficiencia del uso de agua desde una perspectiva diferente a las anteriores. La fruticultura de secano (olivo y vid) está ampliamente extendida en el área mediterránea, normalmente en condiciones muy pronunciadas de déficit de agua. En estas condiciones, una de las metas principales de la gestión de estos sistemas de cultivo trata de alcanzar la máxima eficiencia de este recurso, tanto para optimizar los rendimientos como para maximizar el valor añadido de la producción. En este sentido, los resultados de la viticultura de secano está muy limitados por la variabilidad ambiental, particularmente por la lluvia y su distribución estacional y que, junto al suelo, determinan en buena medida el

rendimiento y la calidad final de la uva y el vino. Los estudios sobre la viabilidad de algunas técnicas para adaptar la planta al medio, como es el manejo del dosel foliar, abren posibilidades para mejorar la calidad del vino y explotar las características que conforman el *terroir* lo que puede contribuir a la mejora de la eficiencia del uso recursos de producción tanto desde una perspectiva económica como agronómica. En esta tesis se presenta un caso de estudio en el que se analizó la influencia de las prácticas de manejo de dosel en el estado hídrico de las plantas y su relación con la calidad de las uvas y el vino resultante. Para ello se dispuso un diseño experimental en una finca, de referencia en la D.O. Somontano, que contempló el efecto del suelo (diferente profundidad y agua disponible), y el manejo de la vegetación, usando tres estrategias que consistieron en realizar pinzamientos en los pámpanos inmediatamente después del cuajado de las uvas para ralentizar su crecimiento vegetativo, deshojar la zona de fructificación, y realizar una poda de rebaje de los sarmientos en la época de envero (siendo esta la técnica habitual de la D.O.). Los resultados indicaron que las diferentes estrategias de manejo del dosel tienen una influencia predominante en el estado hídrico de la planta a lo largo de la estación de crecimiento, modulando el consumo, además de afectar el perfil de aminoácidos del mosto y los atributos sensoriales del vino obtenido. Los pinzamientos de la vegetación realizados en la época de cuajado de las bayas se mostraron eficaces para regular el estado hídrico de la planta durante el período de maduración, incluso en los suelos con baja disponibilidad hídrica. Paralelamente, el deshojado de la zona de fructificación no causó ningún cambio significativo en el estado hídrico de la planta, aunque provocó cambios importantes en la composición de aminoácidos de la baya y los atributos sensoriales del vino. Los resultados, globalmente, pusieron de manifiesto la utilidad de las técnicas de manejo del dosel como medio para gestionar el agua del suelo con el objetivo de fomentar la calidad del vino y facilitar la adaptación del viñedo a diferentes condiciones de disponibilidad de agua.

SUMMARY

Optimizing water productivity of crops is a key objective in modern agriculture. Deficit irrigation is a widely used strategy when water and energy resources are scarce and / or expensive. In the fruit tree crops water stress can affect fruit yield and fruit size, imposing severe restrictions on cropping processes and fruit quality and, in addition, affecting to water productivity from an economic perspective.

Chapter II is devoted to the study of water use efficiency (WUE) from different viewpoints and different ways. The carbon and nitrogen isotope relationship are used widely by agronomists and physiologists on plant breeding and ecophysiological studies involving WUE improvement or behavioral plant response to environmental variability.

However, the use of isotope discrimination techniques in fruit trees has been very limited to detect plant water stress periods and intensity. In this piece of work carbon and nitrogen isotope relationship ($\delta^{13}C$ and $\delta^{15}N$ respectively) were measured in endocarp and mesocarp of peaches from a nitrogen x irrigation experiment: full irrigation throughout the growing season; restricted irrigation during stage-II of fruit growth (70% restriction); and restricted irrigation during stage-III (30% restriction). These irrigation treatments were combined with three different nitrogen fertilization treatments: 0, 60 and 120 kg N ha^{-1}.

The stable carbon isotope ratio ($\delta^{13}C$) in fruit proved a reliable indicator of peach crop water status and physiological water use efficiency. $\delta^{13}C$ in endocarp exhibited a powerful discriminatory capacity for water stress during phase II of fruit growth, whereas $\delta^{13}C$ in the mesocarp was an interesting tracer for water stress in phase III of fruit growth. There was an important relationship between leaf N content and photosynthetic efficiency during period GSII, enhancing leaf WUE. The stable nitrogen isotope ratio ($\delta^{15}N$) in the fruit mesocarp was significantly related to the amount of nitrogen applied and their origin (organic or synthetic). The nitrogen isotope ratio did not, however, exhibit a good discriminative capacity when we studied the influence of water and nitrogen on plant response.

In the same experiment, water use efficiency (WUE) was studied over four years in agronomic terms, as water productivity of fruits and biomass ($WUE_{WPy,b}$), and in physiological terms, as intrinsic and instantaneous leaf WUE (WUE_{int} and WUE_{ins} respectively). Our results show a positive and additive effect of water and nitrogen

application on $WUE_{WP,}$ reflected by yield enhancement. WUE_{int} and WUE_{ins} varied through changes in stomatal conductance and transpiration—two parameters that are determined by plant water status and nitrogen application. Our results suggest that the variation in WUE_{WPy} was caused by complex regulation of plant growth and fruiting, related to water status and nitrogen availability, which both drive plant WUE. $\delta^{13}C$ and $\delta^{18}O$ in wood was studied in order to evaluate WUE at the canopy scale. These indicators were found to be related to leaf stomatal conductance and transpiration and also to the effects of irrigation and nitrogen; however, no clear relationship was observed when were related to WUE_{WPy}. Plant water status was also dependent on crop load and vegetative growth and was strongly associated with WUE_{WPy} and WUE_{WPb}. Taken together, the results show that full irrigation and rational nitrogen application can improve WUE_{WPy}, both in agronomic and economic terms. When water deficit conditions are foreseeable, deficit irrigation in fruit growth phase III (GSIII) is a more profitable strategy that when applied in phase II (GSII). In this regard, flexible deficit irrigation in GSIII is an affordable and practical tool to include in strategies aiming to improve water and nitrogen use efficiency in fruit crops when water is scarce.

In Chapter III we studied the fruit quality for indirect improvement of water use efficiency. Polyphenol oxidase (PPO) is one of the main factors that determine postharvest stability and therefore commercial possibilities of peach orchards. Since the activity of this enzyme depends on several variables of different nature, stating the relationships among them is essential to manage crop inputs so as to achieve the desired quality. For this purposes, a two years experiment in flat peach trees was carried out. The study addressed the effects on fruit quality promoted by different rates of nitrogen (4) under full irrigation. The PPO was measured at different times of the ripening of fruits and variables related to production (yield), fruit quality and nutrient content of leaves and fruits

Multivariate projection techniques have been used to identify these relationships throughout on-tree maturation in flat peach samples that had received different fertilization doses, but harvested in equal conditions of soluble solids, fruit weight, firmness and skin and flesh color. A relatively low fertilization dose that was enough to avoid any lack of nutrients provided the lowest crop yield, but also with the lowest PPO activity. Meanwhile, higher doses resulted in greater yields, but with higher PPO activity. Fruit N/Ca ratio turned out to be a better indicator of PPO activity than isolated nitrogen or calcium contents. Nutrient analyses on leaves did not have strong

correlations with any significant parameter. The most useful variables to predict PPO activity before harvest were fruit firmness and skin color, both of them read at the onset of on-tree maturation.

Chapter IV was devoted to analyses the water use efficiency from a different perspective. Rainfed fruit trees (olives and grapes) are widespread in the Mediterranean basin, usually under severe water deficit conditions. Under these conditions, one of the main goals in these cropping systems is to achieve maximum efficiency of natural resources (mainly rainfall) to optimize yields and to maximize the added value of production. In this sense, the results of rainfed viticulture can be limited by environmental variability, particularly rainfall and the characteristics of the soils, wich largely determine the quality of grapes and wine performance. Studies on the feasibility of some techniques to adapt the plant to the environment, such as the canopy management, open up possibilities for improving wine quality and take advantage of *terroir*. In this way, this chapter focuses on the study of the influence of canopy management practices on water status of the plants and their relationship with the quality of the grapes and wine.

Principal components analysis was used to identify the relationships between a large set of variables, including soil, plant, canopy management, and wine characteristics. Canopy management was found to have a predominant influence on plant response to soil water by modifying plant water status, changing the amino acid profile in berries and, concomitantly, altering the sensorial attributes of the wine obtained.

Grapevine canopy management strategies, such as reiterate shoot trimming to restrict growth during early phases, are effective in adapting plant response to soil water availability. Such strategies affect berry and wine quality, mainly the amino acid profile and sensorial attributes of the wine, without changing yield or grape harvest quality control parameters. Also, in such conditions, nitrogen does not make a significant contribution to grapevine growth or yield or to grape quality. The results showed the usefulness of canopy management techniques as a means to manage soil water content throughout the season, promoting wine quality and adapting the vineyard to different soil water availability.

RESUM

L'optimització de la productivitat de l'aigua dels cultius és un objectiu clau de l'agricultura moderna. El reg deficitari és una estratègia àmpliament utilitzada quan els recursos d'aigua i energia són escassos i/o cars. En el cultiu d'arbres fruiters la severitat i durada de l'estrès hídric poden afectar el rendiment i la mida del fruit, imposant severes limitacions en el resultat dels processos de producció, la qualitat dels fruits, a més de comprometre la productivitat de l'aigua, tant en termes agronòmics com econòmics. El capítol II es dedica a l'estudi de l'eficiència d'ús d'aigua (WUE) des de diferents enfocs i amb diferents mètodes. La relació d'isòtops de carboni i de nitrogen són eines que s'utilitzen àmpliament en els estudis ecofisiològics i en els de millora vegetal. No obstant això, l'ús de tècniques de discriminació d'isòtops en espècies fruiteres ha estat molt limitat. Per conèixer les seves possibilitats a l'efecte de determinar la seva validesa per a la discriminació de períodes d'estrès hídric en fruiters, es va utilitzar un experiment en presseguer en el què es van creuar tres estratègies de reg (reg complet durant tota la temporada de creixement; restringit el reg durant la fase II de creixement del fruit (restricció del 70%), i restringit el reg durant l'etapa-III (restricció 30%), amb tres nivells de nitrogen (0, 60 i 120 kg N ha^{-1}). La proporció d'isòtops estables de carboni (δ13C) en els fruits va resultar ser un indicador fiable de l'estat hídric de la planta i de l'eficiència de l'ús de l'aigua. La relació $\delta^{13}C$ en els teixits de l'endocarpi del fruit va exhibir una bona capacitat discriminatòria d'estrès hídric durant la fase II de creixement, mentre que $\delta^{13}C$ en el mesocarpi va detectar l'estrès d'aigua en la fase III de creixement. Es va trobar una relació significativa entre el contingut de nitrogen de la fulla i l'eficiència fotosintètica durant el període GSII, millorant WUE, tant si va ser mesurada com WUE productiva (WUE_{WPy}) o a partir de les variables d'intercanvi gasós de la fulla (WUE intrínseca i instantània). La proporció d'isòtops de nitrógen (δ15N) en el mesocarpi de la fruita es va relacionar significativament amb la quantitat de N aplicat i l'origen d'aquest. No obstant això, la proporció d'isòtops de nitrógen no va mostrar una bona capacitat per discriminar la influència de l'aigua i el nitrógen en la resposta de la planta.

En el mateix experiment, WUE també va ser estudiat amb el propòsit de realitzar un estudi global de l'eficiència d'ús d'aigua integrant l'efecte any (que no tan sols és atribuïble al clima). Es varen analitzar els resultats, obtinguts durant 4 anys, de l'assimilació neta de carboni i l'intercanvi gasós de fulla, de l'estat hídric de les plantes,

de la biomassa produïda (podes i rendiment de fruita) i de la qualitat dels fruits. En termes agronòmics, WUE es va mesurar com la productivitat de l'aigua de fruits i biomassa (WUEWPy, b), i en termes fisiològics, com l'eficiència d'ús d'aigua intrínseca i instantània de la fulla (WUEint i WUEins respectivament). Com índex integrat de WUE es va utilitzar les relacions isotòpiques de carboni i oxigen ($\delta^{13}C$ i $\delta^{18}O$) en la fusta del tronc un cop identificats els anells de creixement de cada any. Els resultats suggereixen que la variació de la productivitat agronòmica de l'aigua (WUE_{WPy}) va ser causada per una complexa interacció entre el creixement de les plantes i la fructificació, en relació amb l'estat hídric de les plantes i la disponibilitat de nitrogen, mostrant ser dos factors clau de WUE en termes de productivitat (relació pes de fruita per fracció d'aigua transpirada respecte ETo). Les relacions isotòpiques de carboni i oxigen en fusta es van relacionar amb la conductància estomàtica i la transpiració, i també amb els efectes del reg i nitrogen. No es va observar una relació clara entre aquests indicadors i WUEWPy. L'estat hídric de la planta va ser depenent del règim de reg, de la càrrega de fruits i del creixement vegetatiu, i es va relacionar estretament amb WUEWPy (massa de fruits/aigua transpirada respecte ETo) i WUE_{WPb} (massa de vegetació/aigua transpirada respecte ETo).

En conjunt, els resultats mostren que el reg complet i l'aplicació de nitrogen amb dosis suficients tenen efectes positius i additius en WUE_{WPy}, tant des de l'enfoc agronòmic com econòmic. Els resultats també mostren que quan el règim de pluja és molt variable a la primavera i les restriccions d'aigua son més probables a l'estiu, el reg deficitari amb restriccions moderades en la fase de creixement del fruit III, possibilita un estalvi d'aigua similar o superior al reg deficitari de fase II, constituint una estratègia més eficient que aquest. En aquest sentit, el reg deficitari en GSIII, utilitzat en una manera flexible, és un mètode a considerar en les recomanacions destinades a millorar l'eficiència d'aigua i del nitrogen en cultius fruiters en zones on les restriccions d'aigua es produeixen en ple estiu i de manera variable.

El capítol III tracta l'eficiència des del punt de vista de l'optimització de la qualitat de la fruita com a mitjà per incrementar la productivitat econòmica de l'aigua. És conegut que l'alta activitat enzimàtica dels fruits s'associa a condicions indesitjables per mantenir la qualitat necessària d'aquests per a la seva manipulació, conservació i distribució, a més de minvar la qualitat de consum més adequada als estàndards de cada mercat. Una dels enzims que ha estat reconeguda com a causant d'enfosquiments i de la pèrdua de qualitat dels fruits és la *o*-polifenol-oxidasa (PPO), que també ha estat relacionada amb

l'estabilitat dels fruits en postcollita i, per tant, afectant les possibilitats de màrqueting. L'activitat d'aquesta enzim depèn de diverses variables de diferent naturalesa, sent poc estudiades algunes de les agronòmiques amb major pes en la fructicultura mediterrània (aigua i nutrició). El coneixement de la seva influència en l'activitat PPO i la relació entre elles pot arribar a ser interessant per administrar els inputs dels cultius a fi d'aconseguir la qualitat desitjada de la fruita.

Per a això es va plantejar un experiment en presseguer tipus platicarpa, en què van ser aplicats diferents dosis de nitrogen (4) durant dos anys. La PPO es va mesurar en diferents moments de la maduració dels fruits i es va relacionar amb variables productives (rendiment), qualitat dels fruits i contingut de nutrients de fulles i fruits. Els fruits es van recol·lectar esglaonadament segons els tractaments per assolir les mateixes condicions de maduresa, mesurades mijantçant els sòlids solubles, la fermesa i el color de l'epidermis. Utilitzant tècniques d'anàlisi multivariant es van identificar les relacions entre les diferents variables, indicant que una dosi relativament baixa de fertilització va ser suficient per evitar qualsevol deficiència de nutrients, mostrant una activitat baixa de la PPO enfront de les dosis de N elevades. La relació N/Ca en els fruits resultà ser un bon indicador d'activitat de la PPO, més que l'efecte del contingut de N ó Ca analitzats independentment. El contingut de nutrients en les fulles presentà una feble correlació amb PPO dels fruits i amb la qualitat en general. Les variables més útils per predir l'activitat PPO van ser la relació N/Ca, la fermesa de la fruita i el color de la pell. Els resultats van mostrar que les dosis altes de fertilització promogueren majors rendiments de fruits però també amb una major activitat de PPO, el que indicà un risc potencial alt per a les possibilitats comercials de la fruita.

En el capítol IV s'aborda l'eficiència de l'ús d'aigua des d'una perspectiva diferent a les anteriors. La fructicultura de secà (olivera i vinya) està àmpliament estesa a l'àrea mediterrània, normalment en condicions molt pronunciades de dèficit d'aigua. En aquestes condicions, una de les fites principals de la gestió d'aquests sistemes de cultiu tracta d'aconseguir la màxima eficiència de l'aigua, tant per optimitzar els rendiments com per maximitzar el valor afegit de la producció. En aquest sentit, els resultats de la viticultura de secà estan molt limitats per la variabilitat ambiental, particularment per la pluja i la seva distribució estacional i que, amb el sòl, determinen en bona mesura el rendiment i la qualitat final del raïm i el vi. Els estudis de la viabilitat d'algunes tècniques per adaptar la planta al medi, com és el maneig del dosser foliar, obren possibilitats per millorar la qualitat del vi i explotar les característiques que conformen

el *terroir*. En aquesta tesi es presenta un cas d'estudi en el qual es va analitzar la influència de les pràctiques de maneig de dosser en l'estat hídric de les plantes i la seva relació amb la qualitat del raïm i el vi obtingut. Per a això es va disposar un disseny experimental en una finca, de referència a la D.O. Somontano, que va contemplar l'efecte sòl (diferent profunditat i aigua disponible) i el maneig de la vegetació, utilitzant tres estratègies que van consistir en realitzar pinçaments en els pàmpols immediatament després del quallat del raïm per alentir el seu creixement vegetatiu, esfullar la zona de fructificació, i realitzar una poda de rebaix dels sarments en l'època de verol (essent aquesta la tècnica habitual de la D.O.). Els resultats van indicar que les diferents estratègies de maneig del dosser tenen una influència predominant en l'estat hídric de la planta al llarg de l'estació de creixement, modulant el consum, a més d'afectar el perfil d'aminoàcids del most i els atributs sensorials del vi obtingut. Els pinçaments de la vegetació realitzats en l'època de quallat de les baies es van mostrar eficaços per regular l'estat hídric de la planta durant el període de maduració, fins i tot en sòls amb baixa disponibilitat hídrica. Paral·lelament, el desfullat de la zona de fructificació no va causar cap canvi significatiu en l'estat hídric de la planta, encara que va provocar canvis importants en la composició d'aminoàcids dels raims i els atributs sensorials del vi. Els resultats, globalment, van posar de manifest la utilitat de les tècniques de maneig del dosser com a mitjà per gestionar l'aigua del sòl amb l'objectiu de fomentar la qualitat del vi i facilitar l'adaptació de la vinya a diferents condicions de disponibilitat d'aigua.

Capítulo I: Introducción y objetivos

1 Introducción y objetivos

1.1 Marco de partida: recursos y gestión

El siglo XX ha sido crucial en el desarrollo tecnológico de la agricultura en campos como la mejora vegetal, las nuevas técnicas de producción y de post-cosecha, lo que unido al mejor conocimiento del medio productivo, han posibilitado un gran progreso de la capacidad de la producción de alimentos y las expectativas de futuro. Paralelamente, el pasado siglo también ha visto el nacimiento de un fuerte movimiento ambientalista que ha canalizado la creciente sensibilización de la sociedad por la calidad y salud de los alimentos, y por la forma en que deben conservarse adecuadamente los recursos naturales, que en muchos casos han sido objeto de una sobre-explotación. Las declaraciones de intenciones y las acciones específicas en este tema han sido numerosas, incidiendo en una primera etapa en la necesidad de preservar los recursos naturales mediante políticas con un amplio y marcado carácter social. Sin embargo, los cambios globales de las últimas décadas, particularmente los ligados a la economía y la sensibilización de la sociedad hacia la alimentación y el medio ambiente, han propiciado un nuevo enfoque que reconoce la creciente necesidad de acercar al mercado los servicios ambientales y de los recursos naturales para alcanzar su sostenibilidad (Power, 2010). En este sentido, puede parecer banal afirmar hoy que el sector agrario es capaz de asumir el mercadeo de sus producciones, aun con dificultades insoslayables; sin embargo, este mismo sector se muestra aun incapaz de incorporar a su economía los posibles beneficios de los servicios ambientales derivados de la producción, al menos en una cuota significativa que contribuya a su viabilidad (Gómez-Baggethun et al., 2010).

En este punto, es preciso matizar que esta introducción no ha contemplado la opción, casi obligada en estos casos, de resumir la ingente cantidad de información sobre el tema de la gestión de los recursos y su eficiencia en el ámbito agrário, particularmente en el uso del agua. Autores como Hargreaves y Samani (1984), Uphoff (1986) o English (1990), plasmaron magistralmente el estado del arte y el núcleo de las preocupaciones que movió la agricultura de la segunda mitad del siglo XX, incidiendo en la mejora de la eficiencia del uso de recursos mediante la gestión de los procesos (en este caso del agua) y apuntando la necesidad de la gestión integral de los sistemas de producción.

En la línea del progreso, los grandes retos que marcan el desarrollo de la agricultura de nuestro siglo (el XXI) giran en torno a cinco ejes estratégicos: sostener la innovación tecnológica, mantener y mejorar la productividad del suelo, compatibilizar la producción de

alimentos con el ambiente y la seguridad alimentaria, adaptar los sistemas al cambio climático, y promover la gestión intersectorial del agua.

En el trasfondo de los objetivos expuestos se vislumbra un factor común: la necesidad de la gestión eficiente de los recursos –todos-, siendo primordial la gestión del agua. Sin duda, este recurso es uno de los más vulnerables del planeta, que puede comprometer dramáticamente aspectos clave para el futuro de la sociedad, como la degradación y pérdida de recursos (agua y suelo) y su impacto ambiental (Rijsberman, 2006; UNESCO, 2015). Sauer et al. (2010) introdujeron una perspectiva interesante en este tema; a pesar de algunas debilidades y discordancias de las proyecciones existentes de las necesidades futuras de agua y tierra, estos investigadores sostienen que es previsible que se den las condiciones necesarias para incrementar la eficiencia de uso del agua, lo que debe moderar el crecimiento de la necesidad de recursos naturales, tanto de la expansión de las tierras de regadío como el consumo de agua. Concluyendo en la necesidad de implementar modelos a escala que recojan las especificidades locales de la gestión y de la tecnología, favoreciendo la mejora de la eficiencia de uso de agua y tierra. Una visión un tanto crítica que muestra otra realidad de la que podría llamarse la "globalización tecnológica" y que requiere necesariamente de la implementación nuevas políticas para acercar y compatibilizar intereses contrapuestos, lo que probablemente constituye el mayor reto del futuro en estos temas (Libecap, 2008; Cosgrove y Cosgrove, 2012).

1.2 La eficiencia del uso de los recursos (agua y nitrógeno). Un concepto complejo y multifactorial

El título de esta memoria recoge, explícita o implícitamente, algunos de los aspectos tratados anteriormente. La gestión de procesos y de sistemas de producción necesita sin duda un conocimiento adecuado de las tecnologías utilizadas y de la suficiente capacidad metodológica para evaluar tanto la eficiencia de los recursos como la de los procesos utilizados. En una acepción heterodoxa del concepto, la eficiencia se relaciona con la capacidad para evitar pérdidas innecesarias de recursos para alcanzar un resultado concreto. Este término, la eficiencia, se ha prodigado en la investigación desde hace décadas, particularmente en el uso de agua y nutrientes (Tambussi et al., 2007; Hirose, 2011; Fixen et al., 2015).

La eficiencia de uso del agua a nivel de planta se ha abordado desde múltiples enfoques en cuanto a su evaluación, alcance e interpretación (Sinclair et al., 1984; Steduto et al., 2007; Hsiao et al., 2007; Pereira et al., 2012; Levidow et al., 2014), sin que converjan necesariamente en los mismos objetivos ni con el mismo alcance agronómico. Ello ha

promovido algunas voces críticas al reclamar una mayor definición del marco conceptual, y de los objetivos y la interpretación del término "eficiencia", tanto en sus vertientes agronómica como fisiológica (Blum, 2005; Blum, 2009; Lankford, 2012; Van Halsema y Vincent, 2012).

Una cuestión de fondo reside en la generalización de la intepretación del concepto eficiencia de uso del agua desde un enfoque unifactorial (el denominador solo incluye agua), por lo que, al final, el resultado puede ser tan solo una visión parcial del problema global de la eficiencia de uso de este recurso. Este es un tema interesante, y que no es nuevo, ya destacado por Uphoff (1986) en la presentación de su trabajo: "*there has been a growing realization that much of the poor performance stems from fundamental weaknesses in the human processes of planning and management, which no amount of investment in technological hardware is going to overcome on its own [Anthony Bottran cited in Sallam et al. 1984]*". Bajo esta perspectiva, es necesario un mayor conocimiento de la eficiencia del uso de agua en el contexto de las interacciones entre los factores claves del sistema productivo de regadío, como son el riego y la nutrición, constituyendo un capítulo fundamental en la investigación en cultivos frutales (Davis y Quick, 1998; Hatfield et al., 2001; Dordas y Sioulas, 2008).

El binomio rendimiento-calidad es ineludible para la explicación de los resultados de la producción frutal. En las zonas de regadío, particularmente cuando el déficit hídrico es substancial, tanto el riego como la nutrición llegan a constituirse como motores de la producción. Desde hace décadas, se conocen los procesos fisiológicos que muestran la existencia de una sensibilidad diferencial de los órganos y procesos metábolicos de las plantas a la restricción de agua, afectando en diferente grado tanto al crecimiento como a la producción (Hsiao, 1973). Estos incluyen el crecimiento vegetativo y la conformación del dosel foliar, el crecimiento de raíces, la inducción y diferenciación floral, el cuajado, la división celular del fruto, y su crecimiento y maduración, entre otros.

Las ventajas del riego deficitario controlado han sido demostradas ampliamente cuando se producen situaciones de escasez de agua (Chalmers y Van den Ende, 1975; Chalmers et al., 1981; Naor et al., 1999; Boland et al., 2000; Girona et al., 2005; Costa et al., 2007; Geerts y Raes, 2009). Sin embargo, el marco de aplicación de estas estrategias no puede generalizarse, y debe ser delimitada en un contexto más amplio. English (1990) dió una pista clara sobre el tema: dependiendo de los costes del riego y, como mínimo, de los del cultivo, las estrategias de riego deficitario incrementan generalmente la productividad del

agua (entendida como rendimiento del cultivo por unidad de agua utilizada) y pueden mejorar la sostenibilidad del sistema productivo, o no.

La relación del crecimiento estacional de los frutales y su relación con la dinámica del nitrógeno es bien conocida en líneas generales (Rufat y DeJong, 2001; Policarpo et al., 2002), a la vez que existe una exhaustiva información sobre los efectos de la nutrición tanto en el rendimiento como en la calidad. La oferta excesiva de N tiene pocas ventajas y muchos inconvenientes, tales como la reducción de porosidad del dosel debida al exceso de crecimiento vegetativo, los retrasos en maduración de la fruta, la pérdida de calidad, o la pérdida de este nutriente por lixiviación (Crisosto et al., 1997; DeJong, 1999; Daane et al., 2008). Tambien se conoce la interacción agua x nitrógeno, que ha sido ámpliamente estudiada en diferentes cultivos y escalas, y bajo diferentes perspectivas (Brueck, 2008; Rufat et al., 2009; Brueck y Senbayram, 2009; Wang et al., 2010; Sadras y Mcdonald, 2012; Shellie y Brown, 2012; Sadras y Richards, 2014). No obstante, en el ámbito de los cultivos frutales, se detecta aún la necesidad de profundizar en el conocimiento de una cuestión esencial para la producción y, particularmente, para la calidad, como es determinar los efectos de agua y nitrógeno a escala estacional (es decir, los efectos combinados de época, disponibilidad de nutrientes y estado hídrico). En este sentido, han aparecido nuevos enfoques en la evaluación de la eficiencia de uso de nutrientes (nitrógeno), que permiten ciertas posibilidades de integración en un análisis más global de los recursos (Hirose, 2011). Este planteamiento puede contribuir a la implementación de las bases tecnológicas más adecuadas para asegurar tanto la productividad primaria de los recursos (eficiencia) como para incrementar el valor potencial de las producciones (rendimiento y calidad en un sentido amplio).

1.3 Índices de eficiencia de uso de agua

Como se ha dicho, la eficiencia de uso de agua (WUE) ha sido ámpliamente usada tanto en términos de medida de la productividad de este recurso como para determinar la eficiencia del uso del agua a diferentes escalas. Desde un punto de vista fisiológico, a corto plazo, se ha utilizado para evaluar las posibilidades de adaptación de genotipos a diferentes condiciones de disponibilidad de agua (Araus et al., 2002), habitualmente como relación entre la asimilación neta de carbono (An) y la transpiración (T), lo que se conoce como WUE instantánea, y como la relación entre la asimilación neta de carbono y la conductividad estomática (gs), ó WUE intrínseca (Figura 1). En un enfoque clásico, se asume que WUE instantánea se ve influenciada por las condiciones ambientales, ya que la transpiración (T) es muy dependiente del grado de apertura de los estomas (gs) y el déficit

de presión de vapor (VPD) de la atmósfera que rodea la hoja (T = gs ·VPD). Mientras que WUEintrínseca excluye los efectos de los cambios en el flujo de agua de la hoja dependientes de VPD y se supone tan sólo influenciada por la apertura de los estomas (Bierhuizen y Slatyer, 1965). Sin embargo, este tema ha sido ampliamente revisado en la última década, reconociendo otros efectos importantes (Kemanian et al., 2005; Damour et al., 2010).

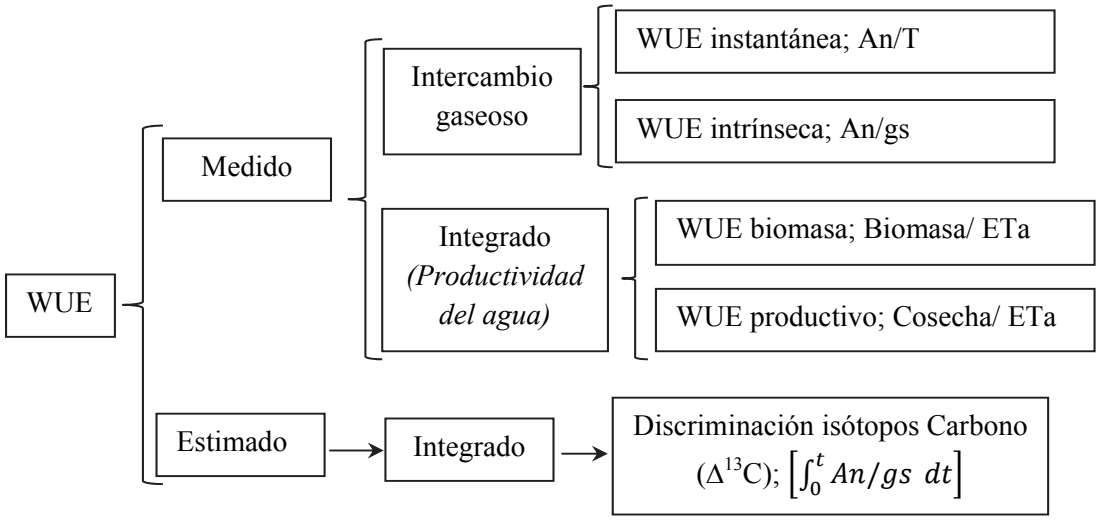

Figura 1. Diferentes formas de evaluar WUE. An, Asimilación neta de CO_2 de hoja; T, transpiración de la hoja; gs, conductancia estomática de la hoja, y ETa, (agua aplicada + lluvia efectiva)/ETo. (modificado de Tambussi et al. 2007)

Los índices de WUE medidos a partir de intercambio gaseoso muestran ser muy útiles en estudios comparativos sobre el comportamiento fisiológico de la planta en respuesta a cambios de corto plazo debidos al agua, la luz, la temperatura o el genotipo (Centritto et al., 2002; Condon et al., 2004; Cabrera-Bosquet et al., 2009); no obstante, un punto débil puede ser atribuído a la poca capacidad de integración de factores tan significativos en especies frutales como son la acción diferencial del medio en diferentes procesos de la planta, la intensidad y duración de los episodios de estrés, los efectos a corto y largo plazo de la tecnología de producción o el propio material vegetal (ciclo de vida y genotipos).

La efciiencia de uso de agua, entendida como la relación entre la cantidad de biomasa producida y el agua utilizada (o productividad del agua), se ha utilizado ampliamente como un índice de integración temporal para evaluar la eficiencia del uso del agua a medio o largo plazo. Sin embargo, el uso de WP como un índice extendido muestra importantes debilidades derivadas fundamentalmente del método de cálculo, dando como resultado un alto grado de variabilidad, particularmente debida al clima y a los métodos de evaluación

(Morison et al., 2008; Pereira et al., 2012). Estos problemas pueden incrementarse en cultivos frutales, en los que el efecto del año, los derivados de su ciclo de vida y de las prácticas culturales pueden ser causa de importantes sesgos en la evaluación de WUE. En este sentido, el enfoque metodológico aportado por Steduto et al. (2007) proporciona mayores posibilidades para un análisis más robusto.

Las variaciones de la proporción de isótopos estables de carbono en los tejidos vegetales ($\delta^{13}C$) se relacionan estrechamente con la relación entre la concentración intercelular de CO_2 y la del atmosférico (Ci/Ca), que varia con el grado de apertura de los estomas (gs) (Farquhar et al., 1982). La relación entre $\delta^{13}C$ en hojas y WUE medido a partir de intercambio gaseoso (gs) se ha demostrado en plantas C3 (Farquhar et al., 1989; de Souza et al., 2005; Cabrera-Bosquet et al., 2007), sugiriendo esta relación la utilidad de $\delta^{13}C$ como indicador integrativo de WUE. Bajo este enfoque, son numerosos los trabajos de investigación relacionando $\delta^{13}C$ con la variación de gs con el estado hídrico (Santesteban et al., 2012), indicando la utilidad de la relación de isótopos de carbono como marcador del estrés hídrico soportado por la planta y la eficiencia de uso del agua.

La respuesta de $\delta^{13}C$ a la interacción nitrógeno- agua ha sido poco estudiada en frutales (Stamatiadis et al., 2007) y, en general no se ha llegado a explicar satisfactoriamente mediante este indicador, posiblemente, entre otros factores, a causa de los tejidos utilizados para la medida de la relación isotópica (hojas y frutos enteros). Otra posible causa se relaciona con el efecto del nitrógeno en la respuesta de la eficiencia fotosintética; la acción combinada de agua-nitrógeno en las variaciones del balance fuente-sumidero a lo largo del ciclo puede inducir una respuesta diferencial de la planta según los niveles de ambos factores. Este planteamiento constituye un aspecto interesante, que puede ser abordado mediante el enfoque que aporta el estudio de la "plasticidad fenotípica" (Sadras y Trentacoste, 2011).

De lo expuesto se deduce que puede tener sentido analizar la variación de $\delta^{13}C$ en tejidos formados en diferentes fases del ciclo anual de la planta para evaluar WUE y los factores influyentes en cada período. En el caso del melocotonero, una especie muy estudiada en cuanto a relaciones hídricas y estrategias de riego, se sabe que el endocarpio del fruto acumula carbono preferentemente en la fase II del crecimiento del fruto (Grossman y DeJong, 1994), mientras que el mesocarpio es el principal sumidero de carbono durante la fase III, o de crecimiento expansivo del fruto. Una hipótesis razonable que puede plantearse hace referencia a la respuesta diferencial de $\delta^{13}C$ en estos tejidos y su posible relación con el estrés hídrico y WUE en diferentes fases del ciclo, lo que conllevaría la posibilidad de

analizar con mayor detalle los efectos concretos del estrés controlado en la eficiencia de uso del agua.

Por otro lado, es frecuente explicar las variaciones interanuales de WUE en base al efecto del clima o de las circunstancias concretas de cada año (carga de fruta, crecimiento vegetativo, etc…), admitiendo en muchos casos, como premisa básica para el análisis, que el riego deficitario provoca siempre cambios sustanciales en la relación fuente-sumidero favorables a WUE, via disminucion de la conductancia estomática. Estos aspectos plantean otra cuestión que trata de la posibilidad de medir $\delta^{13}C$ en la madera del tronco como indicador para el análisis *ex-post* de los resultados experimentales o como índice para la clasificación zonal. Su utilidad potencial puede ser alta, particularmente en la investigación de los efectos a medio plazo del agua y nutrición, y puede constituir una herramienta interesante para la reinterpretación de resultados de la aplicación de estrategias deficitarias en diferentes condiciones (por ejemplo, año o localización).

1.4 La eficiencia de uso de agua y la calidad de los frutos

La percepción y valoración de la calidad organoléptica de la fruta está estrechamente asociada al éxito en el grado de aceptación del consumidor (Delgado et al., 2013); sin embargo, la demanda de los mercados requiere frutos con buenas posibilidades de conservación y de transporte para llegar a satisfacer una demanda cada vez más variada y lejana (Welbaum, 2015). Ello implica frecuentemente la recolección de la fruta en un estado inmaduro, aunque, potencialmente, debe presentar una evolución satisfactoria para llegar hasta el consumidor sin comprometer la calidad del producto (FAO, 2013) o la calidad de consumo (Layne, 2007; Bonany et al., 2014). En la práctica, la madurez del fruto en cosecha (en el caso del melocotón) se determina mediante la concentración de sólidos solubles, la firmeza del mesocarpio, la acidez, y también por observación de los cambios en el color de fondo de la epidermis (Crisosto, 1994; Barrett et al., 2010). La madurez adecuada para los procesos de cosecha, post-cosecha y de consumo forman parte de un preceso cuya evaluación se relaciona con numerosos efectos de diferente naturaleza: los aspectos fisiológicos de la maduración, sea tanto en campo como en postcosecha, los efectos del clima, del suelo, de la gestión del cultivo o de la variedad, se describen a menudo como los principales factores influyentes en la calidad del fruto (Bryla et al., 2005; Wert et al., 2009; Ziosi et al., 2008; Montero-Prado et al., 2011; Legua et al., 2011; Eduardo et al., 2012; Vallverdu et al., 2012).

Sin embargo, los indicadores de madurez utilizados normalmente muestran poca capacidad para evaluar la evolución potencial de la fruta en postcosecha, sobre todo la calidad de consumo (Crisosto, 1994; Ziosi et al., 2008). Al final, la contradicción es que la aceptación de la fruta por los consumidores está más relacionada con la percepción global sensorial (sabor y apariencia) y la vida útil de la fruta (o *shel-life*), que con un atributo particular medido en cosecha. Actualmente, uno de los aspectos fundamentales de la medida de la calidad industrial de la fruta (entendida como calidad necesaria durante el procesado para alcanzar el objetivo de calidad de consumo) es la excesiva simplificación con que es evaluada; la laxitud de la relación entre los indicadores de madurez y la calidad de consumo es un hecho particularmente relevante para la fruta que debe ser preparada para llegar en los mercados de larga distancia.

La maduración exige necesariamente la concurrencia de diferentes enzimas asociadas a la senescencia y ulterior degradación de los tejidos no reproductivos, lo que en ciertas condiciones puede suponer una degradación prematura de la fruta, limitando su calidad o su durabilidad potencial. La Polifenol-oxidasa (PPO) es una enzima ampliamente distribuida entre los seres vivos, que cataliza diferentes reacciones entre el oxígeno y diferentes fenoles mediante la o-hydroxilacion de monofenoles a *o*-difenoles (actividad cresolasa) y la posterior oxidación de *o*-difenoles a *o*-quinonas (actividad catecolasa); la polimerización de estos últimos compuestos, altamente inestables, conduce a la formación de un grupo heterogéneo de polímeros que muestran una coloración oscura. Durante la maduración, los procesos degradativos del fruto conducen a la disminución de la capacidad para eliminar el oxígeno activo, que a su vez conduce en una mayor peroxidación de lípidos, la degradación y pérdida de permeabilidad de las membranas y la disminución de la capacidad para eliminar el oxígeno activo. La consecuencia inmediata es la liberación de PPO de los plastos celulares, reaccionando con los sustratos (fenoles liberados de las vacuolas) y acelerando la degradación de los tejidos, a la vez que se desarrolla, entre otros efectos, el pardeamiento de la fruta (Holderbaum et al., 2010) o el desarrollo de *off-flavors* (Podsedek et al. 2000) . A día de hoy, también es conocido que la actividad alta de la PPO se relaciona con importantes pérdidas de la calidad de la fruta (Murata et al., 1995), afectando su vida útil (Olivos et al., 2012); la importancia de la acción de esta enzima es notoria, estimándose que PPO se halla involucrada, en un porcentaje muy alto, en las pérdidas de calidad de la fruta desde el campo al consumidor, alcanzando hasta el 50% segun Whitaker y Lee (1995).

Bajo esta perspectiva, la posibilidad de utilizar otros indicadores que aporten información sobre la calidad y su posible evolución futura es un reto tan solo parcialmente resuelto. En

esta línea, la medida de la actividad enzimática de los frutos durante la maduración en campo puede mejorar la capacidad de diagnóstico de la calidad, si es posible demostrar que las actividades más caracteristicas de la fruticultura de regadío, como son el riego y la nutrición, afectan a la actividad PPO, y cual es la relación de esta actividad con las posibilidades de conservación y la calidad final de consumo resultante.

Hasta aquí se ha tratado de la evitación de pérdidas de calidad asociadas al proceso de producción. En otra visión del mismo tema, la calidad, se enfoca hacia otra meta de importancia en el sector de la producción de frutas y transformados, como es la consecución de la máxima calidad para conseguir productos de alto valor añadido. Es el caso, por ejemplo, del aceite o del vino. En este caso, la fruticultura de secano merece mayor atención cuando se trata de promover la eficiencia del agua para sostener unos rendimientos que son potencialmente escasos y muy vulnerables al medio, y que a la vez, debe potenciar la máxima calidad posible. No cabe duda que la vid de secano es un cultivo frutal con una importancia, a todos niveles, que lo sitúa de manera destacada en primera línea de la fruticultura, más aun teniendo en cuenta su valor añadido agro-industrial[1] y su alto valor ambiental, social, cultural y paisajístico,[2]. En este contexto, en las viticulturas singulares y bien caracterizadas, es decir, las que se asociarían inequívocamente al concepto de *terroir* (Deloire et al., 2004) una de las oportunidades más significativas para la valorización de sus recursos se encuentran en la capacidad para adaptar la tecnología de la producción a las particularidades del medio a nivel incluso intra-parcelario. La explotación de estas posibilidades pasa por un conocimiento preciso de la respuesta de las plantas a la tecnología de producción o, en otras palabras, responder adecuadamente a qué se debe hacer, cuándo y con qué intensidad.

En el sentido de lo expuesto, la importancia del clima y del suelo sobre la disponibilidad de agua y, en consecuencia, sobre el estado hídrico de la planta son innegables y forman parte de la expresión del estilo característico de la zona (van Huyssteen y Weber, 1980; Ojeda et al., 2001; Ramos y Martínez-Casasnovas, 2014). Paralelamente, y en este entorno, se reconoce la existencia de una fuerte interacción entre disponibilidad hídrica y estado nutricional de la planta y sus consecuencias sobre la calidad (Deloire et al., 2004). Tradicionalmente, el método de formación y el manejo de la vegetación constituyen uno de

[1] La lista de WineSpectator de los 100 vinos de calidad más solicitados en 2014, 94 procedian de viñas no regadas, producidos en zonas con lluvias entre 140 y 900 mm.

[2] Es un buen ejemplo de la incorporación a la economía del cultivo del valor de los recursos naturales y servicios ambientales

los pilares básicos de la sostenibilidad de la vid en secano. Es lo que hoy, muy en boga en sistemas de cultivo más intensivos y estereotipados, se conoce como manejo del dosel en su vertiente tecnológica, formalizado en sus bases cientificas por Shaulis et al. (1966), y hoy profusamente extendido a partir de trabajos como los de Smart y Robinson (1991).

No obstante, en sistemas de secano no queda muy claro aún el impacto del manejo del dosel, diseñado y adaptado frecuentemente bajo condicionantes impuestos por otros sistemas tecnológicos (habitualmente sistemas de mecanización), sobre la disponibilidad de agua y el estado hídrico de la planta durante la estación de crecimiento (Hunter y Archer, 2001;, van Leeuwen et al., 2009), particularmente en la maduración, y sus efectos en la calidad final del vino.

1.5 Objetivos

La finalidad de la tesis de investigación planteada se relaciona con el estudio de la eficiencia del uso de agua desde varias perspectivas, identificadas como objetivos concretos de la tesis, como son: **A**) conocer la aplicabilidad y la interelación de algunas técnicas utilizadas en otros campos de la investigación para la determinación de la eficiencia del uso del agua en cultivos frutales y las interelaciones con la nutrición y la disponibilidad estacional de agua. **B**) Además, en este mismo contexto, y relacionado con el uso de los recursos (como el agua y la nutrición), también se pretende evaluar las posibilidades que ofrece la medida de la actividad de la enzima polifenol-oxidasa como indicador de calidad, para posibilitar la evaluación de la calidad de la fruta y sus posibilidades en cuanto a conservabilidad y vida en estante (o *shelf-life*). **C**) Por último, conocer la respuesta de la vid de secano a la aplicación de técnicas de manejo del dosel foliar, y las relaciones entre estas y el estado hídrico de la planta en diferentes períodos del ciclo, y los efectos combinados en el estado hídrico y nutricional de la planta en la calidad del vino.

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Capítulo II: La eficiencia de uso del agua. Indicadores y estrategias de riego

Stable carbon and nitrogen isotope ratios as indicators of water status and nitrogen effects on peach trees[3]

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Abstract. Carbon and nitrogen isotope ratios are tools that are widely used in ecophysiological studies of forest and herbaceous plants. However, the use of isotope discrimination techniques with fruit trees has been very limited. Three irrigation strategies were applied to peach trees (*Prunus persica* (L.) Batsch. cv. Andross): full irrigation throughout the growing season; restricted irrigation during stage-II of fruit growth (70% restriction); and restricted irrigation during stage-III (30% restriction). These irrigation treatments were combined with three different nitrogen fertilization treatments: 0, 60 and 120 kg N ha^{-1}. The stable carbon isotope ratio ($\delta^{13}C$) in fruit proved a reliable indicator of peach crop water status and physiological water use efficiency. The leaf carbon isotope ratio was not affected by either the irrigation or N treatments. $\delta^{13}C$ in endocarp tissues exhibited a powerful discriminatory capacity for water stress during phase II of fruit growth, whereas $\delta^{13}C$ in the mesocarp was an interesting tracer for water stress in phase III of fruit growth. There was an important relationship between leaf N content and photosynthetic efficiency during period GSII, which enhanced leaf WUE. The stable nitrogen isotope ratio ($\delta^{15}N$) in the fruit mesocarp was significantly related to the amount of N applied and the origin of this N. The nitrogen isotope ratio did not, however, exhibit a good discriminative capacity when we studied the influence of water and nitrogen on plant response.

Keywords: regulated deficit irrigation, leaf gas exchange, water use efficiency, water productivity, N management.

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1. Introduction

Under water stress conditions fruit trees exhibit several short to long-term adaptive responses. Stomata closure and the subsequent reduction in stomatal conductance (gs) and transpiration (E) constitute the first functional adaptations to offset water shortage (Galmés et al., 2007). Midterm adaptive responses are related to carbon partitioning and produce changes in vegetative and fruit growth (Chalmers and Van den Ende, 1975; Hsiao, 1973). The intensity and duration of water stress, whether imposed via irrigation or resulting from natural drought, may affect leaf gas exchange and nutrient uptake (Berry et al., 1992; Boyer, 1976; Boyer, 1996; Flexas and Medrano, 2002). However, according to the intensity of the stress and its duration, it can be difficult to predict the consequences on leaf CO_2 assimilation (An) and variations in gas exchange and their influence on such parameters as growth, yield, fruit quality, plant nutritional status and water use efficiency (WUE) (Ruiz-Sanchez et al., 2010).

WUE is an index that is widely used for the evaluation of plant adaptation to restricted water supply (Araus 2002). This concept has been physiologically defined as the ratio between An and E, which is known as instantaneous water use efficiency, (WUE_{inst} , Polley, 2002), and as the ratio between An and gs, which is known as intrinsic water use efficiency, ($WUE_{intrinsic}$ Boyer 1996). Physiological WUE indices are widely used in comparative studies involving plant responses to changes in water supply and demand (Cabrera-Bosquet et al., 2009; Condon et al., 2004) and provide information about physiological plant behaviour in response to short-term changes in light, temperature and plant water status (Centritto et al., 2002). Biomass water efficiency ($WUE_{biomass}$), which is the ratio between the quantity of plant biomass produced and water used, is widely used as a time integration index for water use efficiency (Boyer, 1996). However, the use of $WUE_{biomass}$ as an extended index has been questioned because it exhibits a high degree of variability and depends on the plant, environment and/or methodology used (Ma et al., 2010; Morison et al., 2008).

The close relationship between the ratio of stable carbon isotopes (δ13C) and the ratio of intercellular CO_2 to atmospheric CO_2 concentration (Ci/Ca) has been explained on the basis that the observed differences in isotopic ratio reflect differences derived from the variation of Ci/Ca ratio in the carboxylation step of photosynthesis in response to environmental constraints, as water stress, that affect stomatal regulation (Farquhar et al., 1982, Farquhar et al., 1989). The relationship between WUE_{inst} and $WUE_{intrinsic}$ and $\delta^{13}C$ has already been extensively studied (Ehleringer et al., 1993; Ennahli and Earl, 2005), providing an interesting time-integration for plant WUE over the period of dry matter plant synthesis (Araus, 2002).

Positive relations between $\delta^{13}C$ in leaves and WUE_{inst} and $WUE_{intrinsic}$ have been reported in C_3 plants (Farquhar et al., 1989) and, particularly, in wheat (Cabrera-Bosquet et al., 2007) and grapes (Souza et al., 2003). Moreover, negative relations between $\delta^{13}C$ and $WUE_{biomass}$ have been reported in poplar (Yin et al., 2005). In addition, the relationship between plant organ $\delta^{13}C$ and yield tends to be somewhat contradictory (Cui et al., 2009) and depends on environmental factors (Ferrio and Voltas, 2005, Stamatiadis et al., 2007, Yousfi et al., 2010), plant organ analysed (Gaudillére et al., 2002) and genotype (Glenn and Scorza, 2006). Moreover, the isotopic ratio $\delta^{13}C$ for leaves and fruits exhibits a close relationship with both leaf water potential in grapevines at midseason (Gaudillére et al., 2002) and with seasonal leaf water potential (Souza et al., 2005).

On the other hand, several studies have shown that the ratio of nitrogen stable isotopes ($\delta^{15}N$) can be used to determine the origin of the N used by plants. $\delta^{15}N$ in plant tissues is related to the isotopic ratio of the main N sources and is extensively used to determine N sources in organic production by different species (Choi et al., 2002; Choi, 2003; Policarpo et al., 2002). N

isotopes are discriminated during the processes of N uptake, assimilation and translocation (Choi et al., 2005; Evans, 2001; Robinson, 2000; Rowland and Lamb, 2005). However, the use of $\delta^{15}N$ as sole indicator of specific effects of irrigation and fertilization is difficult, since its value is affected by several other factors associated to N dynamics in the soil-plant system (Choi et al., 2005; Evans, 2001). The relationships between $\delta^{13}C$, water status and plant N content have been established in wheat (Cabrera-Bosquet et al., 2007), pine (Choi et al., 2005) and poplar (Monclus et al., 2009). Moreover, a relationship between $\delta^{13}C$ and $\delta^{15}N$ has similarly been related to water status and N supply in grapes (Stamatiadis et al., 2007). However, our understanding of $\delta^{15}N$ composition in plants under different water status is not as clear as that of $\delta^{13}C$ (Robinson et al., 1998, Robinson 2001)

Carbon and nitrogen isotope ratios constitute a tool that is widely used to detect environmental effects, particularly in forests and herbaceous plants and to link these effects to water stress, WUE and N availability. However, applications of C and N isotope ratios to fruit trees have, for the moment, still been very limited, and interpretations of these ratios in this context are virtually unknown. In the present research, we applied this technique to peach trees, based on the hypothesis that carbon and nitrogen stable isotope ratios in fruit components (endocarp and mesocarp) would be related to environmental conditions during the formation of both of these tissues. If this were the case, $\delta^{13}C$ and $\delta^{15}N$ would provide a reliable indicator of crop water status and WUE throughout the growing season. That would be a strong tool for detecting, at the time of harvest, water stress episodes that have occurred during the period of fruit growth.

The objective of this study was to assess the use of C and N isotope ratios in peach trees as an indicator of water status and WUE during the growth season and to detect episodes of stress under different nitrogen availability

2. Materials and Methods

Experimental orchard. N and C isotope ratios were analyzed in 2009 with respect to an irrigation and nitrogen experiment, relating to the production of peaches for processing, that had begun in 2006 in a commercial orchard located in Torres de Segre (Lleida, Spain). The climatic classification was continental, semi-arid Mediterranean with a mean annual temperature of 14.7 ºC, annual precipitation of 355 mm and annual reference evapo- transpiration of 1172 mm. Nine-year-old clingstone peach trees (*Prunus persica* (L.) Batsch. cv. Andross) on GF-677 rootstock used for this study were planted at a spacing of 5 x 2.8m.

2.1. Experimental design

A randomized complete block design with four repetitions was established. Three irrigation treatments were evaluated according to fruit growth stages, as defined by Tukey (1938) and Chalmers and Van den Ende (1975): 100% irrigation throughout the season (FI), 70% restriction (DI2) during fruit growth stage-II (GSII) and 30% restriction (DI3) during fruit growth stage-III (GSIII). These were combined with three different nitrogen fertilization treatments: 0 kg ha^{-1} (N0), 60 kg ha^{-1} (N60) and 120 kg ha^{-1} (N120). Nitrogen was applied as Urea Ammonium Nitrate solution (32%N) from beginning of growth until the end of GSII. An automated drip-fertigation system with auto-compensated emitters (18 L h^{-1} $tree^{-1}$) was used to supply daily water requirements based on a simple water budget calculated using the FAO method (Allen et al., 1998) and with data from two nearby meteorological stations. The experiment had 36 plots, and each plot was delimited by three adjacent lines with a total of 30 trees per plot. Plant measurements were carried out on the five central trees in each plot. The volume of irrigation applied to each plot was monitored with a water meter. To minimize leaching and drainage, soil moisture was continuously monitored using ECH2O-20

capacitance probes (Decagon Devices Inc., Pullman, Washington, USA) which provided the volumetric water content of the surrounding soil. Four ECH2O probes were inserted into the soil around one tree per irrigation treatment; these were placed 0.7 m from each side of its trunk and at depths of 0.15 and 0.3 m, within the wetted zone (for complete details of the experiment see Rufat et al., 2011).

2.2. Measurements

In the fourth year of the experiment (2009), the net CO_2 assimilation rate (An) was measured fortnightly, at noon, on three light-saturated leaves per plot (PPFD > 1000 μmol m^2 s^{-1}), using a portable infrared gas analyzer (LI-6200, Li-Cor, Lincoln, Nebraska, USA). At the same time, leaf stomatal conductance (gs) was recorded for three leaves using a steady-state porometer (model LI-1600, Li-Cor). Midday stem water potential (Ψ_s) was also measured with a pressure chamber (model 3005, Soil Moisture Equipment Corp., Santa Barbara, California, USA), following the procedures outlined by McCutchan and Shackel (1992). The minimum values for Ψ_s were obtained on 18 June (stage GSII) and 28 July (stage GSIII) and the data relating to these days were used for the study.

Intrinsic WUE ($WUE_{intrinsic}$) was calculated as the ratio between net photosynthesis (An) and stomatal conductance (gs). Instantaneous WUE (WUE_{inst}) was calculated as the ratio between net photosynthesis (An) and transpiration (E). Biomass water productivity was calculated as the ratio between fruit yield and pruned wood and the amount of water applied plus effective rainfall; they were then expressed as fresh and dry biomass ratios (WUE_{BFM} and WUE_{BDM} respectively).

Fifty leaves per plot were collected at GSII and GSIII and five fruit per plot were sampled at harvest time. Fruit were split into mesocarp and endocarp. Leaves and fruits were dried at 60 ºC to a constant mass and milled to a fine powder. One aliquot was used for nutrient composition analysis and another for stable carbon and nitrogen isotope analyses. Plot pruning mass was recorded and a sample was dried at 60 ºC to a constant mass to calculate the pruning dry matter.

The amount of nitrate-N (N-NO_3) in the soil was measured in each plot at bud-burst, in the middle of GSII, and during the post-harvest period (Post-H), based on a 0-0.3 m composite sample. All samples were extracted with water (1:5 soil/water solution) and colorimetrically analysed for NO_3-N using a Technicon Autoanalyser (Anasol 4P2S1BM2P, ICA Instruments, Tonbridge, Kent, UK).

Carbon and nitrogen stable isotope compositions were analysed by mass spectrometry at Iso-Analytical (Sandbach, Cheshire, UK). Quality control and the reproducibility of the measurements of $\delta^{13}C$ and $\delta^{15}N$ were calibrated against reference materials: IA-R001 (Wheat Flour, $\delta^{13}C_{V\text{-}PDB}$ = -26.44‰ and $\delta^{15}N_{AIR}$ = 2.51‰), IA-R045 (Ammonium Sulphate, $\delta^{15}N_{AIR}$= -4.7‰), IA-R046 (Ammonium sulphate, $\delta^{15}N_{AIR}$ = 22.11‰), IA-R005 (Sugar beet $\delta^{13}C_{V\text{-}PDB}$ = -25.95‰) and IA-R006 (cane sugar $\delta^{13}C_{V\text{-}PDB}$ =-11.59‰). The reference materials used for $\delta^{13}C$ and $\delta^{15}N$ were Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N_2 ($\delta^{13}C_{V\text{-}PDB=}$ -26.40‰ and $\delta^{15}N_{AIR}$ = 2.51‰, respectively). $\delta^{15}N$ for UAN fertilizer application was 1.70‰.

The stable isotope ratios of C and N were determined as $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard} - 1)*1000]$ where the Rs respectively relate to the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. The respective accuracies of the analyses (standard deviation of working standards) were 0.01‰ ($\delta^{13}C$) and 0.4‰ ($\delta^{15}N$).

2.3. Data analysis

Statistical analysis of data was carried out using the SAS-STAT package (SAS®, Version 9.2, SAS Institute Inc., Cary, NC, USA). A mixed procedure was used to evaluate the effects of the

water and nitrogen treatments and their interactions, using a block as a random effect. Analysis of variance (ANOVA) was carried out for Nitrogen and Irrigation effects in $\delta^{13}C$ and $\delta^{15}N$, An, gas exchange (gs and E) and WUE. Analysis of covariance (ANCOVA) was used for the Nitrogen and Irrigation effects on biomass production and related variables. Means were compared using the Tukey HSD test.

Partial least square regression (PLS-R) was carried out to determine the significant variables involved in fruit tissue $\delta^{13}C$ variation. The cross validation method was used to select the number of projection components (Mevik and Cederkvist 2004). The importance of the variables in the PLS model was determined by means of the variance importance in the projection method (VIP), as described by Chong and Jun (2005).

3. Results and Discussion

The periods defined by GSI, GSII and GSIII started on 13 March, 16 May and 19 June, respectively, and harvest was on 12 August; these dates were in line with industry maturity standards.

The amount of irrigation water applied until harvest was 395 mm for the FI treatment and 318 mm and 350 mm for DI2 and DI3 treatments, respectively.The calculated reference evapotranspiration (ETo) was 641 mm during the growing season (in the period from budburst to leaf fall) and the effective rainfall for the same period was 64 mm. The quantities of nitrogen applied were 56.4 kg ha^{-1} for the N60 treatment and 112.7 kg ha^{-1} for the N120 treatment.

3.1. Effects of irrigation and nitrogen on leaf gas exchange, plant water status, $WUE_{intrinsic}$ and WUE_{inst}

Irrigation treatment DI2 had a significantly negative effect on An, gs. E and Ψ_s, whereas no significant differences were observed with respect to WUE_{inst} (Table 1). $WUE_{intrinsic}$ was only significantly affected by irrigation deficit in GSIII. E and Ci/Ca decreased with N application, while applying nitrogen had a clearly positive effect on $WUE_{intrinsic}$ and WUE_{inst} in GSII. In period GSIII, a significant irrigation x nitrogen interaction was observed for stomatal conductance and transpiration. An analysis of interactions showed that E and gs both diminished when N was applied under water deficit conditions during period GSIII (figure 1A and 1B). Improvement of WUE by optimizing nitrogen and water supply has been studied extensively. In general, N supply improve WUE by changes in physiological processes and in leaf anatomy (Brueck and Senbayram, 2009).

However, water and nitrogen have no independent effects. The combined effect of nitrogen and irrigation must be analyzed under the theoretical basis that total assimilation is a scaled linear sum of total N and total plant transpiration (Farquhar et al., 2002). In fact, the present results agree with several research works in other plant species, highlighting the effects of water shortage on N limitation for CO_2 assimilation (Weih et al., 2011) and leaf gas exchange (Cabrera-Bosquet et al., 2009) and, correlatively, on WUE; however, the interaction has been poorly studied. The effect of water and N supply on positive response of An and leaf gas exchange may also be related to seasonal development. Nitrogen induces changes on leaf anatomy across growth season that affect leaf gas exchange characteristics (Marchi et al. 2008). In a similar way, Cui et al. (2009) has shown that deficit irrigation vs. full irrigation exhibit an interaction season time-WUE on pear-jujube trees related to leaf enduring.

Table 1. CO_2 assimilation rate (An, mmol m^{-2} s^{-1}), stomatal conductance (gs, µmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), CO_2 intercellular to air CO_2 ratio (Ci/Ca), WUE_{inst} (An/E), $WUE_{intrinsic}$ (An/gs) and midday stem water potential (Ψ_s, MPa) under different irrigation treatments (Full irrigation, FI, deficit irrigation during the fruit stage II, DI2, and deficit irrigation during the fruit stage III, DI3) and Nitrogen (0, 60 and 120 kg ha^{-1}). Results relate to different growth periods (fruit growth stage II (GSII) and fruit growth stage III (GSIII)).

Period			An	gs	E	Ci/Ca	WUE_{inst}	$WUE_{intrinsic}$	Ψ_s
GSII	Model	R^2	0.58	0.52	0.47	0.52	0.56	0.46	0.94
		P >F	0.004	0.01	0.03	0.01	0.006	0.04	<0.0001
	Fixed effects								
	Irrigation								
		FI	16.4±0.58 a*	219.0±13.2 a	5.74±0.18 a	0.54±0.03	2.87±0.10	0.077±0.004	-0.63±0.01 a
		DI2	13.6±0.69 b	147.3±13.6 b	5.13±0.18 b	0.51±0.03	2.67±0.12	0.079±0.005	-1.08±0.04 b
		DI3	15.3±0.64 a	206.7±12.7 a	5.71±0.17 a	0.53±0.03	2.71±0.10	0.079±0.004	-0.64±0.02 a
		P >F	0.001	0.002	0.01	0.73	0.70	0.81	0.008
	Nitrogen								
		0	14.6±0.60	223.1±13.4	5.94±0.16 a	0.60±0.03 a	2.46±0.10 b	0.066±0.004 b	-0.83±0.12
		60	15.6±1.00	173.3±112.6	5.36±0.18 b	0.47±0.02 b	2.90±0.10 a	0.087±0.005 a	-0.80±0.12
		120	15.3±0.48	182.5±12.8	5.30±0.17 b	0.50±0.03 b	2.88±0.09 a	0.084±0.004 a	-0.79±0.08
		P >F	0.64	0.06	0.03	0.004	0.03	0.01	0.78
	Irrigation x Nitrogen								
		P >F	0.12	0.64	0.70	0.13	0.11	0.06	0.66
GSIII	Model	R^2	0.53	0.72	0.73	0.62	0.54	0.61	0.86
		P >F	0.01	<0.0001	0.0007	0.001	0.09	0.001	0.006
	Fixed effects								
	Irrigation								
		FI	16.2±0.55 a	254.5±11.5	6.59±0.20	0.56±0.01 a	2.45±0.09	0.064±0.003 b	-0.80±0.04 a
		DI2	14.9±0.32 ab	213.3±10.9	6.11±0.17	0.53±0.03 ab	2.35±0.10	0.071±0.003 b	-0.89±0.03 a
		DI3	13.4±0.87 b	169.2±10.4	5.55±0.30	0.48±0.02 b	2.60±0.09	0.083±0.004 a	-1.17±0.08 b
		P >F	0.009	0.01	<0.0001	0.008	0.2	0.003	0.005
	Nitrogen								
		0	15.6±0.42	230.0±10.4	6.42±0.16	0.55±0.01	2.44±0.09	0.068±0.003	-0-90±0.03
		60	14.4±0.90	197.5±10.5	5.82±0.31	0.50±0.02	2.54±0.09	0.078±0.003	-1.02±0.12
		120	14.2±0.66	204.5±11.3	5.97±0.27	0.52±0.03	2.44±0.10	0.074±0.003	-0-99±0.09
		P >F	0.14	0.17	0.08	0.31	0.56	0.19	0.3
	Irrigation x Nitrogen								
		P >F	0.07	0.03**	0.02	0.14	0.52	0.30	0.10

* Values in table are means ± standard error. Values with different letters are significantly different according to the Tukey HSD test (P< 0.05). **When the interaction irrigation x nitrogen was significant, no letters were used in the table.

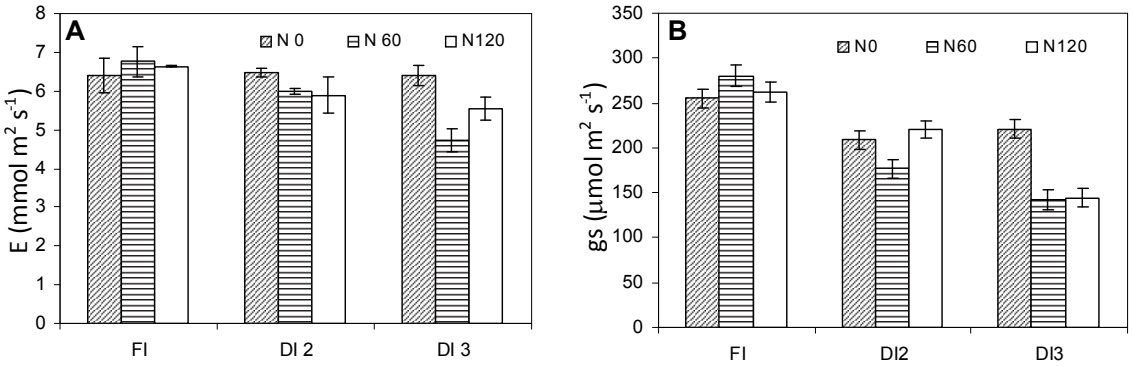

Fig. 1. Transpiration (A) and stomatal conductance (B) for irrigation x nitrogen treatments during the GSIII period. Vertical bars are the means ± standard error.

3.2. *Effects of irrigation and nitrogen application on nitrogen content in fruit tissues and leaves, and $\delta^{13}C$ and $\delta^{15}N$ results*

There was a clear N application effect on N leaf and N fruit mesocarp, but not on N fruit endocarp. Increasing the N rate increased N leaf content (Table 2). Interaction between irrigation and nitrogen was only observed for N leaf content at GSIII (Figure 2). None of the treatments were significant for N content in the endocarp.

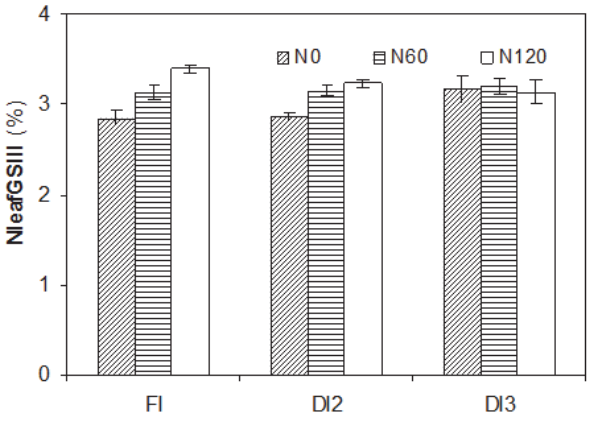

Fig. 2. Nitrogen leaf content during period GSIII (NleafGSIII)) for irrigation x nitrogen treatments. Vertical bars are the means ± standard error.

The irrigation and nitrogen treatments resulted in significant differences in stable isotope composition $\delta^{15}N$ and $\delta^{13}C$ (Table 3). The nitrogen stable isotope ratio in the dry matter of the fruit mesocarp ($\delta^{15}N_{meso}$) was significantly higher when high nitrogen (N120) was applied. Not applying nitrogen or applying only moderate rates (N60) produced similar results. No differences in the nitrogen isotope ratio were found in leaves ($\delta^{15}N_{leaf}$) or endocarps ($\delta^{15}N_{endo}$) and no significant differences were encountered when the different irrigation treatments were applied.

In the N0 treatment, the entire N taken by the plant was from organic matter. According to the analysis of variance shown in Table 3, if it is assumed that with N60 an important fraction of the N uptake was also from this organic source, it can be considered that $\delta^{15}N_{meso}$ discriminated between the origins of the N used by the plants. These findings are partially confirmed by the results obtained by other researchers (Evans 2001). We related this response to N dynamics in plants; in fact, peach leaves and fruit demand

Table 3. Effects of irrigation and Nitrogen treatments on $\delta^{15}N$ and $\delta^{13}C$ (in ‰) in peach leaves, fruit mesocarp and endocarp.

		$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)		
		Mesocarp ($\delta^{15}N_{meso}$)	Endocarp ($\delta^{15}N_{endo}$)	Leaf ($\delta^{15}N_{leaf}$)	Mesocarp ($\delta^{13}C_{meso}$)	Endocarp ($\delta^{13}C_{endo}$)	Leaf ($\delta^{13}C_{leaf}$)
Whole model	R^2	0.67	0.22	0.30	0.69	0.63	0.54
	P >F	0.003	0.82	0.60	0.002	0.005	0.04
Fixed Effects							
Irrigation	FI	-0.57 ± 0.13	-0.60 ± 0.10	-2.81 ± 0.13	-27.24 ± 0.14	-27.59 ± 0.12 b*	-29.03 ± 0.13
	DI2	-0.35 ± 0.08	-0.47 ± 0.09	-2.82 ± 0.09	-26.94 ± 0.09	-26.94 ± 0.14 a	-28.73 ± 0.10
	DI3	-0.42 ± 0.10	-0.51 ± 0.14	-2.89 ± 0.11	-26.59 ± 0.14	-27.49 ± 0.16 b	-28.78 ± 0.10
	P >F	0.33	0.63	0.84	0.0003	0.001	0.11
Nitrogen	0	-0.49 ± 0.10 b	-0.49 ± 0.11	-3.01 ± 0.11	-27.11 ± 0.12	-27.39 ± 0.20	-28.79 ± 0.14
	60	-0.56 ± 0.11 b	-0.49 ± 0.13	-2.75 ± 0.09	-26.79 ± 0.17	-27.29 ± 0.10	-28.92 ± 0.10
	120	-0.28 ± 0.09 a	-0.59 ± 0.09	-2.76 ± 0.11	-26.82 ± 0.13	-27.30 ± 0.18	-28.81 ± 0.11
	P >F	0.002	0.74	0.12	0.06	0.42	0.54
Irrigation x Nitrogen							
	P >F	0.16	0.79	0.41	0.04**	0.09	0.44

* Data are mean values ± standard error. Means with different letters are significantly different according to the Tukey HSD test (P< 0.05). **When the interaction irrigation x nitrogen was significant, no letters were used in the table.

more N than perennial plant tissues. Until full canopy development has been achieved (GSII period), fruit and leaves serve as strong sinks for N. Leaves later act as net N exporters to other tissues and particularly to fruit (Policarpo et al., 2002; Rufat and DeJong, 2001; Thomidis et al., 2007). Until GSII, most of the soil available nitrogen belongs to nitrogen bound to organic matter (Figure 3). However, besides soil uptake, up to this stage the plant uses N from reserves (Policarpo et al. 2002; Rufat and DeJong 2001). Therefore, isotopic analysis is useful to discriminate the origin of N supply mainly in the last stage of fruit growth (GSIII), when the different N doses are applied.Moreover, the different irrigation treatments applied had only insignificant effects on $\delta^{15}N$ because there were no water limitations on the spring dynamics of N (Policarpo et al., 2002; Rufat and DeJong 2001; Tagliavini et al., 1999). This suggested that irrigation had no interaction effects associated with the remobilisation of N and C reserves of the type reported in other studies (Jordan and Habib 1996, Marquat et al., 1999).

The ratio of carbon stable isotope in peach fruit endocarp ($\delta^{13}C_{endo}$) under deficit irrigation DI2 was clearly higher than in treatments that received full irrigation during the same period (FI and DI3) (Table 3). Besides the differences imposed by irrigation deficit, a significant interaction for irrigation x nitrogen was also noted in the results for $\delta^{13}C_{meso}$ (Figure 4). When the water supply was restricted during GSIII (DI3 treatment), the $\delta^{13}C_{meso}$ results were significantly higher for plots on which N fertilizer was applied

than for those which did not receive nitrogen fertilization. There were no significant differences in $\delta^{13}C_{leaf}$ associated with the irrigation or nitrogen treatments.

On the other hand, a significant relationship was observed between $\delta^{13}C_{endo}$ and $\delta^{13}C_{meso}$ (R^2 =0.45, P<0.05), suggesting a uniform carbon allocation in whole fruits up to period GSII for all the treatments. A significant relationship was also observed between $\delta^{15}N_{leaf}$ and leaf N content in periods GSII and GSIII (R^2 = 0.39, P<0.05 and R^2=0.35, P<0.05 respectively) and in fruit mesocarp nitrogen contents (R^2= 0.40, P<0.05). A similar significant relationship was

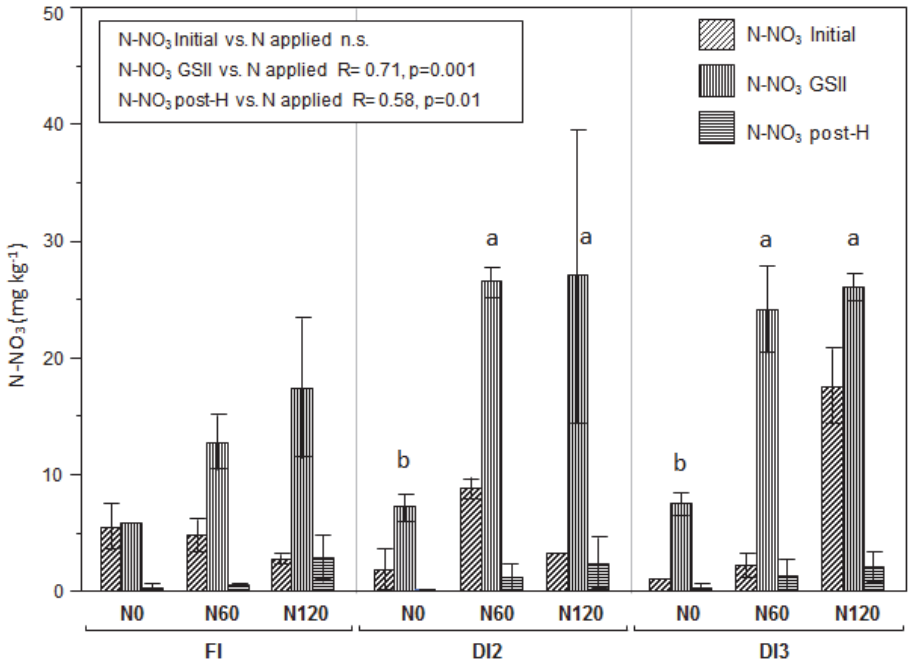

Fig. 3. Nitrogen-nitrate in soil (mg kg^{-1}) at budburst (N-NO_3 initial), during period GSII (N-NO_3GSII) and at postharvest (N-NO_3 post-H) for irrigation x nitrogen treatments. Letters on the N-NO_3GSII bars indicate significant differences between N treatments according to the Tukey HSD test (P=0.05) Vertical bars are the means ± standard error.

noted between N soil availability with foliar N contents. This was pointed to by the significant relationships that were found between nitrogen-nitrate in soil and leaf nitrogen contents for both periods GSII and GSIII (R^2= 0.60, P<0.05; R^2= 0.48, P<0.05 respectively) and between N_{leaf} GSIII and N_{meso} (R^2= 0.42, P<0.05).

The N treatments had a clear effect on nitrate soil availability (Figure 3); in fact, the amount of nitrate in the soil increased until the end of period GSII due to N application and N soil mineralization. The availability of N at bud-burst (in the initial period) was not affected by N treatments. Even so, the N-NO3 soil content during period GSII increased significantly with N applications, whereas after harvest, there was a pronounced decrease in N availability. The overall results suggest that the absence of a clear response of $\delta^{15}N$ to changes in environmental conditions may be attributable to high N availability. According to normal sufficiency ranges (Daane et al. 1995), N was not a limiting factor for plant growth in any of the treatments. Furthermore, $\delta^{15}N$ variation may have been related to both the soil and plant nitrogen dynamics, that – according to Robinson (2001) – it changes with the amount and source of N.

3.3 *Effects of irrigation and nitrogen on yield, fruit quality and* $WUE_{biomass}$

Applying the irrigation and nitrogen treatments did not affect yield variables such as fresh fruit yield (FFY), fruit dry matter yield (FDM), soluble solids content (SS), fruit dry matter in the endocarp (DME) and mesocarp (DMM), fruit fresh mass (FW) or fruit dry matter (FDM) (Table 4). Fruit load, which was used as a covariate, had a significant influence on both fresh and dry fruit yield. Pruning dry matter (DMP) was similarly not significantly affected by the irrigation or nitrogen treatments, while no significant effects were found for biomass water productivity. The absence of significant differences in results may be related to the water status of the trees. In the study, the minimum levels of stem water potential achieved did not exceed -1.1 MPa. Under these conditions, although As, leaf gas exchange and carbon isotope ratios were affected, neither yield nor WUE were significantly altered, as also demonstrated in a recent study (Vera et al., 2012).

3.4 Integration of irrigation and nitrogen effects on $\delta^{13}C_{meso}$ *and* $\delta^{13}C_{endo}$ *variation.*

Partial least squares regression (PLS-R) was used to explain $\delta^{13}C_{endo}$ and $\delta^{13}C_{meso}$ variation using gas exchange variables, An, Ψs and nitrogen tissue contents (Figure 5). The most relevant statistics derived from the PLS-R models are displayed in Table 5; they indicate an adequate model fit.

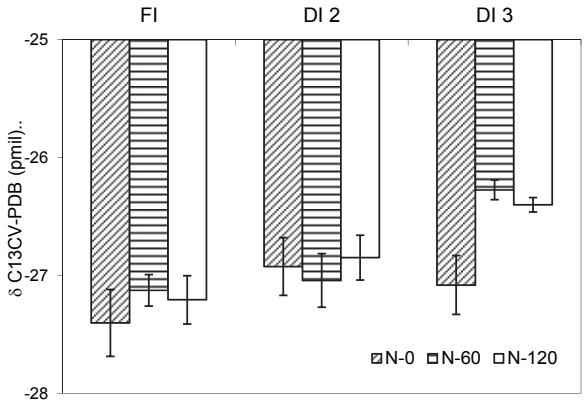

Fig. 4. Fruit mesocarp $\delta^{13}C$ for irrigation x nitrogen treatments. Vertical bars are the means ± standard error.

The results obtained indicate a response of $\delta^{13}C$ to gas exchange variables, An, Ψs and nitrogen contents in the fruit mesocarp and endocarp, which was only partially detected by previous ANOVAS. Moreover, the PLS-R analysis revealed the interaction within the irrigation and nitrogen effects and particularly with respect to the carbon stable isotope ratios and leaf gas exchange responses between the different growth stages. No relationships were found between $\delta^{13}C_{leaf}$ and other variables. It was possible to attribute this lack of relation to the fact that the leaf sampling for carbon isotope analysis was carried out during period GSIII. Under these conditions, the small differences in

$\delta^{13}C_{leaf}$ between treatments could be explained in the terms described by Arndt and Wanek (2002). This was because the most structural carbon allocation in the middle tier peach shoots took place before the deficit irrigation treatments were applied. It was also not possible to detect any difference because the leaves had low non-structural carbon contents (Gordon and DeJong 2007). Despite the results obtained, $\delta^{13}C_{leaf}$ discriminatory capacity related to plant water status has been reported as satisfactory in other species such as pear-jujube (Cui et al., 2009) or grapes (Stamatiadis et al., 2007) when other sampling methods were applied.

Changes in the $\delta^{13}C_{endo}$ ratio were, to a large extent (87.7 %), explained by changes in leaf gas exchange variables (E and gs), plant water status (Ψs), the Ci/Ca ratio and N content, expressed as $N_{endocarp}$, $N_{leaf\ GSII}$, and $\delta^{15}N_{meso}$., that took place during period GSII. The main effects on $\delta^{13}C_{endo}$ are attributable to two effects: firstly, the main effect of water stress on An reduction (Hand et al., 1982, Besset 2001) via Ci/Ca reduction caused by stomatal closure (Farquhar and Richards, 1984) and a correlative decrease in gs and E (Rieger et al., 2003, Massai et al., 2004), and secondly, the effect of nitrogen which increased the CO_2 leaf assimilation rate and which also contributed to reductions in gs, E and the Ci/Ca ratio (Table 1). In fact, the model PLR-S showed the superimposed effects of nitrogen and plant water status and also confirmed the dependence of $\delta^{13}C_{endo}$ on both plant nitrogen contents and water status during GSII.

Although certain relationships have previously been reported between water status and nitrogen in several different crops (Cabrera-Bosquet et al., 2007; Choi et al., 2005; Cui et al., 2009; Monclus et al., 2009), the peach tree response to the irrigation-nitrogen interaction remains unclear (Rufat et al., 2011; Shangguan et al., 2000). The influence of N supply on An enhancement has already been well documented for several crops (Brown et al., 1996, Brueck and Senbayram 2009). This response was related to the yield of the carboxylation process (Marchi et al., 2008). This increased the CO_2 assimilation rate as the leaf N content increased, without having any significant influence on the gs response. The An increase in peach has also been related to several adaptive changes in leaf morphology during the growth period (Le Roux et al., 2001), to leaf N availability in early spring (Lobit et al., 2001), and to nitrogen plant dynamics throughout the season (Policarpo et al., 2002; Rufat and DeJong 2001), all of which suggest a high nitrogen uptake earlier in the growth period. The results obtained in period GSII showed that N had a clearly positive influence on leaf WUE, stimulating the process of photosynthesis, and also had a correlative effect, by reducing the Ci/Ca ratio (NleafGSII vs. Ci/Ca R^2=0.23 P=0.04) and consequently enhancing WUE, which had previously been pointed to by Ripullone et al., (2004) in other species.

$\delta^{13}C_{meso}$ was highly dependent on plant water status in fruit growth period GSIII. Leaf gas exchange variables and plant water status had a positive influence on the mesocarp carbon isotope ratio, while that of nitrogen variables was insignificant. Although the role of plant N content seemed clear in period GSII, in GSIII there was a significant N negative influence on gs, but only when plants were stressed (DI3 treatment, Figure 1).

The interpretation of the $\delta^{13}C_{meso}$ ratio was made on the basis of the directly negative effect that water deficits had on leaf gas exchange variables and Ci/Ca during period GSIII. The effect on fruit mesocarp that had previously been subjected to stress during GSII was also remarkable. The influence of gs, E and Ci/Ca on $\delta^{13}C_{meso}$ during GSII therefore suggested that mesocarp tissues were also sensitive to water stress during this growth period.

Table 4. Effects of irrigation and nitrogen treatments on fruit fresh yield (FFY, t ha^{-1}), fruit dry matter yield (FDY, t ha^{-1}), fruit soluble solids (SS, ºBrix) dry matter in fruit endocarp, in whole fruit and in pruning wood (DME, g $fruit^{-1}$, DMF, g $fruit^{-1}$ and DMP, kg $tree^{-1}$), respectively; Fruit dry matter content (FDM, %), fruit fresh mass (FW, g), WUE in fresh biomass and dry matter biomass (WUE_{BFM} and WUE_{BDM} respectively in kg m^{-3}).

		FFY	FDY	SS	DME	DMF	DMP	FDM	FW	WUE_{BFM}	WUE_{BDM}
Model	R^2	0.87	0.88	0.26	0.15	0.16	0.63	0.78	0.74	0.63	0.28
	P >F	0.02	0.02	0.66	0.94	0.94	0.41	0.11	0.10	0.28	0.87
Fixed effects											
Irrigation	FI	41.9±0.94*	5.06±0.13	9.67±0.21	7.58±0.21	23.07±0.63	3.48±0.28	12.1±0.28	103.8±2.44	12.0±0.65	2.61±0.16
	DI2	41.5±0.95	5.19±0.14	9.51±0.18	7.52±0.17	23.12±0.65	2.79±0.29	12.6±0.29	104.1±2.43	14.2±0.40	2.95±0.15
	DI3	39.0±0.94	5.17±0.13	10.01±0.25	7.95±0.21	23.23±0.75	3.35±0.25	13.2±0.29	96.5±2.45	12.6±0.46	2.92±0.16
	P >F	0.10	0.47	0.22	0.41	0.95	0.18	0.06	0.11	0.11	0.35
Nitrogen	0	40.4±0.95	5.08±0.3	9.63±0.16	7.52±0.15	23.79±0.51	2.90±0.26	12.6±0.29	101.6±2.45	13.3±0.56	2.79±0.15
	60	41.7±0.94	5.27±0.14	9.66±0.19	7.79±0.19	22.65±0.63	3.17±0.26	12.5±0.28	104.2±2.46	13.4±0.55	2.83±0.17
	120	39.0±0.94	5.07±0.13	9.96±0.27	7.49±0.21	22.97±0.81	3.56±0.24	12.8±0.29	98.3±2.44	12.2±0.56	2.82±0.15
	P >F	0.33	0.86	0.51	0.56	0.54	0.24	0.59	0.25	0.34	0.87
Covariate**	*P >F*	0.007	0.002	0.57	0.75	0.86	0.49	0.08	0.13		
Irrigation x Nitrogen											
	P >F	0.36	0.06	0.87	0.92	0.68	0.41	0.14	0.38	0.71	0.90

*Values are means ± SE. Data followed by different letters are significantly different according to the Tukey HSD test (P< 0.05)
**Fruit load was used as a covariate in the ANCOVA model

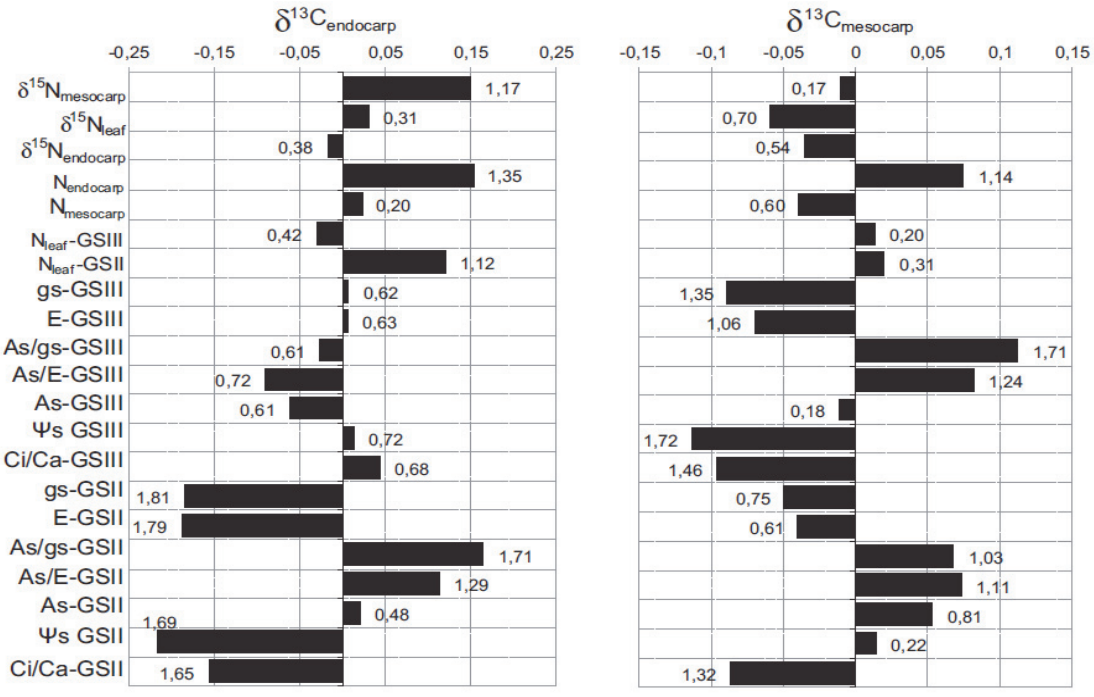

Fig. 5. PLS regression coefficients of $\delta^{13}C_{endo}$ and $\delta^{13}C_{meso}$ versus isotope nitrogen ratios, gas exchange variables, WUE, and Ψs. The values near the horizontal bars are the result of variable importance for projection (VIP).

Table 5. Validation samples of partial least squares regression (PLS) models for prediction of $\delta^{13}C_{endo}$ and $\delta^{13}C_{meso}$

Y-variable	Number of PLS factors	RMSEP*	Correlation **	% of Y variance explained
$\delta^{13}C_{endo}$	2	0.74	0.92	87.7
$\delta^{13}C_{meso}$	1	0.61	0.89	77.5

* Root mean square error of prediction. ** Correlations between predicted and measured values.

4. Conclusions

The ratio of carbon stable isotope ($\delta^{13}C$) in fruit proved to be a reliable indicator of peach crop water status and physiological WUE throughout the growing season, while $\delta^{13}C_{leaf}$ was not affected by the water or N treatments. Overall, the carbon isotope relationship in the mesocarp and endocarp exhibited the ability to detect the physiological constraints imposed by water restrictions throughout the crop season. The results obtained showed the reliability of the carbon isotope ratio in peach fruit endocarp and mesocarp for detecting episodes of stress during different growth phases, even when water restrictions did not produce severe changes in yield and vegetative growth. In fact, $\delta^{13}C$ in endocarp tissues exhibited a powerful discriminatory capacity for water stress in period GSII, whereas $\delta^{13}C$ in the mesocarp was an interesting marker for water stress in fruit growth phase III.

The two restrictive irrigation treatments (the DI2 treatment during GSII and the DI3 treatment during GSIII) had a negative influence on An, gs, E, Ci/Ca and Ψs during the period in which the treatments were applied. Furthermore, during period GSIII, gs and E were affected by the interaction between irrigation and nitrogen with lower values being obtained with higher N applications when the water supply was scarce. Although the irrigation and nitrogen treatments did not affect yield or biomass productivity ($WUE_{biomass}$), a clear irrigation x nitrogen interaction was established for $\delta^{13}C_{meso}$. This showed the effect of water restrictions on both growth stages GSII and GSIII when plant nitrogen content was high. The leaf WUE indices were higher for trees receiving higher N doses, but only during GSII.

The results obtained highlight the important relationship between leaf N content and photosynthetic efficiency during period GSII. This was even evident with decreasing gs and E, which enhanced leaf WUE. The stable isotope nitrogen ratio $\delta^{15}N$ in the mesocarp was significantly related to the amount of N applied and to the origin of the N supply. However, the nitrogen isotope signal did not exhibit a good discriminative capacity when the influences of water and nitrogen on plant responses were studied.

The information obtained from this study was very useful for an overall analysis of the relevant components of physiological and agronomic traits. It showed that stable isotope carbon ratios in fruit tissues are good indicators of the plant response to water stress. This knowledge may also be of use for feedback analysis relating results from fruit tree research under different conditions of imposed or natural water stress.

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Water use efficiency in peach trees over a four-year experiment on the effects of irrigation and nitrogen application[4].

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Abstract

The optimization of crop water productivity is a key objective in modern agriculture. Deficit irrigation is a widely used strategy when water and energy are scarce and/or expensive. However, in fruit tree crops, the severity and duration of water stress can affect yield and fruit size, thus imposing severe limitations on the effectiveness of production processes, fruit quality, and water productivity, both in agronomic and economic terms. Here we analyzed water use efficiency (WUE) of peach trees over four years both in agronomic terms, as water productivity of fruits and biomass ($WUE_{WPy,b}$), and in physiological terms, as intrinsic and instantaneous leaf WUE (WUE_{int} and WUE_{ins} respectively). Our results show a positive and additive effect of water and nitrogen application on $WUE_{WP,}$ reflected by yield enhancement. WUE_{int} and WUE_{ins} varied through changes in stomatal conductance and transpiration, both parameters depending on plant water status and nitrogen application. Results suggest that the variation in WUE_{WPy} was caused by complex regulation of plant growth and fruiting, related to water status and nitrogen availability, both drivers of plant WUE. In order to evaluate WUE at the canopy scale, $\delta^{13}C$ and $\delta^{18}O$ in wood were related to leaf stomatal conductance and transpiration and also to the effects of irrigation and nitrogen; however, no clear relationship was observed with WUE_{WPy}. Plant water status was also dependent on crop load and vegetative growth and was strongly related to WUE_{WPy} and WUE_{WPb}. Altogether, our results show that full irrigation and rational nitrogen application can improve WUE_{WPy} both in agronomic and economic terms. When water deficit conditions are foreseeable, deficit irrigation in fruit growth phase III (GSIII) is a more profitable strategy that when applied in phase II (GSII). In this regard, flexible deficit irrigation in GSIII is an affordable and practical tool to include in policies aimed to improve water and nitrogen use efficiency in fruit crops when water is scarce.

Keywords

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Water stress, carbon and oxygen isotope discrimination, leaf WUE, Water productivity, deficit irrigation

Abbreviations

An: leaf net CO_2 assimilation rate

DI2: deficit irrigation on GSII

DI3: deficit irrigation on GSIII

FI: full irrigation

FN: fruit number (crop load)

FW: fruit weight

FY: fruit yield

gs: leaf stomatal conductance

GSI: Phase from budburst to start of fruit endocarp hardening

GSII: Phase of peach fruit endocarp hardening

GSIII: Phase of expansive fruit growth

Kca: seasonal real (or actual) evapotranspiration

KcGSII: evapotranspiration real (or actual) on GSII

KcGSIII: evapotranspiration real (or actual) on GSIII

PPFD: photosynthetic photon flux density

PW: pruning weight

T: leaf transpiration

WUE_{ins}: instantaneous water use efficiency

WUE_{int}: intrinsic water use efficiency

WUE_{WPb}: normalized water productivity of biomass

WUE_{WPirri}: Water productivity of irrigation

WUE_{WPy}: normalized water productivity of yield

$\delta^{13}C$: carbon isotope relation

Ψ_s: midday stem water potential

$\delta^{18}O$: oxygen isotope relation

1 Introduction

From a conceptual viewpoint irrigated agriculture can reach a high yield potential because it is not limited by essential factors such as CO_2, radiation, water, or temperature However, several technological gaps associated primarily with irrigation, nutrition, and crop management limit the achievable production in these systems (van Ittersum et al., 2013). Given that irrigated agriculture makes a significant contribution to world food production (Sauer et al., 2010), its long-term sustainability clearly calls for the improvement of the efficiency of other aspects of production, such as fertilization (Li et al., 2009 a), crop management, irrigation technology, and water policies (Howell, 2001; Pereira et al., 2002; Ward and Pulido-Velazquez, 2008; Zou et al., 2013).

1.1. Water use efficiency from an agronomic and economic perspective

Water use efficiency (WUE) can be defined in many ways. In agronomic terms, it is usually described as water used per unit of biomass or yield, known also as water productivity (WUE_{WP}) (Table 1). WUE_{WP} has been widely used as a seasonal index for the evaluation of irrigation yield (Boyer, 1996). Despite being an efficient estimator and providing useful information, this parameter has some limitations because of its high variability (Blum, 2005; Blum, 2009; Gleick et al., 2011) and has been questioned mainly because it can show biases related to year, plant, environment, and the method used (Monteith, 1993; Ma et al., 2010; Morison et al., 2008).Years ago, de Wit (1958) provided a robust definition of WUE_{WP} as the relation between biomass produced and crop transpiration (T) to potential water evaporation; in fact, the inclusion of the crop transpiration to potential evapotranspiration ratio makes WUE an integrated index of water efficiency (Kang et al., 2002; Ben-gal et al., 2003; Segal et al., 2006). This approach was improved by Steduto et al. (2007), who introduced a more refined estimation of T and evapotranspiration (ETo). Also, to avoid bias derived from changes in the climate and atmospheric evaporative demand, the same authors defined the concept of normalized WUE_{WP} as the ratio between biomass (WUE_{WPb}) or yield (WUE_{WPy}), to the fraction of ETo used by the crop (Table 1). Thus, normalized WUE_{WP} shows a robust linear relation between biomass and transpiration, and becomes an interesting tool by which to measure and/or predict water productivity.

Table 1. Definitions of water use efficiency (WUE) used. Abbreviations are done in the text.

	Formulae	Font
WUEint	$\mathrm{WUE_{int}} = \frac{A}{gs}$	Boyer (1996)
WUEins	$\mathrm{WUE_{ins}} = \frac{A}{T}$	Polley (2002)
WUE_{WPirri}	$\mathrm{WUE_{WPirri}} = \frac{\mathrm{Yield}}{\mathrm{Irrigation}}$	Boyer (1996); Molden (1997)
WUE_{WPy}	$\mathrm{WUE_{WPy}} = \frac{\mathrm{Yield}}{\frac{\mathrm{T}}{\mathrm{ETo}}}$	Steduto and Albrizio (2005)
WUE_{WPb}	$\mathrm{WP_{WPb}} = \frac{\mathrm{Biomass}}{\frac{\mathrm{T}}{\mathrm{ETo}}}$	Steduto and Albrizio (2005)

From an agro-economic perspective, optimal water efficiency is related to the maximization of marketable yield per unit of water used. Optimal WUE_{WPy} is defined as the dose of water that reaches the maximum value of the first derivative of the yield-water function or, in others words, as the water supply for maximizing marginal productivity (English, 1990). This last premise highlights the incompatibility of water restrictions and high crop yield, thus justifying the need to analyze the economy of irrigation from a holistic perspective (English et al., 2003). In fact, water shortage in crops grown using intensive process inputs and high technology can have negative effects in economic terms (García and Brunton, 2013) as it causes a reduction in the overall efficiency of production resources (Yoo et al., 2009; Rufat et al., 2011; Pascual et al., 2013). Consequently, pursuit of the maximization of WUE_{WPy} can lead

to the paradoxical effect of decreasing the attainable crop yield as a result of water shortage (Blum, 2009; Vera et al., 2013).

1.2. WUE from an agronomic and physiological viewpoint

At the leaf level, the ratio between net CO_2 fixation (An) and transpiration (T) or stomatal conductance (gs) or is defined as instantaneous WUE (WUE_{ins}; Polley, 2002) and intrinsic WUE (WUE_{int}; Boyer, 1996) respectively (Table 1). Both indexes have been extensively used in genotype selection and evaluation for the improvement of water use efficiency (Voltas et al., 1999; Condon et al., 2004; Rajabi et al., 2009).. Despite gs and T being coupled processes, the behaviors of the two leaf WUE indexes do not respond in a similar way to environmental changes, T depends on the gs and VPD, whereas gs depends only on stomatal aperture (Bierhuizen and Slatyer, 1965). Other difference is attributed to stomatal and non-stomatal limitations for CO_2 assimilation, thereby suggesting that plant response differs when confronted with drought (Yoo et al., 2009). Notwithstanding, although both indexes are feasible tools for the study of plant response to environmental factors, they may have several potential limitations related to an integrative time response to the environment (Ouyang et al., 2013), canopy architecture and leaf arrangement (Syvertsen et al., 2003; Weyand and Schultz, 2006) and possibly they are limited use as surrogates of whole plant WUE (Tomás et al., 2014).

In contrast to leaf WUE, isotope discrimi-nation in plant tissues, which is related to stomatal conductance in the case of carbon isotope composition ($\delta^{13}C$) (Farquhar et al., 1982; O'Leary, 1993; Cui et al., 2009) or plant transpiration behavior on the case of oxygen isotope composition ($\delta^{18}O$) (Ferrio and Voltas, 2005; Barbour, 2007), has been used as time integrative whole plant WUE. Consistent behavioral response across environments has been demonstrated for $\delta^{13}C$ (Lajtha and Marshall, 1994; Voltas et al., 2008) in the basis of the relation between $\delta^{13}C$ and ratio between intercellular and atmospheric CO_2 concentration (C_i/C_a). However this ratio is also affected by plant nitrogen content; In fact, when nitrogen availability increases C_i/C_a decreases as $\delta^{13}C$ (Livingston et al., 1999; Garrish et al., 2010; Pascual et al., 2013) and increases leaf WUE_{int} (Warren and Dreyer, 2006). This observation indicates that shifts of $\delta^{13}C$ may be associated with both the effects of water stress and nitrogen induced enhancement of photosynthetic yield (Syvertsen et al., 1997; Brueck, 2008; Cernusak et al., 2009). On the other hand, plant responses to the environment and their relation with $\delta^{18}O$ in tissues are less clear. Distortions may arise related to the variation of $\delta^{18}O$ in source water (which plays a key role in irrigated systems), enrichment of plant water during transpiration and biochemical fractionation on the synthesis of biomass (Yakir, 1992; Barbour, 2007). Despite these limitations, the feasibility of wood $\delta^{18}O$ to trace plant transpiration response to the natural environment has been demonstrated (Ferrio and Voltas, 2005), and like $\delta^{13}C$, this parameter exhibits a robust correlation with seasonal plant water status (Choné et al., 2001; Cernusak et al., 2003; Gebrekirstos et al., 2011).

Finally, WUE cannot be separated from other key resources in agriculture when it comes to achieving maximum efficiency in highly water-dependent food production systems (Foley et al., 2011). Therefore, as a global perspective, the sustainability of irrigated fruit production depends on the development of irrigation strategies better adapted to a wide range of scenarios of water availability. Here we analyzed WUE in order to: (1) evaluate the grade of agreement of various approaches for WUE determination;(2) identify the main agronomic limitations ofWUE_{WPy} optimization under a broad range of irrigation and nutrition strategies; and (3) applying the various concepts of WUE to evaluate irrigation and fertilization strategies in peach.

2 Material and methods

2.1. Experimental orchard.

This study was carried out in a peach orchard located in Torres de Segre (Lleida, Spain). An irrigation and nitrogen experiment was performed over 4 years (2007–2010) to evaluate the effects of water and nitrogen supply on tree growth and yield. Experiment began in 2006 adapting trees to irrigation and nitrogen treatments. The climate in this zone is continental, semi-arid Mediterranean, with a mean annual temperature of 14.7ºC, an annual precipitation of 355 mm, and reference evapotranspiration of 1172 mm. Clingstone peach trees (*Prunus persica* (L.) Batsch. cv. Andross) grafted on GF-677 rootstock and planted 5 x 2.8 m apart were used. The soil is classified as a petrocalcic calcixerept (Petrocalcic Calcisol). This is a shallow soil over a petrocalcic horizon that restricts root penetration and water holding capacity (Ruellan, 2002; Shreve and Mallery, 1932). The effective root depth is between 40 and 45 cm. The soil is loamy textured (20% clay and 40% sand) and the pH (1:2.5) is basic (8.3). The soil bulk density is 1380 kg m^{-3} and its coarse element content 30 % (v/v). The soil has a water holding capacity of 41 mm (approx. 12 mm in wet bulb) and an organic matter content of 3.2 % (Walkey-Black method).

2.2. Experimental design

A 3x3 factorial design in a randomized complete block design with four repetitions was used. The following three irrigation treatments were applied in function of peach fruit growth stages, as defined by Tukey (1938) and Chalmers and Van den Ende (1975): 100% ETc, irrigation throughout the season (FI); 70% restriction of ETc, or 0.3ETc, (DI2) during fruit growth stage II, and 30% restriction, or 0.7ETc, (DI3) during fruit growth stage III. For full irrigation, initial Kc during rapid vegetative growth was 0.25, mid-season Kc was 1.0 – 1.05 during rapid fruit growth, and late-season Kc was 0.55 according Girona et al. (2005). Irrigation treatments were combined with three nitrogen fertilization treatments: 0 kg ha^{-1} (N0); 60 kg ha^{-1} (N60); and 120 kg ha^{-1} (N120). Nitrogen was applied as Urea Ammonium Nitrate solution (UAN, 32%N) since the beginning of growth until the end of GSII. An automated drip-fertigation system with auto-compensated emitters (18 L h^{-1} $tree^{-1}$) was used to supply daily water requirements based on a simple water budget calculated from data from two nearby automated meteorological stations (3 and 4 km) from network of Catalonia meteorological agency, and using the FAO

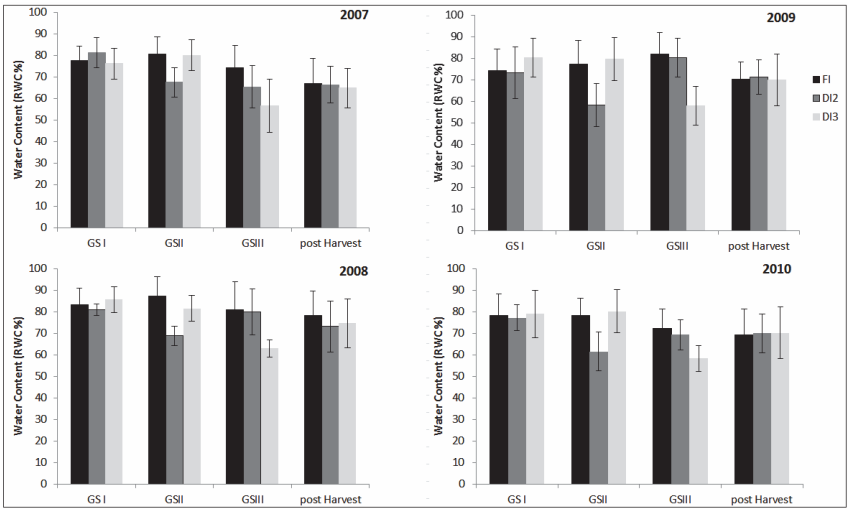

Figure 1. Soil water content as % of soil field capacity) on different periods of tree growth (GSI, from budburst to fruit pit hardening starts; GSII, fruit pit hardening; GSIII, until fruit harvest; and post-harvest period)

method (Allen et al., 1998).

Each experimental plot was delimited by three adjacent tree rows with a total of 30 trees per plot. Plant measurements were made on the five central trees of the middle row. The volume of irrigation applied to each plot was monitored weekly with a water meter. To minimize leaching and drainage, soil moisture was continuously monitored using ECH2O-20 capacitance probes (Decagon Devices Inc., Pullman, Washington, USA), which provided the volumetric water content of the surrounding soil (Figure 1). Four ECH2O probes were inserted into the soil around one tree per irrigation treatment; these were placed 0.7 m from each side of the tree and at depths of 0.15 and 0.3 m, within the wetted zone. Irrigation was managed by weekly programing in function of the ETc-rainfall forecast and trends in soil water content.

2.3 Measurements

The leaf net CO_2 assimilation rate (An) was measured fortnightly, at noon, on three light-saturated leaves per plot (PPFD>1000 μmol m^2 s^{-1}), using a portable infrared gas analyzer (LI-6200, Li-Cor, Lincoln, Nebraska, USA). At the same time, stomatal conductance (gs) was recorded for three leaves using a steady-state porometer (model LI-1600, Li-Cor). Simultaneously, midday stem water potential (Ψ_s) was also measured with a pressure chamber (model 3005, Soil Moisture Equipment Corp., Santa Barbara, California, USA), following the method described by McCutchan and Shackel (1992). The minimum annual Ψ_s reached for the GSII, GSII, and postharvest (postH) periods were recorded, and An and gas exchange measures at this time were used to calculate WUE_{int} and WUE_{ins}. Following Steduto and Albrizio (2005), Eq. (1), biomass and fruit yield water productivity (WUE_{WPb} and WUE_{WPy} respectively) were calculated as the ratio between PW and Y, respectively, by the amount of water applied plus effective rainfall, as a surrogate of tree transpiration, as proposed by Geerts and Raes (2009) on soils with limited water holding capacity.

$$WUEWP_{y\ (or\ biomass)i} = \frac{Yield\ (or\ biomass)i}{\frac{Tij}{ETo}} \qquad (1)$$

Where yield or biomass are yield or biomass for each i-plot, T_{ij} is the sum of irrigation water and effective rainfall, calculated according method proposed by Brouwer and Heibloem (1986) in each i-plot and j interval, and ETo the evapotranspiration calculated (FAO-Penman-Monteith) in each j interval. Tij /ETo is assimilated to real (or actual) mean crop coefficient for each plot and irrigation strategy (Kca) and for each fruit growth phase GSII and GSIII (KcGSII and KcGSIII respectively).

At harvest, the fruit yield (FY) and fruit number (FN) of individual trees were recorded in each plot, and mean fruit weight (FW) was determined. Each year, trees were pruned in winter and excised wood was also weighed (PW).

At the end of the experiment, two core samples of trunk wood from one tree per plot were taken 0.3 m above the ground using a 5-mm diameter tree corer (Pressler, Geosystems Mattson, Mechaniska, AB, Mora, Sweden). One core from each sampled tree was dried for 48h at laboratory air temperature (around 20ºC) and impregnated with resin (Murphy, 1986). Blocks were polished and middle sections were analyzed for annual growth determination by means of ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014). Annual rings were collected from each paired core and dried at 60ºC to constant weight; dry material was ground to a fine powder and handled following the method described by Ferrio and Voltas (2005). Carbon and oxygen stable isotope compositions of all

wood were analyzed by mass spectrometry at the UC Davis Stable Isotope Facility.

2.4 Data analysis

A mixed model of variance and covariance on repeated-measures was built to evaluate the effects of year (repeated effect) and water and nitrogen treatments, and also the interactions of these factors using block as random effect. Tukey HSD test ($P<0.05$) was used to separate all pair means.

Complementary, relations of WUE_{WPy} and WUE_{WPb} with significant variables were established by means of partial least squares regression (PLSR) to quantify the magnitude of each variable avoiding effects of co-linearity and redundancy on Y-variables. The cross validation method was used to select the number of projection components (Mevik and Cederkvist, 2004). The importance of the variables in the model was determined on the basis of the relevance of the variance in the projection method (VIP) (Chong and Jun, 2005). Statistical analysis was carried out using SAS-STAT procedures (SAS®, Version 9.4, SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Water, nitrogen, and year effects on plant water status and on WUE and its components

The start of the growth periods GSI (from budburst to beginning of pit hardening), GSII (pit hardening period), and GSIII (restart of expansive fruit growth until harvest) occurred mid-March, mid-May, and mid-June, respectively. The harvest was between 10 and 12 August, in accordance with fruit maturity standards. The duration of GSI was approximately 65 days, GSII 31 days, and GSIII 54 days. Rainfall during the crop period was scarce and variable, ranging from 136 mm in 2010 to 221 mm in 2009 (Table 2). In contrast, ETo from budburst to fruit harvest was high, oscillating from 573 mm in 2007 to 654 mm in 2009.

Table 2. Duration of tree and fruit growth periods (days), rainfall (mm), and ETo (mm) in each year and by growth stage (GSI, from budburst to start of fruit pit hardening; GSII, fruit pit hardening period; GSIII, from end of pit hardening to harvest).

Year	GS	Days	Rain	Eto	Eto - rainfall
2007	**Year**		246	913	667
	GS I	66	96	188	92
	GS II	28	44	123	79
	GS III	55	6	262	256
	GS I, II & III	149	146	573	427
2008	**Year**		405	997	592
	GS I	67	106	214	108
	GS II	35	89	142	53
	GS III	51	26	262	236
	GS I, II & III	153	221	619	398
2009	**Year**		375	1052	676
	GS I	64	113.	203	89
	GS II	33	14	160	146
	GS III	55	83	289	205
	GS I, II & III	152	211	654	443
2010	**Year**		373	1028	655
	GS I	59	59	207	147
	GS II	32	52	153	100
	GS III	54	25	282	257
	GS I, II & III	145	137	642	5056

Rainfall in 2008 represented 63% ETo in GSII, while in 2009 it accounted for only 9% of ETo in the same period. Similarly, in GSIII, rainfall in 2007 was very low, registering 2.5% of ETo, while in 2009 it was 32% of ETo. Under these conditions, there were significant differences between Kca for irrigation treatments and years. The irrigation water applied until harvest in the FI treatment ranged from 357 mm in 2007 to 463 mm in 2008 (Table 3). Depending on the deficit irrigation treatments and years, between 65% - 70% of seasonal ETc was applied until harvest. Water savings achieved by the deficit irrigation strategies differed depending on the year, accounting, on average, a water saving of 11.8% for the deficit DI2 and DI3 treatments. The

annual nitrogen applied was 55.3±4.1 kg ha^{-1} for the N60 treatment and 116.3±5.3 kg ha^{-1} for the N120 treatment.The results obtained by repeated measures of univariate models (Table 4) show that irrigation promoted significant changes in Kca depending on the irrigation strategy and year, although Kca was also related to the effect of fruit productivity (crop load) and biomass. Along with the Kca variation, similar changes were also observed in Ψ_s, with an interaction between year and irrigation (Figure 2) being detected.

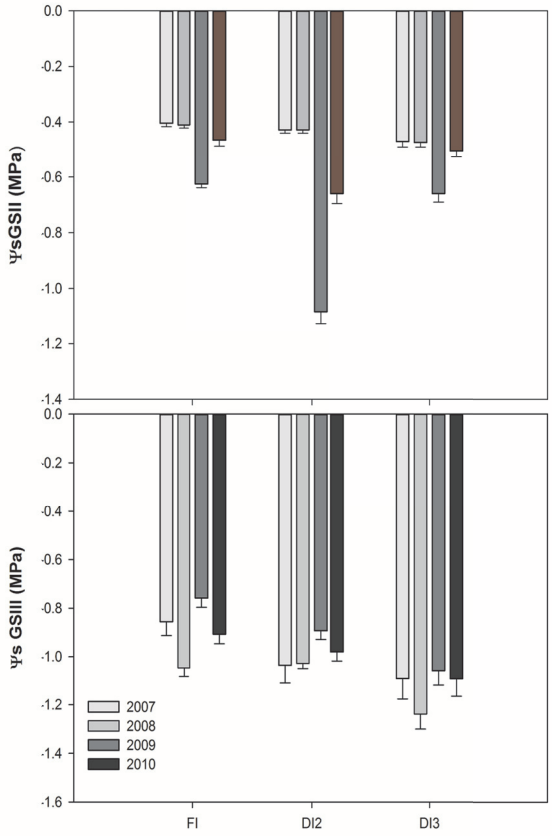

Figure 2. Minimum stem water potential reached in GSII and GSIII by irrigation treatment and year.

These results confirmed that there were no significant differences in Ψ_s during GSII between treatments in 2007 and 2008. In 2009, on GSIII, the lowest Ψ_s values of the four years and the greatest differences between irrigation treatments were achieved (Figure 3). The effect of nitrogen was also observed, revealing that its application had a negative effect on Ψ_s.

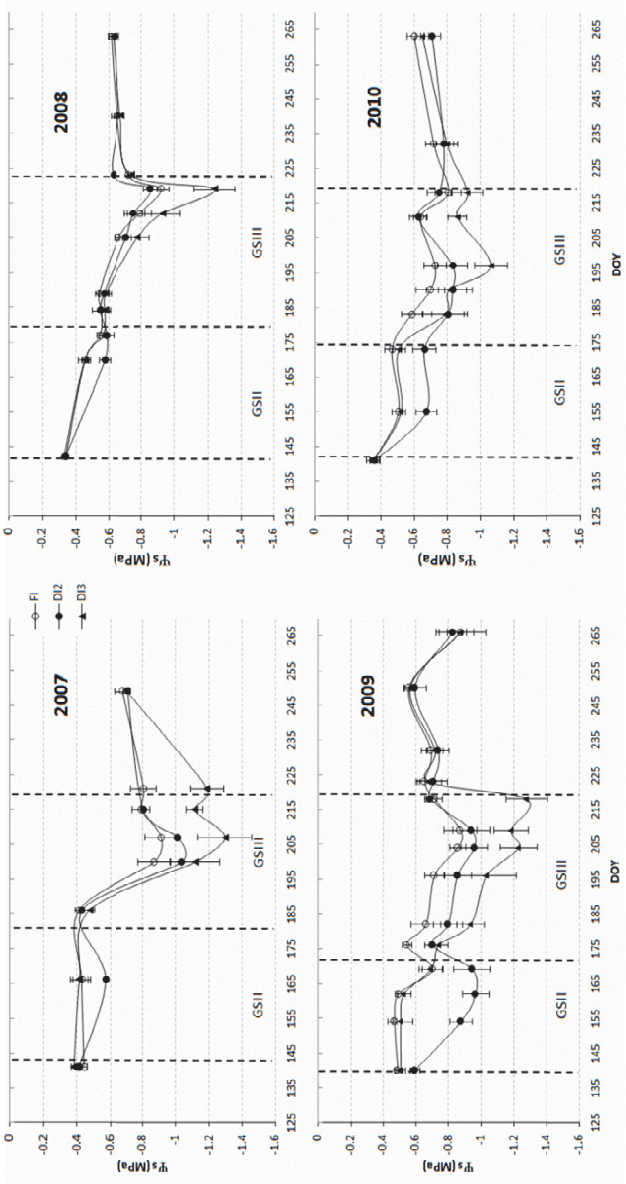

Figure 3. Seasonal trends on midday stem water potential (Ψs) according years and irrigation treatments.

We found significant differences in Ψ_s between irrigation treatments and years. The lowest Ψ_s values between DI3 and the other treatments were reached in 2008 and 2010. Also, yield and biomass were observed to influence plant water status in the same period (GSIII), although the application of nitrogen also promoted water status differences, which were related to the effect of this supplement in plots in the deficit irrigation on GSIII (DI3) treatment (Figure 4).

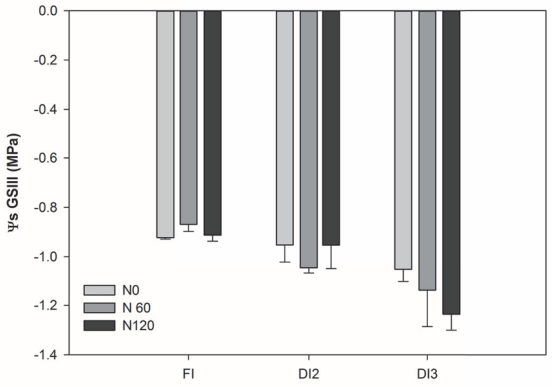

Figure 4. Minimum stem water potential reached in GSIII by irrigation and nitrogen treatments.

The rate of An in GSII was affected by nitrogen, showing a progressive increase as the nitrogen application rate increased. No effect of treatments on An was observed in GSIII, with only the effect of year being significant. In GSII, stomatal conductance was negatively affected by deficit irrigation and it showed and interaction with year. In this way, changes on gs were shown concurrently with the water status of plants in different years, so that gs was not limited by Ψ_s in 2007 or 2008. Similarly, leaf transpiration in GSII showed differences only between years, although its variation was closely related to changes in gs (R^2 = 0.89). In fruit growth phase III, gs varied depending on the year and was also reduced by high doses of nitrogen (N120). A similar behavior was observed for T, which was also negatively affected by nitrogen application in the same phase.

WUE_{int} was clearly affected by nitrogen on GSII, increasing significantly with rising doses of nitrogen, although this index presented a significant nitrogen-irrigation interaction (Figure 5a), which was also affected by year. The response of WUE_{ins} was similar, as it also showed an interaction between nitrogen and irrigation (Figure 5b).

WUE_{int} was sensitive to nitrogen treatments and year in GSIII, while WUE_{ins} varied only with the year in this fruit growth phase. The post-harvest (only two years) results indicated that plants recovered their water status, photosynthetic capacity, and gas exchange after exposure to water restriction.

Seasonal integrative WUE, as variations in $\delta^{13}C$, was related to the interaction between irrigation and nitrogen treatments (Figure 6), resulting in wood $\delta^{13}C$ varying significantly with the rate of nitrogen applied when the plant received less water in GSIII (DI3 deficit irrigation strategy).

Table 3. Irrigation water applied (mm) in each of the growth stages and irrigation treatments by year. Significance of abbreviations is in the text

	2007			2008			2009			2010		
	FI	DI2	DI3	FI	DI2	DI3	FI	DI2	DI3	FI	DI2	DI3
Irrigation until harvest	3574	306	305	462	418	391	433	318	318	444	389	379
Irrigation until end of season	473	423	423	633	595	563	570	459	491	597	535	531
Irrigation fraction until harvest	0.76	0.73	0.72	0.73	0.70	0.70	0.76	0.69	0.65	0.74	0.73	0.71
Irrigation fraction until end of season	0.24	0.27	0.28	0.27	0.30	0.30	0.24	0.31	0.35	0.26	0.27	0.29
Irrigation over R-100	1.00	0.89	0.89	1.00	0.94	0.89	1.00	0.80	0.86	1.00	0.90	0.89
Water saving over R-100 (%)		11	11		6	11		20	14		10	11
Water saving over R-100 (mm)		50	52		44	71		115	115		55	64

Furthermore, the changes in $\delta^{18}O$ were related also to irrigation and nitrogen treatments. This observation thus indicates that the increase in

$\delta^{18}O$ was related to unrestricted water and nitrogen availability (Table 4).

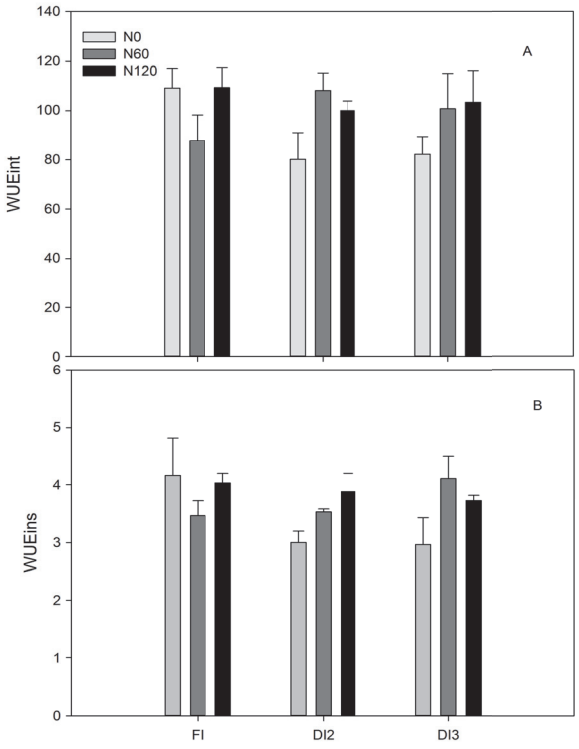

Figure 5. Interaction Irrigation x Nitrogen on WUE_{int} (A) and WUE_{ins} (B) in GSII. Nitrogen rate reveals a significant positive effect when deficit irrigation treatments were applied.

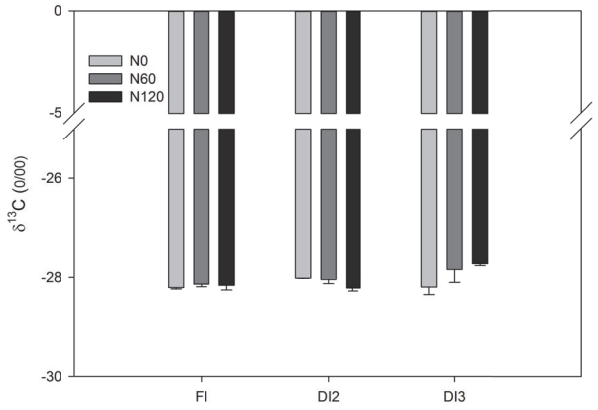

Figure 6. Interaction Irrigation x Nitrogen on $\delta^{13}C$.

3.2 Irrigation, nitrogen, and year effects on yield and vegetative growth.

Table 5 shows the plant response to irrigation nitrogen and year with respect to productivity. Overall, FY, FN, FW, and PW were significantly affected by irrigation treatments and nitrogen application; moreover, there was also an interaction between these factors and year. The most important variables of the productive response were crop load (FN), which was significantly higher in the DI2 treatment, and fruit weight (FW), which was decreased under the irrigation restricted treatments (DI2 and DI3). At the same time, the irrigation x nitrogen interaction was evident in the effect of nitrogen on yield and fruit number when irrigation was limited in GSIII (DI3). Water productivity, as WUE_{WPy}, varied with irrigation strategies, and, in fact, the DI2 irrigation strategy being the most efficient as a result of the FW and FN combination. However, the WUE_{WPb} response was not as clear, showing an interaction between irrigation and nitrogen, in such a way that the nutrient had a positive effect on vegetative growth when there was no water restriction in GSII. The crop load effect was significant in all cases, strongly influencing yield and fruit weight, and being one of the determinants of year effect.

*Table 4. Results on Kca, midday stem water potential (Ψ_s, MPa), CO_2 net assimilation (An, mmol m^{-2} s^{-1}), stomatal conductance, (gs, µmol m^{-2} s^{-1}), Transpiration (T, mmol m^{-2} s^{-1}), intrinsic and instantaneous WUE, and carbon and oxygen isotope discrimination for irrigation and nitrogen treatment and year in each fruit growth phase. Data were analyzed by an ANCOVA repeated measures model with year as repeated effect. Means followed by different letters are different by the Tukey HSD test (P<0.05). The associated probabilities are shown (ns, not significant; * P<0.05; ** P<0.01 and *** P<0.001). When interaction was significant, no letters were used for the separation of simple effects.*

	GSII							GSIII							PostH					
	Kca	ψs	An	gs	T	WUEint	WUEins	Kca	ψs	An	gs	T	WUEint	WUEins	Kca	An	T	gs	$\delta^{13}C$	$\delta^{18}O$
IRRIGATION																				
FI	0.69	-0.47	15.35	156.0	4.64	100.9	3.74	0.95a	-0.90	15.8	196.6	5.51	82.5	3.29	0.37	12.33	3.55	120.0	-28.19	23.13a
DI2	0.47	-0.65	14.67	150.4	4.81	96.5	3.63	0.99a	-0.98	15.5	175.3	5.31	90.8	3.34	0.37	12.26	3.53	123.3	-28.10	22.53b
DI3	0.66	-0.52	15.73	167.3	4.95	99.6	3.59	0.84b	-1.13	14.9	188.8	5.42	92.8	3.16	0.38	11.40	3.34	106.4	-27.95	22.62b
NITROGEN																				
0	0.59	-0.51	14.62b	162.4	4.90	93.7	3.53	0.93	-0.96	15.4	195.2a	5.59a	91.2b	3.24	0.38	11.28	3.36	109.1	-28.16	22.82a
60	0.63	-0.54	15.02b	154.3	4.75	102.8	3.66	0.92	-0.98	16.2	199.1a	5.57a	99.8ab	3.30	0.38	12.75	3.68	123.2	-28.03	22.91a
120	0.62	-0.55	15.97a	157.2	4.74	101.4	3.77	0.92	-1.04	14.8	167.1b	5.09b	104.6a	3.25	0.38	11.71	3.4	117.9	-28.09	22.54b
YEAR																				
2007	0.65	-0.43	15.20	124.1	4.03b	130.3a	3.96a	0.81b	-0.99	15.8ab	126.1b	4.79b	124.3a	3.24ab	0.35	12.87	3.46	98.1	-28.20	22.60
2008	0.67	-0.44	15.47	127.8	4.11b	120,8b	3.72a	0.80b	-1.10	14.3b	200.8a	5.16ab	79,8b	2.72b	0.34	10.96	3.49	133.3	-28.01	22.91
2009	0.52	-0.82	14.75	186.7	5.53a	80.5b	2.70b	1.10a	-0.89	15.2ab	222.3a	6.23a	68.5b	2.46b	0.42				-28.08	22.67
2010	0.55	-0.55	15.38	194.1	5.60a	60.8b	4.20a	1.03a	-0.98	16.6a	192.7a	5.48a	81.8b	4.06a	0.40				-28.10	22.82
ANCOVA																				
IR	***	***	ns	ns	ns	ns	ns	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
N	ns	*	***	ns	ns	**	ns	ns	ns	ns	*	*	**	ns	ns	ns	ns	ns	*	*
Y	*	***	ns	***	***	***	***	***	***	**	***	***	***	***	ns	ns	ns	ns	ns	ns
IRxN	ns	ns	ns	ns	ns	*	**	ns	***	ns	ns	ns	ns	**	ns	ns	ns	ns	**	ns
IRxY	**	***	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IRxNxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Crop load (1)	*	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Biomass	**	***	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns

(1)Crop load and biomass were used as covariates in the ANCOVA model.

*Table 5. Results for irrigation, nitrogen and year factors on fruit yield (FY, kg tree^{-1}), crop load (FN, fruits tree^{-1}), fruit weight (FW, g) pruning weight (PW, kg tree^{-1}) irrigation water productivity (WUE_{irri}, kg tree^{-1} mm^{-1}), yield water productivity (WUE_{WPy}, kg tree^{-1} Kca^{-1}) and biomass water productivity (WUE_{WPb}, kg tree^{-1} Kca^{-1}), and seasonal Kca, An, and ψs. Data were analyzed by an ANCOVA repeated measures model with year as repeated effect. Means followed by different letters are different by the Tukey HSD test (P<0.05). The associated probabilities are shown (ns, not significant; * P<0.05; ** P<0.01 and *** P<0.001)*

	FY	FN	FW	PW	WUEirri	WUEWPy	WUEWPb	Kcaseason	An season	ψs season
IRRIGATION										
FI	62.3	423.5	143.8a	3.50	77.9b	101.7b	70.1	0.60	15.6	-0.70
DI2	61.9	513.6	127.3ab	2.89	88.6a	109.3a	61.80	0.58	15.1	-0.81
DI3	58.9	465.4	130.0b	3.55	83.5ab	104.7ab	73.3	0.58	15.2	-0.85
NITROGEN										
0	59.7	454.1	135.5	2.95	83.9	103.1	63.3	0.58	14.9	-0.76
60	61.5	462.4	137.4	3.41	84.0	105.5	68.5	0.59	15.6	-0.78
120	60.9	489.5	128.3	3.57	82.1	105.0	73.5	0.58	15.4	-0.80
YEAR										
2007	61.7	501.7	127.7b	2.85	100.2a	117.9a	67.4b	0.53b	15.4	-0.72
2008	65.4	440.1	150.3a	3.67	76.5b	126.9a	83.9a	0.51b	14.8	-0.78
2009	55.2	551.0	109.3c	3.24	81.0b	82.2b	58.8b	0.66a	15.1	-0.86
2010	59.2	396.2	142.9bc	3.42	75.8b	93.7b	63.8b	0.64a	15.9	-0.78
FACTOR										
IR	***	***	*	*	***	**	***	ns	ns	ns
N	*	ns	ns	ns	ns	ns	**	ns	ns	ns
Y	***	***	**	**	***	***	***	**	ns	ns
IRxN	***	*	ns	***	ns	ns	*	ns	ns	ns
IRxY	*	*	ns	**	ns	ns	ns	ns	ns	ns
NxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IRxNxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Crop load*	***	-	***	-	***	***	***	ns	ns	ns
Biomass*	ns	*	ns	-	**	***	-	ns	ns	ns

*Crop load and biomass were used as covariates in the ANCOVA model.

4 Discussion

4.1 Changes in water use efficiency depends not only on irrigation

Despite the differences in peach tree response to irrigation and nitrogen treatments, the results reveal the relevance of year effect in them. The variability of spring rainfall greatly affected soil water budget on deficit irrigation strategy in fruit growth phase II (DI2), as reported elsewhere (Girona et al., 2005). Assuming that both fruit load and vegetation growth modulated water demand throughout the cycle, this modulation, along with the rainfall, led to variations in water application, thus affecting the plant water status and productivity in different manners. In this way, WUE_{WPy} and WUE_{WPb} showed a significant and linear relation with seasonal Kca (Figure 7), but less clear in the growth stages (KcGSII and KcGSIII). Moreover, the linearity of these relationships confirms that water losses were negligible under experimental irrigation conditions.

Whole results indicated an overlapping effect of water, nitrogen, and year factors, but relationships between variables, requires the analysis of the combined effects of fruit load and vegetative growth for the interpretation of the overall effects of irrigation regime and nitrogen application through the years.

effect, for example, on pear (Marsal et al., 2002),

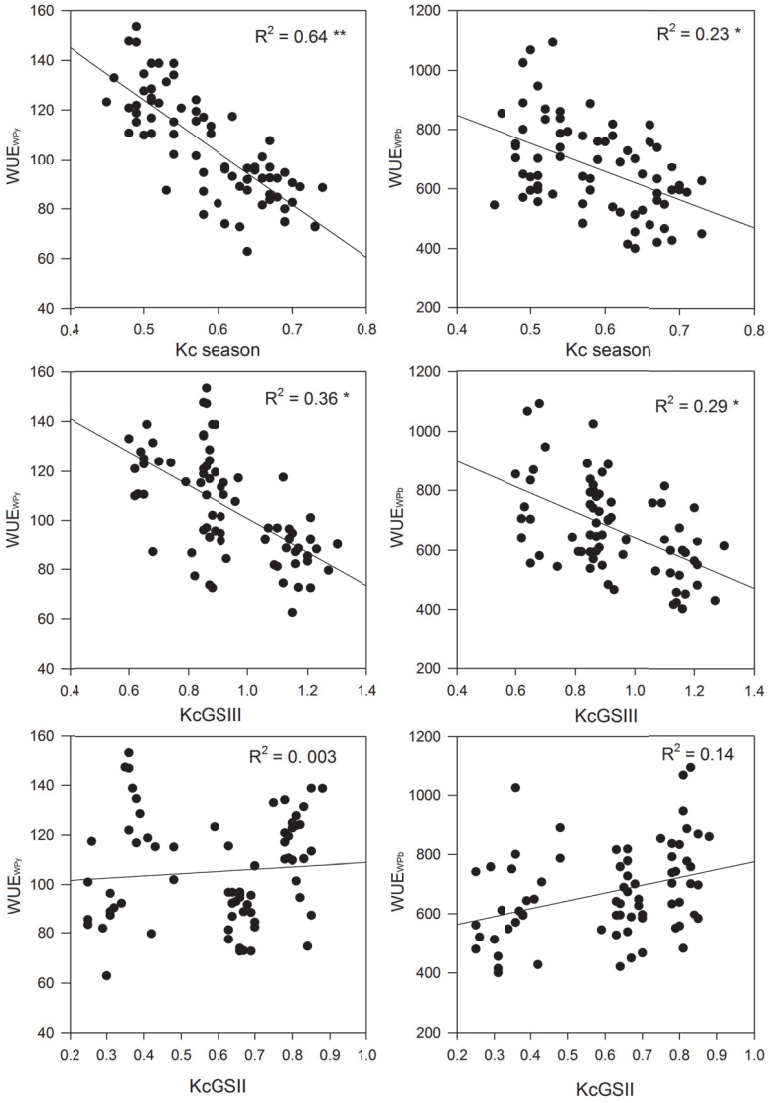

Figure 7. Water use efficiency of yield and biomass by KcGSII and KcGSIII.

4.2 Effects of irrigation strategies on water productivity (WUE_{WPy} and WUE_{WPb})

Such as expected, deficit irrigation in GSII (DI2) significantly decreased vegetative growth and increased crop load (Boland et al., 2000b) (Boland et al., 2000a), even when compared to DI3. This trends in response has been associated with a decrease in internode length, maintaining buds fertility (Girona et al., 2005). However, in return, the DI2 treatment negatively affected fruit weight, as demonstrated as a generalized on several vegetables (Lovelli et al., 2007) or on peach (Boland et al., 2000a; Lopez et al., 2008).

Results also shown that crop load was not proportional to tree growth, thus suggesting that the smaller fruits in the DI2 treatment were related to exacerbation of competition between fruit and vegetative growth under restricted irrigation and high crop load promoted by changes in plant water status (Figure 8). Nevertheless, water restrictions in GSIII (DI3) did not penalize fruit growth as markedly as in the case of the irrigation restriction during fruit growth phase II. Consequently, crop load

appears as a key factor in the WUE_{WPy} and WUE_{WPb} response according to the time when water restriction occurs. In this way, maximum WUE_{WP} was obtained under conditions of high Kca in GSII (i.e., full irrigation) and moderate reductions of irrigation in expansive fruit growth phase. Full irrigation on GSIII enhanced plant water status and photosynthesis during fruit growth, but crop performance and fruit size were limited by vegetative growth restriction if deficit irrigation was applied on GSII, resulting in a sink-source limiting effect in GSII, as has been discussed in Girona et al. (2004).

4.3 Crop load as driver of irrigation strategies expression

As a causal effect of DI2 strategies, besides the well-known vegetative growth reduction, water stress can promote root volume restriction (Girona et al., 1993; Boland et al., 2000a), thus limiting CO_2 assimilation, canopy transpiration, and nutrient absorption (Romero, 2006). Consequently, such limitations can cause a decrease in tree productivity in the mid-term with remarkable effects on WUE_{WPy}. As is stated above, crop load and the canopy development played a key role in the response of WUE_{WPy}, suggesting that the water requirements (Kca) and water status (as Ψ_s) varied with both vegetative growth and crop load (Figure 8).

The variation of crop load and vegetative growth caused by irrigation strategies has been described in other studies and these effects were determinant of yield and fruit size results (Naor et al, 1999; Naor, 2001; Marsal et al., 2002; Trentacoste et al., 2011). In the long term, consequences of the reduction of canopy caused by reiterate application of DI2 strategy can reduce attainable yield via decreasing potential crop load (Romero et al., 2006;Vera et al., 2013) and fruit size (Pérez-Pastor et al., 2014). Therefore, DI2 strategies require usually a rigorous fruit thinning, proportional to canopy, in order to maintain the potential yield of marketable fruit (Lopez et al., 2008; Marsal et al., 2010). In other words, high yield and high WUE_{WPy} in DI2 strategies only can be achieved at the expense of fruit weight, as has been

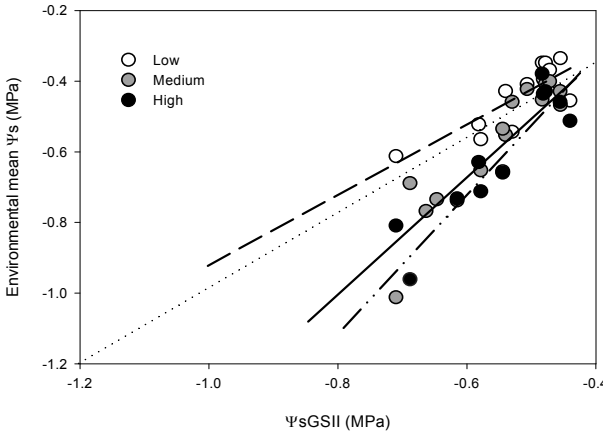

Figure 8. Relationships between stem water potential on GSII, split by yield importance (low, medium and high) and the environmental mean of stem water potential (according Sadras and Trentacoste, 2011).

appointed in others works (Mahhou et al., 2005). The effect of the number of fruits on growth stimulation and leaf photosynthesis appeared to be determinant in WUE_{WPy} in GSIII. During this period, a non-limiting irrigation is particularly relevant, thereby indirectly indicating the close relationship between crop load, fruit growth, canopy functionality, and plant water status (Sadras and Trentacoste, 2011). Therefore reducing water in GSIII is feasible if not compromise plant gas exchange functionality. On the other hand, climate variability effect in WUE_{WP} was associated with changes on Kca during season, attributable to rainfall (Mehta and Wang, 2008). In this way, when KcGSIII decreased and KcGSII increased (year 2008) WUE_{WP} increased, while the opposite results were attained in 2009 falling WUE_{WP}, suggesting a more complex adaptive plant long term response to a changing environment that a causal simple response to irrigation strategy.

In summary, the obtained results suggest that vegetative growth, fruit number, and fruit weight

are major variables to reach high WUE_{WPb} and WUE_{WPy}, and this objective is sustainable both in agronomic and economic terms when canopy volume is not limiting for fruit growth under high crop load conditions (water not restricted).

4.4. Combined effects of irrigation and nitrogen on WUE

Results show the relationship between water and nitrogen application and leaf WUE and WUE_{WP}, and the opposite relationship between vegetative growth and yield. In fact, high doses of nitrogen under full irrigation led to an increase in WUE_{int} and WUE_{ins} both in GSII and GSIII, a finding consistent with other studies (Syvertsen et al., 1997; Ripullone et al., 2004; Brueck and Senbayram, 2009; Garrish et al., 2010) and confirming that nitrogen is a key factor in water productivity at the canopy level (Boland et al., 2000a) promoted by the effects of nitrogen on gs and T (Damour et al., 2010). Meanwhile, in GSIII, increased nitrogen combined with deficit irrigation strategies exhibits a moderate effect in leaf gas exchange (gs and T) and maintained net photosynthesis in similar levels that full irrigation strategy. These observations thus support the feasibility of moderate water restriction at this stage, thus contributing to increased crop load and WUE_{WPy}.

In addition, the low availability of nitrogen was associated with a decrease in An and an increase in gs and T, as a leaf adaptation to canopy development under low nitrogen (Le Roux et al., 2001). Variations on WUE_{ins} and WUE_{int}, was not only associated with changes in Kca or plant water status and it seems to confirm that the irrigation shortage is not solely responsible in their variation. This response seems confirmed by carbon isotope relation; in fact, $\delta^{13}C$, was related to high nitrogen availability when full irrigation was produced on GSII phase. These findings suggest that the variation of $\delta^{13}C$ in wood reflected both nitrogen t and water status effects on Ci/Ca ratio (Brueck, 2008; Pascual et al., 2013) and can lead to biased interpretation of leaf WUE (WUE_{ins} and WUE_{int}) if it is associated with the reduction of gs and T promoted only by water stress.

Results suggest a limited usefulness of $\delta^{18}O$ for irrigation strategies analysis. It was related to vegetative growth and high rates of gas exchange in GSII and GSIII, suggesting that trunk wood $\delta^{18}O$ reflects the behavior of canopy transpiration to water availability as expected (Voltas et al., 2008). In this way, vegetative growth limitation in GSII promoted by the deficit irrigation strategy, together with the $\delta^{18}O$ results, confirm that transpiration was higher in full irrigation treatments during GSII (FI and DI3). So, our findings indicate that $\delta^{18}O$ is an acceptable indicator of the transpiration environment and biomass production at the tree level, as has been pointed by Gessler et al. (2014) but it does not help distinguish the effects of intra seasonal changes in plant water or nitrogen status . We hypothesize that analysis of $\delta^{18}O$ in spring and summer wood fractions could provide more information on plant response to transpiration limitations in specific periods of the growing season.

4.5 Identification of relevant variables in the response of water productivity

The findings indicate a complex relationship between measured WUE from leaf gas exchange, integrated WUE ($\delta^{13}C$) and water productivity (WUE_{WPy} and WUE_{WPb}). The identification and quantification of the relevant variables on the water productivity was performed using a partial least squares regression model (PLSR), which included all analyzed variables as X-variables (standardized), and WUE_{WPy} and WUE_{WPb} as Y-variables. The model indicated the strong dependence of WUE_{WPy} on fruit yield, fruit number, water potential in GSII and in GSIII, and KcaGSIII (number of factors = 2, Y explained= 95%, root mean PRESS = 0.46; Eq. 2). Concerning

WUE_{WPb}, it was related to yield, fruit weight, pruning weight, water potential in GSIII and GSII, KcGSIII and KcGSII (number of factors = 2, Y explained = 99%, Root mean PRESS = 0.42; Eq. 3).

$$WUE_{WPy} = 0.534Yield + 0.235FN + 0.07FW + 0.205\Psi sGSII - 0.198\Psi sGSIII - 0.354KcGSIII \quad \text{(Eq. 2)}$$

$$WUE_{WPb} = 0.175Yield + 0.100FW + 0.598PW + 0.121\Psi sGSII - 0.05\Psi sGSIII + 0.120KcGSII - 0.650KcGSIII \quad \text{(Eq. 3)}$$

The results reveal the dependence of WUE_{WPy} to yield tinged by fruit number and their weight. In turn, the alleviation of water stress in GSII and moderate water reduction in GSIII promote the increase in WUE_{WPy}. Secondly, in agro-economic terms, WUE_{WPy} is not explained in terms of leaf gas exchange and carbon isotope relation.

WUE_{WPb} is affected by the additive effect of vegetative growth (PW), yield and FW under similar water supply conditions to those appropriate to maximize WUE_{WPy}. Along these lines, the results are consistent with those obtained, by others authors (Naschitz and Naor, 2005; Lopez et al., 2006; Lopez et al., 2007; Lopez et al., 2012;) justifying some effects related to crop management, like summer pruning and fruit thinning, as a method to mitigate the negative effects of water stress on fruit weight in advanced stages of the season.

In summary, results shows the compromise between yield and fruit quality (considering fruit weight as a key quality factor) has led in many cases to the recognition of the impossibility of achieving high levels of WUE_{WPy} in DI2 strategies when the goal is to produce high yield and fruit quality (Berman and DeJong, 1996; Naschitz et al., 2010; Naor et al., 2013).

5 Conclusions

The results reveal the peach productive response in different scenarios linked to water availability in irrigated peach crop. Despite the imposed irrigation strategies, the differences in plant water availability were related mainly to the high variability of rainfall in the initial period of fruit growth. Under these conditions, the classic deficit irrigation strategy during pit hardening was found not to promote high water productivity in terms of WUE_{WPy}, fruit quality and, so, in economic value of production, despite achieving high water efficiency in terms of leaf WUE. The weakness of the relation between leaf WUE and productive WUE (WUE_{WPy}) was associated with the effect of crop load and tree canopy, which emerge as decisive factors in modulating plant response to water and nitrogen supply. From the approach of integrative WUE, the isotopic ratios of carbon and oxygen in the trunk wood were associated with the water and nitrogen availability in the case of carbon, and canopy transpiration in the case of oxygen. However, they provide little concrete and useful information about productive WUE.

In terms of WUE_{WPy} optimization, no water restriction is required under no limited nitrogen availability and high crop load potential. In addition, our results suggest that it is not appropriate to address the water supply (or crop water status) independently at different stages of fruit growth. Achievement of a high water productivity (WUE_{WP}) is closely linked to whole seasonal management of water. Thus, high values of KcGSII on trees with well-developed canopy and with high crop load, are positively related to increased canopy and yield, thus contributing to enhanced WUE_{WPy} and WUE_{WPb}. The negative effects on yield water productivity when KcGSII decreases is explained by the mid-term impact of the water and nitrogen restriction on vegetative growth, that limit crop load and causes a progressive reduction of canopy and photosynthetic efficiency. Consequently, under high crop load conditions restrictive irrigation

strategies in GSII decrease the potential results of crop load-fruit weight relationship..

The efficiency of water use, measured at the leaf level (or physiologic level) and as productive efficiency (WUE biomass or yield) do not converge on the same target, although they are complementary. Particularly in fruit trees, in terms of productive efficiency, WUE is strongly influenced by technology, allowing affect harvest index according to specific needs.Thus, although research on deficit irrigation in fruit has deepened with respect to palliative aspects of the consequences of water shortage, it would be pertinent to develop more flexible irrigation strategies to achieve a high degree of plant adaptation to variations in environmental conditions. Therefore, it should be accepted that the deficit irrigation strategies are appropriate in conditions of chronic restricted water supply, and hence systematically limit the productivity of global production resources. In summary, we propose that further research into the effects of long-term water deficit on the growth-performance relationship, change its focus from slogan "more crop per drop" (Kijne et al., 2003) to "more yield per leaf" as a better way of express the improvement of efficiency and sustainability of the production resources.

6 Acknowledgments

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Capítulo III: La eficiencia de uso del agua y la mejora de la calidad. Nuevos indicadores

Relationship between polyphenol oxidase activity and nutrition, maturity and quality parameters in flat peach[5]

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ABSTRACT

BACKGROUND: Polyphenol oxidase (PPO) is one of the main factors that determine postharvest stability and therefore commercial possibilities of peach orchards. Since the activity of this enzyme depends on several variables of different nature, stating the relationships among them is essential to manage crop inputs so as to achieve the desired quality.

RESULTS: Multivariate projection techniques were used to identify these relationships throughout on-tree maturation in flat peach samples that had received different fertilisation doses, but harvested in equal conditions of soluble solids, fruit weight, diameter, firmness and skin and flesh colour. A relatively low fertilisation dose that was enough to avoid any lack of nutrients provided the lowest crop yield, but also with the lowest PPO activity. Fruit N/Ca ratio turned out to be a better indicator of PPO activity than isolated nitrogen or calcium content. Nutrient analyses on leaves did not have strong correlations with any significant parameter. The most useful variables to predict PPO activity before harvest were fruit firmness and skin colour, both of them read at the onset of on-tree maturation.

CONCLUSION: High fertilisation doses resulted in greater yields, but also with higher PPO activity, which is detrimental for the commercial chances of the fruit.

Keywords: flat peach; polyphenol oxidase; fertilisation; nitrogen; N/Ca

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INTRODUCTION

For decades, searching for the best quality of fruit at harvest has focused the interest of agricultural scientists and engineers. However, defining this *quality* concept has often been a matter of discussion, sometimes including different issues beyond nutritional and organoleptic features, such as shelf life or environmental aspects. In this way, strong research efforts have been made and several measuring methods have been developed to ensure that when the fruit reaches its commercial maturity it will be able to fulfil all consumer demands. However, sometimes even the definition of *maturity* is not that clear. In fact, Reid[1] already related *maturity* and *quality*, defining the former as 'the stage at which a commodity has reached a sufficient development that after harvesting and postharvest handling its quality will be at least the minimum acceptable to the ultimate consumer'. In other words, commercial *maturity* depends on what consumers understand as *quality*. Thus defining the most important parameters to quantify this *quality* is an essential first step that turns out to be a challenging process.

In the case of peach, although reaching a minimum concen- tration of soluble solids is often required by processing industries, the variability of this parameter among cultivars (and even from one tree to another due to environmental conditions or crop load) does not make it a reliable indicator of maturity. On the other hand, titratable acidity decreases as ripening develops, but it has been shown not to be related with consumer acceptance of peach or nectarine.[2] Thus at a practical level the moment of harvesting is decided based on ripening-associated changes in ground colour (usually assessed via CIELab's a^*) and flesh firmness[3-5]. Colour variations have been reported to be tightly linked to ethylene and respiratory changes, having at the same time the advantage of being measured by fast and non-destructive methods[6]. However, although colour evolution may be useful to decide the moment of harvest if its changes are well defined for each cultivar, it does not allow predicting postharvest evolution, which in turn determines shelf life and commercial possibilities in new emergent markets. One of the most important factors that determine fruit postharvest evolution is poly- phenol oxidase (PPO) activity.[7,8]

In addition, changes in enzymatic activities during ripening are well known: PPO activity increases 1.5 times after ethylene and respiratory peaks in Calanda peach.[6] Therefore over-mature fruits (with high PPO activity) are likely to undergo physiological changes that make them invalid for handling and long transportation. On the other hand, harvesting at immature stages (with still low PPO activity) to allow long-distance shipment leads to consumer dissatisfaction due to excessive hardness and lack of flavour.[4]

Meanwhile, peach PPO activity has been reported to be affected by several factors that can be divided into those inherent to the kind of fruit and genotype and those concerning environmental issues and crop management. In this way it has been stated that nitrogen fertilisation delays ripening[9] but enhances PPO activity[8], with the subsequent problems in postharvest handling and consumer acceptance[2]. Consequently, peach shelf life begins to be determined long before harvest.

At this point it is clear that crop input management (e.g. nutrition) must be carried out in such a way that avoids increasing PPO activity, so as not to damage commercial chances. However, the entire process relating crop management (preharvest) with PPO activity at harvest and with postharvest possibilities can also be observed the other way round, but to date there are no references on this. Thus the present study had two main aims, namely (1) to assess the effects of different

levels of fertilisation on PPO activity and (2) to search for the relationship between PPO activity in peach fruits at harvest and some of the parameters that have been traditionally used as maturity indicators measured from the beginning of fruit ripening.

MATERIALS AND METHODS

Experimental site and fruit sampling

The trial was conducted in 2012 in a commercial orchard of flat peach (*Prunus persica* (L.) Batsch. var. *platycarpa* (Decne.) LH Bailey cv. Planet Top) grafted on GF-677 rootstock and ridge planted at 2.5 m × 4.5 m in 2009 in the region of Segrià (Lleida, Spain). This area has a semi-arid Mediterranean climate with an average annual rainfall of 360 mm. Irrigation water comes from the canal of Aragon and Catalunya, with low salinity and nitrate content. The soil is saline (electrical conductivity 3.2 dS m^{-1}) with a silty loam texture, a pH of 7.8 and an organic matter content of 21 g kg^{-1}.

The experiment was organised in a complete block design with 24 elementary plots. Four continuous fertilisation treatments were assessed, based on a modified Hoagland solution10 with nutrient concentrations (mg L−1) N 145, Ca 1.4, Mg 17.9, P 9.9, K 172.2, S 25.1, Cu 0.23, B 0.08, Fe 5.60, Mn 0.60, Mo 0.02 and Zn 0.23, with a resulting electrical conductivity of the irrigation water of 1.7 dS m^{-1} . Nutrient solution was applied by means of an automated drip fertigation system with autocompensated emitters in four strategies: (1) throughout the crop cycle (NUT-100); (2) only until the end of phase II of fruit growth (as defined by Chalmers and Van den Ende[11]) (NUT-100-stop); (3) with 50% of the concentration of nutrients throughout the cycle (NUT-50); (4) with 25% of the concentration of nutrients throughout the cycle (NUT-25). The irrigation water dose was the same for all treatments, calculated by means of the FAO method12 considering a leaching fraction of 15%. Each experimental plot consisted of eight control trees. Three samples were taken before harvest on 6, 14 and 20 August. Further samples were taken at harvest (on the appropriate day depending on each treatment, decided upon colour and firmness values) and after 1 week of storage at 0º C.

Physical, chemical and enzymatic analyses

Fruit (mesocarp) firmness (FF, N) was determined with a manual penetrometer (Copa-Technologie /Ctifl, Paris, France). Fruit weight (FW, g), fruit diameter (FD, mm) and trunk perimeter (TP, cm) were also measured. Soluble solids (SS, ◦Brix) content was measured using a thermocompensated refractometer (Atago Bussan Co., Tokyo, Japan). Skin (sk) and flesh (fl) colours were measured with a Chroma Meter CR-400 tristimulus colorimeter (Konica Minolta Sensing, Inc., Tokyo, Japan) in the CIELab colour space. Parameters a^* , b^* and L^* were determined.

For PPO activity determination, 10 g of crushed flat peach flesh was mixed with 10 mL of McIlvaine buffer (pH 6.6) and 0.51 g of polyvinyl- polypyrrolidone as phenolic scavenger. The mixture was homogenised and centrifuged (Avanti J-26XP, Beckman Coulter, Brea, CA, USA) at 5500 × *g* for 10 min at 5 ◦ C. The resulting pellet was discarded and the supernatant was used for PPO analysis with 10 $mmolL^{-1}$ of 4-methylcatechol (Sigma Chemical Co., St Louis, MO, USA) in McIlvaine buffer (pH 6.6) as substrate. The reaction was carried out in a 1 cm light path quartz cell, and the absorbance at 420 nm was recorded for a period of 3 min by means of a Helios Omega spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and associated software. One unit (U) of PPO activity was defined as the amount of enzyme that caused an

increase of one absorbance unit (AU) at 420 nm in 1 min[8].

Nutrient analyses on leaves and fruits

Samples of 50 leaves (le) and five fruits (fr) were dried at 60 ◦ C to a constant weight and milled to a fine powder. Mineral nutrient contents in both tissues were determined by inductively coupled plasma mass spectrometry (Agilent 7700X, Agilent Technologies, Santa Clara, CA, USA).

Statistical analysis

Statistical analysis of data was carried out using SAS-STAT Version 9.2 (SAS Institute Inc., Cary, NC, USA). Means were compared using the Tukey test (at 95% confidence level). In addition, multivariate projection techniques based on principal component analysis (PCA)13 were applied to identify relationships among the different variables using The Unscrambler Version 10.1 (Camo Process AS, Oslo, Norway). Partial least squares (PLS) regression was carried out to determine the significant variables involved in PPO activity variation[14].

The A cross-validation method was used to select the number of projection components[15]. The importance of the variables in the PLS model was determined by means of a variable importance plot[16].

RESULTS AND DISCUSSION

PPO evolution during ripening

Changes in PPO activity during maturation have been described for several fruits, including apple[17], apricot[18], banana[19], grape[20], litchi[21], mango[22], melon[23], olive[24] and also peach[6]. In most cases, PPO activity progressively increases as fruit matures, experiencing the highest rise in the last days before harvest. However, this is not the case for melon, in which the highest activity is found at early maturity stages owing to an accelerated metabolism at that time, confirmed by the highest rate of ethylene production and chlorophyll degradation[23]. In the different treatments of this study, up to harvest date, flat peach PPO activity behaved in a similar way to that reported for Calanda peach[6]. Later, after 1 week of storage at 0 ºC, PPO activity decreased in all samples (Fig. 1). Ding *et al*.[25] also observed a gradual decrease in PPO activity in loquat fruits during cold storage.

As far as the different nutritive dosages are concerned, it can be stated that PPO activity clearly depends on fertilisation, becoming higher as the total amount of applied nutrients increases.

These results are consistent with the conclusions of a previous study, where it was found that application of nitrogen enhances PPO activity in peach[8].

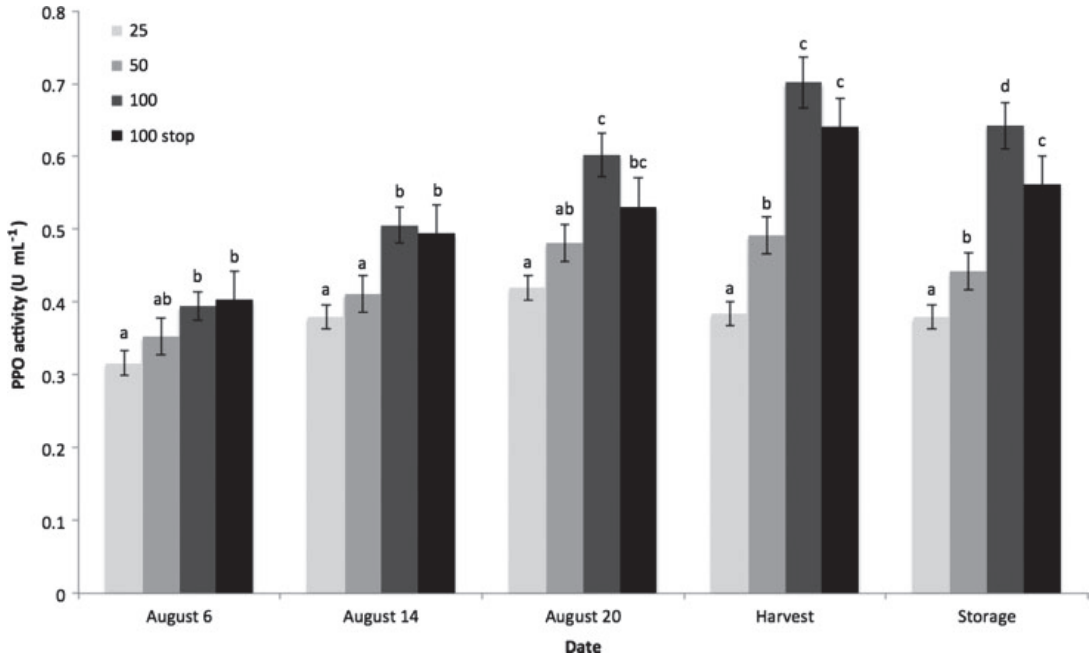

Figure 1. Evolution of PPO activity during on-tree maturation, harvest and postharvest. Within each date, means with different letters are significantly different according to Tukey's HSD test (95% confidence level).

However, in that case, no differences were found at harvest between the two applied doses, while in the present case the three levels (NUT-25, NUT-50 and NUT-100/NUT-100- stop) have been found to be significantly different. Differences between NUT-100 and NUT-100-stop were not significant until post-storage: both samples had the same activity until harvest, but the one that received a higher amount of fertilisation showed a higher activity after storage, even though it was accomplished at 0ºC. Therefore excessive fertilisation levels may result in the inability of the orchard for long shipments, even if it is not observed at the moment of harvest.

Relationships among variables: PCA

In order to find out the possible relationships among such different variables and to identify the ones that contributed the most to the overall variance, a PCA was carried out on a matrix containing 92 variables: the codified treatments (four variables), soluble solids content, fruit weight, fruit diameter, fruit firmness and PPO activity at five different dates (25 variables), number of fruits (one variable), crop yield (one variable), trunk perimeter (as a measure of vegetative growth) at two different dates (two variables), element analyses on fruits at harvest (14 variables), N/Ca ratio in fruits (one variable), element analyses on leaves before harvest (14 variables) and CIELab L^*, a^* and b^* parameters on skin and flesh at five different dates (30 variables).In the loading plot of this preliminary PCA on all data (not shown), soluble solids, fruit weight, diameter, firmness and both skin and flesh colour parameters measured at harvest had low loadings for the first two principal components (PC), proving that the decision on harvesting had been taken in homogeneous conditions for all experimental plots.

In addition, none of these parameters at harvest showed any relationship with PPO activity. The maximum variance direction (PC1) was clearly identified with the four different fertilisation treatments, which appeared homogeneously distributed along this axis. CIELab *a** of both skin and flesh measured before harvest (6, 14 and 20 August) took the highest values of this first PC, surrounding NUT-25 and completely opposite to the high-dose treatments. Therefore it was confirmed that the samples that received a higher amount of nitrogen were greener at the same date (i.e. ripening was delayed)[9]. At the other side of the same axis, PPO activity at the five studied dates appeared close to the NUT-100 loading, reinforcing the statement that high fertilisation doses enhance PPO activity. Furthermore, other data that appeared closely related to high doses included fruit firmness on 6 and 14 August (again, the more fertilisation, the less maturity), fruit N content and the N/Ca ratio in fruits, as the main nutrients related to PPO activity variation. Regarding these last parameters, N/Ca ratio had higher loadings for the first two PCs and therefore seems to be a better indicator of nutritional consequences than fruit N alone. At the same time, fruit Ca was diametrically opposed to PPO activity. Val and Fernández[26] already found that Ca-treated peaches had a lower susceptibility to postharvest internal browning. On the other hand, trunk perimeter appeared tightly linked to crop yield, and both of them were situated at the same side of high fertilisation doses. In other words, fertilisation enhanced vegetative and fruit growth, and the most developed trees provided a greater yield[27,28].

Meanwhile, besides maturity indicators at harvest and postharvest, several other variables had low values for the first two PCs. Among them, soluble solids content was not found to provide significant differences at any time, suggesting its weak utility as a maturity index. Therefore even the lowest fertilisation dose (NUT-25) was enough to ensure a similar sugar accumulation during fruit development, meaning that nutrition was not a limiting factor for this issue.

Table 1. Range, mean and standard deviation (SD) of 20 most important variables according to PCA

Value	FD08/06 (mm)	FD08/14 (mm)	FD08/20 (mm)	PPO08/06 (U mL^{-1})	PPO08/14 (U mL^{-1})	PPO08/20 (U mL^{-1})	PPOha (U mL^{-1})	PPOst (U mL^{-1})	Yield (kg per tree)	TP05/24 (cm)
Min	57.0	66.7	70.9	0.265	0.306	0.322	0.322	0.379	29.5	51.5
Max	75.2	78.8	88.0	0.513	0.673	0.699	0.702	0.664	58.8	79.6
Mean	66.6	71.7	76.8	0.368	0.470	0.494	0.516	0.499	45.6	68.4
SD	4.6	3.6	3.8	0.065	0.094	0.110	0.115	0.081	9.6	7.5
Value (—)	TP06/07 (cm)	frN/Ca (—)	frCa (ppm)	frN (ppm)	08/06ska^* (—)	08/06fla^* (—)	08/14ska^* (—)	08/14fla^* (—)	08/20ska^* (—)	08/20fla^*
Min	54.6	8.4	371.3	5727	−11.97	−15.43	−10.31	−13.66	1.48	−9.28
Max	82.5	30.1	858.2	12847	9.05	−8.00	24.86	0.22	28.39	0.12
Mean	70.4	20.2	506.3	9884	−5.63	−12.53	4.40	−9.07	14.83	−4.86
SD	7.0	5.2	98.9	1863	6.23	2.14	10.05	3.84	7.35	2.80

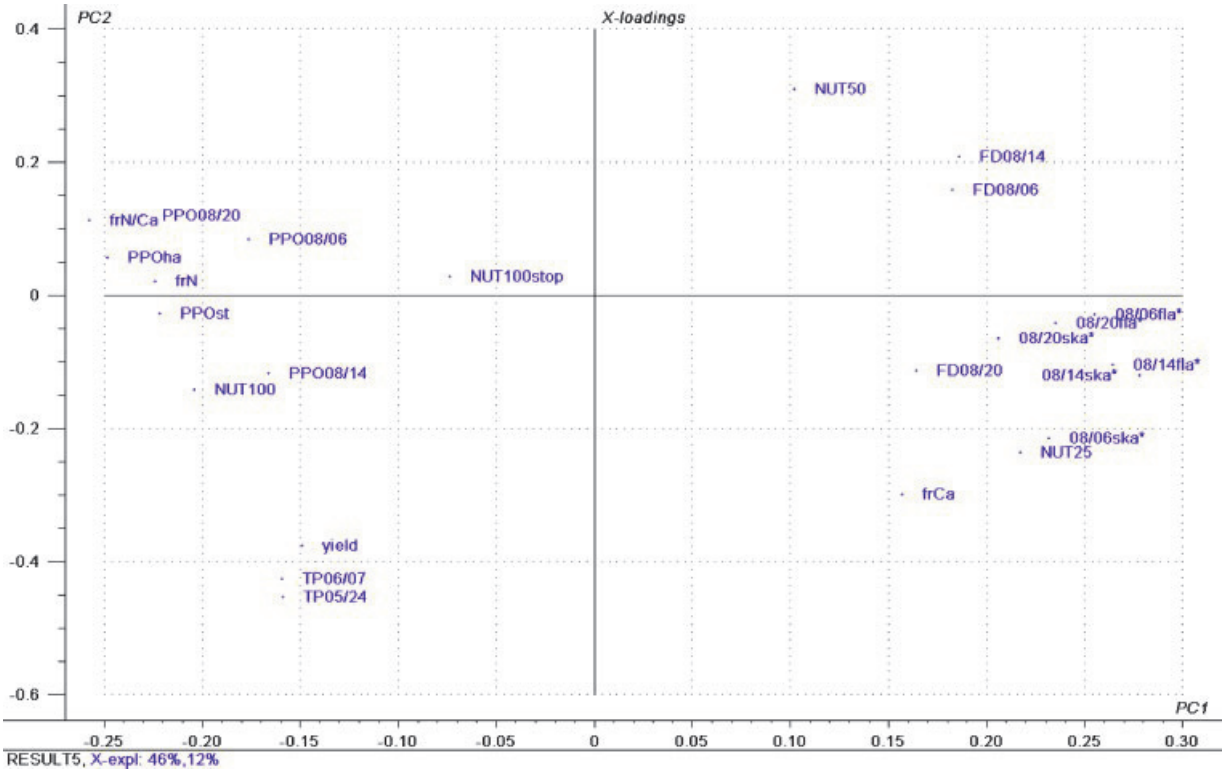

Figure 2. PCA of most important variables, including different treatments (NUT), PPO activity (PPO), fruit diameter (FD), skin and flesh *a** (sk*a** and fl*a**espectively), trunk perimeter (TP), crop yield and nitrogen, calcium and their ratio in fruits at harvest (frN, frCa and frN/Ca respectively).

However, the physiological relationship between pigment changes and PPO is still a matter of speculation. Since ripening is a complex process that may be affected by several parameters,27,29 it is more likely that the different levels of PPO and pigments (most of which are polyphenols) are both consequences of third factors (such as nitrogen uptake) via different physiological processes, rather than being directly related. In fact, some researchers have stated that in certain fruits it is not possible to establish a direct relationship between phenolic content and PPO activity[30,31].

Moreover, leaf and fruit nutrient contents, except fruit N and Ca, were not found to give a valuable contribution to the overall variance. In a similar way, CIELab *b** and *L** also had low loadings for the first two PCs. On the contrary, Ferrer *et al*.[6] found that in Calanda peach *b** could also be used as a ripening indicator. In all cases, red pigment content in the skin (measured through CIELab+*a**) progressively increases as fruit matures, and green pigments (−*a**) disappear in both skin and flesh (this general behaviour can be observed in the mean values reported in Table 1.

In order to reduce data noise, all non-important variables were omitted and a second PCA was carried out. Figure 2 shows the new loading plot of this depurated analysis, for which the 24 most important variables were used (Table 1 shows the main statistics for the 20 important non-coded variables). In this new graph, which represents 58% of the overall variance, all relationships observed in the original PCA and described previously can be seen with more clarity, and correlations between the linked variables were improved. Table 2 shows the aforementioned pairwise correlations between fruit N, fruit Ca and their ratio at harvest and PPO activity at the different dates. The fact that at any date the N/Ca ratio has the highest

correlation with PPO activity confirms that it can be a better indicator than N (positively correlated) or Ca (negatively correlated) alone. The analysis of variance (and the subsequent Tukey's honestly significant difference (HSD) test) revealed that there were no significant differences in Ca content among the fruits from the different treatments. Thus the differences in N/Ca ratio were mainly a consequence of the different N contents (caused by the different fertilisation treatments). Therefore samples with high N/Ca ratios are associated with the effects of high N content, such as delayed ripening[9,27] or enhanced PPO activity[8].

Table 2. Pairwise correlations between PPO activity and fruit content of Ca and N and their ratio. Correlations from PCA of 24 most important variables

PPO activity	Ca	N	N/Ca
PPO 08/06	−0.267	0.446	0.448
PPO 08/14	−0.258	0.220	0.377
PPO 08/20	−0.452	0.470	0.626
PPO harvest	−0.461	0.581	0.683
PPO storage	−0.345	0.497	0.559

Useful variables to predict PPO activity at harvest

A multivariate regression to predict PPO activity was performed by means of the PLS method for a single dependent variable (PLS-1). Only those measurements that were carried out before harvest were used as *X* factors: soluble solids, weight, diameter, firmness, colour parameters and PPO activity assessed on 6, 14 and 20 August. This regression was validated by full cross-validation, obtaining an optimal RMSECV (root mean square error of crossvalidation) value of 0.050 for a model with two PLS factors (with these two PLS factors explaining 90.7% (83.6 + 7.1%) of the *Y* variable variance).

Studying the variable importance (Fig. 3) calculated by this algorithm, it can be observed that, as a general rule, the measures taken on 14 August were more useful than the same variables measured on 6 and 20 August. This is the case for soluble solids, fruit weight, fruit diameter, fruit firmness and skin and flesh colour, i.e. all variables except PPO activity (whose importance increased as time went closer to the harvest date).

This could be explained by the fact that on 6 August all samples were in a very early stage of maturation, while on 20 August they were almost ready to be harvested and, as discussed in the previous section, the measured variables at harvest were not linked to PPO activity. Moreover, in each colour measurement, regardless of the date or the part of the fruit (skin or flesh), *a** had a greater importance than *b** and *L** for predicting PPO activity at harvest.

While (as discussed in the previous section) none of the parameters measured at harvest was related to PPO activity, the most useful variables to predict it were fruit firmness on 14 August (i.e. at the onset of on-tree maturation) and skin colour at the same date. At the other end of the scale, soluble solids were always among the least useful variables to predict PPO activity and therefore fruit durability

CONCLUSIONS

The activity of PPO, which is the main enzyme responsible for postharvest fruit deterioration, is enhanced by fertilisation in flat peach, although these differences may not be found when the fruits are harvested and may only appear after cold storage.

The application of the minimum fertilisation dose that ensures soluble solids accumulation during fruit development (i.e. avoids any lack of nutrients) provides a lower crop yield, but

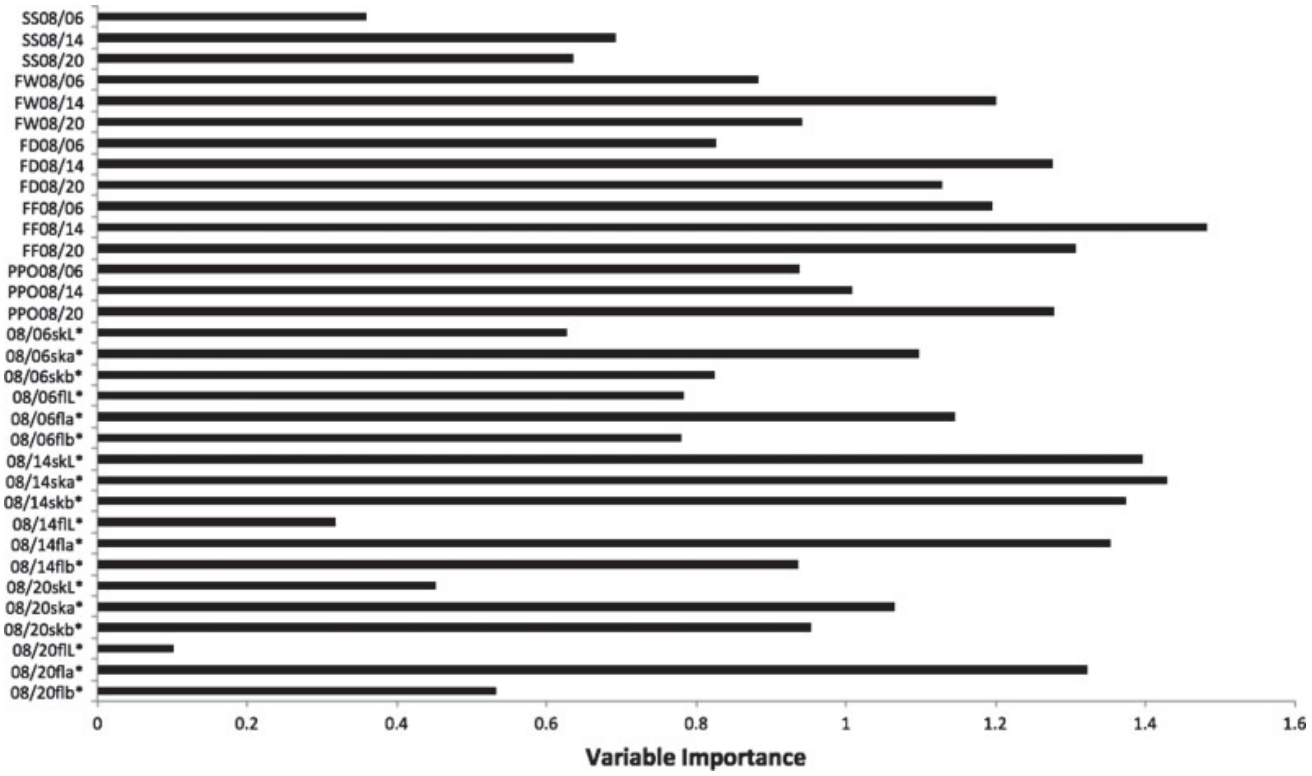

Figure 3. Variable importance plot for first component of PLS regression on PPO activity at harvest

these fruits have a lower PPO activity and therefore are more suitable for long shipments. On the contrary, higher doses give greater yields, but with higher PPO activities that will result in their inability for far marketing and eventually in product losses.

In samples that had received different fertilisation doses but harvested in equal conditions of soluble solids, fruit weight, diameter, firmness and skin and flesh colour, PPO activity was closely and positively correlated with nitrogen content in fruits. In this way, although fruit N/Ca ratio has not been extensively studied in peach cultivars, it turned out to be a better indicator of PPO activity than isolated nitrogen or calcium content, probably owing to the con- tribution of calcium in avoiding internal browning and therefore being negatively correlated with PPO. Nutrient analyses on leaves did not have strong correlations with any significant parameter.

If the measures taken before harvest are analysed as potential predictors of PPO activity, the most useful variables are fruit firmness and skin colour, both of them read at the onset of on-tree maturation. Meanwhile, soluble solids content did not offer any valuable information about this enzyme and therefore is not a good predictor of fruit durability.

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Capítulo IV: La eficiencia de uso del agua.
Efectos del manejo del dosel foliar sobre el estado hídrico y la calidad

Canopy management in rainfed vineyards (cv. Tempranillo) for optimizing water use and enhancing wine quality[6].

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ABSTRACT

BACKGROUND: Rainfed viticulture, mainly in semiarid environments, is limited by environmental variability, particularly precipitation and its seasonal distribution, and soil water availability, thus ultimately determining the final quality of grape and wine. Studies on the feasibility of practices such as canopy management to adapt plant growth and yield to soil water availability open up possibilities to preserve wine quality and reinforce the characteristics of the *terroir*.

RESULTS: Principal components analysis was used to identify the relationships between a large set of variables, including soil, plant, canopy management, and wine characteristics. Canopy management was found to have a predominant influence on plant response to soil water by modifying plant water status, changing the amino acid profile in berries and, concomitantly, altering the sensorial attributes of the wine obtained.

CONCLUSIONS: Grapevine canopy management strategies, such as reiterate shoot trimming to restrict growth during early phases, are effective in adapting plant response to soil water availability. Such strategies affect berry and wine quality, mainly the amino acid profile and sensorial attributes of the wine, without changing yield or grape harvest quality control parameters. Also, in such conditions, nitrogen does not make a significant contribution to grapevine growth or yield or to grape quality.

KEY WORDS: Water stress, soil water content, must amino acids, sensorial attributes, wine quality, climate variability

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INTRODUCTION

Rainfed viticulture, which is practiced mostly under severe water restrictions, is the predominant system worldwide for producing grapes for wine-making purposes. Under the Mediterranean climate, vineyard management strategies aimed to achieve high quality wines are limited by yearly environmental variability[1]. In this regard, there is a need for more extensive knowledge of the environmental and technological factors that constrain vine yield and wine quality. The intrinsic variability associated with various factors (genotype, crop management, soil, and climate) makes it difficult to achieve effective spatial zoning that allows the tailor-made selection of the most suitable processes to enhance the wine quality and strengthen the *terroir* of a given vineyard. *Terroir* -defined as an identifiable spatial region that gives wine its distinctive character[2] - encompasses a large number of variables of diverse nature, such as soil type, climate, and environment, these often showing strong interactions. Therefore, an integrative analysis is required to adapt crop management practices in function of the wine quality potential of each vineyard, rather than applying crop strategies on the basis of information provided by simplified grape quality variables[3].

The importance of soil influence, mainly on plant water status [4,5,6] which plays a basic role in *terroir* expression[7,8], is widely recognized to drive the interaction between water and nutrient availability. Complementary, grapevine canopy management techniques are useful to adapt grapevine water status and nutrient uptake during the growing season, and thus affect wine quality[2]. The marked impact of these techniques are related to the fact that they alter the microclimate of the canopy and plant physiological responses to water status during grape ripening [9,10].

Innovative techniques, such as geographical information systems (GIS)[4], remote sensing[11], and crop response modeling[4], can provide integrated information that contributes to achieving an advanced diagnosis for vineyard management purposes[12]. However, these methods still require proximal analysis to identify the relevant factors contributing to spatial and temporal variability that can be used for the prediction of grape and must properties, thus allowing precise zoning of *terroir* potential. This approach can afford valuable information to trace zonal history and characteristics, and may also provide a powerful tool by which to manage intra-plot variability and therefore to adapt grape yield to the requirements of a given purpose.

On rainfed vineyards, the flexible implementation of canopy management techniques in function of soil water availability may provide the means to achieve the inter-annual grape quality improvement. Therefore, the analysis and characterization of continuous soil-plant expression is the cornerstone for defining the technological requirements needed to attain site-specific responses[13,14]. In addition, climate variability may cause uncertainty to crop management strategies in rainfed viticulture. So, to adapt the vineyard management to environmental variability it is necessary to find strategies that will sustain vineyard productivity and wine quality. The complexity of this scenario calls for diagnostic tools that are more powerful than the simple analysis and interpretation of a limited set of variables[15]. In this regard, here we evaluated the effects of canopy management strategies on grapevine water status during the berry ripening period and on nitrogen in must, wine composition and quality profile.

EXPERIMENTAL

Experimental design

A two-year field experiment (2009–2010) on grapevine was conducted in an 18-year-old vineyard of cv. Tempranillo in the Somontano region (Northeast Spain) planted at 2200 plants ha^{-1} (the total area occupied by the vineyard is 21 ha) and trained on a Royat cordon system (1 m above soil) as vertical shoot positioning by means of mobile iron wires. The vineyard was

selected on the basis of the following three factors: homogeneity of culture practices high soil variability and wine of high quality score. Two plots in the vineyard were delimited on the basis of soil depth and soil water holding capacity: the first (POINT 1) with no limiting rooting depth (> 1.2 m), a loamy texture, and 0.6% organic matter content, and the second (POINT 2) with shallow soil (0.4 m), a sandy loam texture, and 0.9% organic matter. Nitrogen was applied at 30 kg ha^{-1} each year during early spring, and yield was limited by winter pruning to 10-13 clusters per vine.

Three canopy management strategies (also referred to as treatments) were randomly arranged in each point (four replications of 30 plants in each plot, in total near 3000 m^2): canopy management following local common practices, defined as vertical shoot positioning and topping after veraison to limit hedgerow at 1m high (C); leaf removal in fruiting zone at grape veraison, and topping after veraison (LR); and repeated hedgerow trimming at 1m high (ST) -3 times, on dates according shoots regrowth were produced (8-10 cm)- after berry set to minimize vegetative growth from berry set to harvest.

Field measurements

Macro and micro elements were analyzed in leaf petioles at flowering. Petioles of 50 leaves per elemental plot, treatment and replicate were dried at 60ºC to a constant weight and milled to a fine powder. Petiole nutrient contents were determined by inductively coupled plasma optical emission spectrometry (Agilent 7700X, Agilent Technologies, Santa Clara, CA, USA) after closed-vessel microwave digestion (1000W) during 15 min at 120ºC and 25 min at 190ºC. Total petiole nitrogen was determined by Kjeldahl digestion method, and $N\text{-}NO_3$ was determined by ion-chromatography (Dionex, ICS-9). Plant water status was measured as predawn leaf potential (ψ_{pd}) and midday stem water potential (ψ_s) on 5 leaves for each elemental plot at veraison and at harvest, following the method proposed by McCutchan and Shackel [16]. The net CO_2 assimilation rate (An), stomatal conductance (gs), and transpiration (T) were measured on three light-saturated leaves per plot at noon during veraison and at harvest (PPFD > 1000 μmol m^2 s^{-1}), using a portable infrared gas analyzer (LI-6200, Li-Cor, Lincoln, Nebraska, USA). Exposed canopy area was measured by scaled lateral images (Nikon D50/Nikor18-120, Tokio,Japan) and processed with ImageJ software[17]. Yield, clusters, and berries on each plant were determined at harvest on the basis of count or weight.

Must and wine determinations

The parameters related with phenolic fraction were analyzed with a Cromoenos® system (Bioenos, Zaragoza, Spain)[18]. The method involves digesting the grapes in a pectolytic enzyme solution and a sulfur dioxide solution; diluting the solution with diluted acid and spectrophometric reading of the resulting solutions. It has been considered a good method for predicting the color of wines[18]. Briefly, berry samples (100 berries) were collected randomly from harvested clusters of each elemental plot, and were crushed in a mixer and the crush was extracted at 78ºC during 1 minute in a solution of pectolytic enzymes in the presence of an acid solution of sulphurous anhydride. The extracts were centrifuged and diluted 1:100 (v/v) with HCl 2%. The resultant solution is read on a spectrophotometer in an interval of wavelengths between 280-700 nm. Finally, the absorbance at 270 nm was read for expressing the total polyphenol index and the probable color intensity was given by the sum of absorbances at 420, 520 and 620 nm. All these parameters were calculated by means of the software supplied by Bioenos.

The formol titration technique of must was used to provide an estimation of the nutritional status of grape expressed as mg L^{-1} of yeast assimilable nitrogen (YAN). Citric, tartaric and malic acids and glucose and fructose in must were determined

by the methods described in the Compendium of International Methods of Must and Wines Analysis (OIV). The analysis of the amino acids was done using a Li-citrate buffer system with ion exchange chromatography on a Hitachi L-8500 amino acid analyzer (Hitachi, Tokio, Japan). Amino acids were quantified by a postcolumn ninhydrin-reaction detection system. Amino acid concentrations were quantified from peak areas using standard curves. Color intensity (CI), total phenols index (IPT) and tonality-hue of wines were analyzed using OIV standard analytical methods, and total anthocyanins and tannins were measured as described in Fanzone et al.[19], and were expressed as mg L^{-1} of malvidin-3-glucoside and g L^{-1} of (+)-catechin respectively. Must micro- vinification was performed in temperature-controlled fermentative conditions in 50L stainless steel tanks placed in a water bath (18ºC). Wine making and tasting was conducted by technicians of VITEC (Wine Technological Park, University Rovira-Virgili and Catalonia Government consortium) in its facilities, and an expert tasting panel (ISO 17025 certified) composed by 8 trained technicians from VITEC and cellar Bodega Pirineos.

Statistical analysis

Data were analyzed using Univariate analysis of variance (ANOVA). Analysis of variance was carried out with a model considering canopy treatments nested to Point. Replications were randomly arranged as blocks and analyzed in model as random effect. Complementary, Principal Component Analysis was carried out in order to find out the relations among variables. For data analysis were used PROCMIXED and PRINCOM procedures, SAS-STAT package (SAS®, Version 9.2. SAS Institute Inc., Cary, NC, 1989-2009). Tukey Honestly Significant Difference (THSD) post hoc test was used for comparing treatments significance ($p \leq 0.05$) regarding results of studied variables.

RESULTS AND DISCUSSION

Evapotranpiration and rain through studied years

The study area is classified in the upper limit of Winkler Zone III [20], (mean annual temperature was 14.3ºC in 2009 and 14.2ºC in 2010; Winkler-Amerine growing degree days were 1913 for 2009 and 1876 for 2010) in a climatic environment characterized by high evapotranspiration and pronounced water deficit along the growing season (Figure1). Annual reference evapotrans- piration (ETo, FAO-Penman-Monteith) in the Somontano AOC (Controlled designation of origin) region ranges between 1,100 and 1,250 mm, while rainfall varies between 500 and 600 mm $year^{-1}$ (584 mm in 2009 and 538 mm in 2010). The years 2009 and 2010 were representative of this variability. 2009 was rainy during the spring and during berry maturation, while in 2010 most rain fell during flowering and initial berry growth whereas the maturation period was drier than in 2009.

Effects induced by canopy management and zones

The treatments and zones induced significant differences in the crop response and quality of the wine. Little differences were observed in common quality parameters used for harvest time determination. Berry pH, acids, and sugars showed no significant differences, although they showed significant changes at the probable alcohol and on the must color intensity on ST treatment (Table1).

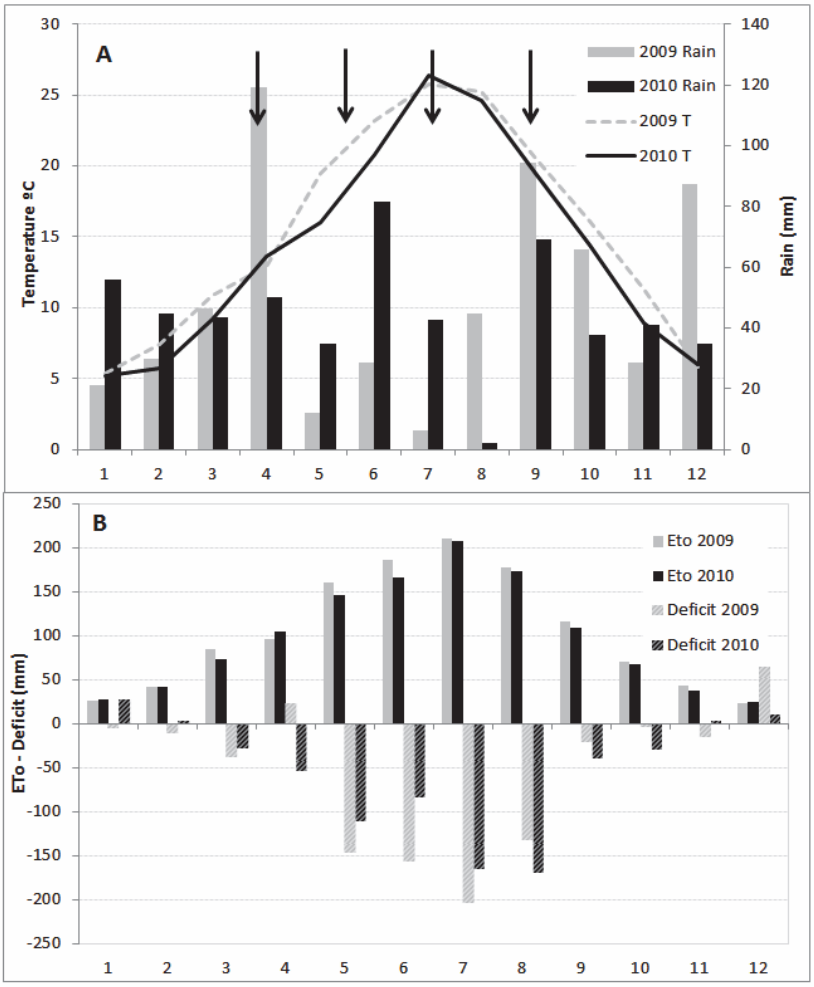

Figure 1. Monthly temperature and precipitation (A), reference evapotranspiration (ETo, mm) and deficit (ETo-Precipitation) for the study area in 2009 and 2010. Arrows indicate the time of budburst, flowering, veraison, and harvest.

Yield components showed differences (cluster and berry weight), attributable to the zone effect (Point), apparently without being influenced by canopy management, which showed lower canopy area in ST treatment. In general, the nutrient content in petioles was affected both by the canopy management and zone (Table 2).

In this way, the petiole nitrates ($N\text{-}NO_3$), calcium and potassium showed different levels according to canopy management and the zone. Petiole nitrate concen- tration was, in a first approximation, higher in the zone of deep soil (Point1) although this effect was not evident for ST treatment, suggesting that limitations imposed on plant growth by shoots trimming reduce $N\text{-}NO_3$ petiole concentration. However, levels of YAN (Table 1) and Arginine and Lysine (main forms for nitrogen accumulation in berries) (Table 3) did not seem to be affected by ST, pointing that petiole $N\text{-}NO_3$ contents may be a questionable tool for diagnostic of berry organic and inorganic nitrogen levels. Predawn water potential and midday stem water potential indicated significant differences between treatments and zones. Ψpd was not significantly affected by canopy treatments in veraison, but significant

Table 1. Means and range of pH, citric acid, tartaric acid, malic acid (all in gL^{-1}), glucose and fructose (gL^{-1}), YAN (mgL^{-1}), probable alcohol (%), probable color intensity, total polyphenol index, and probable color intensity in must for each canopy management strategy and zone (abbreviations are described in the text) (n=48). ANOVA significance is done as probability (P model). Means with different letters are significantly different according to Tukey's HSD test (P<0.05). Letters denotes differences between zones (points).

		Control				**Leaf Removal**				**Shoot Trimming**			
		Point 1		Point 2		Point 1		Point 2		Point 1		Point 2	
	P model	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
pH	0.44	3.28	0.12	3.04	0.18	3.21	0.28	3.28	0.19	2.86	0.14	3.03	0.18
Citric acid	0.58	0.24	0.03	0.26	0.02	0.28	0.05	0.25	0.03	0.26	0.03	0.28	0.05
Malic acid	0.75	3.16	0.17	3.18	0.66	4.35	1.38	3.50	0.41	4.74	3.80	3.45	1.11
Tartaric acid	0.34	5.68	0.62	5.57	0.51	4.83	0.53	5.56	0.51	4.86	0.68	5.63	0.64
Glucose	0.67	98.50	3.98	97.94	18.27	81.09	23.60	105.76	12.36	88.11	28.16	89.44	21.42
Fructose	0.71	116.73	4.69	112.54	20.75	95.29	23.88	122.46	12.13	103.12	29.86	102.84	23.09
YAN	0.37	243	24	284	32	302	26	278	23	317	32	296	31
Probable alcohol	0.008	13.33A	0.50	11.63A	3.80	13.03A	1.00	13.73A	1.90	9.63B	2.30	11.73B	2.40
Color intensity	0.01	12.78A	4.54	11.84A	7.40	11.84A	3.29	10.67A	0.94	7.82B	2.35	8.78B	3.51
Polyphenol index	0.81	27.44	2.44	26.39	7.54	32.36	4.05	31.86	1.89	26.90	4.81	27.84	7.74

Table 2. Results (means and range) (n=48) for number of clusters per vine (CN), yield (Y, kg vine^{-1}), cluster weight (CW, kg cluster^{-1}), berry weight (BW, g berry^{-1}), canopy area projection (CAP, m^2), nutrient petiole contents: nitrate (N-NO_3, mgkg^{-1}), phosphorous (P, g100g^{-1}), Potassium (K, g100g^{-1}), Magnesium (Mg, g100g^{-1}) and Calcium (Ca, g100g^{-1}), wine tonality (Tonality), wine color intensity (CI), wine tannins content (Tannins) , total polyphenols index (IPT and Anthocyanins). ANOVA significance is done as probability (P model). Means with different letters are significantly different according to Tukey's HSD test (P<0.05). Uppercase letters denotes differences between canopy management treatments and lower case letters denotes differences between zones (points). Abbreviations are described in the text.

		Control				**Leaf Removal**				**Shoot Trimming**			
		Point 1		Point 2		Point 1		Point 2		Point 1		Point 2	
	P Model	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
CN	0.54	13.3	2.1	11.3	1.6	11.3	1.9	15.8	2.1	12.5	1.7	13.0	1.8
Y	0.23	4.99	1.50	4.49	2.10	4.50	1.70	6.23	2.00	5.24	1.60	4.49	1.13
CW	0.01	0.381ab	0.112	0.401ab	0.145	0.403ab	0.142	0.395ab	0.113	0.427a	0.150	0.349b	0.117
BW	0.007	2.10a	0.11	1.69b	0.14	2.06a	0.13	1.95a	0.16	1.97a	0.18	2.07a	0.15
CAP	0.003	1.77Aa	0.30	1.60Aa	0.36	1.68ABa	0.28	1.45ABb	0.31	1.37Bb	0.12	1.34Bb	0.09
N-NO_3	0.001	357Aa	31	197Ab	25	403Aa	19	112Ac	20	164Bc	22	180Bc	24
P	0.65	0.11	0.01	0.05	0.01	0.12	0.01	0.12	0.01	0.16	0.01	0.15	0.01
K	0.02	0.49Bb	0.20	0.48Bb	0.12	0.50Ab	0.11	1.39Aa	0.09	0.34Ac	0.08	1.35Aa	1.10
Mg	0.32	1.50	0.70	1.38	0.61	1.45	0.54	1.24	0.63	1.64	0.59	1.05	0.80
Ca	0.02	1.76c	0.08	2.04b	0.09	1.88bc	0.08	2.53a	0.11	2.04b	0.08	2.10b	0.09
Tonality	0.45	37.5	2.1	37.7	3.3	37.5	4.1	37.9	2.4	36.3	2.6	37.8	2.2
CI	0.005	20.2A	0.6	23.0A	0.8	23.2A	0.5	22.2A	0.3	18.1B	0.4	17.8B	0.4
Tannins	0.002	2.6Bb	0.4	2.5Bb	0.5	2.8Ab	0.4	3.6Aa	0.6	2.1Bb	0.7	2.5Bb	0.6
IPT	0.007	37.6b	3.6	50.3a	4.3	49.3a	3.9	44.0a	3.7	39.6b	4.0	42.4ab	4.2
Anthocyanins	0.02	638.4Abc	11.1	731.9Aa	9.0	670.3Bb	10.4	608.8Bc	9.6	597.4Bc	10.5	603.5Bc	9.1

Table 3. Means and range of amino acids in must (gL^{-1}) for each canopy management strategy and zone (abbreviations are described in the text) ANOVA significance was done as probability (P model). Means with different letters are significantly different according to Tukey's HSD test ($P<0.05$). Uppercase letters denotes differences between canopy management treatments and lower case letters denotes differences between zones (points).

		Control						**Leaf Removal**						**Shoot Trimming**					
		Point 1			Point 2			Point 1			Point 2			Point 1			Point 2		
	P Model	Mean		Range	Mean		Range	Mean		Range	Mean		Range	Mean		Range	Mean		Range
Aspartic Ac.	0.34	4.57		1.64	2.56		1.08	4.24		2.59	3.1		1.5	5.33		0.77	4		0.8
Glutaminc Ac.	0.45	15.08		3.31	16.79		5.36	17.42		7.37	12.91		0.81	20.81		3.83	15.23		4.87
Asparagine	0.37	4.16		3.82	2.18		0.6	2.58		0.4	2.3		0.34	4.81		2.66	2.29		1.5
Serine	0.003	44.33	Aa	15.52	49.13	Aa	5.23	39.35	Bab	7.6	27.44	Bb	0.92	40.66	Bab	4.55	31.29	Bb	18.38
Glutamine	0.01	95.92	A	85.18	162.49	A	94.38	60.09	B	10.46	50.5	Bb	0.08	67.09	B	6.69	44.48	B	16.12
Histidine	0.001	19.48	A	11.13	24.74	A	3.45	14.21	B	0.17	14.04	B	1.04	12.49	B	1.2	10.59	B	4.31
Glycine	0.0005	4.19	A	0.31	4.11	A	0.72	3.72	B	0.19	3.41	B	0.31	3.03	B	0.04	3.09	B	0.33
Threonine	0.01	22.44	Aab	15.3	19.25	Aab	3.74	16.63	Bab	5.73	7.74	Bb	1.57	18.08	Bab	1.69	11.61	Bb	13.75
Arginine	0.01	183.62	Aab	151.26	135.39	Aab	50.2	118.46	Bab	32.31	45.88	Bb	0.86	137.92	Bab	20.86	72.13	Bb	103.75
Alanine	0.003	23.54	Aab	9.58	17.13	Aabc	2.56	20.4	Bab	4.74	9.17	Bc	0.35	15.56	Bbc	1.33	11.8	Bbc	4.78
GABA	0.56	45.63		5.85	50.52		16.67	44.41		18.52	54.49		11.03	34.16		0.87	42.93		9.04
Tyrosine	0.0001	3.75	Aa	0.88	5.31	Aa	0.41	3.13	Bbc	0.78	3.03	Bbc	0.16	3.34	Bbc	0.57	2.83	Bc	0.67
Valine	0.0005	17.37	A	5.14	20.5	A	6.55	11.16	B	5.42	9.55	B	0.7	8.94	B	1.46	6.32	B	2.69
Methionine	0.001	1.94	Aa	1.54	4.84	Aa	2.26	1.05	Bb	0.19	1.83	Bb	0.14	1.64	Bb	0.54	1.37	Bb	0.42
Triptophan	0.003	10.36	Aa	7.01	10.91	Aa	4.58	6.6	Bab	0.76	2.58	Bb	1.24	7.48	Bab	0.6	2.59	Bb	5.52
Phenylalanine	<0.0001	3.94	Aa	0.74	7.48	Aa	1.04	2.89	Bbc	1.11	3.76	Bb	0.1	2.38	Bc	0.21	3.02	Bbc	1.28
Isoleucine	0.004	21.55	Aa	13.94	37.55	Aa	14.09	14.34	Bb	2.38	19.71	Bb	0.46	14.66	Bb	1.77	14.47	Bb	12.88
Leucine	0.03	1.6	b	0.6	1.02	b	0.26	1.24	ab	0.1	1.01	ab	0.27	1.18	ab	0.14	1.18	ab	0.32
Lysine	0.0001	208.01	Abc	29.75	149.97	Abc	15.88	197.23	Aab	18.86	94.87	Acd	8.67	115.92	Bcd	22.39	73.65	Bd	15.61
Proline	0.0001	104.43	Abc	15.09	74.98	Abc	8.06	98.96	Aab	9.57	47.02	Acd	4.4	57.7	Bcd	31.66	36.25	Bd	7.92

Table 4. Trends in Tempranillo wine scores according canopy treatments and zone (Points). Mean values for tasting scores (0-5 scale: unacceptable =0, excellent =5) Abbreviations are described in the text. Uppercase letters denotes differences between canopy management treatments and lower case letters denotes significant differences between zones(points) according to Tukey's HSD test (P<0.05).

	Control		Leaf Removal		Shoot Trimming	
	Point 1	Point 2	Point 1	Point 2	Point 1	Point 2
Color	3.3Ab	3.8Aa	3.6Aab	3.5Aab	3.2Bb	3.4Bab
Complexity	3.1B	3.3B	3.0B	3.3B	3.7A	3.6A
Body	3.6A	4.1A	3.3B	3.6B	3.2B	3.4B
Sweetness	3.4Ab	3.9Aa	3.3Bb	3.5Bb	3.2Bb	3.4Bb
Unctuosity	2.1B	2.0B	3.1A	3.0A	2.0B	2.0B
Alcohol equilibrium	2.4B	2.5B	3.0A	2.9A	2.4B	2.3B
Acid equilibrium	2.5B	2.4B	2.9B	2.7B	4.1A	3.1A
Equilibrium	3.0Ab	3.7Aa	3.1Ab	3.5Aa	2.2Bb	2.9Bb
PAI	3.3Ab	3.8Aab	3.0Cb	3.2Cab	3.2Bb	3.4Ba

differences appear between zones, indicating different conditions in soil water availability (Figure 2). At harvest, Ψpd was also significantly different according the zones, reflecting, once again, the changes in soil water availability, smaller in Point 2. Furthermore, at harvest time, there were also differences in plant water status according canopy management treatments, showing higher values in ST treatment, which may suggest the effect of shoot trimming on plant water status, preserving water on first stages of berry growth, being more available on the ripening period, as Ψs results indicated.

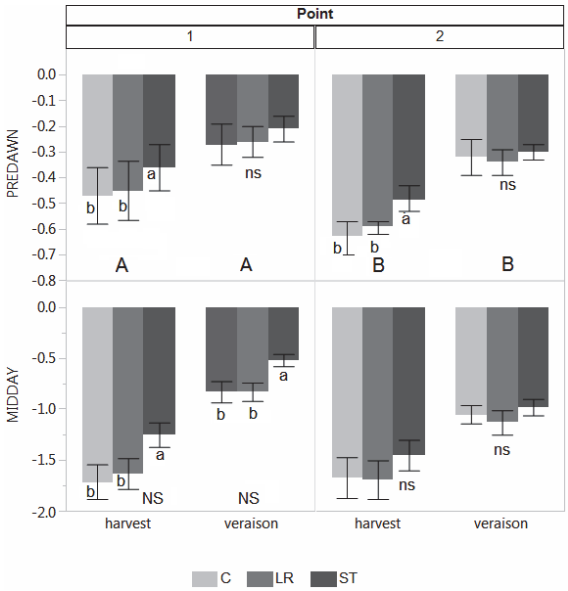

Figure 2. Means of predawn leaf water potential and midday stem water potential (MPa) at veraison and harvest for each zone and canopy management strategy applied. Vertical bars give the total range of results. Uppercase letters shows significant differences between points and lowercase letters shows significant differences between treatments according Tukey HSD test ($p<0.05$).

In fact, canopy restriction imposed by ST treatment show a more favorable water status than the C and LR treatments, under higher water stress conditions (Point 2).

Potassium was found in highest concentration in the ST and LR treatment in Point 2 (shallow soil). Similarly, calcium was significantly affected by zone, reaching higher concentrations in Point 2, without affectation by canopy management. These results agree to the general dynamics of these elements, exhibiting higher concentrations in plants with lower growth or water-limiting conditions. In the case of phosphorus and magnesium, no significant changes were observed.

Net photosynthesis (An) and gas exchange variables (gs and E) do not show significant differences, despite exhibiting a tendency that, paradoxi- cally, suggests best response at Point 2 (Figure 3). The results of photosynthesis and gas exchange at noon on the conditions under which the experiment was conducted (especially restrictive water status), promote high variability in the measures, hindering their interpretation. However, it should be considered that canopy development plays a key role in the response of these variables, modulating the leaf functionality. In fact, the canopy area in Point 2 was significantly smaller than in Point1, limiting whole plant transpiration and suggesting a plausible interpretation for the results obtained.

The must quality (Table 2) also indicate the influence of the canopy treatments and the zone. Color intensity (CI), tannins, total polyphenols (IPT) and anthocyanins were significantly affected, but it is not recognized a clear association with treatments, except on color intensity, affected by repeated trimming treatment (ST).

Amino acid profile of the wine was very significantly affected by both canopy and zone. The amino acid profiles are affected by numerous factors: variety, rootstock, yeasts, and abiotic stress (mainly temperature and water status) are some with a direct and significant effect. The general trend of the results pointed that the amino acid content was higher in the wines from Point 1and for the control treatment (Table 3). Proline, recognized as “amino acid of stress” rise in C and LR treatments, indicating a linkage between amino acids and plant water status. In summary, the results revealed, in an initial step, a strong effect of soil water availability, together canopy control, as driver of grapevine

response. Most of the changes observed, have been widely described, affecting berry and cluster weight[21,22] must acid[23] and amino acid composition [24], and wine quality[10].

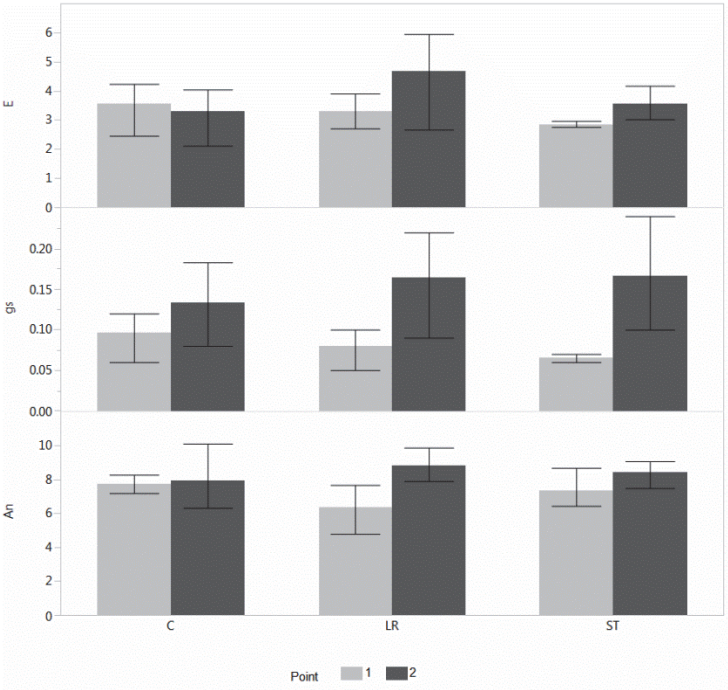

Figure 3. CO_2 net Assimilation rate (An, μmolm^{2}s^{-1}), stomatal conductance (gs, molm^{2}s^{-1}), and transpiration (E, molm^{2}s^{-1}) at midday at pre-harvest for the canopy management strategies and zones (abbreviations are described in the text). Vertical bars give the total variation range of results.

Relations among variables and treatments effects

As discussed in the introduction, only a limited and partial interpretation of results can be achieved by means of univariate data analysis. To overcome this limitation, we performed a PCA analysis to identify the relationships among this diverse group of variables and those most contributing to the overall variance. After a preliminary PCA of all variables (61), 46 variables were retained as relevant. Others that may be interesting in a primary approach, such as yield or cluster number, had no effect because number of clusters was determined by pruning and thinning; also, treatments were harvested when standard quality parameters reached similar levels (sugar content, berry skin color, and acidity) and made little contribution to explaining the total variability of the data set.

First two PCA components accounted for 75.3% of overall variance (Figure 4). The PCA loads distribution and their relevance on each axis shows the complexity of the interaction between canopy management practices and plant water availability, and their influence on grape composition and the sensorial attributes of the wine. The first component (PC1) revealed a strong association of soil and plant water status, measured by predawn water potential and midday stem water potential through the growing season on berry and cluster weight and, correlatively, on yield. In other words, from results it's possible to recognize the well-known effect of plant water availability on weight of berries and clusters considered as important and controversial factors for wine quality[25]. However, although soil water holding capacity plays a key role in plant water status, the results showed an interaction with canopy management strategy, particularly when ST strategy was applied (Figure 3), saving soil water (using Ψ_{pd} as proxy for soil water availability) and improving plant water status at

harvest. Indeed, ST appeared as an interesting strategy to promote conservative plant-soil water behavior in conditions of severe plant water stress, as occurs on studied region (midday stem water potential reached up to -1.7 MPa at harvest time)

.

Effects of plant water status on plant organic and inorganic nitrogen and its relations with canopy management

Results for yeast available nitrogen (YAN) did not show a limitation of the fermentative conditions of juice in any of the treatments. However, PC1 showed that greater water availability in veraison and at harvest promoted an increase in YAN without an apparent relation with plant nitrogen concentration (such as petiole nitrate-nitrogen). This observation is not always consistent with the findings of other authors[26]. Paradoxically, YAN was negatively associated with amino acid contents, thus implying, *a priori*, a contradictory trend in results. This negative relationship can be explained on the basis of the distinct distribution of amino acids and ammonium in the berry. Thus, YAN values may be altered depending on changes in berry development[5] and size[27]. Moreover, amino acids in the must were positively associated with increasing water stress through the growing season, a reduction of plant canopy (CAP), and berry size. In this regard, must from the grapes in the second point, which were more water-limited, exhibited a higher content of amino acids. However, the load projection in PC2 showed that the must from plants subjected to ST also tended to accumulate more amino acids. Also, PC2 indicated a similar response of plants in the C and ST canopy treatments, exhibiting similar trends regarding the reduction in berry size, canopy area, and low plant nitrate-nitrogen. Also, these canopy management strategies were observed to improve the must amino acid profile, changing the importance of several amino acids aroma precursors[28] and relevant traits of wine quality, such as aromatic potential intensity (PAI), wine body, sweetness, and equilibrium.

Soil and plant water status did not appear to play a unique role in wine quality. The observation of a decrease in canopy area and berry weight, together with higher concentrations of amino acids, do not appear to be consistent under the low water potential in the ST treatment.

Also, these canopy management strategies were observed to improve the must amino acid profile, changing the importance of several amino acids aroma precursors[28] and relevant traits of wine quality, such as aromatic potential intensity (PAI), wine body, sweetness, and equilibrium.

Soil and plant water status did not appear to play a unique role in wine quality. The observation of a decrease in canopy area and berry weight, together with higher concentrations of amino acids, do not appear to be consistent under the low water potential in the ST treatment. Furthermore, the inverse relationship was detected between An, gs and E, and ST treatment (PC1 loads). In addition, it is demonstrated that repeated shoot topping reduces the hydraulic conductivity of roots, thus affecting the physiological response of the plant in terms of aquaporin activity in berries, leaf photosynthesis, and gas exchange reduction[29]. In such conditions, consistently with these trends, it can be assumed that the ST strategy constitutes an effective tool for plant growth reduction in the first growing period under high soil water availability resulting a similar wine quality to that achieved when using the C strategy under the driest conditions (PC2 loads). Leaf removal, a widespread practice in viticulture, had variable effects on berry composition and wine quality [30,31,32,33] sometimes without a recognized beneficial effect[34].

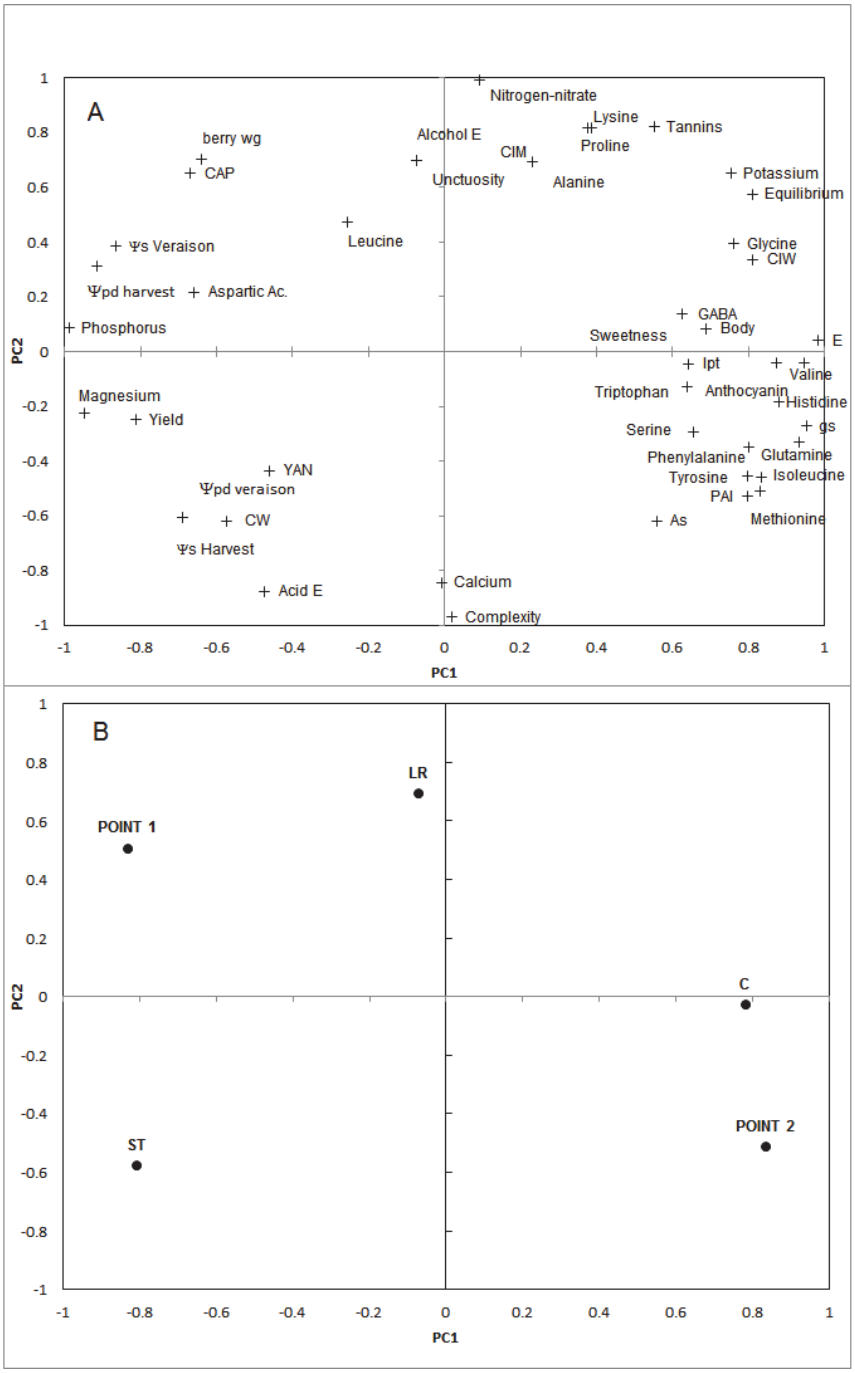

Figure 4. Variable loadings represented in the two first Principal Component space (A) and zone and canopy management strategies (B). Abbreviations for variable names are defined in the abbreviations and Materials and methods section. PC1 account 47.1 and PC2 28.2% of total variance.

In general terms, the cause of this variability may be the integrated effect of water stress and berry temperature, which can affect maturation in various ways. We did not find any clear relation to studied variables typifying results of LR treatment. Alcohol accumulation (sugars), nitrogen-nitrate, tannins, some amino acids, particularly proline, they may be associated with berry stress conditions, and different from the stress caused by water scarcity. In fact, the LR strategy was discriminated in PC3 (accounting only 6.1% of total variance) by the year effect, thus reflecting changes in seasonal water status (pre or post veraison) depending on the year,

resulting in high variability of berry and wine characteristics, as reported in the literature[35]. Furthermore, the beneficial results of LR strategies are closely related to plants with high vegetative growth (high CAP) and they enhance attributes such as wine alcohol equilibrium and unctuosity. In contrast, the wine produced from vines subjected to LR exhibits less complexity and acidity equilibrium. Results obtained on LR treatment suggest the influence of others effects, as berry temperature, promoting stress, no studied in the present work.

Plant nutrients content affected by treatments

The changes observed in plant (petiole) nutrient content could not be satisfactorily explained in the overall context of the analysis. Despite observing some tendencies between nutrients in the PCA analysis, such as high nitrogen and low Ca when growth is not restricted, and more K and less Mg when stem water potential diminishes, a clear cause-effect relationship could not be established. We assume that this is due to the effect of plant water status related to year and intra-year variability. These results suggest that: a) in this research, vineyard nutrient status was not limited by cultural practices and soil fertility; and b) when petiole nutrients content was not limiting for plants, this method do not appear as an accurate tool for predicting the quality of wine produced from the grapes of vines under variable water availability.

CONCLUSIONS

Common practices in AOC regions limit vineyard yield and use a standard set of berry parameters to determine optimal maturity for harvest. Although berries could exhibit similar basic harvest parameters—such as pH, sugar content, acidity and color—,the soil water availability and canopy management strategies clearly influenced wine quality and characteristics. Our results revealed a strong interaction between soil water availability and canopy manipulation as these factors altered plant water status. Shoot topping in early stages of plant growth, namely during berry set, enhance berry quality and the amino acid content of the must, thus resulting in wines more representative of Somontano *terroir* wine style. Moreover, our findings reveal the efficacy of canopy management techniques to adapt vineyards to year-to-year climate variability and to mitigate the combined additive effects of soil and water variability. In non-limiting conditions for plant nutrition, the petiole content of nitrogen, potassium, magnesium, and phosphorous varies in function of the prevailing canopy management strategies and plant water status, thus limiting the usefulness of these parameters for quality evaluation purposes.

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Capitulo V. Discusión General

El análisis global de los resultados presentados en esta tesis pone de manifiesto la complejidad del marco en el que se expresa la eficiencia del uso de agua. A nivel de parcela, los resultados indican que la evaluación de la eficiencia de uso del agua desde diferentes perspectivas debe ser explicada e interpretada más como resultado de diferentes interacciones, como son el agua y la nutrición, que como una respuesta unifactorial asociada tan solo a la disponibilidad hídrica de la planta. Además, también se ha puesto de manifiesto la necesidad de realizar el análisis de la eficiencia incorporando el valor añadido por la calidad a la producción y los costes de no-calidad, con gran importancia en este sector.

5.1. Evaluación de la eficiencia del uso de agua y variables relevantes.

En el Capítulo II se ha puesto de relieve la importancia de la interacción agua y nitrógeno en el resultado de la productividad del agua (WUE_{WPy}) y, indirectamente, de otras tecnologías de la producción, como la poda y el aclareo de frutos, decisivas en el control de las relaciones fuente –sumidero en árboles frutales. Los diferentes efectos del déficit hídrico controlado según el período en el que se aplica, han mostrado la importancia de la carga y de la vegetación en la maximización de WUE a medio o largo plazo. Ello sugiere que la máxima productividad del agua se alcanza en condiciones de alta carga de frutos y crecimiento vegetativo no limitante, como puede interpretarse de otros trabajos en frutales (García-Tejero et al., 2013). Concretamente, en el caso del melocotonero, la respuesta positiva de la eficiencia de uso del agua a largo plazo se muestra estrechamente relacionada con las condiciones no limitantes del crecimiento vegetativo durante la fase II de crecimiento del fruto, que no restringen el volumen de plantación a lo largo del ciclo de vida de las plantaciones. Ello, según los resultados, constituye un factor decisivo para la productividad de esta especie, y ya ha sido apuntado en algunos trabajos sobre los efectos de las estrategias deficitarias de riego en fase II (Naor, 2001, 2004). Por otro lado, las condiciones de estrés hídrico en la fase III de crecimiento del fruto pueden comprometer seriamente la viabilidad de los frutos y, incluso, la de las plantaciones. En estas condiciones tan restrictivas, las podas en pleno verano y el aclareo de frutos en estados avanzados de crecimiento pueden contribuir favorablemente al estado hídrico de las plantas y a la viabilidad de los frutos remanentes (Lopez et al., 2006, 2008; Marsal et al., 2006, 2008, 2010).

Los resultados obtenidos también muestran la aparente discordancia entre la eficiencia, medida como productividad del agua o WUE_{WPy}, y la eficiencia de agua medida a nivel de hoja (WUE_{ins} y WUE_{int}) (Figuras 1 y 2). Las variables de intercambio gaseoso y las variables de eficiencia relacionadas con ellas presentan una capacidad discriminante aceptable para comparar diferentes situaciones en unas condiciones concretas, aunque presentan dificultades como medida integrativa de la eficiencia de uso del agua, relacionadas con la cuantificación de la intensidad y la duración de los episodios de estrés, lo que limita su capacidad potencial para evaluar la eficiencia a una escala estacional o plurianual. En esta línea, la relación isotópica de carbono ha mostrado ser más interesante, particularmente cuando es analizada de manera diferencial en el fruto (tejidos del mesocarpio y del endocarpio), mostrando una menor sensibilidad que cuando fue analizada en los anillos de la madera del tronco derivada de la dificultad para separar la madera de primavera de la de verano.

Las relaciones isotópicas de nitrógeno y oxigeno no han mostrado una relación clara con el estado hídrico y de la intensidad del

estrés, lo que se ha atribuido al efecto del nitrógeno sobre el crecimiento vegetativo, la eficiencia fotosintética y el intercambio gaseoso. Particularmente, el nitrógeno jugó un papel preponderante en la relación de isótopos de N, relacionándose claramente con el origen de éste (orgánico o mineral) y con la productividad de la planta. Ello da pie a una reinterpretación de los resultados desde la perspectiva de eficiencia del uso del nitrógeno en diferentes condiciones de disponibilidad de agua (Dalal et al. 2013), un enfoque que no ha sido tratado en esta tesis y que parece interesante en el ámbito de la fruticultura de regadio. Por otro lado, la relación de isótopos de oxígeno no manifestó una capacidad aceptable para relacionar las diferentes estrategias de riego con la

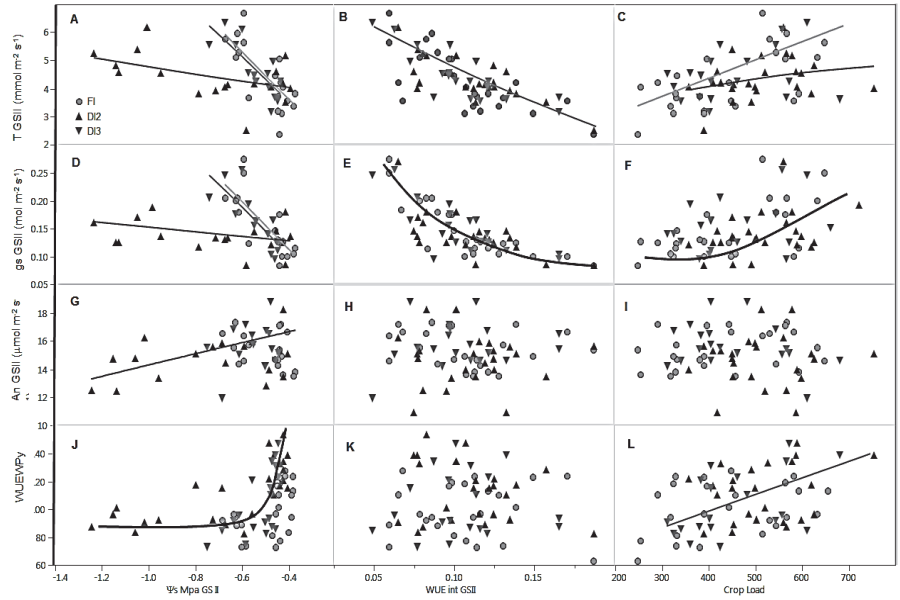

Figura 1. Relaciones entre potencial hídrico, WUE_{int} y carga de frutos con la productividad del agua,(WUE_{WPy}), Asimilación neta (An), conductanca estomática (gs) y Tranpiración (T) en la fase GSII.

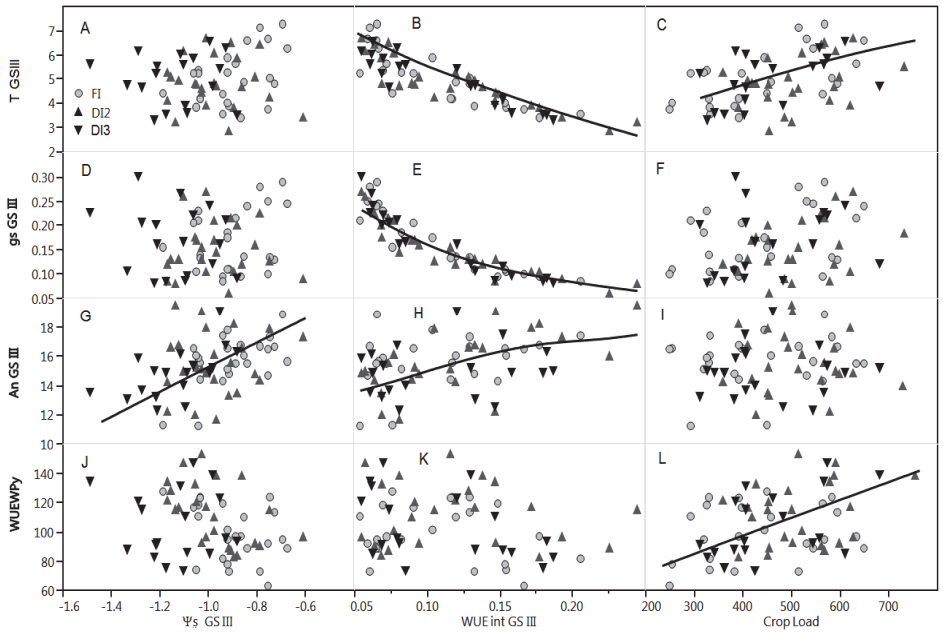

Figura 2. Relaciones entre potencial hídrico, WUE_{int} y carga de frutos con la productividad del agua,(WUE_{WPy}), Asimilación neta (An), conductanca estomática (gs) y Tranpiración (T) en la fase GSIII.

respuesta transpirativa de las plantas al estar también muy influenciado por el nitrógeno a través del crecimiento vegetativo, que aumenta la transpiración de la planta.

5.2. Mejora de la eficiencia de uso de agua a través de la mejora de la calidad de la fruta

En el capitulo III se demuestra que es posible valorar la calidad potencial de la fruta de una manera objetiva. Se ha demostrado la relación entre la actividad polifenol-oxidasa (PPO) y el contenido de nitrógeno en los frutos cosechados en el mismo estado de madurez y también se ha encontrado que existe una estrecha relación entre PPO y la relación N/Ca en el mesocarpo de los frutos. Estas variaciones se relacionaron claramente con las dosis de nitrógeno aplicadas y la época de aplicación del mismo. Aunque la valoración de durabilidad de la fruta no constituyó un objetivo concreto de esta tesis, tanto la actividad PPO como las relaciones N/Ca apuntan hacia un importante efecto en lo concerniente a la conservación y *shef-life* de los frutos, por lo que la cuantificación de la actividad de aquella enzima puede constituir una herramienta de diagnóstico interesante. Algunos resultados obtenidos recientemente (no incluidos en esta memoria) demuestran claramente la relación entre actividad fenoloxidasa y calidad organoléptica de la fruta producida en diferentes condiciones de nutrición. La Figura 3 muestra las diferencias en contenidos de volátiles en melocotones nutridos con diferentes dosis de nitrógeno (en condiciones de fertirrigación), concluyendo que el nitrógeno afecta de modo decisivo la calidad organoléptica de los frutos,

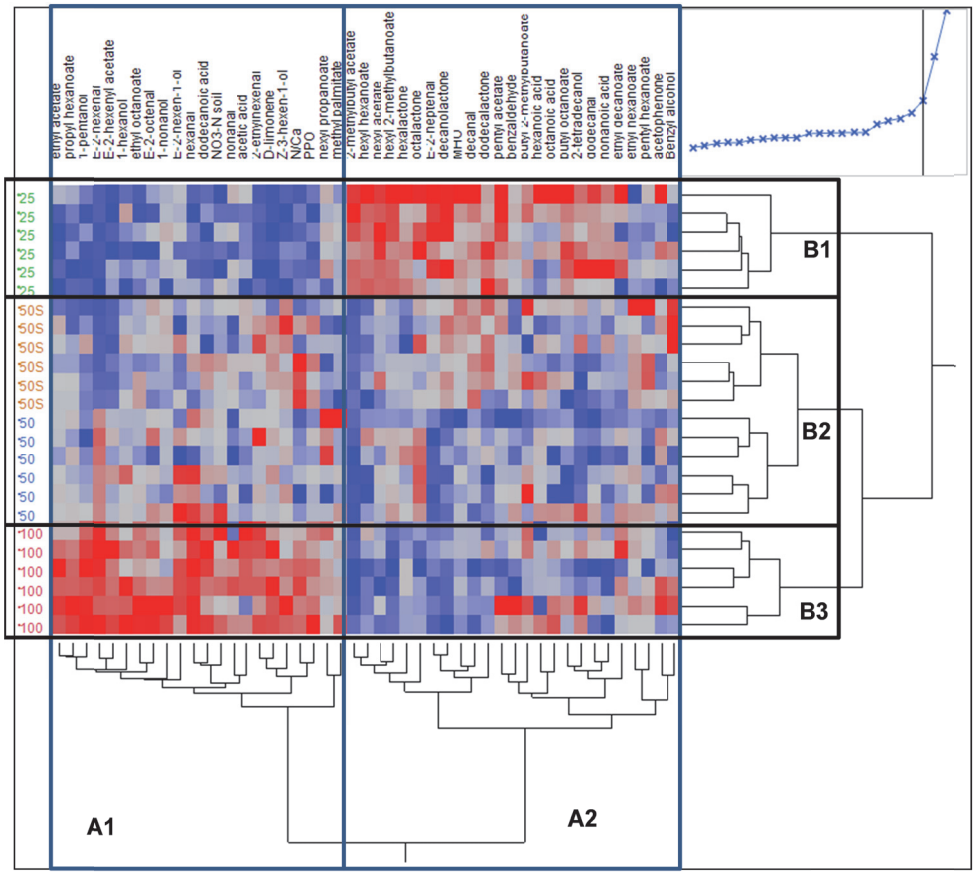

Figura 3. Mapa de color y analisis de cluster bifactorial de los compuestos volátiles (aromas) y sus concentraciones en los frutos, actividad PPO, contenido de nitrógeno y relación N/Ca en frutos. Los colores rojo y azul indican, respectivamente, alto y bajo contenido. El plot de distancias euclidianas se muestra en la esquina superior derecha.El análisis de clusters separa dos grupos en el eje horizontal (A1 y A2) correspondientes a las asociaciones preponderantes de volátiles. En el eje vertical se sepran tres grupos correspondientes a dosis de nitrógeno baja, media y alta (B1, B2 y B3. El análisis y el gráfico se han realizado a partir de resultados obtenidos en 2014 no publicados (obtenidos del experimento expuesto en el capítulo III).

recolectados en el mismo estado de madurez. El nitrógeno se revela como el nutriente clave de estos procesos ya que el contenido de nitrógeno del fruto puede ser determinante en los procesos de degradación de éste. A pesar del conocimiento limitado que se tiene sobre la relación PPO y nitrógeno (Mondy y Klein, 1961; Constabel y Barbehenn, 2008) este nutriente aparece ligado a diversos procesos metabólicos a escala celular que favorecen la respiración, la degradación de quinonas resultantes de la actividad PPO hasta aminoácidos y la posterior síntesis de proteínas a partir de estos. En otras palabras, el contenido de nitrógeno elevado en los frutos promueve una aceleración de la actividad PPO, tanto por degradación del sustrato (quinonas) como por su función de oxidasa terminal acoplada a la actividad citocromo-oxidasa, ligada a la respiración de los tejidos celulares intactos, en presencia de alta concentración de nitrógeno.

Desde una perspectiva agronómica, aparece una cuestión de fondo relacionada con la necesidad de compatibilizar la aplicación de nitrógeno para alcanzar rendimientos altos y, a la vez, preservar la calidad. En este sentido, los resultados obtenidos indican que las aplicaciones moderadas de nitrógeno en fases tempranas son un método aconsejable para alcanzar una alta eficiencia de uso de este nutriente, promoviendo el crecimiento y el rendimiento de fruta a niveles similares a la aplicación de dosis más altas durante periodos más prolongados (hasta mediados de fase III (Figura 4).

En conjunto, los resultados obtenidos indican la necesidad de prospectar con mayor intensidad las interelaciones entre calidad medida en campo (calidad de cosecha) y la calidad de consumo. Las conclusiones obtenidas apuntan que ello puede ser abordado mediante la incorporación de medidas de actividad enzimática de los frutos en fases previas a la cosecha, posibilitando la evaluación de la calidad desde otra perspectiva y contribuyendo a la mejora de ésta, tanto en sus aspectos productivos como para la gestión de la no-calidad.

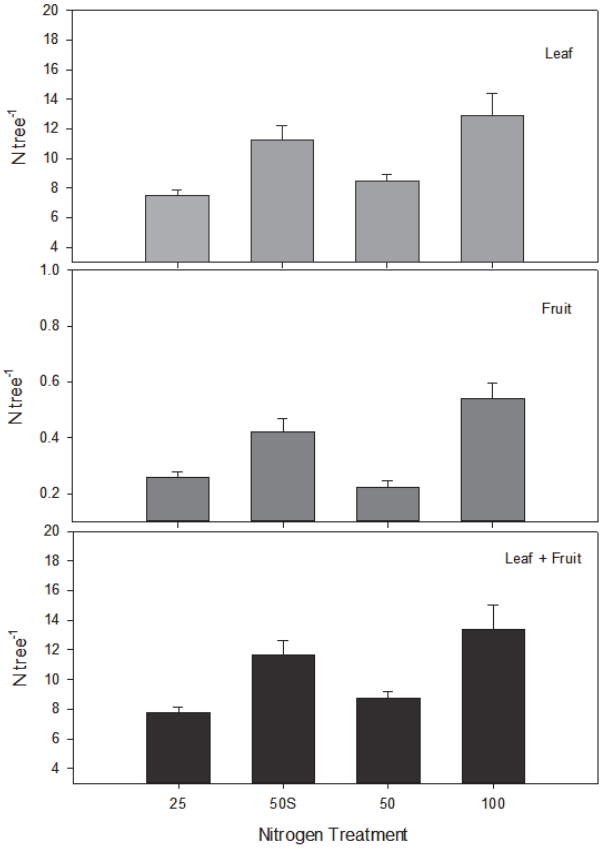

Figura 4. Nitrógeno absorbido por frutos (Kg arbol^{-1}), por hojas (s/u, estimado como producto del área cenital de los árboles y el contenido de nitrógeno) y N absorbido total (s/u). Las líneas verticales representan el error estándar.

5.3. Mejora de la eficiencia de uso de agua a través de la modulación de la demanda hídrica del cultivo promovida por el manejo del dosel foliar.

La disponibilidad de agua del suelo combinada con las estrategias de gestión de dosel, y su influencia en la calidad de la uva y el vino han sido objeto de una atención parcial en la investigación vitícola desde los trabajos de Freeman et al. (1980) y

McCarthy et al. (1983). Sin embargo, pueden citarse un gran numero de trabajos que estudian separadamente sus efectos. Además, la exploración de estas relaciones con la calidad del vino y su estilo presentan un alto interés para valorizar los recursos hídricos de sistemas tan limitados como son los del secano mediterráneo.

Los resultados obtenidos revelaron una fuerte interacción entre la disponibilidad de agua en el suelo y la manipulación del dosel, afectando al estado hídrico de la vid a lo largo del ciclo anual. La evolución del contenido de agua en el suelo apunta a que en las primeras etapas de crecimiento, desde brotación a cuajado del racimo, se produce la absorción de agua prácticamente sin restricciones (Figura 5), mientras que a partir de la primera fase de crecimiento de la uva y durante el período de maduración, el estado hídrico de la planta declina dependiendo de la disponibilidad de agua del suelo y los tratamientos, poniendo de manifiesto la eficacia de las técnicas de gestión del dosel para adaptarse a la variabilidad climática interanual y mitigar los efectos de disponibilidad de agua en el suelo y la variabilidad del agua durante el ciclo, corroborando parcialmente los resultados de Lebon et al. (2003).

En cuanto la infuencia en el estado nutricional, y en condiciones no limitantes para la nutrición, el contenido en el pecíolo de nitrógeno, potasio, magnesio y fósforo varió en función de las estrategias de gestión del dosel y el estado hídrico de la planta, lo

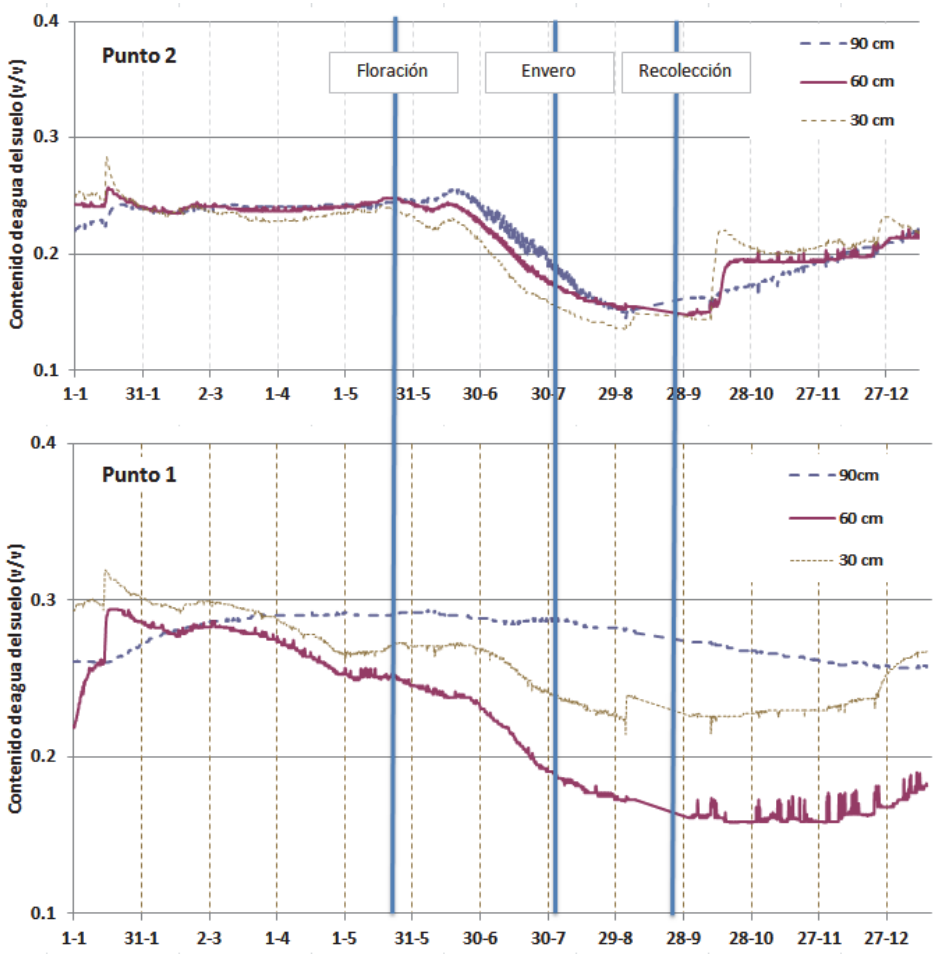

Figura 5. Evolución del agua del suelo a tres profundidades en las zonas 1 y 2, en el tratamiento de control. Realizado a partir de datos de sondas de contenido de agua en suelo (datos del año 2014,Capítulo IV).

que demuestra, por un lado, que el estado nutricional esta muy ligado al estado hídrico de la planta y, por otro, indica las limitaciones del análisis foliar como indicador de estado nutricional (Shellie y Brown, 2012) e indirectamente de la calidad, puesto que los contenidos de nutrientes no se correlacionaron con los parámetros de calidad de uvas y vino.

El perfil de aminoácidos del vino fue afectado significativamente por los tratamientos de manejo del dosel y el suelo, lo que reafiema la utilidad de esta técnica de cultivo para adaptar la planta a diferentes condiciones de disponibilidad de agua. La tendencia general de los resultados señaló que el estrés hídrico puede tener un papel importante en esta respuesta (en efecto, en contenido de prolina en mosto fue mucho mayor en condiciones pronunciadas de estrés hídrico). En conjunto, los resultados indican que el manejo del dosel foliar influye sensiblemente en la calidad sensorial del vino, en parte modulando la disponibilidad de agua en la fase de maduración de la uva, conctityendo una técnica eficaz para la adaptación del cultivo a diferentes condiciones de reserva de agua en el suelo, con el objetivo de alcanzar la mejor calidad.

Los diferentes enfoques que se han utilizado en esta tesis para analizar la eficiencia de uso de agua demuestran la necesidad de abordar su evaluación desde una perspectiva más amplia que la propiamente derivada del agua. Los efectos de la nutrición, de la interacción agua-nutrición y de las operaciones de manejo del foliar son ejemplos de la acción concomitante de diversos factores sobre la eficiencia de uso del agua, tanto a escala fisiológica como productiva y cualitativa. Los diferentes indicadores utilizados han mostrado ser útiles para alcanzar diferentes objetivos en diferentes ámbitos de la investigación en frutales. También, en el caso de la fruticultura, los resultados muestran claramente que es necesario prestar una atención especial en la evaluación de la eficiencia del agua incorporando los aspectos económicos de la calidad, como factor básico para la valorización de las producciones y de los recursos, tanto los impuestos como los medioambientales.

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Conclusiones

e interés para investigaciones futuras

Conclusiones

De acuerdo con los diferentes resultados presentados y discutidos, se ha llegado a las siguientes conclusiones principales:

1. La proporción de isótopos estables de carbono ($\delta^{13}C$) en el endocarpio y mesocarpio de los frutos de melocotonero resultó ser un indicador fiable del estado hídrico de las plantas en la fase II y la fase III de desarrollo del fruto, mostrando ser interesante como índice de eficiencia de uso de agua del cultivo. Como contrapartida, la relación de isótopos estables de carbono en hojas no mostró ser un indicador robusto para estos propósitos.

2. El riego deficitario controlado, aplicado en cualquiera de las fases de crecimiento del fruto, disminuye el intercambio gaseoso de las hojas y el potencial hídrico del tallo durante los períodos en el que se produjo el déficit. No obstante, durante la fase de crecimiento expansivo del fruto, se hizo patente la interacción entre el riego (en condiciones de estrés moderado)) y el nitrógeno, indicando un efecto diferencial del nitrógeno aplicado a dosis altas, que promueve un descenso acusado del intercambio gaseoso de las hojas que puede afectar el rendimiento final del cultivo.

3. Los indicadores de eficiencia de uso de agua se muestran relacionados con el nitrógeno. Tanto los índices de eficiencia de agua medidos a partir del intercambio de gases de la hoja como los índices de eficiencia productiva, fueron mayores en las plantas que recibieron dosis altas de nitrógeno, promoviendo un mayor rendimiento del proceso fotosintético. La debilidad de la relación entre los índices de eficiencia medidos en hojas y la eficiencia en términos productivos se asoció al efecto del número de frutos y al desarrollo del dosel de las plantas, los que se revelan como factores decisivos en la respuesta productiva potencial de la planta al agua y el nitrógeno.

4. El riego deficitario del melocotonero en la fase de endurecimiento del endocarpio se muestra interferido por la variabilidad y la importancia de las lluvias primaverales. En estas condiciones, esta estrategia no llegó a promover una alta productividad del agua en términos de rendimiento de frutos y la calidad de estos. No obstante, la eficiencia de uso de agua se incrementó en términos de indicadores fisiológicos en hoja (fotosíntesis neta, conductancia estomática y la transpiración.

5. La relación entre los isotopos estables de N ($\delta^{15}N$) en el mesocarpio de los frutos se relacionó estrechamente con la disponibilidad de N aplicado y el origen de éste. Sin embargo, la relación de isótopos de nitrógeno no mostró una buena capacidad discriminativa cuando se estudiaron las influencias de agua y nitrógeno en la respuesta del cultivo.

6. La eficiencia de uso de agua, evaluada a través de las relaciones isotópicas de carbono y oxígeno en la madera del tronco, fue asociada con la disponibilidad de agua y de nitrógeno en el caso de carbono, y a la transpiración del dosel en el caso de oxígeno. Sin embargo, ambos indicadores proporcionan poca información concreta y útil sobre la eficiencia productiva, probablemente debido a la metodología de muestreo, que impidió la discriminación de los

efectos diferenciales del estado hídrico en los diferentes períodos del crecimiento de la planta y los frutos.

7. La alta productividad del agua está estrechamente vinculada a su gestión durante todo el ciclo de vida de la planta. No parece apropiado abordar el análisis de la eficiencia de uso de agua de forma independiente, en algúna de las diferentes etapas de crecimiento del fruto. El riego completo de árboles con un dosel bien desarrollado y con una alta carga de frutos se relaciona positivamente con el rendimiento y la eficiencia productiva del agua, mientras que ello no es posible en plantas que presentan restricciones sistemáticas de crecimiento debidas a la restricción de agua, puesto que provocan una reducción crónica del dosel y la disminución de la eficiencia fotosintética, afectando negativamente la eficiencia del agua a medio y largo plazo. En consecuencia, en condiciones de carga de frutos alta, el riego restrictivo en la fase de endurecimiento del endocarpio disminuye el rendimiento potencial esperable. El riego deficitario moderado (hasta 70%ETc) en la fase de crecimiento expansivo de los frutos aparece como más eficiente y puede aportar mayor flexibilidad en cuanto a su capacidad de adaptación a las restricciones extemporáneas de este recurso.

8. La actividad de la enzima o-polifenol-oxidasa en frutos de melocotonero se incrementa con la aplicación de nitrógeno, aunque es indetectable mediante los métodos convencionales de determinación de calidad de cosecha o por diagnóstico visual. La actividad de la enzima es detectable durante el período de maduración y durante la conservación en frío. Los frutos que proceden de àrboles cultivados con dosis moderadas de nutrientes presentan una baja actividad enzimática, lo que puede facilitar la gestión de la postcosecha y mejorar la durabilidad de la fruta. Por el contrario, las dosis más altas de nutrientes aseguran mayores rendimientos de fruta, pero con actividades de la enzima muy superiores, que pueden comprometer su durabilidad del fruto.

9. La actividad *o*-polifenol-oxidasa se correlacionó estrechamente y de forma positiva con el contenido de nitrógeno en las frutas. Paralelamente, el incremento de nitrógeno conllevó variaciones en el contenido de Ca. El ratio N/Ca, poco empleado en melocotón, aparece como un buen indicador de actividad de la PPO. En igual sentido, el contenido elevado de nitrógeno se correlacionó con una pérdida de la calidad organoléptica de los frutos, valorada mediante su composición de componentes volátiles.

10. La interacción entre la disponibilidad de agua en el suelo y el manejo del dosel foliar de la vid mostró un marcado efecto sobre el estado hídrico de las plantas en el período de maduración, afectando el perfil de aminoácidos del mosto y la calidad sensorial del vino obtenido. En esta situación, ni los índices de calidad en cosecha comúnmente utilizados ni el contenido de nutrientes en hoja mostraron una capacidad aceptable para discriminar las posibilidades cualitativas de las uvas a efectos de vinificación.

11. Los resultados ponen de manifiesto la eficacia de las técnicas de gestión del dosel foliar en vid, para adaptar las plantas a la variabilidad climática interanual y mitigar los efectos combinados del suelo y de la variabilidad estacional de la disponibilidad hídrica de las plantas.

12. Los resultados en conjunto indican la necesidad de evaluar la eficiencia de uso de agua a nivel de cultivo desde una perspectiva más amplia, integrando en la medida de lo posible los

efectos del resto de los factores de producción, más allá de la simple relación entre rendimiento y agua utilizada.

Interés en futuras investigaciones

Los resultados obtenidos en los diferentes campos estudiados permiten apuntar algunas indicaciones para definir las líneas de investigación futuras.

- La determinación de las relaciones isotópicas en tejidos específicos de los frutales (frutos y leño) muestran la posibilidad de conocer algunos efectos derivados de las situaciones de estrés o del origen del nitrógeno absorbido. En esta línea, profundizar en el conocimiento de la relación isotópica de nitrógeno como indicador de eficiencia de uso de este nutriente, y de la interacción NUE x WUE (eficiencia de uso de nitrógeno-eficiencia de uso de agua) puede aportar un conocimiento más preciso de las posibilidades de gestión de estos dos recursos.

- La determinación de la actividad *o*-polifenol-oxidasa (PPO) se muestra como un indicador interesante de calidad de la fruta. sin embargo, los métodos más comunes para la evaluación de la actividad son complicados y pueden llegar a ser inabordables a nivel de la gestión de la producción o de la industria de conservación-transformación. La puesta a punto de un método más simple y confiable para analizar la actividad PPO podría constituir un progreso interesante en este campo.

- El 1-metilciclopropeno (1-MCP) es un potente inhibidor de etileno ampliamente utilizado en la conservación de frutas. Las últimas investigaciones acerca de este compuesto indican unos marcados efectos sobre el balance de compuestos oxidantes/antioxidantes en el fruto y, en consecuencia, sobre la senescencia del mismo. Se ha demostrado que PPO es afectada por el 1-MCP, sin que este claro si se trata de una acción inhibitoria temporal o existe algún grado de desactivación de la enzima. En esta línea, puede ser interesante profundizar en la evolución de PPO en frutos conservados con 1-MCP y su relación con la *shelf-life* de los mismos.

- La relación isotópica de carbono ha sido utilizada en viticultura, mostrando una clara relación con el estado hídrico de las plantas. Esta relación se ha determinado en hojas, sarmientos y frutos enteros sin mostrar relaciones claras con la calidad de las uvas o los vinos. En la misma línea que la expuesta en la tesis, la determinación de la relación isotópica de carbono en diferentes partes del fruto puede producir más información para comprender los efectos del momento y la intensidad del estrés hídrico, además de posibilitar el reconocimiento de las variables más influyentes en esta respuesta. Ello puede dar lugar a la interpretación de esta relación isotópica como un índice de zonificación.

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